Textbook of Autoinflammation

Philip J. Hashkes
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Preface

The innate immune system pervades the whole of human life, in all its aspects, and defects in it may have consequences for all organ systems. This makes the autoinflammatory disorders very complex and challenging, with variable clinical presentations and sometimes devastating consequences.

In the two decades since the term 'autoinflammation' was first coined, tremendous strides have been made in both the clinical field and the basic science of this fascinating new area that involves many disciplines including immunology, rheumatology, dermatology and genetics. With the coming of age of the field comes the need for a textbook as a resource to pull together the current knowledge and insights. This is a rapidly changing and developing area of research, with new disorders and pathogenic mechanisms described every year. We hope this book provides a much-needed background to encourage such developments.

This textbook will provide the clinician with detailed clinical information on the monogenic as well as some of the more complex or polygenic autoinflammatory disorders. In addition it provides background information on the cellular, immunologic and genetic mechanisms underlying many of these disorders. The textbook also contains chapters that are meant to give the clinician tools on how to approach patients with a suspected autoinflammatory disorder and how to monitor their course. We have included chapters on genetics, diagnosis, therapeutics and management in general. For basic scientists interested in the field, this book aims to provide a resource which highlights connections between different areas of autoinflammation and gives insight into the consequences of perturbations of the innate immune system in patients, and the relationship with other disorders of the immune system.

We thank the international experts, many of whom are the pioneers and leaders in the field of autoinflammation, who have contributed to this first edition by providing 'state-of-the-art' chapters in their field of expertise.

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Part I

Introduction

Autoinflammation: Past, Present, and Future

1

Daniel L. Kastner

Abstract

The concept of autoinflammation arose from the recognition of monogenic disorders with seemingly unprovoked inflammation without the high-titer autoantibodies or antigen-specific T cells seen in classic autoimmune diseases. During the first decade of the 'autoinflammatory era', a clear connection was established between autoinflammatory disease and the innate immune system, with targeted therapies providing a powerful affirmation of mechanistic hypotheses. Although the 'inflammasomopathies', which are associated with marked interleukin (IL)-1β production, were some of the earliest recognized autoinflammatory diseases, it soon became clear that autoinflammation can be caused by a variety of genetic lesions affecting a range of innate immune pathways, including nuclear factor kappa B (NF-κB) activation and type I interferon production. The advent of next-generation sequencing has resulted in the discovery of multiple new diseases, genes, and pathways, while genome-wide association studies (GWAS) have shed light on the pathogenesis of genetically complex autoinflammatory diseases, such as Behçet disease. During the next decade, the universe of autoinflammatory diseases will continue to expand, but it is likely that distinctions between clinical disease and normal variation will blur, and that treatments developed for autoinflammation will be applied to a much broader range of human illnesses.

Keywords

Autoinflammation · Innate immunity · Inflammasome · Interleukin (IL)-1β · Type I interferon · Next-generation sequencing · Genome-wide association study (GWAS) · Mosaicism · Nomenclature · Targeted therapy · Aphthous ulcers

Abbreviations

CAPS	Cryopyrin-associated periodic syndromes
CINCA	Chronic infantile neurologic cutaneous and articular syndrome
CNO	Chronic non-bacterial osteomyelitis
CRMO	Chronic recurrent multifocal osteomyelitis
DIRA	Deficiency of interleukin-1 receptor antagonist

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FMF	Familial Mediterranean fever
GWAS	Genome-wide association studies
HIDS	Hyperimmunoglobulinemia D with periodic fever syndrome
IL	Interleukin
ISSAID	International Society for Systemic Autoinflammatory Diseases
MKD	Mevalonate kinase deficiency
MWS	Muckle-Wells syndrome
NF-κB	Nuclear factor kappa B
NLR	Nucleotide-binding domain, leucine-rich repeat
NLRP3	NLR family, pyrin domain containing 3
NOMID	Neonatal-onset multisystem inflammatory disorder
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PAPA	Pyogenic arthritis, pyoderma gangrenosum and acne
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
SAVI	STING-associated vasculopathy with onset in infancy
SIFD	Sideroblastic anemia with immunodeficiency, fevers, and developmental delay
STING	Stimulator of interferon genes
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic syndrome

Key Points

- **The autoinflammatory diseases were initially recognized for seemingly unprovoked inflammation, but were soon discovered to be disorders of innate immunity**
- **Next-generation sequencing has led to an explosion of discovery of monogenic autoinflammatory diseases and newly recognized innate immune pathways**
- **Genome-wide association studies (GWAS) provide insight into the etiology of genetically complex autoinflammatory diseases**
- **In addition to continued discovery of new diseases, genes, and pathways, the next decade promises to draw connections between autoinflammatory diseases and the 'range of normal' phenotypes, and to apply the treatments developed for autoinflammatory diseases to a broad spectrum of illnesses**

1.1 'Ancient' History

For over a century, medical science has been fascinated with the questions of if, when, and how the immune system might turn against its host. At the beginning of the twentieth century, the Nobel Prize-winning immunologist Paul Ehrlich proposed the concept of *horror autotoxicus* to argue that the consequences of autoimmunity would be so dire that an organism would have multiple mechanisms in place to prevent self-reactivity from ever happening [1]. However, the subsequent decades provided ample evidence that there are in fact numerous human illnesses in which such safeguards break down, giving rise to either systemic or organ-specific autoimmunity. Self-reactive antibodies and T lymphocytes have been implicated in the pathogenesis of many of these disorders.

By the latter half of the twentieth century there remained a group of illnesses characterized by episodes of seemingly unprovoked systemic or localized inflammation, without the apparent involvement of high-titer autoantibodies or antigen-specific T lymphocytes. Astute clinicians recognized that for several of these illnesses, recurrent fevers were a prominent feature, and that they appeared to be hereditary. These included familial Mediterranean fever (FMF), familial Hibernian fever, hyperimmunoglobulinemia D with periodic fever syndrome (HIDS), Muckle-Wells syndrome (MWS), and familial cold urticaria. The advent of the Human Genome Project provided the tools to search for the underlying genes in a hypothesis-neutral, comprehensive fashion known as *positional cloning*, enabling the discovery of previously unknown regulators of immunity gone awry in these illnesses (see Chap. 2).

1.1.1 First Discoveries: The Birth of Autoinflammation

Owing both to its relatively well-defined phenotype and to the availability of the large numbers of families needed for high-resolution genetic mapping, FMF was the first of the recurrent fever syndromes to be analyzed in this way. In the summer of 1997 two independent consortia discovered recessive mutations in the causative gene, *MEFV*, which encodes what was then a novel protein denoted pyrin (or marenostrin) ([2, 3]; see Chap. 16). Although not known at the time, pyrin forms the nucleus of a macromolecular complex (denoted the pyrin inflammasome) that activates interleukin-(IL)1β, IL-18, and the executioner protein gasdermin D in response to certain bacterial toxins ([4, 5]; see Chap. 5). FMF-associated mutations in pyrin lower the threshold for activation. The ~90 N-terminal residues of pyrin constitute a motif that is the prototype for a cognate interaction domain (the PYRIN domain) found in some 20 immune-related human proteins. The discovery of *MEFV* not only fulfilled the promise of positional cloning, but also allowed the unequivocal determination that certain other periodic fever syndromes were not FMF, thus opening up a new area of clinical investigation. In 1999, mutations in *TNFRSF1A,* encoding the 55 kDa tumor necrosis factor receptor, were shown to define a recurrent fever syndrome now called the tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), which subsumed familial Hibernian fever and several other dominantly-inherited fever syndromes seen in multiple ethnicities ([6]; see Chap. 18).

The authors of the paper describing TRAPS proposed the term 'autoinflammatory' to denote what appeared to be an emerging family of illnesses characterized by seemingly unprovoked systemic or localized inflammation, but without the cardinal features of autoimmunity. A year later the concept was refined and extended, with the proposal of a classification scheme that included the recurrent fever syndromes, certain complement disorders (such as hereditary angioedema), familial urticarial syndromes (familial cold urticaria, MWS—see Chap. 19), granulomatous disorders (Blau syndrome—see Chap. 20), metabolic disorders (crystalline arthropathies—see Chap. 34), storage diseases (Gaucher disease, Hermansky-Pudlak syndrome), fibrosing disorders, and Behçet disease ([7]; see Chap. 35). Recognizing the heterogeneity of human disease, this analysis included both monogenic and genetically complex illnesses. However, at this early stage the schema was based solely on the whimsical notion of a family of diseases manifesting unprovoked inflammation without high-titer autoantibodies or antigen-specific T cells, in the absence of more detailed genetic or functional insight.

1.2 The 'Eureka' Decade

During the next decade, two independent lines of investigation converged to corroborate the concept of autoinflammation. On the one hand, the field of human genetics accelerated the discovery of genes underlying the newly recognized autoinflammatory diseases. On the other hand, advances in basic immunology firmly established the role of the innate immune system in host defense [8]. Whereas the adaptive immune system is mediated by lymphocytes with membrane receptors encoded by genes that somatically rearrange and mutate, the evolutionarily more ancient innate immune system utilizes myeloid effector cells with both extracellular and intracellular receptors that are 'hard-wired' in the genome to recognize 'pathogen-associated molecular patterns' (see Chap. 4). Genetics and immunobiology advanced hand-in-hand, with the growing realization that many of the disorders defined clinically as 'autoinflammatory' are caused by genetic mutations that perturb the innate immune system. Disease-gene discoveries provided clinical relevance for innate immunity, and advances in immunology explained newly recognized autoinflammatory illnesses. Highly successful trials of therapies predicted to target the relevant pathways were the heady affirmation of an emerging understanding of a new field of medicine ([9–11]; see Chaps. 41 and 42).

Nowhere was this paradigm more evident than in the elucidation of the cryopyrin-associated periodic syndromes (CAPS). In 2001 Hal Hoffman and his colleagues discovered dominantly-inherited mutations in the gene encoding a PYRIN domain-containing protein (denoted *cryopyrin*) as the cause of both familial cold autoinflammatory syndrome (formerly familial cold urticaria) and MWS ([12]; see Chap. 19). Within a year, two other groups discovered mutations in the same gene as the cause of neonatal-onset multisystem inflammatory disorder (NOMID; also called (mainly in Europe) chronic infantile neurologic cutaneous and articular [CINCA] syndrome), a devastating disorder manifesting chronic aseptic meningitis [13, 14]. All of these diseases are collectively denoted CAPS. Independently and nearly simultaneously, other groups discovered a role for cryopyrin (alternatively termed 'PYPAF1,' 'NALP3,' and now 'NLRP3') in the activation of IL-1β [15, 16]. The late Jürg Tschopp and his colleagues proposed a macromolecular complex they termed the *inflammasome*, one variant of which includes nucleotide-binding domain, leucine-rich repeat (NLR) family, pyrin domain containing 3 (NLRP3), that leads to the autocatalysis of caspase-1 and the release of biologically active IL-1β from leukocytes ([17]; see Chap. 5). CAPS-associated mutations were soon found to cause constitutive activation of the NLRP3 inflammasome, thus suggesting a possible role for IL-1 inhibition in the treatment of CAPS. The life-altering effects of IL-1 inhibition in CAPS have been a triumph of molecular medicine and a true vindication of the importance of IL-1 in human immunobiology [9–11].

1.2.1 Expanding the Discovery of Diseases Caused by Genetic Mutations

The early years of the 'autoinflammatory era' witnessed the discovery of several new disease-causing genes (Table 1.1), the deepening of our understanding of innate immune pathways, and further therapeutic advances. Given the genomic technologies of the time, the new disease gene discoveries were the result of either positional cloning or candidate gene approaches, sometimes suggesting extensions of known innate immune pathways (see Chap. 2). For example, the discovery of loss-of-function mutations in *IL1RN*, encoding the endogenous IL-1 receptor antagonist (a recombinant form of which is anakinra, a biologic used in the treatment of CAPS), causing the disease deficiency of IL-1 receptor antagonist (DIRA), highlighted the need for tight IL-1 regulation in normal homeostasis ([18, 19]; see Chap. 25). The discovery of dominantly inherited mutations in *PSTPIP1*, which encodes a pyrin-binding protein also involved in regulating the cytoskeleton, in pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome [20, 21], suggested a connection between innate immunity and the cytoskeleton that is still under active investigation (see Chap. 22). The discovery of autoinflammatory phenotypes associated with *CARD15/NOD2* (see Chap. 20) and *NLRP12* (see Chap. 29) expanded the spectrum of disorders associated with this large family of NACHT-domain-containing proteins, raising the possibility of even more [22–24]. The discovery of mevalonate kinase (*MVK*) mutations in HIDS [25, 26], now called mevalonate kinase deficiency (MKD) due to this discovery, established a link between metabolism and autoinflammation that has only recently been explained.

1.2.2 Early Thoughts on Pathophysiologic Mechanisms

During this first decade, many of the advances in disease mechanism and treatment centered on IL-1β and related proteins, leading some to suggest an equivalence of autoinflammation with IL-1-mediated disease (see Chaps. 5 and 10). Evidence emerged that the prototypic autoinflammatory disease, FMF, is driven by IL-1β [27], and that uric acid crystals activate the NLRP3 inflammasome, thus supporting the hypothesis that gout, a genetically complex disorder, is also autoinflammatory and driven by

Table 1.1 Timeline of monogenic autoinflammatory disease gene discoveries

Disorder	Gene	Protein	Year	Chapter
FMF	*MEFV*	Pyrin/Marenostrin	1997	16
TRAPS	*TNFRSF1A*	TNFR1	1999	18
HIDS/MKD	*MVK*	Mevalonate kinase	1999	17
CAPS	*NLRP3*	Cryopyrin/NLRP3	2001	19
Blau	*NOD2*	NOD2	2001	20
Cherubism	*SH3BP2*	SH3BP2	2001	25
PAPA	*PSTPIP1*	PSTPIP1	2002	22
Majeed	*LPIN2*	LPIN2	2005	25
Hydatidiform mole	*NLRP7*	NLRP7	2006	27
FCAS2	*NLRP12*	NLRP12	2008	29
Histiocytosis-lymphadenopathy plus	*SLC29A3*	hENT3	2008	NIB
DIRA	*IL1RN*	IL-1 receptor antagonist	2009	25
VEOIBD	*IL10RA, IL10RB IL10*	IL-10 receptor IL-10	2009 2010	21
DITRA	*IL36RN*	IL-36 receptor antagonist	2011	26
JMP/NNS/CANDLE	*PSMB8*	β5i Immunoproteasome	2010–2012	24
CAMPS/PSORS2	*CARD14*	CARD14	2012	26
APLAID	*PLCG2*	PLCγ2	2012	28
HOIL-1 deficiency	*RBCK1*	HOIL-1	2012	28
DADA2	*ADA2 (formerly CECR1)*	ADA2	2014	23
SAVI	*TMEM173*	STING	2014	24
NLRC4-MAS	*NLRC4*	NLRC4	2014	29
SIFD	*TRNT1*	TRNT1	2014	28
TRAPS11	*TNFRSF11A*	TNFRSF11A	2014	29
HOIP deficiency	*HOIP*	HOIP	2015	28
sJIA	*LACC1*	FAMIN	2015	32
PRAAS	*PSMA3, PSMB4, PSMB9; digenic inheritance*	Proteasome components	2015	24
Adult-onset CAPS	*NLRP3*	NLRP3	2015	19, 37
HA20	*TNFAIP3*	A20	2016	29
PAAND	*MEFV*	Pyrin/Marenostrin	2016	29
Vibratory urticaria	*ADGRE2*	ADGRE2	2016	NIB
MSPC/FKLC	*NLRP1*	NLRP1	2016	29
Otulipenia, ORAS	*OTULIN*	OTULIN	2016	29
NAIAD	*NLRP1*	NLRP1	2017	29
PFIT	*WDR1*	WDR1	2017	28
PRAID	*POMP*	POMP	2018	NIB

Diseases: *FMF* Familial Mediterranean fever, *TRAPS* Tumor necrosis factor receptor associated periodic syndrome, *HIDS* Hyperimmunoglobulinemia D with periodic fever syndrome, *MKD* Mevalonate kinase deficiency, *CAPS* Cryopyrin-associated periodic syndromes, *PAPA* Pyogenic arthritis, pyoderma gangrenosum and acne, *FCAS2* Familial cold autoinflammatory syndrome 2, *DIRA* Deficiency of IL-1 receptor antagonist, *VEOIBD* Very-early onset inflammatory bowel disease, *DITRA* Deficiency of IL-36 receptor antagonist, *JMP* Joint contractures, muscle atrophy, microcytic anemia and panniculitis-induced lipodystrophy syndrome, *NNS* Nakajo-Nishimura syndrome, *CANDLE* Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *CAMPS* Caspase activation and recruitment domain (CARD) 14 mediated psoriasis, *PSORS2* Psoriasis susceptibility locus 2, *APLAID* Autoinflammatory PLCγ2-associated antibody deficiency and immune dysregulation, *HOIL-1* Heme-oxidized IRP2 ubiquitin ligase 1, *DADA2* Deficiency of adenosine deaminase 2, *SAVI* Stimulator of

(continued)

Table 1.1 (continued)

interferon genes (STING)-associated vasculopathy with onset in infancy, *NLRC4-MAS* NLRC: Nucleotide oligomerization domain (NOD)-like receptor family CARD domain-containing protein 4-macrophage activation syndrome, *SIFD* Sideroblastic anemia with immunodeficiency, fevers, and developmental delay, *TRAPS 11* TRAPS due to mutations in *TNFRSF11A*, *HOIP* HOIL-1 interacting protein, *sJIA* systemic juvenile idiopathic arthritis, *PRAAS* Proteasome-associated autoinflammatory syndromes, *HA20* A20 haploinsufficiency, *PAAND* Pyrin-associated autoinflammation with neutrophilic dermatosis, *MSPC* Multiple self-healing palmoplantar carcinoma, *FKLC* Familial keratosis lichenoides chronica, *ORAS* Otulin-related autoinflammatory syndrome, *NAIAD NLRP1*-associated autoinflammation with arthritis and dyskeratosis, *PFIT* Periodic fever, immunodeficiency and thrombocytopenia, *PRAID* Proteasome maturation protein (POMP)-related autoinflammation and immune dysregulation disease

Proteins: *TNFR1* Tumor necrosis factor receptor 1, *NLRP* Nucleotide oligomerization domain (NOD)-like receptor family, leucine rich repeat, pyrin domain, *SH3BP2* SH3 binding protein 2, *PSTPIP* Proline-serine-threonine phosphatase interacting protein, *LPIN2* Lipin 2 gene symbol, *hENT3* Human equilibrative nucleoside transporter-3, *IL* Interleukin, *CARD* Caspase activation and recruitment domain, *PLCγ2* Phospholipase Cγ2, *HOIL-1* Heme-oxidized IRP2 ubiquitin ligase 1, *ADA2* Adenosine deaminase 2, *STING* Stimulator of interferon genes, *NLRC* Nucleotide oligomerization domain (NOD)-like receptor family CARD domain-containing protein, *TRNT* tRNA nucleotidyltransferase, *TNFRSF11A* TNF receptor superfamily 11a, *HOIP* HOIL-1 interacting protein, *FAMIN* Fatty acid metabolic immune nexus, *ADGRE2* Adhesion G protein-coupled receptor E2, *WDR1* WD domain repeat containing protein 1, *POMP* Proteasome maturation protein

NIB *Not in book*

IL-1β ([28]; see Chap. 34). Nevertheless, even during this early era there was mounting evidence for other molecular mechanisms, such as nuclear factor kappa B (NF-κB) activation in Blau syndrome ([29]; see Chap. 20). This is not surprising, given the broad scope of innate immune sensing and signaling. As was noted a decade ago, the autoinflammatory diseases are a sampling from the universe of natural variation in the innate immune system that is severe enough to cause illness, but not so severe to be embryonic lethal [30]. The ensuing decade has given us a glimpse of just how diverse a universe this is.

1.3 *Horror Autoinflammaticus:* The Golden Age of Autoinflammation

The second decade of the autoinflammatory era began in 2009 with the publication of '*Horror Autoinflammaticus:* The Molecular Pathophysiology of Autoinflammatory Disease,' a comprehensive review of the field that proposed a classification scheme based on molecular insights garnered to that point ([30]; see Chap. 10). Autoinflammation had come of age. Building on this foundation, the last decade has witnessed a genomic explosion, catalyzed in large part by a revolution in next-generation sequencing technology that has led to drastic reductions in costs and a concomitant boom in the availability of whole-exome and now whole-genome sequencing (see Chap. 2). The number of monogenic autoinflammatory diseases has gone up dramatically, shedding light on new innate immune pathways and disease mechanisms. While the cases have become ever rarer, they are 'experiments of nature' by which, as Sir William Harvey noted four centuries ago, "Nature is nowhere [more] accustomed to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path" [31].

1.3.1 New Discoveries of Rare Mongenic Autoinflammatory Diseases

Some of the newly recognized disease-causing genes encode known innate immune sensors for which a monogenic human disease had not already been discovered. *NLRC4* encodes the lynchpin of an inflammasome that senses bacterial flagellin; gain-of-function mutations have now been shown to cause colitis, a CAPS-like spectrum, and an increased risk of macrophage activation syndrome (MAS) ([32, 33]; see Chap. 29). *NLRP1* encodes a

protein that nucleates the main inflammasome in the skin; activating mutations were shown to cause dyskeratosis with or without arthritis ([34, 35]; see Chap. 29). *TMEM173* encodes the stimulator of interferon genes (STING), a major sensor of intracellular double-stranded DNA; *de novo* gain-of-function mutations are now known to cause vasculopathy, peripheral gangrene, and interstitial fibrosis (STING-associated vasculopathy with onset in infancy, SAVI) ([36]; see Chap. 24). *MEFV* encodes pyrin, the protein mutated in FMF; mutations in a critical phosphorylation site have been shown to cause a dominantly-inherited chronic neutrophilic dermatosis termed pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) ([37]; see Chap. 29).

In other cases, next-generation sequencing has led to the identification of genes defining entirely new mechanisms of innate immune regulation. *WDR1* encodes a protein that regulates the actin cytoskeleton; loss-of-function mutations lead to activation of the pyrin inflammasome and increased IL-18 production ([38]; see Chap. 28). *ADA2* (formerly *CECR1*) encodes what is thought to be a growth factor expressed in myeloid cells; loss-of-function mutations cause recurrent fevers, early-onset strokes, vasculopathy, and sometimes bone marrow failure and immunodeficiency ([39, 40]; see Chap. 23). *PSMB8* encodes a component of the immunoproteasome that degrades K48-ubiquitinated proteins; biallelic loss-of-function mutations cause a syndrome of fevers, panniculitis, and lipodystrophy ([41–44]; see Chap. 24). *TNFAIP3*, *OTULIN, HOIL-1*, and *HOIP* encode proteins that regulate ubiquitination, a major form of post-translational protein modification. Haploinsufficiency of *TNFAIP3* or biallelic loss-of-function mutations at the other three loci cause a spectrum of autoinflammatory phenotypes ([45–48]; see Chap. 29). *PLCG2* encodes a signaling molecule expressed in hematopoietic cells; heterozygous gain-of-function missense mutations cause an autoinflammatory syndrome of rash, ocular inflammation, mild immunodeficiency, and interstitial lung disease ([49]; see Chap. 28). *ADGRE2* encodes a membrane mechanosensor expressed on mast cells; heterozygous loss-of-function mutations in an autoinhibitory domain cause vibratory urticaria [50]. *LACC1* encodes a key regulator of metabolism in macrophages; biallelic loss-of-function mutations cause a monogenic form of systemic juvenile idiopathic arthritis ([51]; see Chap. 32). Perhaps most surprising of all, *TRNT1* encodes a ubiquitously expressed enzyme that adds the 3-nt CCA sequence to the 3′ ends of all tRNA molecules. Biallelic hypomorphic mutations cause an autoinflammatory syndrome denoted sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD) ([52]; see Chap. 28).

1.3.2 Expanded Understanding of Disease Pathophysiology Related to the Innate Immune System and Novel Genetic Mechanisms

Over the last decade there have also been substantial advances in our understanding of the biology of innate immunity and in targeted therapies, although, not surprisingly, these have not kept pace with new disease gene discoveries. It is no secret that the timeline for functional and mechanistic analysis is much slower than for monogenic disease gene discovery, especially in the world of next-generation sequencing and large clinics dedicated to undiagnosed autoinflammatory patients. As a case in point, it took almost 20 years to understand the role of pyrin in the sensing of bacterial toxins that inactivate RhoA and the pathway by which the pyrin inflammasome is activated [4, 53]. It took an even longer time to discover gasdermin D and its role in IL-1β release from leukocytes [54–56]. Nevertheless, the advances of the last decade have made it abundantly clear that, notwithstanding the great importance of IL-1 in human biology, there is much more to autoinflammation than this cytokine. For example, the type I interferons play a central role in the pathogenesis of several autoinflammatory diseases, such as SAVI and PRAAS [36, 57], and targeted therapies with JAK inhibitors show great promise in a number of these disorders ([58]; see Chap. 24).

The last decade has witnessed not only a dizzying expansion in the *quantity* of monogenic diseases and innate immune pathways, but new *qualitative* insights into broader mechanisms of human disease, driven by the study of autoinflammation. Of extraordinary potential impact is the careful documentation of somatic mosaicism (see Chaps. 2 and 12) not only in infantile-onset forms of NOMID/CINCA [59] but also in adult-onset CAPS and Schnitzler syndrome ([60, 61]; see Chap. 37). We simply do not know how many adult-onset cases of (nonmalignant) unexplained recurrent fever and/or autoinflammation are due to somatic mutations, but the precedent of cancer teaches us that such events are not rare. Of similar general import is the recent documentation of digenic inheritance (see Chap. 12) in the proteasome-associated autoinflammatory syndromes (PRAAS) [57]. Consideration of the multimolecular proteasome complex gave rise to the hypothesis of digenic inheritance in unexplained cases of PRAAS, but it is eminently possible that similar gene-gene interactions are operative in other multistep pathways, offering potential explanations for unsolved cases (see Chap. 24). Finally, and not surprisingly, with the discovery of ever more genes underlying monogenic autoinflammation, there are now an increasing number of cases in which there is an overlap among autoinflammation, autoimmunity, and immunodeficiency ([47, 48]; see Chaps. 28 and 38). In the case of the ubiquitination disorders, this has been shown to be due to the differential effects of regulatory events in multiple cell types. It would be absurd to believe that such overlaps would not be found.

1.3.3 Expansion of Autoinflammation to Non-monogenic and Common Diseases

Nearly since the outset, it has been clear that not all of the illnesses that fit under the autoinflammatory rubric are monogenic. As noted above, some are now known to exhibit a digenic mode of inheritance, but still others are genetically complex. The latter include Behçet disease (see Chap. 35), systemic juvenile idiopathic arthritis, adult-onset Still disease (see Chap. 32), chronic nonbacterial osteomyelitis (CNO), previously called chronic recurrent multifocal osteomyelitis (CRMO) (see Chap. 31), the syndrome of periodic fever with aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) (see Chap. 30), the crystalline arthropathies (see Chap. 34), sarcoidosis, fibrosing diseases, and, by some definitions, atherosclerosis, type 2 diabetes, cancer, and neurodegenerative diseases (see Chap. 39). Probably the best-studied is Behçet disease, which presents with the classic triad of painful oral ulcers, ocular inflammation, and genital ulcers. Advances in genotyping chips have begun to shape our understanding of genetically complex autoinflammatory diseases. Through the careful collection of well-phenotyped patients and ethnically-matched controls, combined with genome-wide association studies (GWAS) and targeted deep-resequencing, a total of 17 susceptibility loci for Behçet disease have been identified: *HLA-B*51, ERAP1, IL10, IL23R, STAT4, CCR1-CCR3, KLRC4, CEBPB-PTPN1, ADO-EGR2, IRF8, RIPK2, LACC1, FUT2, IL12A, MEFV*-p.Met694Val, *IL1A-IL1B,* and *TNFAIP3* [62–66]. Although it often has been observed that most GWAS 'hits' confer relatively little risk to disease susceptibility in any given individual, there nevertheless is a remarkable convergence among GWAS studies in immune diseases, suggesting commonalities in pathogenesis among disorders, and the possibility of targeted therapies. GWAS studies of Behçet disease indicate a role for adaptive immunity (given the remarkable epistasis between *HLA-B*51* and *ERAP1*), shared pathogenesis with spondyloarthropathies and certain infectious diseases, and the possibility of therapies targeting the IL-23 axis (see Chap. 38). As noted below, GWAS also draws a shocking but totally logical connection between Behçet disease and everyday life.

1.4 Nomenclature of the Autoinflammatory Diseases

As a consequence of the burgeoning list of autoinflammatory diseases, there are now vigorous discussions about nomenclature and nosology. Since language is very much a matter of

convention, it would be presumptuous for one individual to impose any specific naming scheme. In any area of discourse, history matters, and thus it would be difficult to advocate against terms like 'familial Mediterranean fever,' regardless of whether all cases are familial, or Mediterranean, or exhibit fever, simply because FMF is thoroughly entrenched in our lexicon. For similar reasons, it is sometimes difficult to dislodge firmly established eponyms. Nevertheless, going forward I do subscribe to the view that eponyms should be avoided, so as not to torment our junior colleagues with a litany of people who didn't actually have the diseases attached to their names. Instead, I favor disease names and classification schemes that reflect the underlying biology, whether that is best reflected in a gene name or the name of its encoded protein – or even a pathway ('inflammasomopathy,' 'interferonopathy')—rather than a string of clinical manifestations that spell out a memorable acronym. Just as we classify and name infectious diseases by their causative microorganisms, so too should we classify and name autoinflammatory diseases according to their underlying etiology. Such a schema shapes our thinking, stimulates hypotheses, and suggests targeted therapies. As noted above, as we learn more there will be an inevitable blurring of the boundaries between *autoinflammatory* and *autoimmune* or *immunodeficiency* (see Chaps. 28 and 38). That is simply the nature of nature, and any useful schema will need to deal with it. The responsibility for establishing naming conventions should rest with the community that uses them most. In this particular case, that is probably the International Society for Systemic Autoinflammatory Diseases (ISSAID) or its designees.

1.5 *Quō vādis?* Autoinflammation and the Human Condition

The third decade of the autoinflammatory era will begin auspiciously with the publication of this, the first medical text on autoinflammation. Anticipating what is in store for this next decade, it is fitting to recall the observation of the twentieth century American 'philosopher', the baseball player Yogi Berra: "It's tough to make predictions, especially about the future." Nevertheless, the developments of the last 10 years likely foreshadow the next ten, and so it would be reasonably safe to predict more disease genes, more pathways, more biology, and more targeted therapies. There has been no evidence that we are approaching an asymptote in new discoveries in this arena, and it is likely that as we peel the onion we will be greeted with successive layers of regulatory complexity. There is nothing wrong in prognosticating 'more of the same' for the next decade. And it would be grand.

However, two recent advances augur additional more profound tectonic shifts. The first is an abstract presented by the direct-to-consumer genomic testing company 23andMe at the 2017 annual meeting of the American Society of Human Genetics [67]. This abstract presented a GWAS of canker sores/aphthous ulcers in 178,409 affected individuals and 66,609 controls. Individuals were scored as affected through their response to a questionnaire ("Have you ever had a canker sore [an open sore on the soft tissue inside the mouth]? Yes/No/Not sure"). There was no medical or dental examination, no review of medical records. Remarkably, 47 loci reached genome-wide significance, including 8 loci known to be associated with Behçet disease (*IL10, STAT4, CCR3, IL12A, RIPK2, NOD2, IRF8, CEBPB*). Whereas the 23andMe study had very large numbers of subjects but little opportunity for clinical observation, the studies of Behçet disease were roughly 100 times smaller, but relied on meticulous phenotyping. The fact that there was significant overlap between the two studies suggests that, at least for some phenotypes, a yes-no questionnaire applied to many subjects may reach the same conclusions as a careful clinical study of a much smaller number of subjects. The overlap between the two GWAS studies also suggests that some of the same loci that confer susceptibility to severe diseases may also confer susceptibility to more common, 'every day' problems like canker sores. It is tempting to speculate that the loci that were *not* in common between the two studies (such as *HLA-B*51, ERAP1*, and *IL23R*), determine who gets Behçet disease rather than simple canker sores. It is also possible that other disorders manifesting with oral ulcers may share some of these susceptibility loci, and that the knowledge

of these loci will eventually lead to targeted therapies for aphthae.

With the increasing dissemination of genomic sequencing and genotyping across the population, and the advent of large cohort studies such as the *All of Us* Research Program, it will be increasingly possible to connect genes and loci associated with autoinflammatory diseases with phenotypes that we would consider in the range of normal experience. While the experience to date with targeted therapies for rare autoinflammatory diseases could certainly be considered to be personalized or precision medicine, the more universal approach will take the field to an entirely new level.

A second advance was the publication, in August 2017, of two papers summarizing the initial results of a randomized, double-blind, placebo-controlled trial of canakinumab (a human monoclonal anti-IL-1β antibody) in 10,061 subjects with a previous myocardial infarction and an elevated C-reactive protein level of 2 mg or more per liter. In a paper published in the *New England Journal of Medicine,* canakinumab at a dose of 150 mg given every 3 months significantly lowered the rate of recurrent cardiovascular events, relative to placebo, regardless of lipid-level lowering ([68]; see Chap. 39). The same research group simultaneously published a paper in *The Lancet* demonstrating reductions in lung cancer and total cancer mortality among subjects treated with canakinumab in the same clinical protocol ([69]; see Chap. 39). Together, these papers suggest an important role for inflammation in both cardiovascular disease and cancer, and the possibility of therapies targeting innate immunity in preventing or treating these common illnesses.

These two advances promise a much greater role for autoinflammation in the general human condition. Not only will the boundaries blur between autoinflammation and autoimmunity or immunodeficiency, but the boundaries between health and disease will also blur.

1.6 Questions for the Next Decade

There also remain a number of questions for the field to address in the next decade. Ten of those I consider of primary importance are listed below:

1. What accounts for the intermittent nature of many of the autoinflammatory diseases?
2. What is the molecular basis of phenotypic heterogeneity among individuals with the same or similar genotypes?
3. What is the penetrance of monogenic autoinflammatory variants in the general population?
4. To what extent does somatic mutation explain late-onset autoinflammatory disease (see Chaps. 2 and 12)?
5. What is the role of the microbiome in autoinflammatory disease?
6. To what extent do epigenetic factors (see Chap. 3) influence the course of monogenic and genetically complex autoinflammatory disorders?
7. How do the various inflammasomes differ in their processing of IL-1β, IL-18, and gasdermin D, and how do these differences correlate with disease phenotype (see Chaps. 5 and 6)?
8. To what extent do monogenic diseases inform our understanding of genetically complex autoinflammatory diseases (see Chap. 38)?
9. How will disease discovery evolve with new technologies, such as whole genome sequencing (see Chap. 2) and metabolomics?
10. What will be the relative roles of biologics, small molecules, and bone marrow transplantation in the therapy of these illnesses (see Chap. 42)?

It is an exciting time to be working in the field of autoinflammation. This textbook offers a multidisciplinary approach to a maturing discipline that truly transcends the arenas of internal medicine, pediatrics, genetics and genomics, clinical and basic immunology, and cell biology, and I expect that practitioners and trainees from all of these fields will derive great benefit from its comprehensive and systematic approach. I hope that you, too, will find yourself as captivated as I am, and that this text will be your passport to an exhilarating journey in autoinflammation.

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Part II

Basic Science and Biology of Autoinflammation

2 Genetic Aspects of Investigating and Understanding Autoinflammation

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Abstract

At present, more than 30 different autoinflammatory diseases have been described at molecular and genetic level. The importance of genetic tests to reach a definitive diagnosis has become evident during the past few years. In parallel to the description of these diseases, several technical changes have occurred that have revolutionized the field of human genetics. Ten years ago, the gold-standard method for genetic studies was the Sanger method of DNA sequencing. Currently, studies based on next generation sequencing (NGS) methods are the standard methods in most genetic laboratories around the world. NGS makes it possible to achieve a diagnosis both by analysis of single families with extremely rare conditions, thus identifying new genes, or simultaneous genotyping of multiple genes in groups of patients. Moreover, in the past few years, different insights demonstrated an unexpected role of post-zygotic mutations and gene mosaicism in the pathogenesis of some monogenic autoinflammatory diseases. The availability of NGS methods in the clinics allows detection of (new) monogenic diseases in a growing number of previously undiagnosed patients with no familial history. This has resulted in the increased awareness of the clinical diversity of these diseases, best therapeutic approaches and follow-up schemes for the patients and appropriate genetic counseling for families.

Keywords

Next generation sequencing (NGS) · Gene discovery · Mutation screening · NGS-based gene panel · Comparative genomic hybridization (CGH) · Gene expression · Real-time polymerase chain reaction (rtPCR) · Gene mosaicism · Post-zygotic mutations Amplicon-based deep sequencing

Abbreviations

ACMG	America College of Medical Genetics and Genomics
CAPS	Cryopyrin-associated periodic syndrome
CGH	Comparative genomic hybridization
CINCA	Chronic infantile neurological, cutaneous and articular
CNV	Copy number variations

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DADA2	Deficiency of adenosine deaminase 2
ddNTP	Dideoxynucleotide
DIRA	Deficiency of IL-1 receptor antagonist
DSAP	Disseminated superficial actinic porokeratosis
FCAS	Familial cold autoinflammatory syndrome
FMF	Familial Mediterranean fever
IL	Interleukin
InDels	Insertions or deletions
JMP	Joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome
LPS	Lipopolysaccharide
MKD	Mevalonate kinase deficiency
MWS	Muckle Wells syndrome
NGS	Next generation sequencing
NOMID	Neonatal-onset multisystem inflammatory disease
PCR	Polymerase chain reaction
PID	Primary immunodeficiency diseases
POADS	Postaxial acrofacial dysostosis
SAVI	STING-associated vasculopathy with onset in infancy
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
STING	Stimulator of interferon genes
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic syndrome
VUS	Variant of uncertain significance
WES	Whole exome sequencing
WGS	Whole genome sequencing

Key Points

- **Next generation sequencing (NGS)-based approaches have replaced previous strategies for discovery of new genes, and mutation detection in already known genes responsible for monogenic autoinflammatory diseases**
- **NGS-based gene panels, allowing parallel sequencing of multiple small fragments of any given DNA target, are suitable to diagnose the many genetically diverse but phenotypically overlapping autoinflammatory disorders, though the success rate is still low**
- **Whole exome sequencing and whole genome sequencing, along with array-comparative genomic hybridization (aCGH), real-time polymerase chain reaction (PCR) and other gene expression studies, represent effective means to identify new genes and new pathogenic mechanisms in autoinflammatory disorders**
- **In the last decade, post-zygotic mutations and gene mosaicism have been described in a growing number of patients with several monogenic autoinflammatory diseases, mainly as a result of using NGS-based methods during the routine genetic screening**

2.1 Introduction

Systemic autoinflammatory diseases are a large and heterogeneous group of disorders of the innate immune system. The search for their underlying genetic causative components has been pursued for decades as a means to discover pathogenic mechanisms, a first step to assess possible medication targets and to develop the most effective pharmacological treatments.

The emerging complexity of the different clinical phenotypes within the autoinflammatory spectrum, ranging from isolated periodic fevers to involvement of the gastrointestinal tract, bone, skin, etc, is not fully accounted for by the many genes identified to date. For this reason, the rate of undiagnosed patients is still remarkably high.

The technological developments during the last decades in the methods of genetic investigations have largely contributed to the evolution of the molecular genetic approaches used to understand autoinflammatory disorders. In this chapter we will review the methods currently used in an attempt to analyze the genetic components of the simple Mendelian autoinflammatory disorders.

We will first describe the Sanger sequencing technique, which was critical in detecting gene mutations both in the first autoinflammatory diseases in which disease-causing genes were identified and even today in performing molecular genetic

diagnosis of single genes or among few candidate genes. The next generation sequencing (NGS)-based approaches have become crucial to speed up the identification of new genes and also for simultaneous multiple gene testing, especially in those patients whose phenotype cannot easily be linked to an already recognized autoinflammatory disorder. The role of additional genetic techniques in the diagnostic process is also going to be reported.

Last, somatic mosaicism has emerged as an important pathogenic mechanism, which is able to account for the development of a full clinical picture despite limited proportions of cells bearing the mutation. The latter part of the chapter will be devoted to understanding this important mechanism of inheritance.

A glossary of genetic terms for readers without a genetics background can be found at https://www.genome.gov/glossary.

2.2 Autoinflammatory Diseases: Approaches to Gene Identification

- **Linkage analysis and homozygosity mapping have allowed the positional cloning strategy to drive the discovery of genes responsible for monogenic autoinflammatory diseases in the past decades**
- **Mutation search in candidate genes has been carried out through Sanger sequencing and more recently by the next generation sequencing (NGS) technology, allowing parallel sequencing of multiple small fragments of any given DNA target**
- **NGS-based gene panels are appropriate to diagnose the many genetically diverse but phenotypically overlapping autoinflammatory disorders. However, the success rate is still low**
- **Whole exome sequencing data can be limited to *in silico* panels, thus avoiding many variants of unknown significance and unwanted detection of secondary findings, and, if no causative variant is found, the entire exome can still be analyzed**

2.2.1 Experimental Methods Used in the Pre-NGS Era

The "positional cloning" strategy has driven the discovery of genes responsible for monogenic diseases for years, before the advent of the NGS-based technologies. To this end, genome-wide genetic linkage analysis was used to identify chromosomal regions involved in the diseases under study. After narrowing the intervals and cloning the chromosomal segments of interest, the disease genes were eventually identified, followed by detection of specific mutations. Despite the great importance and significance that this methodologic approach has had in the discovery of genes causing familial Mediterranean fever (FMF) [1, 2], tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) [3], mevalonate kinase deficiency (MKD) [4, 5], and cryopyrin-associated periodic syndromes (CAPS) [6], such a labor intensive and time consuming strategy has slowly been abandoned, to be replaced by more accurate and powerful techniques [7].

The "homozygosity mapping" approach continues to be successfully applied to the present to complement the search for genes responsible for recessively inherited diseases. Homozygosity mapping relates to the identification of disease causing gene regions in consanguineous families, or in populations subjected to founder effects, where affected individuals are likely to have two "replication" copies of the disease allele, as well as additional identical alleles located near the disease locus from a common ancestor. Rare recessive traits can therefore be identified through regions of homozygosity that are shared by different affected individuals. Informative polymorphic markers have been used to perform homozygosity mapping in the case of FMF [1, 2] and Majeed syndrome [8]. More recently, the genotyping methods have evolved and single nucleotide polymorphism (SNP) arrays have led to the discovery of the deficiency of interleukin (IL)-1 receptor antagonist (DIRA) [9, 10] and the joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome (JMP) [11].

When a linkage based approach cannot be applied to identify a disease-causing gene, for instance in the case of a limited number of available families, the analysis of candidate genes can serve as a possible alternative. Candidate genes can be selected based on different criteria, such as genes whose functions is related to the phenotype, within an already identified chromosomal interval, as in the case of TRAPS [3]; the absence or reduction of enzymatic activity, or any other measurable disease marker, as in the case of MKD [5]; the response to a known treatment, as in the case of DIRA [9] and the homology with genes belonging to the same family and involved in similar autoinflammatory disorders, as in the case of *NLRP12* mutations in familial cold autoinflammatory syndrome 2 (FCAS2) [12], which was discovered due to the known involvement of *NLRP3* gene in FCAS1.

Finally, the presence of chromosomal rearrangements or structural variations occurring in patients, the availability of animal models with human disease phenotypes, and other possible meaningful clues have in many instances driven the identification of the disease-causing gene. Table 2.1 reports the up to date list of genes responsible for autoinflammatory disorders. In addition, Table 2.2 focuses on a more recent subset of autoinflammatory disorders, termed "type 1 interferonopathy", characterized by mutations in genes encoding proteins involved in nucleotide metabolism and resulting in the upregulation of interferon stimulated genes. The advent of whole exome sequencing has greatly facilitated the identification of all these genes (see also Sects. 2.2.4 and 2.3.2.3).

2.2.2 DNA Sequencing: The Sanger Method

DNA sequencing, the process of reading the sequence of nucleotides present in a DNA molecule that verifies the presence of variants in genes of interest, is a crucial final step common to all the genetic approaches.

The gold standard for DNA sequencing has been for many years the so-called "Sanger sequencing", the method developed by the British biochemist Dr. Frederick Sanger. This method makes use of dideoxynucleotides (ddNTPs), chemically modified bases that terminate the chain when incorporated into the new strands while these are synthetized by DNA polymerase during a PCR [48]. In particular, single strand DNA is used as the template in four PCR reactions, each including one different ddNTP labeled with one of four different colored fluorescent tags, besides unmodified nucleotides (dNTPs). By the time the cycling is complete, a ddNTP will have been incorporated at every single position of the target DNA in each tube reaction, namely, the tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original DNA, which will be labeled with final nucleotide specific dyes. In the end, the four reaction mixtures can be combined and applied to a single lane of a capillary electrophoresis. The color of each fragment is detected using a laser beam and the information is collected by a computer that generates chromatograms showing peaks for each color, from which the template DNA sequence can be determined. This sequencing method is accurate for sequences up to a maximum of about 700–800 base-pairs in length (Fig. 2.1).

2.2.3 DNA Sequencing: The NGS Method

NGS is a revolutionary diagnostic tool for genetic investigations, allowing the simultaneous analysis of multiple genes and the effective detection of gene mosaicism (see below). There are a variety of different NGS technologic platforms making use of different sequencing chemistries [49, 50]. However, most share a common set of features concerning sequencing reactions such as: (1) taking place in parallel, at the same time, (2) micro scaled so that a very high number of genes can be accommodated on the same chip, (3) requiring a very tiny amount of DNA per test, (4) cheaper than Sanger sequencing, (5) producing shorter reads (typically 50–700 nt in length).

Table 2.1 List of genes causing systemic autoinflammatory diseases, with experimental approaches used for their first descriptions and initial references[a]

Gene name	OMIM	Disease	OMIM	Trait trans-mission	Experimental approach used for gene identification	References
AP1S3	615781	PSORS15	616106	Dominant	Whole exome sequencing	Setta-Kaffetzi et al., 2014 [13]
CARD14	607211	PSORS2	602723	Dominant	Linkage analysis coupled with targeted whole-exome sequencing and candidate-gene screening	Jordan et al., 2012 [14]
CARD14	607211	PRP	173200	Dominant	Linkage analysis followed by targeted whole-exome sequencing and candidate-gene screening	Fuchs-Telem et al., 2012 [15]
ADA2	607575	PAN/DADA2	615688	Recessive	Whole exome sequencing	Zhou et al., 2014 [16]; Navon-Elkan et al., 2014 [17]
IL10	124092	IL-10D		Recessive	Candidate gene	Glocker et al., 2010 [18]
IL10RA	146933	IBD28/IL-10R1D	613148	Recessive	Linkage analysis and candidate-gene sequencing	Glocker et al., 2009 [19]
IL10RB	123889	IBD25/IL-10R2D	612567	Recessive	Linkage analysis and candidate-gene sequencing	
IL1RN	147679	OMPP/DIRA	612852	Recessive	Candidate gene	Aksentijevich et al., 2009 [9]; Reddy et al., 2009 [10]
IL36RN	605507	DITRA	614204	Recessive	Homozygosity mapping and direct sequencing + whole exome sequencing	Marrakchi et al., 2011 [20]; Onoufriadis et al., 2011 [21]
LPIN2	605519	Majeed syndrome	609628	Recessive	Homozygosity mapping and direct sequencing of genes in the region	Ferguson et al., 2005 [8]
MEFV	608107	FMF	249100	Recessive	Positional cloning	International FMF Consortium, 1997 [1]; French FMF Consortium, 1997 [2]
MVK	251170	HIDS	260920	Recessive	Positional cloning + candidate gene	Drenth et al., 1999 [4]; Houten et al., 1999 [5]
MVK	251170	MEVA/MA	610377	Recessive	Candidate gene	Schafer et al., 1992 [22]
MVK	251170	POROK3/DSAP	175900	Dominant	Linkage analysis and targeted whole exome sequencing	Zhang et al., 2012 [23]
NLRC4	606831	AIFEC	616050	Dominant	Whole exome sequencing	Romberg et al., 2014 [24]; Canna SW, et al. 2014 [25]
NLRP12	609648	FCAS2/NAPS12	611762	Dominant	Candidate gene	Jeru et al., 2008 [12]
NLRP3	606416	FCAS1/FCU/ CAPS1	120100	Dominant	Positional cloning (linkage analysis followed by direct sequencing in the candidate region)	Hoffman et al., 2001 [6]
NLRP3	606416	MWS/CAPS2	191900	Dominant		Dode et al., 2002 [26]

(continued)

Table 2.1 (continued)

Gene name	OMIM	Disease	OMIM	Trait trans-mission	Experimental approach used for gene identification	References
NLRP3	606416	NOMID/CINCA/ CAPS3	607115	Dominant	Candidate gene	Aksentijevich et al., 2002 [27]; Feldman et al., 2002 [28]
NLRP7	609661	HYDM1/RHM	231090	Recessive	Positional cloning	Murdoch et al., 2006 [29]
NOD2	605956	Blau syndrome	186580	Dominant	Linkage analysis and candidate-gene sequencing	Miceli-Richard et al., 2001 [30]
NOD2	605956	Early-onset sarcoidosis	609464	Dominant	Candidate gene	Kanazawa et al., 2004 [31]
NOD2	605956	IBD1	266600	Dominant	Positional cloning based on linkage analysis followed by linkage disequilibrium mapping	Ogura et al., 2001 [32]; Hugot et al., 2001 [33]
OTULIN	615712	AIPDS	617099	Recessive	Homozygosity mapping followed by candidate gene and exome sequencing	Damgaard et al., 2016 [34]; Zhou et al., 2016 [35]
PLCG2	600220	APLAID	614878	Dominant	Whole exome sequencing	Zhou et al., 2012 [36]
PLCG2	600220	FCAS3	614468	Dominant	Linkage analysis, targeted Sanger sequencing, and next generation/whole genome sequencing	Ombrello et al., 2012 [37]
POMP	613386	CANDLE/PRAAS 2	618048	Dominant	Candidate gene	Brehm et al., 2015 [38]
PSMA3	176843	CANDLE/PRAAS			Candidate gene	
PSMB4	176846	CANDLE/PRAAS			Candidate gene	
PSMB8	177046	ALDD/JMP/ NNS/ CANDLE	256040	Recessive	Homozygosity mapping followed by sequencing of candidate genes in the region	Agarwal et al., 2010 [11]; Arima et al., 2011 [39]
PSMB9	177045	CANDLE/PRAAS			Candidate gene	Brehm et al., 2015 [38]
PSTPIP1	606347	PAPA	604416	Dominant	Positional cloning	Wise et al., 2002 [40]
PSTPIP1	606347	Hyperzyncemia and hypercalprotectinemia		Dominant	Candidate gene	Holzinger D et al., 2015 [41]
RBCK1	610924	PBMEI/ HOIL-1D	615895	Recessive	Whole exome sequencing coupled with single nucleotide polymorphism array	Boisson et al., 2012 [42]
SH3BP2	602104	Cherubism	118400	Dominant	Positional cloning	Ueki et al., 2001 [43]
SLC29A3	612373	Histiocytosis-lymphadeno-pathy plus syndrome	602782	Recessive	Genome-wide linkage analysis followed by sequencing of candidate genes in the region	Morgan et al., 2010 [44]
TMEM173	612374	SAVI	615934	Dominant	Whole exome sequencing	Liu et al., 2014 [103]

TNFAIP3	191163	AISBL/ A20 Haploinsufficiency	616744	Dominant	Whole exome sequencing	Zhou et al., 2016 [46]
TNFRSF11A	603499	TRAPS11		Dominant	Identification by means of a structural variant (aCGH) followed by gene sequencing	Jeru et al., 2014 [47]
TNFRSF1A	191190	TRAPS	142680	Dominant	Positional cloning based on linkage analysis followed by linkage disequilibrium mapping	McDermott et al., 1999 [3]

[a]Empty cells refer to information not available yet

PSORS15 Psoriasis 15, pustular, susceptibility to, *HAE1/HANE* Hereditary angioEdema type I/Hereditary angioneurotic edema, *PSORS2/PRP* Psoriasis 2/Pityriasis rubra pilaris, *PAN/DADA2* Polyarteritis nodosa, childhood-onset/Deficiency of adenosine deaminase 2, *IL-10D* IL-10 deficiency, *IBD28/IL10R1D* Inflammatory bowel disease 28, early onset/IL-10 receptor 1 deficiency, *IBD25/IL-10R2D* Inflammatory bowel disease 25, early onset/IL-10 receptor 2 deficiency, *OMPP/DIRA* Osteomyelitis, sterile multifocal, with periostitis and pustulosis/Deficiency of interleukin 1 receptor antagonist, *PSORP/DITRA* Generalized pustular psoriasis/deficiency of interleukin 36 receptor antagonist, *FMF* Familial Mediterranean fever, *HIDS* Hyperimmunoglobulinemia D syndrome (periodic fever, Dutch type), *MEVA/MA* Mevalonic aciduria, *POROK3/DSAP* Porokeratosis 3, disseminated superficial actinic type/Disseminated superficial actinic porokeratosis, *AIFEC* autoinflammation with infantile enterocolitis, *FCAS2/NAPS12* Familial cold autoinflammatory syndrome 2/NLRP12-associated periodic syndrome, *FCAS1/FCU/CAPS1* Familial cold autoinflammatory syndrome 1/Familial cold urticaria/CIAS1-associated periodic syndrome 1, *MWS/CAPS2* Muckle-Wells syndrome/CIAS1 associated periodic syndrome 2, *CINCA/NOMID/CAPS3* Chronic infantile neurological cutaneous and articular syndrome/neonatal-onset multisystem inflammatory disease/CIAS1 associated periodic syndrome 3, *HYDM1/RHM* Hydatidiform mole, recurrent, 1/Recurrent hydatiform moles, *BLAU-JABS/EOS/IBD1* Blau and Jabss Syndromes/Early onset sarcoidosis/Inflammatory bowel disease 1(Crohn disease), *AIPDS* Autoinflammation, panniculitis, and dermatosis syndrome, *APLAID* Autoinflammation, antibody deficiency, and immune dysregulation, PLCG2-associated syndrome, *FCAS3* Familial cold autoinflammatory syndrome 3, *CANDLE/PRAAS* Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome/Proteasome-associated autoinflammatory syndromes, *ALDD/JMP/NNS/CANDLE* Autoinflammation, lipodystrophy, and dermatosis syndrome/Joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome/Nakajo-Nishimura syndrome/Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome, *PAPA* Pyogenic sterile arthritis, pyoderma gangrenosum, and acne, *PBMEI/HOIL-1D* Polyglucosan body myopathy, early-onset, with or without immunodeficiency/HOIL1 deficiency, *SAVI* STING-associated vasculopathy, infantile-onset, *AISBL* Autoinflammatory syndrome, familial, Behçet-like

Table 2.2 List of interferonopathies and corresponding causative genes

Disease	OMIM	Inheritance	Gene (s)	OMIM	Protein function/pathway
CANDLE (Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature) syndrome; also referred to as Proteasome associated autoinflammatory syndromes (PRAAS)	*256040*	*AR (digenic transmission has also been described)*	*PSMA3, PSMB4, PSMB8, PSMB9, POMP*	176,843, 602177, 177046, 177045, 613386	Proteasome pathway: responsible for regulating proteolysis in eukaryotic cells
STING associated vasculitis with onset in infancy (SAVI)	*615934*	*AR*	*TMEM173*	612374	Adapter molecule involved in IFN production
Aicardi-Goutières syndromes (types 1–7)	*225750, 610329, 610181, 610333, 612952, 615010, 615846*	*AD/AR AR AR AR AR AR AD*	*TREX1, RNASEH2C, RNASEH2B, RNASEH2A, SAMHD1, ADAR, IFIH1*	606609, 610330, 610326, 606034, 606754, 146920, 606951	Regulation of cytoplasmic DNA/RNA
Retinal vasculopathy with cerebral leukodystrophy (RVCL)	*192315*	*AD*	*TREX1*	606609	Regulation of cytoplasmic DNA/RNA
Spondyloenchondrodysplasia (SPENCD)	*607944*	*AR*	*ACP5*	171640	Lysosomal acid phosphatase activity/ osteoclastic dysfunction
Singleton-Merten Syndrome (types 1–2)	*182250, 616298*	*AD AD*	*IFIH1, DDX58*	606951, 609631	Cytosolic sensor of ds-RNA
ISG15 deficiency (immunodeficiency 38)	*616126*	*AR*	*ISG15*	147571	Negative regulator of type I IFN by stabilisation of USP18
USP18 deficiency (pseudo-TORCH syndrome)	*617397*	*AR*	*USP18*	607057	Negative feedback regulator of type I IFN signalling
Trichohepatoenteric syndrome 2	*614602*	*AR*	*SKIV2L*	600478	RNA helicase

The NGS technology is therefore based on the parallel sequencing of multiple small fragments of a given DNA target [49, 51], which are ligated to proper adaptors and pooled in so-called "libraries" for the successive sequencing, rather than on the sequencing of single fragments like in the Sanger sequencing technology. Next generation methods of DNA sequencing have therefore three main steps: (1) creation of DNA libraries including the whole target DNA, first captured in the form of DNA segments that are then ligated to custom linkers, (2) amplification of the libraries using clonal methods to separate each fragment, and (3) sequencing of each fragment of the library using one of several different approaches.

Due to high speed and remarkably rich outputs, NGS can be defined as a "high-throughput technology" where the effective costs are significantly reduced and the quantity of information/sequences produced markedly increased [50, 52]. Due to these great improvements, NGS can be used to sequence very large DNA targets, such as specific regions of interest that may span even hundreds of thousands of base pairs, the whole "exome", representing the coding portion of the genome (around 30–40 Megabases), up to the whole genome, corresponding to ≈3 × 10E9 base pairs.

2.2.4 NGS-Based Techniques for New Gene Discovery

Whole exome sequencing (WES) is the most widely used application of NGS when searching

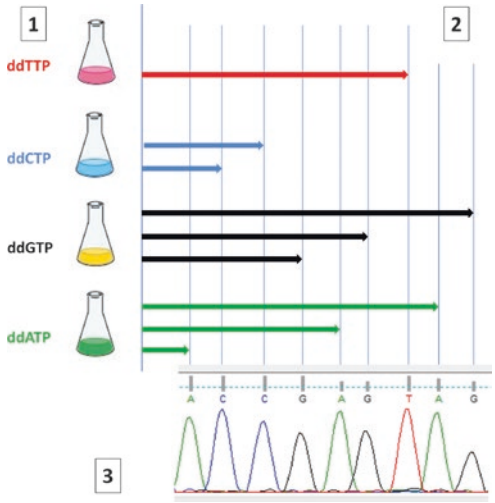

Fig. 2.1 Diagram schematically showing the Sanger sequencing procedure. Molecular steps start with the establishment of four PCR reactions, each including one different ddNTP labeled with one of four different colored fluorescent tags. Single strand DNA is used as the template (not shown) (Step 1). Fragments of different lengths, ending at each of the nucleotide positions in the original DNA template, will be labeled with nucleotide specific dyes (Step 2). In the end, the four reaction mixtures are combined and applied to a single lane of a capillary electrophoresis coupled with a laser beam so that the DNA fragments can be detected according to their length fluorescent tag, thus determining the template DNA sequence (Step 3)

for the gene responsible for a rare disease [53]. Patients with rare diseases benefit from WES application that can facilitate gene discovery, thus attaining a correct and timely clinical diagnosis, providing insights into biological mechanisms, and increasing therapeutic opportunities.

Despite the great versatility of the NGS method and its applications, the bio-informatics data analysis, critically needed to interpret the huge amount of sequences obtained, can be complicated. WES is typically used to detect single nucleotide variants (SNVs) and small insertions or deletions (InDels). But the identification of causative variants responsible for phenotypes under study requires careful filtering and ranking (prioritizing) candidate genes, according to several criteria [54]. In particular, public databases should be searched for each variant in order to obtain information regarding allele frequencies, impact at protein level, pathogenicity prediction, degree of conservation of the protein domain(s), and possible associations with disease phenotypes, etc (Table 2.3). Moreover, the most likely pattern of disease inheritance should be considered in order to filter for *de novo*, heterozygous, homozygous, compound heterozygous or hemizygous variants. To this end, the analysis of unaffected members of the patient's family may become indispensable for the segregation study of selected variants. Finally, a thorough investigation of the gene function, if already known, and how it is affected by the presence of the variant will help to correlate the genotype thus selected with the clinical phenotype of the patient under study. For this reason, patients' history and clinical phenotypes should be also carefully assessed [55].

Despite the tangible progress made in the technology underlying massive sequencing, the genetic interpretation of the results still remains a critical aspect.

An early application of WES has been the rapid discovery of new genes in patients affected by simple Mendelian disorders [56]. Among the first publications of a genetic diagnosis achieved by WES is the report of congenital chloride diarrhea, based on the finding of a homozygous missense variant at the *SLC26A3* gene in a patient referred with a different diagnosis [57], as well as the discovery of the gene responsible for Miller syndrome. This syndrome also known as postaxial acrofacial dysostosis (POADS) is characterized by mandibulofacial dysostosis with postaxial limb anomalies. The discovery started from four patients in three independent kindreds, finding that all shared homozygous or compound heterozygous mutations of the *DHODH* gene [58]. Since then, many other reports have demonstrated the great utility of WES [56]. WES has been particularly successful in the case of rare diseases where identifying a gene or a potentially altered pathway may be extremely difficult and often otherwise impossible.

Nearly one third of the genes responsible for hereditary autoinflammatory disorders have been identified using NGS technology (Table 2.1). In

Table 2.3 Public databases that can be used to annotate variants found

Information that can be retrieved	Database	URL
Allele frequencies	The Exome Aggregation Consortium (ExAC)	exac.broadinstitute.org/
	The Genome Aggregation Database (gnomAD)	http://gnomad.broadinstitute.org/
	The Single Nucleotide Polymorphism database (dbSNP)	https://www.ncbi.nlm.nih.gov/SNP/
	NHLBI Exome Sequencing Project (ESP): Exome Variant Server	http://evs.gs.washington.edu/EVS/
	The 1000 Genomes Project	http://www.internationalgenome.org/data
Impact of variants at protein level	ENSEMBL	https://www.ensembl.org/
	The Protein Variation Effect Analyzer (PROVEAN)	http://provean.jcvi.org
	PANTHER	http://www.pantherdb.org/
	SNPs&GO	http://snps.biofold.org/snps-and-go
Prediction of variant pathogenicity	Combined Annotation Dependent Depletion (CADD)	http://cadd.gs.washington.edu/
	Sorting Intolerant From Tolerant (SIFT)	http://sift.jcvi.org/ift
	Polymorphism Phenotyping v2 (Polyphen 2)	http://genetics.bwh.harvard.edu/pph2/
	SNPeff	http://snpeff.sourceforge.net/SnpEff_manual.html
	MutationTaster	http://www.mutationtaster.org/
Degree of conservation of the protein domain(s)	The Genomic Evolutionary Rate Profiling (GERP)	http://mendel.stanford.edu/SidowLab/downloads/gerp/
Single nucleotide variants (SNV) annotation tools	Variant Effect Predictor (VEP)	http://www.ensembl.org/
	VarSome: The Human Genomic Variant Search Engine	https://varsome.com/
	ANNOVAR	http://annovar.openbioinformatics.org/
	Jannovar	https://github.com/charite/jannovar
Overlaps with regulatory elements, known segmental duplications, etc	AnnTools	http://anntools.sourceforge.net/
	SCAN	http://www.scandb.org/newinterface/about.html
Phenotypic abnormalities encountered in human disease	The Human Phenotype Ontology (HPO)	http://human-phenotype-ontology.github.io/
Collections of disease genes and human genetic diseases	DisGeNET	http://www.disgenet.org/web/DisGeNET/menu
	Online Mendelian Inheritance in Man (OMIM)	https://www.omim.org/

many cases, an unbiased WES protocol was applied and the gene variants causative of the corresponding patients' phenotype were assessed upon the application of the filters mentioned above. As reported in Table 2.1, this was the case for the *AP1S3*, *ADA2*, *NLRC4*, *PLCG2*, *TMEM173*, and *TNFAIP3* genes and corresponding, mostly dominant, disorders. On the other hand, *IL36RN*, *OTULIN*, and *RBCK1*, responsible for three recessive diseases, have been discovered by targeted massive sequencing after identifying the chromosomal segment containing the disease-causing gene through genome-wide linkage analysis. Similarly, in two diseases, linkage analysis followed by targeted NGS approach has expanded the phenotypic spectrum associated with mutations of the *MVK* and *PLCγ2* genes, after detecting new damaging variants of

these genes in two unexpected clinical entities, disseminated superficial actinic porokeratosis (DSAP) and FCAS3 syndrome, respectively (Table 2.1).

One of the great powers of WES is its versatility. Creation of *in silico* panels, including the genes already known to be responsible for most cases of the disease under study (i.e. autoinflammatory disease), allows for a wide number of genes to be examined simultaneously. If no pathogenic variants are found, the entire exome can then potentially be analyzed. *In silico* panels are particularly suitable when the involvement of a specific pathway can be postulated or when the disease under study belongs to a very large phenotypic spectrum known for a wide genetic heterogeneity. Moreover, use of *in silico* panels can avoid the unwanted detection of secondary or incidental findings from the whole exome data.

2.3 Autoinflammatory Diseases: Approaches to Molecular Genetic Diagnosis

The accuracy of the NGS approach [59–61] is high, though dependent on the platform and chemistry used, thus making this the method of choice for the detection of causative mutations in already known genes, no matter whether typical, atypical or new variants. A diagnostic application of NGS is very much feasible, though limited by a few circumstances which must be kept in mind: (1) patients should be well characterized in term of clinical phenotype, a condition not always satisfied because of the strong heterogeneity of autoinflammatory disorders and the difficulties to correctly classify patients suffering from different disorders with overlapping symptoms, and (2) genetic heterogeneity may exceed our expectations thus preventing the identification of the causative variant. Indeed, in many instances, an expensive and time consuming NGS search for mutations in the candidate gene(s) might lead to an inconclusive result.

Based on the considerations previously discussed, and despite having been used for years as the only means to screen patients for gene mutations [62–66], the application of Sanger sequencing in the diagnosis of hereditary autoinflammatory disorders should nowadays be limited to those patients showing unequivocal clinical phenotypes and whose diagnosis can be predicted with a reasonably high confidence. This has recently been confirmed in more than 2000 patients who were screened by Sanger sequencing for three autoinflammatory genes, namely *NLRP3*, *MVK* and *TNFRSF1A* in addition to some portions of other genes, without finding any mutations in 86% of samples [67]. In general clinical genetic settings, it has been reported that the diagnostic rate of Sanger sequencing is not more than 50%, and the rate becomes much lower for patients who have already been through one unsuccessful genetic evaluation [68, 69]. Possible explanations may be, among others, the limited number of genes tested but also possible clinical misdiagnosis, a wider than expected genetic heterogeneity, complex modes of inheritance, gene mosaicism, poor yield of the Sanger sequencing approach for specific gene portions and missed mutations.

For all these reasons, other methodologies, enabling the testing of multiple genes or detecting genetic defects other than SNVs or small insertions/deletions (indels), should be preferred to Sanger sequencing for most patients with autoinflammatory disorders.

2.3.1 NGS-Based Gene Panels

Allowing the sequencing of several genes simultaneously, the use of NGS-based gene panels can facilitate the diagnosis in patients with autoinflammatory disorders, often hampered by the wide heterogeneity of the many genetically diverse but phenotypically overlapping diseases belonging to the autoinflammatory spectrum.

The development of NGS-based gene panels represents a perfect application of scientific knowledge gained about autoinflammatory disorders and genes involved in their pathogenesis for diagnostic purposes. Indeed, knowing genes associated with pathogenesis allows for the *ad hoc* creation of panels, including all genes or gene portions of interest. Early commercial panels,

limited to ≤10 genes, were developed and applied to patients with autoinflammatory disorders taking advantage of new NGS technologies ([70], https://www.genedx.com/). More recently, these panels have been revised with updated gene sets, up to 166 genes, to study both systemic autoinflammatory disorders and vasculitis [71]. These panels uncovered a number of unexpected technological drawbacks, that potentially limit the diagnostic performance of the new tool. This is the case, for instance, for the degree of representation of the submitted target (in other words, the effectiveness of target capture), for the sequencing depth (i.e. the mean coverage of samples), for the ability of the panel to recognize variants present in the sample under analysis (sensitivity) and not to find variants that in fact are not present in the sample (specificity). Therefore, several technologic aspects can affect the performance of a NGS panel, such as (1) the sequencing chemistries adopted by different available commercial platforms, (2) the selection methods applied to capture of the desired targets, and (3) unlike in Sanger sequencing, the bioinformatics step can make a difference in data analysis needed to reach a genetic diagnosis [70]. This highlights the need to first validate panels with DNA samples from patients already diagnosed.

This new experimental approach for the diagnosis of heterogeneous autoinflammatory disorders has given a great impulse to the study not only of simple cases, with a clear clinical phenotype, but also and especially of the so-called "undifferentiated" patients, namely those clinically undiagnosed patients, with non-confirmatory genetic test and/or atypical presentations, complicated by unexpected symptoms.

The demand for NGS-based testing has grown rapidly without a corresponding increase in the rate of detection of causative mutations, a circumstance that has had a strong impact on the yield of NGS panels, in terms of the proportion of patients that have been diagnosed through the molecular approach. Indeed, in the literature, the yield of the most focused disease panels varies from a higher yield (40–50%) to a lower yield (15–25%), likely depending on the phenotype and genetic heterogeneity of the included disorders, as well as, for recessive diseases, on the level of consanguinity of parents (correlated to the degree of inbreeding of the populations which patients belong to). For instance, different targeted gene panels for primary immunodeficiency diseases (PID) including between 162 and 170 genes allowed a definitive diagnosis in 15–25% in a total of 165 patients [72], while the diagnostic yield was much more heterogeneous in patients with epilepsy ranging from 10 to 48.5% using panels including from 35 to 265 genes [73]. In autoinflammatory disorders, the gene panels published to date report a satisfactory validation of positive controls which in all the cases revealed high sensitivity and specificity of the panels under development [70, 71]. Omoyinmi and colleagues tested 50 patients with undefined autoinflammatory disorders using their "vasculitis and inflammation panel" that contains 166 genes, finding either pathogenic or likely pathogenic mutations in 16 samples, corresponding to a yield of 32% [71]. A further panel with a set of 41 genes specific for autoinflammatory disorders was applied to 50 undifferentiated patients, without any improvement in the mutation detection rate compared to Sanger sequencing. This again suggests that patients with undifferentiated phenotypes may have either a complex multifactorial/multigenic etiology or the involvement of still unknown genes (IC, personal observation). Finally, among 246 children with a suspected primary immunodeficiency, a NGS-based panel containing 302 genes, including 23 genes for autoinflammatory disorders, revealed 15 subjects with a likely genetic diagnosis of NLRP12-associated autoinflammatory disorder and primary immunodeficiency, thus highlighting still undisclosed associations and confirming the powerfulness of the NGS tools to investigate complex and/or heterogeneous disorders like autoinflammatory disorders [74].

Therefore, an NGS-based gene panel for autoinflammatory disorders may often be unsatisfactory in routine confirmation of a genetic diagnosis in clinical practice or to solve complicated phenotypic pictures. Nevertheless, NGS-based autoinflammatory gene panels are still widely used,

as there are many patients suspected to have an autoinflammatory disorder and the turnover time of the test is quite fast. Gene panels might be regarded as a first screening test before deciding about further investigations. Only a limited number of unsolved patients can ultimately undergo further analysis, like WES, as this is still quite demanding in terms of both costs and time.

According to present data, there does not seem to be a strict correlation between the number of genes in NGS-panels specific for autoinflammatory disorders and the diagnostic success rate, as stringency of inclusion criteria and tested genes also affect the final performance of the panel. In addition, both small and large panels have advantages and also drawbacks: if small panels have an undersized representation of disease-related genes, the number of variants of unknown significance thus detected is limited and the panel results are more "manageable", the opposite happens with large panels. To overcome such an inconvenience, the use of sub-panels has been introduced in many labs, each covering a different class of autoinflammatory disorders (recurrent fevers, skin related diseases, chronic urticaria, syndromes with intestinal involvement, etc) with highly specific genes, some of which can be redundant, overlapping different sub-panels.

The use of NGS-panels has lead to the detection of an enormous number of sequence variants whose significance is often uncertain and whose correlation with the phenotype is anything but straightforward. Indeed, as the number of gene variants detected is continuously growing, we have realized the need of a consensus or agreement for scoring variant pathogenicity. A role, either pathogenic or benign, can be assigned to each variant based on a number of observations within the framework of the American College of Medical Genetics and Genomics (ACMG) recommendations [75]. These include (1) the allele frequency of the variant in large control populations, (2) the type of gene variant (splice, stop codon, missense, etc), (3) whether the variant is already reported in relevant patients or registered in databases (publications, Infevers, ClinVar, etc), (4) whether it affects a mutational hotspot, namely a codon in which a mutation has already been detected previously, (5) *in silico* predictions, including evolutionary conservation of the codon, location in putative functional sites of the protein, type of amino acid substitution, (6) familial co-segregation of the variant with the phenotype, and finally (7) results from available functional *in vivo* and *in vitro* studies (Fig. 2.2).

Some studies have reported variant classifications for selected genes causing autoinflammatory disorders [76, 77]. One of these studies

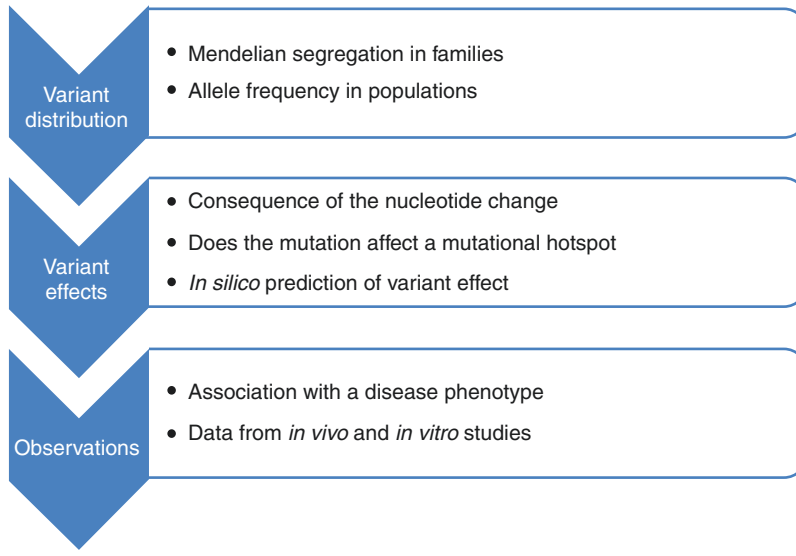

Fig. 2.2 List of criteria used mostly for variant prioritization

described a consensus-driven process by experts for the pathogenicity assessment, which resulted in the classification of almost all variants reported so far in the four main genes causing hereditary recurrent fever syndromes (*MEFV*, *TNFRSF1A*, *NLRP3* and *MVK* genes). According to the ACMG recommendations, these variants have been classified as (1) benign, (2) likely benign, (3) variants of uncertain significance (VUS), (4) likely pathogenic, and (5) pathogenic. The results of this classification have been made available on the INFEVERS database at https://fmf.igh.cnrs.fr/ISSAID/infevers/ [77].

Discussion between laboratories involved in the study of patients affected with autoinflammatory disorders about variants detected in autoinflammatory-related genes is crucial to increase the quality and speed of the interpretation of NGS data, to minimize discordant variant classifications between laboratories, to limit misinterpretation of DNA variants, and, finally, to recognize variants detected sporadically in diagnostic labs that are not yet contained in public databases.

As discussed above, NGS is an approach that, for technical reasons, may be prone to yield both false positive and false negative results. Therefore, despite the high reliability of the method, it is of utmost importance to validate the detected variants by the Sanger sequencing method, still the gold standard for the DNA sequencing, to avoid wrong conclusions, especially in a diagnostic setting.

Last, the costs associated with the new NGS technological approach represent another advantage. Indeed, it has been estimated that a NGS panel of more than 150 genes costs as much as the screening of one single gene performed by the Sanger sequencing approach ([71], IC, personal observation). However, the time and cost spent on bioinformatics analysis should also be taken into account when comparing Sanger sequencing and NGS-based tests.

In conclusion, NGS is a reliable diagnostic tool for autoinflammatory disorders, which can be applied when the Sanger sequencing approach is not appropriate, testing progressively larger targets according to needs (Fig. 2.3). Though lacking a high diagnostic yield in the very heterogeneous field of the autoinflammatory disorders, target resequencing, namely the (re)sequencing of a small subset of the genome such as with a gene panel, results in a higher diagnostic power and a reduction of costs, compared to the classical method of investigation [78].

The still low diagnostic yield might be due to mutations in regions not included in the NGS panel used (either non-coding regions or alternative transcripts) or in other genes. When these circumstances are suspected, either whole exome or whole genome sequencing should be considered. Indeed, additional genes causing autoinflammatory disorders and still undisclosed genetic mechanisms need to be identified to explain the full genetic heterogeneity of autoinflammatory disorders and NGS is already contributing to this.

2.3.2 Other Approaches Employed in Patients with Autoinflammatory Disorders

2.3.2.1 Array-Comparative Genomic Hybridization (aCGH)

Microarray-based comparative genomic hybridization (Array-CGH, also known as aCGH) is a molecular cytogenetic technique that allows for the detection of chromosomal imbalances involving either loss or gain of genomic regions. Balanced structural variations, such as balanced chromosomal translocations, cannot be revealed by aCGH. The principle of the method is based on the comparison (hybridization) of two DNA samples, typically one patient and one control, labeled with different fluorescent tags, to detect any difference in the relative quantity of individual regions of the genome. For this reason, aCGH is the test of choice to investigate so-called "copy number variations" (CNVs), with a resolution ranging from about 20 to 200 Kb [79]. After denaturation, the two DNA samples are mixed and loaded onto a so-called array containing thousands of synthetic short single-stranded immobilized DNA fragments representing the whole

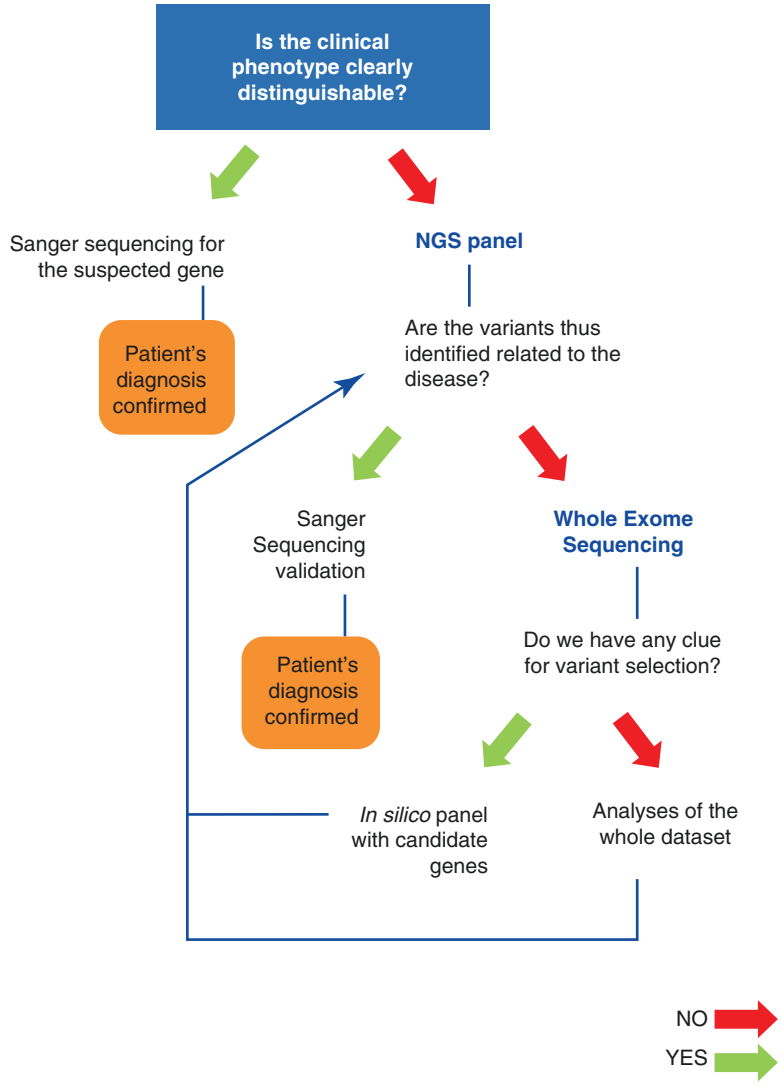

Fig. 2.3 A suggested diagnostic workflow for the genetic analyses of a candidate patient. In case Sanger sequencing and next generation sequencing (NGS)-based gene panels do not identify a consistent variant, whole exome or whole-genome sequencing should be considered

genome. Because the fluorescently labeled DNA of the patient and the control compete with these oligonucleotides, conclusions can be drawn from the ratio of the color signals of the patient and control DNAs regarding their relative gene dosage. For instance, when the color signal of the patient DNA prevails on the control DNA in a given genomic region, a gain of DNA is suspected, and *vice versa* for DNA loss. There are a few examples of patients with autoinflammatory disorders that were identified through this method. One is the case of the already mentioned 175 kb large homozygous deletion of the interleukin-1 family gene cluster, including the *IL1RN* gene, identified in an infant of Puerto Rican origin affected with DIRA [9, 10]. Another, is the recessive HOIL1 deficiency, reported in Table 2.1 as a clear example of the combined use of WES and aCGH, found a patient with a compound heterozygosity for a stop mutation of the *RBCK1* gene and a genomic 30 Mb deletion that includes this gene [42]. In this case the HOIL1 deficiency derives from the failure of either allele to produce a correct and functioning RBCK1 protein as one allele carries a nonsense mutation and the other a null allele, namely no gene is present in the corresponding chromosomal region due to an interstitial deletion. While the first mutation was

detected by WES, the second genetic defect was assessed by aCGH [42]. Finally, very recently, a 13.13 Mb deletion on chromosome 6, encompassing 53 genes including the *TNFAIP3* gene, has been identified by using aCGH in a patient with a complex phenotype consistent with the dominantly inherited A20 haploinsufficiency [80]. Therefore, it has been recommended to include CGH arrays in the routine diagnostic methods for comprehensive analysis of patients with syndromic features and immune dysregulation.

2.3.2.2 Real-Time Polymerase Chain Reaction (PCR)

Quantitative polymerase chain reaction (Q-PCR), sometimes referred to as real-time PCR, is a method by which the amount of the PCR product can be determined, in real-time, by the use of fluorescent or DNA intercalating dyes, typically used to measure gene expression. A further application of Q-PCR is to estimate the copy number of a gene or a genomic region, a quantification that allows to detect CNVs, either deletions or duplications [81]. This technique was successful in the case of two sisters with a phenotype consistent with DADA2, who were initially found to be only heterozygous for a missense pathogenic variant at the *ADA2* (previously known as *CECR1*) gene. Q-PCR revealed an additional heterozygous deletion of exon 7 predicted to lead to a frameshift and truncated protein in the opposite *ADA2* allele [82].

2.3.2.3 Gene Expression in Autoinflammatory Disease

Analysis of gene expression in autoinflammatory diseases has often provided a powerful means to obtain hints about pathogenic mechanisms of disease. A microarray (also chip) is used for such studies. Such a microarray contains a large set (up to millions) of DNA probes attached to a solid surface that can be hybridized with transcripts (targets), thus assessing, simultaneously, through fluorescence or chemiluminescence signals, the expression levels of large numbers of genes.

Taking advantage of such technology, gene expression patterns were analyzed to define a specific gene expression signature able to distinguish patients with cryopyrin-associated periodic syndromes (CAPS) from controls. Interestingly, several differentially expressed genes turned out to be shared among other systemic inflammatory diseases [83]. A similar study increased knowledge of pathogenic mechanisms in TRAPS. Gene expression profiles in resting monocytes from TRAPS patients confirmed the patients' chronic inflammatory condition, while additional pathways, not yet associated with the disease, were discovered, such as interferon types I and II response to lipopolysaccharide (LPS) stimulation and a downregulation of the transforming growth factor (TGF)-β pathway in the basal condition [84].

Analysis of gene expression has become crucial in suspected interferonopathies, a group of Mendelian diseases associated with an upregulation of interferon and consequently with a specific "signature" given by the simultaneous upregulation of genes whose expression is stimulated by interferon (see Table 2.2 and Chap. 24) [85]. In particular, the expression of interferon-stimulated genes is measured by quantitative PCR, and the median fold change is used to create an interferon score. This interferon score is higher in patients than among controls. Type 1 interferonopathies have emerged during the latest few years and their number is still growing as interferon signatures are often recognized in patients with novel autoinflammatory and/or autoimmune phenotypes [86–88].

2.4 Gene Mosaicism

- **According to the tissue distribution, gene mosaicism can be divided into gonadal mosaicism, somatic mosaicism and gonosomal mosaicism. In this latter case the post-zygotic mutation affects both gonadal and somatic cells**
- **The allele frequency of post-zygotic (somatic) mutations ranges from 1 to 40%, and often less than 20%. These mutations can be missed when using conventional methods of genetic analyses (i.e Sanger method of DNA sequencing), and their**

detection usually requires NGS-based methods with great depth

- **During the last few years various patients with somatic *NLRP3* mosaicism restricted to cells from myeloid lineage (neutrophils and monocytes) have been described.**
- **Somatic gene mosaicism has also been described as a disease-causing mechanism in monogenic autoinflammatory diseases other than cryopyrin-associated periodic syndromes (CAPS), but in smaller numbers of patients**
- **The presence of post-zygotic mutations in gonadal tissue may cause an unexpected recurrence of a dominantly-inherited disease in a subsequent child of a healthy couple with no mutations detected in previous standard genetic analyses, thus emphasizing the importance of considering the possibility of parental gene mosaicism in gene counseling of families**

In genetics, the term mosaicism describes an individual who has developed from a single zygote, but carries two, or more than two, cell types with distinct genotypes [89]. In a strict sense, gene mosaicism should be clearly distinguished from the related phenomenon of chimerism, which describes an individual who carries cell types with distinct genotypes, but these cells derived from distinct fertilized eggs (i.e. a recipient of an allogeneic transplant or cell fusion from an aborted dizygote twin early in embryogenesis).

2.4.1 Germline and Post-zygotic Mutations

In an individual with gene mosaicism, the differences observed among genetically different cells are a consequence of mutational events that occur post-zygotically, either during the embryonic development in the $\approx 10^{16}$ mitotic cell divisions required to generate an adult organism, or later, after birth, in a similar manner as in the case of somatic gene variants involved in carcinogenesis. The post-zygotic (or somatic) mutations are clearly different from germline mutations. Germline mutations are already present in the first fertilized egg, and consequently are present in all cells of the body from conception. Moreover, these germline mutations can easily be detected by conventional methods of genetic analysis (i.e. Sanger sequencing) in any analyzed tissue, and their expected allele frequency in heterozygosity is around 50%. On the contrary, post-zygotic mutations are strictly *de novo* mutations, which are absent in the individual's parents. Their body distribution may differ among individuals carrying mosaicism, the main factor that determines this distribution being the precise time when the post-zygotic mutational event occurred. The allele frequency of post-zygotic mutations is less than 50%, ranging from 1 to 40%, and often less than 20%. This low or extremely low frequency of the mutant allele means that these mutations can be missed when using conventional methods of genetic analyses, and their detection usually requires the use of novel technologies such as NGS-based methods with great depth.

2.4.2 Tissue Distribution of Gene Mosaicism

As mentioned, the precise time when the post-zygotical mutational event occurs will determine the body distribution of gene mosaicism in a given individual. When the mutational event occurs early during embryonic development, the post-zygotic mutation will probably be present in tissues derived from all three embryonic layers and provoke a type of mosaicism called extended gene mosaicism. By contrast, the mosaicism could be tissue-restricted when the post-zygotic mutation occurs later, during post-natal life.

According to the tissue distribution of post-zygotic mutations, three main types of gene mosaicism can be distinguished, each having different clinical consequences. When the post-zygotic mutation is restricted to the gonadal tissue, the gene mosaicism is named **gonadal mosaicism**. In this case, the individual carrying the mosaicism is healthy, but at moderate-to-high risk to transmit

the mutant allele to his/her offspring, who will receive it as a germline mutation because it will be already present at the first zygote.

When the post-zygotic mutation is restricted to body (somatic) cells, the gene mosaicism is called **somatic mosaicism**. In this case, the individual carrying the post-zygotic mutation could be healthy or affected, depending on different variables such as the frequency of the mutant allele, the precise type of mutation and its consequences on the function of the normal protein, and the precise relationship among tissues carrying the post-zygotic mutation and the tissues where the specific mutated gene is expressed. However, unlike individuals with gonadal mosaicism, individuals with pure somatic mosaicism are not at risk of transmitting the mutant allele to their offspring.

Finally, when the post-zygotic mutation affects both gonadal and somatic cells, the gene mosaicism is termed **gonosomal mosaicism**, representing the most complex type among gene mosaicism. In this case, the individual carrying gonosomal mosaicism is at moderate-to-high risk to transmit the mutant allele to his/her offspring, and also could develop clinical symptoms depending on the variables mentioned previously in the definition of somatic mosaicism.

2.4.3 State of the Art in Monogenic Autoinflammatory Diseases

The history of gene mosaicism in the field of monogenic autoinflammatory diseases started in 2005, when a Japanese group identified for the first time a somatic *NLRP3* mosaicism as the underlying disease-causing mechanism in a patient diagnosed with CAPS [90]. Since then, at least 68 individuals, most with clinical illness, have been identified carrying post-zygotic mutations in different genes associated with monogenic autoinflammatory diseases. Most of reported gene mosaicisms belong to the group of somatic mosaicism, with only seven cases belonging to the group of gonosomal mosaicism. Interestingly, no pure gonadal gene mosaicism has been described to date. With regard to the genes causing autoinflammatory diseases, post-zygotic mutations have been detected in six different genes, with most of the cases (87%) identified in the *NLRP3* gene (Fig. 2.4). Table 2.4 contains a summary of the cases of gene mosaicism in the *NLRP3* gene reported to date, whereas Table 2.5 contains those reported in genes other than *NLRP3*.

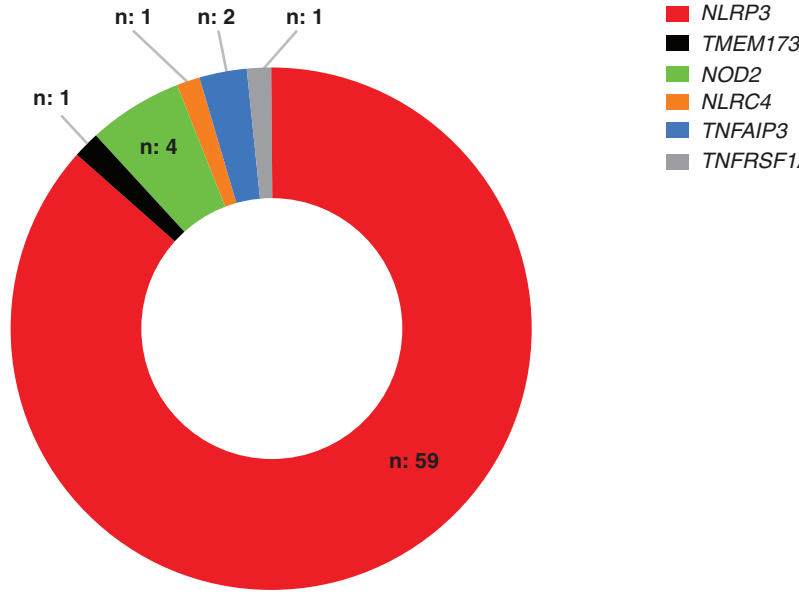

Fig. 2.4 Summary of genes related to autoinflammatory diseases in which gene mosaicism has been identified. The numbers for each gene indicate the total number of unrelated individuals carrying a specific gene mosaicism

Table 2.4 Summary of the currently known individuals carrying *NLRP3* mosaicism

Gene	Exon	Nucleotide exchange	Amino acid exchange	MAF (%) in whole blood	Phenotype	Country	Type of mosaicism	Reference	Germline counterpart phenotype
NLRP3	3	c.779G>C	p.Arg260Pro	10.9	MWS	Italy	Somatic	[91]	Yes—NOMID
NLRP3	3	c.790C>T	p.Leu264Phe	4.3	NOMID	Japan	Somatic	[92]	Yes—NOMID
NLRP3	3	c.906C>A	p.Phe302Leu	9.8	NOMID	Japan	Somatic	[93]	No
NLRP3	3	c.907G>A	p.Asp303Asn	7.2	Asymptomatic	Mexico	Gonosomal	Personal unpublished data	Yes—NOMID
NLRP3	3	c.907G>C	p.Asp303His	19.1	NOMID	Spain	Somatic	[94]	Yes—NOMID
NLRP3	3	c.907G>C	p.Asp303His	4.2	NOMID	France	Somatic	[95]	Yes—NOMID
NLRP3	3	c.907G>C	p.Asp303His	11.9	NOMID	Japan	Somatic	[95]	Yes—NOMID
NLRP3	3	c.907G>C	p.Asp303His	7.1	NOMID	Japan	Somatic	[93]	Yes—NOMID
NLRP3	3	c.907G>C	p.Asp303His	13.8	NOMID	Spain	Somatic	Personal unpublished data	Yes—NOMID
NLRP3	3	c.908A>C	p.Asp303Ala	31.3	MWS	Spain	Somatic	[96]	No
NLRP3	3	c.918A>T	p.Gln306His	5.1	Late-Onset MWS	Spain	Somatic	Personal unpublished data	No
NLRP3	3	c.919G>A	p.Gly307Ser	4.3	NOMID	Japan	Somatic	[92]	No
NLRP3	3	c.920G>A	p.Gly307Ala	4.5	CAPS	Turkey	Somatic	[97]	No
NLRP3	3	c.920G>T	p.Gly307Val	9.6	NOMID	Spain	Somatic	[95]	Yes—NOMID
NLRP3	3	c.1000A>G	p.Ile334Val	34.9	MWS	Japan	Somatic	[96]	Yes—NOMID
NLRP3	3	c.1040C>T	p.Thr347Ile	4.9	MWS	USA	Somatic	Personal unpublished data	No
NLRP3	3	c.1043C>T	p.Thr348Met	2.8	Asymptomatic	Spain	Gonosomal	[98]	Yes—MWS
NLRP3	3	c.1054G>A	p.Ala352Thr	14.6	Late-Onset MWS	UK	Somatic	[99]	Yes—NOMID
NLRP3	3	c.1054G>A	p.Ala352Thr	21.3	Late-Onset MWS	Spain	Somatic	Personal unpublished data	Yes—NOMID
NLRP3	3	c.1064A>C	p.Lys355Thr	20.2	MWS	Japan	Somatic	[96]	No
NLRP3	3	c.1065A>T	p.Lys355Asn	18.8	NOMID	USA	Somatic	[95]	No
NLRP3	3	c.1216A>G	p.Met406Val	9.2	NOMID	France	Somatic	[95]	No
NLRP3	3	c.[1231C>T;1233G>T]	p.Leu411Phe	14.4	MWS	Spain	Somatic	[96]	No
NLRP3	3	c.1298C>T	p.Thr433Ile	5.2	NOMID	France	Somatic	[95]	No
NLRP3	3	c.1298C>T	p.Thr433Ile	3.2	NOMID	Italy	Somatic	[100]	No

(continued)

Table 2.4 (continued)

Gene	Exon	Nucleotide exchange	Amino acid exchange	MAF (%) in whole blood	Phenotype	Country	Type of mosaicism	Reference	Germline counterpart phenotype
NLRP3	3	c.1298C>T	p.Thr433Ile	5.5	NOMID	Italy	Somatic	[91]	No
NLRP3	3	c.1303A>G	p.Lys435Glu	27.0	Variant-type SS	Netherlands	Myeloid-restricted, somatic	[101]	No
NLRP3	3	c.1305G>T	p.Lys435Asn	9.0	MWS	Brazil	Somatic	Personal unpublished data	No
NLRP3	3	c.1315G>C	p.Ala439Pro	21.9	NOMID	France	Somatic	[95]	Yes—NOMID
NLRP3	3	c.1564A>T	p.Thr522Ser	28.9	NOMID	Colombia	Somatic	Personal unpublished data	No
NLRP3	3	c.1569C>A	p.Phe523Leu	8.7	MWS	Spain	Somatic	[96]	Yes—NOMID
NLRP3	3	c.1569C>G	p.Phe523Leu	8	Variant-type SS	Netherlands	Myeloid-restricted, somatic	[101]	Yes—NOMID
NLRP3	3	c.1688A>G	p.Tyr563Cys	2.7	NOMID	Italy	Somatic	[91]	No
NLRP3	3	c.1688A>G	p.Tyr563Cys	5.1	Late-Onset MWS	UK	Somatic	[99]	No
NLRP3	3	c.1688A>G	p.Tyr563Cys	3.2	Late-Onset MWS	UK	Somatic	[99]	No
NLRP3	3	c.1688A>G	p.Tyr563Cys	11.1	Late-Onset MWS	UK	Somatic	[99]	No
NLRP3	3	c.1688A>G	p.Tyr563Cys	8.0	Late-Onset MWS	Spain	Somatic	Personal unpublished data	No
NLRP3	3	c.1690G>A	p.Gly564Ser	8.1	NOMID	Italy	Somatic	[91]	No
NLRP3	3	c.1691G>A	p.Gly564Asp	5.0	Late-Onset MWS	UK	Somatic	[99]	No
NLRP3	3	c.1691G>A	p.Gly564Asp	8.5	MWS	Belgium	Somatic	Personal unpublished data	No
NLRP3	3	c.1698C>A	p.Phe566Leu	11.5	NOMID	France	Somatic	[95]	No
NLRP3	3	c.1698C>A	p.Phe566Leu	14.6	NOMID	USA	Somatic	[95]	No
NLRP3	3	c.1698C>A	p.Phe566Leu	14.5	NOMID	UK	Somatic	[102]	No
NLRP3	3	c.1699G>A	p.Glu567Lys	6.5	MWS	Japan	Somatic	[92]	No
NLRP3	3	c.1699G>A	p.Glu567Lys	6.3	NOMID	Netherlands	Somatic	[95]	No
NLRP3	3	c.1699G>A	p.Glu567Lys	5.8	NOMID	Japan	Somatic	[93]	No
NLRP3	3	c.1699G>A	p.Glu567Lys	18.3	NOMID	Japan	Somatic	[93]	No
NLRP3	3	c.1699G > A	p.Glu567Lys	5.6	MWS	Japan	Somatic	[96]	No

NLRP3	3	c.1699G>A	p.Glu567Lys	5.5	MWS	Japan	Somatic	[96]	No
NLRP3	3	c.1699G>A	p.Glu567Lys	5.4	Late-Onset MWS	UK	Somatic	[99]	No
NLRP3	3	c.1700G>C	p.Glu567Gln	15.0	Late-Onset MWS	UK	Somatic	[99]	
NLRP3	3	c.1704G>C	p.Lys568Asn	9.4	NOMID	USA	Somatic	[95]	No
NLRP3	3	c.1706G>T	p.Gly569Val	21.1	Late-Onset MWS	UK	Somatic	[99]	No
NLRP3	3	c.1708T>C	p.Tyr570His	11.9	Severe CAPS	Turkey	Somatic	[97]	No
NLRP3	3	c.1709A>G	p.Tyr570Cys	16.7	NOMID	Japan	Somatic	[90]	Yes—NOMID
NLRP3	3	c.1709A>G	p.Tyr570Cys	10.9	Late-Onset MWS	USA	Myeloid-restricted, somatic	[103]	Yes—NOMID
NLRP3	3	c.1906C>G	p.Gln636Glu	18.4	Late-Onset MWS	Spain	Myeloid-restricted, somatic	[104]	No
NLRP3	4	c.2263G>A	p.Gly755Arg	35.8	NOMID	USA	Somatic	[95]	Yes—NOMID
NLRP3	4	c.2263G>A	p.Gly755Arg	6.3	NOMID	Netherlands	Somatic	[95]	Yes—NOMID

MAF Mutant allele frequency, *MWS* Muckle-Wells syndrome, *NOMID* Neonatal-onset multisystem inflammatory disease, *CAPS* Cryopyrin-associated periodic syndromes, *SS* Schnitzler syndrome, *USA* United States of America, *UK* United Kingdom

Table 2.5 Summary of the individuals carrying gene mosaicism in autoinflammatory diseases-associated genes other than *NLRP3*

Gene	Exon	Nucleotide exchange	Amino acid exchange	MAF (%) in whole blood	Phenotype	Country	Type of mosaicism	Reference
NLRC4	4	c.529A>G	p.Thr177Ala	30.5	NOMID	Japan	Somatic	[105]
NOD2	4	c.1001G>A	p.Arg334Gln	2.7	Asymptomatic	Spain	Gonosomal	Unpublished personal data
NOD2	4	c.1001G>A	p.Arg334Gln	7.7	Mild BS	Spain	Somatic	[106]
NOD2	4	c.1001G>A	p.Arg334Gln	12.9	Mild BS	Malaysia	Gonosomal	[107]
NOD2	4	c.1001G>A	p.Arg334Gln	40.5	BS	Spain	Somatic	Unpublished personal data
TMEM173	5	c.461A>G	p.Asn154ser	Unknown	SAVI	USA	Somatic	[45]
TNFAIP3	8	c.1245_1248del4	p.Lys417SerfsX4	16.7	Asymptomatic	Japan	Gonosomal	[108]
TNFAIP3	Intron 8	c.2088+5G>C	p.His636GlufsX55	10.06	Asymptomatic	Japan	Gonosomal	[108]
TNFRSF1A	3	c.255_278del	p.Ser86_Glu93del	21.0	TRAPS	UK	Gonosomal	[109]

MAF Mutant allele frequency, *NOMID* Neonatal-onset multisystem inflammatory disease, *BS* Blau syndrome, *SAVI* STING-associated vasculopathy with onset in infancy, *TRAPS* Tumor necrosis factor receptor associated periodic syndrome, *USA* United States of America, *UK* United Kingdom

2.4.3.1 Somatic *NLRP3* Mosaicism in Cryopyrin-Associated Periodic Syndromes (CAPS)

CAPS are dominantly-inherited autoinflammatory diseases consequence of *gain-of-function* mutations in the *NLRP3* gene, which encodes for the cryopyrin protein (see Chap. 19). They encompass three different clinical phenotypes of increasing severity along a clinical spectrum, with FCAS at the less severe end, Muckle-Wells syndrome (MWS) as the intermediate phenotype and neonatal-onset multisystem inflammatory disease (NOMID), also known as chronic infantile neurological, cutaneous and articular (CINCA) syndrome at the most severe end [110]. Initial genetic studies in NOMID using the Sanger method of DNA sequencing yielded positive results in the *NLRP3* gene in ≈55–60% of patients [27, 28].

A Japanese group reported a small group of patients with CAPS who carried post-zygotic, *gain-of-function NLRP3* mutations as the underlying disease mechanism [90, 92]. This first evidence of somatic *NLRP3* mosaicism as a disease-causing mechanism in NOMID was corroborated by a clinical case [94] and by an international multicenter study [95]. This study enrolled 26 patients with NOMID, *NLRP3*-negative by Sanger sequencing, and post-zygotic *gain-of-function NLRP3* mutations were detected in 18 (69%). These studies clearly established gene mosaicism as the disease-causing mechanism in a large proportion of patients with NOMID [95].

Four years later, a collaborative study also showed that somatic *NLRP3* mosaicism is the underlying mechanism in the MWS. A total of 56 patients with a clinical phenotype compatible with MWS were enrolled, identifying 7 patients (12.5%) harboring post-zygotic *NLRP3* mutations with variable mutant allele frequency (5.5–35%). Three out of 6 *NLRP3* mutations detected in this work were novel. A comparative analysis of phenotypes of patients with germline or post-zygotic *NLRP3* mutations revealed that those patients carrying post-zygotic *NLRP3* mutations had a milder disease, which seemed to start slightly later than in patients with germline mutations [96].

This observation has recently been reinforced by the description of 8 British adult patients with late-onset CAPS (median age at disease-onset 50 years; range 31–71 years) who carried different post-zygotic *NLRP3* mutations (Table 2.4) [99]. In this work, the stability of mosaicism in blood samples over time was also analyzed. The percentage of mutant allele did not change in all patients except for one woman (patient 1), in whom the percentage of the mutant allele increased over time, despite the fact that she was being treated with anti-IL-1 medications. Interestingly, the increased presence of the mutant allele was associated with worsening of her clinical symptoms and the need to up-titrate the dose of her anti-IL-1 medications to control her symptoms. From a technical point of view, in Sanger chromatograms the mutant allele in the oldest blood sample (mutant allele frequency 5%) was interpreted as "background noise", while in the later blood samples (mutant allele frequencies 27% and 45%, respectively), the mutant allele was easily detectable in Sanger chromatograms [99].

Myeloid-Restricted Somatic NLRP3 Mosaicism

In all previous cases, the distribution of the *NLRP3* mosaicism was either not analysed or, when analysed, equally represented in all tissues. However, several patients with somatic *NLRP3* mosaicism restricted to cells from myeloid lineage (neutrophils and monocytes) have been recently described. This conclusion was drawn from complex genetic studies using NGS-based methods in DNA extracted from isolated leukocyte subpopulations (neutrophils, monocytes, T cells, B cells).

The first cases of myeloid-restricted somatic *NLRP3* mosaicism were detected in 2 Dutch patients diagnosed with the variant-type of Schnitzler syndrome, a late onset autoinflammatory disease characterized by neutrophilic urticaria-like rash, bone pain, monoclonal gammopathy and extremely responsive to treatment with anti-IL-1 medication [111] (see Chap. 37). Interestingly, during treatment with anti-IL-1 medication, these two patients experienced resolution of their clinical symptoms as well as the disappearance of the monoclonal gammopathy [101].

Some months later, two nearly identical clinical cases, a woman and a man with recurring episodes starting in their 50s, were shown to have somatic *NLRP3* mosaicism restricted to neutrophils and monocytes [103, 104].

Overall, the somatic *NLRP3* mosaicism data strongly suggests that CAPS could have two different types in relation to the age of disease onset. Those patients with germline *NLRP3* mutations and most of the patients with extended somatic *NLRP3* mosaicism develop symptoms during childhood. By contrast, those individuals carrying myeloid-restricted *NLRP3* mosaicism present with a late-onset CAPS, as late as their 50s, with the possibility that they had received another diagnosis including Schnitzler syndrome, chronic urticaria, adult-onset Still disease, other rheumatic conditions or even some hematological neoplastic disorder as a consequence of the unexplained and extremely high leukocyte and neutrophils counts.

Localization of Post-zygotic Mutations in the NLRP3 Gene

In addition to the differences of the phenotypes of CAPS between patients with germline or with post-zygotic *NLRP3* mutations, a novel insight has been obtained about post-zygotic mutations regarding their localization in the gene. Three regions located in or around nucleotide positions 906–920, 1298–1315 and 1688–1699 seem to concentrate most of already known post-zygotic mutations (Fig. 2.5). This observation may facilitate the identification of these mutations through a detailed analysis of these regions when using the Sanger method of DNA sequencing.

2.4.3.2 Somatic Mosaicism in Other Monogenic Autoinflammatory Diseases

Somatic gene mosaicism has also been described as a disease-causing mechanism in monogenic autoinflammatory diseases other than CAPS, but

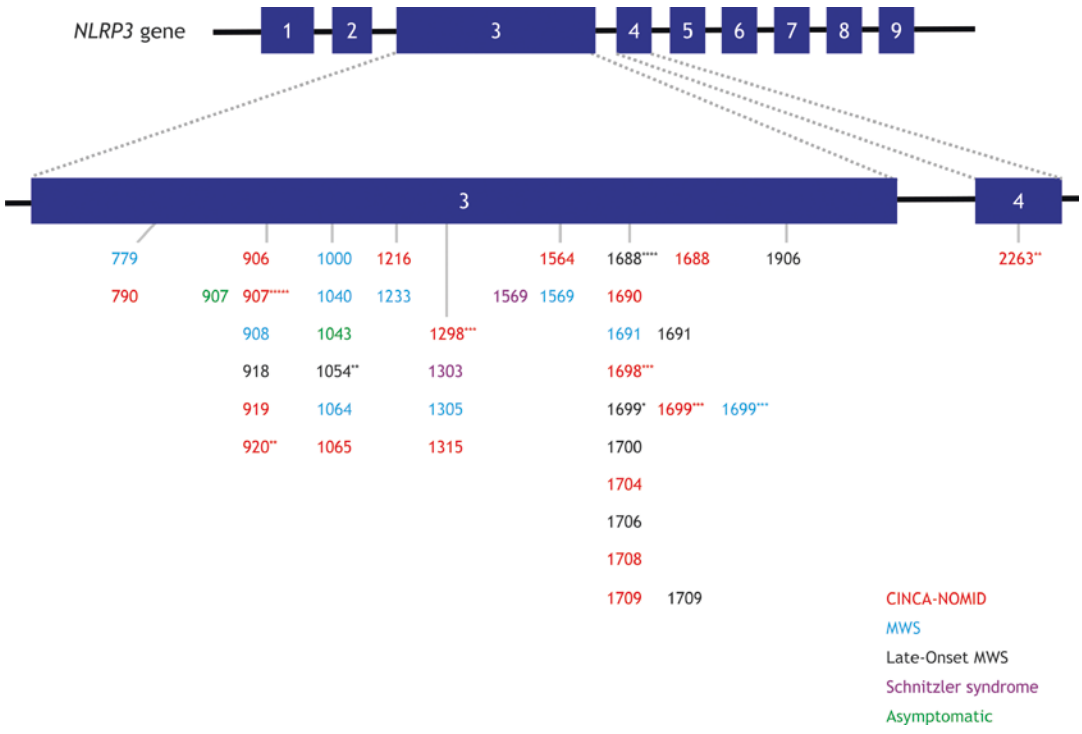

Fig. 2.5 Distribution of known post-zygotic mutations on the *NLRP3* gene. Asterisks indicate total number of unrelated individuals in whom this particular mutation has been detected. *MWS* Muckle Wells syndrome, *NOMID* neonatal-onset multisystem inflammatory disease

in smaller numbers of patients. From a chronological point of view, the first description of a somatic mosaicism affecting a gene other than *NLRP3* was in 2014, when the stimulator of interferon genes (STING)-associated vasculopathy with onset in infancy (SAVI) syndrome was first published [45]. This disease is characterized by a vascular inflammation restricted to capillaries, with cutaneous and pulmonary manifestations as the main features (see Chap. 24). SAVI is caused by dominantly-inherited *gain-of-function* mutations in the *TMEM173* gene, which encodes for the STING protein. Among the six patients included in the original article, one (patient 6) carried the p.Asn154Ser *TMEM173* mutation, the most common among those causing SAVI syndrome, as a post-zygotic mutation. The authors detected this mutation by analyzing the differences in the intensity of fluorescence of the peaks obtained in the Sanger sequencing when they analyzed DNA samples from different origins (whole blood, isolated neutrophils, buccal mucosa, keratinocytes) [45].

Blau syndrome is an early-onset disease (before 4 years of age) characterized by the triad of dermatitis, oligo-polyarthritis with tenosynovitis and uveitis (see Chap. 20). Its hallmark is the presence of non-caseating granulomata in the affected tissues. It is inherited as a dominant trait as a consequence of dominantly-inherited *gain-of-function* mutations in or around the central NACHT domain of the *NOD2* gene [110]. Interestingly, two different mutations located in the 334 amino acid residue (p.Arg334Gln and p.Arg334Trp) have been detected as the pathogenic defect in 80–85% of all reported patients. In 2015, the first somatic low-level mosaicism affecting the *NOD2* gene was described in a patient with a clinical suspicion of Blau syndrome [106]. Since then, only one additional patient with severe Blau syndrome was discovered to have a somatic mosaicism (personal communication). Both patients carried the p.Arg334Gln as the post-zygotic *NOD2* mutation, with differences in the mutant allele frequency (8% in the first case and 40.5% in the last patient). These differences may explain, at least partially, the marked phenotypic differences observed among both patients, with a milder disease in the first patient carrying the lowest mutant allele frequency in comparison to the patient with the highest mutant allele frequency. The phenotype of the last patient was indistinguishable to patients carrying germline mutations. Interestingly, in both patients the gene mosaicism was first suspected by the results of Sanger sequencing due to the presence of small peaks in the chromatograms and subsequently confirmed by NGS-based methods. These observations reinforce the need to carefully review the Sanger chromatograms to identify those small peaks that may represent post-zygotic mutant alleles.

Dominantly-inherited mutations in the *NLRC4* gene have been associated with a complex autoinflammatory disease characterized by early-onset enterocolitis, recurrent fever, urticarial-like skin rash and recurrent, life threatening episodes of macrophage activation syndrome [110] (see Chap. 29). In 2017, a Japanese group reported the first patient carrying a somatic mosaicism in the *NLRC4* gene, with a complex study involving generation of induced pluripotent stem cells and whole-exome sequencing [105]. Interesting, the clinical diagnosis of the first patient with somatic *NLRC4* was NOMID. This insight raises the possibility that patients with a clinical suspicious of CAPS with neither germline nor somatic mutations in the *NLRP3* gene could be in fact a consequence of (germline or somatic) mutations in the *NLRC4* gene.

2.4.3.3 Gonadal and Gonosomal Gene Mosaicism

The presence of post-zygotic mutations in gonadal tissues leads to a moderate to high risk to transmit the mutant allele to the offspring. When this phenomenon occurs, the mutation will be present at the time of conception of the new individual, and consequently the mutation in the offspring will be of germline type. The appearance of clinical symptoms of the disease in the new descendant will mainly depend on the Mendelian inheritance pattern, and also may be slightly modified by the penetrance of the mutation. In the case of dominantly inherited diseases, as in many monogenic autoinflammatory

diseases, the presence of the germline mutation in the new descendant will be strongly associated with the development of clinical symptoms, independently of his/her sex, and these symptoms will be more severe than those observed in the parent carrying mosaicism. In X-linked diseases, the scenario for male new descendants are similar to that previously described for dominant diseases. By contrast, female new descendants will be asymptomatic, but potential carriers of a germline mutation. Finally, in classic recessive diseases, the new descendant will be a carrier of a germline mutation on one allele, and the development of clinical symptoms will depend on the presence or absence of an accompanying mutation in the opposite allele.

In monogenic autoinflammatory diseases, no cases of pure gonadal mosaicism have been reported to date. By contrast, seven cases of gonosomal mosaicism (post-zygotic mutation present in gonadal tissue as well as extragonadal tissue) have been detected (Tables 2.4 and 2.5). These cases have been identified in four different diseases, including CAPS, Blau syndrome, TRAPS and haploinsufficiency of A20 ([98, 107–109]; JIA, personal unpublished data). Beyond the risk of transmitting the mutant allele to the offspring, individuals with gonosomal mosaicism are also at risk to develop clinical symptoms due to the presence of the mutation in somatic cells. Among the seven individuals with gonosomal mosaicism detected to date, five were asymptomatic and two displayed clinical symptoms of the respective disease with varying degrees of severity.

In asymptomatic individuals, the identification of post-zygotic mutations was achieved by genetic analyses performed during genetic counseling of families of patients carrying apparent *de novo* mutations ([98, 108]; JIA, personal unpublished data). Finding a mutation previously detected in the patient in the peripheral blood of one of the parents as a post-zygotic mutation strongly supports the presence of somatic mosaicism. Moreover, the transmission of the mutation to his/her offspring strongly suggests that it is also present in the gonadal tissue with the consequent risk of recurrence of the disease in future pregnancies.

In the two symptomatic individuals, the first genetic analyses identified the post-zygotic gene mutation in their peripheral blood, as this sample is the most commonly obtained in genetic studies. An accompanying gonadal mosaicism in these individuals was identified by direct analysis of gonadal tissue [107, 109]. To date, these studies have only been performed in males. The phenomenon of gonadal mosaicism may also occur in women, but in women experimental confirmation is usually not performed, because the collection of gonadal tissue is an invasive procedure with potential undesirable adverse effects.

2.4.4 Conclusions on Genetic Mosaicism

Post-zygotic mutations and gene mosaicism have been described during the last decade in around 70 patients with several monogenic autoinflammatory diseases, mainly as a result of using NGS-based methods in routine genetic screening, and it is expected that this figure will increase. The identification of gene mosaicism in patients expanded the clinical diversity of some diseases towards milder phenotypes or late onset, in some cases as late as the fourth, fifth or sixth decade. Moreover, gene mosaicism may have other serious clinical consequences, some related to therapeutic approaches, allowing to start treatments under the labelled indication. Other consequences are related to gene counseling since the presence of post-zygotic variants in gonadal tissue may provoke an unexpected recurrence of a dominantly-inherited disease in a healthy couple with no mutations detected in previous standard genetic analyses. This emphasizes the importance of considering the possibility of gene mosaicism in the differential diagnosis of patients with suspected autoinflammatory diseases, and comprehensive genetic analysis using NGS methods is strongly recommended in these cases.

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Epigenetics in Autoinflammation

3

Clara Lorente-Sorolla, Mihai G. Netea, and Esteban Ballestar

Abstract

The molecular mechanisms of inflammation involve a series of processes that start as extracellular signals that interact with membrane-bound receptors, cell signaling cascades, nuclear factors, and epigenetic enzymes that activate a specific gene expression program. Environmental factors and/or genetic defects can result in constitutive activation of this program. Recent studies highlight the relevance of epigenetic (dys) regulation in these processes and suggest several implications of these mechanisms and alterations in the clinical management of patients with autoinflammatory diseases. In this chapter, we provide an overview of the latest findings related to the epigenetic control in the function of myeloid cells as main effectors of inflammation, as well as the latest findings in the field of autoinflammatory diseases.

Keywords

Autoinflammation · Epigenetics · DNA methylation · Myeloid cells

Abbreviations

5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
AID	Activation-induced cytidine deaminase
AIM2	Absent in melanoma 2
AP-1	Activator protein
ASC	Apoptosis-associated speck-like protein
C/EBPα	CCAAT/enhancer binding protein
CAPS	Cryopyrin-associated periodic syndromes
CD	Crohn disease
CNO	Chronic non-bacterial osteomyelitis
CREB	cAMP response element-binding protein
DAMPs	Danger-associated molecular patterns
DNMTs	DNA methyltransferases
EBF1	Early B cell factor 1
ETS	E26 transformation-specific
FCAS	Familial cold autoinflammatory syndrome
FMF	Familial Mediterranean fever
HATs	Histone acetyltransferases

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HDACs	Histone deacetylases
HIDS	Hyperimmunoglobulinemia D syndrome
HMTs	Histone methyltransferases
HSCs	Hematopoietic stem cells
IKKs	IκB kinases
IL	Interleukin
IRAK	Interleukin-1 receptor-associated kinases
IRF	Interferon-regulatory factors
I-SRE	Intronic enhancer element
JmjC	Jumonji domain-containing proteins
JNK	c-Jun N-terminal kinases
LPS	Lipopolysaccharide
MAPKs	Mitogen-activated protein kinases
MKD	Mevalonate kinase deficiency
MWS	Muckle-Wells syndrome
NLR	NOD-like receptor
NOMID	Neonatal-onset multisystem inflammatory disease
PAMPs	Pathogen-associated molecular patterns
PAX5	Paired box protein 5
PGE2	Prostaglandin E2
PRRs	Pattern-recognition receptors
STAT	Signal transducer and activator of transcription
TET	Ten-eleven translocation
TNF	Tumor necrosis factor
TRAF	Tumor necrosis factor receptor-associated factor

Key Points

- **Inflammation involves a series of linked processes that range from extracellular stimulation to transcription factor-mediated and epigenetic control**
- **Epigenetic alterations have been associated with both monogenic and genetically complex autoinflammatory diseases**

3.1 Introduction

Inflammation is an adaptive response triggered by infection or tissue damage. It is induced as host defense against invading pathogens, as tissue-repair response, or as homeostatic state restoration [1]. The inflammatory response is very complex and controlled by different regulatory networks which are responsible for modulation of inflammation and its resolution. However, inflammation is sometimes dysregulated and becomes detrimental; there are many diseases such as autoimmune and autoinflammatory disorders, sepsis, atherosclerosis, type II diabetes, or cancer that lead to inflammation [2].

The initial phase of typical inflammatory response is induced by pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). PAMPs are molecular structures or molecules that are shared by most pathogenic bacteria and some viruses, whereas DAMPs are molecules that are actively excreted or passively released by stressed or dying cells and further enhance inflammatory or cell-death signaling. PAMPs and DAMPs act through germ-line encoded pattern-recognition receptors (PRRs) which are important in microbial recognition and in regulation of inflammatory response [1]. These receptors, which act as key components in this initial process, are mainly expressed by myeloid cells including monocytes, macrophages, neutrophils and dendritic cells (see Chap. 4). Cellular activation drives the release of inflammatory cytokines (tumor necrosis factor-TNF, interleukin- IL-1β, IL-6), chemokines (such as chemokine (C-C motif) ligand 2, CCL2, and chemokine (C-X-C motif) ligand 8, CXCL8) as well as prostaglandins (like prostaglandin E2, PGE2). Cytokines and chemokines not only activate these innate immune cells but also can exert induction of acute-phase proteins in the liver, fever and fatigue by acting on the hypothalamus, platelet activation, and a multitude of cellular processes [3] (see Chap. 6).

In particular, engagement of extracellular or intracellular PRRs triggers cell signaling pathways that lead to the recruitment of signaling proteins including members of the tumor necrosis factor receptor-associated factor (TRAF) family and various protein kinases such as IL-1 receptor-associated kinases 1 and 4 (IRAK1 and IRAK4). These molecules activate several effector molecules and transcription factors. The most important activated signal routes are mitogen-activated protein kinases (MAPKs) and IκB kinases (IKKs) (Fig. 3.1a). MAPKs include, among others, c-Jun N-terminal kinases (JNKs) and p38 which

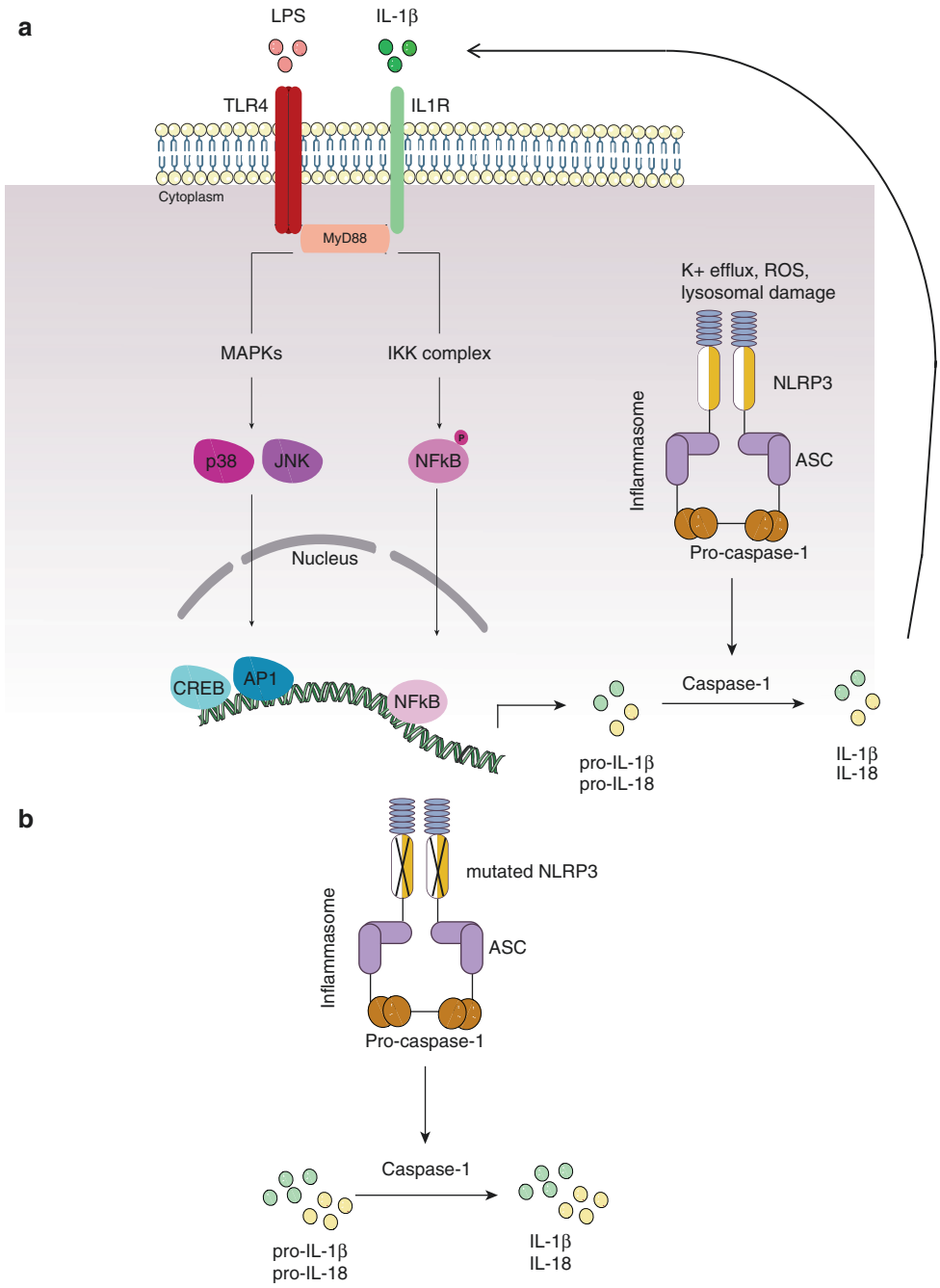

Fig. 3.1 Mechanisms of inflammation and links with epigenetic changes (**a**) Scheme depicting cellular pathways in monocytes/macrophages related to inflammation. Toll-like receptor 4 (TLR4) is a pattern recognition receptor (PRR) that recognizes bacterial antigens, such as lipopolysaccharide (LPS). Interleukin-1 receptor (IL-1R) is a cytokine receptor which binds interleukin 1 (IL-1). Activation of TLR4 and IL-1R result in activation of myeloid differentiation primary response 88 (MYD88), which subsequently activates transcription factor NF-kB and mitogen-activated protein kinase (MAPKs). Both have an effect on the activation of specific genes either by direct binding to chromatin or through the activation of downstream transcription factors, such as cAMP response element-binding protein (CREB) or activator protein (AP1). Transcription factors influence the acquisition of epigenetic changes. Increased transcription of the *IL1B* gene leads to an amplification loop involving the activity of the inflammasome (**b**) Mutations in the inflammasome subunit NLRP3 lead to an increase of IL1-β production and therefore impacts the nuclear effects of inflammation

phosphorylate and activate several transcription factors such as activator protein (AP-1) and cAMP response element-binding protein (CREB). Secondly, IKKs participate in the activation of the NF-κB transcription factor and subsequently all their gene targets [4]. Altogether, inflammation is able to activate several crucial signaling pathways to immune cells.

A key signaling pathway that controls the innate immune response by regulation of inflammation and tissue repair is mediated by the inflammasome. Inflammasomes are multimeric complexes that assemble following the detection of microbial pathogens and DAMPs (potassium efflux, reactive oxygen species, monosodium urate crystals, cathepsin) [5]. Inflammasomes consist of a sensor molecule [including members of the NOD-like receptor (NLR) or absent in melanoma 2 (AIM2)], an adaptor molecule (apoptosis-associated speck-like protein, ASC) and the effector molecule pro-caspase-1. Once inflammasomes are assembled, they activate caspase-1 which processes inactive proinflammatory cytokine precursors of IL-1β and IL-18 into their mature forms. In addition, inflammasome activation leads to an inflammatory cell death pathway known as pyroptosis [5, 6] (see Chap. 5). The inflammatory response activates various signaling pathways that regulate expression of numerous mediators. As mentioned above, reversal of this response is crucial to return to homeostasis; if inflammasome activation persists over time and there is a lack of inhibition, inflammation-related disorders occur. Therefore, several mechanisms inhibit or attenuate inflammation including anti-inflammatory cytokines (IL-10, IL-37, etc), receptor antagonists (IL-1R, TNFR), complement inhibitors, negative regulators of Toll-like-receptor signaling, prostaglandins and lipid mediators [2].

During the past 25 years, a group of disorders characterized by a dysregulated inflammatory response has been established under the term of autoinflammatory diseases. Autoinflammatory diseases, often linked to genetic defects, are characterized mainly by systemic or organ specific inflammation and recurrent fever in the relative absence of autoreactive T cells, high autoantibody titers or any detectable pathogen [7]. Thereby, the term autoinflammation is connected with dysregulation in the innate immune system [8]. Episodic fever, rash, swelling of joints and other tissues and overproduction of IL-1β are common findings associated with these diseases [8, 9].

New insights into the pathogenesis of autoinflammatory diseases have recently provided increasing evidence that epigenetic modifications are involved. For this reason, the identification of these epigenetic changes is crucial for patient diagnosis and new therapies.

3.2 Epigenetic Control in Immune Cells

Key Points

- **Epigenetic mechanisms involve the establishment of transcriptional activity states through the chemical and reversible modification of DNA and histones**
- **Epigenetic control participates both in the acquisition of cell identity and activation of inflammatory cells**

Epigenetics has been defined as the set of mechanisms that register, signal or perpetuate altered activity states without changing the DNA sequence. Epigenetic modifications play an important role in the regulation of gene expression [10]. In general, epigenetic mechanisms mainly involve modification of amino acid residues in the histone N-terminal ends, and DNA methylation (Fig. 3.2). Some also include non-coding RNAs-mediated processes as epigenetic mechanisms [11].

3.2.1 DNA Methylation and Histone Modifications

In mammals, DNA methylation generally refers to the addition of a methyl group to cytosine (5mC) and it takes place in CpG dinucleotides, although methylation has recently been demonstrated to occur in other nucleotides, in very low

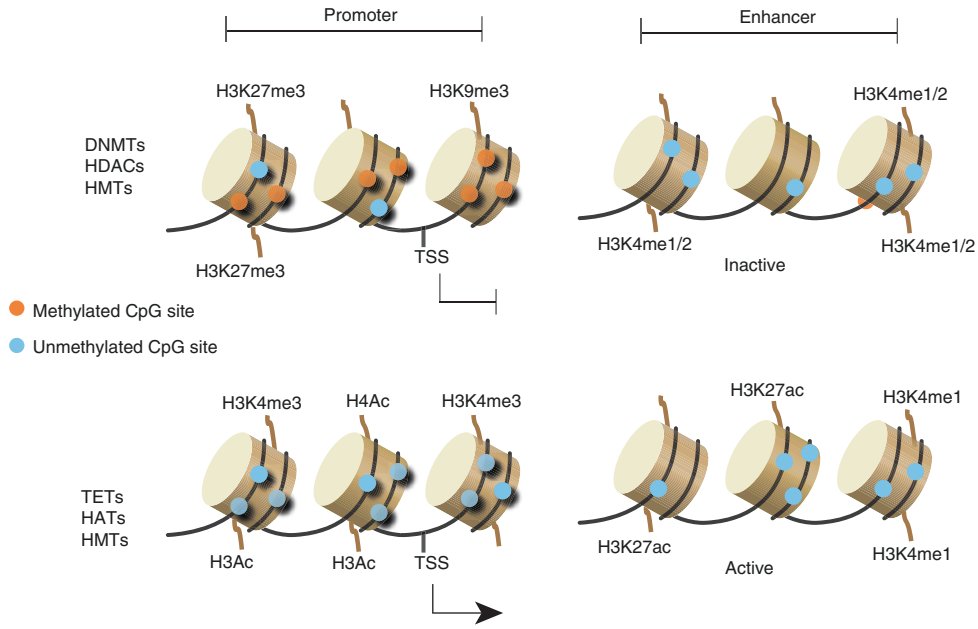

Fig. 3.2 A summary of epigenetic modifications and their relationship with transcriptional activity. Two groups of modifications are presented: methylation of cytosines, that occurs at CpG dinucleotides and post-translational modifications of histones that mainly occur at the N-terminal tails. Although these modifications occur along the entire genomic DNA sequence, in this figure two specific genomic regions have been chosen: gene promoters (left) and enhancers (right). Inactive promoters (top) are enriched in histone modifications such as trymethylation of lysine 27 of histone 3, H3K27me3 (and sometimes H3K9me3), are devoid of acetylated histones and can be heavily methylated for some CpG island containing promoters. Active promoters are enriched in trymethylation of lysine 4 of histone 3, H3K4me3, hyperacetylated histones (H3Ac and H4Ac) and low levels of 5mC. Enhancers regulate transcription at a long distance and are marked by H3K4me1 (in both active and inactive enhancers) and with H3K27Ac only in active ones. Enzymes implicated in these processes are DNA methyltransferases (DNMTs), histone acetyltranferases (HATs) and deacetylases (HDACs), ten eleven translocation (TET) and histone methyltransferases (HMTs)

proportion. The incorporation of a methyl group to cytosines is catalyzed by a family of enzymes known as DNA methyltransferases (DNMTs). DNMT1 is responsible for the maintenance of DNA methylation during replication whereas DNMT3A and DNMT3B are involved in the establishment of *de novo* methylation [12]. The reversion or loss of methyl groups, known as DNA demethylation, can be passive, caused by the inefficient maintenance of methylation during DNA replication, or active. Active DNA demethylation involves the activity of ten-eleven translocation (TET) enzymes that catalyze the conversion of 5mC to 5-hydroxymethylcytosine (5hmC), and further oxidize it into other intermediate forms that are then excised by thymine DNA glycosylase (TDG). The activity of the DNA repair machinery later restores the incorporation of an unmethylated cytosine [12, 13]. DNA methylation can influence gene expression through different mechanisms including the interference of transcription factor binding or the recruitment of histone modifiers or chromatin remodeling complexes containing methyl-CpG binding domain proteins. These effectors can alter chromatin accessibility or competence and modulate gene transcription. DNA methylation gains in promoters are generally associated with transcriptional repression. However, the effects of DNA methylation changes on gene transcription can be different depending on the genomic location.

Histone proteins interact closely with DNA and are responsible for packing DNA, by wrapping it around the histone octamer. Amino acid residues in histones are subjected to covalent post-translational modifications that include methylation, phosphorylation, acetylation and others [14]. Histone acetylation is the addition of an acetyl group carried out by histone acetyltransferases (HATs); acetylation plays important roles in chromatin dynamics, gene silencing, DNA repair, etc. Histone deacetylases (HDACs) catalyze the removal of the acetyl group to histone residues providing an equilibrium reaction. Another important modification is the methylation of histone lysine or arginine residues by different families of histone methyltransferases (HMTs). Methylation also affects gene transcription, promoting activation or repression, depending on the modified residue. Histone demethylation is the removal of methyl groups in modified histones via histone demethylases as Jumonji domain-containing proteins (JmjC). These modifications participate in regulating gene expression in very different ways depending on various factors, such as genomic location. For instance, acetylation of histones H3 and H4 and trimethylation of each K4, K36 and K79 of histone H3 are associated with a more open chromatin structure and correlate with active transcription. By contrast, histone deacetylation and methylation of K9 and K27 of histone H3 mark silenced regions [15].

3.2.2 Epigenetic Control of Differentiation of Hematopoietic Stem Cells

Epigenetic control is crucial to determine cell fate decisions. Extracellular signals that are internalized through receptors and signaling cascades establish a crosstalk with lineage-specific transcription factors, which interact with epigenetic complexes. Immune cells are a very good example of this interplay between extracellular signals, signaling pathways, transcription factors and epigenetic enzymes. Immune cell differentiation involves the differentiation from hematopoietic stem cells (HSCs) to a large number of cell types, which are mainly grouped in two branches, lymphoid and myeloid cells. In the past few years a number of epigenomic studies have delineated the range of both DNA methylation and histone modification changes that occur during the differentiation of HSCs [16]. In human hematopoiesis, distinct DNA methylation changes are pivotal to promoting the commitment to lymphoid or myeloid differentiation. A genome-wide methylation analysis during hematopoietic cell differentiation revealed an increase in DNA methylation levels during lymphoid cell differentiation whereas a loss of methylation is associated with myeloid cell differentiation [17]. In addition, DNMT3A and DNMT3B are needed to *de novo* methylate and repress genes encoding transcription factors involved in the self-renewal capacity of hematopoietic stem cells, subsequently allowing cell differentiation [16]. Analysis of the epigenome of HSCs has shown that important transcription factors for hematopoiesis, including CCAAT/enhancer binding protein (C/EBPα), early B cell factor 1 (EBF1) and paired box protein 5 (PAX5) are demethylated and are also enriched in both activating H3K4me3 and repressive H3K27me3 (bivalent) histone marks [18]. One of the conclusions of the aforementioned studies is that myeloid and lymphoid cells are very different in relation to the participation of the epigenetic machinery. For instance, TET2 is a key enzyme in the acquisition of myeloid cell identity since the discovery that C/EBPα activates TET2 during C/EBPα-mediated B cell to macrophage reprogramming of pre-B cells. Furthermore, mutated TET2 has been described to be related with several myeloid malignancies [19]. Moreover, activation-induced cytidine deaminase (AID), comprehensively studied for its role in class-switch recombination and somatic hypermutation in B lymphocytes, has also been reported to participate in promoting DNA demethylation changes during B cell differentiation [20], although this role remains controversial. Histone modifiers are also important in myeloid- or lymphoid-specific cell development and identity. For example, the histone demethyltransferase KDM2B acts as key

regulator during lymphoid differentiation since ectopic expression of KDM2B favors lymphoid commitment [21]. It is also necessary to take into account that mutations in genes involved in epigenetic regulation are very common in leukemia. Finally, class II histone deacetylase HDAC7 has a transcriptional repression role of myeloid specific genes and its downregulation is crucial during C/EBPα-mediated reprogramming of B cells into macrophages [22].

3.3 Epigenetic Control in Inflammation

Although inflammation encompasses the activity of both innate and adaptive immune cells, myeloid cells are the main effectors of the inflammatory process; monocytes, macrophages, neutrophils or dendritic cells are very plastic and can display epigenetic modifications. Therefore, it is crucial to understand epigenetic changes in these cells that can contribute to chronic inflammation and disease.

Many myeloid transcription factors, including signal transducer and activator of transcription (STAT) family members, interferon-regulatory factors (IRFs), NF-κB family and members of the ETS (E26 transformation-specific or E-twenty-six) family such as PU.1, can recruit or associate DNMTs and histone modifying enzymes. This implicates a role of epigenetic mechanisms in the differentiation into inflammatory cell types, as well as in immune-related gene transcription [23, 24]. In this regard, chromatin structure is crucial to control NF-κB-regulated genes such as proinflammatory cytokines. As an example, following lipopolysaccharide (LPS) stimulation of macrophages, TLR-induced genes have been categorized into two classes: tolerized genes, which include inflammatory genes, show repressed expression whereas non-tolerized genes, which include antimicrobial mediators, increase their levels of expression. These changes in expression are related to the fact that histone acetylation and H3K4 methylation are only maintained on the promoters of the non-tolerized genes [4].

Activation of dendritic cells and macrophages is also regulated by epigenetic modifications. For instance, dendritic cell development and maturation is accompanied by significant DNA demethylation [25]. During macrophage polarization, lower expression of DNMT3B promotes a shift towards the M2 (anti-inflammatory) macrophage phenotype [26]. Furthermore, chromatin remodeling is also important in the acquisition of the M2 phenotype; demethylation of H3K27 by Jmjd3 and the absence of HDAC3 lead to M2 polarization [16]. Also, in the case of monocyte to macrophage differentiation, epigenetic reprogramming associated with the acquisition of specific epigenetic signatures has been observed. For example, priming of monocytes or macrophages by an initial stimulus (such as LPS or β-glucan) renders a tolerized or trained phenotype, respectively, both associated with epigenetic alterations in H3K4me3, H3K27Ac and H3K4me1. In this context, initial engagement of PRRs leads to a global acetylation and H3K4me3 mark in proinflammatory genes. Subsequent challenges produce loss of H3K4me3 and acetylation in the case of tolerized genes and leads to silent gene expression. By contrast, trained immunity retains initial histone marks in promoters of important genes (such as TNF and IL-6) facilitating gene transcription [23, 27].

3.4 Perspectives on Autoinflammatory Diseases

Key Points

- **Untreated CAPS patients show exacerbated DNA demethylation of several inflammasome-related genes whereas this demethylation is reverted in CAPS treated with anti-IL-1 drugs**
- **Upregulation of miR-4520a has been observed in FMF patients**
- **In CNO, a complex autoinflammatory disorder, the expression of IL-10 and IL-19 is decreased through impaired chromatin remodeling**

Autoinflammatory disorders comprise a wide range of pathologies characterized by hyperinflammation and recurrent attacks of fever as well as activation of innate immune cells [8]. Advances in genomic techniques (such as next-generation sequencing) have resulted in the inclusion as autoinflammatory syndromes both hereditary monogenic disorders such as cryopyrin-associated periodic syndromes (CAPS) (see Chap. 19) and familial Mediterranean fever (FMF) (see Chap. 16), as well as multifactorial and complex diseases such as Behçet disease (see Chap. 35), chronic non-bacterial osteomyelitis (CNO) (see Chap. 31) and Crohn disease (CD) among others [7]. The implication of epigenetic factors in monogenic and complex inflammatory diseases might be very different.

Several studies have reported that epigenetic modifications may participate in the development and pathogenesis of autoinflammatory diseases. Altered DNA methylation, covalent histone modification and miRNAs dysregulation have been linked as additional factors in these pathologies (Table 3.1).

3.4.1 Cryopyrin-Associated Periodic Syndromes (CAPS)

Despite the fact that monogenic autoinflammatory syndromes are caused by mutations in specific inflammatory-related genes, heterogeneous patient phenotypes and diverse drug response within the same disorder may suggest contribution of epigenetic factors [28]. For instance, cryopyrin-associated periodic syndromes (CAPS) are described as a spectrum of heterogeneous phenotypes with different degrees of severity; this suggest that additional factors as epigenetic modifications may contribute to the disease [29]. CAPS are monogenic autoinflammatory diseases which, in increasing order of severity, include: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease (NOMID). Gain-of-function mutations of *NLRP3* gene (Fig. 3.1b), a well-known member of the inflammasome family, cause this rare disease, resulting in markedly increased IL-1β production and secretion [30]. Furthermore, *NLRP3* mosaicism (in the myeloid compartment) has been described in some CAPS patients with diverse disease severity, including adult-onset CAPS [28]. A recent study showed that DNA demethylation of several inflammasome-related genes is more efficient in monocytes from untreated CAPS patients than those of healthy counterparts (Fig. 3.3). Interestingly, monocytes from patients with CAPS treated with IL-1 inhibitors display methylation levels similar to those seen in control subjects, suggesting the effectiveness of the drug in preventing the exacerbated demethylation of inflammasome genes [30]. Another study found that NOMID patients present significantly down-regulated expression of miR-29c and miR 103-2

Table 3.1 Evidence of epigenetic contributions to autoinflammatory disease

Disease	Gene/protein	Epigenetic alterations	References
FMF	*MEFV*/ pyrin	Gains of DNA methylation of *MEFV* gene/Upregulation of miR-4520a	[32, 33]
CAPS	*NLRP3*/ NLRP3	DNA demethylation of inflammasome-related genes in untreated CAPS patients/miRNAs regulation in NOMID patients	[30, 31]
MKD	*MVK*/MVK	Trained immunity phenotype of monocytes	[34]
BD	Complex	Different DNA methylation patterns in monocytes and CD4+ cells	[35]
CNO	Complex	Failure of H3 phosphorylation at serine residue 10 (H3S10p) at the *IL-10* proximal promoter	[36]
CD	Complex	*DNMT3A* as a susceptibility gene/Differential methylation in several immune-related genes	[37, 38]

FMF Familial Mediterranean fever, *CAPS* Cryopyrin-associated periodic syndromes, *MKD* Mevalonate kinase deficiency, *BD* Behçet disease, *CNO* Chronic non-bacterial osteomyelitis, *CD* Crohn disease

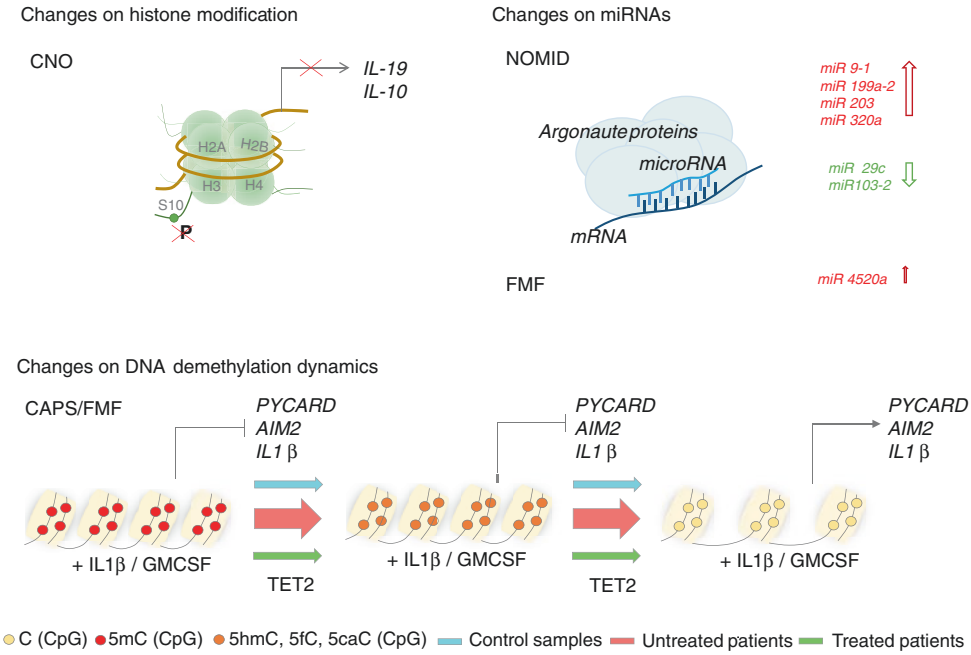

Fig. 3.3 Epigenetics in autoinflammatory diseases. Several autoinflammatory diseases have been associated with epigenetic changes. In chronic non-bacterial osteomyelitis (CNO), IL-19 and IL-10 expression is impaired due to a failure of histone H3 phosphorylation at serine residue 10 (H3S10p) in the promoter region. Changes in miRNA levels have been associated with neonatal-onset multisystem inflammatory disease (NOMID) (increase of miR 9-1, miR 199a-2, miR 203 and miR 320a, and a decrease of miR 29c and miR103-2 in their skin) and with familial Mediterranean fever (FMF) (increase in miR-4520a). In cryopyrin- associated periodic syndromes (CAPS) and FMF, changes in DNA demethylation dynamics have been reported. DNA demethylation (in light circles) is associated with increased gene expression of some inflammasome-related genes (*PYCARD, AIM2* and *IL1B*). Figure from Álvarez-Errico D, Vento-Tormo R, Ballestar E (2017) Genetic and epigenetic determinants in autoinflammatory diseases. Front Immunol. https://doi.org/10.3389/fimmu.2017.00318 [7]

but an increase of miR 9-1, miR 199a-2, miR 203 and miR 320a in skin lesions, suggesting the involvement of miRNA-mediated dysregulation in this disease (Fig. 3.3). Furthermore, several genes encoding histone modifiers were found to display aberrant expression levels in lesional skin compared to normal skin [31].

3.4.2 Familial Mediterranean Fever (FMF)

Another example of the role of epigenetics in monogenic autoinflammatory diseases involves familial Mediterranean Fever (FMF), an autosomal recessive disorder characterized by recurrent attacks of fever, caused by mutations in the *MEFV* gene, which encodes the pyrin protein [29]. Pyrin, which is expressed mainly in myeloid cells, is implicated in inflammation by the activation of caspase-1, which is responsible for the maturation of IL-1β and IL-18. Changes in DNA methylation dynamics have been described in FMF (Fig. 3.3). A slightly increased methylation of the second exon of *MEFV* in peripheral leukocytes from FMF patients is associated with reduced *MEFV* expression level [32]. In addition, upregulation of miR-4520a expression levels has recently been reported in patients with FMF (Fig. 3.3) [33].

3.4.3 Mevalonate Kinase Deficiency (MKD)

Mevalonate kinase deficiency (MKD), also known as hyperimmunoglobulinemia D syndrome (HIDS), is caused by loss-of-function mutations in *MVK,* which lead to accumulation of mevalonate [29] (see Chap. 17). Monocytes from patients with MKD have a trained immunity phenotype; analysis of H3K27ac histone mark by ChIP-sequencing also shows different peaks in patients compared to healthy controls [34].

3.4.4 Behçet Disease

There are also studies implicating epigenetic dysregulation on the disease course of multifactorial and complex autoinflammatory diseases. It has been suggested that changes in global DNA methylation may be responsible for the pathology of Behçet disease. Behçet disease is a chronic multi-systemic inflammatory disorder characterized by complex and numerous symptoms (recurrent oral and genital ulcers, skin lesions, uveitis, among others) [8]. Genome-wide DNA methylation analysis in monocytes and CD4+ cells of patients with Behçet disease shows different methylation levels in comparison to healthy controls; specifically, 383 differentially methylated CpGs were identified in BD monocytes, and CD4+ lymphocytes displayed 125 differential CpGs sites. This aberrant methylation is associated with important genes for structural and functional cytoskeletal proteins in monocytes and for antigen processing and presentation in CD4+ cells. Importantly, patients with Behçet disease in remission following treatment show a partial restoration of the DNA methylation pattern, similar to controls [35].

3.4.5 Chronic Non-bacterial Osteomyelitis (CNO)

There is evidence of epigenetic contribution in chronic non-bacterial osteomyelitis (CNO). CNO is an autoinflammatory disorder that mainly affects bones and it is occasionally associated with inflammatory bowel disease. CNO presents an imbalance of pro- and anti-inflammatory cytokines and regulatory signals; in particular, decreased IL-10 and IL-19 expression has been reported in these patients. This repression is suggested to be caused by chromatin remodeling; an altered histone H3 phosphorylation at serine residue 10 (H3S10p) in the promoter region impairs cytokines expression (Fig. 3.3). Moreover, a differential DNA methylation in IL-10 intronic enhancer element (I-SRE) has also been observed, giving strong support to the hypothesis of epigenetic contribution to pathophysiology in CNO [36].

3.4.6 Crohn Disease

Epigenetic modifications have also been observed in Crohn disease (CD). CD, which is one of the main types of inflammatory bowel disease, is a polygenic disease that presents a dysregulated response to intestinal microbiota in genetically susceptible individuals. In 2010, *DNMT3A* was identified by genome-wide association studies (GWAS) as a susceptibility gene for CD, suggesting a possible relationship between altered DNA methylation and the disease [37]. Further genome-wide methylation analysis in CD patients shows a specific methylation pattern in peripheral blood of patients compared to controls, with methylation changes in several important immune response genes including *MAPK13*, *FASLG*, *PRF1*, *S100A13*, *RIPK3*, and *IL-21R* [38]. Moreover, miRNA expression profiles of CD have also been studied in tissue and peripheral blood, although it is still necessary to further study the implications of their dysregulation to understand the possible role of miRNAs in CD diagnosis or therapy [7].

3.5 Conclusions

Based on the observations we described, it is becoming clear that epigenetic mechanisms likely contribute to the pathophysiology of

autoinflammatory diseases (Table 3.1 and Fig. 3.3). An increasing number of studies has addressed the participation of different epigenetic mechanisms involved in differentiation and function of myeloid cells, including their role in inflammation. Future efforts in the knowledge of autoinflammation could establish epigenetic modifications as crucial factors in related diseases, allowing the identification of attractive targets for novel therapeutic interventions. Although we are still far from understanding the complete extent of epigenetic alterations in autoinflammatory syndromes, a better knowledge of epigenetic deregulation in these patients will help to open new therapeutic approaches in these diseases.

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Pattern Recognition Receptors in Autoinflammation

4

Victor Saavedra, Fiona Moghaddas, Eicke Latz, and Seth L. Masters

Abstract

The immune system is essential for maintenance of tissue homeostasis. This task requires that immune cells detect and respond to dyshomeostatic states (when homeostasis has broken down) that can occur during invasion of the host with pathogenic microbes, after sterile trauma of tissues or during metabolic derangements. Research in the field of innate immunity has uncovered many molecular mechanisms by which the immune system can prevent the spread of infection, restore damaged tissues and respond to altered metabolism. These pathways involve different classes of pattern recognition receptors, some of which can directly detect minimal motifs (patterns) that are common to multiple pathogens or types of damaged cells. Here, we summarize the general concepts that have been developed to explain how immune recognition of dyshomeostasis is achieved and discuss our current knowledge of the innate immune signaling receptors that are known to directly bind ligands.

Keywords

Toll-like receptor (TLR) · Nucleotide-binding oligomerization domain (NOD) · NOD-like receptor (NLR) · C-type lectin receptor (CLR) · RIG-I-like receptor (RLR) · Pattern recognition receptor (PRR)

Abbreviations

ADAR1	Adenosine deaminase acting on RNA 1
AGS	Aicardi-Goutières syndrome
AIM2	Absent in melanoma 2
ASC	Apoptosis related speck-like protein containing CARD

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ATP	Adenosine triphosphate
BS	Blau syndrome
CARD	Caspase activation and recruitment domain
CD	Crohn's disease
CDN	Cyclic dinucleotides
cGAMP	Cyclic GMP-AMP
cGAS	cGAMP synthase
CLR	C-type lectin receptor
CRISPR	Clustered regularly interspaced short palindromic repeats
CTLD	C-type lectin like domain
DAMP	Damage associated molecular patterns
DAP	Diaminopimelic acid
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA
EOS	Early onset sarcoidosis
FcRγ	Fc receptor gamma chain
GTP	Guanosine triphosphate
HA	Hyaluronic acid
HAMP	Homeostasis-altering molecular processes
HIN	Hematopoietic expression, interferon-inducible nature, and nuclear localization
HIV	Human immunodeficiency virus
HMGB	High mobility group box 1
HMW	High molecular weight
HSE	Herpes simplex encephalitis
HSP	Heat shock protein
IBD	Inflammatory bowel disease
IFN	Interferon
IFNAR	Interferon alpha/beta receptor 1
IKK	IκB kinase
IL	Interleukin
IRF3	Interferon regulatory factor 3
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibition motif
IκB	Inhibitor of NF-κB
JAK	Janus kinase
KO	Knock-out
LGP2	Laboratory of genetics and physiology 2
LMW	Low molecular weight
LOX-1	Lectin-like oxidized LDL receptor 1
LPS	Lipopolysaccharide
LRR	Leucine-rich-repeat
MAL	MyD88 adaptor like (= TIRAP)
MAPK	Mitogen-activated protein kinase 1
MAVS	Mitochondrial antiviral signaling
MCMV	Mouse cytomegalovirus
MD2	Myeloid differentiation factor 2
MDA5	Melanoma differentiation-associated protein 5
MDP	Muramyl dipeptide
MICL	Myeloid inhibitory C-type lectin
Mincle	Macrophage-inducible C-type lectin
miRNA	Micro RNA
mRNA	Messenger RNA
MSU	Monosodium urate
MyD88	Myeloid differentiation primary response gene 88
NBS	Nucleotide binding site
NFAT	Nuclear factor of activated T-cells
NF-κB	Nuclear factor–κB
NK	Natural killer
NLR	NOD-like receptor
NLRP	NOD-like receptor protein
NOD	Nucleotide-binding oligomerization domain
oxPAPC	Oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine
PAMP	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
pDCs	Plasmacytoid dendritic cells
PID	Primary immunodeficiency
POP	PYD-only protein
PRR	Pattern-recognition receptors
PYD	Pyrin domain
RIG-I	Retinoic acid-inducible gene 1
RIPK2	Receptor-interacting serine/threonine kinase 2
RLR	RIG-I-like receptor
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SARM	Sterile α- and armadillo motif containing protein
SAVI	STING-associated vasculopathy with onset in infancy
SH2	Src homology region 2

SHP	SH2 domain-containing phosphatase
siRNA	Small interfering RNA
SMS	Singleton-Merten syndrome
SNP	Single nucleotide polymorphism
ssRNA	Single stranded RNA
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
TBK1	TANK-binding kinase 1
TFAM	Mitochondrial transcription factor A
TIR	Toll/IL-1 receptor
TIRAP	TIR domain containing adaptor protein (= MAL)
TLR	Toll-like receptor
TMEM173	Transmembrane protein 173
TRAM	TRIF-related adaptor molecule
TREX	Three-prime repair exonuclease 1
TRIF	TIR-domain containing adaptor protein inducing IFN-β (=TICAM1)
tRNA	Transfer RNA
UNC93B1	Unc-93 homologue B1
WES	Whole exome sequencing
WT	Wild type

Key Points

- **A series of germline encoded pattern recognition receptors (PRRs) directly detect the presence of ligands associated with disease or infection**
- **Activating mutations in PRRs can trigger autoinflammatory disease, loss of functions mutations are associated with immunodeficiency**
- **Inappropriate clearance or modification of ligands can potentiate PRR signaling and autoinflammation**

4.1 Introduction: Molecular Patterns and Processes

Key Points

- **Pathogen-associated molecular patterns (PAMPs) directly engage PRRs on the cell surface or in the cytoplasm**
- **Damaged host cells generate danger-associated molecular patterns (DAMPs) that also directly engage PRRs**
- **Cells that are damaged also trigger homeostasis altering molecular processes (HAMPs) that activate innate immune sensors indirectly**

Every organ and tissue contains a large number of tissue resident immune cells, which monitor their environment for molecules that signify invasion by pathogenic microorganisms, tissue damage or a combination thereof. Once a microbial or injurious threat has been detected, immune cells need to integrate multiple signals they receive in these dyshomeostatic situations (i.e. when homeostasis has been disrupted). Upon interpretation of these signals, the activation state of these immune cells changes and a balanced, situation-adjusted immune response is orchestrated to fend off the intruding pathogen and initiate tissue repair mechanisms. The blood and lymphatic compartments additionally harbor a large number of diverse immune cells that can be attracted to the endangered tissue and provide support in pathogen defense. The recruited immune cells can mature or polarize locally, aid in the removal of damaged tissue, and provide support for the repair and reconstitution of the dyshomeostatic tissue.

The immune system can be divided into the innate and adaptive immune systems. The innate immune system, the first line defense, is evolutionarily conserved, developing before the separation of vertebrates and invertebrates. The majority of organisms depend solely on the innate immune system for their response to pathogens and tissue dyshomeostasis. In contrast to cells from the adaptive immune system which provide immunological memory and specificity through an almost infinite variability of receptors due to gene rearrangement, innate immune cells express a large array of germline-encoded signaling receptors able to respond quickly and without previous exposure to a threat.

These predefined innate immune signaling receptors can recognize and signal to the presence of molecular patterns specific to microbes, or to host molecules that change subcellular localization or are modified upon tissue damage. Innate immune signaling receptors, also called

pattern recognition receptors (PRRs), are located in all subcellular localizations in cells, including the cell surface, the endolysosomal compartment, the cytoplasm and the nucleus. Of note, it is not only immune cells that express PRRs. Most other cell types that have different primary functions express these sensors as they are also subject to invasion by pathogens, or are necessary for the surveillance of the tissue environment.

This chapter gives an overview on the different families of PRRs and highlights their roles in disease settings in which the presence of activators of these receptors does not disappear, thereby resulting in chronic inflammatory conditions associated with several diseases. Other chapters in this book focus on the diseases associated with mutations in the PRR genes that lead to the development of disease.

4.1.1 Pathogen-Associated Molecular Patterns (PAMPs)

Initially proposed by Janeway in 1989 [1], pathogen-associated molecular patterns (PAMPs) are highly conserved structures that are shared by a specific class of pathogens and are recognized by PRRs. Essential features of PAMPs are their specificity to microbes and the necessity of the structures for survival and pathogenicity of the organism. Yet, since most PAMPs can also be found in non-pathogenic microbes, the molecular patterns recognized by PRRs are not necessarily specific for pathogens. Lipopolysaccharide (LPS), also known as endotoxin, is a prototypical bacterial molecular pattern or PAMP consisting of an O-linked polysaccharide attached to the lipid A moiety that is necessary for viability and virulence of most gram-negative organisms [2, 3]. Although the lipid A component is accepted as the potent PAMP of LPS, response by toll-like receptor (TLR) 4 is affected by the length of the polysaccharide portion [4]. A range of bacterial patterns can also end up in the cytosol and to mediate innate immune detection in this locale, additional pathogen receptors such as NOD1 and NOD2 can detect other bacterial cell wall products.

Viruses are the most prominent group of pathogens that humans have to deal with. The genetic material of viruses varies in chemical structure of the nucleic acid (DNA or RNA) and number of strands (single or double). During a viral infection, the enzymatic machinery of the host cell is hijacked to produce replicates of the virus, with the help of viral enzymes. To create new copies of the virus, the viral nucleic acids must be replicated in the host cell and also converted into messenger RNA (mRNA) that the host protein-synthesizing machinery can translate into new viral proteins. Unlike bacterial PAMPs, viral PAMPs are most often nucleic acids, although components of the capsid or phospholipid envelope can also be recognized by the innate immune system. As a defense mechanism, host cells have evolved different ways to discriminate pathogen-derived from host-derived nucleic acids in the cytoplasm. Sensing of viral nucleic acid leads to changes in gene expression, including the production of the type I IFNs, IFN-α and IFN-β. Type I IFNs are key in orchestrating the antiviral response by the immune system.

4.1.2 Damage- or Danger-Associated Molecular Patterns (DAMPs)

The innate immune system can recognize both pathogen-induced as well as sterile tissue damage resulting from trauma or metabolic disturbances. The immune stimulatory substances appearing during these ‘danger’ situations are called danger-associated molecular patterns (DAMPs). DAMPs represent endogenous molecules that are often modified or exposed during or shortly after cellular death [5, 6]. There are numerous classes of DAMPs, such as modified lipids or nucleic acids, metabolites such as adenosine triphosphate (ATP) or uric acid/monosodium urate (MSU), or proteins such as high mobility group box 1 (HMGB1) or low molecular weight hyaluronic acid (HA). HMGB1, for example, is a nuclear protein that is actively released by activated monocytes and

macrophages, or passively released by damaged cells. It was identified as an important mediator in LPS-induced inflammation in mice [7] and in human monocytes [8]. Of mechanistic importance, Scaffidi et al., showed HMGB1 to be important in the distinction of non-programmed cell death from apoptosis [9], suggesting that HMGB1 may signal unexpected cell death. MSU, the end product of the catabolism of purines, is a well-studied DAMP. In this context it was identified as a small molecule that accumulates in the setting of injured cells [10]. MSU has also been shown to have a role in gout, with MSU crystals activating the nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3), resulting in inflammasome activation and release of IL-1β [11]. High molecular weight HA is a glycosaminoglycan produced by fibroblasts, present in the extracellular matrix and is part of the basement membrane of many organs [12]. High molecular weight HA is degraded to a low molecular weight form at sites of inflammation and in response to inflammatory cytokines [13, 14]. HA was shown to be a ligand of TLR2 using both a HEK293T luciferase model and *TLR2*$^{-/-}$ mice [15].

4.1.3 Homeostasis-Altering Molecular Processes (HAMPs)

The concept of homeostasis-altering molecular processes (HAMPs) was introduced by Liston and Masters in 2017 as distinct from PAMPs and DAMPs [16]. They proposed that those PRRs that can detect alterations in cellular homeostasis, as an indirect indication of infection, could be termed as sensors for HAMPs. Frequently, this is observed for cytoplasmic innate immune sensors that form inflammasomes, such as pyrin and NLRP1. This allows for a response to a broad range of changes without the requirement for direct or specific DAMP/PAMP ligand-PRR interaction. For further detail about inflammasomes and associated autoinflammatory disease, please see Chap. 5. This following chapter will narrow its discussion to those PRRs that are known to directly bind ligands.

4.2 Toll Like Receptors (TLRs)

Key Points

- **TLRs signal from the cell surface or endosome via MyD88 and/or TRIF for inflammatory cytokine production**
- **Deficiencies in TLR signaling pathway can cause immunodeficiency**
- **Aberrant recognition of host molecules by TLRs may trigger sterile inflammation**

TLRs, type I transmembrane glycoproteins, were the first PRRs to be described and significant insight in this area was made through the study of *Drosophila melanogaster*. The *toll* gene was initially identified in 1985 with a role in setting polarity during embryonic development in the fruit fly, but its role in the nuclear factor kappa B (NF-κB) response to both fungal and bacterial stimuli was documented over a decade later [17–19]. Janeway and colleagues confirmed the relevance of *toll* to humans through the discovery of its human homologue [20]. Using a dominant positive mutant *toll* stably expressed in a THP-1 monocyte cell line, the authors showed that there was induction of NF-κB controlled cytokine genes such as IL-6 [20].

There are currently ten known human TLRs (Fig. 4.1) and, although recognizing a variety of ligands, they share the structural framework of a horse-shoe shaped N-terminal leucine rich repeat (LRR) ligand binding domain, a single transmembrane α-helix and a C-terminal cytoplasmic toll/IL-1 receptor (TIR) signaling domain [21–24]. Upon ligand and receptor interaction, there is conformational change and dimerization of the TIR domain, and subsequent activation of downstream signaling pathways [23, 25, 26]. A spontaneous murine missense mutation encoding Pro712His in the TIR domain of TLR4 rendered mice resistant to endotoxin, highlighting the necessity of the cytoplasmic domain for signaling [27]. This was further explored functionally with an NF-κB luciferase assay through transfection of HEK293T cells with human TLR4 [28]. A number of mutations in the region of the TIR domain thought to interact with downstream adaptors were made, and all showed reduced

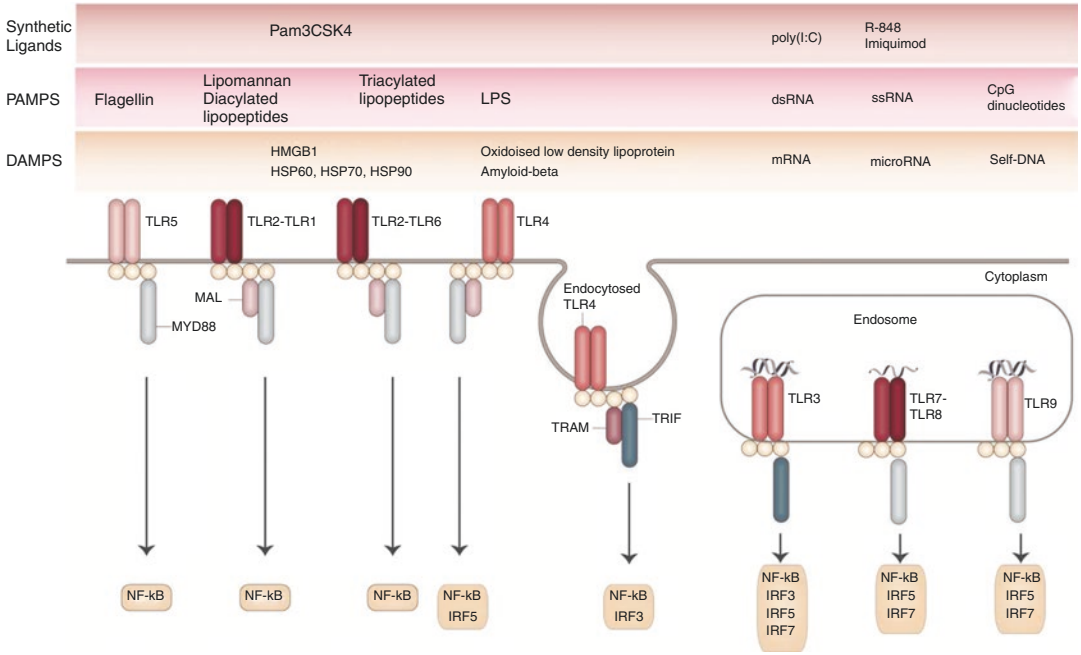

Fig. 4.1 Schematic diagram of TLR ligand recognition at the cell membrane. No ligand is currently known for TLR10. *DAMPs* damage associated molecular patterns, *dsRNA* double-stranded RNA, *HMGB1* high mobility group box 1, *HSP* heat shock protein, *IRF* interferon regulatory factor, *LPS* lipopolysaccharide, *MAL* MyD88 adaptor like, *mRNA* messenger RNA, *MYD88* myeloid differentiation primary response gene 88, *NF-κB* nuclear factor–κB, *PAMPS* pathogen-associated molecular patterns, *ssRNA* single-stranded RNA, *TLR* toll-like receptor, *TRAM* TRIF-related adaptor molecule, *TRIF* TIR-domain containing adaptor protein inducing IFN-β. Adapted from [269]

NF-κB activity when compared to wild type (WT).

With the exception of TLR3, all TLRs associate with the adaptor myeloid differentiation primary response gene 88 (MyD88), which allows for recruitment of components of the downstream pathway (Fig. 4.1). MyD88 was initially characterized as a marker of macrophage differentiation but was later noted to have sequence and amino acid homology with important cytoplasmic domains of TLRs and the IL-1 receptor [29]. MyD88 is composed of an N-terminal 'death domain' and a C-terminal signaling TIR domain [30]. The recruitment of MyD88 to a complex and involvement in the signaling pathways of TLRs was explored by various groups [31–34] and its importance highlighted with the endotoxin resistance shown in *MyD88*$^{-/-}$ mice [35]. The significance of Myd88 function in humans was documented by the identification of MyD88 deficiency in seven individuals with invasive pyogenic infections caused by homozygous inframe deletions, a homozygous missense mutations or compound heterozygous missense mutations [36]. Von Bernuth et al., proposed certain redundancy in the human TLR/MyD88 pathway as, although susceptible to *Streptococcus pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*, patients did not have increased viral or other bacterial infections.

There are four additional adaptor proteins with various roles in the appropriate functioning of TLRs: MyD88 adaptor like (MAL), TIR-domain containing adaptor protein inducing interferon (IFN)-β (TRIF), TRIF-related adaptor molecule (TRAM) and sterile α- and armadillo motif containing protein (SARM). MAL, also known as TIR domain containing adaptor protein (TIRAP), functions in the TLR4 [37] and TLR2 pathways [38]. Importantly, Horng et al. note a difference in the requirements for MyD88 of TLR2 and TLR4, with TLR4 still able to activate

NF-κB in the absence of MyD88, suggesting a MyD88-independent pathway. This difference was clarified with the description of TRIF (also known as TICAM1).

The role of TRIF was elucidated when transfected into HEK293T cells along with an NF-κB or an IFN-β reporter [39]. Transfection of TRIF significantly induced the IFN-β reporter, and also associated with TLR3 in immunoprecipitation experiments [39, 40]. *Trif*$^{-/-}$ mice did not respond to the TLR3 specific ligand poly(I:C) (see Sect. 4.2.4), but, importantly, were also impaired in their response to LPS, suggesting a MyD88-independent TRIF-dependent arm of TLR4 signaling [41]. Mutations in *TRIF* were found through the sequencing of this gene in patients with herpes simplex encephalitis (HSE). Two patients were identified with mutations in *TRIF* [42]. The first patient harbored homozygous missense mutations resulting in a premature stop codon with a resulting null allele and deficient responses to TLR3 stimuli. The second was a heterozygous missense mutation. The functional significance of the heterozygous mutation was not seen in overexpression experiments, but through investigation of retrovirally transduced fibroblasts. Furthermore, as the heterozygous mutation was also found in family members, incomplete penetrance was suggested.

In vivo exploration of the role of TRAM revealed it to be an adaptor for TLR4 important in the MyD88-independent pathway [43]. The authors showed that in the absence of TRAM, although there was evidence of activation of the MyD88 pathway as demonstrated by phosphorylation of downstream pathway proteins, the pro-inflammatory cytokine response was impaired. Further studies of TRAM using dominant negative forms of the protein as well as knock-down studies suggested that whilst TRIF was an adaptor for both TLR3 and TLR4, TRAM was exclusively involved in TLR4 signaling through its interaction with TRIF [44]. The endocytosis of TLR4 was previously considered to be part of downregulation of this TLR signaling pathway, however Kagan et al. determined this localization to be necessary for the TRAM-TRIF pathway [45]. TLR4 was shown to engage with TRAM-TRIF in early endosomes.

The final adaptor protein to be characterized was SARM [46]. Although expression of SARM in HEK293Ts transfected with NF-κB reporter failed to drive the transcription factor activation, stimulation of primary human peripheral blood mononuclear cells (PBMCs) with TLR4 ligand LPS (see Sect. 4.2.2) increased expression of SARM. The possibility of SARM being a negative regulator has been explored. SARM was able to inhibit TRIF dependent NF-κB activation in a dose dependent manner as seen when using TLR3 ligand poly(I:C) (see Sect. 4.2.4). The authors used RNA mediated interference to knock-down *SARM* in HEK cells stably expressing TLR3 and stimulated with poly(I:C), demonstrating enhanced TRIF dependent gene expression. Although not yet reported, it is possible that patients with loss-of-function mutations in *SARM* could present with an inflammatory phenotype.

The compartmentalization of TLRs is important, with TLRs 1, 2, 4, 5, 6 and 10 localizing to the cell surface. TLRs recognizing nucleic acid PAMPs, TLRs 3, 7, 8 and 9, are stabilized by the protein Unc-93 homologue B1 (UNC93B) [47] and are trafficked to endosomes after transport to the Golgi apparatus [48, 49]. Loss-of-function homozygous mutations in *UNC93B1* were reported in two patients from consanguineous families diagnosed with HSE [50]. The mutations, one a frame shift mutation (c.1034del4), the other a single nucleotide substitution (c.781G>A), resulted in significantly reduced expression of UNC93B1. Both patients' PBMCs had defects in response to TLRs 3, 7, 8 and 9, highlighting the importance of correct localization of TLRs for their function.

4.2.1 TLRs 1, 2, 6, 10

Through its association with either TLR1 or TLR6, TLR2 recognizes and responds to a variety of ligands [51] including PAMPs mycobacterial lipomannan [52–54], bacterial lipoteichoic acid [55], diacylated (TLR2/6) and triacylated (TLR1/2)

bacterial lipopeptides [56, 57], as well as DAMPs HMGB1 and heat shock proteins (HSP) 60, 70 and 96. A single nucleotide polymorphism (SNP) in the TIR domain of TLR2 was originally thought to be associated with susceptibility to *Mycobacterium leprae* [58]. Sequencing of an amplified fragment of TLR2 revealed a p.Arg677Trp SNP in 10 of 45 patients with lepromatous leprosy. No functional studies were performed. However, since this time there is evidence to suggest that it is not a true polymorphism but rather a variation in a pseudogene [59]. Although this highlights the complexity of genetic sequencing, it also prompts consideration of potential primary immunodeficiencies (PIDs) in other mycobacterial diseases. A retrospective case control study of Turkish patients with *M. tuberculosis* (TB) determined a sixfold increase in risk of disease in patients with a homozygous p.Arg753Gln substitution [60], also shown in a small Tunisian cohort [61]. A direct causal link has not, however, been determined. To date no monogenic PID or autoinflammatory disorders have been identified that result from mutations in these TLRs, potentially reflecting the requirement of appropriate function of this group of TLRs for life.

TLR10 was isolated in 2001 and, when compared with other known TLRs, has the most amino acid identity with TLRs 1 and 6 [62]. Although a ligand for TLR10 has not been determined, it has been shown to associate with MyD88, the same downstream adapter used by TLRs 1, 2 and 6 [63].

4.2.2 TLR4

Spontaneous mutations in *Tlr4* in two mice strains tolerant to endotoxin, C3H/HeJ [64, 65] and C57BL/10ScCr [66], prompted the hypothesis and subsequent confirmation that TLR4 is the receptor for LPS [67]. Interestingly, in an NF-κB luciferase reporter system with various TLRs transfected in to HEK293T cells, TLR4 was unable to generate a significant response to LPS [68]. Given this inconsistency, it was proposed that TLR4 may require a cofactor for its function, and this protein was later identified as myeloid differentiation factor 2 (MD-2) [69, 70]. MD-2 associates with TLR4 in the endoplasmic reticulum and the complex is transported to the cell surface [71, 72]. MD-2 is vital in the correct localization of TLR4, as shown in a murine MD2$^{-/-}$ model. In this model, TLR4 resided only in the Golgi apparatus [73].

Similar to TLRs 1, 2, 6 and 10, no pathogenic mutations in the human population has been found. Much interest has been taken in polymorphisms p.Asp299Gly and p.Thr399Ile in the extracellular domain of TLR4, with an allele frequency of 0.061 and 0.056 respectively [74]. The initial publication investigating the role of these variants documented no response to LPS in THP-1 cells transfected with p.Asp299Gly TLR4 when compared with WT, but an intermediate response with p.Thr399Ile TLR4 [75], suggesting that even common polymorphisms may alter a host's response to an infective challenge.

4.2.3 TLR5

Tlr5 was initially mapped as a genetic locus that may be involved in modulation of response to Salmonella infection [76, 77]. In an elegant series of experiments including mass spectroscopy of concentrated and fractionated *Listeria monocytogenes* culture assessed for TLR5 stimulating activity, the specific ligand for TLR5 was determined to be flagellin. Flagellin was shown to bind to TLR5 and induce a MyD88 dependent response [78]. TLR5 has a conserved concavity in the extracellular domain that is responsible for recognition of a highly conserved site on flagellin [79, 80]. More recently, alterations in gut microbiota and metabolic profile, including insulin resistance, have been noted in *Tlr5*$^{-/-}$ mice [81]. This phenotype was transferred with transfer of gut microbiota, suggesting that TLR5 is important in microbiome homeostasis.

4.2.4 TLR3

TLR3, only expressed in dendritic cells [82], recognizes and then assembles along double stranded RNA (dsRNA), a PAMP seen in viral infections [83–85]. HEK293T cells transfected with an NF-κB reporter along with various TLRs were stimulated with poly(I:C), a synthetic

dsRNA. An NF-κB response to poly(I:C) was only seen with the transfection of TLR3 [83]. Importantly, TLR3 did not confer a response to single stranded RNA (ssRNA) suggesting specificity to dsRNA [84]. This finding was confirmed in *Tlr3*$^{-/-}$ mice, with abrogation of the IFN cytokine response to poly(I:C), which was determined to be independent of MyD88. A missense mutation p.Pro554Ser in TLR3 was identified in two unrelated patients with HSE [86]. A fibroblastic cell line derived from one of these patients was hyporesponsive to poly(I:C), a dominant negative effect that corrected in vitro with treatment with IFN-α and IFN-β. The clinical penetrance of this monogenic susceptibility to HSE is incomplete and Zhang et al., hypothesized on potential implicating factors. Of note, this heterozygous mutation has now been identified in 115 'healthy' individuals with an allele frequency of 0.0004 [74], and it is interesting to consider whether these individuals are also at risk of HSE.

4.2.5 TLRs 7, 8, 9

TLRs 7, 8 and 9 localize to the endosome and recognize nucleic acid components. The specific role of TLR9 was the first to be elicited, with *Tlr9*$^{-/-}$ mice failing to mount an inflammatory response to unmethylated CpG dinucleotides [87]. CpG dinucleotides are absent from mammalian cells and hence TLR9 allows distinction between bacteria and self DNA [88]. Following on from this, human TLR9 was shown to recognize the same PAMP [89]. Recent structural studies have characterized the mode by which TLR9 recognizes the CpG motif forming a symmetric complex with two CpG molecules binding two TLR9 molecules [90]. The role of TLR7 in response to pathogens was determined with clues from its response to synthetic guanosine analogs such as imiquimod [91, 92]. In HEK293T cells transfected with an NF-κB reporter and various TLRs, Lee et al. tested a number of these analogs and determined that the response seen was specific for TLR7. The authors also showed that the response was dependent on endosomal processing. It was this last observation that prompted consideration of TLR7 as a potential receptor involved in the sensing of influenza [93]. It was shown that endosomal acidification as well as MyD88 were completely required for an IFN response to influenza. Following from this, after exclusion of the role of TLR3, TLR9 and TLR4, *Tlr7*$^{-/-}$ plasmacytoid dendritic cells (pDCs) failed to response appropriately to influenza. Using multiple synthetic RNAs, the specificity of TLR7 response was determined to be ssRNAs. The role of TLR8 was difficult to determine, highlighted in a paper by Jurk et al. [94]. The NF-κB activation seen in response to stimulation with the small molecule imidazoquinoline agonist R848 was shown to be mediated by either TLR7 or TLR8 in human cells. It was previously published that this response was specific to TLR7 when using murine TLRs in a HEK293T system [91]. Jurk et al. reproduced this result and suggested that TLR8 is nonfunctional in mice [94]. Both TLR7 and TLR8 were shown to be involved in the recognition of guanosine (G)- and uridine (U)- rich ssRNA [95]. GU-rich ssRNA are not unique to viral pathogens and are found in endogenous RNA, suggesting that the compartmentalisation of the RNA, as well as the receptor, is important in discriminating self from non-self. Recent structural biology work on TLR7 and 8 has demonstrated that both recognize nucleic acid degradation products rather than RNA itself. Notably, this work has established the molecular basis for the recognition of nucleic acids by these TLRs, and identified both allosteric mechanisms of receptor activation and potential mechanisms for interference with small molecule compounds [96–99].

4.3 NOD-Like Receptors (NLRs)

Key Points

- **At least two NLRs are thought to detect pathogens directly, with NOD1 and NOD2 responding to fragments of the bacterial cell wall**
- **NOD2 is particularly relevant for the progression of inflammation in Crohn disease and Blau syndrome**

Similar to TLRs, NLRs are PRRs that play an important role in PAMP and DAMP sensing.

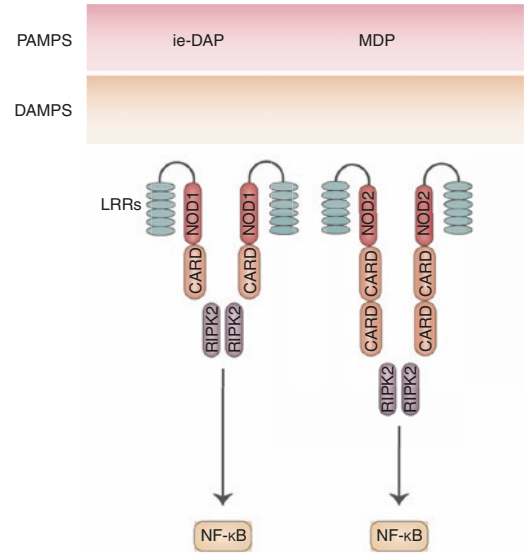

Fig. 4.2 Schematic diagram of NOD1 and NOD2 ligand recognition in the cytoplasm. *CARD* caspase activation and recruitment domain, *DAMPs* damage associated molecular patterns, *LRRs* leucine-rich-repeats, *NF-κB* nuclear factor–κB, *NOD* nucleotide-binding oligomerization domain, *PAMPs* pathogen-associated molecular patterns, *RIPK2* receptor-interacting serine/threonine kinase 2

These cytoplasmic proteins are characterized by a NOD and an LRR domain (Fig. 4.2). The standardized classification of the 22 NLRs described to date is based on the N-terminal domain, either a pyrin domain, caspase recruitment domain (CARD), acidic transactivation domain or baculoviral inhibitory repeat-like domain [100]. Here, two NLRs that are thought to directly bind ligands will be discussed, NOD1 and NOD2. For discussion of other NLRs, please see Chap. 5.

4.3.1 NOD1

Human NOD1, also called CARD4, was first identified in 1999 as a protein with an N-terminal CARD, a NOD and multiple C-terminal LRRs [101, 102]. Co-immunoprecipitation experiments in HEK293T cells revealed that NOD1 interacted with a number of regulators of apoptosis and NF-κB activation, including caspase-8 and caspase-9 [102]. Functionally, however, NOD1 overexpression resulted in cleavage of caspase-9 only. Importantly, in the same study, NOD1 was shown to interact with receptor-interacting serine/threonine kinase 2 (RIPK2) via CARD-CARD interactions, and that both synergistically induce the activation of NF-κB as shown in luciferase assays. RIPK2 has been shown to be an activator of the NF-κB pathway and cell death [103]. NOD1 was later shown to self-associate via the nucleotide binding domain, and that deleting the LRR domain enhanced oligomerization, suggesting an inhibitory role of the LRR [104]. This self-association was deemed important for its interaction with RIPK2 [104]. The demonstration of NF-κB response to HEK293T cells transfected with NOD1 stimulated with LPS prompted exploration of the ligand or motif recognized by NOD1 [105]. By determining the amino acid sequence of the fraction of LPS that activated NOD1, diaminopimelic acid (DAP) type peptidoglycan, found in common Gram-negative bacteria and several Gram-positive bacteria, was considered to be important for NOD1 stimulation [106]. Using a synthetic DAP (ie-DAP), Inhorara and colleagues stimulated macrophages from WT mice and showed robust TNF-α response [105]. This response was completely abrogated in the macrophages from *Nod1*$^{-/-}$ mice. At the same time, Girardin and colleagues published experiments using purified peptidoglycans from various bacteria, and determine a unique diaminopimelate-containing *N*-acetylglucosamine-*N*-acetylmuramic acid (GlcNAc-MurNAc) tripeptide motif was the specific motif recognized by

NOD1 [107]. Using dominant-negative forms of MyD88 and RIPK2, the authors also provide evidence that this pathway is MyD88-independent and RIPK2-dependent. A subsequent finding has been the recognition that activation of Rho GTPases by bacterial virulence factors can lead to NOD1 signaling [108]. This finding suggests that both bacterial peptidoglycan and alterations in the host cell resulting from a pathogen can lead to NOD1 signaling**.**

4.3.2 NOD2

By searching for a region with sequence homology with *NOD1*, the gene now known as *NOD2* was identified and expression mapped to monocytes [109]. Similar to NOD1, expression of NOD2 cDNA in HEK293T resulted in increased NF-κB luciferase reporter activity, which was entirely dependent on expression of its two CARDs. Furthermore, the CARDs are essential for the interaction between NOD2 and RIPK2. Broad interest in NOD2 was sparked by the publication of two papers linking variants in NOD2 to Crohn disease (CD) [110, 111]. Hugot et al., observed linkage disequilibrium amongst markers in a previously identified CD susceptibility locus, and went on to identify *NOD2* as the candidate gene. They mapped most of the SNPs tested with significant linkage disequilibrium to the LRR domain, and predicted these to result in decreased inhibitory function of this domain and subsequent activation of NOD2 [110]. Ogura et al. provided evidence that the role of NOD2 in CD may be more complex than this [111]. This group identified an insertion of cysteine at position c.3020 resulting in premature truncation of NOD2 that was preferentially transmitted to patients with CD. The resulting protein was predicted to be 1007 amino acids, compared to 1040 in WT NOD2. By transfecting WT or mutant NOD2 construct into a HEK293T system with an NF-κB luciferase reporter, these authors showed a similar amount of NF-κB activation with NOD2 overexpression. Interestingly, when they used the same HEK293T cells with the NF-κB reporter, but transfected in low amounts of NOD2 and then stimulated the cells with LPS from a variety of bacteria, the mutant NOD2 had a diminished NF-κB response to the stimuli when compared to WT. The authors had a number of hypotheses to account for the unexpected result and acknowledge the complexity of the association between this variant and disease.

The finding by this last group that LPS from various Gram-negative bacteria resulted in WT NOD2 dependent NF-κB activity prompted consideration of ligand specificity for this immune sensor. Girardin et al., purified peptidoglycans from various bacteria and added those to cells transfected with NOD2 and NF-κB luciferase reporter [112]. There was a NOD2-dependent activation of the NF-κB pathway with synthetic muramyl dipeptide (MDP), the minimal peptidoglycan signature found in all bacteria, with no NF-κB response seen when NOD1 or TLR2 were transfected instead of NOD2. The authors went on to test NOD2 c.3020insC and showed that the NF-κB response to MDP was abrogated when compared to WT NOD2. This is consistent with the finding by Ogura et al., [111] and suggests that an abnormal response or processing of microbial insult may be part of the pathogenesis of CD.

The idea of abnormal processing by the CD-associated NOD2 is strengthened by work of Simmons and colleagues looking at the role of NOD2 in autophagy [113]. Autophagy, the process of sequestration of cytoplasmic material into autophagosomes and subsequent fusion with lysosomes allowing for degradation, is important for the appropriate presentation of antigens on major histocompatibility complex II (MHC II) (see Chap. 8). The authors induced autophagy in dendritic cells by treatment with MDP and quantified formation of autophagosomes by electron microscopy. Using dendritic cells with NOD2 knocked down through short interfering RNA, this formation was determined to be dependent on NOD2 and RIPK2. Interestingly, when dendritic cells expressed the CD-associated NOD2 c.3020insC, they displayed defective autophagosome formation and antigen processing when compared with WT NOD2.

Initially described by Blau et al., in 1985, the eponymous syndrome was noted to be dominantly inherited based on family pedigree, and later defined as caused by mutations in NOD2 [114, 115]. This monogenic autoinflammatory disorder is characterised by uveitis, rash and arthritis with histological evidence of non caseating granulomatous changes [114] (see Chap. 20). Linkage analysis was used to identify the responsible locus of Blau Syndrome (BS) as chromosome 16q12 in a 74-member pedigree [116]. Through screening of each exon of NOD2, also located at this locus, in four families with BS, three novel variants in NOD2 were identified that segregated with disease [115]. This finding prompted consideration as to whether patients with early-onset sarcoidosis (EOS) have similar genetic origins. Although EOS and BS share clinical and histological findings, children with EOS lack a significant family history. A cohort of patients with EOS had all exons of NOD2 sequenced and compared with 100 healthy controls [117]. Nine of the ten patients sequenced had heterozygous mutations in NOD2. Each mutation was then tested in an NF-κB luciferase assay and displayed increased NF-κB activity when compared with WT NOD2 when overexpressed in HEK293T cells. The mutant NOD2 was comparable to WT NOD2 in its ability to respond to MDP stimulation with NF-κB activity induction. This evidence suggests that constitutive induction of NF-κB activity by mutant NOD2 is part of the pathogenesis of BS and EOS.

4.4 Other Pattern Recognition Receptors (PRRs)

Key Points

- **Various classes of PRR detect DNA and RNA**
- **Inappropriate detection of host DNA/RNA is restricted by molecules that degrade or edit host nucleic acids**
- **C-type lectin receptors (CLRs) are triggered by carbohydrates, especially of fungal origin**
- **Caspase-4/-5 may directly detect LPS in the cytosol, acting as PRRs that also recognize host lipid moieties**

4.4.1 RIG-I Like Receptors (RLRs)

RIG-I like receptors (RLRs) are a family of DExD/H-box RNA helicases that detect the presence of foreign RNA in the cytoplasm. This is in contrast to other innate immune receptors that are specialized in the recognition of cytosolic DNA, like cGAMP synthase stimulator of interferon genes (cGAS-STING) (see Sect. 4.4.4) and absent in melanoma 2 (AIM2) (see Sect. 4.4.3), or of nucleic acids in endosomal compartments, like TLRs (see Sect. 4.2). To date, three RLRs have been described: RIG-I (retinoic acid-inducible gene 1, also known as DDX58), MDA5 (melanoma differentiation-associated protein 5, also known as IFIH and Helicard) and LGP2 (laboratory of genetics and physiology 2, also known as DHX58) [118]. RIG-I and MDA5 consist of an RNA helicase domain and a C-terminal domain that together constitute the binding site for viral RNA, and two CARDs at the N-terminus for protein-protein interaction and signal transduction. Interestingly, LGP2 contains the ligand-binding domains but lacks the CARD domains, so it is thought to work as a regulatory receptor for the other two [119–121].

RLRs can recognize chemical features of viral RNA that are not found in normal host-derived RNA. During typical synthesis of messenger RNA (mRNA) in mammalian cells, the transcriptional machinery attaches a covalent cap to the 5′ end of mRNA before export to the cytoplasm. RIG-I can mainly detect the presence of cytoplasmic RNA with an unprotected 5′ tri- or diphosphate group [122, 123], a sign that the RNA might have been synthesized using a foreign viral mechanism. dsRNA is associated with different kinds of viral infections and can also be recognized by RLRs. Specifically, RIG-I and LGP2 tend to bind dsRNA ends, while MDA5 binds along the dsRNA molecule [124–126]. Since LGP2 does not signal by itself, it is thought that LGP2 binds to dsRNA ends to assist further

MDA5 oligomerization along the dsRNA stem [127]. Shorter dsRNA sequences seem to be preferentially recognized by RIG-I, while long sequences are typically sensed by MDA5, which suggests a different involvement of the two receptors depending on the length of the viral genome [128–130].

RIG-I and MDA5 are normally expressed in the cytoplasm as monomers. Binding of dsRNA ligand to the helicase domain of RIG-I or MDA5 induces the oligomerization of the receptor via CARD domains, resulting in an RNA-RLR oligomer complex [124, 126, 129, 131, 132]. It has been shown that non-covalent interaction of this complex with chains of lysine-63-linked polyubiquitin further contributes to the stability of RLR oligomers [133, 134]. The resulting complex, containing active RIG-I or MDA5, can then associate with the mitochondrial antiviral-signaling (MAVS; also known as IPS-1, VISA, and Cardif) adaptor protein via CARD domain on the membrane of mitochondria and peroxisomes [135–139]. Subsequent polymerization of MAVS on the organelle membrane [140, 141] results in activation of the downstream effectors inhibitor of NF-κB kinase (IKK) and TANK-binding kinase 1 (TBK1), two signaling molecules that activate NF-κB and interferon regulatory factor 3 (IRF3) pathways, respectively [142], as seen for other innate immune receptors**.** IKK complex phosphorylates the inhibitor of NF-κB (IκB) and allows the transcription factor NF-κB to be released and translocated into the nucleus. In parallel, activation of TBK1 leads to phosphorylation and translocation of the transcription factor IRF3 into the nucleus. Both NF-κB and IRF3 induce expression of pro-inflammatory cytokines and type I IFNs upon binding to their target DNA sequences.

Several different mechanisms exist to avoid spontaneous activation of RLRs by self-RNA in the cytosol [143]. First of all, self-RNA passes through a series of chemical modifications that reduce recognition by these receptors. Modifications at the 5′ end prevent the binding of RIG-I to self-RNA. They include addition of a 5′ cap on mRNA, cleavage of 5′ end to a monophosphate group on transfer RNA (tRNA), and the presence of 3′ overhangs of two nucleotides on micro RNA (miRNA) and small interfering RNA (siRNA) [144]. Also, although mRNA is mainly formed by single-stranded RNA, the enzyme adenosine deaminase acting on RNA 1 (ADAR1) can deaminate adenosine to inosine in order to prevent base-pairing and recognition of Alu duplexes by MDA5 [145–147]. Secondly, in the case of RIG-I, intramolecular interaction of CARD and RNA-binding domain keeps the receptor in an autoinhibited state. The binding of RNA to RNA-binding domain releases CARD and allows for downstream signaling [125]. In the case of MDA5, although the receptor does not show autoinhibition, it seems to require the assistance of LGP2 and further oligomerization along dsRNA to become active [148]. Third, different post-translational modifications of the RLRs are known to affect their functionality, including acetylation, phosphorylation and stabilization by lysine-63-linked polyubiquitin [143]. Finally, binding of ATP to RLRs seems to play a major role in assuring the specificity towards the ligand. Evidence suggests that both RNA and ATP binding to the RLR are necessary for downstream signaling and that ATP hydrolysis is a mechanism to unbind RLR from dsRNA. Signaling upon engagement of RLRs and self-RNA becomes kinetically favorable only in case of high affinity binding, when oligomerization of the RLRs on the ligand is fast enough to overcome the inhibitory effect of ATP hydrolysis [149–151].

Type I interferonopathies, a heterogeneous group of genetic disorders characterized by high levels of type I IFNs, are derived from monogenic mutations that render a constitutively active antiviral response [152] (see Chap. 24). Aicardi-Goutières syndrome (AGS), the prototypic type I interferonopathy, is a rare genetic encephalopathy that shows similar symptoms to congenital viral infection. Several mutations associated with AGS have been found in *IFIH1*, the gene encoding MDA5, and also in *ADAR1*, which prevents recognition of self-RNA by MDA5 [153, 154]. In these cases, aberrant activation of MDA5 results from an increased susceptibility of the receptor to activation by self-RNA, or from an improper masking of self-RNA by ADAR1 [145, 146, 153].

Gain-of-function mutations of both *IFIH1* and *DDX58*, encoding RIG-I, have been found in Singleton-Merten syndrome (SMS), another rare interferonopathy characterized by teeth alterations, calcification in aorta and heart valves and osteoporosis [155, 156].

4.4.2 C-Type Lectin Receptors (CLRs)

C-type lectin receptors (CLRs) are a super-family of proteins characterized by the presence of a C-type lectin-like domain (CTLD) in their sequence [157]. The fact that CLRs can recognize a broad variety of ligands confers these receptors a large number of physiological roles, including the regulation of coagulation, development, cell death and the innate and adaptive immune responses [157]. CLRs are expressed either as secreted or transmembrane proteins, some of them in very specific cell types or under a narrow range of circumstances and can therefore be used as markers of cellular populations or cellular state. For instance, L-selectin is expressed by most circulating leukocytes, serving as an adhesion molecule to vascular endothelium to mediate transmigration into tissues [158]. CD69 is expressed short after stimulation of lymphocytes and natural killer (NK) cells and can be used to distinguish cells in their activated state [159].

Most research on CLRs has focused on their role as PRRs. Soluble CLRs, such as collectins, can bind to the microbial surface and work as opsonins to block the microorganism, induce its destruction by phagocytosis and the complement system, and modulate inflammation [157]. Transmembrane CLRs can transduce the signal to the cytoplasm upon binding of their ligand and can therefore mediate multiple cellular functions. For example, CLRs can induce production of anti-microbial effectors, assist in antigen presentation for the development of adaptive immunity, and activate the inflammasome [160–162].

Dectin-1 and dectin-2 belong to a subgroup of transmembrane CLRs that can signal into the cytosol via the tyrosine kinase Syk. The SH2 (Src Homology 2) domain of Syk provides a docking site that can bind to phospho-tyrosine residues on either ITAM (immunoreceptor tyrosine-based activation motif) or hemi-ITAM motifs, which can be found in the cytoplasmic domain of some adaptor molecules and CLR receptors, respectively. Upon binding of ligand to dectin-1, Syk is recruited to the hemi-ITAM domain of the receptor and becomes active. Syk subsequently induces the recruitment of CARD9, Bcl-10 and Malt-1, which leads to downstream signaling to the canonical NF-κB pathway and expression of different pro-inflammatory molecules [163–165]. Syk also activates the non-canonical NF-κB, MAPK (mitogen-activated protein kinase 1) and NFAT (nuclear factor of activated T-cells) cascades, as well as the production of reactive oxygen species (ROS) that can help in the formation of NLRP3 inflammasome [164, 166–168]. In a Syk-independent mechanism, activated dectin-1 also regulates NF-κB signaling via RAF1 [169]. Other CLRs share signaling steps with dectin-1. For example, binding of dectin-2 to its ligand also leads to recruitment of Syk, but this requires mediation by the ITAM motif on an adaptor protein, Fc receptor gamma chain (FcRγ). Activation of Syk by dectin-2 induces many of the same pathways as seen for dectin-1. Macrophage-inducible C-type lectin (Mincle) also signals downstream via FcRγ-Syk-CARD9 to activate NF-κB, and is normally expressed at low levels in macrophages and highly up-regulated under pro-inflammatory conditions [170]. In contrast, myeloid inhibitory C-type lectin (MICL) recruits SHP1 (Src homology region 2 domain-containing phosphatase-1) and SHP2 via its ITIM motif (immunoreceptor tyrosine-based inhibition motif) and acts as an inhibitory receptor of the Syk-couple CLRs [171]. DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin), LOX-1 (lectin-like oxidized LDL receptor 1) and others do not signal through ITAM or ITIM motifs [165].

CLRs play an important role in different kinds of infections and in maintaining homeostasis. These receptors are especially important in the immune response to fungi, and as such, different carbohydrate components on the wall of fungi are

preferentially recognized by individual CLRs. For example, dectin-1 and dectin-2 can recognize β-1,3 glucans and α-mannans, respectively. Accordingly, it has been shown that mutations in CLRs and their downstream effectors predispose mice to fungal infections [172]. The interplay between microbiota and immune system in the intestine has a strong influence in health and disease. Sensing of commensal fungi via dectin-1 has been shown to be important for the maintenance of intestinal homeostasis and protection against colitis. Indeed, lack of dectin-1 was linked to a higher susceptibility to colitis in a mouse model, while polymorphisms in the gene for dectin-1 are associated with more severe cases of ulcerative colitis [173]. Dectin-1 has been proposed to modulate homeostasis in the intestinal immune system by controlling the microbial composition to induce differentiation of regulatory T cells [174]. Thus, sensing of fungi by dectin-1 may help in the maintenance of a non-inflammatory status in the intestine and modulate the severity of inflammatory bowel disease (IBD).

4.4.3 Absent in Melanoma 2 (AIM2)

AIM2 is a cytosolic receptor that senses double-stranded DNA (dsDNA) and induces the formation of an inflammasome complex [175–178]. AIM2 is able to sense a wide variety of cytosolic dsDNA regardless of the sequence, either from microbial sources during certain infections, or from host cells upon disruption of the mitochondrial or nuclear envelope. Thus AIM2 plays an important role in the immune response to a variety of intracellular infections, as well as in cancer and autoinflammatory disease [179].

AIM2 displays a simpler structure than other inflammasome-forming receptors, containing only a C-terminal HIN (hematopoietic expression, interferon-inducible nature, and nuclear localization) domain, for binding of dsDNA, and an N-terminal PYD domain, for downstream signaling via the inflammasome adaptor ASC. The ligand of AIM2 is double-stranded DNA, with no specificity for the nucleotide sequence. A positively charged surface on the HIN domain can interact electrostatically with the negatively charged sugar-phosphate backbone of dsDNA [180]. AIM2 in its monomeric form stays inactive in an auto-inhibited state, with HIN and PYD domains of the same molecule interacting with each other. Binding of dsDNA to HIN releases the PYD [180, 181], allowing for oligomerization of AIM2 along the dsDNA molecule and recruitment of ASC [180, 182, 183]. Therefore, engagement of dsDNA and AIM2 results in assembly of the canonical inflammasome, caspase-1 maturation and consequently the secretion of the pro-inflammatory cytokines IL-1β and IL-18 and cell death by pyroptosis [184, 185]. This outcome stands in contrast with the cGAS-STING axis, which also senses cytosolic DNA but induces secretion type I IFNs and pro-inflammatory cytokines via the transcription factors IRF3 and NF-κB [186] (see Sect. 4.4.4).

Expression of AIM2 protein occurs basally in the spleen, small intestine and peripheral blood [187], although expression in different tissues can be up-regulated in response to type I IFNs [188–190]. Thus, complete activation of the AIM2 inflammasome is achieved by the previous recognition of the stimulus by parallel pathways, such as cGAS-STING, which result in secretion of type I IFNs and subsequent expression of AIM2 [191]. Mechanisms common to other inflammasomes keep control of ASC activity, including phosphorylation and ubiquitination [192, 193]. Additionally, AIM2 can be degraded via autophagy [194] or by binding of inhibitory proteins, such as PYD-only protein 1 (POP1) and POP3 in human cells [195, 196].

Several pathogens are known to induce AIM2 inflammasome formation. The list includes the bacteria *Francisella tularensis*, *Listeria monocytogenes*, *Streptococcus pneumonia*e, *Mycobacterium*, *Staphylococcus aureus*, and *Brucella abortus*; viruses like mouse cytomegalovirus (MCMV), vaccinia virus and human papillomaviruses; the fungal pathogen *Aspergillus fumigatus* and the protozoan *Plasmodium berghei*. In the case of bacterial infections, the pathogen must escape the endosome and undergo bacteriolysis to release dsDNA and activate AIM2 [179].

In the colon, AIM2 has shown a regulatory role by blocking the proliferation of tumorigenic stem cells and maintaining a balanced microbiota [197]. Mice deficient in AIM2 showed stronger development of tumors in a model of colon cancer, in a mechanism independent of the inflammasome [197, 198]. Accordingly, patients with colorectal cancer with lower levels of AIM2 generally have a worse prognosis than patients with normal levels [199].

Accumulation of self-DNA in the cell cytosol is a well-known trigger of some autoinflammatory or autoimmune diseases. In a mouse model of polyarthritis-like disease caused by a deficiency of DNAse II, an improper degradation of dsDNA leads to over-activation of dsDNA receptors. The maintenance of basal inflammation in these mice is mediated by both AIM2 and cGAS [200, 201]. Furthermore, keratinocytes expressing higher levels of AIM2 and containing cytosolic DNA have been found in psoriatic lesions of human patients. Chronic activation of the AIM2 inflammasome in these cells results in higher levels of the pro-inflammatory cytokine IL-1β, linking AIM2 to the pathogenesis of this skin disorder [202].

Contrary to the NLRs and other receptors, there is not yet evidence of gain-of-function mutations in AIM2 that cause autoinflammatory disease. This difference might exist because AIM2 requires dsDNA as a nucleation center to oligomerize and further recruit ASC for inflammasome assembly, as opposed to NLR oligomers that do not seem to require the ligand to stay stable and form the inflammasome [191].

4.4.4 cGAMP Synthase (cGAS) and Stimulator of Interferon Genes (STING)

Together with the AIM2 inflammasome, the cGAS-STING axis is a key sensor of cytosolic dsDNA. STING, encoded by *TMEM173,* was originally identified as an adaptor protein residing on the endoplasmic reticulum that, through conformational changes, facilitated the activation of NF-κB and IRF3, as well as the production of type I IFN in a TBK1-dependent manner [203, 204]. STING was subsequently characterized as an essential component of the host's response to cytoplasmic DNA [205]. Although Vance and colleagues determined that STING binds directly to cyclic dinucleotides (CDNs) produced by bacteria, the means by which STING responded to cytoplasmic dsDNA was elucidated in two seminal papers published in 2013 [206–208]. Cytoplasmic DNA, whether host or pathogen derived, may be converted to an endogenous second messenger CDN, a non-canonical cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), by the sensor cGAS [207–209]. Once formed, cGAMP binds to and activates STING, leading to both an NF-κB and IFN-β response. Interestingly, cGAMP enhances the antiviral response independently of this response by passing from the initial cell to surrounding cells through gap junctions and thus triggering STING activation [210].

Since the first description of cGAS, a cytosolic enzyme with a DNA binding site, there have been a number of key discoveries that shed light on how it recognizes dsDNA. cGAS exists as an inactive monomer at rest. The binding of canonical dsDNA causes a conformational change, allowing oligomerisation and activation of cGAS [211, 212]. cGAS has two DNA binding sites, both important for optimal enzymatic activity of cGAS. The first induces a conformational change and the second enhances cooperative binding of DNA [212]. Although cGAS was proposed at this time to bind to a long strand of dsDNA or two short strands of DNA, this was further clarified by Hopfner and colleagues in 2017 [213]. Not only did they document a DNA length-dependent activation of cGAS, they observed that each dimer attaches to two strands of DNA like a protein step of a DNA ladder. This positioning facilitates the recruitment of further cGAS dimers to the protein-DNA complex. Importantly, a number of bacterial and mitochondrial proteins including mitochondrial transcription factor A (TFAM) and HMGB1 are known to induce U-turns or bends in DNA. These bends were shown to enhance the sensing of DNA by cGAS

by 'pre-arranging' DNA in an optimal position for cGAS binding.

The cGAS-STING pathway has a well-recognized role in combating viral infections. Several DNA viruses can induce type I IFNs via cGAS-STING, including herpes virus and cytomegalovirus [214–216]. cGAS is also important in the detection of retroviruses, such as human immunodeficiency virus (HIV), after the reverse transcriptase converts the viral RNA into cDNA [217, 218]. However, retroviruses can escape cGAS detection by different mechanisms, including the direct injection of the cDNA into the nucleus, or the infection of cells with poor cGAS-STING signaling, like T cells [218, 219]. Evidence suggests that cGAS-STING can also contribute in the response against infections by RNA viruses, when the infection triggers cell damage and the release of host DNA [220]. Additionally, infections by several intracellular bacteria have been found to induce interferon signaling via the cGAS-STING pathway, including *Chlamydia*, *Mycobacterium*, *Neisseria* and *Listeria* [221–226].

Human cells have a number of mechanisms in place to remove cytosolic DNA and prevent the inappropriate and unwanted inflammatory response that would otherwise be induced. This is highlighted by the description of monogenic disorders caused by dysfunction of the regulatory mechanisms, such as mutations in *TREX1* causing AGS1, an interferonopathy (see Chap. 24). Since the first description in 2006, the phenotypic spectrum of loss of function mutations in *TREX1* has expanded to include familial chilblain lupus, systemic lupus erythematosus and an autosomal dominant retinal vasculopathy with cerebral leukodystrophy [227–232]. *TREX1* encodes the 3′-to-5′ DNA endonuclease three-prime repair exonuclease (TREX1) that has been shown to be important in the metabolism of DNA fragments, preventing subsequent inflammatory response [233–235]. The role of cGAS-STING in the pathophysiology of loss-of-function mutations in *TREX1* was elucidated some time later. Using a murine *Trex*$^{-/-}$ model of AGS, the severe inflammatory phenotype was abrogated when crossed with mice lacking Sting (*Tmem173*$^{-/-}$) [236]. Furthermore, the IFN signature of *Trex*$^{-/-}$ mouse embryonic fibroblasts was lost when cGAS was deleted using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 gene editing techniques [237].

More recently, loss-of-function mutations in *DNAS2* have been reported in patients with a syndrome of severe anemia, glomerulonephritis, liver fibrosis, deforming arthropathy and the presence of autoantibodies [238]. The encoded lysosomal deoxyribonuclease DNase II is important for the hydrolysis of DNA phosphodiester linkages during cell death [239]. Even prior to the description of these patients, murine models highlighted that deficiency in DNase II could result in anemia [240, 241] and arthritis [242, 243] that was cGAS-STING dependent [244–246].

Only one monogenic disorder has been described that affects the sensors of this pathway directly. Originally documented in six unrelated children, heterozygous gain-of-function mutations in *TMEM173* were reported to cause an autoinflammatory disease with an IFN gene signature, termed STING-associated vasculopathy with onset in infancy (SAVI) (see Chap. 24) [247]. Infants presented before the age of 8 weeks with peripheral vascular inflammation, nail dystrophy and paratracheal adenopathy. The majority of patients has evidence of interstitial lung disease. Transient low-titre autoantibodies associated with vasculitis were present in three children. None of the children responded to corticosteroids or disease modifying antirheumatic drugs. Whole exome sequencing (WES) performed on one patient and both parents identified a candidate mutation in *TMEM173,* and subsequent Sanger sequencing in other patients identified a total of three novel *de novo* mutations in *TMEM173*. These mutations affected amino acid residues near the dimerization domain of STING, resulting in a more stable dimer when compared with WT STING. This idea was developed by Jeremiah et al., through an exploration of the 3D structure of STING, with the more stable dimer was predicted to behave in a similar manner to ligand bound STING [248].

Patients with gain-of-function mutations in *TMEM173* expressed increased IFN-related genes at baseline, but diminished *IFNB1* transcription in response to cGAMP [247]. At baseline, unstimulated patient PBMCs expressed constitutively high signal transducer and activator of transcription 1 (STAT1) phosphorylation in CD4+ T and CD19+ B cells. Patient monocytes failed to phosphorylate STAT1 in response to cGAMP stimulation. An important finding in this publication was the reduction in phosphorylated STAT1 expression in CD4+ T cell and CD19+ B cells in response to the janus kinase (JAK) inhibitors tofacitinib, ruxolitinib and baricitinib. Each JAK inhibitor prevented the phosphorylation of STAT1 downstream of the Interferon alpha/beta receptor 1 (IFNAR) and reduced the transcription of IFN response genes. Work is currently underway to optimize dosing of JAK inhibitors for the treatment of patients with rare IFN mediated disorders, also known as interferonopathies [249]. More recently, three novel mutations located distal to the dimerization domain and away from the cGAMP binding site were identified by Crow and colleagues [250]. All mutations tested, including the previously described p.Val155Met, were dependent on phosphorylation of STING. Mutation of the phosphorylation site p.Ser366 STING to alanine abolished the downstream phosphorylation of IRF3 as well as increased IFN-β luciferase activity.

An interesting addition to this field was made through the evaluation of the T cell phenotype of patients with SAVI [251]. In this cell type, STING was determined to have antiproliferative activity that was distinct from and independent to interactions with TBK1 and IRF3. This report was quickly followed by a murine model of SAVI using p.Asn153Ser STING knock-in mice generated through CRISPR/Cas9 techniques [252]. Heterozygous mice developed spontaneous inflammatory disease that mimicked certain features of SAVI, including ulcerative skin lesions. There was no evidence of pulmonary fibrosis, but lung histology suggested marked inflammatory infiltrate. Remarkably, breeding mutant STING mice to *Irf3*$^{-/-}$ mice did not rescue the inflammatory phenotype, raising questions, at least in the murine model, of the role of IRF3 in the inflammation associated with SAVI. A similar incomplete resolution of inflammation was seen in mice deficient in DNase II when crossed with *Irf3*$^{-/-}$ mice [253]. As STING also activates NF-κB, the potential role of this pathway in disease pathogenesis cannot be discounted.

4.4.5 Non-canonical Inflammasome

An additional pathway for detection of Gram-negative bacteria has been revealed in the last years. Bacterial LPS in the cytosol of myeloid cells can be sensed by murine caspase-11, or its human homologues caspase-4 and caspase-5, and this ultimately leads to cell death by pyroptosis, activation of caspase-1 and secretion of IL-1β [254, 255]. To distinguish the activation of caspase-1 in this setting, which does not require ASC or an upstream inflammasome sensor, it was termed as a *non-canonical inflammasome.* This has been shown to play an important role in the response against *Escherichia coli, Vibrio cholerae, Salmonella* Typhimurium and *Legionella pneumophila* infection, among others [256, 257]. Non-canonical inflammasomes show several particularities compared to the canonical inflammasome. First, caspases-4/-11 bind LPS directly via their CARD domains and subsequently oligomerize and become catalytically active [255]. Secondly, caspases-4/-11 seem to induce two complementary pathways. While they can induce pyroptosis by themselves via activation of gasdermin D [258], they require the mediation of the NLRP3 inflammasome to induce caspase-1 cleavage and secretion of IL-1β [259, 260]. Non-canonical activation of the NLRP3 inflammasome occurs via K^+ efflux from the cell, seemingly when the cell membrane integrity is disrupted by pyroptosis [258, 259].

Human caspase-4 is constitutively expressed, while murine caspase-11 protein level remains low in resting conditions and requires a pro-inflammatory priming signal to become expressed to effective levels [254]. Murine caspase 11 expression is up-regulated by sensing of different TLR ligands, including LPS (TLR4), Pam3CSK4

(TLR2) and R837 (TLR7) [261]. After priming, LPS ligand needs to be delivered into the cytosol for activation of the non-canonical inflammasome, a situation that can arise when an intracellular bacteria escapes the vacuole [262] or by delivery from extracellular bacteria via outer membrane vesicles [263]. Ultimately, this pathway results in pyroptosis of the cell and release of pro-inflammatory cytokine IL-1β.

Studies have recently shown that the oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC) may be an endogenous ligand for caspase-4/-5/-11. Under inflammatory conditions, oxidative stress generates oxidized phospholipids, such as oxPAPC, that can act as DAMPs to regulate the inflammatory response. Oxidized phospholipids can form in apoptotic and necrotic cells, and accumulate during infection and inflammatory conditions including atherosclerosis and rheumatoid arthritis [264–266]. On one hand, OxPAPC may compete with LPS for the binding to caspase-4/-11, effectively blocking pyroptosis and secretion of IL-1β [267]. However this antagonistic effect of oxPAPC on the non-canonical inflammasome may be specific to macrophages, since a weak induction of IL-1β release rather than an inhibitory effect was observed in human dendritic cells [267, 268]. OxPAPC also partially antagonizes TLR4 signaling by LPS in macrophages [267]. In conclusion, accumulation of endogenous oxidized lipids like oxPAPC, might strongly influence the course of inflammation and is likely to have a role in autoinflammatory disease.

4.5 Conclusion

Two decades ago little was known about the molecular mechanisms that govern innate immune cell activation and it was thought that innate immunity was a rather primitive immune system providing non-specific protection against infections. Basic research in this field has rapidly progressed our molecular understanding of how immune cells sense our environment and can detect not only infectious organisms but also surveil the environment for signs of tissue damage. Several families of signaling receptors that sense microbial products and molecules liberated or formed during danger or metabolic stress have been identified. Of note, gain- and loss-of-function mutations in multiple members of these receptors have been associated with diseases, which validates the concept and provides exciting new avenues for pharmacological interference and precision immunologic approaches. While much has been learned about the strategies immune cells take to recognize and respond to threats and dyshomeostasis, translation of these concepts into the clinic has been hampered by; (1) the fact that it remains a challenge to identify which particular pathways are indeed activated in defined patient populations and; (2) the risk associated with disabling these pathways, which are potentially required to prevent infection. It is of critical importance to better understand the interplay between the different innate immune signaling pathways and to be able to reveal when and why these pathways are overly activated in disease settings. Such studies would enable patient stratification for interference with promising specific modulators of innate immune pathways.

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Inflammasomes and Autoinflammation

5

Lori Broderick

Abstract

Inflammasomes are large intracellular multiprotein polymeric complexes comprised of sensors, adaptor proteins and caspases. As innate immune sensors capable of recognizing and rapidly responding to pathogen and metabolic danger signals, inflammasomes act as key modulators of initial immune responses. This chapter focuses on the known inflammasome complexes, how they assemble into a molecular platform for caspase-1 activation and ultimately lead to the release of pro-inflammatory cytokines in the context of the innate immune response. A brief discussion of the role for the inflammasomes in disease is included.

Keywords

Inflammasome · NLR · Interleukin-1 Interleukin 18 · Pyrin · Caspase-1 · ASC

Abbreviations

AIM2	Absent in melanoma 2
ALR	AIM2-like receptor
ANA	Antinuclear antibody
ASC	Apoptosis related speck-like protein containing CARD
ATP	Adenosine triphosphate
Bid	BH3 interacting-domain death agonist
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase activation and recruitment domain
CRP	C-reactive protein
DAMP	Damage associated molecular patterns
FADD	Fas-associated death domain
FCAS	Familial cold autoinflammatory syndrome
FIIND	Function to find domain
FMF	Familial Mediterranean fever
GTP	Guanosine-5′-triphosphate
GWAS	Genome-wide association study
ICE	Interleukin-1β converting enzyme
IFN	Interferon
IGIF	Interferon-gamma inducing factor
IL	Interleukin
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
LRRs	Leucine-rich-repeats
MAS	Macrophage-activation syndrome
MDP	Muramyl-dipeptide
MSU	Monosodium urate

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NAIP	NLR family, apoptosis inhibitory protein
NASH	Nonalcoholic steatohepatitis
NBS	Nucleotide binding site
NEK	Nima-related kinase
NF-κB	Nuclear factor–κB
NLR	NOD-like receptor
NLRC	NLR family CARD domain-containing protein
NOD	Nucleotide-binding oligomerization domain
NOMID	Neonatal-onset multisystem inflammatory disease
PAMP	Pathogen-associated molecular patterns
PRR	Pattern-recognition receptors
PYD	Pyrin domain
ROS	Reactive oxygen species
SNP	Single-nucleotide polymorphism
TLR	Toll-like receptor

Key Points

- **Inflammasomes are innate, multimeric sensors key to recognition of pathogenic and metabolic danger signals**
- **Activation of the inflammasome ultimately results in the release of IL-1β and IL-18, and extension of an inflammatory cascade**
- **The varied triggers of inflammasomes solidify their role in driving inflammation**
- **Mutations in inflammasome components lead to rare autoinflammatory diseases, and are increasingly recognized as players in common diseases**

5.1 Introduction

Inflammation is a necessary, physiologic response to infection and tissue damage, leading to the elimination of pathogens and guiding tissue repair processes. In vertebrates, two distinct but interacting immune systems have evolved to protect the host: the innate and the adaptive immune systems. Initially labeled as non-specific, the innate immune system is the first to be activated, recognizing and rapidly responding to pathogen and metabolic danger signals by sensing nearly 1000 conserved protein and nucleic acid patterns through a limited number of germline encoded receptors, or pattern-recognition receptors (PRRs) (see Chap. 4). These conserved microbial signatures, called pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS) and flagellin, are biochemically distinct from host proteins. The role of these innate immune sensors has extended to also detect metabolic danger signals (damage associated molecular patterns or DAMPS) that are upregulated with cell activation and cell death, including adenosine triphosphate (ATP). Many of these danger signals are detected by intracellular pattern recognition sensors, known as the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). The NLRs form the core of multimeric protein scaffolds called inflammasomes [1, 2] that activate when the scaffold protein senses or directly binds its activating stimulus. Together with adaptor proteins and caspases, these inflammasomes assemble in the cytosol, and subsequently lead to proteolytic cleavage of caspase and pro-cytokines. In this chapter, I focus on the assembly of the inflammasomes, and the checkpoints necessary to regulate these intracellular modulators of inflammation.

5.2 The Inflammasome

- **Inflammasomes consist of defined component proteins that interact with each other through specific protein domains, resulting in activation, oligomerization, and formation of filamentous structures**
- **Regulation of inflammasome activation occurs at multiple levels from transcription to post-translational modification to receptor signaling**
- **Activation of caspase-1 leads to cytokine release and pyroptotic cell death, with new regulatory roles in other cellular processes under ongoing study**

Inflammasomes consist of three separate molecules: a sensor molecule for which the complex is named, the adapter molecule apoptosis related speck-like protein containing caspase activation and recruitment domain (ASC) and the effector molecule caspase-1. These sensor molecules are either members of the NLR or the absent in melanoma 2 (AIM2)-like receptor (ALR) families. While there are 22 known NLRs in humans, and at least 34 NLRs in mouse, each identified by the arrangement of PYRIN-NACHT-LRR domains [3, 4], only a subset (NLRP1, NLRP3, NLRP6, NLRP7, NLRP12, and NLRC4) have been shown to form functional inflammasomes to date. In the ALR family, AIM2 and interferon-inducible protein 16 (IFI16) can form functional inflammasomes [5].

Activation of inflammasome sensors (Table 5.1) leads to the recruitment of the adapter molecule ASC, which is required for the activation of pro-caspase-1 [2]. This multimerization leads to auto-proteolysis and the generation of enzymatically active subunits [1, 2, 6–10]. Active caspase-1 induces the cleavage of the pro-forms of IL-1β and IL-18, and may induce pyroptosis. Secretion of mature IL-1β and IL-18 mediates the release of additional inflammatory factors which through non-canonical secretion allows for binding to specific receptors leading to signal transduction, expression of downstream cytokines, chemokines, and adhesion molecules resulting in an inflammatory cascade and recruitment of additional inflammatory cells [11] (see Chap. 6).

Table 5.1 Summary of inflammasomes

Inflammasome	Associated proteins	Activators	Associated human syndrome	References
NLRP1	ASC Pro-Caspase-1 Pro-Caspase-5	MDP, *T. gondii*, proteases	*Monogenic*: *NLRP1*-associated autoinflammation with arthritis and dyskeratosis, palmoplantar carcinoma, familial keratosis lichenoides chronica, corneal intraepithelial dyskeratosis *Polymorphisms*: Vitiligo	[1, 54, 55, 57–59]
NLRP3	ASC Pro-Caspase-1 Caspase-8 NEK7 Cardiolipin	Cold, LPS, amyloid beta, potassium efflux, ROS, calcium, pyrophosphate dehydrate, cholesterol, monosodium urate crystals, MDP	CAPS Schnitzler syndrome	[60, 61, 78, 79, 83–90]
Pyrin	ASC Pro-Caspase-1 14-3-3	Rho GTPases	Familial Mediterranean fever Familial autoinflammation with neutrophilic dermatosis	[101–105]
NLRC4	ASC Pro-Caspase-1 NAIP	Flagellin, bacterial type 3 (T3SS) rod/needle proteins	MAS-like syndrome FCAS-like syndrome NOMID-like syndrome	[107–113]
NLRP6	ASC Pro-Caspase-1		None to date	[115–117]
NLRP7	ASC Pro-Caspase-1	Acylated lipopeptides	Familial biparental hydatidiform mole	[125–130]
NLRP12	ASC Pro-Caspase-1		Periodic fevers with cold-associated urticaria (CAPS-like syndrome), ulcerative colitis	[133–138]
AIM2	ASC Pro-Caspase-1	dsDNA	SLE, psoriasis	[142–146]

Abbreviations: *AIM2* absent in melanoma 2, *ASC* apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD), *CAPS* cryopyrin-associated periodic syndromes, *FCAS* familial cold autoinflammatory syndrome, *FMF* familial Mediterranean fever, *LPS* lipopolysaccharide, *MAS* macrophage-activation syndrome, *MDP* muramyl-dipeptide, *NEK* nima-related kinase, *NOMID* neonatal-onset multisystem inflammatory disease, *NLR* NOD-like receptors, *NAIP* NLR family, apoptosis inhibitory protein, *ROS* reactive oxygen species, *SLE* systemic lupus erythematosus

5.2.1 Assembly of the Inflammasome

While the assembly of the sensor molecule, the adaptor ASC and the effector caspase-1 appears simple, identification of the precise assembly steps long eluded investigators. It was established early on that these components form a much larger complex than the simple 3-protein structure would be predicted to generate. Rather, the first described inflammasome, NLRP1, assembled into a complex of approximately 700 kD [1]. Upon activation, the complex increased further in size with the recruitment of ASC and caspase-1 [1]. Numerous theories postulated that the inflammasome complex contained additional unrecognized proteins that contributed to its large size, or that substantial post-translational modifications added to the molecular mass of the complex. Only recently has the structure of the inflammasome been revealed.

Inflammasomes are made up of defined component proteins. The protein components of the NLR inflammasomes contain death domains including a PYRIN domain (PYD) or caspase activation recruitment domain (CARD). Computational modeling and NMR spectroscopy have demonstrated that the PYRIN domain [12, 13], is the fourth member of the death domain-fold superfamily [14–19] which also includes death domains, death effector domains, and CARDs. All four of these proteins facilitate protein-protein interactions via electrostatic charges to form an inducible, six alpha helix structure with key roles in cell death and inflammation [3, 20]. The PYRIN domain is considered the effector domain, by acting as a docking motif driving self-assembly into characteristic disk like complexes consisting of homotypic and heterotypic oligomers, involving seven or more of the same protein molecules.

5.2.1.1 Apoptosis-Associated Speck-Like Protein with a CARD (ASC)

ASC or PYCARD is an adaptor protein consisting of an N-terminal PYRIN domain and a C-terminal CARD in tandem [21–23]. Through its CARD, ASC binds caspase-1 (also known as IL-1β converting enzyme (ICE)) and other adaptor proteins. During inflammasome polymerization, ASC assembles into a large protein aggregate or 'speck' that localizes to the paranuclear area of the cell, and is visible by microscopy [24]. Advances in microscopy have revealed more details about inflammasome assembly. *In vitro* reconstitution experiments indicate that the PYD domains of inflammasome sensors (such as NLRs) self-nucleate and induce the assembly of organized helical ASC PYD filaments [25]. Full-length ASC then induces the formation of caspase-1 filaments. Using NLRP3 and AIM2 as distinct sensors, these studies suggest that different core inflammasome molecules result in the same downstream assembly of ASC and caspase-1 into filaments via nucleation-induced protein polymerization. Interestingly, recent data demonstrated that these macromolecular specks can be passively released into the extracellular space and influence and activate neighboring cells [26].

5.2.1.2 Caspase-1

Caspases are a family of conserved cysteine proteases. Caspase-1, caspase-4 and caspase-5 comprise a subset of inflammatory caspases. Activation of the inflammasome centers on activation of caspase-1, with formation of an α2β2 tetramer consisting of two p20 (containing the active site) and two p10 subunits. The most studied functions of caspase-1 are the proteolytic activation of pro-inflammatory cytokines IL-1β, IL-18 [5], and cleavage of the soluble cytosolic protein gasdermin-D [27–29], though proteomic evaluations have identified more than 100 independent substrates [30–32]. Upon proteolysis, the 31 kD gasdermin-D N-terminal fragment inserts into the plasma membrane to mediate the non-conventional secretion of IL-1β and IL-18, both of which lack the signal peptide, and to induce a pro-inflammatory type of cell death known as pyroptosis [16, 33]. Cell lysis is not necessary for cytokine release.

Caspase-1 in Cytokine Release

While release of IL-1β was linked early to inflammasome activation, the release of IL-1β and IL-18, both of which lack a signal peptide, has

only recently been described. Two molecules of the caspase heterodimer form a tetramer with two molecules of pro-IL-1β for cleavage at amino acid aspartic acid 116 of the pro-IL-1β, from its 31 kD precursor form to its 17 kD mature form. IL-1β activity is limited by expression and competitive binding of IL-1R antagonist (IL-1Ra) to the IL-1 receptor (see Chap. 6).

Similar to IL-1β, caspase-1 cleaves pro-IL-18 from its 24 kD immature form to a 17.2 kD active molecule after aspartate 36 [34]. Initially named interferon-gamma inducing factor (IGIF), IL-18 is best known for promoting the production of interferon-γ (IFN-γ), T helper type 1 cell proliferation and natural killer cell activation. It can also enhance T helper type 2 cells and promote local inflammatory responses, with activity dramatically enhanced with exposure to IL-12. This multifaceted activity and inherent interaction with other components of the immune system often leads IL-18 to be called a "double edged sword".

Caspase-1 in Pyroptosis

Pyroptosis is a type of inflammatory cell death, similar to necrosis, in which pore formation results in cell swelling and disruption of the plasma membrane with release of cytosolic components [35]. The key executor of pyroptosis is caspase-1 or caspase-11 cleaved gasdermin-D, which is then released from self-inhibition [28, 29]. Oligomers of gasdermin-D then migrate to the cell membrane forming pores with an inner diameter of 10–15 nm, wide enough for IL-1β and caspase-1 to pass easily through [33]. Thus, pyroptosis plays a dual role in both eliminating the infected/compromised cell and inducing a reactive inflammatory response. Interestingly, pyroptosis itself is not bactericidal, but plays an important role in the host anti-microbial defense by releasing intracellular bacteria from macrophages. Now unprotected, microbes are susceptible to uptake and killing by neutrophils, thereby preventing further dispersion and host damage [36].

Many questions remain regarding the molecular process from inflammasome assembly to caspase-1 cleavage to IL-1β and IL-18 release and/or pyroptosis. Not all stimuli that activate caspase-1 result in pyroptosis, and not all cell types undergo pyroptosis [37, 38]. In addition, caspase-1 can activate caspase 3 and BH3 interacting-domain death agonist (Bid), key components in apoptotic cell death [5, 32]. *In vitro* studies using live cell, time-lapse imaging have attempted to resolve these questions. Fernandes-Alnemri et al. [39] demonstrated that in THP-1 cells, stimuli such as LPS, monosodium urate (MSU) and Pam3CSK4 resulted in potassium depletion and the formation of an oligomeric ASC complex. This ASC oligomer recruited caspase-1, and led to the induction of pyroptosis with release of IL-1β. Given the large molecular assembly and inflammatory stimuli, this inflammasome-sensor independent assembly was termed the pyroptosome [39]. In murine macrophages, release of mature IL-1β release only occurred with speck formation, and every cell possessing a speck proceeded to undergo pyroptosis [40]. Future investigations will determine if these *in vitro* experiments represent *in vivo* molecular physiology and potential therapeutic targets for autoinflammatory disease.

Additional Roles for Caspase-1

Caspase-1 has been described to have functions beyond its classic roles in the inflammasome. Diagonal gel proteomics have shown that caspase-1 could use several glycolysis enzymes as targets, including aldolase, GAPDH, and enolase. This "digestosome" resulted in reduction of the cellular glycolytic rate in models of sepsis and *Salmonella* infection, suggested to be a definitive first step towards cellular death [41]. Elevated levels of reactive oxygen species (ROS) has been examined as a trigger for NLRP3 activation, and caspase-1 has been shown to activate several pathways leading to disassembly of the mitochondria via dissipation of the membrane potential, permeabilization, fragmentation and inhibition of mitophagy, all resulting in mitochondrial damage [42, 43]. Finally, caspase-1 may play a regulatory role in cell signaling and inflammation via protein degradation by recognizing PEST motifs: segments rich in proline (P), glutamic acid (D), aspartic acid (E) and serine (S)

or threonine (T) residues. Cleavage leaves exposed terminal loops resulting in ubiquitin-proteasome dependent and independent degradation of proteins [44]. Some proteins, including ubiquitin E2 conjugating enzyme (UBE2L3), are involved in ubiquitylation (also known as ubiquitination) and proteasomal turnover of pro-IL-1β, thereby modulating the levels of pro-cytokine available for maturation. Caspase-1 driven proteasome-dependent degradation of UBE2L3 enhances inflammation by increasing pro-IL-1β levels intracellularly. This effect was found to be similar in all sensor proteins examined including NLRP1, NLRP3, NLRC4, pyrin, and AIM2 [45]. Perhaps given the number of functions and potential substrates, it is not surprising that caspase-1 undergoes rapid inactivation upon formation of the mature enzyme (half-life approximately 9 min), thereby limiting its abundance within a cell [32].

5.2.1.3 Inflammasomes as DAMPs

Inflammasome activation leads to the caspase-1 dependent, inflammatory cell death termed pyroptosis. As described above, pore formation results in a compromised plasma membrane with leakage of intracellular content. It was therefore postulated that cytosolic ASC specks, which normally form in response to activation of inflammasomes, could be passively secreted into the extracellular environment. In two seminal papers, Latz, Pelegrin and colleagues used advances in cell-free systems and fluorescence imaging to identify extracellular, active inflammasome components following their release from pyroptotic cells [26, 46]. The released ASC specks could act as bioactive inflammasomes in the extracellular space leading to further processing of pro-caspase-1 and pro-IL-1β. These findings were important in prolongation of the immune response in two contexts. First, it implied that ASC specks could act as inflammatory mediators in the extracellular space, by continuing to cleave pro-caspase-1 and pro-IL-1β. Second, the released extracellular ASC specks can be ingested by neighboring macrophages, leading to inflammasome activation in the recipient cells, and act as danger signals, further amplifying inflammation. These new extracellular activities of ASC could thereby lead to a chronic perpetuation of immune responses in autoinflammatory diseases.

5.2.1.4 Inflammasome Triggers: An Overview

Despite the existing dogma that the innate immune system acts as a non-specific defense, NLRs demonstrate specificity for particular PAMPS or DAMPs, though some appear to be more restricted than others (Table 5.1). As described in Chap. 4, PAMPs are biochemically distinct from host proteins, and are identified by germline encoded pattern recognition receptors, such as the toll-like receptors. Beyond pathogens and microbial products, the innate immune sensors also detect host danger signals known as DAMPs. DAMPs include metabolites that are upregulated with cell activation and cell death such as ATP, nucleotides, or uric acid. How such different stimuli can specifically activate one pathway leading to IL-1β and IL-18 release needs further investigation.

5.2.1.5 Inflammasome Regulation

Inflammatory reactions are tightly controlled, with failure to eliminate the danger signal resulting in ongoing inflammation or persistent local tissue damage and subsequent development of chronic inflammatory disease states. Given the autopropagation of inflammasome-driven inflammation, it is not surprising that regulation of inflammasome activation occurs at multiple steps. Expression of sensor proteins is regulated both at the RNA level, and by post-translational proteolysis at the protein level. Chaperone and scaffolding proteins may stabilize intra-protein domain interactions in the inactive state, and control inflammasome priming. Inflammasome activation is dependent on the inter-protein domain oligomerization. Activation of caspase-1 and release of mature IL-1β and IL-18 are dependent on proteolytic cleavage to form mature, biologically active forms. Activity of released cytokines is dependent on binding to their respective receptors leading to a self-driven positive feedback loop of increased transcription of the

inflammasome component proteins as well as the pro-cytokines. Competitive inhibition by IL-1 receptor antagonist and IL-18 binding protein regulates cytokine binding to the respective receptors. Errors at any of these steps would be predicted to drive chronic inflammation and disease. Indeed, mutations in genes that code for inflammasome components or related proteins result in hyperactivation or constitutive activation of inflammasomes and have been described in a growing group of autoinflammatory diseases.

5.2.1.6 Non-canonical Inflammasomes

While undisputedly informative, differences between human and mouse inflammasomes, and *in vitro*, *ex vivo* and *in vivo* models have driven some controversial findings in our understanding of the inflammasomes. Caspase-1 deficient mice used in early studies were subsequently shown to also be deficient in caspase-11 due to chromosomal proximity. Caspase-11 was found to respond to intracellular LPS, independently of toll-like receptor (TLR)4, resulting in IL-1β and IL-18 secretion and cell death via a "non-canonical" inflammasome. The authors proposed that caspase-11, rather than caspase-1, may be the critical effector of inflammatory responses in models of murine sepsis [47]. In humans, however, it remains unclear whether caspase-4 and caspase-5 are functional orthologs of the caspase-11 seen in mice. Studies have shown both caspase-4 and caspase 5 to act as functional components of inflammasomes, but the tissue expression patterns differ. Caspase-4 was necessary for mature cytokine secretion in vitro in human intestinal epithelial cells and in keratinocytes [48, 49]. It may be that in certain tissues, such as epithelium, certain stimuli preferentially activate non-canonical inflammasomes.

5.3 Individual Inflammasomes

The challenges and successes of understanding the inflammasome have long been tied to the translational "bench to bedside" approach, with patient characterization driving *ex vivo* and *in vitro* studies to reveal molecular cellular dysfunction [50]. The coinciding discovery of the inflammasome and mutations in *NLRP3* solidified the pathogenic role of the inflammasome in the classic autoinflammatory disorders, and contributed to the development of novel, targeted therapies. More importantly, it also solved several questions regarding the mechanisms of IL-1β release and increased our understanding of basic innate immune mechanisms. Additional details for the role of each known functional inflammasomes are described in the sections below.

5.3.1 NLRP1

- **The first inflammasome to be described, NLRP1 provided early evidence of inflammasome oligomerization and dependence on ASC**
- **Mutations in NLRP1 result in NLRP1-associated autoinflammation with arthritis and dyskeratosis (NAIAD)**

NLRP1 (synonyms CARD7, NALP1) was the first NLR to be identified to form a cytosolic complex, termed the inflammasome by Tschopp, Martinon and colleagues, given the similarities in domain structures to the apoptosome [1]. This was the first paper to demonstrate the molecular details of IL-1β processing, and the first suggestion of ASC as an adapter protein connecting the CARD domain of caspase-1 to the PYD domain of the NLRP1 protein (Fig. 5.1). Unique to NLRP1 are function to find domain (FIIND) and CARD domains which appear to be critical to NLRP1 inflammasome formation and function [51].

5.3.1.1 Regulation of NLRP1

Martinon's seminal paper was also the first to discuss the regulation of the inflammasome [1]. In HEK293 transfection systems, they observed that deletion of LRR and PYD led to increased NLRP1 oligomerization, and they proposed that both domains were required to maintain NLRP1 in an autoinhibited state. In addition to dependence on ASC, further studies have demonstrated that NLRP1 activity requires autolytic cleavage at Ser1213 within

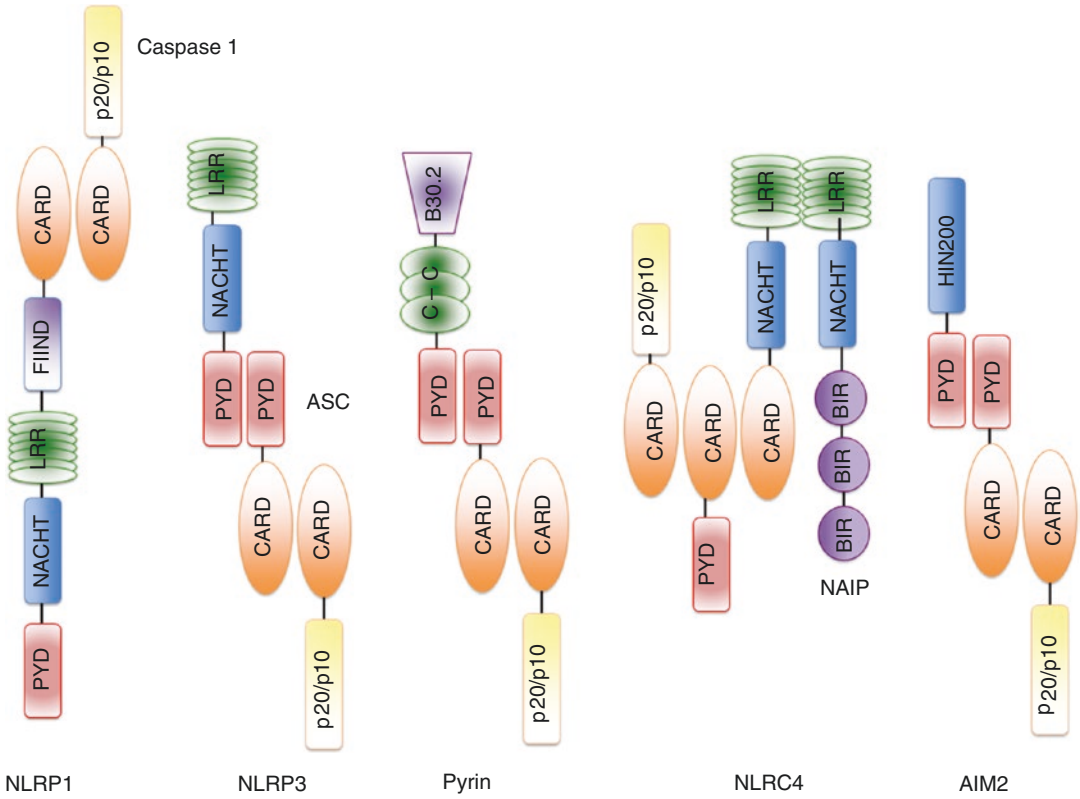

Fig. 5.1 Schematic of inflammasome structure. NLRP6, NLRP7 and NLRP12 inflammsomes are proposed to have structures similar to the NLRP3 inflammasome. *BIR* baculovirus inhibitor of apoptosis protein repeat, *CARD* caspase activation and recruitment domain, *C-C* coil-coil domain, *LRR* leucine-rich-repeats, *NAIP* NLR family, apoptosis inhibitory protein, *PYD* PYRIN domain

the FIIND [51]. This post-translational event is dependent upon the highly conserved residue His1186, which three-dimensional modeling demonstrated is held in close proximity to the cleavage site. Interestingly, a naturally occurring splice variant results in excision of exon 14, possibly increasing the distance between the catalytic histidine and the cleavage site, and resulting in blockage of IL-1β release. NLRP1 activity is further regulated by two anti-apoptotic Bcl-2 family proteins, B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma extra-large (Bcl-X_L), which bind to NLRP1 in the inactive state and suppress activation of caspase-1 [52].

5.3.1.2 Activators of NLRP1

Activators of human NLRP1 have been somewhat elusive in part due to the *in vitro* systems established for study of human NLRP1 and the differences between human and murine NLRP1. While *in vitro* studies have long used MDP as a synthetic ligand to induce NLRP1 inflammasome formation in an ATP dependent fashion [53], patients with polymorphisms in NLRP1 have guided the search for natural activators of the inflammasome. For example, i*n vitro* data, driven by cohorts of patients with susceptibility to toxoplasmosis, has shown that human NLRP1 is important for the cleavage of pro-IL-1β in response to *T. gondii* [54]. More recently, Vance and colleagues have demonstrated that N-terminal proteolysis is sufficient to activate NLRP1, and proposed that NLRP1 variants evolved to recognize diverse pathogen-encoded proteases [55].

5.3.1.3 NLRP1 in Autoinflammatory Disease

NLRP1 is widely expressed with increased expression in multiple immune cells, including T cells and Langerhans cells [56]. Polymorphisms in *NLRP1* have long been associated with autoimmune disorders such as vitiligo, type 1 diabetes, systemic lupus erythematosus and rheumatoid arthritis, but only recently has the NLRP1 inflammasome been implicated in monogenic human disease. Dominant mutations in *NLRP1* were linked to three independent conditions: palmoplantar carcinoma, familial keratosis lichenoides chronica [57] and inherited corneal intraepithelial dyskeratosis [58]. The variants, all identified in the PYD and LRR domains of NLRP1, resulted in increased spontaneous ASC speck formation and increased pro-IL-1β in *in vitro* studies. These data suggest that NLRP1 mutations disrupt the PYD and LRR domains which are essential for maintaining an inactive state, and subsequently lead to increased self-oligomerization.

A novel monogenic disorder was described in 2017 by Grandemange and colleagues, which they termed *NLRP1*-associated autoinflammation with arthritis and dyskeratosis (NAIAD) [59] (see Chap. 29). This new syndrome bridges the gap of autoinflammatory and autoimmune disorders, with patients displaying elevated serum C-reactive protein (CRP), as well as positive antinuclear antibody (ANA) levels, and abnormal B cell subsets. While the patients had elevated serum levels of caspase-1, IL-1β and IL-18, the mechanisms of activation of the NLRP1 inflammasome activation leading to the syndrome features were not determined.

5.3.2 NLRP3

- **Of the inflammasomes, NLRP3 is activated by the most highly diverse triggers**
- **Regulation of NLRP3 includes a 2-step process of initial priming (signal 1), followed by an inflammasome formation step (signal 2), as well as interactions with scaffold and accessory proteins**
- **NLRP3 was initially implicated in the rare cryopyrinopathies, and now recognized to play a role in more common diseases including cardiovascular disease, diabetes and gout**

NLRP3 was first described in 2001 in a rare autosomal dominant fever disorder known as familial cold autoinflammatory syndrome (FCAS), see Chap. 19 [60, 61]. The NLRP3 protein was initially named cryopyrin to emphasize the presence of an N-terminal PYRIN domain, and the association with cold-induced symptoms in patients with FCAS [60]. Similar to other NLRs, NLRP3 contains a central NACHT domain [62], and seven, C-terminal leucine-rich repeats (LRRs). NLRP3 is primarily expressed in leukocytes and chondrocytes, with lower levels in other tissues and cells [56, 60, 63–65]. It is the most-well studied of the inflammasomes.

The PYRIN domain of cryopyrin has been shown to interact specifically with ASC. This macromolecular complex of cryopyrin, ASC, and caspase-1, and possibly a cardinal, forms the NLRP3 inflammasome [8, 9]. The NACHT domain contains seven conserved motifs, including an ATPase-specific P loop and a magnesium binding site, and is involved in protein oligomerization [62]. This domain has been found to have ATPase activity and, disease-associated mutations are found primarily in the NACHT domain, suggesting an important role for this motif in the function of NLRP3 [66]. NLRP3 regulates IL-1β, IL-18 secretion [7, 10], NF-κB activation [21–23, 67, 68], and cell death [23, 69–72]. However, the mechanism by which diverse triggers tailor a specific immune response, remain under investigation.

5.3.2.1 Regulation of NLRP3

Under normal conditions, the NLRP3 inflammasome is regulated by a 2-step process: an initial priming step (signal 1), followed by an inflammasome formation step (signal 2, Fig. 5.2). During signal 1, PAMPs or DAMPs are recognized by TLRs, leading to activation of NF-κB with transcriptional upregulation of sensor proteins,

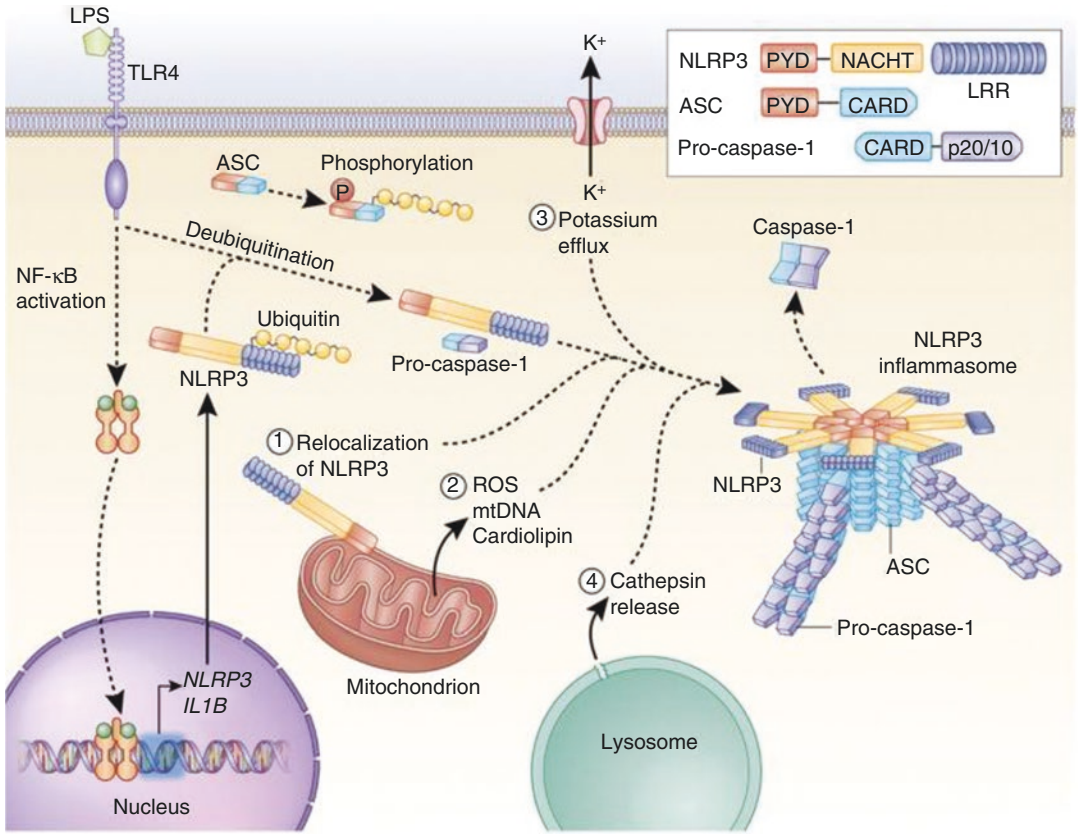

Fig. 5.2 NLRP3 inflammasome activation and regulation. The NLRP3 inflammasome components: NLRP3, ASC and pro-caspase-1 are shown in the inset (upper right). The NLRP3 inflammasome is activated in a two-step process. Priming and licensing of NLRP3 occurs by first inducing expression of NLRP3 and IL-1β by NF-κB–activating stimulus, as well as deubiquitination of NLRP3. Step two, inflammasome formation, is induced by a variety of triggers, including potassium efflux, mitochondrial factors, and cathepsin release. Activated NRLP3 subsequently nucleates ASC forming filaments via PYD-PYD interactions, and drives pro-caspase-1 filament formation through CARD-CARD interactions. This complex ultimately results in autoproteolytic activation of pro-caspase-1, and downstream, cleavage of pro-IL-1β and pro-IL-18. Inset shows domain arrangement of the NLRP3 inflammasome components. *CARD* caspase activation and recruitment domain, *LRR* leucine-rich-repeats, *PYD* PYRIN domain, *ROS* reactive oxygen species. *Reprinted by permission from Springer Nature*, from*: Guo H, Callaway JB and J P-Y Ting. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nature Medicine 2015; 21:677*

accessory proteins and pro-cytokines. In addition, post-translational modifications of NLRP3 itself have been investigated as regulatory steps during signal 1. While deubiquitination was suggested as a necessary step in activation of the NLRP3 inflammasome nearly 15 years ago in murine macrophages [73], this mechanism has only recently been replicated in human cell lines. Kawashima and colleagues have demonstrated that an E3 ligase, Ariadne homolog 2 (ARIH2), acts as a post-translational negative regulator of NLRP3 inflammasome activation. Unlike the initial studies which identified the LRR domain of NLRP3 to be the target for deubiquitination, ARIH2 interacted with NLRP3 via its NACHT domain [74]. Others have suggested that inflammasome assembly can be prevented by S-nitrosylation of NLRP3 and caspase-1 by nitric oxide [75]. The authors suggest that nitric oxide induced by IFN-γ during infection generated a potential negative feedback loop to prevent tissue damage that results from excessive

inflammasome signaling. Finally, several groups have identified serine phosphorylation of NLRP3 as an important regulator, though the results are conflicting. Stutz and colleagues propose a model in which phosphorylation drives electrostatic repulsion of the PYRIN domains, and phosphatase 2A dephosphorylation licenses NLRP3 for activation. In contrast, others suggest that serine phosphorylation by JNK1 is required for activation of the inflammasome [76, 77]. These differences may be due to variations in experimental conditions, i.e. length of exposure to priming stimuli, the inflammasome triggers used, and the experimental cell lines. Alternatively, NLRP3 regulation through phosphorylation may be thought of as a dynamic process, allowing the cell to rapidly respond to threats.

Several models have been proposed for signal 2 and assembly of the NLRP3 sensor with ASC and pro-caspase-1, and inflammasome activation [78]. Extracellular nucleotides such as ATP increase in the extracellular space during inflammation, and activate the P2X7 receptor, thereby driving ion efflux [79]. Mitochondrial derived signals including ROS and calcium signaling, indicative of mitochondrial damage, can activate NLRP3. Finally, lysosomal rupture induced by crystals can activate NLRP3. It is likely that many of these pathways work cooperatively to drive NLRP3 activation. This variability in signal 2 has led investigators to believe that NLRP3 does not directly bind to its activators, but rather senses a common, downstream, intracellular signal, such as potassium efflux.

Beyond ion efflux, nima-related kinase 7 (NEK7), a serine-threonine kinase with a role in microtubule spindle formation and linked to mitosis, has recently been proposed as a regulator of NLRP3 activity. Potassium efflux leads to the association of NLRP3 with NEK7, and the subsequent assembly and activation of the NLRP3 inflammasome, in murine studies and HEK293 cell assays [80, 81].

Finally, scaffolding proteins such as caspase-8 and its adapter Fas-associated death domain (FADD) have been shown to be recruited to the inflammasome. Initial studies also demonstrated that caspase-8 could induce activation of NF-κB, thereby maintaining levels of pro–IL-1β mRNA and protein after microbial stimulation. In vitro studies further suggested that caspase-8 is able to directly and specifically cleave caspase-1, confirming a role for caspase-8 both in priming and post-transcriptional activation of the inflammasome components [82].

5.3.2.2 Activators of NLRP3

Of all the NLR inflammasomes, NLRP3 has the most diverse collection of activators. NLRP3 detects intracellular bacteria or bacterial products, such as peptidoglycan and LPS via LRRs [83–85], as well as the synthetic PAMP muramyl-dipeptide (MDP), a common PAMP [86]. Intriguingly, most of the activators of the NLRP3 inflammasome do not interact directly with NLRP3. Rather, mechanisms involving cold, potassium efflux, generation of ROS in mitochondria, and membrane disruption by crystalline (calcium pyrophosphate dehydrate, cholesterol, MSU crystals) and peptide aggregates (amyloid beta) have been proposed to mediate inflammasome activation [87]. Uptake of these conglomerates may drive lysosomal rupture leading to the cytosolic release of lysosomal proteases such as cathepsins B and L. It is undisputed that complex processes regulating NLRP3 activation exist, and investigations into the molecular-atomic details are ongoing.

5.3.2.3 NLRP3 in Autoinflammatory Disease

NLRP3 mutations were first described in patients with a cold-induced, autoinflammatory syndrome, initially called familial cold urticaria, and later FCAS [61, 88]. Mutations were subsequently identified in more severely affected patients with Muckle Wells syndrome [89], and neonatal-onset multisystem inflammatory disease (NOMID) [90]. This autoinflammatory spectrum of disease, all resulting from gain of function mutations in NLRP3, was named the cryopyrin-associated periodic syndromes (CAPS) or the cryopyrinopathies. While patients share symptoms of recurrent fever, urticarial-like rash, malaise, headaches, joint pain, and conjunctivitis,

clinical features can be used to further delineate where patients fall on the CAPS inflammatory spectrum. See Chap. 19 for a further description of CAPS.

Variants in NLRP3 have also been described in another rare autoinflammatory disease, Schnitzler syndrome (see Chap. 37). Schnitzler syndrome is characterized by chronic, neutrophilic urticaria, monoclonal gammopathy, and systemic inflammation [91]. Identified variants include novel amino substitutions, as well as modifications previously observed in atypical (variant) CAPS and NOMID. The response of these patients to IL-1 blockade suggests that these variants may play a pathologic role in disease, but further investigations are needed.

NLRP3 activation has more recently been described in common diseases including cardiovascular disease, gout (see Chap. 34), pseudogout, type 1 diabetes (see Chap. 39), Alzheimer (reviewed in [92]) and Crohn disease [93]. Given the diversity of metabolic stimuli that trigger the NLRP3 inflammasome, these pathologic associations are not surprising. Complicating the delineation of the pathophysiologic pathway, however, is that numerous key activators are often present. For example, oxidized LDL, cholesterol crystals, and elevated ROS species, each of which trigger NLRP3 activation independently, have all been described in atherosclerotic plaque-derived macrophages (see Chap. 39). Co-morbidities including obesity may contribute to the metabolic danger signals driving NLRP3 activation, by adding to the abundance of free fatty acids and oxidative stress. The identification of roles for NLRP3 and common downstream inflammation driven by IL-1 in each of these disorders, however, provides new opportunities for therapeutic intervention (see Chaps. 41 and 42).

5.3.3 Pyrin

- **Regulation of pyrin occurs primarily by phosphorylation and constitutive inhibition, requiring interactions with 14-3-3 proteins**
- **The pyrin inflammasome underlies the pathology of familial Mediterranean fever, and pyrin-associated autoinflammation with neutrophilic dermatosis**

Pyrin is expressed in granulocytes, cytokine-activated monocytes, and synovial and peritoneal fibroblasts [94–97]. Similar to NLRP3, the N-terminal PYRIN domain of pyrin interacts specifically with the homologous domain of ASC [6, 14, 23, 24, 98], resulting in co-localization to actin polymers [99], and ultimately leads to release of mature IL-1β in a caspase-1 dependent fashion [1, 2, 6–10]. In addition, the interaction between pyrin and ASC plays regulatory roles in leukocyte apoptosis and NF-κB activation [6, 14, 23, 98], though these processes are less well understood.

5.3.3.1 Activation of Pyrin

Much of our knowledge of the pyrin inflammasome has resulted from murine models of *MEFV* knockouts, a truncated, hypomorphic form of pyrin, and knockins. *Ex vivo* studies from hypomorph and knockout mice demonstrated increased caspase-1 activation, increased IL-1β processing and secretion, and a defect in apoptosis [6, 100]. While knockin murine models solidified the role of the inflammasome *in vivo* [101], until recently the stimulus for activation of pyrin itself remained difficult to discern. First described in response to *Clostridium difficile* toxin b (TcdB), bacterial toxins mediate the glucosylation, adenylylation, ADP-ribosylation, or deamidation of various residues in RhoA, thereby inhibiting guanine nucleotide binding and GTPase activity. Inhibition of RhoA signaling pathways leads to activation of the pyrin inflammasome [102].

5.3.3.2 Regulation of Pyrin

Pyrin is regulated primarily by phosphorylation and constitutive inhibition. Recent work has shown that phosphorylation at two serine sites maintain the inactive state through binding by 14-3-3 proteins. Pyrin dephosphorylation is triggered by toxin stimulation and bacterial infection with 14-3-3 dissociation, resulting in activation of the pyrin

inflammasome. It is not yet known whether pathogenic signals inhibit the relevant kinase or activate a specific phosphatase [103, 104].

5.3.3.3 Pyrin in Autoinflammatory Disease

Familial Mediterranean fever (FMF) is likely the most well-known of the autoinflammatory syndromes, and is caused by mutations in the pyrin gene, *MEFV*, a 10 exon gene on human chromosome 16p13.3 [105]. More than 300 variants have been described to date, mostly in exons 2 and 10. Disease is classically considered to be autosomal recessive in inheritance, but increasing reports of patients with only one or no identifiable *MEFV* mutations are available. FMF is characterized by discrete, short episodes of fever with serositis, synovitis, and occasionally an erysipeloid skin rash localized to the lower extremities. Amyloidosis is the most worrisome long-term complication of FMF (see Chap. 16).

More recently, a distinct, autosomal dominant syndrome caused by mutations in pyrin has been identified by Masters and colleagues (see Chap. 29). From early childhood, patients experienced recurrent episodes of fever, neutrophilic dermatosis, arthralgia, myalgia/myositis and elevated serum acute-phase reactants, which they subsequently named pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND). A single mutation was identified, resulting in the loss of a 14-3-3 binding motif at phosphorylated S242, reminiscent of bacterial triggers such as TcdB described above, thereby inducing inflammasome activation and IL-1β production [104]. Further investigations of a second family with PAAND, with the mutation E244K, again demonstrated decreased 14-3-3 binding, with increased ASC speck formation [106]. Interestingly, cells transfected with the E244K variant showed significantly greater caspase-1 dependent IL-1β and IL-18 secretion, and pyroptotic cell death, compared to wild-type and FMF mutations. Differences in serum cytokine protein expression between PAAND and FMF patients are reflective of the clinical presentations, and further highlight the complexities in regulation of the pyrin inflammasome.

5.3.4 NLRC4

NLR family CARD domain-containing protein (NLRC)4, ICE-protease activating factor, (*IPAF/CARD12*) is unique among the inflammasomes in that it complexes with another NLR family member, NLR family, apoptosis inhibitory protein (NAIP) [107]. The NAIP-NLRC4 inflammasome plays a critical role in anti-bacterial defenses with NAIP acting as a cytosolic receptor for bacterial flagellin and T3SS rod/needle proteins, while NLRC4 acts as an adapter for caspase-1 activation [108, 109]. Like the other inflammasomes, NLRC4 is held in an inactive state by steric domain interactions. NAIP recognition of the bacterial ligand initiates the assembly of the NLRC4 inflammasome, with early studies in mice demonstrating that NLRC4 induced activation of caspase-1 and IL-1β in an ASC dependent fashion in response to *S. typhimurium* infection [110]. New electron microscopy studies have further revealed the assembly of the NLRC4 inflammasome in which 11-12 polymers take on the characteristic disk shape, with the LRR domains at the perimeter and the NLRC4 CARD domain exposed for activation of caspase-1 or ASC.

5.3.4.1 NLRC4 in Disease

While studies have largely focused on murine knockout models to demonstrate a role for caspase-1 activation to various bacteria through NLRC4 (reviewed in [111]), only recently have specific autoinflammatory syndromes been linked to mutations in NLRC4 (see Chap. 29). Two groups independently described *de novo* mutations in NLRC4 associated with macrophage activation syndromes (MAS) and elevations in IL-1β and IL-18. Gain-of-function mutations have been described which lead to recurrent episodes of fever, periodic urticarial rash, enterocolitis, splenomegaly and MAS [112, 113]. A third group has described a less severe phenotype in a Japanese family with similarities to FCAS: episodes of recurrent fever and urticaria-like rash induced by cold exposure [32]. Adding to the clinical heterogeneity of these patients, an infant with a NOMID phenotype, with wild-type NLRC4 alleles was

subsequently described by Kawasaki et al. [114]. In novel experiments, induced pluripotent stem cells were derived from patient fibroblasts, and then differentiated into monocytic lineage cells. They subsequently identified a novel heterozygous, somatic mutation in NLRC4. Whether the differences between these phenotypes represents a spectrum of disorders similar to the cryopyrinopathies, or the effect of different mutations in the context of different genomes, remains to be seen.

5.4 Other Inflammasomes

- **Structurally related multimeric proteins have been identified indicating that additional inflammasomes exist**
- **Additional work is needed to understand the role of these inflammasomes in health and disease**

Several additional inflammasomes have been identified based on similar structures, nucleated by NLRP6, NLRP7, NLRP12 and AIM2. However, due to differences in murine and human genes, proteins and experimental models, as well as the lack of monogenic disease associations, far less is known about these inflammasomes. Models have shown that these inflammasomes, such as NLRP6 and NLRP7 may have roles in regulation of homeostasis with host microbiota and embryologic development. Clearly, much remains to be learned regarding their role in the innate immune response.

5.4.1 NLRP6

NLRP6 is primarily expressed in the duodenum, ileum, and colon specifically in the epithelial cells of the gut, where it plays an essential role in maintaining intestinal homeostasis via mucosal self-renewal and proliferation [115]. NLRP6 forms an ASC-caspase-1 dependent inflammasome, leading to the cleavage of pro-IL-1β and pro-IL-18. However, most of the functional information to date has been derived from studies of Nlrp6 knockout mice, and our knowledge of human NLRP6 remains limited. Studies in *Nlrp6−/−* mice indicate that its role is primarily as a negative regulator of gut microbiota [116–118].

5.4.1.1 NLRP6 in Autoinflammatory Disease

A monogenic autoinflammatory disease has not been ascribed to NLRP6. Association in human gastrointestinal disease has been limited. Patients with nonalcoholic steatohepatitis (NASH) and portal fibrosis demonstrated increased expression of NLRP6 and circulating IL-18 compared to patients without portal fibrosis [119]. Contrary to murine data, in which studies of colorectal cancer showed a protective role for NLRP6, no differences were observed in NLRP6 expression in humans with colorectal cancer compared to controls. Beyond the gut, genome-wide association studies (GWAS) and single-nucleotide polymorphism (SNP) analysis have linked identified *NLRP6* to platelet count [120], and susceptibility to essential hypertension [121], suggesting that more investigation into the non-intestinal function of NLRP6 may be warranted.

5.4.2 NLRP7

NLRP7 (synonym NALP7) was first identified by Okada et al. in testicular germline tumors [122], but little is known about the NLRP7 inflammasome. Similar to other NLRs, NLRP7 contains a nucleotide-binding domain (consisting of the NACHT domain and the NACHT associated domain (NAD)). Recently, Radian et al. demonstrated that the nucleotide-binding domain of NLRP7 is an ATP binding domain with ATPase activity, required for oligomerization, and NLRP7 inflammasome formation and activity [123]. NMR spectroscopy has shown that the PYD surface of NLRP7 is unique compared to ASC and NLRP1, with significantly different electrostatic surfaces that may partly account for the differential downstream and disease-related effects of NLRP7 [124].

5.4.2.1 Regulation of NLRP7

Transcription of NLRP7 is induced in response to inflammatory stimuli, including LPS and IL-1β in peripheral blood mononuclear cells. While NLRP7 is highly expressed in thymus, spleen and

bone marrow, the nervous system, lung, testis and ovaries also have high expression levels. NLRP7 has been shown to co-localize intracellularly with the Golgi complex and microtubule-organizing center. This data has led to the suggestion of a role for NLRP7 in the negative regulation of inflammation by modulating cytokine secretion via disruption to their trafficking networks, though the use of different *in vitro* reconstitution models has produced conflicting results [125].

5.4.2.2 NLRP7 Triggers

In vitro studies of human macrophages have demonstrated that NLRP7 forms an active inflammasome, triggered by acylated bacterial lipopeptides [126], leading to the release of IL-1β and IL-18 in a caspase-1 dependent fashion, downstream of TLR2. The authors postulate that NLRP7 and TLR2 contribute to the host defense against intracellular Gram positive bacteria, inhibiting replication of *L. monocytogenes* and *S. aureus* infection. In THP-1 cells, *M. bovis* has also been show to activate the NLRP7 inflammasome, but this has not been extrapolated to primary human macrophages [127].

5.4.2.3 NLRP7 in Disease

The role of NLRP7 in disease has been restricted to disorders of the reproductive system. Patients with recurrent hydatiform moles have been found to have variants in NLRP7 [128] (see Chap. 27). When cells from these patients were investigated *ex vivo*, IL-1β release was low to mildly elevated compared to controls [129]. New studies suggest a role for NLRP7 in imprinting through chromatin programming, specifically in establishment and/or maintenance of the maternal imprint. In NLRP7 variants, loss of methylation was restricted to the maternal loci, and led to autosomal dominant aberrant imprinting marks in the offspring. These epigenetic functions have not previously been associated with NLRP family members [130, 131].

5.4.3 NLRP12

NLRP12 (Monarch-1, Pypaf7) is expressed by hematopoetic, gastrointestinal cells, and the nervous system [132, 133]. While it was among the first NLRs to be identified, the formation of a complete inflammasome has been controversial. Similar to the other NLRs, the PYD of NLRP12 forms a six-helical bundle death domain fold [134]. However, gene-silencing studies suggest that NLRP12 acts as a negative regulator of tumor necrosis factor receptor driven NF-kB signaling [135].

5.4.3.1 NLRP12 in Disease

Similar to NLRP3, gain of function variants in NLRP12 have been linked to enhanced inflammation in cold-associated periodic fevers, with enhanced speck formation and caspase-1 activation, and increased IL-1β secretion (see Chap. 29) [136–138]. Beyond these rare variants, however, much of our knowledge about the function of NLRP12 stems from murine knockout lines and investigations of colitis and colon tumorigenesis, similar to NLRP6.

These models showed that NLRP12 has a protective role in intestinal inflammation by suppressing NF-κB activation, and promotes microbial symbiosis, which results in reduced colitis susceptibility [135, 139]. However, data in human disease have been lacking. In meta-analysis of human 16S RNA data from pairs of twins affected by ulcerative colitis, *NLRP12* expression was found to be significantly down-regulated in cohorts with active ulcerative colitis, compared to healthy controls and patients with inactive disease. Since microbial dysbiosis is associated with inflammatory bowel disease, it is proposed that NLRP12 plays an anti-inflammatory role by regulating gut microbial communities [140].

In other tissues, new murine data have suggested that NLRP12 plays a role in myelopoiesis, especially under conditions requiring emergency reconstitution as seen in radiation and thermal combined injury and infection [141]. Similarly, NLRP12 has been shown to ameliorate inflammation in experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis [142]. Though consistent with its proposed role as an attenuator of inflammation, it remains to be seen whether similar tissue-specific functions can be attributed to human NLRP12.

5.4.4 Absent in Melanoma 2 (AIM2)

AIM2 is perhaps the most well known non-NLR protein capable of forming an inflammasome. In contrast to the NLRs, AIM2 forms an intracytoplasmic sensor, but rather than use adaptor proteins, directly binds cytosolic bacterial or viral dsDNA [143, 144], leading to IL-1β secretion [83, 84]. A critical question is how well AIM2 is able to distinguish bacterial or viral dsDNA from self-DNA. The seeming lack of regulatory and adaptor proteins has made the AIM2 inflammasome a target for studies of autoimmunity.

5.4.4.1 AIM2 in Disease

The level of AIM2 expression has been associated with severity of disease in patients with systemic lupus erythematosus [145]. Similarly, IL-1β and AIM2 expression are increased in keratinocytes in active psoriatic skin lesions compared to skin from healthy donors [146]. Furthermore, studies of human keratinocytes have demonstrated, at least *in vitro*, that stimulation with dsDNA activates AIM2 in these cells leading to IL-1β secretion [147].

5.5 Summary

In just two short decades, patient-centered research followed by the use of advances in *in vitro* and *ex vivo* assays, as well as murine models, has significantly increased our understanding of the inflammasomes as master modulators of inflammation. We know that a variety of triggers can activate the different inflammasomes, ranging from cold temperatures, to ion fluxes to microbial products and metabolites, but how such different stimuli can specifically activate one pathway leading to IL-1β and IL-18 release, needs further investigation. Subsequently, the fact that one signaling pathway can drive such different human pathologies indicates that the initial activation of the inflammasome may play a role in guiding the downstream inflammatory effects, and a specific immune response, as well as how the host re-establishes homeostasis following insult. In addition, it is becoming increasingly clear that NLRs regulate the innate immune system independent of forming inflammasomes (reviewed in [148]). Ongoing investigations will be instrumental in completely understanding the role of the inflammasome in the scope of an innate immune response, and leveraging that knowledge in the development of new therapies.

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Cytokines in Autoinflammation

6

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Abstract

Autoinflammatory diseases represent an expanding spectrum of diseases characterized by recurrent episodes of fever and systemic inflammation, due to disorders of innate immunity. Since the concept of autoinflammation was first affirmed in 1999 to define TNF-receptor associated periodic syndrome (TRAPS), many other monogenic autoinflammatory diseases have been identified. Uncontrolled secretion of IL-1β is responsible for the many of these syndromes, as also confirmed by the dramatic clinical response to IL-1 blockade. More recently, different mechanisms have been implicated in the pathophysiology of several monogenic autoinflammatory diseases, including cell stress, dysregulation in NF-κB signaling, ubiquitination, protein folding, type I interferon production and complement activation. In this chapter, we discuss IL-1β and other members of the IL-1 family and their inhibitors involved in monogenic autoinflammatory diseases, focusing on the mechanisms underlying their secretion in health and disease. Furthermore, we describe type I interferons and their role in autoinflammation.

Keywords

IL-1 family · Inflammasome · Caspases
Gasdermin D · Autoinflammatory diseases

Abbreviations

ADAR	Adenosine deaminase
AGS	Aicardi–Goutières syndrome
AIM2	Absent in melanoma 2
ASC	Associated speck-like protein containing a CARD
CAPS	Cryopyrin-associated periodic syndromes
CARD	C-terminal caspase recruitment domain
cGAMP	cyclic GMP-AMP
cGAS	cyclic GMP-AMP synthase
COX-2	Cyclooxygenase type 2
DAI	DNA-dependent activator of IFN-regulatory factors
DAMPs	Damage-associated molecular patterns
DIRA	Deficiency of IL-1Ra

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DITRA	Deficiency of IL-36Ra
FMF	Familial Mediterranean fever
GSDMD	Gasdermin-D
IFN	Interferon
IFNAR	Interferon alpha/beta receptor
IL	Interleukin
IL-1F	IL-1 family
iNOS	Type 2 phospholipase A and inducible nitric oxide synthase
ISG	Interferon stimulated gene
MAS	Macrophage activation syndrome
MDA5	Melanoma differentiation associated gene 5
NK	Natural killer
NL	Nucleotide-binding domain leucine-rich repeat containing
NO	Nitric oxide
PAMPs	Pathogen associated molecular patterns
PGE2	Prostaglandin-E2
PRAAS	Proteasome-associated autoinflammatory syndromes
PYD	Pyrin domain
Ra	Receptor antagonist
REDD1	Regulated in development and DNA damage responses 1
RIG-I	Retinoic acid inducible gene I
RIP	Receptor interacting protein
RNASEH	Ribonuclease H2 subunits
ROS	Reactive oxygen species
SAMHD1	Sam domain- and HD domain containing protein
SAVI	STING-associated vasculopathy with onset in infancy
SOCS	Suppressor of cytokine signaling
STING	Stimulator of interferon genes
TRAPS	TNF-receptor associated periodic syndrome
TREX 1	Three prime repair exonuclease 1

6.1 Introduction

Autoinflammatory diseases represent an expanding spectrum of diseases characterized by recurrent episodes of fever and systemic inflammation, due to disorders of innate immunity. Since the concept of autoinflammation was first affirmed in 1999 to define TNF-receptor associated periodic syndrome (TRAPS), many other monogenic autoinflammatory diseases have been identified. Uncontrolled secretion of IL-1β is responsible for the many of these syndromes, as also confirmed by the dramatic clinical response to IL-1 blockade. More recently, different mechanisms have been implicated in the pathophysiology of several monogenic autoinflammatory diseases, including cell stress, dysregulation in NF-κB signaling, ubiquitination, protein folding, type I interferon production and complement activation**.** In this chapter, we discuss IL-1β and other members of the IL-1 family and their inhibitors involved in monogenic autoinflammatory diseases, focusing on the mechanisms underlying their secretion in health and disease. Furthermore, we describe type I interferons and their role in autoinflammation.

6.2 The Interleukin (IL)-1 Family

Key Points

- **The IL-1 family comprises cytokines involved in innate immunity. Most of them lack a secretory signal sequence and are secreted through unconventional pathways**
- **IL-1β and IL-18 are produced as inactive precursors that require inflammasome-mediated proteolytic processing to acquire bioactivity**
- **IL-1β is the most powerful inflammatory mediator in the IL-1 family and plays a causative role in several autoinflammatory disorders**
- **In autoinflammatory diseases, secretion of IL-1β is dysregulated due to the genetic defects and/or to the cell stress of IL-1β producing cells**

The IL-1 family (IL-1F) is composed of 11 members, based on conservation of amino acid sequence, identity of gene structure, and three-dimensional structure [1]. Except for IL-18 and IL-33, IL-1F members map on chromosome 2 between the IL-1α and IL-1 receptor antagonist (IL-1Ra) loci, suggesting their origin from the

duplication of a common ancestral gene. All the IL-1F genes code for proteins made of a single structural domain composed by 12 beta strands connected by loop regions arranged in a beta-trefoil structure. The various members of the IL-1F have different biologic activities, but all are involved in innate immunity [2]. Seven of them display agonist activity (IL-1α, IL-1β, IL-18, IL-33, IL-36α, β, and γ), three are receptor antagonists (IL-1Ra, IL-36Ra and IL-38), and one is an anti-inflammatory cytokine (IL-37).

IL-1F cytokines are essential for the correct development and outcome of innate immune responses, but if their production is increased or dysregulated, the positive role of IL-1 cytokines as a defense against pathogens and sterile injuries switches to a detrimental role [1, 2]. Thus, some of them are involved in the pathogenesis or pathophysiology of chronic disorders [2]. The availability of specific IL-1 targeting therapies indeed unveiled an increasing list of diseases where IL-1F members (especially IL-1α and β) mediate pathologic inflammation [1, 2].

The common biologic effect of pro-inflammatory IL-1 cytokines is the stimulation of the expression of genes associated with inflammation, either cytokines, adhesion molecules or enzymes such as cyclooxygenase type 2 (COX-2), type 2 phospholipase A and inducible nitric oxide synthase (iNOS). This accounts for the large amount of prostaglandin-E2 (PGE2), platelet activating factor and nitric oxide (NO) produced by cells exposed to IL-1. IL-1 and IL-18 also increase the expression of adhesion molecules such as intercellular adhesion molecule-1 on mesenchymal cells and vascular-cell adhesion molecule-1 on endothelial cells. This latter property promotes the infiltration of inflammatory and immunocompetent cells into the extravascular space. IL-1 is able to induce itself as well as downstream inflammatory cytokines such as IL-6 thus amplifying the inflammatory response. As most cell types express the IL-1 receptor that binds and is activated by either IL-1α or β, the two cytokines, directly or through induction of other mediators, induce inflammatory responses on virtually all tissues in human body [1, 2]. While IL-1α works mostly locally, IL-1β displays several systemic effects (Table 6.1) that are commonly observed in patients affected by autoinflammatory diseases. First of all, IL-1β enters the circulation and triggers IL-1 receptors on the hypothalamic vascular network leading to synthesis of COX-2, followed by increased brain levels of PGE2 that activate the thermoregulatory center for fever production. In the periphery, IL-1β triggers IL-1 receptors on endothelial cells resulting in rashes and production of IL-6. IL-1β-induced IL-6 stimulates hepatocytes to synthesize several acute phase proteins, which accounts for the increase in erythrocyte sedimentation. IL-1β also acts on the bone marrow with enhanced mobilization of granulocyte progenitors and mature neutrophils, and subsequent peripheral neutrophilia. IL-1–induced IL-6 increases platelet production, which results in thrombocytosis. IL-1 also causes decreased response to erythropoietin and anemia [1, 2]. Most IL-1F members are synthesized as precursor proteins that subsequently undergo proteolytic cleavage by converting enzymes. Some members of the IL-1F, such as IL-1β, IL-18 and IL-37, strictly require proteolytic maturation in order to unlock their full biological potential [3]. Other IL-1F members such as IL-1α and IL-33, bind to their specific receptors and trigger a response on target cells also in their uncleaved molecular form.

Table 6.1 Major systemic effects of IL-1β

Hypothalamus	COX-2 synthesis → increased PGE2 → activation of thermoregulatory center → fever
Endothelium	Induction of IL-6, rashes
Hepatocytes	Induction of IL-6 → acute phase protein synthesis
Bone marrow	– Increased mobilization of granulocyte progenitors and mature neutrophils → peripheral neutrophilia – Decreased response to erythropoietin → anemia – Induction of IL-6 → increased platelet production → trombocytosis

IL-1β interleukin-1β, *IL-6* interleukin-6, *COX-2* cyclooxygenase type 2, *PGE2* prostaglandin-E2

Another characteristic of most IL-1F members is that, unlike other cytokines, they lack a secretory signal sequence, a peculiar feature for proteins that act extracellularly [3].

6.2.1 Processing of IL-1F Cytokines: Canonical and Non-canonical Inflammasome Activation

IL-1β is absent in cells of the innate immune system but is rapidly induced by triggering of pattern recognition receptors (PRR) by microbial molecules (pathogen-associated molecular patterns, PAMPs) or molecules associated with tissue components that are released upon tissue injury and cell death (damage-associated molecular patterns, DAMPs) (see Chap. 4). This is considered to be the first signal in the induction of IL-1β production. In contrast, IL-18 is constitutively produced by inflammatory cells. Both proteins however accumulate in the cell cytosol and require a second signal to be secreted [3].

6.2.1.1 Canonical Inflammasomes

The second signals promote indirect activation of intracellular multiprotein complexes, named inflammasomes (reviewed in [4], see Chap. 5), resulting in cleavage of pro-caspase-1 to active caspase-1, the major converting enzyme responsible for processing of IL-1β and IL-18 [5]. Different types of inflammasomes exist, each composed by a member of the nucleotide-binding domain leucine-rich repeat containing (NLR) gene family, including NLRC4, NLRP1, NLRP3, or pyrin domain (PYD)-containing non-NLRs, such as absent in melanoma (AIM)2 and pyrin. Upon stimulation, these proteins assemble and recruit pro-caspase-1 molecules, bringing them close enough to induce their autoprocessing with generation of bioactive caspase-1 that triggers the maturation of IL-1β and IL-18. While NLRP3 and AIM2 interact with procaspase-1 through ASC (associated speck-like protein containing a CARD), an adaptor protein containing a C-terminal caspase recruitment domain (CARD), NLRP1 and NLRC4 may form canonical inflammasomes with pro-caspase-1 in the absence of ASC whereas pyrin has been recently shown to form a unique inflammasome, dependent on ASC but independent of NLRP3 or other NLRs. The different inflammasomes are activated by specific stimuli. For instance, direct or indirect binding to various pathogens has been proposed to trigger NLRP1 and NLRC4; cytosolic DNA from virus and some intracellular bacteria activates AIM2; recently, the pyrin inflammasome has been found to be activated by alterations of actin cytoskeleton. The NLRP3 inflammasome is activated by extracellular stimuli (PAMPs, DAMPs, UV radiations, pore-forming toxins, crystals, ATP) that converge to perturb intracellular processes, ultimately responsible for NLRP3 activation. The processes and how they affect NLRP3 are still debated. The most plausible events leading to NLRP3 inflammasome activation include mitochondrial reactive oxygen species (ROS) production, K^+ efflux, and cytosolic release of cathepsin B by damaged lysosomes. In any case, NLRP3 is the major sensor of non-microbial stimuli, therefore responsible for sterile inflammation (reviewed in [6]).

6.2.1.2 Non-canonical Inflammasome

More recently, a non-classical inflammasome activation pathway that participates in the IL-1β secretory process has been discovered [4]. Earlier studies showed that caspase-11 and the human orthologues caspase-4/caspase-5 are components of the 'non-canonical inflammasome' that senses intracellular LPS derived from Gram-negative bacteria during macrophage-mediated inflammatory responses [7]. Direct recognition of intracellular LPS facilitates the rapid oligomerization of caspase-11/4/5, which results in pyroptosis. This is a highly inflammatory form of programmed cell death associated with secretion of IL-1β and IL-18. Caspases-11/4/5 rather than caspase-1, were proposed as the enzymes responsible for non-canonical inflammasome-triggered pyroptosis [7]. More recently, gasdermin D (GSDMD), a substrate of both caspase-1 and caspase-11/4/5, was identified as the pyroptosis executioner [8]. LPS-activated caspase-11/4/5 cleaves GSDMD, generating its N-terminal pore-forming domain that oligomerizes and forms pores in the cell membrane (see Fig. 6.1c). The diameter of pores is estimated to be in the range of 10–15 nm, which allows the passage of small proteins, including mature IL-1β (4.5 nm diameter) [9]. The simultaneous entry of sodium and water in the cell drives swelling and membrane rupture. Thus, pyroptosis may be re-defined as gasdermin-mediated programmed necrosis [10].

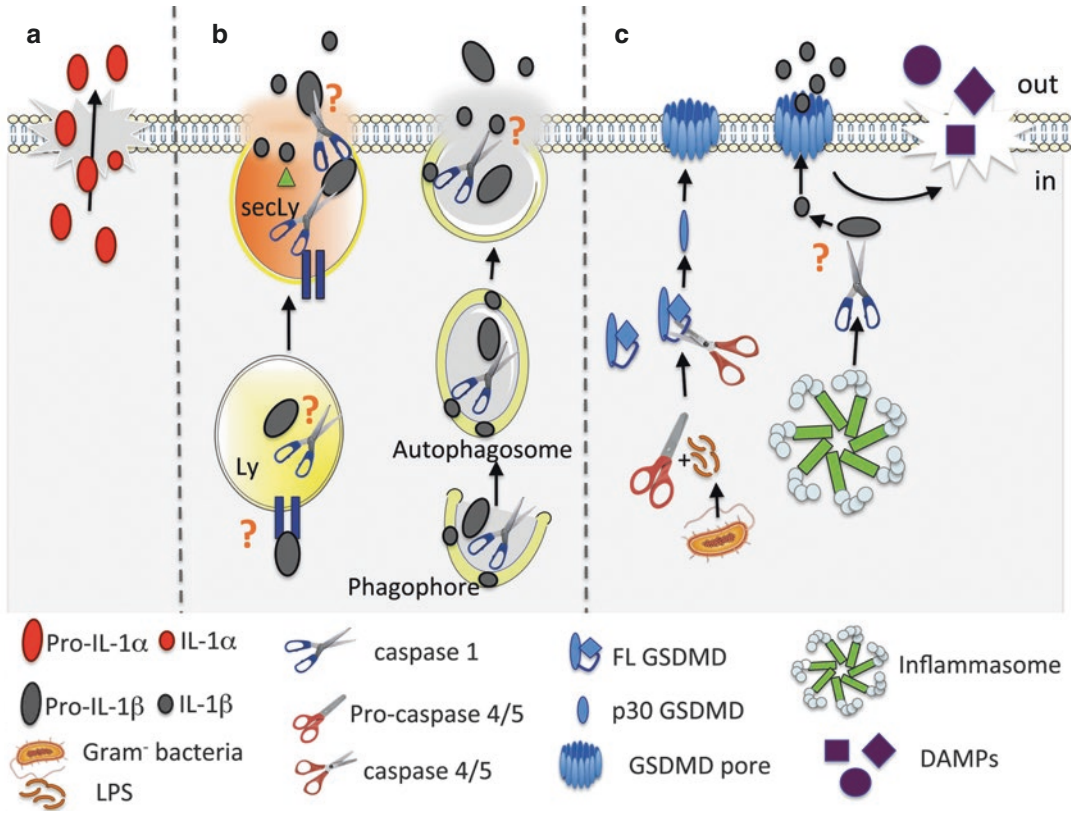

Fig. 6.1 Mechanisms of secretion of leaderless IL-1 family members *(reviewed in* [12]*)*. (**a**). IL-1α (active both as precursor protein or after cleavage by calpain) is released from necrotic cells, such as during hypoxic death. A similar way to exit the cell is exploited by IL-33. (**b**) Numerous vesicular pathways mediating the export of IL-1β have been described, involving, among others, secretory lysosomes and possibly autophagic vesicles. Several questions remain open (marked by a question mark). Among others: Where does pro-IL-1β processing occur? Which is the molecular machinery that translocates pro-IL-1β (or IL-1β) into lysosomes or across the internal membrane of the phagophore? (**c**) Inflammatory human caspases 4/5 (orthologues of caspase 11 in the mouse, non-canonical inflammasome) can be activated in the cell cytosol by lipopolysaccharide (LPS) released upon infection with Gram negative bacteria. The active caspases cleave the full length gasdermin D (FL GSDMD) with generation of p30 GSDMD. About 16 monomers of p30 GSDMD oligomerize into ring-shaped structures that bind to inner plasma membrane lipids, forming pores with a diameter of about 10–15 nm, able to accommodate small proteins such as IL-1β, but not larger proteins such as pro-IL-1β. However, the transit of ions and water rapidly increases the cell volume, causing membrane ruptures larger in size than the gasdermin pores, through which the remaining soluble cytosolic contents including DAMPs are released, promoting pyroptosis. The link between non-canonical and canonical inflammasome activation remains to be clarified. *Ly* Lysosome, *secLy* secretory lysosome

6.2.2 Secretion of IL-1F Cytokines

A peculiar feature of IL-1F proteins is that only IL-1Ra is a classical secretory protein endowed with a secretory signal peptide and secreted through the ER-Golgi exocytotic route; all the other members are leaderless [3]. This peculiarity, unexpected for soluble mediators, raised the questions of how these cytokines exit the cell in a different way than the classical secretory pathway.

Some IL-1F members, including IL-1α and IL-33, have an intracellular (nuclear) function in addition to the extracellular one [3]. Interestingly, unlike IL-1β and IL-18, IL-1α and IL-33 activate their receptors on target cells as full-length molecules: thus, when released from injured cells they can exert their biological activity in the

absence of proteolytic processing (see Fig. 6.1a). IL-1 family members can be divided into two groups: a group of cytokines that retain some intracellular function and are passively externalized upon cell lysis (the prototype being IL-1α), and a second group composed by cytokines that are stored in the cell cytosol before secretion, but do not have an intracellular function, and undergo regulated processing and secretion (the prototype being IL-1β).

6.2.2.1 Secretory Mechanisms for IL-1β (and IL-18)

The first hypothesis for IL-1β externalization was that it is passively released by cells dying at the site of inflammation. However, early observations demonstrated that IL-1β is selectively released by LPS activated human monocytes, with no bulk externalization of other cytosolic proteins, and cell viability is required for secretion of the processed, bioactive form of IL-1β [11]. Since then, different mechanisms have been proposed for IL-1β secretion, which can be classified in two groups, namely secretory mechanisms mediated by specialized membrane vesicles or employing direct protein passage across the plasma membrane, through translocators or pores [11] (Fig. 6.1).

Vesicle Mediated Secretion

These mechanisms include exocytosis of IL-1β-containing secretory lysosomes, IL-1β-release from micro-vesicles shed from plasma membrane, fusion of multi-vesicular bodies with the plasma membrane and subsequent release of IL-1β-containing exosomes. Involvement of autophagy in IL-1β-secretion has also been reported: however, the literature reports discrepant results on whether autophagy promotes or inhibits IL-1β secretion [12] (see Chap. 8). A different type of autophagy, chaperone mediated autophagy (CMA), mediates the degradation in lysosomes of a selective subset of cytosolic proteins featured by the presence of a pentapeptide motif biochemically related to KFERQ in their amino acid sequence (see Chap. 8). These proteins dock at the lysosomal membrane through interaction with the cytosolic tail of the lysosomal protein LAMP2a [13]. The primary sequence of IL-1β contains three KFERQ-like motifs [14] and pro-IL-1β accumulates in the cytosol but is also present in lysosomes [15], suggesting that IL-1β may exploit this mechanism to reach the lysosomal lumen. Although the fate of the translocated proteins is usually degradation, there is evidence that IL-1β containing lysosomes are exocytosed, thus releasing IL-1β out of the cell (Fig. 6.1b).

Direct Transport Across the Plasma Membrane

As introduced above (Sect. 6.2.2), several recent reports revealed yet another route for IL-1β release, involving pyroptosis (Fig. 6.1c). Infection with intracellular pathogens or transfected LPS activate murine caspase 11, or its human homologues caspase 4/5 to cleave GSDMD, generate a toxin-like peptide that forms pores on the plasma membrane through which secretion of mature IL-1β, but not of the 33 kDa precursor, occurs [8, 9, 16]. As detailed in Sect. 6.2.2, the plasma membrane rupture responsible for pyroptosis is likely delayed with respect to IL-1β secretion, suggesting that pyroptosis is associated but not causative of IL-1β secretion.

Although less studied, IL-18 is also actively secreted through pathways different from the ER-Golgi one, in most cases corresponding to the IL-1β secretory routes [12].

6.2.2.2 IL-1α and IL-33 Extracellular Release

Death as a mechanism of secretion was proposed for other members of the IL-1F, such as IL-1α and IL-33, and confirmed by various studies. However, several reports indicate the possibility that IL-1α and IL-33 are also actively released by cells that maintain their integrity (reviewed in [3]). IL-1α was reported to be secreted in response to heat shock and through an unknown mechanism requiring caspase-1. In the case of IL-33, intracellular calcium increase, regulated in an autocrine fashion by ATP and purinergic receptor stimulation, induces translocation from nucleus to cytoplasm and release of full-length IL-33. Extracellular ATP is a well-known inducer of

inflammasome activation and IL-1β/IL-18 processing. The mechanism through which ATP induces IL-33 secretion seems to be different, since the unprocessed, full-length molecular form of IL-33 is secreted (reviewed in [3]). However, we have previously observed that in human monocytes ATP drives exocytosis of pro-IL-1β containing vesicles also if caspase-1 is inhibited, resulting in secretion of the precursor form of the cytokine [17]. Moreover, both in monocytes and in dendritic cells, calcium influx induces secretion of pro-IL-1β and pro-IL-18 [17, 18]. Thus, it is conceivable that the pathway described for IL-33 makes use of mechanisms (purinergic receptor stimulation and calcium influx) which are old, conserved during evolution and exploited for different processes of molecule externalization from cells. The ATP-mediated signaling may then have further specialized adding to the older function of inducing exocytosis the newer function of controlling inflammasome activation and hence bioactivity of cytokines such as IL-1β and IL-18.

Importantly, another report [19] indicates that, in fibroblasts, newly synthesized IL-33 first moves to the nucleus and then is translocated to cytoplasmic vesicles, a pathway reminiscent of that followed by the DAMP HMGB1 [20]. Secretion of uncleaved IL-33 is then induced by mechanical strain (i.e. application of a physical deformation) in the absence of cellular necrosis. Extracellular release of IL-33 is also observed in mice subjected to acute trans-aortic constriction, which causes mechanical stress in the left ventricle [20]. Together, these data suggest that IL-1α and IL-33, in addition of being released by necrotic cells, may be secreted by cells that are subjected to nonlethal stress.

6.2.3 IL-1F Member-Linked Autoinflammatory Diseases

In this section, we will briefly discuss autoinflammatory diseases linked to mutations in IL-1F genes or genes directly controlling the secretion of IL-1F [21]. Discussion of other autoinflammatory diseases is covered in the relevant chapters.

6.2.3.1 IL-1-Mediated Autoinflammatory Diseases

Cryopyrin-Associated Periodic Syndromes (CAPS)

CAPS is the prototype of IL-1-mediated diseases (see Chap. 19). In 2001 the *CIAS1/cryopyrin* gene was found responsible for the disease, although the function of the gene remained unknown. A few years later, the group of Jurg Tschopp showed that CIAS1 (renamed NLRP3) is part of the inflammasome, thus disclosing the connection between CIAS1/cryopyrin and IL-1β [22]. In CAPS, gain of function mutations of *NLRP3* lead to increased inflammasome assembly and IL-1β secretion, in turn responsible for the devastating inflammatory manifestations displayed by patients affected by this syndrome as demonstrated by the dramatic effectiveness of IL-1 blocking agents, such as IL-1Ra (anakinra), human monoclonal antibody targeted at interleukin-1 β (canakinumab) or rilonacept [23].

More recently, cell stress in CAPS inflammatory cells was proposed to participate in the pathophysiology of the disease, based on the following observations: (1) monocytes from CAPS patients have higher basal ROS levels than monocytes from healthy donors even before PAMP stimulation, but also display higher expression of antioxidant systems that allow them to maintain a redox poise. However, stimulation by minute amounts of TLR agonist, unable to activate IL-1β secretion in healthy monocytes, further induces ROS production with loss of the precarious redox equilibrium, resulting in extracellular release of huge amounts of endogenous ATP. Since extracellular ATP, through activation of its P2X7 receptor, is a strong inducer of NLRP3 inflammasome assembly, the high levels of released ATP in CAPS monocytes stimulate processing and secretion of large quantities of IL-1β in an autocrine fashion. (2) After a few hours of TLR stimulation, the antioxidant system collapses, and oxidative stress arises. Stressed monocytes slow down protein translation; accordingly, the production of IL-1Ra, normally secreted by activated monocytes a few hours after IL-1β to limit inflammation is impaired. Thus, deficient IL-1Ra

production likely colludes with the enhanced IL-1β secretion in increasing the severity of the disease (reviewed in [24]).

Deficiency of IL-1Ra (DIRA)

In agreement with the above considerations that the successful outcome of an inflammatory response is ensured by a balance between IL-1 and IL-1Ra, in DIRA the lack of IL-1Ra due to loss-of-function-mutations of the gene *IL1RN* causes the disequilibrium, allowing unopposed action of IL-1 with dramatic consequences (see Chap. 25). Thus, patients affected by DIRA display autoinflammatory clinical features, including neonatal onset, cutaneous and osseous manifestations [21]. Skin manifestations in DIRA are more severe than in other IL-1β-mediated autoinflammatory syndromes and consist of severe neutrophilic pustular skin eruptions, skin pathergy, and nail dystrophy. Since IL-1RA and IL-1α are highly expressed in keratinocytes, whereas IL-1β is not, these differences may be due to the loss of control of IL-1α bioactivity, rather than IL-1β, in skin of DIRA patients. Thus, while in CAPS the disease phenotype is mostly linked to hyperactivity of IL-1β, in DIRA, especially at the skin level, IL-1α could also play a relevant role.

IL-1α in Autoinflammatory Diseases

The role of IL-1α in autoinflammatory diseases is rather controversial. However, some studies indicate a possible implication also of this IL-1F member in autoinflammation. In fact, not only IL-1β, but also IL-1α secretion is enhanced in CAPS monocytes [25]. This finding was quite unexpected as IL-1α processing does not depend on the inflammasome. However, several studies indicate that IL-1α -and other leaderless secretory proteins not cleaved by caspase-1- are secreted after inflammasome activation through an undefined mechanism (reviewed in [3]). IL-1α secretion in CAPS is increased even by low doses of LPS: this finding suggests that IL-1α may contribute to the promotion and progression of inflammatory episodes and should be considered when IL-1 blockade is applied as a therapeutic strategy. Along this line, a prominent role of IL-1α in brain could explain in part the better results obtained with anakinra than with canakinumab in CAPS patients with central nervous system inflammation [26].

In addition to this potential role in CAPS, dysregulated, receptor interacting protein (RIP)-1-dependent IL-1α secretion, but not IL-1β, has been found involved in a severe murine inflammatory syndrome that resembles neutrophilic dermatosis in humans, and is developed by mice carrying mutations in SHP-1, a tyrosine phosphatase that controls RIP-1 activity [27].

6.2.3.2 IL-18 in Autoinflammatory Diseases

Although activated identically to IL-1β by canonical inflammasomes, a direct implication of IL-18 in the pathogenesis of hereditary autoinflammatory disorders has not been demonstrated. However, IL-18 has been proposed to mediate macrophage activation syndrome (MAS) [28]. This is a systemic immune dysregulatory condition associated with uncontrolled macrophage activation and hemophagocytosis, and represent a life-threatening complication of some rheumatic diseases, most commonly systemic juvenile idiopathic arthritis (sJIA), and, less frequently, of autoinflammatory syndromes (see Chaps. 32 and 33).

In support of a major role of IL-18 in MAS, a syndrome of recurrent MAS with early-onset enterocolitis has been shown to be linked to a gain-of-function mutation in NLRC4, causing constitutive caspase-1 cleavage [29] (see Chap. 29). Patients with recurrent MAS have dramatic chronic elevation of serum IL-18; administration of IL-18 binding protein (IL-18BP), an endogenous protein that binds tightly to IL-18 preventing its activation of target cells [30], rapidly improved the overall health of an individual patient and maintained its therapeutic efficacy for several months [29].

Recently, an autoinflammatory disease driven by IL-18 has been identified in mice [31]. The disease is due to inactivating mutation of the actin-depolymerizing cofactor Wdr1 with perturbation of actin polymerization; IL-1β secretion is unaltered but IL-18 production is greatly enhanced.

Interestingly, the inflammasome sensor of actin dynamics in this system requires pyrin, suggesting the involvement of the pyrin inflammasome. IL-1β and IL-18 are generated by the same enzymatic reaction; thus, it remains to be seen how the selective increase of IL-18 may arise.

6.2.3.3 Deficiency of IL-36 Receptor Antagonist (DITRA)

DITRA is due to deficiency of the IL-36 receptor antagonist (IL-36Ra) (see Chap. 26). This cytokine antagonizes the proinflammatory signals of IL-36 molecules at the IL-36 receptor, with a mechanism analogous to the way IL-1Ra blocks IL-1. The main manifestation of DITRA is generalized pustular psoriasis, in agreement with the predominant expression of IL-36 receptor in epithelial cells in direct contact with the environment, including the skin. Interestingly, efficacy of IL-1 blockers has been reported in some, but not all patients [32]. This observation, together with the similarity of the cutaneous lesions in DIRA and DITRA, suggests the existence of a functional loop involving IL-36 and IL-1α at the level of the skin.

6.2.4 Role of Stress in Autoinflammatory Diseases Mediated by IL-1F Members

Inflammation is an important component of many hereditary disorders, whose pathogenesis has been long debated. At present, it is largely accepted that inflammation is due to the state of stress in cells expressing mutant genes due to the proteotoxic effects of the encoded proteins [24]. When expressed in non-immune cells, any mutant protein may cause the release of signals that trigger and propagate inflammation. As discussed above, secretion of most IL-1F members is modulated by stress. Thus, in cells expressing IL-1F cytokines upon activation—mostly inflammatory cells—the mutant protein responsible for a given autoinflammatory disease will induce autoinflammation not only by (directly or indirectly) causing the increased and dysregulated secretion of a given IL-1F member but also by inducing stress that strongly enhances the secretion of these cytokines, resulting in the explosive responses that are the hallmark of autoinflammatory diseases. Support for this view came from studies on monocytes from patients with CAPS [33] and TRAPS [34], which display redox alterations at baseline and exhibit enhanced responsiveness to LPS. Blocking ROS production in these cells strongly reduces IL-1β secretion [25, 34]. A link between stress and initiation of inflammatory attacks has also been shown in patients with familial Mediterranean fever (FMF), where the stress-related protein REDD1 was found to be significantly overexpressed during FMF attacks. REDD1 is a regulator of neutrophil function upstream of pyrin and is involved in the regulation of neutrophil extracellular traps and IL-1β release [35].

6.3 Type I Interferons

Key Points

- **Type I interferons are produced by almost all cell types and production is tightly regulated**
- **Type I interferons have antiviral, antitumor and immunomodulatory activity**
- **Elevated type I interferons are involved in pathogenic mechanisms of autoinflammatory disorders**

Interferons (IFN) are divided into three different families. The type I IFN family includes 13 partially homologous IFNα subtypes, a single IFNβ and the less well studied IFNε, IFNτ, IFNκ, IFNω, IFNδ and IFNζ. The type II IFN family only consists of IFNγ. IFNγ is produced mainly by T cells and natural killer (NK) cells, and affects a wide group of IFNγ receptor expressing cells. IFNλ1, IFNλ2 and IFNλ3 (IL-29, IL-28A and IL-28B, respectively) and the newly identified IFNλ4 are members of the type III IFN family. They have restricted activity on epithelial cells since their receptors are mainly expressed on these cells [36].

Type I IFNs have antiviral, antitumor and immunomodulatory activity. They are produced

by almost all cell types. In addition, type I receptor components (IFNAR1/2) are expressed on most nucleated cells, indicating a broad importance of type I IFNs (Hall & Rosen, 2010).

Induction of type I IFN production depends on TLR dependent and independent pathways (see Chap. 24, Fig. 6.1). TLR3 is responsible for endosomal RNA recognition. Retinoic acid inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA5) play a role as cytoplasmic sensors of RNA. Their activation results in type I IFN production. Endosomal TLR9 senses foreign DNA. In addition, simulator of interferon genes (STING) and DNA-dependent activator of IFN-regulatory factors (DAI) act in the pathways of cytoplasmic DNA sensing [36]. Endoplasmic STING protein is activated by its ligand cyclic GMP-AMP (cGAMP) which is produced by cyclic GMP-AMP synthase (cGAS) upon detection of cytoplasmic dsDNA. Transcription of IFN genes is upregulated by TBK1-induced IRF-3 activation. Type 1 IFNs are released and bind to type I interferon receptor (IFNAR) which activates interferon stimulated gene (ISG) transcription by activating JAK/STAT pathways [37]. Heterodimeric IFNAR (composed of IFNAR1 and IFNAR2) is expressed in a wide range of cells. After ligand binding its dimerization induces phosphorylation of TYK2 and JAK1. IFNAR1 is constitutively associated with TYK2 while IFNAR2 is associated with JAK1. The phosphorylated IFNAR can bind to STAT proteins which are then phosphorylated as well. pSTAT1 and pSTAT2 dimerize and bind to IRF9 in the nucleus to form the transcription factor complex ISGF3. ISGF3 can induce transcription of interferon stimulated genes (ISGs).

Negative regulation of type I IFN activation can be divided into three steps. (1) Proinflammatory cytokines, TLR activation and oxidative and/or metabolic stress induce internalization of IFNAR. (2) Suppressors of cytokine signaling (SOCS) proteins counterbalance IFN-I signaling in a negative feedback loop e.g. by blocking enzymatic activity of JAKs by direct binding. In addition, SOCS proteins induce proteasomal degradation of JAKs and IFNAR components. Mice lacking SOCS develop lupus-like autoimmune disease [38]. (3) Gene transcription can be regulated by microRNAs [39].

6.3.1 Diseases Associated with Elevated Type I Interferons

The prototype interferonopathy Aicardi–Goutières syndrome (AGS) is typically associated with an upregulated IFN gene signature (see Chap. 24). AGS is a genetically heterogeneous disorder with inflammation of skin and brain resembling congenital viral infections and SLE [40]. Mutations in genes encoding three prime repair exonuclease 1 (TREX1), ribonuclease H2 subunits A, B and C (RNASEH2A/B/C), Sam domain- and HD domain containing protein (SAMHD1) [40], IFIH1 (MDA5), as well as adenosine deaminase (ADAR1) have been detected in AGS patients [41]. Accumulations of endogenous nucleic acids that are sensed by cytoplasmic sensors induce type I IFN release [40, 42].

Proteasome-associated autoinflammatory syndromes (PRAAS) are associated with high levels of interferon-induced genes (see Chap. 24). They are induced by loss-of-function mutations in genes encoding immunoproteasome components like PSMB8 [43].

De novo gain-of-function mutations in TMEM173/STING were shown to cause STING-associated vasculopathy with onset in infancy (SAVI), a severe pediatric condition with poor prognosis [44–46] (see Chap. 24).

All patients carried heterozygous de novo mutations in exon 5 of the TMEM173 gene causing missense mutations at highly conserved amino acid positions of the STING protein. Transcriptional analyses of PBMCs derived from SAVI patients revealed a strong upregulation of type I IFN and interferon-inducible genes (ISGs) suggesting a gain-of-function of STING. Indeed, mutant STING protein of SAVI patients was found to constitutively signal in the absence of the STING ligand cyclic GMP-AMP (cGAMP). Activated STING recruits TBK1 which phosphorylates IRF-3. pIRF-3 then shuttles to the nucleus and activates transcription of ISGs.

Constitutive activation of the type 1 interferon signaling pathway by mutated STING in SAVI patients is thought to be perpetuated by enhanced activation of the interferon receptor IFNAR1 which results in constitutive phosphorylation of STAT1 downstream of the receptor and hence, pSTAT1 mediated constitutive transcription of further ISGs [44].

STING is expressed in endothelial cells. In the lung, it is also expressed in alveolar type 2 pneumocytes, bronchial epithelium and alveolar macrophages. Biopsies of skin lesions showed markers of endothelial activation associated with small-vessel inflammation with signs of neutrophil decay (leukocytoklasia) [44]. cGAMP-induced STING activation of endothelial cells increased expression of ISGs and markers of endothelial activation like inducible nitric oxide synthase (iNOS), E-selectin and tissue factor (TF). Most of these markers were also detected in immunofluorescence staining of SAVI skin biopsies.

Taken together, these data suggest uncontrolled activation of the type 1 IFN pathways due to gain-of function of mutated STING. Some groups attempted blocking enhanced IFN signaling by treating SAVI patients with JAK/STAT inhibitors [47–49] which interfere with essential components in the signaling of IFNAR. This treatment reduced upregulation of ISGs [48] and induced some improvement of the clinical symptoms. Efficacy of ruxolitinib treatment was documented in three SAVI patients who received treatment for 6–18 months [47]. The authors reported an almost complete resolution of the cutaneous lesions and a major improvement of pulmonary function. However, the clinical improvement was not systematically associated to a decrease of the IFN signature. Treatment of another patient for 3 months with tofacitinib also improved the skin phenotype but did not resolve pulmonary manifestations [48] (Seo et al., 2017). JAK/STAT inhibitors can be associated with severe adverse effects like growth arrest, anemia, neutropenia, reactivation of tuberculosis, viral infections, progressive multifocal leukencephalopathy, and non-melanoma skin cancer. Hence, therapeutic approaches to chronic STING activation urgently need significant improvement.

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Proteasomes in Autoinflammation

7

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Abstract

The cellular proteostasis network integrates all signals controlling protein synthesis, folding, trafficking, and clearance machineries in multiple subcellular compartments to maintain the integrity of the proteome and to ensure the survival of cells and tissues under varying proteotoxic insults. We here review the proteostasis network that controls the adaptation of the ubiquitin proteasome system (UPS) to cellular demands and its perturbations in autoinflammation. Proteotoxic stress of various physiological origins such as inflammation can be typically counteracted by the shut-down of global protein translation, or the up-regulation of protein quality control and degradation machineries including stress specific sets of ubiquitin-conjugation and deconjugation factors as well as alternative proteasome isoforms. The loss of controlled adaptation and/or impairment of proteasome function represent a hallmark of various proteinopathies including proteasome associated autoinflammatory syndromes (PRAAS), which are accompanied by oxidative stress and induction of endoplasmic reticulum (ER) stress. A common and surprising feature of such diseases is the initiation of chronic inflammation under pathogen-free conditions through the release of various mediators, particularly type I interferon (IFN). Recent work in this field has highlighted a possible role of ER-membrane located signaling cascades originating from TCF11/Nrf1 as well as the PERK and IRE1α arms of the unfolded protein response (UPR) in this process. Their precise implication in the pathogenesis of proteinopathies as well as their relevance for the design of novel drug targets will be discussed.

Keywords

Proteostasis · Ubiquitin · Proteasome · Inflammation · Unfolded protein response · Proteinopathy · Interferonopathy · PRAAS

Abbreviations

ALIS	Aggresome-like induced structures
ARE	Antioxidant response elements
ATF	Activated transcription factor
CANDLE	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature

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CHOP	C/EBP-homologous protein 10
COX	Cyclooxygenase
CP	Core particle
DALIS	Dendritic aggresome-like induced structures
DC	Dendritic cell
DDI2	DNA damage-inducible protein homolog 2
DRiP	Defective ribosomal product
DUB	Deubiquitinating enzyme
eIF2	eukaryotic translation initiation factor 2
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
GADD34	Growth arrest and DNA damage-inducible protein 34
IFN	Interferon
IP	Immunoproteasome
IRE-1	Inositol-requiring protein 1
IRF3	Interferon regulatory factor 3
ISG	Interferon-stimulated gene
JMP	Joint contractures muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy
KLICK	Keratosis linearis with ichthyosis congenita and sclerosing keratoderma syndrome
MHC	Major histocompatibility complex
NOX	NADPH oxidase
NNS	Nakajo-Nishimura syndrome
Nrf1	Nuclear factor erythroid 2-related factor 1
OASL	2′-5′-Oligoadenylate synthase-like protein
PA200	Proteasome activator 200
PA28	Proteasome activator 28
PAC	Proteasome assembly chaperone
PAMP	Pathogen-associated molecular pattern
PERK	Protein kinase R-like endoplasmic reticulum kinase
PKR	Protein kinase R
POMP	Proteasome maturation protein
PRAAS	Proteasome-associated autoinflammatory syndrome
PRR	Pathogen recognition receptor
RIDD	IRE1α-dependent decay
RIG-1	Retinoic acid-inducible gene 1 protein
RNS	Reactive-nitrogen species
ROS	Reactive oxygen species
RP	Regulatory particle
Rpn	Regulatory particle non-ATPase subunit
SP	Standard proteasome
TCF11	Transcription factor 11
UPR	Unfolded protein response
UPS	Ubiquitin-proteasome system
XBP-1	X-box-binding protein 1
XO	Xanthine oxidase

Key Points

- **The ubiquitin proteasome system (UPS) is responsible for selective, energy-dependent protein degradation of ubiquitin-modified protein substrates to ensure protein homeostasis, regulatory protein function and antigen presentation**
- **The UPS can be adjusted to cellular demands due to perturbations of proteostasis by up-regulation of stress specific sets of ubiquitin-conjugation and deconjugation factors as well as alternative proteasome isoforms**
- **The unfolded protein response acts as a central hub integrating cellular responses to proteotoxic stress of physiological or pathological origin in order to decide cell fate between repair and death**
- **Impairment of such adaptation processes are a hallmark of various proteinopathies including proteasome associated autoinflammatory syndromes (PRAAS), which are accompanied by oxidative and proteotoxic stress as well as production of type I IFNs**

7.1 Concept of Protein Homeostasis and Its Importance in Preserving Cell Function and Integrity

Key Points

- **Substrates for degradation are damaged, misfolded, and unwanted proteins as well as defective ribosomal products most of them modified with ubiquitin**

- **The proteasome is a proteolytic multi-subunit complex, which is assembled in a modular manner with different catalytic core- and regulatory-particles**
- **The catalytic capacity is adapted to the cellular requirements by differential gene expression to adjust the amount, kind of incorporated subunits, or used regulatory particles in proteasome complexes**

The protein homeostasis of a cell, also referred to as proteostasis, describes the maintenance of a healthy proteome, which depends on a smooth interplay of multiple processes of a protein life cycle including protein synthesis, folding, quality control, stability, trafficking, and degradation. This concept of interplay and adaptation preserves the cellular function and viability by ensuring cell metabolism, organelle biogenesis, and stress adaption to maintain tissue and organismal viability and function. External stresses (including infection), aging, and inherent instability of proteins can cause proteins to misfold and aggregate to an increasing degree. To combat these problems protein homeostatic mechanisms are used, including up-regulation of protein folding catalysts, reduction of the overall protein synthesis and the induction of protein degradation systems [1–3].

7.1.1 The Ubiquitin-Proteasome System

Besides autophagy, the main proteolytic system in eukaryotic cells is the ubiquitin-proteasome system (UPS), which is localized in the nucleus and the cytosol, but also in the extracellular space and is associated to the endoplasmic reticulum (ER) by the ERAD (ER-associated degradation) system. It plays a crucial role in many cellular pathways including cell cycle regulation, antigen presentation, regulation of gene expression, differentiation and many others. Accordingly, the multi-protein machinery serves to remove continuously misfolded and damaged proteins to recycle amino acids for new protein synthesis. In parallel, some key regulatory proteins fulfill their biological functions via selective or partial degradation and furthermore, proteins are digested into peptides for MHC class I antigen presentation. To meet such complex demands in biological processes, protein substrates must be recognized, recruited, and eventually hydrolyzed in a well-controlled manner. Most target proteins are attached to ubiquitin by a cascade of three types of enzymes known as E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzymes), and E3 (ubiquitin ligases) whereby poly-ubiquitination occurs in a branched or linear manner (Fig. 7.1). Poly-ubiquitination with linkages through the amino acid K48 of the ubiquitin protein is recognized by the proteasome and predominantly serves as a marker for degradation. However, other poly-ubiquitination types along amino acids K6, K11, K27, K29, K33, K63, and M1 exist and can change the outcome of the modified protein. The complexity of the ubiquitin code is larger than initially anticipated and is under intensive investigation. K63 poly-ubiquitination can serve as signal for autophagy, protein trafficking, and DNA repair whereas chains linked by K6, K11, K27, K29 and M1 seem to allow proteasomal degradation as well. A further regulation of the UPS is performed by the family of de-ubiquitinases (DUBs), which can selectively remove ubiquitin chains from substrates and thus prevent proteasomal degradation [4–6].

7.1.2 Structure of the Proteasome

The proteasome itself is a multi-catalytic subunit complex, has a cylindrical shape and comprises a proteolytic core particle (CP or 20S proteasome) covered on one or both sides by a regulatory particle (RP or 19S) (Fig. 7.1). The 19S complex in turn is structurally divided into a base and a lid, each with nine subunits, where ubiquitinated proteins are recognized, bound and de-ubiquitinated which allows a recycling of ubiquitin molecules. Afterwards, unfolding and delivery of the target protein into the CP proceeds. These complex functions are realized by different subunits of the 19S complex. **R**egulatory **p**article subunit **n**on-ATPase (RPN)10 and RPN13 are the two subunits which possess ubiquitin recognition domains and rec-

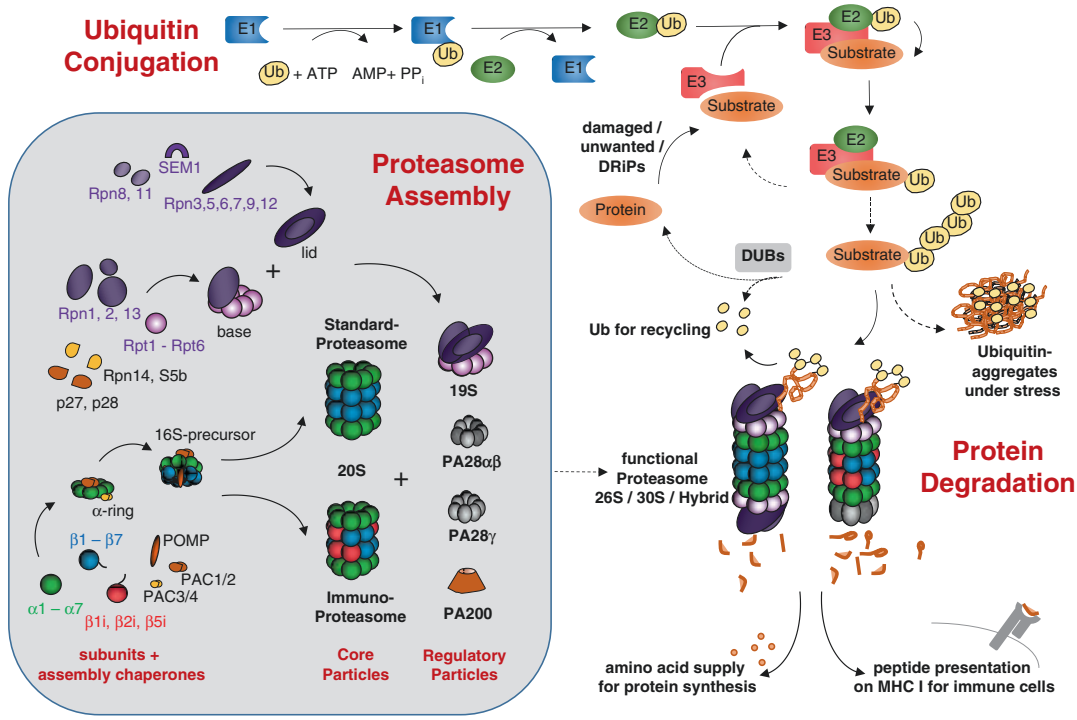

Fig. 7.1 Organization of the ubiquitin-proteasome-system (UPS). The degradation of unwanted, damaged or misfolded proteins including defective ribosomal products (DRiPs) by the UPS starts with an ubiquitin-labeling of the substrate. Herein the label ubiquitin, a small protein with 76 AA, is fused first to E1 (ubiquitin-activating enzyme) under ATP usage. Afterwards ubiquitin is transferred to E2 (ubiquitin-conjugating enzymes), which in turn is used by E3 (ubiquitin ligases) to transfer the ubiquitin on the substrate specific bound to E3. This conjugation of ubiquitin is prolonged by repeating conjugation events resulting in a polyubiquitination of the substrate. With this kind of tag the substrate is recognized by the 19S regulatory particle or associated polyubiquitin receptors for degradation by the proteasome. Ubiquitin is cleaved off for recycling, the substrate gets unfolded and transferred into the catalytic chamber of the proteasome. The resulting peptides are further degraded by peptidases and serve as amino acid pool for new protein synthesis. Peptides can also be used for loading on MHC class I complexes in the ER, which are then presented as antigens on the cell surface for immune cells. Instead of being degraded, ubiquitinated substrates can be deubiquitinated by deubiquitinases (DUBs), a mechanism used for regulatory proteins, or under stress conditions ubiquitin aggregates are transiently or stably formed, to prevent toxic effects of misfolded proteins. The proteasome itself consists of one or two regulatory particles (19S/PA28αβ/PA28γ/PA200) associated to one or both ends of a cylindrical catalytic core particle, containing two heptameric α-rings and two heptameric β-rings harboring the catalytic subunits β1, β2, and β5. Alternatively, the catalytic subunits β1i, β2i, and β5i can be incorporated forming the immune proteasome. 19S- and 20S-complex formation follows a stepwise assembly process with precursor stages in between. Assembly chaperones (brown and yellow/lined) are essential for this process

ognize poly-ubiquitin chains. Furthermore, RPN1 and RPN2 bind ubiquitinated substrates indirectly via extrinsic ubiquitin receptors and on the other hand it was recently shown, that RPN1 can bind ubiquitinated substrates directly as well. The de-ubiquitination and the resulting recycling of ubiquitin are performed by RPN11. Further unfolding of the target protein into a linear conformation requires the coordinated action of six ATPases (proteasome regulatory particle subunit triple A-ATPase—RPT1-6) of the base which ensure 20S proteasome gate opening as well as the guidance of the substrate into the proteolytic chamber. They are ordered in a ring, belong to the ATPases associated with various cellular activities (AAA) family and hydrolyze nucleotide triphosphates [7, 8].

The 20S CP resembles a cylinder formed by four rings with seven subunits in each ring. Herein two identical peripheral α-rings with the subunits α1-α7 cover the two also identical inner β-rings with the subunits β1-β7. Within the β-ring three enzymatic activities, a caspase-like (post-acidic residues cleavage), a trypsin-like (post-basic residues cleavage), and a chymotrypsin-like (post-hydrophobic residues cleavage) activity, are present and reside in the subunits β1, β2, and β5, respectively, to allow fragmentation of each kind of amino acid sequence into peptides with a length of 4–14 amino acids. The α-rings have a regulatory function; they prevent randomly protein degradation and protect the catalytic channel. The C-termini of the α-subunits form a trellised gate-like structure, where only small proteins can enter it without being ubiquitinated (closed conformation). To achieve full processing activity of the proteasome, the interaction with a RP that induces conformational changes in the α-ring is necessary (open conformation). For a comprehensive overview of subunit nomenclature including synonyms see [7, 9].

7.1.3 Proteasome Assembly

The assembly of the proteasome complex is an ordered process and is assisted by proteins that leave the complex during maturation (Fig. 7.1). For the CP these are proteasome assembly chaperone (PAC)1-PAC4 and proteasome maturation protein (POMP). At the beginning, the α-ring is formed with the assistance of a PAC1-2 dimer and a PAC3-4 dimer, in which they ensure the right positioning of the α-subunits. The PAC1-2 dimer further prevents α-ring dimerization and premature binding of a RP through the binding at the later RP interaction face. In the middle of the α-ring POMP is localized and with its help the β-subunits are recruited sequentially to the α-ring to form a precise β-ring. Two of those half proteasomes (16S) dimerize and a final maturation step of the β-subunits via autocatalytic cleavage of pro-peptides is performed. With this, the catalytic active sites of the threonine proteases (β1, β2, and β5 subunits) become accessible. During the 19S assembly, the base and the lid are formed in separate sequential processes, which are still under investigation. The base formation is assisted by the assembly chaperones p27, p28, RPN14, and S5b to form a hexameric ring with the AAA-ATPases RPT1-RPT6 combined with RPN1, RPN2, and RPN13. In parallel the lid is formed, whereby the subunit SEM1 has an assembly factor-like function and promotes a propeller like structure out of RPN 3, 5, 6, 7, 9, 12 with RPN8 and RPN11 in the middle. At the end RPN10 comes into the complex together with the base [7, 10].

7.1.4 Alternative Proteasomes

Beside the standard proteasome (SP) which consists of 20S and 19S, alternative compositions of CP and RP are possible. These different types of proteasomes reflect the capacity of a cell to adapt to its proteolytic needs. One way of adaptation is the preferred incorporation of alternative catalytic subunits into newly formed proteasomes. These subunits designated β5i, β1i, and β2i confer a higher processing rate for peptide hydrolysis and alter the population of peptides generated for MHC class I antigen presentation. They are constitutively expressed in immune cells and are inducible in other cell types after exposure to type I and type II inteferons (IFN) or under stress conditions. This occurs for example during immune reponses; therefore this proteasome type is called “immunoproteasome” (IP). Besides SP and IP, some proteasomes assemble as mixed proteasomes containing both standard β and βi subunits. A further alternative subunit is β5t, which is only expressed in the thymus. There β5t is exclusively assembled with β1i and β2i to form the thymoproteasome, which plays an essential role in positive selection of CD8+ T cells. Apart from the 19S, alternative RPs can associate with the CP, whereby also the combination of two different RPs on one CP is possible forming a so-called hybrid-proteasome. Alternative RPs include PA200 and the heptameric PA28αβ- (11S) and PA28γ- rings. Their detailed function is not well understood, but they are not able to recognize ubiquitinated proteins and lack ATPase

activity. Hence, they are capable of targeting small proteins with simple tertiary structures such as inhibitors of cyclin dependent kinases p16, p19, and p21. PA28αβ is inducible during IFN signaling and is mostly assembled with the IP, suggesting a function in peptide production for immune presentation. PA28γ is restricted to the nucleus and was shown to play a role together with PA200 in the oxidative stress response and male fertility [9, 11, 12].

7.1.5 Further Sources of Proteasome Substrates

During inflammation, the production of peptides for MHC class I antigen presentation is rapidly increased. About 30–40% of all peptides presented on the cell surface do not arise from mature full-length proteins degraded via the normal process of protein aging (retirees). Rather, they derive from defective ribosomal products (DRiPs). These DRiPs can have different sources. On the one hand, they are prematurely terminated or misfolded full-length proteins produced from *bona fid*e mRNA. This part is especially thought to be increased during inflammation, because of higher oxidative damage to nascent proteins as result of induction of reactive oxygen species (ROS) and NO as normal innate response to inflammation. On the other hand, DRiPs are produced from defective gene products resulting from errors in transcription and translation. In the past years, the source of DRiPs was expanded and it was hypothesized these are translation products of "immunoribosomes", ribosomes specialized for generating antigenic peptides for immunosurveillance. Although there is evidence for cotranslational ubiquitination and degradation [13], this process warrants further investigation.

7.2 Protein Homeostasis Perturbations

Key Points

- **Disruption of protein homeostasis under both physiological and pathological conditions is characterized by the accumulation of intracellular ubiquitin-positive inclusions**
- **The higher proteolytic capacity of immunoproteasomes greatly contributes to the progressive clearance of ubiquitin-protein conjugates**
- **(Immuno-)proteasome dysfunction may lead to the development of proteasome-associated autoinflammatory syndromes (PRAAS) in which sustained protein aggregation is associated with a type I-IFN signature**

Unbalanced protein homeostasis is mostly, if not always, characterized by the cytosolic accumulation of ubiquitin-modified proteins, which reflects the inability of the cells to cope with damaged proteins in a given situation. Depending on the transient or sustained character of such aggregation, one can easily distinguish between physiological and pathological protein homeostasis perturbations, respectively.

7.2.1 Physiological Perturbations

One prime example of a physiological process which is associated with cellular disturbed protein homeostasis is the innate immune response, as originally evidenced by the transient aggregation of ubiquitin-modified proteins (also referred to as dendritic cell aggresome-like induced structures or DALIS) in dendritic cells (DC) following LPS exposure [14]. Meanwhile, the cytosolic accumulation of similar ubiquitin-positive inclusions upon inflammation and/or infection has been described in many other cells types such as macrophages [15] as well as tumor cells [16] and has been termed aggresome-like induced structures (ALIS) to discriminate them from those taking place in DC. One major explanation for the increased accumulation of ubiquitin aggregates upon pathogenic and/or inflammatory stimuli is the increased translation rate that typically occurs following binding of pathogen-associated molecular patterns (PAMP) to pattern recognition receptors (PRR) (see Chap. 4). Consequently, the elevated protein biosynthesis results in a sudden increased production of misfolded proteins

(i.e. DRiPs) whose levels surpass the degradation capacity of the cells thereby leading to the accumulation of ubiquitin-positive aggregates. Besides, exposure of pro-inflammatory mediators results in the upregulation of a flurry of enzymes using oxygen (O_2) as co-substrate including xanthine oxidase (XO), cyclooxygenase-2 (COX-2), and NADPH oxidase (NOX) which give rise to a variety ROS and reactive-nitrogen species (RNS) such as superoxide radicals as well as hydroxyl radicals and peroxynitrite. While radical formation is beneficial to host antimicrobial defense, at high concentration it leads to oxidative and nitrosative stress and may result in various types of cellular damage to structures including membrane lipids, proteins and nucleic acids. Herein, excessive production of ROS actively contributes to unbalanced protein homeostasis, as it causes oxidation and subsequent cross-linking of cellular proteins and therefore augments the intracellular pool of damaged proteins. Finally, during infections, pathogens often hijack the host translation machinery to support the synthesis and folding of their own proteins. This in turn leads to a potential lack of molecular chaperones for the proper assembly of host proteins, thereby increasing the cellular pool of misfolded proteins. Overall, the disturbed protein homeostasis observed in the early phases in inflamed and/or infected cells mainly occurs as a consequence of increased protein synthesis and damage, while the degradation rate remains constant.

However, the harmful effects of inflammation and/or infection on protein homeostasis are counteracted under normal conditions by an increased protein degradation rate in the later phase of the innate immune response. Indeed, pro-inflammatory mediators, and type I and II IFN in particular, promote the upregulation of the three inducible proteasome subunits β5i, β1i and β2i whose progressive incorporation into newly synthetized proteasomes allows the formation of IP. Due to their higher proteolytic activity, IP are more effective than their standard counterparts in the removal of ubiquitin-modified proteins. Herein, it has been shown that β5i-knock out cells are characterized by a sustained aggregation of ubiquitin-protein conjugates, when exposed to IFN and/or pathogens [16, 17]. This formally demonstrates that IPs are key players in the maintenance of protein homeostasis by clearing ubiquitin inclusions during inflammation and/or infections. Interestingly, further investigations in β5i-free mice have unveiled that the failure to efficiently eliminate ubiquitin aggregates in response to pathogens causes a substantial alteration of the cytokine profile [17], thereby unveiling a possible close relationship between protein homeostasis and inflammation.

Noteworthy, IFNs are also potent inducers of the proteasome activator subunits PA28-α und –β, thereby favoring the association of the PA28 ring with 26S proteasomes to form so-called hybrid proteasomes. The observation that PA28-depeleted cells exhibit higher levels of oxidant-damaged proteins (protein carbonyls) strongly suggests participation of these complexes in the elimination of damaged proteins during inflammation [18, 19]. Generally, protein aggregation in such physiological processes largely occurs as a consequence of the time delay between protein damage and assembly of fully functional 26S hybrid IP.

7.2.2 Pathological Protein Homeostasis Perturbations

The sustained aggregation of ubiquitin-modified proteins is a hallmark of pathological perturbations of protein homeostasis and a typical feature of numerous neuronal disorders such as Huntington and Parkinson diseases. It is believed that such disturbances may have a genetic origin with point mutations being responsible for the generation of an aberrant proportion of misfolded proteins including huntingtin and α-synuclein [20, 21]. A growing body of evidence suggests that protein degradation might be affected by genetic alterations as well. Over the past 5 years, an increasing number of loss-of-function mutations affecting inducible subunits, in particular β5i and β1i, but even standard subunits (β7 and α7) and the assembly helper POMP have been described in skin disorders such as keratosis linearis with ichthyosis congenita and sclerosing

Table 7.1 List of disorders associated with genetic alterations of proteasome genes

Disease	Altered proteasome gene(s)	Aggregation of Ub-modified proteins	Inflammation markers	Reference
Parkinson's disease (PD)	PSMC3	Not addressed	Not addressed	[30]
Keratosis linearis with ichthyosis congenita and sclerosing keratoderma syndrome (KLICK)	POMP	Not addressed	ER stress (CHOP)	[22]
Joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy (JMP)/Nakajo-Nishimura syndrome (NNS)/ Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE)/Proteasome-associated autoinflammatory syndrome (PRAAS)	PSMB8	Yes	IL-6, IFN-γ, IP-10	[25]
	PSMB8		Infiltration of immune cells in the dermis	[26]
	PSMB8	Not addressed	IP-10, MCP-1, IL-6	[28]
	PSMB8	Not addressed	Yes	[29]
	PSMB8	Not addressed	IL-6, IL-8, IFN-γ	[23]
	PSMB8	Yes	IL-6	[24]
	PSMA3, PSMB4, PSMB9, POMP	Yes	IFN Signature	[27]
Syndromic neurodevelopmental disorder	PSMD12	Yes	Not addressed	[31]

keratoderma syndrome (KLICK) genodermatosis [22] as well as in various auto inflammatory syndromes including joint contractures muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy (JMP) [23], Nakajo-Nishimura syndrome (NNS) [24–26], chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) [27–29] and neuronal disorders [30, 31] (Table 7.1) (see Chap. 24). Importantly, it has been shown that such mutations lead to proteasome malfunctions including impaired proteasome assembly and/or decreased chymotrypsin-like activity, ultimately resulting in the accumulation of ubiquitin-protein conjugates as a consequence of the incapacity of the cell to cope with damaged proteins. Because of the obvious correlation between proteasome defects and the induction of inflammation, such diseases are also referred to as proteasome-associated autoinflammatory syndromes (PRAAS). CANDLE/PRAAS syndromes are characterized by the constitutive production of pro-inflammatory cytokines, in particular type I IFN, which places them in the category of the interferonopathies. Importantly, the production of IFN-α/β exacerbates the protein homeostasis perturbation, as it favors the production of ROS/RNS which in turn augment the pool of misfolded proteins. It therefore gives rise to a pathological vicious circle of events in which inflammation persists (Fig. 7.2). The observation that such syndromes are characterized by a type I IFN signature with upregulation of typical IFN-stimulated genes (ISG) including the 2′-5′-oligoadenylate synthase-like protein (OASL) and protein kinase R (PKR) is somehow surprising given that IFN-α/β is usually induced following Toll-like receptor (TLR)-dependent sensing of viral pathogens (see Chap. 6). The precise functions of these ISG as response to unbalanced protein homeostasis in a viral-free context also remain unclear. One could argue that the liberation of type I IFN may represent a compensation mechanism aiming to downregulate protein synthesis through the (1) degradation of host mRNA via the OAS/RNase L pathway or (2) inhibition of translation via phosphorylation of eIF2α by the PKR, so that homeostasis could be restored. Alternatively, it is conceivable that the increased type I IFN in patients with proteasome mutations might reflect an inability to resolve inflammation in response to viral pathogens. In any case, the effects and underlying mechanisms by which ubiquitin-protein aggregates and type I IFN interact with each other warrants further investigations.

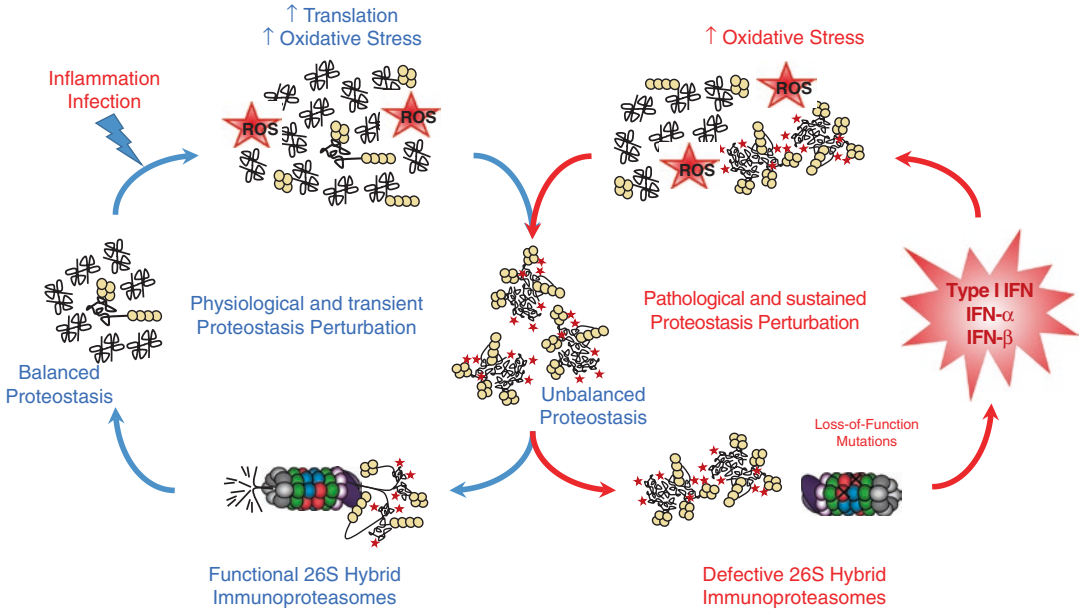

Fig. 7.2 Dynamics of the protein homeostasis cycle under physiological and pathological conditions. Inflammation and/or infection lead to increased protein translation and production of reactive oxygen species (ROS) which in turn augment the pool of misfolded and/or damaged proteins. The formation of immunoproteasomes following inflammatory and/or pathogenic stimuli results in the acceleration of the proteolytic degradation rate. This allows the progressive clearance of ubiquitin-protein aggregates, thereby completing the physiological protein homeostasis cycle. By contrast, genetic-based immunoproteasome defects are accompanied by ineffective removal of ubiquitin-positive inclusions which persist and promote and/or favor a type I interferon (IFN) response. This leads to increased oxidative stress which further strengthens the aggregation of ubiquitin-modified proteins. Both protein aggregates and type I IFN amplify each other, culminating in a vicious pathological cycle which cannot restore protein homeostasis

7.3 Cellular Responses to Unbalanced Protein Homeostasis

Key Points

- **Proteotoxic stress and proteasome impairment induces the TCF11/Nrf1-antioxidant response element (ARE)-driven activation of the ubiquitin proteasome system (UPS) and the unfolded protein response (UPR)**
- **Three arms of the unfolded protein response (IRE-1α; PERK; ATF6) result in modulation of cellular systems for protein synthesis, folding, quality control and degradation**
- **The unfolded protein response and cell autonomous innate immune signaling are closely interconnected**

As outlined before, proteotoxic stress of physiological or pathological origin can lead to accumulation of damaged proteins and thus demands a higher proteolytic capacity of the UPS degradation machineries to eliminate these non-functional proteins [32]. The inducible expression of alternative proteasome isoforms such as immune- and hybrid proteasomes along with the up-regulation of certain subsets of UPS factors represent such adaptation mechanisms to altered proteolytic demands. The initiation of autophagic removal of protein aggregates is another prime example of these adaptation processes [33]. Of note, the UPS and autophagy are closely interconnected and can partially compensate for one another. Main intercrossing signaling cascades are the unfolded protein response (UPR) in the ER (UPR^{ER}) or in mitochondria (UPR^{mt}), the

PI3K/Akt/mTOR pathway, and the formation of aggresomes accompanied by induction of detoxifying pathways for oxidative stress [34, 35].

7.3.1 The Unfolded Protein Response

In recent years we have made progress in understanding how cells and tissues integrate different proteotoxic insults to an adequate cellular stress program that will induce survival and repair pathways, stimulate immune responses, or ultimately induce cell death to eliminate irreversibly damaged cells. Nevertheless, our understanding of the molecular processes that control the biogenesis, folding, trafficking and degradation of proteins is still very limited. The ER which transports 30–40% of newly synthetized proteins represents a main check-point and acts as a major sensor of proteostasis perturbations [36].

Three arms of ER-stress signaling integrate all signals into the UPR, the function of which is to restore cellular homeostasis or stimulate apoptosis and thus determine cell fate. PERK, IRE1α, and ATF6 are ER-membrane bound receptors that survey the ER-lumen for problems with protein integrity. Under homeostatic conditions all three sensors are kept inactive by binding to the ER-chaperone BiP. Upon proteotoxic stress BiP dissociates from the stress receptors via titration by misfolded proteins. The activated stress sensors then transduce the signals of protein-misfolding from the ER into the cytosol to finally modulate cellular systems for protein synthesis, folding, quality control and degradation by activation of different transcription factors (Fig. 7.3). Other UPR target genes, whose products are involved in amino acid metabolism, calcium homeostasis, mRNA metabolism, redox metabolism, secretion and export, as well as lipid synthesis, can be induced by these signaling pathways in parallel. PERK (pancreatic ER-kinase) directly phosphorylates the initiation factor eIF2α to shut down global translation and to initiate translation of special factors such as transcription factor ATF4. In addition, this arm finally activates CHOP and GADD34, which is a phosphatase subunit for eIF2α, thereby counteracting this process. The IRE1α receptor, as the second arm, exhibits endonuclease activity that is required to splice the transcription factor XBP1 for its activation and cleaves mRNA from arrested translation initiation complexes into small fragments. The third arm is controlled by the cleavage of ATF6, an ER-membrane tethered transcription factor that traffics to the Golgi apparatus upon ER-stress where it is processed and released from the membrane by the proteases S1P and S2P. Proteostasis can be re-established by induction of protein quality control and degradations systems and reduction of translation. To that end, UPR signaling provokes the upregulation of chaperones and other folding catalysts, as well as the ER-associated degradation (ERAD) and autophagy. ERAD ensures the removal of terminally misfolded proteins from the ER by their detection, retro-translocation into the cytosol, their ubiquitin conjugation and final clearance by degradation by the proteasome [37].

As mentioned before, physiological or pathological proteotoxic insults in immune responses perturb proteostasis by the production of radicals, other tissue damaging molecules and/or the massive synthesis of pathogen proteins. Thus, inflammation and cytokine signaling represent not only potent innate responses to pathogens, but also confer severe proteotoxic stress to cells and tissues by radicals or the release of many degradative enzymes from their granula. An adequate adaptation of cellular clearance pathways to the increased burden of damaged proteins is thus of fundamental importance to prevent protein aggregation, inclusion body formation and ultimately cell death. In this context, it is important to note that immune cells of either myeloid or lymphoid origin permanently express immuno- and hybrid proteasomes [33]. In addition, these cell types use arms of the UPR for their maturation and function. For example, B cells require XBP1 for their differentiation in plasma cells and immunoglobulin production. In a similar way, dendritic cells require XBP1 for their maturation and function in antigen presentation [37].

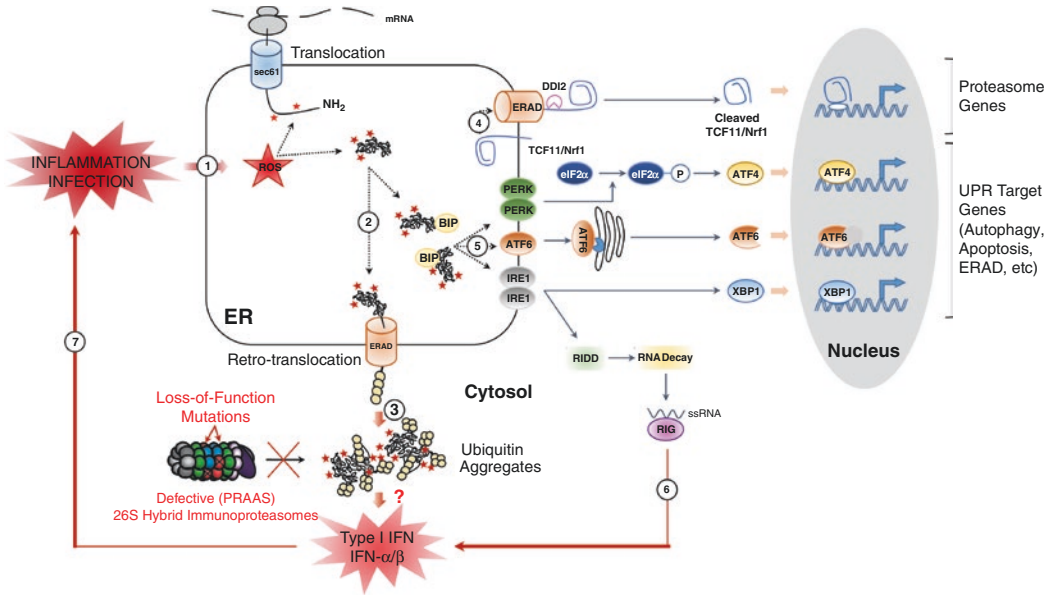

Fig. 7.3 Transcription factor (TCF) 11/Nuclear factor erythroid 2-related factor (Nrf) 1 and the unfolded protein response (UPR) as the main cellular responses to disturbed protein homeostasis in proteasome-associated autoinflammatory syndromes (PRAAS) patients. (1) The increased production of reactive oxygen and/or nitrogen species following inflammation and/or infection results in elevated levels of oxidized proteins trafficking in the endoplasmic reticulum (ER). (2) These misfolded proteins are then retro-translocated into the cytosol through ER-associated degradation (ERAD) and undergo ubiquitination during the extraction process. (3) PRAAS proteasomes bearing loss-of-function mutations are defective, and cannot effectively clear the increasing amount of ubiquitin-protein conjugates which in turn aggregate as ubiquitin-positive inclusions in the cytosol. (4) Impaired proteasome activity promotes the extraction of the TCF11/Nrf1 ER-resident protein from the ER into the cytosol via ERAD whereby it is cleaved by the DNA damage-inducible protein homolog 2 (DDI2) protease at the cytosolic side. Cleaved TCF11/Nrf1 acts as an active transcription factor that enters the nucleus in which it stimulates the expression of proteasome genes. (5) The accumulation of misfolded/oxidized proteins in the ER favors their interaction with the BIP chaperone protein which itself dissociates from its membrane receptors inositol-requiring protein 1 (IRE1), activated transcription factor 6 (ATF6) and protein kinase R-like endoplasmic reticulum kinase (PERK), thereby initiating the UPR. This leads to the activation of the ATF4, ATF6 and X-box-binding protein 1 (XBP1) transcription factors, which following nuclear translocation stimulate the expression of genes encoding proteins involved in apoptosis, ERAD and autophagy. (6) IRE1 also activates the IRE-1α-dependent decay (RIDD) pathway which may lead to excessive production of ssRNA that activates pathogen recognition receptors (PRR) such as retinoic acid-inducible gene-1 (RIG-1) and initiate a type I interferon (IFN) response. (7) IFN-α/β secretion aggravates the initial inflammation process and establishes a vicious circle in which perturbed protein homeostasis and type I IFN reinforce each other

7.3.2 Cellular Responses to Proteasome Inhibition

Proteasome impairment by either chemical inhibitors, depletion of subunits by siRNAs, or mutations/deletions of proteasome subunits results in a defined cellular stress response program. This program is first characterized by induction of proteotoxic stress responses comprising the TCF11/Nrf1-antioxidant response element (ARE)-driven activation of UPS gene expression [38–43]. This transcriptional feed-back-loop regulates UPS-dependent protein degradation in response to proteotoxic and oxidative stress via activation of the ER-tethered transcription factor TCF11/Nrf1. Under non-inducing conditions TCF11/Nrf1 resides in the ER membrane, where its low abundance is ensured by the ERAD system requiring the E3-ubiquitin ligase HRD1 and the AAA-ATPase p97. Upon exposure to proteotoxic stress by proteasome inhibitors or oxidants, cells activate the cleavage of TCF11/Nrf1 by

DDI-2 and its membrane detachment (Fig. 7.3). The released TCF11/Nrf1 in turn translocates into the nucleus and activates the gene expression of almost all proteasome subunits, the assembly factor POMP, and other UPS-related genes by binding to AREs in their promoter regions. This TCF11/Nrf1-dependent increase of proteasomes is essential to prevent cell death. Activation of the TCF11/Nrf1-dependent gene expression has also been observed in cells of PRAAS patients bearing digenic or compound heterozygous mutations. This upregulation may partly compensate for impaired proteasome capacity by induction of the healthy allele of the mutated subunit gene [27, 40].

The activation of the TCF11/Nrf1 transcriptional pathways by proteotoxic stress is accompanied by induction of typical $UPR^{(ER)}$ downstream events such as inactivation of translation by phosphorylation of eIF2α, induced transcription of ER chaperones and the processing of XBP1. As a final event, up-regulation of type I IFN induction has been observed both in hematopoietic and non-hematopoietic cells in response to proteasome impairment by inhibitors as well as in cells from PRAAS patients.

Transcriptomic analysis using microarrays from cultured cells treated with proteasome inhibitors [38] or RNA-sequencing analysis of PBMCs from PRAAS patients [27] strongly indicates ER-stress and the induction of the PERK and the IRE1α arm of the UPR. Current concepts of UPR signaling connect both receptors with innate immune responses. The endonuclease activity of IRE1α is not only responsible for splicing of XBP1, but also for the decay of other mRNAs located near the ER membrane (most likely installed translation initiation complexes) mediating regulated IRE1α-dependent decay (RIDD). The production of small RNA fragments by RIDD is thought to activate the RNA-virus pattern recognition system involving the RNA-helicase RIG1 and the mitochondrial antiviral signaling protein (MAVS) that in turn activates IRF3 and IFN-β production. Translational inhibition by PERK action triggers the rapid degradation of inhibitor of nuclear factor (NFκB) IκB and thus gene expression of many NF-κB dependent down-stream events including production of cytokines such as IFN-β and other pro-inflammatory factors [44].

In this context, it is also important to note, that many differentiation or innate signaling cascades depend on the timely degradation of factors by the UPS including iκBα, NFκB, IRF3 or MAVS. Impairment of UPS degradation capacity as shown in PRAAS may stabilize these factors in addition to activation of their signaling cascades by the UPR [45]. Thus, such fine-tuned pathways can be prolonged by these imbalances that ultimately favor pro-inflammatory signaling.

In summary, type I IFN-production in PRAAS patients, as part of the cell autonomous innate immune response, drives a vicious cycle of protein damage by radical production and dysfunction in clearance of these damaged proteins in (auto)inflammation. Interestingly, type I IFNs and downstream interferon-stimulated genes (ISGs) were identified as drivers of inflammation in other proteinopathies such as neurodegenerative diseases as well. The understanding of the molecular details of type I IFN induction in PRAAS patients and other proteinopathies remains to be defined for the aforementioned mechanistic possibilities. This understanding is necessary for pharmacologic intervention. This is underlined by the fact that most PRAAS patients are successfully treated with inhibitors of the IFN-signaling pathway [27]. Potential targets within the UPR or other intercrossing signaling cascades may open new avenues for treatment of these diseases.

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8 Disruption of Protein Homeostasis and Activation of Cellular Stress Pathways in Autoinflammation

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Abstract

In addition to being a critical part of host defense against pathogens, the inflammatory response can also be triggered by a number of perturbations to cellular homeostasis, including responses to protein misfolding and endoplasmic reticulum (ER) stress. Physiologically, these responses can lead to activation of tissue repair pathways, but when not properly regulated, these stress response pathways can lead to chronic inflammation. ER stress and other inflammatory pathways triggered by misfolded proteins have been implicated in the pathogenesis of several monogenic autoinflammatory diseases, and also may play a role in other conditions such as neurodegenerative diseases, where increasing evidence has accumulated about the contribution of inflammation to disease pathogenesis. Alterations in protein homeostasis can trigger autoinflammatory diseases in a number of ways, including (1) a pathogenic protein is itself misfolded, primarily activating inflammatory signaling pathways, as with the mutant tumor necrosis factor receptor 1 (TNFR1) protein in TNF receptor-associated periodic syndrome (TRAPS), or triggering an intracellular ER stress response, such as the human leukocyte antigen (HLA)-B27 protein in spondylarthropathies; (2) inflammatory responses can also be triggered by extracellular misfolded proteins, and (3) genetic defects in protein homeostasis pathways which lead to inflammatory diseases. Examples of this mechanism are proteasome mutations in chronic atypical neutrophilic dermatitis with lipodystrophy and elevated temperature (CANDLE) and related syndromes, and variants in the gene encoding ATG16L which reduce the efficiency of autophagy and related secretory pathways in inflammatory bowel disease.

Keywords

Protein homeostasis · Autophagy · LC3-associated phagocytosis · Autoinflammatory disease · Spondyloarthropathy · Endoplasmic reticulum (ER) stress response · Reactive oxygen species

Abbreviations

AD	Alzheimer disease
AIM	Absent in melanoma
AMPK	AMP-activated protein kinase
AS	Ankylosing spondylitis
ATG	Autophagy-related genes
Bcl-2	B-cell lymphoma 2

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CANDLE	Chronic atypical neutrophilic dermatitis with lipodystrophy and elevated temperature
cGAMP	cyclic guanosine monophosphate–adenosine monophosphate
cGAS	cyclic guanosine monophosphate-adenosine monophosphate synthetase
FIP200	Family interacting protein of 200
HLA	Human leukocyte antigen
IRF	Interferon regulatory transcription factor
ISG	Interferon-stimulated gene
LAP	LC3-associated phagocytosis
LC3	Microtubule-associated protein light chain 3
MHC	Major histocompatibility complex
mTOR	mammalian target of rapamycin
NEDD	Neural precursor cell expressed, developmentally down-regulated
NF-κB	Nuclear factor kappa B
NK	Natural killer
NLRP	NOD-like receptor family pyrin domain containing
NMDA	*N*-methyl-D-aspartate
NOD	Nucleotide-binding oligomerization domain
PARKIN	Parkinson kinase
PDA	Protein disulfide isomerase
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PINK	PTEN-induced putative kinase 1
ROS	Reactive oxygen species
SAVI	STING-associated vasculopathy with onset in infancy
STING	Stimulator of interferon genes
SUMO	Small ubiquitin-like modifier
TBK	TANK binding kinase
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TORC	Target of rapamycin complex
TRAPS	TNF receptor-associated periodic syndrome
TRIM	The superfamily of tripartite motif-containing
ULK	unc-51 like autophagy activating kinase
UPR	Unfolded protein response
UPS	Ubiquitin–proteasome system
VPS	Vacuolar protein sorting
WIP	WPP domain–interacting proteins

Key Points

- **Autophagy and the proteasome control protein homeostasis, and also regulate inflammation and immunity**
- **Dysregulation or disruption of these processes can contribute to pathology of a variety of diseases**
- **Accumulation of misfolded proteins triggers ER stress responses and can contribute to the pathogenesis of monogenic autoinflammatory diseases, and other conditions including neurodegenerative disorders and type II diabetes**
- **Genetic alterations in the efficiency of autophagy or proteosome function can contribute to autoinflammatory disease**
- **Better understanding of these pathways may aid the design of therapeutic interventions in both monogenic and more complex autoinflammatory diseases**

8.1 Cellular Mechanisms Maintaining Protein Homeostasis and Links to Inflammation Biology

Key Points

- **Damaged or ubiquitinated proteins are degraded via the proteasome, which also has essential roles in antigen presentation, the cellular stress response, and regulating cell death**
- **Autophagy is primarily responsible for the degradation of long-lived proteins and cellular organelles (mitochondria, peroxisomes), and is essential for cell growth and the response to nutrient deprivation**
- **The proteasome and autophagy proteins also regulate intracellular metabolism and inflammatory signaling pathways**

Balancing protein synthesis, degradation and secretion is an essential part of cellular physiology, and multiple molecular mechanisms exist to maintain protein homeostasis, with over 1000 proteins estimated to participate in this process. Feedback mechanisms control the rate of protein synthesis. Separate networks of chaperones

control protein folding and sensing of unfolded proteins, both in the cytoplasm and inside the vesicular network of the secretory pathway. Two major mechanisms of protein degradation, the proteasome (see also Chap. 7) and autophagy, are responsible for homeostasis of most proteins and other cellular components. Together, these systems maintain cellular viability amidst dramatic changes in protein output which occur particularly in immune cells. These cells can dramatically upregulate their biosynthetic protein flux to support rapid growth and secretion of large quantities of cytokines and antibodies. This is most evident in B lymphocytes, where in as little as 1 week, cells can differentiate from a resting B lymphocyte with little secretory capacity to plasma cells, which synthesize and secrete up to 175 million antibody molecules per day.

8.1.1 The Proteasome

The proteasome, a multi-subunit cytoplasmic protein complex, is capable of rapid degradation of proteins marked with small molecules in the ubiquitin family, which share a structural β-grasp fold. K48-linked ubiquitin was the first identified modifier protein, which has expanded to include other related molecules including: small ubiquitin-like modifier (SUMO), neural precursor cell expressed, developmentally down-regulated 8 (NEDD8), interferon-stimulated gene 15 (ISG15), F adjacent transcript 10 (FAT10), and monoclonal non-specific suppressor factor beta (MNSFB) proteins. A special set of proteasome subunits is induced in antigen-presenting cells and forms a so-called 'immunoproteasome' that facilitates processing of peptides with hydrophobic C-termini that are optimized to be presented in the groove of major histocompatibility complex (MHC) class I molecules. The immunoproteasome also plays an important role in eliminating protein aggregates which can accumulate under inflammatory conditions [1]. Ubiquitination of target proteins is accomplished through three sets of enzymes termed E1, E2 and E3 ubiquitin ligases, with E1 and E2 having catalytic activity and E3 proteins serving to link the target protein to the ubiquitination machinery. Ubiquitin-like proteins are conjugated to target proteins through parallel sets of enzymes, and like ubiquitin, these proteins also have functions beyond mediating recognition of target proteins by the proteasome. Once recognized by the proteasome, target proteins are loaded into the proteolytic core of the proteasome where adenosine triphosphate (ATP)-dependent proteases digest proteins into short oligopeptides suitable for further catabolism and antigen presentation (for details on proteasomes see Chap. 7).

8.1.2 Autophagy

The other major proteolytic mechanism in cells is autophagy, a process in which organelles and cytoplasmic contents are enveloped in lipid membrane-enclosed vesicles which fuse with lysosomes to degrade proteins into smaller fragments. Autophagy was originally considered to be a constitutive process by which long-lived proteins and damaged organelles are degraded, but more recently, it has been found to be regulated by a series of proteins which allow environmental conditions, particularly nutrient starvation, to induce autophagy to maintain cellular protein homeostasis and function [2].

Two key nutrients sensing pathways regulate autophagy (Fig. 8.1a). Depletion of ATP or other stressors (starvation, hypoxia, oxidative stress, ER stress, infections) can activate AMP-activated protein kinase (AMPK), which phosphorylates substrates that activate autophagy. Another key nutrient sensor, the target of rapamycin complex (TORC), represses autophagy, but under conditions of amino acid starvation or removal of growth factors, TORC is repressed, which activates autophagy. Biochemically, TORC and AMPK regulate the pre-initiation complex, consisting of unc-51 like autophagy activating kinase 1/2 (ULK1/2), autophagy-related genes 13 (ATG13) and family interacting protein of 200 (FIP200) proteins (Fig. 8.1b) which regulate a class III phosphatidylinositol-3 kinase (PI-3 kinase) enzyme complex termed the initiation complex, consisting of the proteins ATG14L, beclin1, vacuolar protein sorting 34 (VPS34) and VPS15. Full activation of the initiation complex

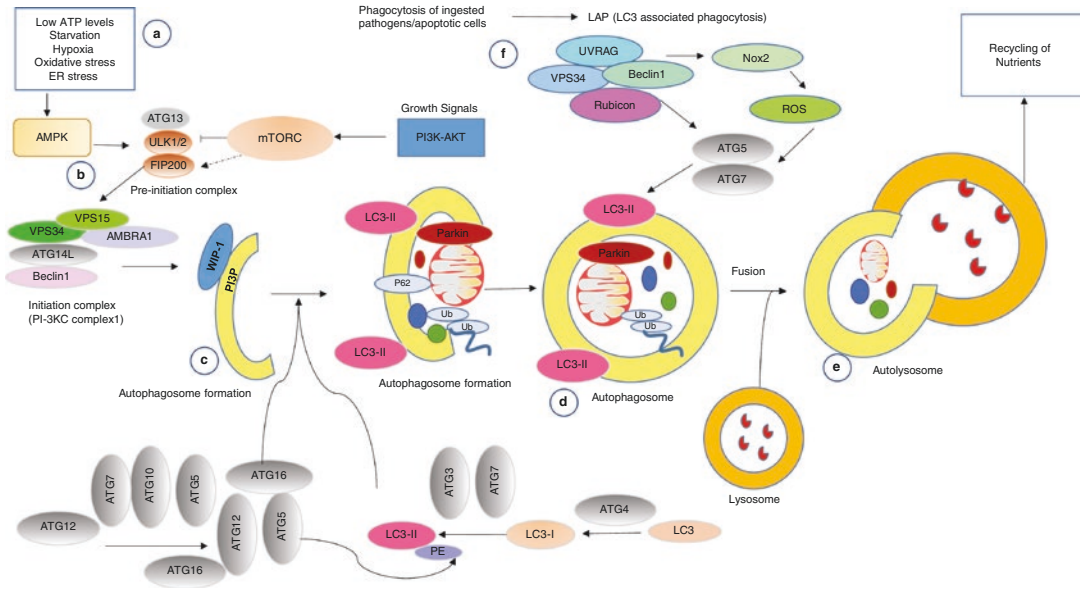

Fig. 8.1 Cellular autophagy pathways. Lettered steps described in the main text. *AMBRA1* activating molecule in BECN1-regulated autophagy protein 1, *AMPK* AMP-activated kinase, *ATG* autophagy-related genes, *FIP200* family interacting protein of 200, *LC3* Microtubule-associated protein 1A/1B-light chain 3, *mTORC* mammalian target of rapamycin, *Nox2* NAPDH oxidase 2, *PI3K* phosphatidylinositol-3 kinase, *PI3P* phosphatidylinositol 3-phosphate, *ROS* reactive oxygen species, *Ub* ubiquitin, *ULK1/2* unc-51 like autophagy activating kinase 1/2, *UVRAG* UV radiation resistance-associated gene protein, *VPS* vacuolar protein sorting, *WIP* WPP domain–interacting proteins

requires dissociation of the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) from the beclin-1 protein, linking autophagy to regulation of apoptotic cell death. The PI-3 kinase activity of the initiation complex phosphorylates lipids on intracellular membranes, creating a substrate for binding of proteins such as WPP domain–interacting proteins 1(WIP-1 or ATG18). This converts the nascent autophagic vesicle into a crescent shaped isolation membrane (Fig. 8.1c). The completion of autophagic lipid vesicles is accomplished through a ubiquitin-like chain-reaction of protein modification termed the elongation reaction, in which a lipid modified protein LC3, takes the place of ubiquitin. The protease ATG4 cleaves LC3 to produce LC3-I, which in turn is bound by the ATG7 molecule and transferred to ATG3. A protein complex consisting of ATG5, ATG12, and ATG16L1 transfers a phosphatidylethanolamine (PE) molecule onto LC3-I, forming LC3-II, which is incorporated into the growing isolation membrane and assists in the formation of the completed double-walled autophagic vesicle (Fig. 8.1d). Fusion of autophagosomes with lysosomes to degrades the organelles and proteins within (Fig. 8.1e), providing substrates for synthesis of new macromolecules in nutrient limiting conditions.

In addition to being a generalized mechanism for recycling cellular components, autophagy and components of the autophagic machinery can perform targeted degradation of damaged cellular components and control exocytic and endocytic processes where lysosomes are coupled to vesicular trafficking. Even under non-starvation conditions, certain organelles, including peroxisomes and mitochondria, can be recycled through autophagy, which performs the valuable cellular function of removing sources of reactive oxygen species (ROS) and toxic lipids. In damaged mitochondria, loss of the mitochondrial electrochemical gradient causes the accumulation of the PTEN-induced putative kinase 1 (PINK1) kinase on the cytosolic-facing outer mitochondrial membrane. PINK1 activates Parkinson kinase (Parkin), an E3 ligase, which catalyzes

ubiquitination of multiple substrates on the mitochondrial membrane. These substrate proteins recruit molecules such as p62/sequestrome and optineurin, which in turn recruit LC3 to the surface of these particular mitochondria and results in their selective elimination.

During exocytosis, fusion of exocytic and other intermediate vesicles to lysosomes can regulate the secretory process. Certain components of the autophagy machinery also participate in the LC3-mediated fusion of endocytic vesicles with lysosomes, which enhances the degradation of phagocytosed apoptotic cells and dampens the inflammatory response triggered by apoptotic cells, a process termed LC3-associated phagocytosis (LAP) [3] (Fig. 8.1f). Mice deficient in components of LAP have heightened inflammatory responses to apoptotic cells, and develop features of both autoinflammatory and autoimmune disease [4].

8.2 Protein Homeostasis in the Pathogenesis and Regulation of Monogenic Autoinflammatory Diseases

Key Points

- **Autophagy degrades activated NLRP3 inflammasomes**
- **Autophagy and proteasome-mediated degradation regulate production of type I interferons via the cGAS-STING pathway**
- **In TRAPS, mutant TNFR1 protein are retained in ER and are able to activate MAPK signaling via mitochondrial ROS**

8.2.1 Degradation of Inflammasomes Through Autophagy (Fig. 8.2)

Protein homeostasis regulate many key inflammatory pathways which are disrupted in monogenic autoinflammatory diseases (Table 8.1, Fig. 8.2). For the group of syndromes caused by activating mutations in the nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 3 (NLRP3) inflammasomes, a key link was made by the observation that activated inflammasomes are degraded through autophagy [5]. This was first observed during activation of NLRP3 and absent in melanoma 2 (AIM2) and inflammasomes, where ubiquitination of activated inflammasome components led to recognition by the p62 autophagy adaptor proteins and targeted delivery of activated inflammasome components to the autophagolysosome [6]. The superfamily of tripartite motif-containing (TRIM) family proteins also specifically target NLRP3, NLRP1 and caspase-1 for autophagic degradation. Other mechanisms regulate autophagic degradation of pro-interleukin (IL)-1β [7, 8]. Multiple activators of autophagy, including amino acid starvation, have been shown to reduce production of IL-1β whereas inhibitors of autophagy or genetic deficiency of genes encoding autophagosome components enhance inflammatory responses [9, 10]. Autophagy also inhibits the release of mitochondrial DNA into the cytoplasm in cells triggered to undergo inflammatory responses, likely due to the rapid degradation of mitochondria in which the proton gradient has collapsed after triggering of inflammatory responses, short circuiting the amplification loop in inflammation [11]. Autophagy itself can also be induced by inflammasome-regulated processes, generating a feedback loop which keeps inflammasome-mediated pathology under control, while allowing appropriate activation of innate immunity for host defense.

8.2.2 Regulation of Cyclic Guanosine Monophosphate-Adenosine Monophosphate Synthetase (cGAS) and Stimulator of Interferon Genes-(STING) Pathway by Autophagy

Autophagy can also regulate the activity of the cyclic guanosine monophosphate-adenosine monophosphate synthetase (cGAS)-stimulator of interferon genes (STING) pathway, another key intracellular sensor of pathogens

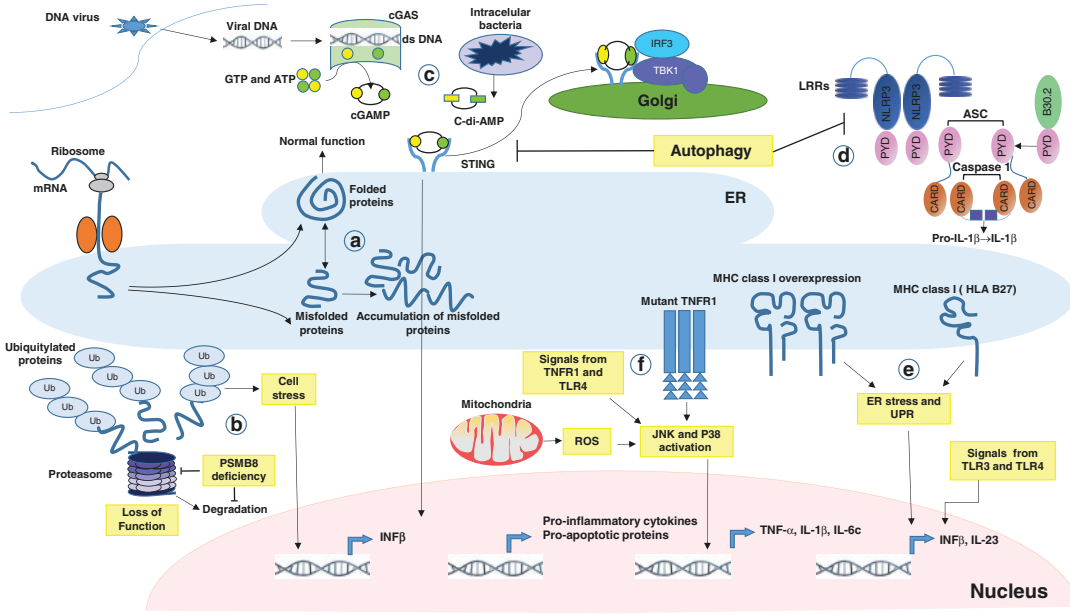

Fig. 8.2 Consequences of protein misfolding and intracellular signaling complexes that play a role in the pathogenesis of specific autoinflammatory disease. (**a**) The effects of the misfolding of secretory proteins in the endoplasmic reticulum (ER) are depicted at the bottom of the figure. The degradation of misfolded proteins can cause a loss-of-function, whereas the accumulation of misfolded proteins can trigger abnormal intracellular signaling or, at higher levels, the induction of the unfolded-protein response (UPR), which can also lead to the induction of inflammation and programmed cell death. Different foci of abnormal cellular signaling that trigger autoinflammatory diseases are depicted in the cell. (**b**) In proteasome subunit beta 8 (PSMB8) deficiency, reduced degradation of misfolded proteins and peptides by the immunoproteasome leads to the accumulation of ubiquitylated proteins and cellular stress. This can lead to the production of interferon-β (IFN-β), which in turn upregulates the synthesis of immunoproteasome subunits, perpetuating the abnormalities. (**c**) stimulator of interferon genes (STING) senses cyclic dinucleotides generated by cyclic guanosine monophosphate-adenosine monophosphate synthetase (cGAS) from endogenous and viral DNA or directly from bacteria, and triggers induction of interferon production through TBK1 and IRF3. STING is degraded in an autophagy-dependent manner. (**d**) In the cryopyrin-associated periodic syndromes (CAPS), mutations in nucleotide-binding oligomerization domain (NOD) leucine-rich repeats (LRR) and pyrin domain-containing 3 (NLRP3) enhance the activation of the NLRP3 inflammasome and the processing of pro-interleukin (IL)-1β into its active form. In familial Mediterranean fever, mutant pyrin is thought to associate with the inflammasome adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and increase IL-1β processing. The NLRP3 inflammasome can also be degraded through autophagy. (**e**) In the spondyloarthropathies, human leukocyte antigen (HLA)-B27 is expressed at a high level (which is enhanced in inflammation), fails to fold properly and is retained in the endoplasmic reticulum (ER), triggering a partial ER stress response that leads to type I IFN and IL-23 production. (**f**) In tumor necrosis factor receptor-associated periodic syndrome (TRAPS), mutations in the extracellular region of the TNF receptor 1 (TNFR1) leads to accumulation of the mutant receptor in the ER, which triggers an abnormal inflammatory response that is amplified by TNF or lipopolysaccharide (LPS) signaling through cell-surface receptors. *CARD* caspase recruitment domain, *JNK* c-Jun N-terminal kinase, *LRR* leucine-rich repeat, *PYD* pyrin domain, *ROS* reactive oxygen species, *TLR* Toll-like receptor. Adapted from [42]

and ectopic double stranded DNA (Fig. 8.2c). The enzyme (cGAS) is activated by cytosolic dsDNA to synthesize the dinucleotide second messenger cyclic guanosine monophosphate–adenosine monophosphate (cGAMP). The sensor protein STING is activated by cGAMP and activates the production of type I interferons through the kinase TANK binding kinase (TBK1) and the transcription factor interferon regulatory transcription factor 3 (IRF3) [12].

Table 8.1 Disruption and regulation of protein homeostatic mechanisms in genetic autoinflammatory diseases and complex diseases with an autoinflammatory component

Disease/Model	Causative gene /Mechanism	Induction of ER stress/UPR	Inflammasome activation by misfolded protein	Defective autophagy	Defective proteasome	Regulated by autophagy	Regulated by proteasome
Mendelian Diseases							
TNF receptor- associated periodic syndrome (TRAPS)	*TNFRSF1A*						
Familial Mediterranean fever (FMF)	*MEFV*						
Proteasome-associated autoinflammatory syndromes: JMP, NNS, CANDLE, JASL	*PSMB8*						
Hyperimmunoglobulinemia D with periodic fever syndrome (HIDS)	*MVK*						
Neonatal-onset multisystem inflammatory disease (NOMID)/ Muckle-Wells syndrome (MWS)/Familial cold autoinflammatory syndrome (FCAS)	*NLRP3*						
Deficiency of IL-1 receptor antagonist (DIRA)	*IL1RN*						
Deficiency of IL-36 receptor antagonist (DITRA)	*IL36RN*						
Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis	*CD2BP1(PSTPIP1)*						
Sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD)	*TRNT1*						
STING-associated vasculopathy with onset in infancy (SAVI)	*TMEM173*						
NLRC4-associated Autoinflammatory Disease	NLRC4						
Haploinsufficiency of A20 (HA20) syndrome	*TNFAIP3*[†]						
Otulin Deficiency	*FAM105B(OTULIN)*[†]						
Immunodeficiency and Autoinflammatory Disease associated with C-terminal NEMO mutations	*IKBKG*						
Immunodeficiency, autoinflammation and amylopectinosis	*HOIL1/RBCK1, HOIP/RNF31*						
Pediatric Onset Inflammatory Polyarthritis	*Myd88*						
Complex Diseases							
Polygenic inflammatory diseases Inflammatory bowel disease (IBD): Crohn,UC	ATG16L						
HLA-B27 associated AS	HLA-B27						
Gout and calcium pyrophosphate disease	NRLP3 inflammasome						
Type-2 diabetes (T2D)	IAPP						
Alzheimer's disease (AD)	APOE, APP, ADAM10						
Parkinson's disease (PD)	LRRK2, Parkin, PINK1, PS1, PS2, SNCA						
Amyotrophic lateral sclerosis (ALS)	SOD1, TDP-43, PDI						
Huntington's chorea	HTT						

Diseases are listed with causative and associated genes, and shading indicates the strength of evidence linking to the mechanisms in the columns. *TNF* tumor necrosis factor, *TNFRSF1A* TNF receptor super family, member 1A, *MEFV* familial Mediterranean fever gene, *TRNT1* tRNA nucleotidyltransferase, CCA-adding 1, *JMP* joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced childhood-onset lipodystrophy, *NNS* Nakajo-Nishimura syndrome, *CANDLE* chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *JASL* Japanese autoinflammatory syndrome with lipodystrophy, *MVK* mevalonate kinase, *PSMB8* proteasome subunit beta type 8, *IAPP* islet amyloid polypeptide, *APP* amyloid precursor protein, *APOE* apolipoprotein E, *ADAM10* A disintegrin and metalloproteinase domain 10, *PS1* preselenin 1, *PS2* preselenin 2, *LRRK2* leucine rich repeat kinase 2, *SNCA* synuclein alpha, *PARKIN* Parkinson protein 2, *PINK1* PTEN-induced putative kinase 1, *SOD1* superoxidase dismutase 1, *TDP-43* TAR DNA binding protein 43, *PDI* protein disulfide isomerase, *HTT* huntingtin. Adapted from [41]

Activating mutations in STING trigger the syndrome of STING-associated vasculopathy with onset in infancy (SAVI) which is marked by excess production of type I interferon in response to cGAMP and a strong in vivo interferon gene transcriptional signature [13] (see Chap. 24). Bacterial dinucleotide metabolites can activate STING directly without cGAS [14]. In parallel with activating interferon synthesis, dsDNA also activates autoph-

agy through cGAS, and a pathway requiring activation of AMPK and the downstream kinases ULK1/2 [15]. Activated STING is also ubiquitinated, leading to recruitment of the p62/sequestrin which mediates its delivery to autophagocytic vesicles and degradation [15]. This regulatory mechanism is likely important in preventing sustained activation of STING and type I interferon and the severe clinical consequences that are seen in the SAVI syndrome.

8.2.3 Accumulation of Misfolded Mutated Proteins

In addition to being regulated by autophagy, inflammatory signaling pathways can be activated in monogenic autoinflammatory disease by the altered protein encoded by the causal genetic mutation. The tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is caused by autosomal dominant missense mutations in the extracellular domain of TNFR1, the key pro-inflammatory receptor for TNF [16] (see Chap. 18). In cells from patients with TRAPS and mice engineered to express TRAPS-associated TNFR1 mutations, the mutant protein misfolds and is retained in the endoplasmic reticulum (ER), where it signals in a ligand-independent manner to activate MAPK signaling through a pathway dependent on mitochondrial ROS [17]. Autophagy may also play a role in degrading TNFR1 [18]. Cells from patients with TRAPS have enhanced pro-inflammatory responses to innate immune stimuli and sensitivity to *in vivo* lipopolysaccharide (LPS) challenge in a manner dependent on the wild-type TNFR1 [19]. Accumulated TNFR1 also triggers a low but detectable activation of the unfolded protein response (UPR) [20]. Upregulation of NLRP3 and enhanced inflammasome activation and IL-1β production ensues in myeloid cells, likely explaining the clinical responsiveness of TRAPS to blockade of IL-1β [21].

8.3 Alteration in Protein Homeostasis Mechanisms and Triggering of Inflammatory Responses by Misfolded Proteins in Complex Diseases

Key Points

- **Intracellular misfolded proteins are key players in inducing the inflammation and ER stress responses in various diseases including ankylosing spondylitis**
- **Extracellular protein aggregates can also induce ER stress and are important players in pathogenesis of Alzheimer disease and type II diabetes**

In addition to genetic variants in specific proteins which can trigger ER stress responses or altered signal transduction, alterations in protein homeostasis mechanisms themselves can lead to enhanced inflammation. For diseases associated with misfolded proteins, pathogenesis can further be divided into intracellular vs. extracellular proteins, as the mechanisms by which inflammation is triggered vary depending on the location of the misfolded protein.

8.3.1 Defects in the Autophagy Pathway

Genetic deficiencies in components of the autophagy pathway such as ATG5 and ATG7 result in accumulation of damaged mitochondria, as do mutations in parkin, which are associated with hereditary forms of Parkinson disease linking defective mitophagy to neurodegeneration. In studying the susceptibility allele for Crohn disease linked to the gene encoding ATG16L1, it was discovered that exocytosis by intestinal Paneth cells can be regulated by autophagy, with the disease susceptibility variant reducing secretion of antimicrobial peptides [22].

8.3.2 Accumulation of Intracellular Misfolded Proteins (Fig. 8.2)

The human leukocyte antigen (HLA)-B27 protein is a well-studied example of an abundant protein where misfolding contributes to induction of an ER stress response and inflammation in spondylarthropathies. Ankylosing spondylitis (AS) is a polygenic immune mediated multisystem inflammatory chronic disorder characterized by inflammation centered on the axial spine with syndesmophyte formation resulting in the fusion of vertebral facet joints, involvement of sacroiliac and peripheral joints, enthesitis and extra-articular manifestations including inflammatory bowel disease and acute anterior uveitis. AS has a strong genetic predisposition, and presence of the HLA-B27 MHC class I allele remains the greatest genetic risk factor identified to date, conferring a relative risk of more than 80-fold in AS and somewhat lower, but significant risk in other spondylarthropathies [23]. It was initially presumed that the pathogenesis involved presentation of pathogenic peptides to class-I restricted T cells, but the failure to identify these putative peptides, and the persistence of AS-like disease in HLA-B27 transgenic rats in the absence of $CD8^+$ T cells has refocused research into roles for HLA-B27 in triggering inflammation independent of the adaptive immune system, thus supporting the concept of AS as an autoinflammatory disease. Compared to other HLA molecules, HLA-B27 is predisposed to form homodimers in the absence of β2-microglobulin. A role in recognition of surface HLA-B27 through KIR3DL2, an activating receptor found on the surface of natural killer (NK) cells, T cells and myeloid cells has been hypothesized in AS [24, 25], and the recent development of therapeutic antibodies against KIR3DL2 may allow clinical testing of this hypothesis. Another property of HLA-B27 dimers is accumulation in the ER, likely due to protein misfolding. Accumulation of misfolded HLA-B27 can trigger the ER stress response, also known as the UPR which in turn can increase the expression of proinflammatory mediators, including IL-23 in myeloid cells. IL-23 is a powerful costimulatory signal for the development of IL-17 secreting lymphocytes [26, 27]. A possible target cell for IL-23 has emerged from studies of non-classical T cells which reside in the tendon sheath and respond to IL-23 by secreting IL-17 [28]. These findings correlate with the therapeutic efficacy of antibodies blocking the activity of IL-23 and IL-17 in AS in clinical trials [29]. Inflammatory signals including toll-like receptor (TLR) ligation can also enhance activation of the UPR, constituting a positive feedback loop [30].

8.3.3 Accumulation of Extracellular Misfolded Proteins

Inflammation has recently been implicated in the pathogenesis of neurodegenerative and other diseases associated with organ failure, with specific misfolded proteins triggering inflammation and cell death through shared and individual pathways. Alzheimer disease (AD) is a neurodegenerative disease that is the most common cause of dementia in the elderly. The main histopathological features of AD are intracellular deposits of neurofibrillary tangles made of hyperphosphorylated Tau proteins and extracellular aggregates of amyloid-β, which form amyloid plaques. This accumulation is associated with glial activation, increased brain inflammation in the hippocampus and the cerebral cortex and neuronal toxicity. Several studies have identified abnormal levels of ER stress in the human brain of patients with AD. Markers of dysfunctional ER proteostasis correlate with the progression of AD and are associated with an activation of the UPR machinery [31]. Amyloid-β oligomers can induce ER stress in cultured neurons via interaction with *N*-methyl-D-aspartate (NMDA) receptors leading to alteration of ER calcium homeostasis and neuronal dysfunction [32]. The study of brain tissue from patients with AD as well as those with Parkinson disease also revealed s-nitrosylation

and inactivation of protein disulfide isomerase (PDA), a protein critical for folding in the ER. Inactivation of PDA leads to accumulation of misfolded proteins, ER stress and neuronal cell death [33]. A connection to inflammation was made through the observation that the UPR activates the production of pro-inflammatory cytokines through the nuclear factor kappa B (NF-κB) signaling pathway [34]. Some misfolded extracellular proteins, such as islet amyloid polypeptide, can directly activate the NLRP3 inflammasome, triggering IL-1β release [35]. These findings may partly explain the responsiveness of type II diabetes to therapeutic blockade of IL-1β [36], and are inspiring clinical trials of anti-inflammatory and anti-cytokine agents for the treatment of these diseases.

8.4 Targeting Protein Homeostasis for the Therapy of Autoinflammatory Diseases: Future Perspectives

Although cytokine blocking therapies have been notably successful in the treatment of some autoinflammatory diseases, preventing protein misfolding and triggering of ER stress by mutant proteins in monogenic disease or misfolding-prone protein isoforms such as HLA-B27 remains an important therapeutic goal. 'Molecular chaperones' which bind misfolded proteins and attenuate ER stress responses to them have been successful in some animal models of diseases such as in α-crystallin mutations which cause cataracts [37]. The discovery that autophagy and proteasome-mediated degradation control expression of inflammatory mediators and pathways involved in autoinflammatory as well as other diseases has spurred research into enhancing proteolysis of key inflammatory mediators or autophagy in general as a therapeutic strategy [38, 39]. Pharmacological agents that activate AMPK, such as methotrexate, or inactivate mTOR, such as rapamycin, can promote autophagy and degradation of aggregate-prone proteins, but what portion of their anti-inflammatory or immunosuppressive effects are due to this mode of action is not clear. Using bifunctional molecules to enhance degradation of targeted proteins through the recruitment of E3 ligases may be a more selective strategy to remove key inflammatory mediators from the cell [40]. If these strategies succeed, it will be a good example of how harnessing the powerful physiological mechanisms of protein homeostasis can lead to therapeutic benefit.

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S100 Proteins in Autoinflammation

9

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Abstract

Among the putative markers for autoinflammatory diseases, studies on phagocyte-derived S100 proteins (S100A8/A9, S100A12: calgranulins) are the most advanced to date. Translational studies have suggested an important role for these danger-associated molecular pattern (DAMP) molecules as robust inflammation biomarkers.

S100A8/A9 and S100A12 can be released from monocytes and granulocytes via so-called alternative secretory pathways. When extracellular, they can operate as proinflammatory endogenous toll like receptor (TLR)4-ligands. Tissue and serum concentrations of S100 proteins correlate with disease activity, both during local and systemic inflammatory processes. In autoinflammatory diseases such as familial Mediterranean fever (FMF), PSTPIP1-associated inflammatory diseases (PAID) or systemic juvenile idiopathic arthritis (SJIA), dysregulation of alternative secretory pathways may be involved in the pathogenesis. Resulting calgranulin-hypersecretion can then aggravate disease in a feed-forward loop together with IL-1β.

Analysis of S100A8/A9 and A12 concentrations in patients' specimens is a valuable supportive tool in the difficult diagnosis of SJIA and FMF and in investigating fever of unknown origin. Furthermore, calgranulins can be used to monitor disease activity to subclinical level, as their serum concentrations decrease with successful treatment. Their expression and function in disease may provide a better understanding of autoinflammatory mechanisms and calgranulins may pose novel therapeutic targets for future treatments.

Keywords

S100 proteins · Autoinflammation · Danger associated molecular patterns · Biomarker · Fever of unknown origin · Diagnosis · Monitoring · TLR agonist · Calgranulins

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Abbreviations

AIDAI	Autoinflammatory disease activity index
AOSD	Adult-onset still disease
CAPS	Cryopyrin associated periodic syndromes
DAMP	Danger-associated molecular pattern
FMF	Familial Mediterranean fever
FUO	Fever of unknown origin
IL	Interleukin
LPS	Lipopolysaccharide
MMP	Matrix metalloproteinase
MRP	Myeloid-related protein
MWS	Muckle-Wells syndrome
NET	Neutrophil extracellular trap
NOMID	Neonatal-onset multisystem inflammatory disease
PAID	PSTPIP1-associated inflammatory diseases
PAMI	PSTPIP1-associated myeloid-related proteinemia inflammatory syndrome
PAMP	Pathogen associated molecular pattern
PAPA	Pyogenic sterile arthritis, pyoderma gangrenosum, and acne syndrome
PBMC	Peripheral blood mononuclear cell
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, adenitis syndrome
RAGE	Receptor for advanced glycation end products
sJIA	Systemic juvenile idiopathic arthritis
TLR	Toll like receptor

Key Points

- **Phagocyte-derived S100 proteins (calgranulins) are endogenous proinflammatory TLR-4 agonists**
- **Dysregulation and hypersecretion of S100 proteins might be involved in the pathogenesis of autoinflammatory diseases**
- **S100 serum levels correlate with disease activity during local and systemic inflammation**
- **Monitoring S100 serum levels can support the diagnosis of systemic juvenile idiopathic arthritis and familial Mediterranean fever in the investigation of fever of unknown origin**

9.1 Functions of Phagocyte-Specific S100 Proteins

Key Points

- **Phagocyte-specific S100 proteins are abundantly expressed by monocytes (S100A8/A9) and neutrophils (S100A8/A9, S100A12)**
- **S100 proteins can bind divalent metal ions and subsequently arrange into homo- (S100A12) or heteromultimeric (S100A8/A9) oligomers**
- **S100A8/A9 and S100A12 can be released upon cellular necrosis or active, non-classical transport**
- **Extracellularly, these proteins can act as damage associated molecular pattern (DAMP) molecules by triggering toll like receptor (TLR)4-dependent pro-inflammatory immune responses**

The S100 protein family represents the largest subgroup within a protein superfamily, which binds Ca^{2+} via a structural motif named 'EF-hand'. Their name has derived from the observation that the first identified S100 proteins were obtained from the soluble bovine brain fraction upon fractionation with saturated (100%) ammonium sulfate [1] while their systematic nomenclature (S100A1-16, S100B, S100G, S100P, S100Z) relates to their genomic organization and location [2, 3].

Constitutive expression of the phagocyte-specific S100 proteins A8 (also termed calgranulin or myeloid-related protein, MRP8) and A9 (calgranulin B, MRP14) as well as A12 (calgranulin C, MRP6) is largely restricted to granulocytes and monocytes. Because of their functional similarities and localization S100A8, A9 and A12 have been designated as calgranulins within the large family of S100 proteins. Apart from expression by monocytes, S100A8/A9 is abundantly expressed in neutrophils, comprising approximately 40% of the cytosolic protein

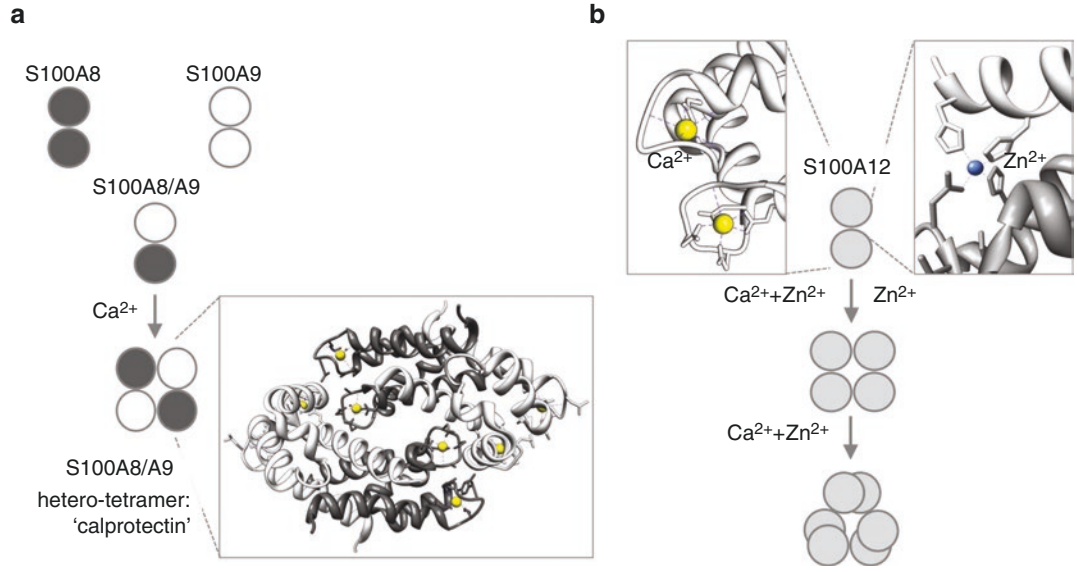

Fig. 9.1 Ion-induced oligomerization of S100A8/A9 and S100A12. (**a**) Calcium (Ca^{2+}) binding to S100A8 or A9 homo- or heterodimers can facilitate oligomerization to a hetero-tetrameric complex also known as calprotectin. (**b**) In its dimeric form S100A12 binds two Ca^{2+}-ions per monomer via loop structures named 'EF-hands'. Ca^{2+}-binding to S100A12 controls the protein's Zn^{2+}-sequestering. Either Zn^{2+} alone or Zn^{2+} together with Ca^{2+} can trigger oligomerization of S100A12 to homotetramers. Additional Ca^{2+} (and Zn^{2+}) can promote the protein's structural re-arrangement to form hexamers. All S100-oligomerization is highly transient. Once removed from a respective ion-environment the complexes disintegrate

content. In contrast, physiological S100A12 expression is largely restricted to human neutrophils, accounting for approximately 5% of cytosolic protein [4].

As all other S100 proteins, calgranulins share key structural motifs [5]. In the proteins' monomeric forms these motifs comprise a *C*-terminal EF-hand containing the classical Ca^{2+}-binding motif as well as an *N*-terminal EF-hand ("pseudo EF hand") with comparably lower Ca^{2+}-affinity. Apart from Ca^{2+}, calgranulins can bind Zn^{2+} as well as other divalent metal ions such as Cu^{2+}, Mn^{2+} or Fe^{2+} with high affinity [6–11]. Following ion-binding as a hallmark of calgranulins as well as other S100 proteins, is their organization into homo- (S100A12: di-, tetra-and hexamer [9, 12, 13]) or hetero-multimeric complexes (S100A8/A9: dimer and tetramer, also termed 'calprotectin' [14]) (Fig. 9.1). Metal-ion binding-induced changes in quaternary structure are thought to confound functional diversity [15–19].

9.1.1 Intracellular Functions

A vast number of different functions have been proposed for S100A8/A9 (reviewed in [20]). Comparably, there are very little data demonstrating an intracellular role of S100A12 in human neutrophils. Yet, the cytoplasmic abundance of calgranulins in monocytes (S100A8/A9) and neutrophils (S100A8/A9, S100A12) suggests important functions in cellular homeostasis, functions or differentiation [20].

Although S100A8/A9 expression correlates with the development of the myeloid lineage and is downregulated in the course of monocyte-to-macrophage differentiation [21] the myelopoietic potential of bone marrow cells obtained from S100A9 knock out mice is reportedly unaltered [22–24].

The Ca^{2+}-binding capability of calgranulins implies the proteins operate as Ca^{2+}-store or sensors. For S100A12 a similar involvement in buffering intra-versus extracellular Zn^{2+} has been

proposed [9]. In mice, S100A9^{null} neutrophils revealed less intracellular Ca^{2+}-mobilization in response to chemoattractant stimulation [25]. In human cell-line derived neutrophils, S100A8/A9 has been observed to support phagocytosis and reactive oxygen species (ROS) production [26–28].

Cytoplasmic S100A8/S100A9 can translocate to the cell membrane upon phagocyte activation and Ca^{2+}-dependent interaction with microtubules, vimentin, keratin and actin filaments are suggested to be important for migration, degranulation and phagocytosis of activated monocytes and neutrophils. While the S100A8/A9 tetramer promotes microtubule polymerization and F-actin cross-linking, S100A9 reduces this action [15, 16, 29, 30].

9.1.2 Release from Phagocytes

S100A8, A9 and A12 lack structural elements required for secretion via the classical endoplasmic reticulum and Golgi dependent secretory pathway. Thus, one of the primary, though passive, release 'mechanisms' is necrotic cell death [16, 30, 31]. In this way, S100A8/A9 and S100A12 can be released from neutrophils alongside with active release of the neutrophilic DNA content in a process termed neutrophil extracellular trap (NET) formation (NETosis) and NET-derived S100A8/A9 can promote interleukin 1 (IL-1) expression [32, 33].

Furthermore, there is evidence for active non-classical secretion following cytoskeleton-dependent alternative secretory pathways [16, 30, 31], which are similarly used by cytokines such as IL-1 [34]. Secretion along microtubules may involve an energy-dependent process requiring protein kinase C activation in combination with a second Ca^{2+}-dependent signal [16, 30]. Ca^{2+}-promoted translocation of S100A12 to granulocytic cytoskeletal and membrane fractions (unpublished data from our group) as well as cell membrane binding has been demonstrated [35].

9.1.3 Extracellular Functions

Once released from cells, S100 proteins exert numerous extracellular functions. These can be roughly grouped into processes triggered upon binding to pattern recognition receptors (chemotaxis, cell migration, myeloid/lymphocyte/endothelial activation), functions depending on the proteins' ion binding capacities (regulation of matrix metalloproteinase (MMP) activity, antimicrobial/antifungal activity) as well as anti-inflammatory functions (reviewed in [20, 36] (Fig. 9.2).

9.1.4 Function as Danger Associated Molecular Pattern (DAMP)

In the context of autoinflammation the extracellular role of calgranulins as DAMPs is potentially most relevant. DAMPs comprise endogenous cellular proteins, lipids or nucleic acids, which, comparable to pathogen associated molecular patterns (PAMPs) such as lipopolysaccharides (LPS) or flagellin, are recognized by pattern recognition receptors (see Chap. 4). This triggers an inflammatory response, which in the case of PAMPs should initiate clearance of the pathogen, while DAMP-signaling is meant to alert the immune system to remove DAMP-releasing necrotic cells [38, 39] (Fig. 9.2).

In the literature, there is an ongoing debate which cellular receptor is most relevant for calgranulins. Both S100A8/A9 and S100A12 have been reported to bind to the multi-ligand receptor for advanced glycation end products (RAGE) [19, 40, 41]. Hexameric S100A12 has been reported as RAGE ligand, although this is largely based on biochemical binding and computational modelling data [9, 19]. RAGE ligation by S100A12 is proposed to trigger a pro-inflammatory cascade in microvascular endothelial cells, macrophages and lymphocytes, culminating in NFκB-B activation. It is suggested that this amplifies inflammation by triggering

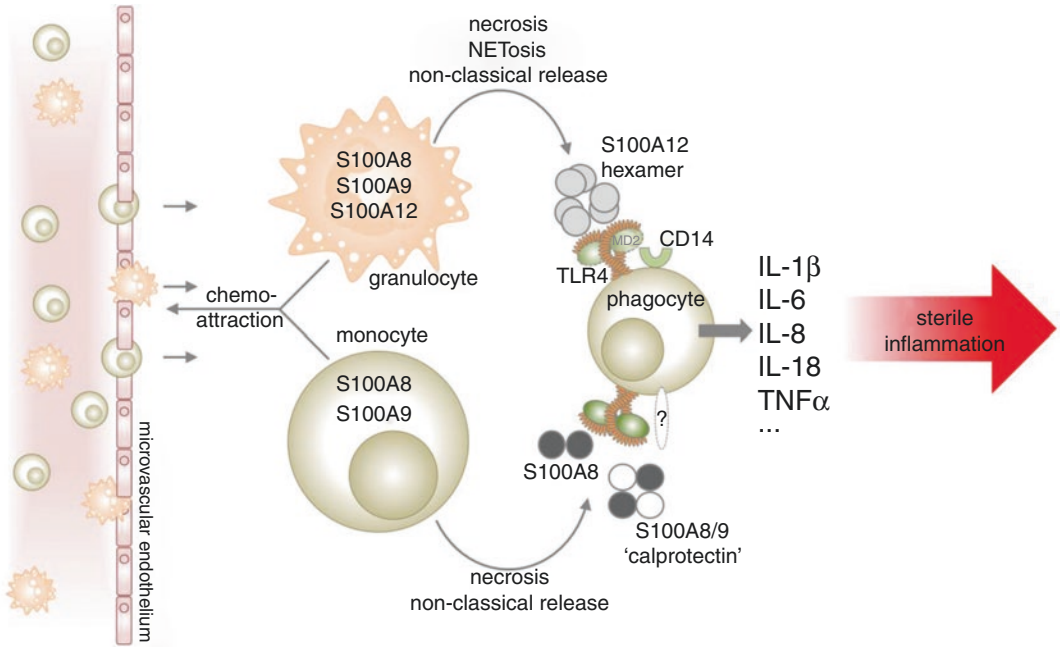

Fig. 9.2 Calgranulins as DAMPs. S100A8/A9 and S100A12 released by neutrophils and monocytes can bind and signal through TLR4 expressed on phagocytes but also other cells. TLR4-binding and signaling by S100A12 requires the protein's hexameric quaternary structure as well as surface expression of CD14, but seems independent from the MD2-subunit of TLR4 [37]. Receptor-complex elements required for S100A8/A9-signalling are yet unclear but TLR4-binding is suggested to occur via S100A8, while S100A9 is speculated to antagonize this. TLR4-dependent phagocyte activation by calgranulins triggers expression of chemoattractants, resulting in further cell-influx, as well as pro-inflammatory cytokines, which promotes sterile inflammation

further RAGE expression and thus drives a feed-forward loop that can potentiate inflammation [42, 43].

However, most studies limit receptor binding and inflammatory signaling of calgranulins to toll-like receptor 4 (TLR4). TLR4 binding is supposedly primarily mediated by S100A8 as part of the S100A8/A9 complex, whereas S100A12 needs to be arranged into its hexameric quaternary structure to bind and signal through TLR4 expressed on human monocytes [37, 44]. TLR4-signalling by S100A8/A9 and S100A12 can result in proinflammatory cytokine expression by myeloid as well as lymphoid cells and promote myeloid cell migration [31, 45–47] (Fig. 9.2).

Although, overexpression of S100A8/A9 and S100A12 appears concordant in certain autoinflammatory diseases, the proteins may exert divergent DAMP-functions. While monocytic gene expression profiles induced by S100A8 and LPS as primary TLR4-ligands are very similar [48], S100A12 induces a partly different gene expression profile compared to LPS [31].

9.2 S100 Proteins in Autoinflammatory Diseases

Key Points

- **Markedly elevated S100 protein levels are a hallmark of systemic juvenile idiopathic arthritis (SJIA), familial Mediterranean fever (FMF) and PSTPIP1-associated inflammatory diseases (PAID)**
- **Hypersecretion of S100 proteins triggers autoinflammatory processes by acting as an**

Table 9.1 Serum concentration of phagocyte-specific S100 proteins in systemic inflammatory diseases (adapted and updated from Kessel et al. 2013 [36])

	S100A8/A9 levels (ng/mL)	N[c]	Ref.	S100A12 levels (ng/mL)	N[c]	Ref.
Healthy controls	340 ± 70	50	[61]	50 ± 10	45	[62]
				50 (5)[b]	74	[73]
Monogenic autoinflammatory diseases						
FMF	110,000 ± 82,000		[50]	6720 ± 4960	17	[62]
				33,500 (22,200)[b]	7	[81]
PAPA	116,000 ± 74,000			–		
PAMI	2,045,000 ± 1,300,000			–		
NOMID	2830 ± 580	18	[61]	720 ± 450	18	[62]
MWS	4390 (2535)[a]	12	[65]	150 ± 60	17	[62]
FCAS	3600 (4610)[a]	5	[65]	–	–	–
Polygenic autoinflammatory diseases						
Systemic-onset JIA	14,920 ± 4030	60	[61]	7190 ± 2690	60	[62]
	24,750 ± 11,410	20	[86]	3700 (1080)[b]	33	[18]
Polyarthritis JIA	2380 ± 530	89	[18, 97, 98]	395 (45)[b]	89	[18]
PFAPA	3846 ± 1197	15	[71]	685 ± 210	15	[71]
Vasculitis						
Kawasaki disease	3630 ± 480	21	[99]	398 (294)[a]	67	[100]
				450 ± 106	50	[84]
IgA vasculitis/ Henoch-Schoenlein nephritis	881 ± (670)[a]	30	[101]	–	–	–
Infections						
Severe febrile infections	3720 ± 870	66	[61]	470 ± 160	83	[62]

All other data are mean ± 95% confidence interval
FCAS familial cold autoinflammatory syndrome, *FMF* familial Mediterranean fever, *JIA* juvenile idiopathic arthritis, *MWS* Muckle Wells syndrome, *NOMID* Neonatal onset multisystem inflammatory disorder, *PAMI* PSTPIP1-associated myeloid-related proteinemia inflammatory, *PAPA* pyogenic sterile arthritis, pyoderma gangrenosum, and acne syndrome, *PFAPA* periodic fever, aphthous stomatitis, pharyngitis, adenitis syndrome
[a]Mean (standard deviation)
[b]Mean (standard error of the mean)
[c]*N* number of patients studied

endogenous TLR-4 ligand, e.g. by a feed-forward loop together with IL-1β

- **Mutations in pyrin and PSTPIP1 are associated with enhanced calgranulin levels pointing to an involvement of cytoskeletal structures**

S100 proteins are not specific for autoinflammatory processes, however, hypersecretion of calgranulins in certain autoinflammatory diseases can result in a sterile inflammatory environment, which triggers proinflammatory cytokine as well as further S100A8/A9 and S100A12 expression and thus can perpetuate disease activity [36, 49]. Markedly elevated S100 levels are a hallmark of SJIA, FMF and PSTPIP1 associated inflammatory diseases (PAID) such as pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome or PSTPIP1-associated myeloid-related proteinemia inflammatory (PAMI) syndrome [50] (see Chaps. 16, 22 and 32) and differentiate these conditions from other infectious or autoinflammatory conditions (Table 9.1). In contrast, in the cryopyrin associated periodic syndromes (CAPS, see Chap. 19) or periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) syndrome (see Chap. 30) S100 levels are lower and within the range of usual proinflammatory levels. In these entities S100 proteins are not able to differentiate from infectious diseases; however, they do correlate with disease activity.

9.2.1 Monogenic Autoinflammatory Syndromes

9.2.1.1 Familial Mediterranean Fever (FMF)

FMF is an autoinflammatory syndrome associated with the activation of phagocytic cells and oversecretion of IL-1β (see Chap. 16) Mutations in pyrin are the genetic basis of a complex pathogenesis of dysfunction of intracellular processes, e.g. alternative secretory pathways, and immune dysregulation involving inflammasome-dependent recruitment and processing of IL-1β [51]. During inflammatory attacks serum levels of S100A8/A9 and S100A12 are massively elevated and are significantly higher than in CAPS [50, 52]. The excessive amount of S100A12 in FMF as opposed to other autoinflammatory diseases suggests that the neutrophil-derived S100 proteins may be involved in pathogenesis of this disease and that their release is independently regulated from inflammasome activation. As mentioned above, S100A8/A9 co-localizes with the cytoskeleton and a Golgi-independent, but tubulin-dependent release, has been shown [16, 30]. Interestingly, pyrin likewise is associated with these subcellular structures while colchicine blocks tubulin-dependent processes at the molecular level and is therefore a possible inhibitor of alternative secretion [53].

9.2.1.2 PSTPIP1 Associated Inflammatory Diseases (PAID)

In the last 3 years, the spectrum of autoinflammatory diseases due to mutations in PSTPIP1 with distinct clinical phenotypes has been expanded (see Chap. 22), indicating that the PAPA syndrome is only one clinical entity within the spectrum of PAID [54]. Recently, PAMI syndrome (PSTPIP1 E250K mutation) has been defined as a distinct autoinflammatory disorder presenting with clinical and biochemical features not found in patients with classical PAPA syndrome. This syndrome has been formerly described as hypercalprotectinemia and hyperzincemia [50]. Mutated PSTPIP1 markedly increases pyrin binding and IL-1ß production by peripheral blood leukocytes from patients with PAPA and in cell lines transfected with both PAPA associated mutants [55]. Moreover, PAPA-associated PSTPIP1 mutants activate pyrin, thereby allowing it to interact with ASC and facilitate ASC oligomerization into an active ASC pyroptosome [56].

A hallmark of PAID are very high (PAPA:116 ± 74 μg/mL vs. 0.48 ± 0.1 μg/mL in healthy controls) or exorbitant (PAMI: 2070 ± 1190 μg/mL) S100A8/A9 serum concentrations [50]. Although the exact role of these molecules in the pathogenesis of these syndromes is not yet clear, there are several interesting links to the molecular processes described above for PSTPIP1 and pyrin. S100A8/A9 serum levels are highly elevated also in FMF (110 ± 82 mg/mL) [50]. Like PSTPIP1 and pyrin, S100A8 and S100A9 are highly expressed in phagocytes. Both proteins bind to both the subcellular actin network and microtubules in a calcium dependent manner [16]. Interestingly, Il-1ß secretion is only apparent in monocytes of PAPA patients after stimulation with the exogenous TLR-4 ligand LPS [57], which points to a putative role of endogenous TLR-4 ligands S100A8 and S100A9 for the release of IL-1ß from PAPA monocytes.

9.2.1.3 Cryopyrin-Associated Periodic Syndromes (CAPS)

CAPS comprise a group of rare autoinflammatory diseases, in which uncontrolled pro-IL-1β processing results in a constitutive excess of IL-1β release from phagocytic cells of CAPS patients [58–60]. IL-1 hypersecretion is not easy to determine *in vivo*, and is obviously only one factor among others involved in a complex immune dysregulation including phagocyte activation during autoinflammation [61]. (see Chap. 19) Although the exact role of the S100 proteins in CAPS has not yet been fully unraveled, these proteins are promising markers of IL-1ß-driven inflammation in CAPS. Accordingly, S100A12 has been shown to be elevated in patients with active neonatal-onset multisystem inflammatory disease (NOMID) and Muckle-Wells syndrome (MWS) [62]. In patients with CAPS treated with IL-1-blockers, S100A12 and S100A8/A9 both showed a rapid decline along with a normalization of neutrophil counts [63]. In a broader

approach, S100A8/A9 was demonstrated to be sensitive biomarker of disease activity in CAPS, which also indicated subclinical inflammation when CRP and ESR already were normalized [64, 65].

9.2.2 Polygenic Autoinflammatory Diseases

9.2.2.1 Systemic Juvenile Idiopathic Arthritis (SJIA)

SJIA is defined as a subtype of JIA which can be classified as an autoinflammatory syndrome rather than presenting as classical autoimmune arthritis (see Chap. 32). Serum of SJIA patients induces the transcription of genes of the innate immune system including IL-1 in peripheral blood mononuclear cells (PBMCs). In addition, activated monocytes from patients with SJIA secrete significantly higher amounts of IL-1β in comparison with monocytes of healthy controls [66].

The predominant role of the innate immune system in SJIA is furthermore underscored by the high expression and serum concentrations of S100A8, S100A9 and S100A12. The extraordinarily high serum concentrations in SJIA are closely associated with disease activity and can be found neither in other forms of inflammatory arthritis, nor in other autoimmune or infectious diseases [18, 67, 68]. Furthermore, extracellular S100A8 and S100A9 form a positive inflammatory feedback loop with IL-1ß and depletion of these proteins from SJIA patient's serum diminishes the IL-1ß inducing capacity of this serum [61]. The hypersecretion of IL-1, IL-18, S100A8, S100A9 and S100A12 points to a novel aspect regarding the pathogenesis of SJIA since they are all released by the alternative secretory pathway. In contrast to IL-1 and IL-18, S100 proteins are not processed by caspase-1 prior to release [30]. Thus, a loss of control of the alternative secretory pathway downstream of caspase-1 has been proposed to be involved in release of pro-inflammatory proteins leading to the inflammatory process of SJIA [69]. However, at present, it is not known whether secretion of IL-1β, IL-6 or S100 proteins is a primary or secondary step in the cause-and-effect chain of SJIA [70].

9.2.2.2 Periodic Fever, Aphthous Stomatitis, Pharyngitis, Cervical Adenitis (PFAPA) Syndrome

The pathogenic mechanism of PFAPA syndrome is not known, but studies indicate that the production of IL-1β by monocytes is dysregulated in these patients (see Chap. 30). Approximately 20% of patients with PFAPA were found to have *NLRP3* variants, suggesting that inflammasome-related genes might be involved in this autoinflammatory syndrome. S100A8/A9 and S100A12 are upregulated during flares and are within the range of healthy controls during symptom-free intervals. The levels of patients with disease flares are within those of systemic infections [71].

9.3 S100 Proteins in Clinical Practice

Key Points

- **S100 proteins are biomarkers of local and systemic inflammation**
- **S100 proteins are helpful tools in the differential diagnosis of fever of unknown origin**
- **S100 levels reflect disease activity and are therefore meaningful surrogate markers of therapeutic response and subclinical disease activity**

9.3.1 Use as Biomarkers

To be useful, immunological biomarkers, such as DAMPs or cytokines, should be able to support initial diagnosis, accurately reflect disease activity, and predict the further outcome of inflammatory diseases with high diagnostic accuracy through non-invasive, easily performed, reproducible and cost-effective procedures. In recent years, patient outcomes have dramatically improved in autoinflammatory diseases due to

the availability of effective therapies. However, autoinflammatory syndromes are heterogeneous, with variable progression and treatment response. While some patients respond to a single therapy, others need more intensive treatment strategies. Better biomarkers for this heterogeneity are required in all aspects of patient care. The assessment of disease activity is currently based on a combination of clinical (e.g. Autoinflammatory Disease Activity index-AIDAI, see Chap. 13) and conventional laboratory parameters such as C-reactive protein (CRP) and serum amyloid A (SAA) levels or the erythrocyte sedimentation rate (ESR). These measures have little predictive value for the future course of the disease.

DAMPs have been proven useful as mechanistic biomarkers, which are part of the local inflammatory process and reflect the disease activity when measured in the serum. The close correlation of S100 protein serum concentrations with inflammatory activity and their stability, makes these proteins useful as biomarkers for the monitoring of various diseases. Phagocyte-specific S100 proteins have been established as useful markers of both local and systemic inflammation. They correlate with disease activity in rheumatic diseases, vasculitis, inflammatory bowel disease, pulmonary diseases and infections [64, 67, 72–82]. S100 proteins have been associated with several autoinflammatory diseases and allow some prediction of relapses, probably due to the ability to detect subclinical inflammation [52, 62, 65, 83–86]. Figure 9.3 shows the different applications of S100 proteins as biomarkers during the course of autoinflammatory disor-

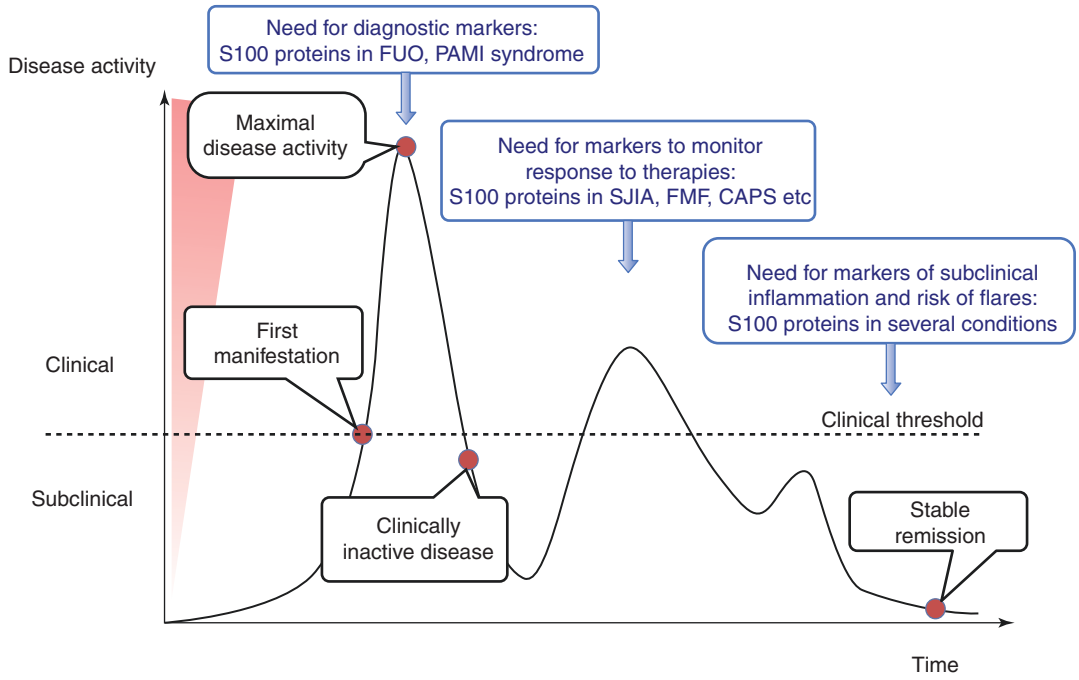

Fig. 9.3 Application of S100 proteins as biomarkers**.** There are different needs of biomarkers during the course of autoinflammatory disorders, which are addressed by measuring S100 proteins. They can be used as markers during the diagnostic work-up, e.g. in fever of unknown origin (FUO) to detect systemic juvenile idiopathic arthritis (SJIA) or familial Mediterranean fever (FMF) and when suspecting pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome or PSTPIP1-associated myeloid-related proteinemia inflammatory (PAMI) syndrome. During the future course of the disease, they can help stratifying patients for the need to initiate additional therapies (e.g. in FMF) or help monitor therapeutic responses (e.g. in SJIA or cryopyrin autoinflammatory periodic syndrome (CAPS)). Finally, in cases of clinical disease remission, S100 proteins can detect subclinical inflammatory activity that correlates to the risk of disease flares

ders. Assays are commercially available, especially for the detection of S100A8/A9. Some of these assays are certified for use in clinical diagnostics. However, these assays are not strictly comparable and especially the resultant concentrations vary among different products. For some assays validation studies have been performed and these assays can be used in clinical practice [87]. Validation and standardization remains crucial before introducing commercial assays in clinical practice. S100 proteins are stable at room temperature for several days in separated serum, so serum samples can be sent at room temperature [88]. Serum concentrations of S100 proteins are independent of age and gender [88]. Normalization of S100 levels can take 8 (in CAPS with effective canakinumab treatment [65]) to 30 days (in SJIA with effective anakinra treatment [89]). S100 proteins can be detected in the tissue of various diseases [75]. This is of scientific interest to study the role of S100 proteins in local inflammation, but is not recommended in clinical practice.

In most instances, the use of S100 proteins as biomarkers is restricted to monitoring inflammatory activity, without any specificity for a particular disease during the work-up of the differential diagnosis. Two exceptions are their application in the situation of fever of unknown origin (FUO), where they can help differentiate SJIA and FMF from other causes (see below), and their use as a disease marker in patients presenting with PAMI [50].

9.3.2 Differential Diagnosis of Fever of Unknown Origin (FUO)

FUO and unexplained signs of inflammation are challenging medical problems which are predominantly caused by infections, malignancies, immune deficiency syndromes and autoimmune or autoinflammatory diseases [90]. FUO is defined as a temperature higher than 38.3 °C on several occasions and lasting longer than 3 weeks in an immunocompetent patient, with a diagnosis that remains uncertain after extensive investigations [91, 92]. Although there is no standard definition of pediatric FUO, fever lasting anywhere from 10 days is generally accepted as the working definition of FUO in children [93].

In such a scenario, it is important to exclude other possible causes. The diagnostic approach in patients with FUO is extensive, ranging from physical examination, standardized laboratory tests to various forms of imaging, biopsies and exploratory treatment attempts with antibiotic and corticosteroids.

The differential diagnosis of FUO includes autoinflammatory syndromes, in particular SJIA and adult-onset Still disease (AOSD). At initial presentation, SJIA is difficult to differentiate from severe systemic infections. Biomarkers could be useful to make the correct diagnosis when facing a patient with suspected SJIA/AOSD. An IL-1-dependent gene expression profile exists in SJIA, but IL-1 is an unstable cytokine and thus not useful as a biomarker [66]. As IL-1 is hard to detect in patient samples, the link to S100A8/A9 provides a potential disease marker differentiating SJIA from other forms of JIA and also from other diagnoses—in contrast to markers like CRP, which are not able to differentiate SJIA from other causes of FUO [61]. The same applies for neutrophil-derived protein, S100A12 [62, 94]. Although SJIA cannot be differentiated from FMF in this context (they can be differentiated on clinical basis), these autoinflammatory diseases may at least be differentiated from other causes of FUO. To categorize a patient into one of the major subcategories autoinflammation, infection or malignancy is already very helpful and provides clues to further diagnostic and therapeutic approaches at early checkpoints.

9.3.3 Monitoring Therapies

In patients with an established diagnosis of an autoinflammatory disorder, rapid commencement of effective therapy is essential to avoid damage and complications. However, the therapeutic response is often not immediate, and among individuals, subclinical disease can have a major impact on out-

comes. Biological drugs such as IL-1-inhibitors are effective treatment options for auto-inflammatory diseases. Especially in complex-genetic diseases such as SJIA or in autoinflammatory syndromes without a clear pathogenic role of a specific gene, a cytokine or a target pathway, the response to a given choice of therapeutic interventions is therefore variable and must be monitored carefully. The identification of biomarkers that predict disease response could have a huge impact. Biomarkers can help to easily monitor inflammatory activity or to detect subtle immune disturbances and subclinical activity, which can be used to adjust the treatment to individual needs.

In autoinflammatory diseases, acute phase reactants are commonly elevated, including SAA and CRP, as markers of inflammation [95]. As more sensitive biomarkers of inflammation, the levels of S100A12 have been demonstrated to track the activity of the clinical disease and are therefore meaningful surrogate markers of the therapeutic response in MWS [64]. Various states of subclinical disease activity were demonstrated in all categories of CAPS, depending on the type of anti-IL-1 therapy. In this context, S100A8/A9 proved to be a sensitive biomarker for monitoring disease activity, and response to IL-1 blockade in patients with CAPS [65]. Here, S100 levels were compared with CRP and ESR and seemed to have a higher sensitivity to detect subclinical inflammation. In FMF, S100A12 shows an excellent correlation to disease activity [62, 81]. S100A12 may also allow stratification of FMF patients according to disease severity [49]. During acute attacks serum levels of S100A8/A9 and S100A12 in FMF are massively elevated. Interestingly, S100A12 was also useful in demonstrating subclinical inflammation in heterozygous carriers of MEFV gene mutations [96]. Moreover, patients with FMF well controlled with anti-inflammatory treatment have significantly decreased serum levels. The same applies for SJIA, where S100A8/A9 serum concentrations correlate closely with response to drug treatment and disease activity and therefore might be an additional measurement for monitoring anti-inflammatory treatment of individual patients with SJIA [86].

9.3.4 Prediction of Relapses

As in many other chronic inflammatory diseases, various triggers can cause flare-ups following periods of clinical remission. The triggers can be as trivial as cold, stress or a simple infection. An optimal treatment aim is to reach a status of stable remission with no remaining subclinical inflammation and with only minimal risk of flares. In the case of autoinflammatory diseases without a genetic background leading to constitutive immune activation (e.g. SJIA), treatment can be stopped if a stable remission has been achieved. Identifying in advance patients at particular risk for disease flares after tapering or withdrawing anti-inflammatory therapies may improve patient care and reduce stress and side effects.

S100 proteins have been shown to be markers of subclinical disease activity not detectable by clinical investigation or other laboratory tests. They are indicators of systemic inflammation but also markers of local disease activity (e.g. in the joint or tissue), and indicate subclinical inflammation [83, 88]. In SJIA, S100A8/A9 serum concentrations are the first predictive biomarker indicating subclinical disease activity and stratifying patients at risk of relapse during times of clinically inactive disease [86]. S100A8/A9 and S100A12 can thus be used as surrogate markers not only to monitor therapeutic responses at initiating therapies with the goal of inducing remission, but also during maintenance therapies.

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Part III

General Approach to Autoinflammatory Diseases

10 Classification of Genetically Defined Autoinflammatory Diseases

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Abstract

Autoinflammatory diseases are hyperinflammatory, immune-dysregulatory conditions that typically present in early childhood with fever, rashes and disease-specific patterns of sterile organ inflammation of predominantly innate immune cells. The identification of disease-causing genetic mutations in key innate immune pathways that regulate proinflammatory cytokines, paired with the impressive clinical responses to cytokine blocking therapies has led to the concept that cytokine activation drives "cytokine amplification loops" that lead to the development of systemic and organ-specific disease manifestations of autoinflammatory diseases. While the initial discoveries of the genetic causes of autoinflammatory diseases and the clinical treatment successes centered around conditions that were presumed to be caused by interleukin (IL)-1 overproduction and signaling, more recent studies are providing insights into proinflammatory cytokine dysregulation, that includes Type-I interferon (IFN), IL-17, IL-18 or IL-36 and more generally ubiquitination disorders that affect nuclear factor kappa B (NF-kB) dysregulation. Characteristic clinical findings such as fever patterns, type of skin lesions and pattern of organ inflammation track with specific innate immune pathways. In this chapter we use two different classification systems of the known genetically-defined autoinflammatory diseases, a clinical classification system based on skin lesions, other characteristic clinical features and the pattern of the inflammatory episodes (i.e. fever pattern), and a pathophysiological classification based on innate immune sensor and cytokine pathways that are dysregulated. The clinical and pathophysiological classification systems can be integrated.

Keywords

Classification · Autoinflammatory diseases · Dermatologic manifestations · Key inflammatory and regulatory pathways · Interleukin (IL)-1 · Type-I interferon (IFN) · IL-18 · IL-23/IL-12/IL-17 axis · Tumor necrosis factor (TNF) · Nuclear factor kappa B (NF-kB)

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Abbreviations

ACH	Acrodermatitis continua of Hallopeau
AGS	Aicardi-Goutières syndrome
AIM	Absent in melanoma
AISLE	Autoinflammatory syndrome associated with lymphedema
AMPS	AP1S3 mediated psoriasis
APLAID	PLCG2-associated autoinflammation, antibody deficiency and immune dysregulation
ASC	Apoptosis related speck-like protein containing CARD
CAMPS	CARD14-mediated psoriasis
CANDLE	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase activation and recruitment domains
CINCA	Chronic infantile neurologic, cutaneous and articular syndrome
CTLs	Cytotoxic CD8+ T cells
DADA2	Deficiency of adenosine deaminase 2
DC	Dendritic cells
DIRA	Deficiency of the interleukin 1 receptor antagonist
DITRA	Deficiency of the interleukin 36 receptor antagonist
DUB	Deubiquitinases
FCAS	Familial cold autoinflammatory syndrome
FCAS2	Familial cold autoinflammatory syndrome 2
FDA	Food and Drug Administration
FKLC	Familial keratosis lichenoides chronica
FLH	Familial hemophagocytic lymphohistiocytosis
FMF	Familial Mediterranean fever
GOF	Gain-of-function
GPP	Generalized pustular psoriasis
HA20	Haploinsufficiency of A20
HIDS	Hyperimmunoglobulinemia D and periodic fever syndrome
HLH	Hemophagocytic lymphohistiocytosis
HSCT	Hematopoietic stem cell transplantation
IL	Interleukin
IL-1Ra	IL-1 receptor antagonist
IFN	Interferon
IRF	Interferon regulatory factor
IRS	IFN response gene signature
ISGF	Interferon stimulated gene factor
JAK	Janus kinase
JMP	Joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy
LACC1	Laccase (multicopper oxidoreductase) domain-containing 1
LOF	Loss-of-function
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
LUBAC	Linear ubiquitination chain assembly complex
MAP	Mitogen-activated protein
MAS	Macrophage activation syndrome
MDA5	Melanoma differentiation-associated protein 5
MDFIC	MyoD family inhibitor domain containing
MKD	Mevalonate kinase deficiency
MPO	Myeloperoxidase
MSPC	Multiple self-healing palmoplantar carcinoma
MWS	Muckle-Wells syndrome
NAIAD	NLRP1-associated autoinflammation with arthritis and dyskeratosis
NAIP	NLR family apoptosis inhibitory protein
NDAS	NEMO deleted exon 5 autoinflammatory syndrome—X-linked
NEMO	NF-κB essential modulator
NF-κB	Nuclear factor kappa B
NISBD1	Neonatal inflammatory skin and bowel disease-1
NK	Natural killer
NLR	NOD-like receptor

NLRC	NOD-like receptor family CARD domain containing
NLRP	NOD-like receptor family pyrin domain containing
NOD	Nucleotide-binding oligomerization domain
NOMID	Neonatal-onset multisystem inflammatory disease
NSAIDs	Non-steroidal anti-inflammatory drugs
ORAS	Otulin-related autoinflammatory syndrome/Otulipenia
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PAMP	Pathogen-associated molecular pattern
PAPA	Pyogenic arthritis, pyoderma gangrenosum and acne (syndrome)
PFIT	Periodic fever, immunodeficiency, and thrombocytopenia
PGA	Pediatric granulomatous arthritis
PLAID	PLCG2-associated antibody deficiency and immune dysregulation
PPP	Palmoplantar psoriasis
PRAAS	Proteasome-associated autoinflammatory syndrome
PRR	Pattern recognition receptors
PYD	Pyrin domain
RIG	Retinoic acid-inducible gene
RLR	RIG-like receptor
SAVI	STING-associated vasculopathy with onset in infancy
SCAN	Syndrome of enterocolitis and autoinflammation associated with mutation in NLRC4
SDH	Succinate dehydrogenase
SIFD	Sideroblastic anemia with B-cell immunodeficiency, periodic fevers, and developmental delay
SIRS	Systemic inflammatory response syndrome
SMS	Singleton-Merten syndrome
SPENCD	Spondyloenchondrodysplasia with immune dysregulation
STAT	Signal transducer and activator of transcription
STING	Stimulator of IFN genes
TACE	TNF-α convertase enzyme
TNF	Tumor necrosis factor
TORCH	Toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex
TRAP	Tartrate-resistant phosphatase
TRAPS	TNF receptor-associated periodic syndrome
TRAPS11	TNFRSF11A-associated hereditary fever disease
TYK	Tyrosine kinase
USP	Ubiquitin-specific peptidase
VEOIBD	Very early-onset inflammatory bowel disease
XIAP	X-linked inhibitor of apoptosis

Key Points

- **This chapter describes two classification systems of the genetically-defined autoinflammatory diseases, one based on characteristic clinical features and another on disease pathogenesis**
- **Most autoinflammatory diseases present with disease-specific clinical manifestations and can be characterized on the presence of absence of skin rashes and/or the histology of the skin biopsies, and the pattern of systemic inflammatory episodes, including the fever pattern**
- **Many but not all genetically-defined autoinflammatory diseases are caused by mutations that dysregulate innate immune sensor pathways and lead to excessive pro-inflammatory cytokine signaling**
- **Clinically overlapping features correlate with patterns of cytokine dysregulation (i.e. interleukin (IL)-1 and Type-I interferon (IFN) and suggest a specific role for key cytokines in causing or amplifying organ pathology that can be used to reconcile a clinical and pathogenesis based classification system**

10.1 Introduction

The term "autoinflammatory diseases" was introduced in 1999 in a seminal paper by Drs. Michael McDermott and Daniel Kastner et al., that reported the genetic cause for the tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), 2 years after the discovery of the gene for the first and most prevalent monogenic periodic fever syndrome, familial Mediterranean fever (FMF) [1–3]. This concept aimed to distinguish FMF and TRAPS as members of a new class of autoinflammatory diseases, from autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. The latter conditions were thought to be caused by adaptive immune dysregulation, marked by high-titer autoantibodies and auto-reactive lymphocytes (see Chap. 1).

The recognition of a key role for IL-1 dysregulation in autoinflammatory diseases started with the discovery by Dr. Hal Hoffman's group that gain-of-function (GOF) mutations in the gene encoding the first recognized human intracellular sensor of microbial danger signals, the nucleotide-binding oligomerization domain (NOD)-like receptor with a pyrin domain (*NLRP3*), cause the disease spectrum of cryopyrin-associated autoinflammatory syndrome (CAPS) [4]. Work by several groups foremost Drs. John Bertin and Jorg Tschopp established the NLRP3 inflammasome as a molecular platform that tied danger recognition to the activation and release of the pro-inflammatory cytokine interleukin (IL)-1 (see Chaps. 4, 5, 6, and 19). These discoveries led to the successful use of IL-1 blocking strategies as treatments in patients with CAPS [5, 6] that culminated in the approval of three IL-1 blocking agents for the treatment of CAPS: rilonacept (Arcalyst®) in 2008, canakinumab (Ilaris®) in 2009, and anakinra (Kineret®) in 2012 by the Food and Drug Administration (FDA) and ignited a paradigm shift in our understanding and treatment of autoinflammatory diseases.

The discovery that loss-of-function (LOF) mutations in *IL1RN*, that lead to the absence of functional IL-1 receptor antagonist causes deficiency of the interleukin-1 receptor antagonist (DIRA) [7] demonstrated the potent role of uninhibited IL-1 signaling and amplification in causing a systemic inflammatory response syndrome leading to organ failure and death. These findings in CAPS and DIRA forged the concept of IL-1/cytokine amplification as contributing to the systemic and organ-specific disease manifestations, clinically confirmed by the successful use of IL-1 blocking agents [8]. More recently, discoveries of mutations in pathways that affect the production of Type-I interferons (IFNs), or increase the production of IL-18, or amplify IL-17 and IL-36 signaling, point to additional innate cytokine amplification loops beyond IL-1 and additional targets for treatment and justified a classification system based on additional innate immune pathway dysregulation [9].

Figure 10.1 depicts a schema of key components of an innate immune response including an innate immune sensor, an effector pathway that links innate immune sensing to the production of an inflammatory mediator, negative regulatory molecules of the inflammatory pathway and cell metabolic and/or homeostatic pathways that when dysregulated can trigger innate immune sensors and responses. The genetic causes of autoinflammatory diseases are organized by the component of the innate immune response that is affected, i.e. disease-causing mutations that affect the innate immune sensing, signaling and its negative regulation, and those that link dysregulation of critical homeostatic mechanisms to innate immune pathways.

First, GOF mutations (depicted in red) that increase cytoplasmic pattern recognition receptors (PRR), either NOD-like receptors (NLRs) or retinoic acid-inducible gene (RIG) like recep-

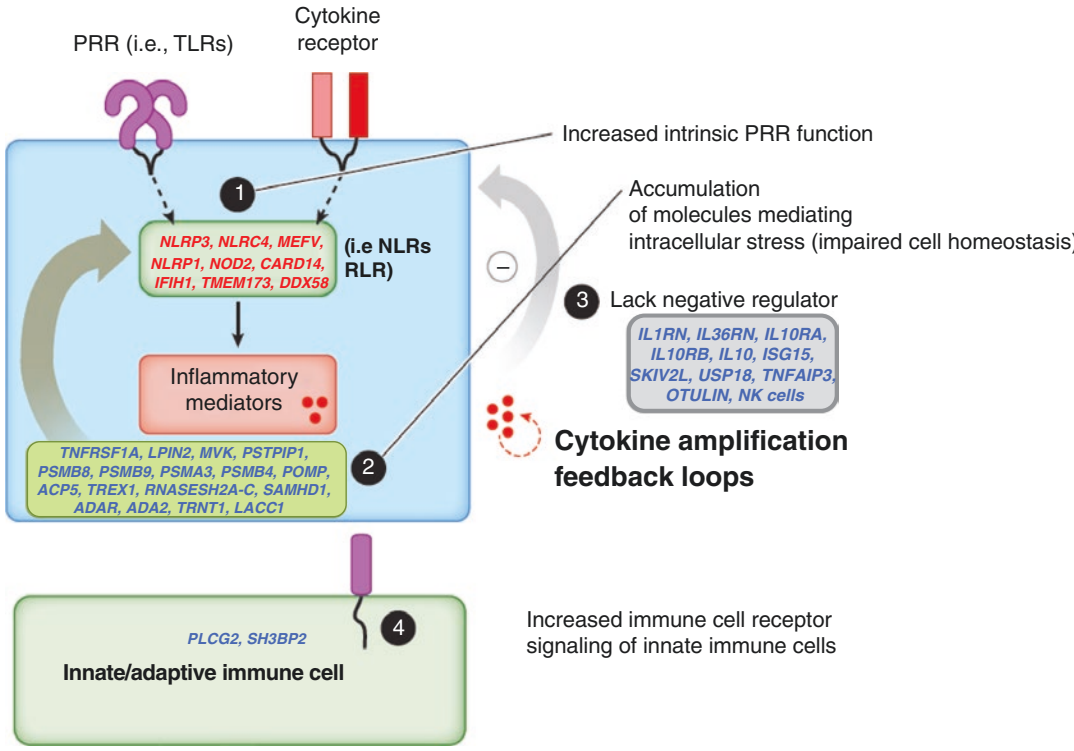

Fig. 10.1 Principles of immune dysregulation in autoinflammatory diseases: The innate immune system relies on danger recognition by germline-encoded pattern recognition receptors (PRRs) such as membrane bound receptors (i.e. Toll-like receptors-TLRs), and intracellular sensors (nucleotide-binding oligomerization domain (NOD)-like receptors-NLRs and retinoic acid-inducible gene 1 receptor (RIG)-like receptors-RLRs). Genes that are mutated and lead to autoinflammatory phenotypes contribute to the innate immune dysregulation in specific ways as outlined below. (**1**) *Gain-of function (GOF) mutations in genes encoding PRRs, referred to as "sensors"* that recognize and or respond to microbial or intracellular danger signals, *or mutations in their adaptors* lead to increased production of inflammatory mediators and cause autoinflammatory disease phenotypes. (a) GOF-mutations in the intracellular sensors that form interleukin (IL)-1 activating inflammasomes and cause activation of caspase-1: *NLRP3, MEFV*, *NLRC4* and *NLRP1* are linked to increased IL-1 production. (b) GOF-mutations in the viral RLRs, *IFIH1/MDA5* and *DDX58/RIG-I*, or in the adaptor molecule, *TMEM173/STING* are linked to increased Type-I interferon (IFN) production. (c) GOF mutations in *NLRC4* that lead to high IL-18 production, prime for the development of macrophage activation syndrome (MAS). (d) GOF mutations in *CARD14* lead to nuclear factor kappa B (NF-κB) activation in keratinocytes and recruitment of IL-17 producing cells that perpetuate epidermal inflammation. (e) Whether mutations in *NOD2/CARD15* that cause Blau syndrome are GOF or loss-of-function (LOF) is still not fully clarified (**2**) *LOF mutations in molecules that control cellular homeostatic pathways result in cell maladaptation and stress* and cause autoinflammatory phenotypes. (a) LOF mutations in enzymes or molecules that affect protein homeostasis (i.e. protein misfolding, endoplasmic reticulum transport, protein degradation and clearance (i.e. proteasome components, *PSMB8, PSMB9, PSMA3, PSMB4, POMP*); (b) Mitochondrial function (i.e. *TRNT1*) and oxidative stress production/signaling (i.e. *LACC1*); (c) Intracellular trafficking (i.e. *MVK, TNFRSF1A, LPIN2*); autophagy (i.e. *NOD2*); (d) Cell differentiation (i.e. *ADA2*); (e) Nucleotide metabolism/degradation (i.e. *TREX1, SAMHD1*, *RNASEA-C, ADAR*). (**3**) *LOF mutations resulting in negative regulators of an immune response* can also lead to autoinflammatory phenotypes. (a) Negative regulators of cytokine receptor function (i.e. *IL1RN, IL36RN, USP18, ISG15, SKIV2L)* or loss of an anti-inflammatory cytokine or its function *(IL10, IL10RA, IL10RB)*; (b) Deubiquitination defects that increase NF-κB signaling *(i.e. TNFAIP3, OTULIN)*; (c) Loss of natural killer (NK) cell function. (**4**) *Increased signaling through receptors that control innate immune cell function* lead to hyperresponsiveness to immune signals. As the signaling abnormalities affect innate and adaptive immune cells, patients with these latter mutations often present with overlapping clinical features of autoinflammation, mild immunodeficiencies, and/or autoimmunity [145]

tors (RLRs) that are linked to proinflammatory cytokine production (see Chap. 4). Second, LOF mutations (depicted in blue) in enzymes or molecules that are critical in cell homeostatic processes generate "cell stress molecules" that activate cytoplasmic sensor platforms and proinflammatory cytokines. Third, LOF mutations in negative regulators of innate immune responses (depicted in blue), that when mutated fail to dampen or downregulate an innate immune response. Last, mutations in signaling molecules that modulate innate immune cell function can cause autoinflammatory phenotypes [9]. As many of these signaling molecules affect innate and adaptive immune cells and in some instances tissue specific cells, mutations in these genes often cause mixed clinical phenotypes of innate and adaptive immune cell dysfunction and clinical features of autoimmunity and immunodeficiency including infections (see Chaps. 28 and 38).

We focus disease classification on genetically defined autoinflammatory diseases for which sufficient mechanistic and treatment data suggest a prominent primary role of innate immune (autoinflammatory) dysregulation. We did not include presumed "complex autoinflammatory diseases" as we currently do not have sufficient knowledge nor biomarkers to define these conditions as "autoinflammatory diseases" based on clinical or immunological grounds. Furthermore, evidence emerges that several complex or presumed polygenic diseases are caused by a mixture of innate (autoinflammatory), adaptive (autoimmune) immune dysregulatory mechanisms, and/or by various immunodeficiencies (see Chap. 38). Further, secondary innate immune (or autoinflammatory) dysregulatory responses accompany and often aggravate common metabolic, degenerative or proliferative conditions. Examples include coronary artery disease, Alzheimer disease or hematologic or solid organ malignancies (see Chap. 39). Some authors refer to these conditions are "autoinflammatory" to underline the innate immune responses observed, as the outcomes of these conditions may even be dependent on the magnitude or absence of an innate or adaptive immune response.

In this chapter we used two classifications systems: clinical and pathophysiologic and attempt to integrate both systems. The *clinical classification* is based on skin involvement and fever pattern. The skin is the largest barrier organ with critical inherent innate immune defense mechanisms to maintain a homeostatic balance of skin microbiome and pathogenic organisms. It is perhaps the multitude of innate immune mechanisms that operate in the skin, that when dysregulated bring forth the various skin rashes in autoinflammatory diseases. Skin rashes (e.g. urticaria, psoriasis, livedo pattern, plaque–like rashes and others) have proven to be clinically useful to differentiate between the monogenic autoinflammatory diseases based on the type of skin lesion and the pattern of skin involvement. The *pathophysiologic classification* is based on the cytokine or the innate immune pathway predominantly involved in causing the disease, which in most instances has been validated based on the response to specific, cytokine-blocking therapies. As the cytokine dysregulation causes organ dysregulation and damage, it is perhaps not too surprising that pathophysiologic pathways correlate with specific skin manifestations (e.g. the Type-I IFN pathway with vascular, livedo-like rashes). Thus, in Table 10.1 and Supplementary Data 1 we attempt to reconcile and join both classification systems.

Table 10.1 Classification of genetically defined autoinflammatory diseases based on disease pathogenesis and characteristic clinical features

			Group No	Gene (chromosome region)	Clinical Findings	
					Mucocutaneous	Other / Specific features
Autoinflammatory diseases caused by excessive interleukin (IL)-1 signaling, production and secretion						
Intrinsic PRR activation	**CAPS**	**NOMID**	Group 2b	*NLRP3* (1q44)	Neutrophilic urticaria	Sensorineural hearing loss, bony overgrowth, cognitive impairment, hydrocephalus and brain atrophy, visual nerve atrophy and blindness
		MWS	Group 2b	*NLRP3* (1q44)	Neutrophilic urticaria	Sensorineural hearing loss
		FCAS	Group 2a	*NLRP3* (1q44)	Neutrophilic urticaria	Sensorineural hearing loss (very rare)
	FMF		Group 1a	*MEFV* (16p13.3)	Erysipelas-like erythema	Serositis and abdominal adhesions, pericarditis, epididymitis
	PAAND		Group 3f	*MEFV* (16p13.3)	Severe acne, pyoderma gangrenosum, neutrophilic small vessel vasculitis	Pyoderma gangrenosum without pyogenic arthritis
Extrinsic PRR activation	**HIDS/MKD**		Group 1a	*MVK* (12q24.11)	Maculopapular or purpuric exanthema	Abdominal pain, recurrent and or severe infections in up to 30% of the patients
	TRAPS		Group 1b	*TNFRSF1A* (12p13.31)	Erysipelas-like erythema	Pericarditis, scrotal pain, prolonged fever episodes
	Majeed syndrome		Group 3a	*LPIN2* (18p11.31)	Pustular dermatitis	Sterile osteomyelitis, dyserythropoietic anemia
Loss of negative regulation	**DIRA**		Group 3a	*IL1RN* (2q13)	Pustular dermatitis	Osteomyelitis and periostitis, bone deformities, absence of odontoid process, venous thrombosis, vasculitis (rare)
Interferon mediated autoinflammatory diseases						
Intrinsic PRR or adaptor molecule activation	**SAVI**		Group 5a	*TMEM173* (5q31.2)	Erythematous-purpuric lesions, ischemic ulcerative skin disease, necrosis of extremities, loss of tissue	Acral and cold sensitive area vasculitis (chilblain distribution), nasal septum perforation, anemia, lymphopenia, hypergammaglobulinemia
	AGS7		Group 5b	*IFIH1* (2q24.2)	Chilblain lesions, livedo reticularis	Variable severity of CNS disease, thrombocytopenia
Extrinsic PRR activation	**AGS1**		Group 5b	*TREX1* (3p21.31)	Chilblain lesions, livedo reticularis	Thrombocytopenia, neonatal-onset, higher mortality

Table 10.1 (continued)

		Group No	Gene (chromosome region)	Clinical Findings	
				Mucocutaneous	Other / Specific features
	AGS2	Group 5b	*RNASEH2B* (13q14.3)	Chilblain lesions, livedo reticularis	Later onset, lower morbidity and mortality
	AGS3	Group 5b	*RNASEH2C* (11q13.1)	Chilblain lesions, livedo reticularis	Thrombocytopenia, neonatal-onset, higher mortality
	AGS4	Group 5b	*RNASEH2A* (19p13.2)	Chilblain lesions, livedo reticularis	Thrombocytopenia, neonatal-onset, higher mortality
	AGS5	Group 5b	*SAMHD1* (20q11.23)	Chilblain lesions, livedo reticularis	Later onset, lower morbidity and mortality, inflammatory intracranial large-vessel vasculitis
	AGS6	Group 5b	*ADAR* (1q21.3)	Chilblain lesions, livedo reticularis	Later onset, lower morbidity and mortality
	CANDLE	Group 4a	*PSMB8* and other genes (6p21.32)	Nodular exanthema, panniculitis, lipodystrophy	Myositis, arthritis, dyslipidemia, growth delay, anemia, cytopenias
	SPENCD	Group 5c	*ACP5* (19p13.2)	Hyperpigmentation, vitiligo, hemangioma	Recurrent infections (pneumonia and URI), spondylometaphyseal dysplasia, short stature, autoimmunity (AIHA, AITP, positive ANA, thyroiditis)
Loss of negative regulation	**USP18 deficiency**	Group 5b	*USP18* (22q11.21)	Petechiae with thrombocytopenia	Thrombocytopenia, early mortality (1 week-22 days of life)
Autoinflammatory diseases caused by nuclear factor kappa B (NF-κB) dysregulation in keratocytes					
Intrinsic PRR or adaptor molecule activation	**CAMPS**	Group 3d	*CARD14* (17q25.3)	Plaque or pustular psoriasis	Rare systemic manifestations
Extrinsic PRR activation	**AMPS**	Group 3d	*AP1S3* (2q36.1)	Generalized or palmo-plantar pustular psoriasis, nail dystrophy	Severe nail dystrophy and digit tapering may be observed
Loss of negative regulation	**DITRA**	Group 3d	*IL36RN* (2q13)	Generalized pustular psoriasis	Fever of elevated temperature, secondary skin infections
Autoinflammatory diseases caused by nuclear factor kappa B (NF-κB) also affecting interferon (IFN) signaling					
Loss of negative regulation	**ORAS**	Group 4b	*OTULIN* (5p15.2)	Nodular panniculitis and lipodystrophy, pustular and scarring rash (n=1)	Failure to thrive, early age of onset (1-4.5 months-old)

Table 10.1 (continued)

			Group No	Gene (chromosome region)	Clinical Findings	
					Mucocutaneous	Other / Specific features
	NDAS		Group 4b	*IKBKG* (Xq28)	Lymphohistiocytic panniculitis, lipodystrophy	Non-caseating granulomas in skin, lymph nodes and liver, conical teeth (n=1), hypogammaglobulinemia, thrombocytopenia
Loss of negative regulation	**HA20**		Group 3c	*TNFAIP3* (6q23.3)	Oral ulcers, genital ulcers, erythematous papules, folliculitis, pathergy	Hemolytic anemia, thrombocytopenia, positive ANA
Autoinflammatory diseases caused by nuclear factor kappa B (NF-κB) dysregulation and granulomatous diseases						
Intrinsic PRR or adaptor molecule dysregulation	**Blau syndrome/PGA**		Group 6a	*NOD2/CARD15* (16q12.1)	Ichthyosis-like exanthema	Granulomatous arthritis, parotitis, pericarditis (rare)
Extrinsic PRR dysregulation	**LACC1**		Group 7a	*LACC1* (13q14.11)	Erythematous maculopapular rash	Quotidian fever, arthritis
Autoinflammatory diseases caused by systemic macrophage activation (with and without high IL-18 levels)						
Intrinsic PRR or adaptor molecule activation (high IL-18 levels)	**NLRC4-MAS**		Group 7a	*NLRC4* (2p22.3)	Rare dermographism / urticarial rash	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
	XLP2-MAS		Group 7a	*XIAP* (Xq25)	Uncommon	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
Impaired cytotoxicity	**Without albinism**	**FHL2**	Group 7b	*PRF1* (10q22.1)	Uncommon	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
		FHL3	Group 7b	*UNC13D* (17q25.1)	Uncommon	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
		FHL4	Group 7b	*STX11* (6q24.2)	Uncommon	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
		FHL5	Group 7b	*STXBP2* (19p13.2)	Uncommon	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
	With albinism	**CHS**	Group 7b	*LYST* (1q42.3)	Skin and hair hypopigmentation, photosensitivity	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
		GS2	Group 7b	*RAB27A* (15q21.3)	Skin and hair hypopigmentation, photosensitivity	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides

Table 10.1 (continued)

			Group No	Gene (chromosome region)	Clinical Findings	
					Mucocutaneous	Other / Specific features
		HPS2	Group 7b	*AP3B1* (5q14.1)	Skin and hair hypopigmentation, photosensitivity	Coagulopathy, pancytopenia, ↑ferritin, ↑triglycerides
Autoinflammatory diseases caused by enzymatic defects in innate and adaptive immune cell signaling pathways						
PLAID			Group 6b	*PLCG2* (16q23.3)	Cold-induced urticaria and/or granulomatous skin rash	Positive autoantibodies and autoimmune manifestations, recurrent and/or severe infections, allergic disease
APLAID			Group 6b	*PLCG2* (16q23.3)	Erythematous plaques and vesico-pustular lesions, cellulitis	Mild immunodeficiency
Cherubism			NA	*SH3BP2* (4p16.3)	No rash reported	Oligodontia, agenesis of teeth, displaced teeth
Unclassifiable autoinflammatory diseases based on pivotal inflammatory mediators						
Accumulation of intracellular stress	**PAPA**		Group 3b	*PSTPIP1* (15q24.3)	Pyoderma gangrenosum, severe acne	Thrombocytopenia and neutropenia (rare) deforming aseptic pyogenic arthritis
	PFIT		Group 3f	*WDR1* (4p16.1)	Severe recurrent oral ulcers with scarring and microstomia, recurrent perianal ulceration	Hyperferritinemia, thrombocytopenia, neutropenia, recurrent infections
	DADA2		Group 5d	*ADA2* (22q11.1)	Livedo reticularis, purpuric lesions and ischemic and necrotic skin disease	Testicular pain, portal hypertension, lymphopenia, low IgM, recurrent infections
Loss of negative regulator	**IBD28**		Group 3e	*IL10RA* (11q23.3)	Folliculitis	Colitis, recurrent infections
	IBD25		Group 3e	*IL10RB* (21q22.11)	Folliculitis	Colitis, recurrent infections
	IBD with IL-10 deficiency		Group 3e	*IL10* (1q32.1)	Folliculitis	Colitis, recurrent infections
	NISBD		Group 3e	*ADAM17* (2p25.1)	Perioral and perianal erythema and generalized pustular rash	Cardiomyopathy, secondary skin infections, paronychia, increased IgE

Table 10.1 (continued)

Predominantly dysregulated cytokine	
	IL -1
	Type 1 IFN
	IL-17/IL-23/IL-36
	TNF but also IL-1 (HA20) and IFN (NDAS, ORAS)
	IL-18
	Unclear but likely loss of cytokine signaling
	IL-10 deficiency

PRR pattern recognition receptors; *IL* interleukin; *CAPS* cryopyrin-associated periodic syndrome; *FCAS* familial cold autoinflammatory syndrome; *MWS* Muckle-Wells syndrome; *NOMID* neonatal-onset mutlisystem inflammatory disease; *CINCA* chronic infantile neurological cutaneous and articular syndrome; *FMF* familial Mediterranean fever; *PAAND* pyrin-associated autoinflammation with neutrophilic dermatosis; *HIDS/MKD* hyperimmunoglobulinemia D with periodic fever syndrome/mevalonate kinase deficiency; *TRAPS* tumor necrosis facor receptor-associated periodic syndrome; *DIRA* deficiency of the interleukin-1 receptor antagonist; *SAVI* STING-associated vasculopathy with onset in infancy; *AGS* Aicardi-Goutières syndrome; *CANDLE* chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; *SPENCDI* spondyloenchondrodysplasia with immune dysregulation; *CAMPS* CARD14-mediated psoriasis; *AMPS* AP1S3 mediated psoriasis; *DITRA* deficiency of the IL-36 receptor antagonist; *ORAS* otulin-related autoinflammatory syndrome/otulipenia; *NDAS* NEMO deleted exon 5 autoinflammatory syndrome—X-linked; *HA20* haploinsufficiency of A20; *LACC1* laccase (multicopper oxidoreductase) domain-containing 1; *MAS* macrophage activation syndrome; *FHL* familial hemophagocytic lymphohistiocytosis; *CHS* Chediak-Higashi syndrome; *PLAID* PLCG2-associated antibody deficiency and immune dysregulation; *APLAID* PLCG2-associated autoinflammation, antibody deficiency and immune dysregulation; *PAPA* pyogenic arthritis, pyoderma gangrenosum and acne syndrome; *PFIT* perioidc fever, immunodeficiency, and thrombocytopenia; *DADA2* deficiency of adenosine deaminase 2; *IBD* inflammatory bowel disease; *AR* autosomal recessive; *AD* autosomal dominant; *URI* upper respiratory infection; *AIHA* autoimmune hemolytic anemia; *AITP* autoimmune thrombocytopenic purpura; *ANA* anti-nuclear antibody; *IFN* interferon; *TNF* tumor necrosis factor

10.2 Clinical Classification of Autoinflammatory Diseases

Genetic analyses are the gold standard for the diagnosis of the genetically defined autoinflammatory diseases, but it can take weeks to months to obtain the genetic results particularly in patients with somatic mutations, in whom disease causing mutations are still challenging to identify (see Chaps. 2 and 12).

However, clinical characteristics, particularly the fever pattern, characteristic skin rashes and specific inflammatory organ manifestations distinguish the different autoinflammatory diseases and can be used in clinical diagnosis (see Chap. 11), to obtain early clues to the underlying pathogenesis and to initiate treatment. They can also be used to classify autoinflammatory diseases [10]. Skin biopsies of various rashes associated with autoinflammatory diseases have pointed to skin infiltration of predominantly innate immune cells, and even if absent or only sporadically present (e.g. FMF, a Behçet-like disease) can help in disease classification. Table 10.2 is an expansion of a previously proposed clinical classification that includes novel autoinflammatory diseases [11].

Each of the diseases listed in Table 10.1 is discussed in detail in the respective chapters, therefore the description of the diseases below is concise and focused on the skin and on characteristic organ manifestations that are used for the clinical classification. "Group 8" comprises a group of conditions that have too little clinical and mechanistic data for classification; therefore details are not included in Table 10.1.

Table 10.2 Clinical classification of genetically defined autoinflammatory diseases

Clinical presentations	Sensor	Inflammation-triggering molecules and pathways	Cytokine dysregulation
Group 1. Recurrent/episodic fever and abdominal pain with absence or sporadic presence of maculopapular rashes (hereditary periodic fever syndrome)	Pyrin, other?	Inactive RhoGTPase due to prenylation defect	IL-1
Group 2. Neutrophilic urticaria (cryopyrin-associated periodic syndrome—CAPS)	NLRP3		IL-1
Group 3. Pustular skin rashes and episodic fevers	NLRP3 and amplification of cytokine signals	Decreased phosphatase activity and cholesterol deposition in cell membrane	IL-1, IL-17, IL-36
Group 4. Vasculopathy and panniculitis/lipodystrophy syndromes	Unknown	Ubiquitinated proteins	Type-I IFN, NF-kB dysregulation
Group 5. Vasculopathy and/or vasculitis with livedo reticularis syndromes	Viral sensors, STING, other?	Nucleic acids, decreased adenosine deaminase activity	Type-I IFN, TNF
Group 6. Autoinflammatory disorders with granulomatous skin diseases	NOD2, LACC1		Multiple including IL-1, TNF, IL-6, unknown
Group 7. Autoinflammatory syndromes presenting with macrophage activation syndrome and hyperferritinemia	NLRC4, LACC1		IL-18, IL-1, IL-6
Group 8. Other autoinflammatory syndromes			

IL interleukin; *CAPS* cryopyrin-associated periodic syndrome; *NLRP* NOD-like receptor family pyrin containing; *NF-κB* nuclear factor kappa B; *IFN* interferon; *TNF* tumor necrosis factor; *NOD* nucleotide-binding oligomerization domain; *STING* stimulator of IFN genes; *NOD* nucleotide-binding oligomerization domain; *LACC* laccase (multicopper oxidoreductase) domain-containing 1; *NLRC* NOD-like receptor family CARD domain containing

10.2.1 Group 1. Recurrent/Episodic Fever and Abdominal Pain with Absence or Sporadic Presence of Maculopapular Rashes (Hereditary Periodic Fever Syndromes)

(a) **Recurrent fever attacks of short duration (typically ≤7 days)**
- **Familial Mediterranean fever (FMF; *MEFV*) (Chap. 16)**
- **Hyperimmunoglobulinemia D with periodic fever syndrome/Mevalonate kinase deficiency (HIDS/MKD; *MVK*) (Chap. 17)**

(b) **Recurrent fever attacks with longer duration (typically >7 days)**
- **TNF receptor-associated periodic syndrome (TRAPS; *TNFRSF1A*) (Chap. 18)**

10.2.1.1 Recurrent Fever Attacks of Short Duration (Typically ≤7 days)

Familial Mediterranean fever (FMF) and hyperimmunoglobulinemia D with periodic fever syndrome (HIDS)/mevalonate kinase deficiency (MKD) are characterized by episodes of high fever that are accompanied by abdominal and/or chest pain in the context of elevation of acute phase reactants (erythrocyte sedimentation rate-ESR, C-reactive protein-CRP, haptoglobin, fibrinogen). Inflammatory attacks are followed by periods of remission or reduced inflammation. In FMF and HIDS/MKD, the recurrent fever flares are of short duration, 1–3 days in FMF, and 3–7 days in HIDS/MKD [12, 13].

Familial Mediterranean Fever (FMF)

FMF is the most prevalent monogenic autoinflammatory disease worldwide with more than 100,000 affected persons. Characteristic skin lesions in FMF are typically absent during an FMF attack and can vary among different populations, therefore rashes are not obligatory for diagnosis. However, erysipelas-like erythema of the distal extremities when present is the pathognomonic rash in FMF and can help with diagnosis [14]. Lesions are tender, warm, swollen, and the erythematous plaques develop with prolonged walking and subside within 24 h to 1 week. Scattered nonspecific purpuric papules or nodules can rarely be seen. Lesional biopsies show dermal edema with a perivascular and interstitial dermal infiltrate composed of neutrophils and lymphocytes. Mild hyperkeratosis and acanthosis can be seen in the epidermis [15, 16].

Hyperimmunoglobulinemia D with Periodic Fever Syndrome (HIDS)/ Mevalonate Kinase Deficiency (MKD)

HIDS/MKD is caused by autosomal recessive LOF mutations in *MVK* gene [17]. More than 2/3 of patients with HIDS/MKD have skin lesions which are maculopapular, morbilliform, nodular, and purpuric. Behçet-like aphthae with or without genital ulcerations develop in up to 50% of patients [18, 19]. Lesional skin biopsies show endothelial cell swelling, fibrinoid necrosis of vessel walls, and a perivascular neutrophilic and lymphocytic infiltrate. Other features may include leukocytoclastic vasculitis. Direct immunofluorescence shows perivascular deposits of IgD and C3 in a granular staining pattern in some patients [20].

10.2.1.2 Recurrent Fever Attacks with Longer Duration (Typically >7 days)

TNF Receptor-Associated Periodic Syndrome (TRAPS)

TRAPS is caused by LOF mutations in the *TNFRSF1A* gene, which encodes the p55 TNF receptor [3]. Febrile episodes in TRAPS are longer-lasting, from 1 week up to several weeks. Clinical manifestations include conjunctivitis and periorbital edema and rarely uveitis. Patients with TRAPS develop focal migratory myalgia that underlies centrifugal, migratory, tender, well demarcated, blanchable, erythematous plaques, often on the lower legs. Lesional skin biopsies show a mild perivascular lymphocytic infiltrate in the edematous areas of the papillary dermis. Perivascular complement (C3 and C4) deposition in the dermis has been described [21]. A fascial biopsy underlying a characteristic rash shows a dense inflammatory infiltrate (predominantly monocyte/macrophage and scattered T lymphocytes) surrounding connective tissue, focal panniculitis, fasciitis, and perivascular chronic inflammation [22].

While FMF is responsive to treatment with colchicine, the responses of all three diseases to IL-1 blocking treatments suggest an important role of IL-1 in the pathogenesis of these conditions [23].

10.2.2 Group 2. Syndromes Presenting with Neutrophilic Urticaria (e.g. Cryopyrin-Associated Periodic Syndrome—CAPS)

(a) **Recurrent fever attacks of short duration (typically <24–72 h)**
- **Familial cold autoinflammatory syndrome (FCAS; *NLRP3*) (Chap. 19)**

(b) **Continuous low-grade inflammation with exacerbations of febrile episodes**
- **Muckle-Wells syndrome (MWS; *NLRP3*) (Chap. 19)**
- **Neonatal-onset multisystem inflammatory disease (NOMID)/Chronic infantile neurological cutaneous and articular syndrome (CINCA; *NLRP3)* (Chap. 19)**

The three forms of cryopyrinopathies or CAPS, *familial cold autoinflammatory syndrome (FCAS)*, *Muckle-Wells syndrome (MWS)*, and *neonatal-onset multisystem inflammatory disease (NOMID)* also called chronic infantile, neurological, cutaneous and articular (CINCA) syndrome [24] comprise a clinical disease severity continuum (see Chap. 19). CAPS is caused by autosomal dominant GOF mutations in *NLRP3/CIAS1* [4]. The hallmark of all forms of CAPS includes a non-pruritic neutrophilic urticaria on the trunk, extremities, and face associated with low-grade fevers, conjunctivitis, arthralgia, and marked elevation of acute phase reactants during active disease.

10.2.2.1 Recurrent Fever Attacks of Short Duration (Typically <24–72 h)

Familial Cold Autoinflammatory Syndrome (FCAS)

The inflammatory attacks in FCAS are cold-induced and subside within several hours, [25]. The skin lesions are intermittent, migratory, and non-scarring. The ice cube test is negative. Lesional skin biopsies show no epidermal pathology. Mild papillary dermal edema and dilatation of superficial dermal capillaries can be present. Predominantly neutrophilic, peri-eccrine, and perivascular infiltrates are noted throughout the dermis. Features of vasculopathy or vasculitis are absent.

10.2.2.2 Continuous Low-Grade Inflammation with Exacerbations of Febrile Episodes

Muckle-Wells Syndrome (MWS) and Neonatal-Onset Multisystem Inflammatory Disease (NOMID)

The clinical manifestations in MWS and NOMID is usually continuous due to low-grade inflammation, and fever which can exacerbate due to various stressors. In MWS and NOMID, patients present with neutrophilic urticaria often within hours of birth. In contrast to patients with FCAS, the rash is typically not cold-induced. The histologic features do not differ in FCAS, MWS and NOMID.

10.2.3 Group 3. Syndromes Presenting with Pustular Skin Rashes and Episodic Fevers

(a) **IL-1-mediated pyogenic disorders *with sterile osteomyelitis***
- **Deficiency of the interleukin-1 receptor antagonist (DIRA; *IL1RN*) (Chap. 25)**
- **Majeed syndrome (*LPIN2)* (Chap. 25)**

(b) **Partially IL-1-mediated pyogenic disorders *with pyogenic arthritis***
- **Pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPA; *PSTPIP1*) (Chap. 22)**

(c) **Pustular disorders *with a Behçet disease-like phenotype***
- **Haploinsufficiency of A20 (HA20; *TNFAIP3*) (Chap. 29)**

(d) **Pustular, *psoriasis-like disorders* caused by IL23/IL-17 mediated cytokine dysregulation**
- **Deficiency of the IL-36 receptor antagonist (DITRA; *IL36RN*) (Chap. 26)**
- **Caspase activation and recruitment domains (CARD)14-mediated psoriasis (CAMPS; *CARD14*) (Chap. 26)**

- **AP1S3-mediated psoriasis (AMPS; *AP1S3*) (Chap. 26)**

(e) **Pustular disorders *with inflammatory bowel disease***
- **Very early-onset inflammatory bowel disease (VEOIBD; *IL10, IL10RA, IL10RB*) (Chap. 21)**
- **Neonatal inflammatory skin and bowel disease 1 (NISBD 1; *ADAM17*) (Chap. 21)**

(f) **Pyogenic disorders *caused by a variety of mechanisms***
- **Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND; *MEFV*) (Chap. 29)**
- **Periodic fever, immunodeficiency, and thrombocytopenia (PFIT; *WDR1)* (Chap. 28)**

10.2.3.1 IL-1-Mediated Pyogenic Disorders with Sterile Osteomyelitis

Deficiency of the Interleukin 1 Receptor Antagonist (DIRA)

DIRA is caused by autosomal recessive LOF mutations in *IL1RN*, which encodes the IL-1 receptor antagonist (IL-1Ra) [7, 26]. Patients with DIRA present with pustular rashes in isolated crops or as generalized eruptions in the context of increased acute phase reactants (see Chap. 25). Lesional skin biopsies show dense myeloperoxidase positive (MPO) neutrophilic infiltrates in the epidermis and superficial dermis, formation of pustules around the hair shaft, acanthosis, and hyperkeratosis. Deep connective tissue may show evidence of vasculitis and perivascular neutrophilic infiltration [7]. Treatment with IL-1 inhibitors leads to complete remission, which is maintained when IL-1 blocking treatment is continued [27].

Majeed Syndrome

Majeed syndrome is caused by autosomal recessive LOF mutations in *LPIN2*, which encodes a phosphatase that catalyzes the conversion of phosphatidic acid to diacylglycerol in the endoplasmic reticulum membrane [28]. Patients present with early-onset recurrent osteitis and multifocal osteomyelitis, and with congenital dyserythropoietic anemia (see Chap. 25). Skin involvement is more variable than in DIRA. Skin lesions can be absent, but various forms of neutrophilic dermatoses including pustulosis, severe psoriasis and Sweet syndrome–like rashes have been described [28]. Skin lesions can be pruritic with serosanguinous discharge [29]. Lesional skin biopsies show a dermal neutrophilic infiltrate with edema of the upper dermis without histologic evidence of vasculitis [30]. Remission obtained with use of IL-1 inhibitors confirms the important role of IL-1 [31].

10.2.3.2 Partially IL-1-Mediated Pyogenic Disorders with Pyogenic Arthritis

Pyogenic Arthritis, Pyoderma Gangrenosum and Acne (PAPA) Syndrome

PAPA syndrome is caused by autosomal dominant mutations in *PSTPIP1* [32]. Patients develop pyoderma gangrenosum and sterile pyogenic arthritis (see Chap. 22). Cystic acne and pustules or skin abscesses can develop at trauma or needle injection sites (pathergy) [32]. Lesions form chronic, poor-healing ulcers with granulation tissue and poorly healing borders. These lesions and the severe cystic acne heal with hypertrophic scars. Histologic findings include inflammatory infiltrates of predominantly neutrophils in the dermis and superficial ulcerations. Treatment of PAPA is challenging but responses of the pyogenic arthritis and mild skin lesions to IL-1 blocking agents demonstrate a role of IL-1 in PAPA. However, the need for the need for combination with TNF blocking therapies and high doses of corticosteroids point to additional pathways that are necessary to control the disease [33].

10.2.3.3 Pyogenic Disorders with Behçet Disease-Like Phenotype

Haploinsufficiency of 20 (HA20)

HA20 is caused by autosomal dominant LOF mutations in *TNFAIP3,* which causes haploinsufficiency in the NF-κB regulatory protein A20 leading to prolonged signaling after TNF stimulation (see Chap. 29). Skin eruptions in HA20 cannot be differentiated from Behçet disease and include papules, folliculitis, erythema nodosum-like lesions, and pathergy [34].

10.2.3.4 Pyogenic, Psoriasis-Like Disorders Caused by IL23/IL-17 Mediated Cytokine Dysregulation

Deficiency of the Interleukin-36 Receptor Antagonist (DITRA)

DITRA is caused by homozygous LOF mutations in *IL36RN*, that encodes the IL-36 receptor antagonist [35] (see Chap. 26). Most patients develop a generalized scaly erythematous pustular skin lesions and or erythematous scaly plaques, associated with fever up to 40–42 °C during childhood; lesions are often triggered by viral or bacterial infections [35]. Secondary skin infections and sepsis are frequent complications [35]. The initial erythematous pustular lesions often precede desquamation of the skin over the legs and scalp. Psoriasis vulgaris, acral pustular lesions of the digits, nail dystrophy, impetigo herpetiformis, geographic tongue, and scrotal lesions have been described [35]. Lesional skin biopsies show spongiosis with subcorneal pustules, acanthosis and parakeratosis, and a dense neutrophilic and lymphocytic infiltrate with CD3 and CD8 positive T cells. Dermal CD68 positive macrophages and MPO positive neutrophils are seen.

Caspase Activation and Recruitment Domains (CARD)14 Mediated Psoriasis (CAMPS)

CAMPS is caused by autosomal dominant GOF mutations in *CARD14* causing a monogenic form of plaque and pustular psoriasis (see Chap. 26). It is also the cause of familial pityriasis rubra pilaris, once thought to be an early-onset "psoriasis mimic" with resistance to many psoriasis treatments [36–38]. Patients present with a variable clinical severity spectrum reaching from localized plaque and pustular psoriasis to the severe generalized manifestations of familial pityriasis rubra pilaris. Fever and other systemic manifestations are generally not present but can occur with superinfections [36, 37]. Histologic features of lesional skin biopsies include acantholysis, hypergranulosis, follicular plugging, and the absence of psoriatic capillary alterations, granular layer diminution, and epidermal pustulation [36].

AP1S3-Mediated Psoriasis (AMPS)

AMPS is caused by autosomal dominant LOF mutations in *AP1S3*, the gene encoding AP-1 complex subunit σ1C (see Chap. 26). Patients with AMPS present with pustular psoriasis that often manifests with concomitant plaque psoriasis, mostly in adulthood (25–76 years-old) [39]. The pustular manifestations can range from a more severe form, generalized pustular psoriasis (GPP), to a chronic pustular involvement of palmar plantar psoriasis (PPP) or acrodermatitis continua of Hallopeau (ACH) [39].

10.2.3.5 Pyogenic Disorders with Inflammatory Bowel Disease

Very Early-Onset Inflammatory Bowel Disease (VEOIBD)

VEOIBD is caused by autosomal recessive LOF mutations in the IL-10 receptor and IL-10 encoding genes [40] (see Chap. 21). Patients present before 3 months of age with severe enterocolitis associated with recurrent fever and failure to thrive [41]. Dermatologic manifestations include recurrent folliculitis [41].

Neonatal Inflammatory Skin and Bowel Disease 1 (NISBD1)

NISBD1 is caused by autosomal recessive variants in *ADAM17*, which encodes the TNF-α convertase enzyme (TACE). Patients with NISBD1 present with generalized pustular rashes that develop into psoriasiform erythroderma with erythema and scaling [42, 43].

10.2.3.6 Pyogenic Disorders Caused by a Variety of Mechanisms

Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND)

PAAND is caused by heterozygous missense mutations either at position p.S242 or p.E244 in *MEFV* (see Chap. 29). The disease has a different phenotype from FMF and is clinically characterized by neutrophilic dermatosis, recurrent episodes of long-lasting fever, arthralgia and myalgia or myositis. The spectrum of the neutrophilic dermatosis includes severe acne, sterile skin

abscesses, pyoderma gangrenosum and neutrophilic small vessel vasculitis [44, 45].

Periodic Fever, Immunodeficiency and Thrombocytopenia (PFIT) Syndrome

PFIT syndrome is caused by autosomal recessive variants in actin-regulatory gene *WDR1* in actin-regulatory gene WDR1, which encodes the actin-interactin protein-1 (AIP1). Patients with PFIT present with recurrent infections, neutropenia, impaired wound healing and severe stomatitis with oral stenosis [46].

10.2.4 Group 4. Syndromes of Vasculopathy and Panniculitis/ Lipodystrophy (Unresponsive to IL-1 Blockade)

(a) **Type-I interferon (IFN) mediated**
- **Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome (CANDLE) or proteasome-associated autoinflammatory syndromes (PRAAS; *PSMB8, PSMB9, PSMA3, PSMB4, POMP*) (Chap. 24)**

(b) **Other pathways, partially TNF-dependent**
- **Otulin-related autoinflammatory syndrome (ORAS)/otulipenia (Chap. 29)**

10.2.4.1 Type-I Interferon (IFN) Mediated

Chronic Atypical Neutrophilic dermatosis with Lipodystrophy and Elevated Temperature Syndrome (CANDLE) or Proteasome-Associated Autoinflammatory Syndromes (PRAAS)

CANDLE/PRAAS also called Nakajo-Nishimura syndrome and joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy (JMP) syndrome is caused by recessive or digenic mutations in proteasome subunits, *PSMB8* [47] *PSMB9*, *PSMA3, PSMB4* and the proteasome assembly molecule, POMP [48] (see Chap. 24). Patients present with early-onset recurrent fever, violaceous cutaneous rashes, periorbital edema and erythema, lipodystrophy, arthritis or arthralgia, myositis and with increased acute phase reactants [49]. CANDLE is characterized by early-onset nodular erythematous eruptions on the trunk and extremities, and facial rashes that include red edematous, heliotrope-like rashes and progressive facial lipodystrophy. Histologic findings include perivascular and interstitial mononuclear inflammatory infiltrates with karyorrhexis in the reticular dermis with extension into the subcutis. Strong and diffuse MPO and chloroacetate esterase staining in skin biopsies support the presence of myeloid cells. Intense positivity of CD68, and CD163, indicate the presence of histiocytes and macrophages. Moderate amounts of CD123+ cell infiltrates in the dermis represent the presence of plasmacytoid dendritic cells [50].

10.2.4.2 Other Pathways, Partially TNF Dependent

OTULIN-Related Autoinflammatory Syndrome (ORAS)/Otulipenia)

ORAS/Otulipenia is an autoinflammatory disease linked to homozygous LOF mutations affecting the deubiquitinase OTULIN [51, 52] (see Chap. 29). The disease presents with features similar to CANDLE with early-onset recurrent fevers, relapsing neutrophilic nodular panniculitis, diarrhea and arthritis. Preliminary treatment data suggest a response to TNF blocking therapies which distinguishes this condition from CANDLE [51, 52].

10.2.5 Group 5. Syndromes of Vasculopathy and/or Vasculitis with Livedo Reticularis

(a) **Vasculopathy and/or vasculitis without significant demyelination and with interstitial lung disease (Type-I IFN mediated)**
- **Stimulator of IFN genes (STING)-associated vasculopathy with onset in infancy (SAVI; *TMEM173*) (Chap. 24)**

(b) **Vasculopathy and/or vasculitis with severe demyelinating central nervous system disease (Type-I IFN mediated)**
- **Aicardi-Goutières syndromes (AGS) 1-7 (*TREX1, RNASEH2A-C, SAMHD1, ADAR, IFIH1, DDX58)* (Chap. 24)**

- **Pseudo—TORCH (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex)—ubiquitin specific peptidase (USP)18 deficiency**

(c) **Vasculopathy and/or vasculitis with spondyloenchondrodysplasia (Type-I IFN mediated)**
- **Spondyloenchondrodysplasia with immune dysregulation (SPENCD; *ACP5*) (Chap. 24)**

(d) **Vasculopathy and/or vasculitis associated with strokes (responsive to TNF inhibition)**
- **Deficiency of adenosine deaminase 2 (DADA2; *ADA2, formerly CECR1*) (Chap. 23)**

Several syndromes presenting with vasculopathies, vasculitis and severe livedo reticularis are caused by dysregulation of viral sensing pathways and are linked to Type-I IFN production marked by the presence of a strong IFN response gene signature (IRS) in the blood.

10.2.5.1 Vasculopathy and/or Vasculitis with Livedo Reticularis without Significant Demyelination and with Interstitial Lung Disease (Type-I IFN Mediated)

Stimulator of IFN Genes (STING)-Associated Vasculopathy with Onset in Infancy (SAVI)

SAVI is caused by *de novo* GOF mutations in the adaptor protein in the cytosolic DNA-sensing pathway *STING*, encoded by *TMEM173* [53], pointing to the important role of IFN in several diseases. Patients with SAVI present with early-onset vasculitis that affects small dermal vessels in cold sensitive acral areas including fingers, toes, ears and patellae that lead to vaso-occlusion and gangrene. Most patients develop progressive interstitial lung disease with variable severity [53, 54]. Skin lesions include telangiectasia; pustular, blistering rashes; vasculitic ulcers; or plaques that predominantly developed on cheeks, nose, fingers, toes, and soles of feet. Large eschars and secondary painful crusts on the cheeks and the tip of the helix were reported and are thought to be secondary to localized superinfection of the skin [53, 55]. Lesional skin biopsies show features of small-vessel vasculitis with fibrin deposits and microthrombosis. Skin biopsy samples from some patients show evidence of IgM and C3 depositions [53].

10.2.5.2 Vasculopathy and/or Vasculitis with Livedo Reticularis with Severe Demyelinating Central Nervous System Disease (Type-I IFN Mediated)

Aicardi-Goutières Syndromes (AGS)

AGS 1–7 are a rare group of diseases caused by autosomal recessive LOF mutations in genes encoding enzymes that are involved in nucleotide metabolism, the exonuclease *TREX1*, the ribonucleases *RNASEH2A, RNASEH2B*, and *RNASEH2C*; a nuclease, *SAMHD1*, and the dsRNA-specific adenosine deaminase *ADAR1* (see Chap. 24). More recently, autosomal dominant GOF mutations in the viral sensors, *IFIH1*, encoding melanoma differentiation-associated protein 5 (MDA5), and *DDX58* encoding RIG-I, have been found to cause AGS [56]. Patients who develop disease in early infancy present with subacute encephalomyelitis mimicking viral infections that rapidly leads to demyelination, spastic paraplegia and neurological decline which dominates the clinical picture. When the disease manifests later in life or presents in an autosomal dominant form, the central nervous system manifestations can be more variable and some patients may present with prominent vascular manifestations including stokes (Supplementary Data 1).

Later-onset conditions include a syndrome referred to in the literature as Singleton-Merten syndrome (SMS) characterized by abnormalities of blood vessels, teeth, and bone that is caused by autosomal dominant mutations in *IFIH1* or *DDX58*. Calcifications of the aorta, and the aortic and mitral valves, glaucoma and acro-osteolysis are typical features. Cutaneous manifestations include digital vasculitis and or necrosis, chilblains, skin mottling, sometimes with panniculitis, necrotic cheek eruptions, and lipodystrophy.

10.2.5.3 Vasculopathy and/or Vasculitis with Livedo Reticularis with Spondyloenchondrodysplasia (Type-I IFN Mediated)

Spondyloenchondrodysplasia with Immune Dysregulation (SPENCD)

SPENCD is a syndrome of bone dysplasia; central nervous system involvement (cerebral calcifications) and immune dysregulation that is caused by LOF mutations in tartrate-resistant alkaline phosphatase (TRAP; encoded by *ACP5*) causing a prominent IRS response [57, 58] (see Chap. 24). Patients present with features of immunodeficiency, including upper respiratory and pulmonary infections and interstitial fibrosis, fulminant hemorrhagic chickenpox, and autoimmunity including idiopathic thrombocytopenic purpura and thyroid disease [58]. Cutaneous manifestations include palpable purpura, petechiae on the lower limbs, severe eczema, hyperpigmented macules, vitiligo, Raynaud phenomenon with dilated loops of capillaries, livedo reticularis, and sclerodermatous or acrocyanotic changes of hands and feet with edema and digital vasculitis that leads to necrosis and amputation. Affected skin biopsies show a perivascular polymorphonuclear infiltrate without evidence of deposition of complement or immunoglobulin, consistent with a nonspecific leukocytoclastic vasculitis [57, 58].

10.2.5.4 Vasculopathy and/or Vasculitis with Livedo Reticularis Associated with Strokes and/or Cytopenias and Immunodeficiency (Responsive to TNF Inhibition)

Deficiency of Adenosine Deaminase 2 (DADA2)

DADA2 is caused by autosomal recessive mutations in *ADA2 (formerly CECR1)*, encoding the enzyme adenosine deaminase 2 (see Chap. 23). DADA2 classically presents with early-onset vasculopathy resembling polyarteritis nodosa [59, 60]. Early-onset stroke, livedo reticularis/racemosa, recurrent fever, hepatosplenomegaly, arterial hypertension, ophthalmologic manifestations and myalgia are frequently observed. Histologic findings of skin biopsies include a predominantly interstitial inflammatory infiltrate composed of neutrophils and macrophages with perivascular T lymphocytes and evidence of vasculitis with fibrinoid necrosis [59]. Intravascular thrombosis and necrotizing or nonspecific leukocytoclastic vasculitis and panniculitis have been seen [60]. In lesional skin and brain biopsies, endothelial damage and endothelial cell activation (E-selectin upregulation) are also seen. The marked responses to TNF blockade, particularly the prevention of new strokes, points to an important role of TNF signaling in DADA2.

10.2.6 Group 6. Autoinflammatory Disorders with Granulomatous Skin Diseases

(a) **Without significant immunodeficiency**
- **Blau syndrome (pediatric granulomatous arthritis, PGA) (*NOD2*) (Chap. 20)**

(b) **With variable features of immunodeficiency**
- **Cold-induced urticaria and or granulomatous rash (PLAID) *(PLCG2)* (Chap. 28)**
- **PLCγ2 associated antibody deficiency and immune dysregulation (APLAID) (Chap. 28)**
- **Nuclear factor (NF)-κB essential modulator (NEMO) deleted exon 5 autoinflammatory syndrome—X-linked (NDAS; *IKBKG*) (Chap. 29)**

10.2.6.1 Autoinflammatory Disorders with Granulomatous Skin Diseases without Significant Immunodeficiency

Blau Syndrome or Pediatric Granulomatous Arthritis (PGA)

Blau syndrome is inherited in an autosomal dominant pattern [61] or can occur sporadically (referred to as early-onset sarcoidosis) [62, 63] (see Chap. 20). Patients with Blau syndrome present with granulomatous inflammation of the eyes (often presenting as panuveitis), joints and skin leading to the classical triad of chronic uveitis, arthritis and dermatitis. Most patients with Blau

syndrome develop an ichthyosis-like exanthema [62, 63]. The rash may initially appear as a generalized erythematous micropapular eruption. In later stages the rash persists longer and develops into a papulonodular, tender, reddish brown, dirty-looking, and sometimes scaly exanthema that symmetrically affects the trunk and extremities. The lesions can also appear as erythema nodosum–like. Histologic findings include an inflammatory infiltrate of non-necrotizing, noncaseating, sarcoid-type granulomas, composed of epithelioid and multinucleated giant cell granulomas that are typically found in the subpapillary dermis or in the vicinity of a hair follicle. The granulomata are referred to as "Blau granuloma". Other findings include leukocytoclastic vasculitis. Skin biopsies offer a good diagnostic yield [64].

10.2.6.2 Autoinflammatory Disorders with Granulomatous Skin Diseases with Variable Features of Immunodeficiency and Significant Central Nervous System Disease

PLCγ2 Associated Antibody Deficiency and Immune Dysregulation (PLAID/APLAID)

PLAID/APLAID is caused by autosomal dominant mutations in different domains of *PLCG2* (see Chap. 28). PLAID is clinically characterized by a cold-induced urticarial and/or a granulomatous skin rashes with atopic manifestations, positive autoantibodies, and recurrent opportunistic infections while APLAID presents with recurrent erythematous plaques and vesicopustular skin lesions, granulomas on skin biopsy, arthralgia, uveitis, and recurrent sinopulmonary infections [65, 66].

NF-κB Essential Modulator (NEMO) Deleted Exon 5 Autoinflammatory Syndrome—X-Linked (NDAS)

NDAS is caused by *de novo* variants in *IKBKG* that affect splicing and lead to deletion of exon 5 (see Chap. 29). These patients present with clinical manifestations that were not previously observed in NEMO deficiency, such as panniculitis, subdural hemorrhage and uveitis. Granulomas were present in skin and lymph node biopsies in one of 3 male patients.

10.2.7 Group 7. Autoinflammatory Syndromes Associated with Macrophage Activation Syndrome (MAS), Hyperferritinemia and Various Skin Rashes

(a) **Diseases associated with macrophage activation syndrome (MAS)**
- ***NLRC4*-associated autoinflammatory syndromes (*NLRC4*) (Chap. 33)**
- ***LACC1*-mediated monogenic Still disease (*LACC1*) (Chap. 32)**

(b) **Diseases associated with natural killer (NK) and cytotoxic T cell defects that cause familial hemophagocytic lymphohistiocytosis (FHL) often with prominent immunodeficiency**
- ***FLH 2–5 (PRF1, UNC13D, STX11, STXBP2*)**
- ***Chediak-Higashi* syndrome (*LYST*)**
- ***Griscelli* syndrome (*RAB27A*)**
- ***Hermansky-Pudlak* syndrome II (*AP3B1*)**

10.2.7.1 Autoinflammatory Syndromes Associated with Macrophage Activation Syndrome (MAS)

NLRC4-associated autoinflammatory syndromes

NLRC4-associated autoinflammatory syndromes including NLRC4-macrophage activation syndrome (MAS) are caused by activating heterozygous GOF mutations in the innate immune sensor *NLRC4*, which assembles a caspase-1 activating inflammasome [67, 68] (see Chap. 29). Patients have variable, early-onset enterocolitis followed by recurrent febrile episodes. Attacks of MAS can be triggered by infections or physical stress. The presentation of MAS includes pancytopenia, hepatitis, splenomegaly, and hyperferritinemia (see Chap. 33). In contrast to patients with CAPS with *NLRP3* mutations, extraordinary elevation of serum IL-18 levels (10- to 100-fold higher than in CAPS) are detected even during clinical quiescence, a finding also seen in patients with systemic juvenile idiopathic arthritis/adult-onset Still disease at risk for MAS [69, 70]. Cutaneous manifestations of

NLRC4-associated autoinflammatory syndromes vary in different patients and may correlate with genotype-phenotype associations. Some patients present with an urticaria-like rash and inflammatory febrile episodes similar to those seen in patients with CAPS with *NLRP3* mutations [71].

Monogenic Still Disease Caused by LACC1

This subset of Still disease is caused by homozygous LOF mutations in *LACC1*, which encodes the enzyme laccase (multicopper oxidoreductase) domain-containing 1 (LACC1). Patients present clinically with features of systemic juvenile idiopathic arthritis (JIA) including fever, erythematous maculopapular rashes, chronic polyarthritis, leukocytosis, thrombocytosis, and elevated markers of inflammation [72]. The development of MAS appear to be rare but too little data are available to fully characterize this condition.

10.2.7.2 Diseases Associated with Natural Killer (NK) and Cytotoxic T Cell Defects that Cause Familial Hemophagocytic Lymphohistiocytosis (FHL) Often with Prominent Immunodeficiency

These pathogenesis of these conditions and genetic causes are listed in Chap. 33.

10.2.8 Conditions with Too Little Data to Classify Based on Pathogenic Mechanisms

Cherubism is caused by autosomal dominant mutations in *SH3BP2* and approximately 50% of the cases occur *de novo* (see Chap. 25). Patients develop symmetrical multilocular and radiolucent lesions in the mandible and the maxilla that expand and first appear in childhood in the presence of submandibular and cervical lymphadenopathy [73, 74].

Congenital sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD) is a mitochondrial disease caused by autosomal recessive LOF mutations in *TRNT1* [75] (see Chap. 28). Patients present in infancy with transfusion-dependent sideroblastic anemia, recurrent noninfectious fever episodes, B-cell lymphopenia with hypogammaglobulinemia causing recurrent sinopulmonary bacterial infections, and with progressive developmental delay [76].

Autoinflammatory syndrome-associated with lymphedema, (AISLE) is caused by homozygous frameshift variants in the transcription regulator, *MDFIC* (MyoD family inhibitor domain containing) and was reported in two families of Turkish and Italian origins. The patients presented with recurrent fever, urticarial rash, myalgia, chylous serosal effusions and chronic facial and extremities lymphedema [77].

NLRP12 autoinflammatory disease is an autosomal dominant autoinflammatory disease clinically characterized by periodic fever, cold-induced urticarial rash, arthralgia/arthritis, myalgia and lymphadenopathy (see Chap. 29) [78, 79].

TNFRSF11A related autoinflammation was described in 2014 by Jeru et al. who reported 3 patients from two families with long-lasting recurrent fever and abdominal pain (see Chap. 29) [80].

NLRP1-associated autoinflammation with arthritis and dyskeratosis (NAIAD) was initially reported in three families with multiple self-healing palmoplantar carcinoma (MSPC) and in one family with familial keratosis lichenoides chronica (FKLC) [81] (see Chap. 29). Subsequently, 3 patients from two families with dyskeratosis, arthritis, recurrent fever and increased inflammatory markers were reported [82].

10.3 Pathogenesis-Based Disease Classification

- **Autoinflammatory disease-causing genetic defects reveal mechanisms that perpetuate sterile inflammation which can be used for a pathogenesis-based classification system**
- **Diseases caused by mutations that lead to the activation of the IL-1 activating inflammasomes and respond to IL-1 blocking agents, are referred to as IL-1 mediated autoinflammatory diseases**
- **Mutations leading to activation of Type-I IFN pathways that cause diseases with chronic IFN signatures and respond to IFN blocking agents are referred to as autoinflammatory interferonopathies**

- **Disorders that present with extremely elevated IL-18 overproduction have a predisposition to the development of MAS**
- **Mutations that lead to activation of the IL-23/IL-17/IL-36 axis of cytokine amplification in the skin cause psoriasiform diseases**
- **Mutations that lead to ubiquitination disorders that modify NF-kB signaling cause a spectrum of autoinflammatory phenotypes**
- **NOD2 mutations can cause granulomatous inflammation**
- **While single cytokine amplification and characterization of sensor pathways is helpful in understanding the pathogenesis of some diseases, other autoinflammatory diseases cannot currently be characterized based on a single major inflammatory pathway**

Many disease-causing mutations resulting in autoinflammatory phenotypes activate proinflammatory cytokine pathways that respectively amplify pathologic innate immune cells and organ responses. Differences in patterns of clinical disease manifestations, that often track with the dysregulated cytokine pathways (i.e. the IL-1, Type-I IFN and IL-18 mediated diseases), suggest that the specific cytokine dysregulation contributes to the respective disease manifestations. In Supplementary Data 1 we attempt to reconcile a clinically- and pathogenic-based disease grouping.

In the cryopyrinopathies, cellular assays in *NLRP3* knock down or knock out cells showed that lipopolysaccharide (LPS)-stimulated IL-1 production was dependent on NLRP3 [6, 83] which suggested a potential role for IL-1 in human disease. In other instances, the clinical response to empiric treatment with cytokine blocking agents clinically confirm or suggest a pivotal role of that cytokine in various diseases. Examples include the use of IL-1 blocking therapies in treating DIRA, Majeed syndrome, FMF, HIDS/MKD and TRAPS [7, 8]. More recently, the use of Janus kinase (JAK) inhibitors to reduce Type-I IFN signaling in patients with CANDLE and SAVI [84, 85], and the empiric use of anti-TNF agents in preventing stroke recurrence in patients with DADA2 [86, 87] confirm the pathogenic roles of Type-I IFN and TNF, respectively, in disease pathogenesis. The successful treatment with IL-23 or anti-IL-17 blocking therapies in psoriasiform skin inflammatory diseases [88–90] and lastly, early studies in using IL-18 targeting treatments in the patients prone to MAS [91, 92] similarly suggest a role of cytokine amplification of these proinflammatory cytokines. These clinical and pathomechanistic data therefore allow for the grouping of autoinflammatory diseases based on the cytokine dysregulatory pathways that drive pathology.

10.3.1 Stimulation of IL-1 Activating Inflammasomes and Response to IL-1 Blocking Agents Define IL-1 Mediated Autoinflammatory Diseases (Fig. 10.2a)

IL-1β is a potent endogenous pyrogen that coordinates immune responses by the recruitment and activation of neutrophils, macrophages and other immune cells (see Chap. 6). Its role in causing systemic and organ-specific immunopathology in infections [93] and in IL-1-mediated autoinflammatory diseases is well established [8]. In contrast to IL-1α, IL-1β requires proteolytic cleavage to become activated and secreted, a process that is tightly regulated by IL-1 activating inflammasomes (see Chaps. 5 and 6). IL-1α and IL-1β signaling is also regulated at the receptor level. IL-1Ra competes for IL-1 receptor binding [93] and therefore competitively inhibits IL-1α and IL-1β signaling. Many autoinflammatory diseases are caused by mutations that lead to increased IL-1 release or signaling. Patients with LOF mutations in *IL1RN,* the gene that encodes IL-1Ra (see Chap. 25), can develop a life-threatening systemic inflammatory response syndrome (SIRS), thus confirming the potent role of excessive IL-1 [7].

GOF mutations in four IL-1 activating inflammasomes, three NLR proteins, NLRP3, NLRP1 and NLRC4, and in pyrin, cause autoinflammatory diseases (see Chap. 5). Inflammasome activation results in caspase-1 activation and IL-1β maturation and in a specialized form of caspase-mediated inflammatory cell death called pyroptosis, mediated by cleavage of gasdermin D [94]. Cleaved gasdermin D forms membrane pores that leak intracellular contents and cause pyroptotic cell death [94, 95]. Other autoinflammatory diseases,

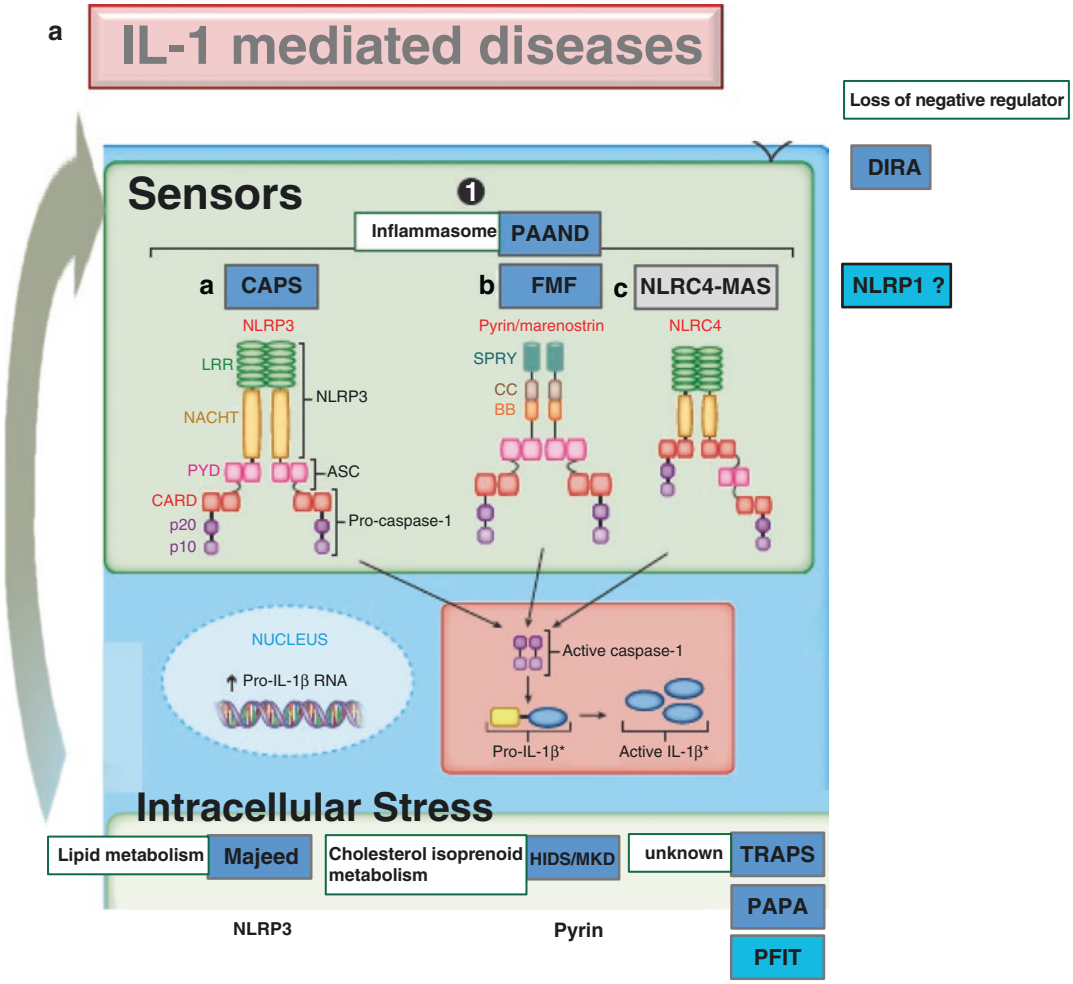

Fig. 10.2 (**a**) Interleukin (IL)-1 mediated autoinflammatory diseases: IL-1 mediated diseases caused by gain-of-function (GOF) mutations in sensors are highlighted in yellow and loss-of-function (LOF) mutations in cellular/metabolic pathways that result in stress are highlighted in green. The diseases caused by LOF mutation in a negative regulator are highlighted in red. *CAPS* cryopyrin-associated periodic syndrome (*FCAS* familial cold autoinflammatory syndrome, *MWS* Muckle-Wells syndrome, *NOMID* neonatal-onset multisystem inflammatory disease); *FMF* familial Mediterranean fever; *PAAND* pyrin-associated autoinflammation with neutrophilic dermatosis; *DIRA* deficiency of the interleukin-1 receptor antagonist; *NLRC4-MAS* NOD-like receptor family CARD domain containing 4—macrophage activation syndrome (also IL-18 mediated or SCAN syndrome) or enterocolitis and autoinflammation associated with mutation in *NLRC4; HIDS/MKD* hyperimmunoglobulinemia D and periodic fever syndrome/mevalonate kinase deficiency; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *PAPA* pyogenic arthritis, pyoderma gangrenosum and acne; *PFIT* periodic fever, immunodeficiency, and thrombocytopenia; *ASC* apoptosis-associated speck-like protein containing CARD; *NLRP3* NOD-like receptor protein 3; *PYD* pyrin domain; *LRR* leucine rich repeat

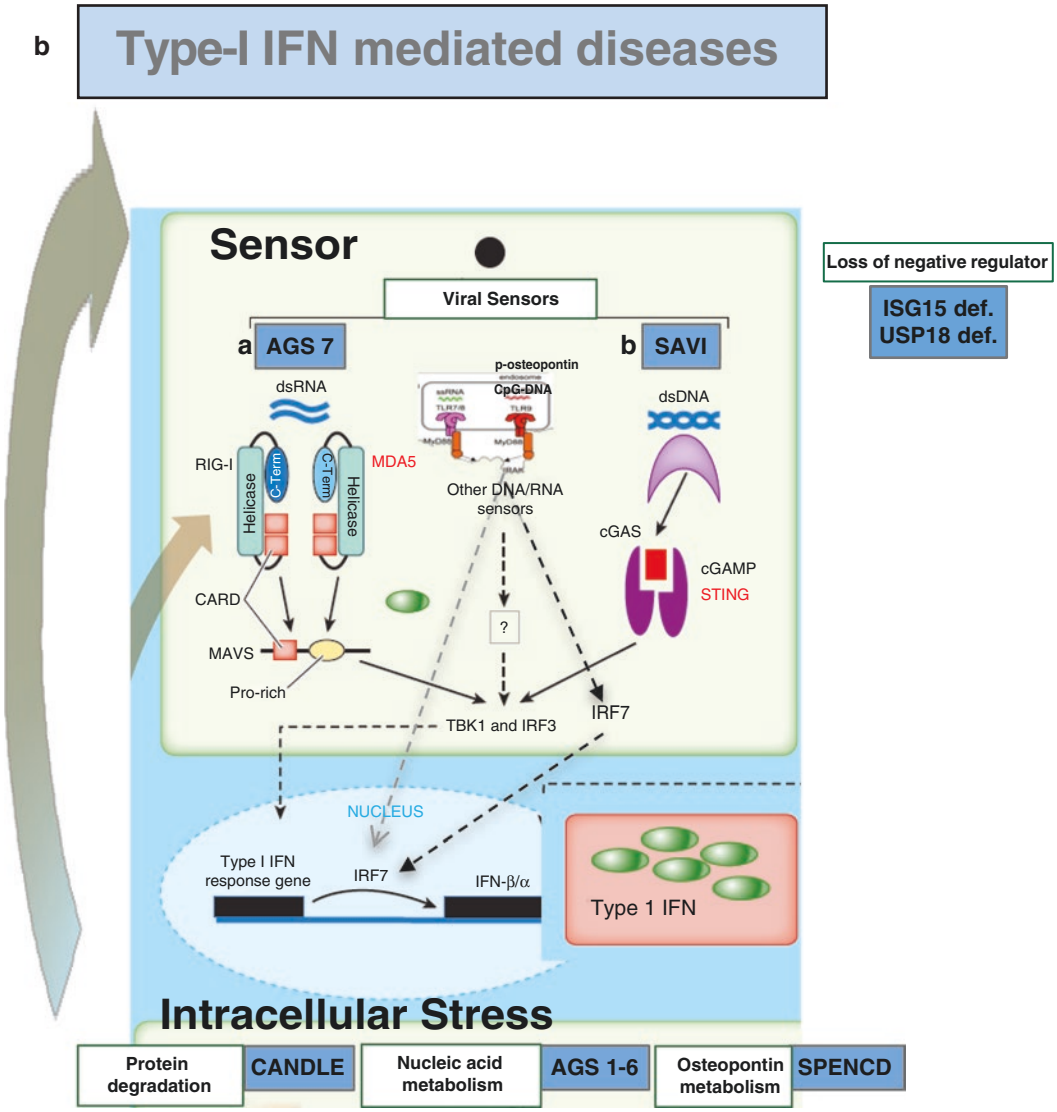

Fig. 10.2 (continued) (**b**) Type-I interferon (IFN) mediated autoinflammatory diseases or autoinflammatory interferonopathies. *AGS* Aicardi-Goutiéres syndrome; *SMS* Singleton-Merten syndrome; *STING* stimulator of interferon genes; *SAVI* STING-associated vasculopathy with onset in infancy; *CANDLE* chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature/PRAAS—proteasome-associated autoinflammatory syndrome; *SPENCD* spondyloenchondrodysplasia with immune dysregulation; *INF* interferon

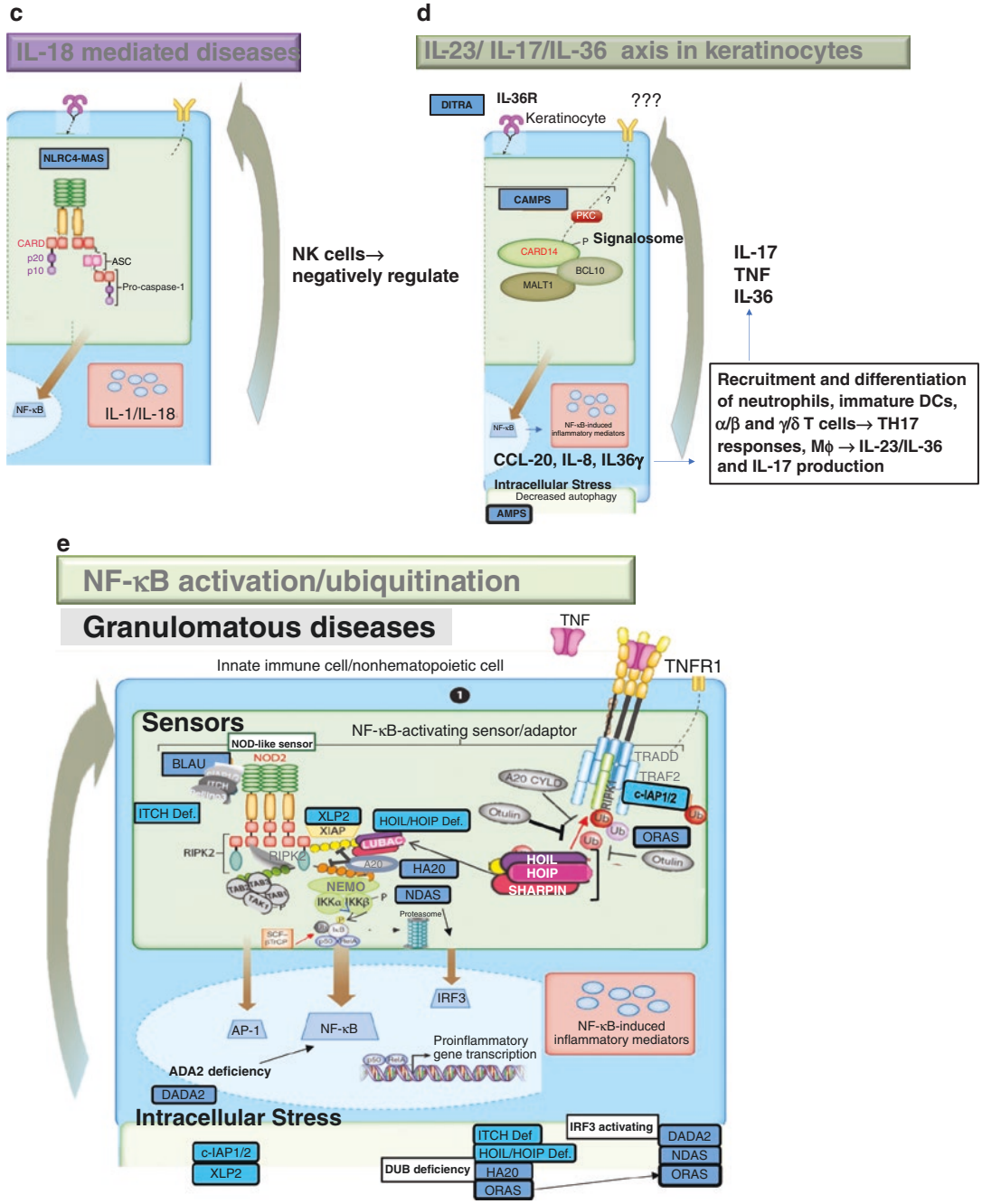

Fig. 10.2 (continued) (**c**) IL-18 overproduction and predisposition to macrophage activation syndrome (MAS). *NLRC4-MAS* NOD-like receptor family CARD domain containing 4—macrophage activation syndrome (also IL-18 mediated or SCAN syndrome) or enterocolitis and autoinflammation associated with mutation in *NLRC4*; *IL* interleukin; *NK* natural killer; *CARD* caspase activation and recruitment domains; *ASC* apoptosis-associated speck-like protein containing CARD. (**d**) NF-KB dysregulation and IL-17 amplification in the skin. *IL* interleukin; *DC* dendritic cells; *TNF* tumor necrosis factor; *CCL* CC chemokine ligands; *DITRA* deficiency of the interleukin-36 receptor antagonist; *CAMPS* CARD14-mediated psoriasis; *AMPS* A1S3-mediated psoriasis. (**e**) NF-KB dysregulation caused by dysregulated NOD2 signaling and by ubiquitination disorders. *NK-κB* nuclear factor kappa B; *TNF* tumor necrosis factor; *XLP2* X-linked lymphoproliferative syndrome 2; *HA20* haploinsufficiency of A20; *NDAS-NEMO* deleted exon 5 autoinflammatory syndrome; *ORAS* otulin associated autoinflammatory syndrome; *DADA2* deficiency of adenosine deaminase 2. *Turquois: immunodeficiencies with and without autoinflammation. Blue: autoinflammatory diseases*

including Majeed syndrome and HIDS/MKD, are associated with extrinsic activation of the NLRP3 inflammasome. In Majeed syndrome membrane permeability and sensitivity of the P2X7 receptor that controls K+ efflux regulate NLRP3 inflammasome activation [96]. In HIDS/MKD, the LOF mutations in *MVK* leads to reduced production of geranyl pyrophosphate, a substrate critically important for the prenylation and activation of RhoGTPase. RhoGTPase inactivation causes constitutive activation of the pyrin inflammasome thus linking MVK to the activation of the pyrin inflammasome (see Chap. 17). Pathways that link PAPA syndrome and TRAPS to IL-1 production have been proposed [97–99] and are discussed in Chaps. 22 and 18, respectively.

Understanding the cell- and organ-specific sources and effects of IL-1, particularly in human cells, is critical to understanding the organ-specific disease manifestations in patients with autoinflammatory syndromes.

10.3.2 Activation Pathways that Lead to Type-I IFN Production and/or a Chronic Presence of an IFN Response Gene Signature Define Type-I IFN Mediated Autoinflammatory Diseases or Autoinflammatory Interferonopathies (Fig. 10.2b)

IFNs have anti-virus and anti-tumor effects and potent immune-modulating functions, including enhancing the antigen-presentation function of dendritic cells, promoting T lymphocyte response and B lymphocyte antibody production, and restraining proinflammatory cytokine production [100–102] (see Chap. 6). Type-I IFNs signal by binding with Type-I IFN receptors, through activation the JAK/signal transducer and activator of transcription (STAT) pathway including the JAK kinases JAK1 and tyrosine kinase (TYK) 2 which phosphorylate and recruit STAT1 and STAT2. Activated STAT1 and STAT2 bind to interferon regulatory factor (IRF) 9 and form the interferon stimulated gene factor (ISGF) 3 transcriptional complex that translocates to the nucleus and promotes expression of IFN-response genes. Similar to IL-1, IFN induces its own amplification and blocking IFN signaling is emerging as an effective treatment strategy for patients with upregulated IFN signaling [84].

Recent progress in the identification of viral sensors that sense DNA or RNA nucleic acids in different cellular compartments, including the endosomal, TLR 3, 7, 8, and 9, and cytosolic DNA and RNA sensors, cGAS and RIG-I/DDX58 and MDA-5/IFIH1 provided insights into viral nucleic acids recognition and signaling pathways, many of which are tied to IFN signaling and provided an understanding of autoinflammatory conditions with distinct clinical phenotypes that present with a Type-I IFN gene signature, suggesting chronic IFN signaling. The detection of DNA or RNA by cytosolic sensors results in activation and recruitment of TBK1, leading to phosphorylation/activation of IRF3 and transcription of IFN-β. Genetic defects in endo- and exonucleases that lead to accumulation of self-DNA and RNA in the cytoplasm and cause Aicardi-Goutieres syndrome (AGS) have taught us that the cytoplasmic sensors sense DNA and RNA regardless of the source (viral or self) and provide insights into the pathomechanism that regulates IFN signaling.

The dsDNA sensor cGAS signals through the ER-residing adapter protein STING that activates the shared end pathway to IFN signaling. Mutations that constitutively activate STING cause SAVI, a disease that illustrates the role of the STING pathway in the development of peripheral vasculitis and in lung fibrosis [53, 54]. Finally, activation of the endosomal TLR9 by phosphorylated osteopontin that cannot be cleaved in patients with a mutation in the acid phosphatase, *ACP5*, causes another interferonopathy, SPENCD. Intracellular pathways that link mutations in the proteasome to IFN production are still poorly characterized.

The role of Type-I IFN in driving adaptive immune dysregulation is well established [103, 104] and the presence of antinuclear antibodies and other disease-specific antibodies are more common in patients with autoinflammatory interferonopathies than in patients with IL-1 mediated autoinflammatory diseases. Autoantibody levels are higher in SAVI, AGS and SPENCD than in CANDLE, suggesting a dose-effect of IFN signaling. However, genetic variants that influence autoimmunity [105] likely play a role in the variability of autoimmune features even in patients with the same disease-causing mutations.

10.3.3 Disorders Associated with IL-18 Overproduction, Hyperferritinemia and a Predisposition to the Development of MAS via NK Cell Dysfunction and Increased IL-18 Axis (Fig. 10.2c)

FLH-causing mutations that impair the cytotoxic function of natural killer (NK) cells and cytotoxic CD8+ T cells (CTLs) illustrate the importance of NK cells and CTLs as regulators of dendritic cell (DC) and macrophage function in infections and inflammation (see Chap. 33). The regulatory feedback mechanism involving competent NK cells and CTLs in killing and eliminating infected DCs has recently been described in murine models of hemophagocytic lymphohistiocytosis (HLH)/MAS [106]. During infection, antigen-loaded antigen presenting cells stimulate CTLs and perforin-deficient mice show persistence of infected DCs, which was not observed in wildtype mice [107, 108]. Immunization of perforin-deficient and wildtype mice with DCs showed that DCs drive a progressive increase in antigen-specific CTLs that produce IFN-γ, which drives systemic macrophage activation and the HLH/MAS phenotype [109–111]. However, CTLs from perforin-containing wildtype CTLs eliminated infected and activated DCs from peripheral tissues, but not those present in the lymph node, and thus prevented further migration of antigen-loaded DC from the peripheral tissues to the lymph nodes where they can continue to activate antigen-specific T cells. In contrast, perforin-deficient CTLs failed to eliminate antigen-laden DCs in peripheral tissue and the continued migration of antigen-loaded DC from the peripheral tissues to the lymph node led to sustained activation of antigen-specific T cells in the lymph nodes and exuberant IFN-γ production. Successful treatment of HLH and MAS with anti-IFN-γ antibodies suggest a role of IFN-γ in these conditions [112].

The recent discovery that GOF mutations in *NLRC4* (also known as IPAF, CARD12 or CLAN) predispose to the development of MAS in patients with no primary defects in cytotoxicity suggest a role of the NLRC4 inflammasome in the pathogenesis of MAS. In contrast to non-NLRC4 inflammasomopathies, GOF mutations in *NLRC4* are associated with a high risk of MAS in the context of elevated free IL-18 levels, which distinguishes this condition from other inflammasomopathies and from FLH [113]. Mechanisms that lead to preferential IL-18 cleavage by the NLRC4 inflammasome are not fully understood (see Chap. 29). Intracellular flagellin that is sensed by the NLR family apoptosis inhibitory protein (NAIP) [114, 115] is critical in nucleating and activating the NLRC4/NAIP inflammasome [113]. However, the role of chronically elevated IL-18 levels on NK and CTL function and pathways of how primary NK and CTL defects converge with pathways causing MAS in patients with GOF mutations in NLRC4, which are the hallmark of FLH, MAS and murine models of HLH, is an area of active investigation [116–119]. Preliminary data have shown that using recombinant IL-18 binding protein to decrease the concentration of free IL-18 in serum of patients with NLRC4-MAS have prevented recurrent MAS and provide preliminary evidence of the therapeutic utility of blocking this pathway in patients with chronically elevated IL-18 levels [92].

10.3.4 Mutations Leading to IL-23/IL-17 Cytokine Amplification in the Skin (Fig. 10.2d)

The importance of the IL17/23 cytokine amplification in causing psoriasis and psoriasis-like skin inflammation has been established in basic research models and is confirmed by the magnitude of the clinical responses to treatments with IL-17 and IL-23 signaling blockade in psoriasis. Inflammation resulting in psoriasis is thought to be precipitated by skin injury through infectious triggers or trauma that leads to activation of keratinocytes and their production of IL-8, IL-36γ and CCL20, all cytokines and chemokines which recruit neutrophils, and immature myeloid DCs. The activation of DCs leads to the release of IL-12, IL-23 and CCL20 [35, 120–122]. These cytokines drive polarization of T cells into effector TH17 cells including αβ T-cells and dermal γδ T-cells that produce IL-17 and IL-23 as well as TNF-α and IFN-γ, which further induce keratinocyte hyperproliferation and the production of chemokines to

sustain a vicious cycle of immune cell activation and cutaneous pathology [123, 124]. GOF mutations in *CARD14*, which encodes a protein that is predominantly expressed in keratinocytes, lead to constitutive activation of NF-kB and the release of proinflammatory cytokines and chemokines, IL-8 and CCL20 [36, 37]. Furthermore, LOF mutations in *IL36RN* also cause severe pustular psoriasis and point to the critical role of IL-36 signaling in propagating keratinocyte activation [35]. Among the keratinocyte activating signals, activation of TLR3 in keratinocytes increases expression of genes involved in formation of the epidermis, lipid accumulation, and epidermal organelles [125]. Interestingly, mutations in *AP1S* genes disrupt formation and function of the AP-1 complex that has been associated with the development of autophagosomes. In AP1S3-deficient keratinocytes, autophagy is disrupted, causing abnormal accumulation of p62, an adaptor protein that mediates NF-kB activation (see Chaps. 8 and 26). As a consequence, up-regulation of IL-1 signaling and overexpression of IL-36α were observed. These abnormal immune profiles were recapitulated by pharmacological inhibition of autophagy and verified in patient keratinocytes, where they were reversed by IL-36 blockade in vitro [126, 127]. The findings of LOF mutations in *IL36RN,* an inhibitor of IL-36α,β and IL-36γ signaling, that cause DITRA, point to the important role of IL-36 signaling in causing psoriasis. Clinical data that blockade of the IL-17/IL-23 axis is efficacious not only in patients with *CARD14* mutations [128] but also in patients with DITRA and *AP1S3* deficiency, confirm the vicious cycle of immune activation in the skin that converges on NF-κB activation and can be effectively blocked by preventing the differentiation of IL-17 producing T cells and the action of IL-17 on keratinocytes [126, 129].

10.3.5 Ubiquitination Disorders That Modify NF-kB Signaling Cause Autoinflammatory Phenotypes (Fig. 10.2e)

Ubiquitination of proteins is an evolutionarily conserved process that marks proteins post-translationally for degradation. Therefore, a wide array of physiological processes that include cell survival and differentiation, innate and adaptive immunity can be affected by defects in ubiquitination and deubiquitination. Deubiquitination defects have been implied in the cause and progression of cancer, metabolic syndromes, neurodegenerative diseases, autoimmunity, inflammatory disorders, infection and muscular dystrophies [130]. Accumulation of ubiquitinated proteins in CANDLE is proposed to drive IFN signaling. Stimulation of PRRs through bacterial triggers leads to assembly of multi-protein complexes of ubiquitin ligases (E3s) and deubiquitinases (DUBs) that assemble and disassemble polyubiquitin chains, respectively. These ubiquitin chains orchestrate activation of kinase signaling pathways, and modify inflammatory responses mediated by NF-κB transcription factors and by transcription factors activated by mitogen-activated protein (MAP) kinases [131, 132]. Recently, ubiquitination defects have been described to cause immune dysregulatory phenotypes presenting with immunodeficiency and autoinflammatory phenotypes [133, 134]. In humans, there are 102 putative DUB genes [135].

Interestingly, mutations in genes that regulate ubiquitin chain-assembly at the NOD1/2 signaling complex can cause autoinflammatory phenotypes, including LOF mutations in X-linked inhibitor of apoptosis (XIAP), which give rise to the familial and often fatal immunodeficiency X-linked lymphoproliferative syndrome-2 (XLP2) [136]. LOF mutations in components of the linear ubiquitination chain assembly complex (LUBAC) complex, a multimeric E3 ligase that assembles Met1-linked (linear) ubiquitin chains, including *HOIL* and *HOIP* [133, 137, 138] cause immunodeficiencies with immune dysregulation, and more recently LOF mutations in OTULIN, a deubiquitinase which selectively removes Met1-linked ubiquitin chains causes otulin-related autoinflammatory syndrome (ORAS)/otulipenia [51, 52] (see Chap. 29). LOF mutations in another DUB, TNFAIP3/A20, that removes linear Lys63 (K63) Ub moieties, results in a Behçet-like disease with mucosal barrier defects [139]. TNFAIP3/A20 contains both ubiquitin ligase and deubiquitinase activities. While mutations in the OTU domain of A20 cause a Behçet disease-like phenotype, mutations outside of the OTU domain have recently been asso-

ciated with the development of a lymphoproliferative syndrome [140]. A20 regulates immune and inflammatory responses signaled by cytokines, such as TNF-α and IL-1β, or pathogens via TLRs through terminating NF-κB activity. Treatment responses to IL-1 and TNF inhibiting agents result in clinical benefit in some but not all patients (see Chap. 29) [34], pointing to a complex pathomechanism that causes HA20. However, the clinical similarities to Behçet disease may point to shared pathomechanisms.

10.3.6 NOD2 Mutations and Granulomatous Inflammation (Fig. 10.2e)

Blau syndrome is caused by de novo or autosomal dominant mutations in the NACHT domain of *NOD2* [61, 141] and was the first autoinflammatory disease described that presents with granulomatous inflammation (see Chap. 20). Together with observations that mutations in the leucine-rich repeat (LRR) domain of *NOD2* cause granulomatous colitis in Crohn disease suggested an important role of *NOD2* signaling in causing granuloma formation. However, to date, the mechanisms of how *NOD2* causes granuloma formation remain poorly understood. While transient overexpression of transfected mutant and wild-type *NOD2* constructs suggest excessive NF-kB and MAP kinase activation with mutant compared with the wild-type constructs [141], blood samples from patients with Blau syndrome do not spontaneously release inflammatory cytokines, nor were inflammatory responses observed when peripheral blood monocytes cells were stimulated with muramyl dipeptide [142, 143]. Interestingly, *LACC1,* the gene mutated in monogenic systemic juvenile idiopathic arthritis cooperates to enhance NOD2-induced succinate dehydrogenase (SDH) activity by constitutively associating with SDH-A and leading to increased mitochondrial reactive oxygen species (mtROS) and cytokine secretion [144]. Relative to macrophages with the *LACC1* variant Ile254, macrophages with the Val254 disease-risk variant demonstrate decreased PRR-induced mtROS, signaling, cytokine secretion and bacterial clearance [144], thus linking *LACC1* to NOD2 signaling and to granuloma formation. Although partial clinical responses to anti-cytokine therapies with anti-IL-1, anti-TNF and anti-IL-6 therapies are observed, mostly in the context of being able to decrease the dose of corticosteroids, far better therapies are needed for granulomatous diseases.

10.4 Conclusion

In the past 20 years, the genetic causes and pathogenic pathways of a growing number of immune dysregulatory disorders that present with fever and systemic/organ-specific inflammation define an increasing spectrum of novel immune-dysregulatory diseases.

After early data pointed to the importance of IL-1 inflammasome dysregulation in perpetuating sterile inflammation, the discovery of dysregulation in intracellular pathways that regulate Type-I IFN production, IL-18 over-secretion, the absence of anti-inflammatory cytokine IL-10 signaling, unopposed IL-36 signaling and IL-17 secretion, in patients who were unresponsive to IL-1 blocking treatments, point to a role of other innate immune cytokines, and expand the scope of autoinflammatory conditions that can be classified based on innate immune cytokine dysregulation.

Insights into disease pathogenesis allows to meaningfully classify diseases based on innate immune sensor platforms that link danger sensing to dominant innate immune cytokine production. However, there is a growing number of genetically defined conditions that regulate multiple effector pathways including innate and adaptive immune function, and cell differentiation of hematopoietic and tissue specific cells. Genetic defects that regulate NF-kB signaling (i.e. deficiency of adenosine deaminase in DADA2 and ubiquitination defects in ORAS/otulipenia), and mutations in genes that drive chronic Type-I IFN amplification (i.e. the autoinflammatory interferonopathies) modify inflammatory pathways and cell differentiation, and autoinflammatory interferonopathies drive innate and adaptive immune dysregulation. Furthermore, mutations in genes that affect molecules that regulate and modify immune cell receptor signaling of innate but also adaptive immune cells (i.e. *PLCG2*), point to the

limitations of an innate cytokine-centric classification system, in describing the pathomechanisms that amplify sterile inflammation and drive autoinflammatory phenotypes [145].

A classification system based on characteristic clinical phenotypes as suggested above can be updated as new knowledge is acquired and allows for classification of conditions that are not yet genetically characterized; it may further help with delineating pathomechanisms that drive certain phenoptyes and in making treatment decisions based on clinical similarities to known, genetically defined conditions. However, each classification system remains transitional. As novel insights into the many pathways that drive and amplify sterile inflammation become identified and characterized, our understanding of pathogenic pathways is becoming refined, which will translate into novel ways to classify autoinflammatory and, in general, immune dysregulatory conditions.

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Clinical Approach to the Diagnosis of Autoinflammatory Diseases

11

Philip J. Hashkes, Karyl S. Barron, and Ronald M. Laxer

Abstract

In this chapter, we present the clinical approach to patients with a suspected autoinflammatory syndrome. We describe a systematic approach to the history, physical examination, system involvement and laboratory testing and offer an algorithm on investigative pathways for these patients. We discuss the various diagnostic criteria currently used for specific autoinflammatory diseases. Finally, we propose an approach, including empiric treatment and management, to the many patients with suspected autoinflammatory syndromes who remain undiagnosed, despite a comprehensive work-up, including genetic testing.

Keywords

Autoinflammatory syndrome · Diagnosis · Management · Diagnostic criteria

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Abbreviations

AGS	Aicardi-Goutières syndrome
AIDAI	Autoinflammatory diseases activity index
AP1S3	Adaptor related protein complex 1 sigma 3 subunit
APLAID	Autoinflammation and PLAID
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase activation and recruitment domain
CNO	Chronic non-bacterial osteomyelitis
DADA2	Deficiency of adenosine deaminase 2
DIRA	Deficiency of the IL-1 receptor antagonist
DITRA	Deficiency of the IL-36 receptor antagonist (generalized pustular psoriasis)
FCAS	Familial cold autoinflammatory syndrome
FMF	Familial Mediterranean fever
IBD	Inflammatory bowel disease
IL	Interleukin
MAS	Macrophage activation syndrome
MKD	Mevalonate kinase deficiency
MWS	Muckle-Wells syndrome
NLRC	Nucleotide-binding oligomerization (NOD), leucine rich repeats and CARD domain containing
NLRP	Nucleotide-binding domain, leucine-rich repeat, and pyrin domain containing

NOMID	Neonatal-onset multisystem inflammatory disease
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PAPA	Pyogenic sterile arthritis, pyoderma gangrenosum, and acne
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
PFIT	Periodic fever, immunodeficiency and thrombocytopenia
PLAID	PLCγ2-associated antibody deficiency and immune dysregulation
PSTPIP1	Proline-serine-threonine phosphatase-interacting protein 1
SAPHO	Synovitis, acne, pustulosis, hyperostosis and osteitis
SAVI	STING-associated vasculopathy with onset in infancy
SIFD	Sideroblastic anemia, immunodeficiency, fevers, and developmental delay
sJIA	Systemic juvenile idiopathic arthritis
TNF	Tumor necrosis factor
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome

Key Points

- **A detailed history and physical examination are crucial in reaching the correct diagnosis in a patient with a suspected autoinflammatory syndrome**
- **There are often specific demographic and clinical clues for each of the autoinflammatory diseases**
- **Diagnostic and classification criteria have been developed for the major autoinflammatory diseases and these are undergoing refinement based on real cases from registries**
- **Many patients with a suspected autoinflammatory syndrome remain undiagnosed even after a complete clinical and genetic evaluation**

11.1 Introduction

Autoinflammation is a term used to describe a group of illnesses characterized by attacks of mostly unprovoked inflammation without the presence of either autoantibodies or autoreactive T cells more characteristic of autoimmune diseases ([1], see Chap. 1). Many of the autoinflammatory syndromes (previously called hereditary periodic fever syndromes) are Mendelian genetic disorders manifesting with discrete episodes of recurrent fever and inflammation. The spectrum of autoinflammatory diseases has expanded broadly over the last several years, in part due to the increased recognition of these disorders and the advances in and availability of genetic testing [2]. Autoinflammation can occur at any age and the presentation in children and adults can differ.

In children, the most common, but not exclusive, presentation of autoinflammatory syndromes is with recurrent fever. A history of recurrent fever is a common presenting complaint in children, less so in adults. Most often, the fever episodes are acute and of short duration. Upper respiratory infections account for most fevers of childhood. It is not unusual for young children attending daycare centers or kindergarten to present with repeated fever episodes. Viral infections are the most common cause of fever in this setting. When fever episodes are prolonged in all age groups, infection should still be considered in the differential diagnosis. Unusual or opportunistic infections should prompt an evaluation for immune deficiency, especially in the setting of failure to thrive, weight loss or other clinical signs of underlying pathology. Localization of infections to the same organ should raise the suspicion of an anatomic defect. Once infection is excluded, a search for possible malignancy or inflammatory illness is warranted.

Once infection, malignancy, immune deficiency and other inflammatory illnesses are excluded from consideration, unexplained episodes of fever with a constellation of symptoms are considered to fall under the category of auto-

inflammatory syndromes, formerly called *recurrent or periodic fever syndromes*. These conditions may demonstrate strict periodicity or recur with variable intervals between episodes. It is important to note that in adults, recurrent fever is a less common presentation of these syndromes.

Autoinflammatory diseases vary widely in their presentation and clinical manifestations. While the definitive diagnosis is supported by genetic testing in those disorders shown to be monogenic (see Chap. 12), in many other diseases, including some of the monogenic diseases, the diagnosis remains clinical. Even in the monogenic diseases a clinical approach is first necessary to narrow the genetic search for the correct disease/condition [3, 4]. It is important to note that genetic analysis is not always available or is limited to specific gene testing in many regions and countries. Thus, a systematic clinical approach for these diseases is critical.

Furthermore, it appears that the clinical manifestations for many of the diseases are not disease-specific and may overlap with those of other diseases. For example, symptoms of patients with periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome may overlap with symptoms characteristic of other autoinflammatory diseases [5].

Most of the currently recognized autoinflammatory diseases do not have validated clinical diagnostic criteria but in recent years, diagnostic and/or classification criteria have been developed for several of the classic autoinflammatory diseases, mainly based on data from multicenter/multinational registries ([6–14], see below and for the Eurofever registry, see Chap. 14).

Many papers have been published offering a clinical approach to the diagnosis of autoinflammatory syndromes [15–23]. In this chapter, we summarize our clinical approach, including detailed history, pertinent physical findings, laboratory testing and imaging in patients with suspected autoinflammatory syndromes. We offer an algorithm which can be helpful for diagnosing many patients (Fig. 11.1). We discuss some of the most important clinical features and diagnostic clues of the classic autoinflammatory diseases. We also describe the efforts to develop diagnostic and classification criteria and our approach to the patient with an undiagnosed or unspecified autoinflammatory syndrome.

11.2 When to Suspect an Autoinflammatory Syndrome

There is still a considerable delay in the diagnosis of patients with autoinflammatory syndromes [24–27]. Thus, early suspicion is crucial. These syndromes should be suspected in patients with recurrent fever unexplained by infections and/or with episodic stereotypic symptoms in various organs and tissues, especially the skin, gastrointestinal tract, musculoskeletal system, eyes, mucous membranes and central nervous system. While most syndromes begin in childhood, it is not unusual for patients to present in adulthood, either with initial manifestations or more commonly with a long history dating back to childhood. In some of the diseases the clinical course is chronic and persistent rather than episodic. Sometimes the clues to the existence of these syndromes are unexplained, multisystem involvement, with elevated acute phase reactants, commonly starting very early in life. In some of the newly described diseases, recurrent infections, indicative of an immunodeficiency, are seen along with autoinflammatory-related symptoms.

Autoinflammatory syndromes should also be considered in patients with unexplained elevations of acute phase reactants, even in the absence of symptoms. A family history often reveals relatives with similar symptoms, including unexplained cases of renal failure (as a feature of amyloidosis from long-standing inflammation) or hearing loss.

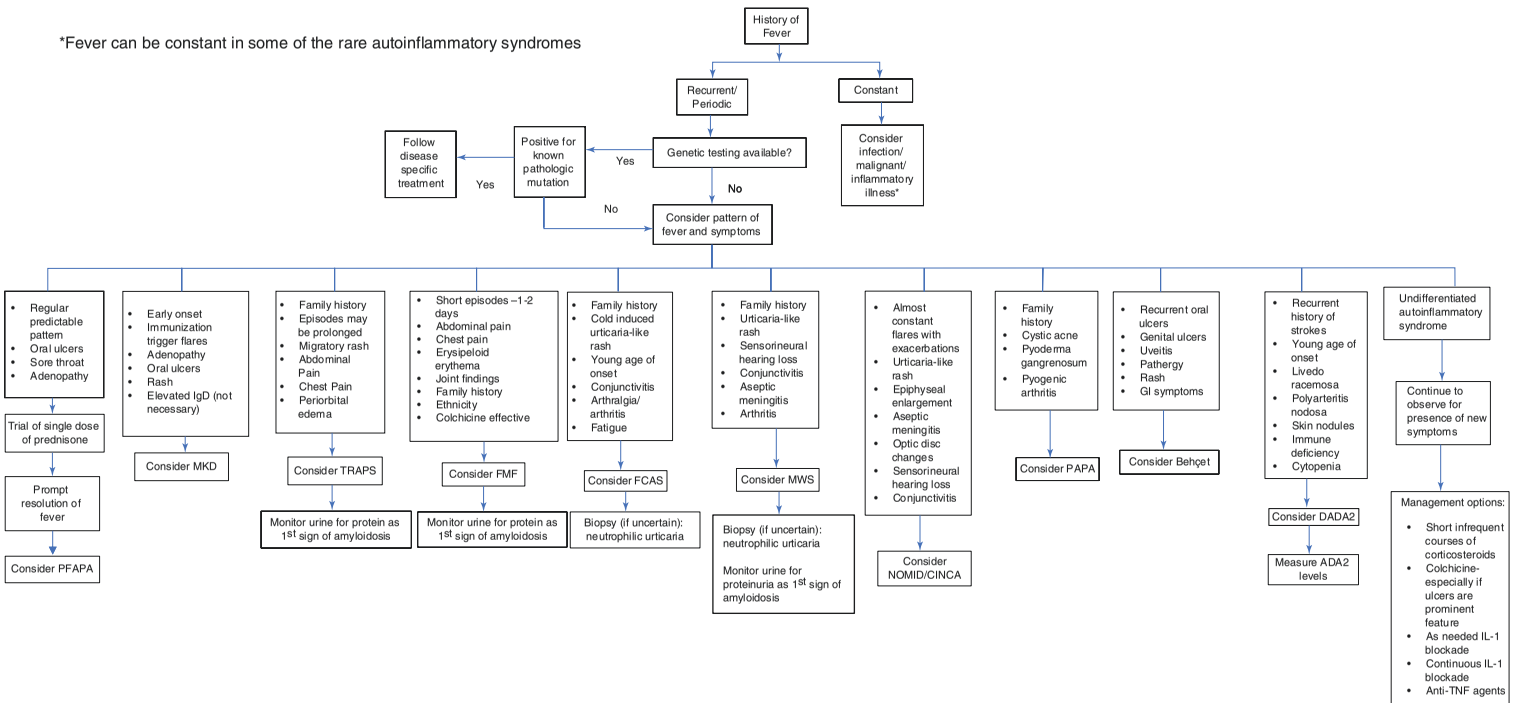

Fig. 11.1 Algorithm for diagnosis of the major recurrent febrile autoinflammatory diseases. *PFAPA* Periodic fever with aphthous stomatitis, pharyngitis and cervical adenitis, *MKD* Mevalonate kinase deficiency, *TRAPS* TNF receptor-associated periodic fever syndrome, *FMF* Familial Mediterranean fever, *FCAS* Familial cold autoinflammatory syndrome, *MWS* Muckle-Wells syndrome, *NOMID* Neonatal-onset multisystem inflammatory disease, *CINCA* Chronic infantile neurological cutaneous articular syndrome, *PAPA* Pyogenic arthritis, pyoderma gangrenosum and acne, *DADA2* Deficiency of adenosine deaminase 2, *ADA2* adenosine deaminase 2, *CNS* Central nervous system, *TNF* Tumor necrosis factor, *IL* Interleukin

11.3 Systematic Clinical Approach

- **A systematic history based on pertinent demographic and clinical symptoms should be obtained**
- **It is important for patients to document attacks in a diary, including potential triggers, and photograph physical findings such as rash and joint swelling**
- **Laboratory tests are mostly non-specific and less helpful in reaching a specific diagnosis**
- **Occasionally, treatment trials can be helpful in confirming a diagnosis**

The approach to autoinflammatory syndromes often resembles solving a complex puzzle with many pieces. The more pieces that fit help identify patterns enabling an earlier diagnosis. Complete histories and physical examinations are crucial. Several pertinent questions can help characterize and differentiate between syndromes and often point to the correct diagnosis before genetic test results are available (Tables 11.1 and 11.2). It is often very helpful to examine patients during an attack. If this is not possible, patients or caretakers should be asked to carefully document attacks in a diary (the autoinflammatory diseases activity index—AIDAI can be used [28], see Chap. 13) and take pictures of relevant physical findings, especially rashes, joint/soft tissue swelling and even the appearance of the eyes, mouth and throat. For example, it would be useful to see a red eye or periorbital swelling as well as oral ulcers and tonsillar exudates. It is also useful to measure markers of inflammation during and between attacks, as in some diseases attacks are only the "tip of the inflammatory iceberg" and patients consistently have increased inflammatory indices. In some of the diseases, persistent inflammatory states increase the risk of developing amyloid A (AA) amyloidosis (see Chap. 15).

11.3.1 Age at Onset

Determination of the age of onset may help differentiate some of the disorders, although occasionally, especially in older patients or those with a long history, an accurate answer is not possible. Table 11.2 details the age of onset of the major autoinflammatory diseases.

11.3.2 Ethnicity/Geographic Origin

Determining ethnicity may be helpful in supporting the diagnosis of some of the autoinflammatory diseases, for example, for familial Mediterranean fever (FMF) (see Chap. 16). In some of the rare diseases cases and families are detected in specific localities (Table 11.2). It is clear, however, that the vast majority of autoinflammatory diseases can be found in nearly all ethnicities and geographic areas.

11.3.3 Family History/Consanguinity

Questions related to family history and consanguinity are important in determining the genetic inheritance (mainly autosomal recessive vs. dominant). Drawing a family pedigree is very useful. It must be remembered that in many of the autosomal dominant diseases mutations may arise de-novo or as a somatic mutation, in which case the family history will not be informative. Even in non-monogenic diseases (e.g. PFAPA syndrome) a family history may be informative [29].

Table 11.1 Important historical points to consider during investigations of autoinflammatory syndromes

Age of onset
Ethnicity (e.g. Mediterranean)
Consanguinity
Family history
Triggers for attack (e.g. infection, cold, vaccines, stress, exercise, menstrual periods, pregnancy)
Duration of attacks
Frequency of attacks (including periodicity)
Clinical features/system involvement (e.g. fever, rash, serositis, gastrointestinal, chest, musculoskeletal, ophthalmologic, eye, neurologic [including hearing], infections)
Response to therapy (colchicine, corticosteroids, tumor necrosis factor, interleukin-1 inhibitors and other)

Table 11.2 Helpful clues to assist in the diagnosis of the autoinflammatory syndromes

Common age of onset	Disease
Neonatal	NOMID, DIRA, FCAS, SAVI, TRAPS11
Infancy/first year of life	MKD, FCAS, NLRP12, other interferonopathies, Very early-onset IBD, DADA2, NLRP1
Toddler	PFAPA, Blau/Early-onset sarcoidosis
Late childhood	PAPA
Adolescence/Adulthood	Schnitzler, Gout, Recurrent pericarditis, Behçet
Most common of primarily childhood syndromes to have onset in adulthood	TRAPS, DITRA, some forms of AGS
Variable (mostly in childhood)	All others
Typical ethnicity/geography	
Armenians, Turks, Italian, Greek, Sephardi > Ashkenazi Jews, Japan	FMF
Arabs	FMF, DITRA (Arab Tunisian), Majeed syndrome, DIRA (Lebanon)
Dutch, French, German, Western Europe	MKD, MWS, NLRP12, PAPA
Scottish/Irish	TRAPS
North America	FCAS
Eastern Canada, Puerto Rico	DIRA
Georgian Jews	DADA2
Pakistani	PFIT
Worldwide	All others
Classic triggers	
Vaccines	MKD
Cold exposure	FCAS, NLRP12, NLRC4, PLAID, SAVI (worsening of lesions)
Menses	FMF
Minor Trauma	PAPA, TRAPS, MKD, Behçet (skin)
Exercise	FMF, TRAPS
Pregnancy	DITRA
Infections	All, especially DITRA
Stress	All
Usual duration of attack	
Less than 24 h	FCAS, FMF, NLRP12
One to three days	FMF, MWS, DITRA (fever)
Three to seven days	MKD, PFAPA, PFIT
Longer than 7 days	TRAPS, PAPA, PAAND
Months	CNO
Chronic	NOMID, DIRA, interferonopathies, systemic JIA, Schnitzler syndrome
Interval between attacks	
Three to six weeks	PFAPA, MKD
More than six weeks	TRAPS
Mostly unpredictable	All others
Truly periodic	PFAPA, cyclic neutropenia
Useful laboratory tests	
Acute phase reactants must be normal between attacks	PFAPA
Anemia (disease specific)	Majeed (dyserythropoetic), MKD (dyserythropoetic), DADA2 (aplastic), SIFD (sideroblastic), MAS/NLRC4
Thrombocytopenia	MAS/NLRC4, PFIT

(continued)

Table 11.2 (continued)

Common age of onset	Disease
Urine mevalonic acid in attack	MKD
IgD >100 mg/dl	MKD (see text for limitations)
Low immunoglobulin levels	PLAID, APLAID, HOIL-1 (memory B cells), PFIT, DADA2
Proteinuria (amyloidosis)	FMF, TRAPS, MWS, NOMID, MKD (rare)
Response to therapy	
Corticosteroid dramatic	PFAPA, pericarditis
Corticosteroid partial	TRAPS, MKD, FCAS, MWS, NOMID, sJIA, CNO, PAPA[a]
Colchicine	FMF, gout, pericarditis, PFAPA (30–50% effective), Behçet
Cimetidine	PFAPA (30% effective)
Etanercept	TRAPS, FMF (arthritis), CNO, DADA2
Anti-TNF (antibodies)	FMF (arthritis), DADA2, Behçet (gastrointestinal, eyes), CNO, PAPA, Blau/Early-onset sarcoidosis, IBD
Anti IL-1 dramatic	DIRA (anakinra), FCAS, MWS, NOMID, PFAPA, Schnitzler
Anti-IL-1 mostly	sJIA, gout, FMF, TRAPS, MKD, pericarditis, DITRA
Anti-IL-1 partial	PAPA, Behçet
Anti-IL-6	sJIA, Schnitzler
Janus kinase inhibitors	Interferonopathies

AGS Aicardi-Goutières syndrome, *CNO* Chronic non-bacterial osteomyelitis, *DADA2* deficiency of adenosine deaminase 2, *DIRA* Deficiency of the IL-1 receptor antagonist, *DITRA* Deficiency of the IL-36 receptor antagonist (generalized pustular psoriasis), *FCAS* Familial cold autoinflammatory syndrome, *FMF* Familial Mediterranean fever, *IBD* Inflammatory bowel disease, *IL* interleukin, *MAS* Macrophage activation syndrome, *MKD* Mevalonate kinase deficiency, *MWS* Muckle-Wells syndrome, *NLRP* Nucleotide-binding domain, leucine-rich repeat, and pyrin domain containing, *NLRC* Nucleotide-binding oligomerization (NOD), leucine rich repeats and CARD domain containing, *NOMID* Neonatal-onset multisystem inflammatory disease, *PAAND* Pyrin-associated autoinflammation with neutrophilic dermatosis, *PAPA* Pyogenic sterile arthritis, pyoderma gangrenosum and acne, *PFAPA* Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis, *PFIT* Periodic fever, immunodeficiency and thrombocytopenia, *PLAID* PLCγ2-associated antibody deficiency and immune dysregulation, *APLAID*, autoinflammation and PLAID, *SAVI* STING-associated vasculopathy with onset in infancy, *SIFD* Sideroblastic anemia, immunodeficiency, fevers, and developmental delay, *sJIA* Systemic juvenile idiopathic arthritis, *TNF* Tumor necrosis factor, *TRAPS* Tumor necrosis factor receptor-associated periodic syndrome, *TRAPS 11* TRAPS due to mutations in TNFRSF11A
[a]Intraarticular corticosteroids

Occasionally the family history uncovers members who have suffered organ damage caused by the disease (e.g. hearing loss, renal failure) or "treatment-response" (e.g. tonsillectomy in PFAPA) may be elicited.

11.3.4 Triggers

The trigger for attacks can be an important diagnostic clue (Table 11.2). Some of the triggers are quite specific for a particular disease (e.g. vaccines for mevalonate kinase deficiency-MKD; cold exposure for several conditions) but others like stress and infections are non-specific. Other important triggers include physical exertion, minor trauma, menstrual periods and pregnancy, and may be seen in a variety of diseases.

11.3.5 Duration of Attack

There is marked overlap between diseases but when the duration is very short (<24 h) or long (more than 2 weeks) the differential diagnosis becomes much narrower (Table 11.2). While autoinflammatory diseases have been called periodic fever syndromes, many of the diseases,

particularly those newly described, are chronic diseases, and not all have fever as part of their clinical picture.

11.3.6 Interval Between Attacks

Similar to the duration of attacks, there is marked overlap in the interval between attacks among the different autoinflammatory diseases (Table 11.2). In some conditions, such as PFAPA, the interval between attacks is often very predictable. The interval between attacks may vary markedly in individual patients, for example, by the season of the year or stress levels, and there may be even long periods without attacks.

11.3.7 Periodicity

There are very few diseases that are truly periodic (Table 11.2) with parents/patients able to predict when the attack will occur. In PFAPA attacks may become less periodic over time, especially after corticosteroid treatment is commenced.

11.3.8 Laboratory Testing

The results of laboratory testing in the evaluation of the autoinflammatory syndromes is usually non-specific. However, it is important to test for inflammatory markers both during an attack and between attacks. In almost all of the autoinflammatory diseases inflammatory markers will be elevated during an attack. The diagnosis of an autoinflammatory disease must be questioned in patients with a consistent lack of inflammatory marker elevation during attacks; however, this does rarely occur, especially in the interferonopathies. It is also important to measure inflammatory markers between attacks. For example, in patients with suspected PFAPA, one most consider other diagnoses that mimic PFAPA if inflammatory markers between attacks are elevated [30]. An elevation of these markers between attacks suggests ongoing sub-clinical inflammation.

More specific laboratory tests include urinary mevalonic acid levels during an attack of MKD (Table 11.2). Serum levels of immunoglobulin D are neither sensitive nor specific for MKD, as they may be slightly elevated in many conditions (see Chap. 17). In patients with suspected cyclic neutropenia (periodic fever, gingivostomatitis occurring every 3–4 weeks, and recurrent bacterial infections) testing of neutrophil counts at least weekly (and perhaps more often) for at least 1 month is helpful, as the nadir of neutrophils occurs prior to the attack. The presence of autoantibodies does not rule out autoinflammatory conditions, particularly those involving the interferon pathway. Periodic urinalysis is important to test for proteinuria, an early sign of amyloidosis.

In several diseases, specific findings of common laboratory tests may aid in the diagnosis. For example, sideroblastic anemia is seen in the sideroblastic anemia, immunodeficiency, fevers, and developmental delay (SIFD) syndrome and dyserythropoetic anemia is seen in Majeed syndrome and rarely in MKD. Thrombocytopenia is seen in the periodic fever, immunodeficiency and thrombocytopenia (PFIT) syndrome. Low immunoglobulin levels or abnormalities in lymphocyte flow cytometry may be seen in the syndromes combining autoinflammation and immunodeficiency (e.g. deficiency of adenosine deaminase 2-DADA2, see Chaps. 23 and 28).

In some diseases, advanced testing may be helpful in the diagnostic process or in assessing disease damage (Table 11.3). Other testing such as serologies, endoscopy/colonoscopy, other imaging, biopsies (e.g. lymph node, bone marrow, bone, etc.) may be performed as part of the work-up for the differential diagnoses of the autoinflammatory diseases.

11.3.9 Response to Treatment

The response to treatment can be useful in the diagnostic process of the autoinflammatory diseases and is even included in some of the diagnostic criteria for FMF (Table 11.2) [4]. A trial of colchicine can be useful in patients with suspected

Table 11.3 Specialized investigations in the autoinflammatory diseases

Test	Disease
Lumbar puncture	Cryopyrin-associated periodic syndromes
Joint aspiration	Gout, [a]FMF, [a]PAPA, rule-out infectious arthritis
Audiometry	Cryopyrin-associated periodic syndromes
Skin biopsy	Many conditions with rash
Echocardiography	FMF, TRAPS, Recurrent pericarditis
Whole body MRI	CNO, Schnitzler syndrome
Other MRI	TRAPS (fasciitis), Cryopyrin-associated periodic syndromes (inner ear, brain), DADA2 (brain)
Endoscopy/ colonoscopy	Very early-onset inflammatory bowel disease

FMF Familial Mediterranean fever, *PAPA* Pyogenic sterile arthritis, pyoderma gangrenosum and acne, *TRAPS* Tumor necrosis factor receptor-associated periodic syndrome, *CNO* Chronic non-bacterial osteomyelitis, *DADA2* Deficiency of adenosine deaminase 2
[a]Not diagnostic, but reveals massive neutrophil counts

FMF, although this is not completely specific as patients with PFAPA and Behçet disease may also respond to colchicine. While corticosteroids are beneficial in the treatment of several autoinflammatory diseases, a dramatic response to one dose of corticosteroid is characteristic of PFAPA. Failure to respond to corticosteroids may also be helpful in making a diagnosis. For example, classic FMF attacks do not respond to corticosteroids. A lack of response may lead to searching for monogenic mimics of PFAPA such as MKD. Response to IL-1 inhibitors (and there are initial indications that perhaps IL-6 inhibitors-see Chaps. 41 and 42) is not specific and common to many of the autoinflammatory syndromes and may be useful in confirming that an autoinflammatory syndrome is likely [31]. Tumor necrosis factor (TNF) inhibitors may be useful in many autoinflammatory diseases (Table 11.2). Janus kinase (JAK) inhibitors may be useful in treating interferonopathies. Readers are referred to the disease specific chapters for greater detail on treatments.

11.4 System Involvement

- **A careful history of system involvement is probably the most important element in differentiating between the various autoinflammatory syndromes**
- **The major systems involved include the skin, musculoskeletal system, eyes, gastrointestinal and central nervous system**

While there is a great deal of overlap between diseases, the overall pattern of system involvement is the most helpful clue in advancing the diagnosis. The involvement of different organs and systems may evolve over time. For example, younger patients with FMF often present only with fever and the more classic picture of FMF may take years to develop [32]. In cryopyrin-associated periodic syndromes (CAPS) hearing loss will usually start to develop in adolescence.

The clinical characteristics of each disease are specified in their individual chapters. Our approach in this chapter is to link the diseases to specific organs and/or systems and to the specific features of the diseases within each system/organ (Table 11.4).

11.4.1 Dermatologic Manifestations

Almost all the autoinflammatory diseases involve the skin (Table 11.4). While almost none are pathognomonic for a specific disease, the pattern of the rash is important [33]. A skin biopsy is often helpful in the diagnostic process. CAPS, Schnitzler syndrome and other less common autoinflammatory diseases manifest an urticarial-like rash showing a neutrophilic infiltrate, unlike allergic rashes, where the urticaria are associated with mast cells and histamine. Plaque-like lesions are seen particularly in FMF and TRAPS. Pustular lesions are seen in Behçet disease and chronic non-bacterial osteomyelitis (CNO) as well as in many of the rare syndromes, including those associated with psoriasis (see Chap. 26). Ulcerative lesions, including

Table 11.4 System involvement in the autoinflammatory syndromes

Dermatology manifestations	
Urticarial-like rash	FCAS, MWS, NOMID, sJIA (occasional), MKD (occasional), Schnitzler, NLRP-12, PLAID, NLRC4
Fasciitis/plaque	FMF ("erysipelas-like"), TRAPS (painful, centrifugal, migratory fasciitis), APLAID (cellulitis)
Neutrophilic dermatosis	PAAND, Majeed, Otulipenia, Behçet, SAPHO
Maculopapular	sJIA, MAS, MKD, TRAPS11, NLRC4
Nodular	Gout (tophi), DADA2
Multiforme/mobiliform	MKD
Granulomatous (waxy) rash	Blau/Early-onset sarcoidosis, PLAID
Pustular rash	Behçet, CNO, DIRA, DITRA, AP1S3, Majeed, HA20, SAVI, APLAID, CARD14, Otulipenia
Pathergy	Behçet, PAPA, HA20
"Abscesses"	Behçet, PAPA, PAAND
Blister	APLAID
Psoriatic	CNO, PAPA, DITRA, CARD14, AP1S3
Acneiform	Behçet, CNO (SAPHO), PAPA, PAAND
Panniculitis	Behçet, Interferonopathies, Blau/Early-onset sarcoidosis, Otulipenia
Lipodystrophy	PRAAS/CANDLE, Very early-onset IBD, Otulipenia
Ulcerative (including pyoderma)	Behçet, PAPA, Very early-onset IBD, HA20, PAAND, NLRP1
Livedo-like	DADA2, Interferonopathies
Pernio/chilblains	Interferonopathies, DADA2
Vasculitis	FMF, Behçet, DADA2, MKD, PAPA, SAVI, PAAND, Otulipenia
Atopy	PLAID
Other	CARD14 (pityriasis rubra piliaris), NLRP1 (dyskeratosis, self-healing palmoplantar carcinoma), SAVI (nail dystrophy)
Musculoskeletal manifestations	
Arthralgia	Most conditions
Arthritis	sJIA, Gout, FMF, Behçet, CNO, MKD, TRAPS, MWS, NOMID, PAPA, Very early-onset IBD, NLRP1, Blau/Early-onset sarcoidosis, PRAAS/CANDLE, HA20
Inflammatory bone disease	CNO (SAPHO), Schnitzler, DIRA, Majeed, Cherubism, PAPA
Epiphyseal overgrowth	NOMID, DIRA
Joint deformities	sJIA, NOMID, Blau/Early-onset sarcoidosis, DIRA, PRAAS/CANDLE
Severe prolonged myalgia	FMF, TRAPS
Ophthalmologic manifestations	
Conjunctivitis	TRAPS, FCAS, MWS, NOMID, MKD
Uveitis	Behçet, MWS, NOMID, Blau/Early-onset sarcoidosis, HA20
Papillitis	NOMID
Periorbital swelling	TRAPS
Glaucoma	AGS
Corneal ulcers/scarring	APLAID, NLRP1
Retinitis pigmentosa	SIFD, MKD
Central nervous system manifestations	
Headache	Many syndromes, especially MWS, NOMID, NLRP12
Hearing loss	MWS, NOMID, NLRP12, SIFD
Recurrent meningitis	MWS, NLRC4, FMF, MKD
Meningoencephalitis	Behçet
Chronic meningitis	NOMID
Stroke	DADA2
Vasculopathy/vasculitis	DADA2, AGS, Behçet, HA20
Calcifications	AGS, PRAAS/CANDLE
Developmental/intellectual delay	AGS, NOMID, Mevalonic aciduria (severe subset of MKD), SIFD

Table 11.4 (continued)

Seizures	Many febrile syndromes, especially PFAPA, Mevalonic aciduria, AGS, SIFD
Ataxia (cerebellar)	SIFD, Mevalonic aciduria
Gastrointestinal manifestations	
Peritonitis	FMF, TRAPS
Abdominal pain	Behçet, MKD, PFAPA, Very early-onset IBD, DADA2, HA20
Constipation	FMF, TRAPS11
Diarrhea/enterocolitis	MKD, TRAPS, FMF, Behçet, HA20, Very early-onset IBD, NLRC4, APLAID, Otulipenia
Oral ulcerations	PFAPA, Behçet, MKD, HA20, Very early-onset IBD, NLRP12, PFIT, DITRA (geographic tongue)
Reticuloendothelial manifestations	
Lymphadenopathy—cervical	PFAPA, MKD
Lymphadenopathy—general	sJIA, MKD, MAS, NLRC4, NLRP12, TRAPS11, HOIL-1
Hepatomegaly	sJIA, MAS, Blau/Early-onset sarcoidosis, NLRC4, DADA2, SIFD, HOIL-1 (amylopectinosis)
Splenomegaly	FMF, TRAPS, MKD, sJIA, MAS, NLRC4, PRAAS/CANDLE, SIFD, HOIL-1
Tonsillitis	PFAPA (and overlap with FMF, MKD, TRAPS)
Portal hypertension	DADA2
Chest/respiratory manifestations	
Pleuritis	FMF, TRAPS, sJIA
Pericarditis	FMF, TRAPS, sJIA, Recurrent pericarditis
Cardiomyopathy	SIFD
Lung disease	sJIA, SAVI, DIRA, APLAID
Immune disturbances	
Immunodeficiency	DADA2 (usually mild, B-cell disease), APLAID (sino-pulmonary), PLAID (sino-pulmonary), SIFD (sino-pulmonary), HOIL-1 (pyogenic, viral), PFIT (pyogenic, *Pneumocystis jirovecii)*, NLRP1
Autoimmunity (with antibodies)	Interferonopathies, PLAID (thyroid)
Other	
Genital ulcerations	Behçet, MKD, HA20, Very early-onset IBD
Scrotal swelling	FMF, TRAPS
Lymphangiectasia	HOIL-1
Amyloidosis	FMF, TRAPS, MWS, NOMID, MKD (rare), sJIA (in past)

AGS Aicardi-Goutières syndrome, *AP1S3* Adaptor-related protein complex 1 sigma 3 subunit, *CARD* Caspase activation and recruitment domains, *CNO* Chronic non-bacterial osteomyelitis, *DADA2* Deficiency of adenosine deaminase 2, *DIRA* Deficiency of the IL-1 receptor antagonist, *DITRA* Deficiency of the IL-36 receptor antagonist (generalized pustular psoriasis), *FCAS* Familial cold autoinflammatory syndrome, *FMF* Familial Mediterranean fever, *HA20* A20 haploinsufficiency, *IBD* Inflammatory bowel disease, *IL* interleukin, *MAS* Macrophage activation syndrome, *MKD* Mevalonate kinase deficiency, *MWS* Muckle-Wells syndrome, *NLRP* Nod-like receptor family, pyrin domain, *NLRC* NLR family CARD domain-containing protein, *NOMID* Neonatal-onset multisystem inflammatory disease, *PAAND* Pyrin-associated autoinflammation with neutrophilic dermatosis, *PAPA* Pyogenic sterile arthritis, pyoderma gangrenosum and acne, *PFAPA* Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis, *PFIT* Periodic fever, immunodeficiency and thrombocytopenia, *PLAID* PLCγ2-associated antibody deficiency and immune dysregulation, *APLAID* Autoinflammation and PLAID, *PRAAS/CANDLE* Proteasome-associated autoinflammatory syndromes/Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *SAPHO* Synovitis, acne, pustulosis, hyperostosis and osteitis, *SAVI* STING-associated vasculopathy with onset in infancy, *SIFD* Sideroblastic anemia, immunodeficiency, fevers and developmental delay, *sJIA* systemic juvenile idiopathic arthritis, *TNF* Tumor necrosis factor, *TRAPS* Tumor necrosis factor receptor-associated periodic syndrome, *TRAPS 11* TRAPS due to mutations in TNFRSF11A

The less common features of the various diseases are not listed. The more common diseases are generally listed earlier in each manifestation

pyoderma gangrenosum, can be seen in several syndromes, like pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome and very early-onset inflammatory bowel disease. The interferonopathies are associated with panniculitis, lipodystrophy, pernio/chilblains and livedo rashes. The presence of granulomatous inflammation in a skin biopsy may be helpful, especially in identifying Blau syndrome/early-onset sarcoidosis. Vasculitis is often a secondary skin feature (e.g. in FMF, MKD) but is a prime feature in some syndromes (e.g. DADA2, some of the interferonopathies). Non-specific maculopapular rashes are seen in many syndromes and undifferentiated disease. In some of the syndromes multiple types of rashes can be seen. The location of the rashes may be helpful. For example, the lesions seen in STING-associated vasculopathy with onset in infancy (SAVI) are mostly acral. Centrifugal migrating plaque lesions are seen in TRAPS.

11.4.2 Musculoskeletal Manifestations

Arthralgia and/or myalgia are common to most autoinflammatory syndromes (Table 11.4). In some diseases (e.g. TRAPS and FMF) myalgia can be particularly prolonged and severe. Arthritis can vary from monoarthritis (most common type of arthritis in FMF and PAPA) to polyarthritis (MKD, systemic juvenile idiopathic arthritis [sJIA]). The arthritis may become chronic in some diseases like sJIA, Blau syndrome/early-onset sarcoidosis and in 5–10% of patients with FMF (for the latter mainly in the hip and sacroiliac joints). In some disease (e.g. Blau syndrome/early-onset sarcoidosis, sJIA and proteasome-related diseases) arthritis often leads to joint contractures.

Bone deformities related to epiphyseal and/or metaphyseal abnormal growth, including clubbing, may be seen in some of the diseases, particularly in the more severe CAPS phenotypes and deficiency of the IL-1 receptor antagonist (DIRA). Lytic/sclerotic bone lesions are common in CNO and other monogenic forms of chronic osteomyelitis.

11.4.3 Ophthalmologic Manifestations

Ophthalmologic involvement is one of the common manifestations of the autoinflammatory diseases (Table 11.4). The severity ranges from mild features (conjunctivitis) to severe uveitis and papillitis and can result in blindness, particularly in Behçet disease and neonatal-onset multisystem inflammatory disease (NOMID). The eye is most commonly involved in CAPS, with severity parallel to the severity of the phenotype, and TRAPS. In some of the very rare syndromes there are unusual eye manifestations such as corneal ulceration and retinitis pigmentosa. Periorbital swelling in the context of prolonged recurrent fever is almost pathognomonic for TRAPS.

11.4.4 Central Nervous System (CNS) Manifestations

There are many forms of CNS involvement. The most specific and crucial are the issue of strokes, often starting early in life in DADA2, vasculitis in Behçet disease and chronic meningitis in NOMID and some interferonopathies. Hearing loss, particularly in the severe CAPS phenotypes, is also an important damaging outcome. In several syndromes like NOMID and some of the interferon diseases (also the severe MKD phenotype of mevalonic aciduria) patients may have developmental delays and intellectual impairment.

11.4.5 Gastrointestinal Manifestations

Peritonitis is the hallmark in FMF and TRAPS. Non-specific abdominal pain and diarrhea is seen in many syndromes. Post-prandial pain can be seen in DADA2 as part of the polyarteritis nodosa phenotype. Enterocolitis is commonly seen in very early-onset inflammatory bowel disease (IBD) and NLRC4-macrophage activation syndrome (MAS), but also may occur in MKD (especially in early years) and other syndromes.

11.4.6 Reticuloendothelial Manifestations

Hepatosplenomegaly is common to many autoinflammatory syndromes (Table 11.4). Lymphadenopathy can be regional as in PFAPA or generalized as in MKD and other conditions.

11.4.7 Chest and Respiratory Manifestations

Lung disease is relatively uncommon in autoinflammatory conditions (Table 11.4) except in SAVI. Pleural and pericardial involvement are seen in diseases with serositis, for example FMF and TRAPS. Pericarditis can also be seen as an isolated phenomena (see Chap. 36).

11.4.8 Immune Disturbances

The combination of autoinflammation, autoimmunity and immunodeficiency is seen in some of the newly discovered diseases (Table 11.4). Chapters 28 and 38 expand on the science behind these interesting phenomena.

11.5 Clues to the Diagnosis of Specific Classic Autoinflammatory Diseases

- **Periodicity is a major clue for diagnosis of the periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome**
- **Ethnicity/geographic origin as well as serositis are the most important clues for familial Mediterranean fever (FMF)**
- **The age of onset as well as vaccine triggers of an attack are important clues for suspecting mevalonate kinase deficiency (MKD)**
- **Attacks that last longer than 1 week and include periorbital edema as well as a migratory painful rash are suggestive of tumor necrosis factor receptor-associated periodic syndrome (TRAPS)**
- **An urticarial-like rash, eye involvement and hearing loss are crucial elements of the cryopyrin-associated periodic syndromes (CAPS)**

11.5.1 Periodic Fever, Aphthous Stomatitis, Pharyngitis, Cervical Adenitis (PFAPA) Syndrome

Regular, clocklike and predictable patterns of fever episodes, along with oral ulcers, pharyngitis, and cervical adenopathy are suggestive of the PFAPA syndrome (see Chap. 30). Episodes generally last 3–6 days. Patients may have nonspecific abdominal and joint pain but do not have peritonitis, and only occasionally have diarrhea, arthritis, conjunctivitis or a rash. While currently there are no known genetic mutations associated with PFAPA, a family history of PFAPA including successful tonsillectomies in close family members with a similar clinical picture may be helpful in diagnosis [34, 35].

The response to a trial of a single dose of corticosteroid may be informative. Prompt resolution of fever and possible shortened intervals between fever episodes is classic. As noted previously, the clinical presentation of PFAPA may overlap with other monogenic diseases such as FMF and MKD. Many Middle Eastern patients with PFAPA may have a heterozygous mutation/polymorphisms of the *MEFV* gene associated with FMF (more than in the general population) and some of these patients may respond to treatment with colchicine [36].

11.5.2 Familial Mediterranean Fever (FMF)

Short episodes (1–3 days) associated with severe abdominal and/or chest pain (often severe), representing serositis, should raise the suspicion for FMF, usually an autosomal recessive disease (see Chap. 16). Other features include erysipeloid-like erythema, often on the ankle or dorsum of the foot, joint pain, and a family

history of similar episodes. An important feature is the ethnicity/geographic origin of the patients, who are usually from the Mediterranean basin. A history of constipation during the flare, followed by diarrhea is common. Acute scrotal pain reflects the serosal inflammation associated with this disease. Acute arthritis, mainly in the knees and ankle may last as long as 1 week, longer than the febrile attack. Elevation of acute phase reactants are seen in the vast majority of attacks, and sometimes between attacks. Leukocytosis is common during attacks. Colchicine treatment is highly effective for most patients in preventing febrile episodes (and amyloidosis) and the response to colchicine may be a useful diagnostic aid [2].

11.5.3 Mevalonate Kinase Deficiency (MKD)

Onset of episodes within the first year of life, triggering of flares with childhood immunizations (the most useful clues), gastrointestinal symptoms, cervical (and sometimes generalized) painful lymphadenopathy, oral ulcers, and a generalized erythematous rash suggest a diagnosis of MKD (see Chap. 17). Serum levels of immunoglobulin D levels may be elevated but this is not a consistent finding and does not correlate with disease activity. Urinary mevalonic acid levels are usually highly elevated during an acute episode.

11.5.4 Tumor Necrosis Factor Receptor-Associated Periodic Syndrome (TRAPS)

A history of prolonged attacks, lasting more than 1 week, associated with a painful migratory rash (representing underlying fasciitis), chest and/or abdominal pain, myalgia and periorbital edema suggests the diagnosis of TRAPS, especially in combination with an autosomal dominant family history (see Chap. 18). Similar to FMF, abdominal pain may be severe and mimic a surgical abdomen. The presence of aphthous stomatitis and tonsillitis is not characteristic of classic TRAPS. Acute phase reactants are increased during flares and often remain elevated between flares, suggesting an elevated level of baseline inflammatory activity. Unlike FMF, colchicine is not effective for symptoms or preventing the development of amyloidosis.

11.5.5 Cryopyrin-Associated Periodic Syndromes (CAPS)

A history of short episodes of cold-induced urticaria-like rash and early age of onset suggests the diagnosis of familial cold autoinflammatory syndrome (FCAS), the mildest form of CAPS (see Chap. 19). Patients with FCAS also complain of fever, conjunctivitis or arthralgia and often overwhelming fatigue. Age of onset is usually less than 6 months and duration of most attacks is less than 24 h. The rash is migratory, maculopapular, urticaria-like and usually non-pruritic. Biopsy of the rash shows a predominant perivascular neutrophilic infiltrate as opposed to the eosinophil infiltrate seen in classical urticaria. Patients with FCAS usually respond very well to blockade of the IL-1 pathway [2].

A family history of an urticaria-like rash, sensorineural hearing loss (starting with high frequency tones) and recurrent aseptic meningitis should raise the suspicion for Muckle-Wells syndrome (MWS), the intermediate form of CAPS. Attacks last longer than FCAS, usually up to 3 days. Patients may also develop conjunctivitis (they may also have uveitis) or arthralgia/arthritis. Unlike FCAS, these patients are at risk to develop amyloidosis, so monitoring of urine for proteinuria is recommended. As with the other forms of CAPS patients usually respond to treatment with IL-1 blockade.

A neutrophilic urticaria-like rash can also be seen in patients with NOMID, the most severe form of CAPS. Unlike FCAS or MWS, these patients have almost continuous flares with periods of worsening. They also demonstrate epiphyseal enlargement (in about 50%), chronic aseptic meningitis, papilledema and sensorineural hearing loss.

11.5.6 Pyogenic Arthritis, Pyoderma Gangrenosum and Acne (PAPA) Syndrome

A history of severe, scarring, cystic acne, pyoderma gangrenosum and recurrent episodes of sterile, painful, erosive arthritis is suggestive of a diagnosis of the PAPA syndrome (see Chap. 22).

11.5.7 Behçet Disease

The clinical presentation of recurrent oral ulcers, genital ulcers, uveitis, pathergy, rash and gastrointestinal symptoms suggests the diagnosis of Behçet disease (see Chap. 35).

11.5.8 Deficiency of Adenosine Deaminase 2 (DADA2)

Recurrent history of strokes, early age of onset, livedo racemosa, polyarteritis nodosa, skin nodules, immune deficiency and cytopenias should raise suspicion for the diagnosis of DADA2 (see Chap. 23). Where available, measurement of ADA2 levels may be helpful in making the diagnosis. It is important to start anti-TNF therapy early, as this decreases the chance of further strokes.

11.6 Diagnostic vs. Classification Criteria for Differentiating Autoinflammatory Diseases

- **It is important to recognize the difference between diagnostic and classification criteria**
- **While several sets of diagnostic and classification criteria have been proposed for several autoinflammatory syndromes they have not been validated in the general population therefore limiting their applicability**
- **A recent Eurofever initiative is underway to develop diagnostic criteria that can be applied with and without the results of genetic testing**

Autoinflammatory diseases are often difficult to differentiate both from other disorders as well as from one another. It would therefore be extremely helpful to have criteria to help clinicians establish a specific diagnosis and institute effective treatment. It is important in this regard to differentiate "diagnostic" from "classification" criteria, a subject recently reviewed by the American College of Rheumatology [37].

Diagnostic criteria are used to guide the care of individual patients. They must have both very high sensitivity and specificity in order that patients receive the correct diagnosis and appropriate treatment. On the other hand, classification criteria are primarily used to define cohorts of patients that can be included in clinical research; they are broader with a very high specificity, but a lower sensitivity. This may result in some patients with the disease not being captured (false negative); however, the chances of patients not having the indicated diagnosis (false positive) are very low. When a gold standard exists for a specific disease (e.g. previously identified pathogenic mutations in *NLRP3* in CAPS), and that element is included in classification criteria, then classification criteria can serve as diagnostic criteria as well. In some autoinflammatory diseases (e.g. TRAPS), the presence of identified pathogenic mutations in patients with characteristic clinical signs and symptoms may define some (although not all) diseases and therefore can serve as a gold standard for diagnosis. However, factors such as reduced penetrance, epigenetics, environment and somatic mosaicism, among others, may affect the interpretation of genetic test results (see Chap. 12). Furthermore, genetic testing is not available in most parts of the world. As a result, a gold standard is not available for the majority of patients with autoinflammatory diseases, making both diagnostic and classification criteria difficult to validate.

In this section of the chapter we will summarize the diagnostic and classification criteria that have been developed for autoinflammatory diseases. To date, these include the diseases characterized by recurrent and / or periodic fevers: FMF, MKD, TRAPS, CAPS and PFAPA syndrome. The Eurofever registry is currently

analyzing the results of a consensus conference to develop classification criteria for these periodic fever syndromes.

11.7 Diagnostic Criteria for Specific Diseases

11.7.1 Criteria for Familial Mediterranean Fever (FMF)

FMF was the first autoinflammatory disease for which criteria were proposed. In 1967, Sohar et al. published the first Tel Hashomer criteria (Table 11.5) [38]. These were developed primarily in an adult population with a specific ethnic background, limiting their generalizability as well as its use in the pediatric population, the age group in which most cases of FMF present. Challenges of using the Sohar Tel Hashomer criteria in children are that peritonitis and pleuritis are very difficult to characterize, fever attacks were often less than 12 h and in this group 20% of the patients did not have a temperature above 38°. Furthermore, some of the variables that were included are not very common (such as erysipeloid-like erythema). Including a response to colchicine implied that the diagnosis may be delayed. Livneh et al., recognizing this gap, established a set of diagnostic criteria (also called the Tel-Hashomer criteria) that incorporated some of the lesser-known features of FMF [6]. They defined clinical criteria common to patients with FMF into major and minor (Table 11.6) and determined that the presence of at least one major criterion or two minor criteria resulted in an overall sensitivity of 99% and specificity of 98%. These criteria were felt to be better than the ini-

Table 11.5 Diagnostic criteria of familial Mediterranean fever—FMF (adapted from reference [38])

Sohar Tel-Hashomer criteria
Major criteria
• Recurrent febrile episodes associated with peritonitis, pleuritis or synovitis
• Amyloidosis of AA-type without a predisposing disease
• Favorable response to daily colchicine
Minor criteria
• Recurrent febrile episodes
• Erysipelas-like erythema
• Positive history of familial Mediterranean fever in a first degree relative
Definite diagnosis: 2 major or 1 major +2 minor criteria
Probable diagnosis: 1 major +1 minor criteria

Table 11.6 Livneh long Tel Hashomer criteria for the diagnosis of familial Mediterranean fever- FMF (adapted with permission from reference [6])

Major criteria
Typical attacks
1. Peritonitis (generalized)
2. Pleuritis (unilateral) or pericarditis
3. Monoarthritis (hip, knee, ankle)
4. Fever alone
Minor criteria
1–3. Incomplete attacks involving 1 or more of the following sites:
1. Abdomen
2. Chest
3. Joint
4. Exertional leg pain
5. Favorable response to colchicine
Supportive criteria
1. Family history of FMF
2. Appropriate ethnic origin
3. Age < 20 years at disease onset
4–7. Features of attacks
4. Severe, requiring bed rest
5. Spontaneous remission
6. Symptom-free interval
7. Transient inflammatory response, with 1 or more abnormal test result(s) for white blood cell count, erythrocyte sedimentation rate, serum amyloid A, and/or fibrinogen
8. Episodic proteinuria/hematuria
9. Unproductive laparotomy or removal of 'white' appendix
10. Consanguinity of parents

The requirements for diagnosis of FMF are ≥1 major criteria, or ≥2 minor criteria, or 1 minor criterion plus ≥5 supportive criteria, or 1 minor criterion plus ≥4 of the first 5 supportive criteria. Typical attacks are defied as recurrent (≥3 of the same type), febrile (rectal temperature of 38 °C or higher), and short (lasting between 12 h and 3 days). Incomplete attacks in 1 or 2 features, as follows: (1) the temperature is normal or lower than 38 °C; (2) the attacks are longer or shorter than specified (but not shorter than 6 h or longer than a week); (3) no signs of peritonitis are recorded during the abdominal attacks; (4) the abdominal attacks are localized; (5) the arthritis is in joints other than those specified. Attacks are not counted if they do not fit the definition of either typical or incomplete attacks

tial Tel Hashomer criteria as the features of the attack required for diagnosis were accurately defined, incomplete attacks could be diagnosed and nephropathic amyloidosis, a rather late manifestation, was excluded.

Following the publication of the Livneh Tel Hashomer criteria, a Turkish group proposed diagnostic criteria that could be applied uniquely to the pediatric population [7] (Table 11.7). These criteria, like the Livneh and Sohar Tel Hashomer criteria, were also developed in a population with a high prevalence of FMF, thus potentially limiting their generalizability. While the sensitivity was slightly lower than the Livneh Tel Hashomer criteria [98.8%], the specificity was low [54.6%].

Demirkaya et al., examined the performance of these different diagnostic criteria using data from the Eurofever registry [10]. Patients who did not have biallelic *MEFV* mutations (including at least one in exon 10) had to have been diagnosed with FMF based on one of the three above-described diagnostic criteria. Control patients had CAPS, TRAPS, MKD, PFAPA or an undefined periodic fever syndrome. They reported that positivity for at least 2 Turkish criteria was associated with the highest sensitivity among the three sets of criteria (Table 11.8). This was counterbalanced by a low specificity. The Sohar Tel Hashomer criteria displayed a very high specificity but a much lower sensitivity, likely due to the rarity of amyloidosis and erysipeloid-like erythema in the pediatric population. Many would prefer not to include the treatment response to colchicine, suggesting this element might be more important in classification criteria. In addition, some patients with other forms of periodic fever syndrome might also respond to colchicine [36, 39, 40]. The Livneh Tel Hashomer criteria demonstrated a sensitivity of 77.3% and a specificity of 40.7%, rather close to the Turkish criteria.

Of note, the Turkish and Livneh Tel Hashomer criteria performed similarly well in this cohort that included patients from multiple countries and multiple ethnicities. In contrast, the Sohar Tel Hashomer criteria had a lower sensitivity in European patients. In patients with biallelic exon 10 mutations, the Sohar Tel Hashomer criteria had the highest accuracy.

Kondi et al. evaluated both the Sohar Tel Hashomer criteria and the Turkish criteria in a group of 100 children with FMF, 70% of whom were of Sephardic Jewish background, 11% North African and 9% Turkish. Interestingly, the sensitivity, specificity and positive and negative predictive values were almost the same using either set of criteria [8].

Table 11.7 Turkish pediatric criteria for diagnosis of familial Mediterranean fever-FMF (adapted from reference [7])

1. Fever: axillary temperature > 38 °C, duration 6–72 h and ≥3 attacks
2. Abdominal pain: duration 6–72 h and ≥3 attacks
3. Chest pain: duration 6–72 h and ≥3 attacks
4. Arthritis: duration 6–72 h, ≥3 attacks, and oligoarthritis
5. Family history of FMF
Definitive diagnosis: The presence of at least 2 out of 5 criteria

Table 11.8 Sensitivity, specificity, PPV and NPV of the Turkish and Tel Hashomer diagnostic criteria (adapted with permission from reference [10])

Criteria	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Yalchinkaya-Ozen					
1. Criterion	99.3	5.7	53.4	88.2	54.5
2. Criteria	87.4	40.7	61.6	74.8	65.0
3. Criteria	52.4	88.2	82.9	63.0	69.6
4. Criteria	24.1	99.6	98.6	54.7	60.3
5. Criteria	5.6	99.6	94.1	49.2	50.6
Sohar Tel Hashomer criteria	45.0	97.2	93.8	65.0	71.8
Without colchicine response	16.6	99.6	97.8	55.7	59.2
Livneh Tel Hashomer criteria	77.3	41.1	58.8	62.4	59.9

FMF familial Mediterranean fever, *PPV* positive predictive values, *NPV* negative predictive values

To date there is no agreement on which set of criteria should be used, especially in populations of different ethnicities than those that were studied to develop the criteria. Thus, clinicians will still need to rely on a careful history and physical examination, appropriate laboratory investigations and genetic testing where available, to make a diagnosis of FMF.

11.7.2 Criteria for the Cryopyrin-Associated Periodic Syndromes (CAPS)

Recently, diagnostic criteria have been developed for CAPS including FCAS, MWS and NOMID and those with overlapping features [14]. The proposed diagnostic model was validated in a group of children and adults with CAPS (based on expert diagnosis) and true CAPS controls that included patients with sJIA, Schnitzler syndrome, Kawasaki disease (classic and incomplete forms), FMF and unclassified fever syndromes. The best diagnostic criteria model resulted in a specificity of 94% and a sensitivity 81% (Table 11.9). These criteria are generalizable and do not require the identification of a genetic mutation, therefore making them easy to use worldwide. However, as the control group only included patients with inflammatory diseases, and the proposed cases of CAPS were developed by a group of experts on CAPS, the authors noted that they will perform best in the context of suspected inflammatory disease, where disorders such as malignancy and infection have already been excluded.

Table 11.9 Cryopyrin-associated periodic syndrome (CAPS) diagnostic criteria (adapted with permission from reference [14])

Raised inflammatory markers (C-reactive protein/serum amyloid A) as mandatory criteria
Plus
≥2 of 6 CAPS typical signs/symptoms
Urticaria-like rash
Cold/stress triggered episodes
Sensorineural hearing loss
Musculoskeletal symptoms (arthralgia/arthritis/myalgia)
Chronic aseptic meningitis
Skeletal abnormalities (epiphyseal overgrowth/frontal bossing)

11.7.3 Criteria for the Periodic Fever, Aphthous Stomatitis, Pharyngitis, Cervical Adenitis (PFAPA) Syndrome

A syndrome initially described by Marshall et al. in 1987 [41] was named PFAPA in 1989 and initial diagnostic criteria were proposed [42]. These proposed diagnostic criteria were updated in 1999 to include evaluation for cyclic neutropenia and exclude an elevated erythrocyte sedimentation rate and leukocytosis [43]. Elevations in the erythrocyte sedimentation rate and white blood cell count were removed from the diagnostic criteria as they are frequently raised in febrile children and were not thought to provide added specificity for the diagnosis. The updated proposed criteria are listed in Table 11.10.

These criteria have served patients and their caregivers well. However as many of the symptoms of PFAPA can overlap with other febrile childhood diseases, it may be difficult to discriminate on clinical grounds alone. A significant proportion of patients with PFAPA in some regions carry mutations for *MEFV* [44] and many patients with the R92Q variant in the *TNFRS1A* gene behave more like patients with PFAPA than with TRAPS [5]. Furthermore, Gattorno et al., have reported that a significant number of patients with other mono-

Table 11.10 Diagnostic criteria for periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome (with permission from reference [43])

1. Regularly recurring fever with an early age of onset (< 5 years old)
2. Constitutional symptoms in the absence of upper respiratory tract infection with at least one of the following clinical signs:
(a) Aphthous stomatitis
(b) Cervical lymphadenitis
(c) Pharyngitis
3. Exclusion of cyclic neutropenia
4. Completely asymptomatic interval between episodes
5. Normal growth and development

genic periodic fever syndromes also meet the criteria for PFAPA [5]. Additionally, an increasing number of adults with the PFAPA have been reported for which these criteria cannot be applied.

11.8 A New Approach to Classification Criteria

The initial diagnostic and classification studies for periodic fever autoinflammatory diseases suffered from being based on expert opinion, using single ethnic populations limiting generalizability or studied clinical manifestations in patients affected by only a single disease. The autoinflammatory recurrent fever syndromes are marked by overlapping features and it would therefore be helpful to study clinical manifestations of each of these diseases in comparison to each other. The Eurofever registry, composed of patients with all recurrent fever syndromes from multiple countries with multiple ethnicities has enabled such comparisons to be done (see Chap. 14). Provisional classification criteria for FMF, MKD, TRAPS and CAPS have been developed by studying patients with confirmatory genetic studies compared to the disease control PFAPA (Table 11.11) [13]. These provisional criteria should be applied only after exclusion of other diseases that present with recurrent fever such as

Table 11.11 The Eurofever clinical diagnostic/classification criteria for the classic hereditary autoinflammatory syndromes (with permission from reference [13])

FMF		MKD		CAPS		TRAPS	
Presence	Score	Presence	Score	Presence	Score	Presence	Score
Duration of episodes <2 days	9	Age at onset <2 years	10	Urticarial rash	25	Periorbital edema	21
Chest pain	13	Aphthous stomatitis	11	Neurosensorial hearing loss	25	Duration of episodes >6 days	19
Abdominal pain	9	Generalised enlargement of lymph nodes or splenomegaly	8	Conjunctivitis	10	Migratory rash[a]	18
Eastern Mediterranean[b] ethnicity	22	Painful lymph nodes	13			Myalgia	6
North Mediterranean[b] ethnicity	7	Diarrhea (sometimes/often)	20			Relatives affected	7
		Diarrhea (always)	37				
Absence		**Absence**		**Absence**		**Absence**	
Aphthous stomatitis	9	Chest pain	11	Exudative pharyngitis	25	Vomiting	14
Urticarial rash	15			Abdominal pain	15	Aphthous stomatitis	15
Enlarged cervical lymph nodes	10						
Duration of episodes >6 days	13						
Cutoff	≥60	Cut-off	≥42	Cut-off	≥52	Cut-off	≥43

CAPS cryopyrin-associated periodic syndromes, *FMF* familial Mediterranean fever, *MKD* mevalonate kinase deficiency, *TRAPS* TNF receptor-associated periodic fever syndrome

The clinical features should be related to the typical fever episodes (i.e., exclusion of intercurrent infection or other comorbidities)

[a]Centrifugal migratory, erythematous patches most typically overlying a local area of myalgia, usually on the limbs or trunk

[b]Eastern Mediterranean: Turkish, Armenian, non-Ashkenazi Jewish, Arab; North Mediterranean: Italian, Spanish. Greek

infection, malignancy and other inflammatory disorders. Work is continuing that will incorporate genetic data into the classification.

11.9 The Approach to Undiagnosed Patients with an Unspecified Autoinflammatory Disorder

Most patients that present with features suggesting an autoinflammatory syndrome will not have one of the currently known disorders. In a large cohort of autoinflammatory patients at the National Institutes of Health, only about 40% have a known genetic association, while 60% are still not diagnosed genetically. In some patients the disease will resolve and others are awaiting new gene discoveries. Even in the absence of a known genetic association, it may be helpful to find a known syndrome that overlaps in presentation. It is reasonable to extend the treatment options for the known diseases to those situations where the clinical presentation is similar, thus, the following treatment guidelines for that disease may be warranted. Continued observance for the presence of new symptoms may be informative. There is no specific treatment plan for patients with undifferentiated autoinflammatory syndromes. Short infrequent courses of corticosteroids may be helpful in some patients. In patients with PFAPA-like symptoms, there may be a shortened interval before the next flare. Colchicine may be helpful, especially if oral ulcers are a prominent feature of the disease flare. In some diseases, "on-demand" IL-1 blockade with anakinra is frequently effective, especially if given at the first sign of a flare [31]. For patients with frequent flares, continuous IL-1 blockade may be indicated. Anti-TNF agents may also be used in this setting, and in our experience, are often helpful when oral ulcers predominate the complaints.

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Genetic Approach to the Diagnosis of Autoinflammatory Diseases

12

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Abstract

Over the past 20 years, almost 30 new genes associated with autoinflammatory disorders have been identified. The genetic diagnosis is based on the identification of clearly pathogenic mutations, even in atypical cases (i.e. patients not meeting recognized clinical criteria). Therefore, genetic testing should be preferably ordered by healthcare providers who are familiar with the spectrum of clinical presentation of autoinflammatory diseases.

The constellation of clinical findings will guide the choice of genetic testing. The prerequisites that are usually asked before requesting a genetic test for suspected autoinflammatory disease are: evidence for systemic inflammation, suspicion of a monogenic trait i.e. for example early-onset disease or familial inheritance, and existence of a plausible candidate causal gene.

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Sequencing approaches remain the gold-standard techniques. Sanger sequencing is a rapid, inexpensive method to test for mutations in a single gene in a single patient. However, its performance is low (10–20%) when examined in all patients tested for autoinflammatory disorders, including those who did not fulfil the criteria discussed above. The indications for Sanger sequencing should be restricted to prevalent mutations and/or mutational hot spots in a gene (e.g. extracellular domain of TNFRSF1A responsible for tumor necrosis factor receptor-associated periodic syndrome—TRAPS), or to patients with a complementary biochemical diagnosis (e.g. mevalonate kinase deficiency—MKD, deficiency of adenosine deaminase 2—DADA2).

If Sanger method is not confirmatory, large scale next generation sequencing (NGS) approaches should be contemplated. NGS allows investigations of multiple genes in multiple patients at a time. Targeted NGS sequencing of gene panels has become more time- and cost-effective and is commonly used today for phenotypically undifferentiated autoinflammatory diseases, genes known to harbour mosaic mutations (e.g. *NLRP3*) and oligogenism (e.g. proteasomopathies). New concepts regarding modes of inheritance and interpretation of variant pathogenicity will require a systematic

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review and update of genetic diagnostic strategies and new guidance on standardization and reporting of genetic tests.

Keywords
Genetic diagnosis · Autoinflammatory diseases · Inheritance · Sanger · Next generation sequencing (NGS)

Abbreviations

ADA2	Adenosine deaminase 2
APLAID	Autoinflammation, PLCG2-associated antibody deficiency, and immune dysregulation
CAPS	Cryopyrin-associated periodic syndrome
CRP	C reactive protein
DADA2	Deficiency of adenosine deaminase 2
DSAP	Disseminated superficial actinic porokeratosis
ESR	Erythrocyte sedimentation rate
FMF	Familial Mediterranean fever
ISSAID	International Society for Systemic Autoinflammatory Diseases
LUBAC	linear ubiquitin chain assembly complex
MAF	Minor allele frequency
MEFV	Mediterranean fever (gene)
MKD	Mevalonate kinase deficiency
NGS	Next generation sequencing
NLRP3	NLR pyrin domain containing protein 3
NOMID/CINCA	Neonatal-onset multisystem inflammatory disease/chronic infantile neurological cutaneous and articular syndrome
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PLAID	PLCG2-associated antibody deficiency and immune dysregulation
PRAAS	Proteasome-associated autoinflammatory syndromes
SAA	Serum amyloid A
SAVI	STING-associated vasculopathy with onset in infancy
SIFD	Sideroblastic anemia with immunodeficiency, fevers and developmental delay
SNP	Single nucleotide polymorphism
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome
VUS/VOUS	Variant of unclear significance
WES	Whole exome sequencing
WGS	Whole genome sequencing

Key Points

- **Genetic diagnosis has dramatically improved with the discovery of the causative genes**
- **Novel sequencing approaches have become routine and are expected to advance genetic diagnosis**
- **Limitations remain regarding interpretation of genetic variants, and understanding of unexpected modes of inheritance**
- **Specific guidelines for genetic diagnosis are necessary**

12.1 Introduction

The first reported autoinflammatory disease gene, *MEFV*, responsible for familial Mediterranean fever (FMF), was discovered in 1997 based on the studies of families with clearly recessively inherited highly-penetrant disease and using the tedious positional cloning approach [1, 2]. The entire project lasted for about 7 years. In the 20 years since then, almost 30 new genes associated with autoinflammatory diseases have been identified through the

Table 12.1 Genetic and molecular description of autoinflammatory diseases

Recessive diseases	Gene/protein	Dominant diseases/*de novo* mutations	Gene/protein
Aicardi-Goutieres syndrome (AGS)	TREX1, RNASEH2A, RNASEH2B, RNASEH2C,SAMHD1, ADAR, DNAse II	Aicardi-Goutieres syndrome (AGS)	ADAR, IFIH1/ MDA5
Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE)	*PSMB8, PSMB9, PSMB4, PSMA3*	PLCG2-associated diseases (APLAID/ PLAID)	*PLCG2*/PLCγ2
Deficiency of ADA2 (DADA2)	*ADA2/CECR1*/ADA2	Blau/Early-onset sarcoidosis	*NOD2*/NOD2
Deficiency of the IL-1 receptor antagonist (DIRA)	*IL1RN*/IL-1Ra	Cryopyrin associated periodic syndromes (CAPS; FCAS1; MWS; NOMID/CINCA)	*NLRP3*/NLRP3
Deficiency of IL-36 receptor antagonist (DITRA)	*IL36RN*/IL36Ra	CARD14-mediated pustular psoriasis (CAMPS/PSORS2)	*CARD14*
Early-onset inflammatory bowel diseases/IL-10 Deficiency	IL-10/IL-10 receptors	Familial cold autoinflammatory syndrome (FCAS2)	*NLRP12*/NLRP12
Familial Mediterranean fever (FMF)	*MEFV*/Pyrin	NLRP1-associated diseases (FKLC/MSPC)	*NLRP1/NLRP1*
Hyper-IgD/Mevalonate kinase deficiency (HIDS/MKD)	*MVK*/MVK	Haploinsufficiency of A20 (HA20)	*TNFAIP3*/A20
LUBAC deficiency	HOIL-1/HOIP	NLRC4-associated diseases (MAS/FCAS)	*NLRC4*/NLRC4
Majeed syndrome	*LPIN2*/LPIN2	Pyrin-associated neutrophilic dermatosis (PAAND)	*MEFV*/Pyrin
Monogenic form of systemic juvenile arthritis	*LACC1*/LACC1	Pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPA)	*PSTPIP1*/PSTPIP1
OTULIN deficiency	*OTULIN*/OTULIN	STING-associated vasculopathy (SAVI)	*TMEM173*/STING
Periodic fever, immunodeficiency, and thrombocytopenia (PFIT)	*WDR1*/WDR1	TNFR-associated periodic syndrome (TRAPS)	*TNFRSF1A*/TNFR1
Sideroblastic anemia, B-cell immunodeficiency, periodic fevers, developmental delay developmental delay (SIFD)	*TRNT1*/TRNT1	TNFRSF11A-associated hereditary fever disease	*TNFSF11A*/RANK

combination of candidate gene screening and next-generation whole exome sequencing (WES) [3, 4]. The use of powerful next generation sequencing (NGS) technology has fuelled the discovery of novel genes in the past 5 years. The current list of monogenic autoinflammatory diseases and associated causal genes is shown in Table 12.1. We will describe and discuss how the screening of mutations in these genes can help in the diagnosis of autoinflammatory diseases. This chapter will not describe the pathophysiology and phenotypes of autoinflammatory diseases; these topics are discussed elsewhere in this book.

12.1.1 Relevant Dedicated Websites

- A list of the genes, phenotypes, classical modes of inheritance, acronyms and alias is freely accessible on the website of the International Society for Systemic Autoinflammatory Diseases (ISSAID) at: https://fmf.igh.cnrs.fr/ISSAID/Classification_AID/page1.html
- A registry of mutations is available online at https://fmf.igh.cnrs.fr/ISSAID/infevers

12.1.2 Clinical Versus Genetic Diagnosis

A clinical diagnosis is based on a set of defined criteria and does not require molecular confirmation i.e. identification of mutation(s) in the corresponding gene. Clinical criteria are not available for all autoinflammatory diseases or might not be applicable in non-classic populations, as is the case with FMF outside of the classically affected populations [5]. For recessively inherited disorders such as FMF, variable disease expression is likely explained by an allelic heterogeneity of causal mutations in different populations. Moreover, the differential diagnosis for autoinflammatory diseases can be difficult because patients with different diseases may present with nonspecific manifestations and often overlapping symptoms (see Chap. 11).

A genetic diagnosis is based on the identification of clearly pathogenic mutations, even in atypical cases (i.e. patients not meeting recognized clinical criteria). In such atypical cases, the diagnosis often relies on genetic confirmation. Regrettably, many clinicians inexperienced in recognizing autoinflammatory diseases will order numerous individual molecular tests, which might delay accurate diagnosis and relevant patient care.

Therefore, the test should ideally be ordered by physicians who are experienced in caring for patients with autoinflammatory diseases.

Genetic diagnosis of autoinflammatory diseases can assist in the patient's management and therapy. The prerequisites before requesting a genetic test for suspected autoinflammatory disease are:

1. Evidence for systemic inflammation (elevated acute phase reactants during disease flare e.g. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and serum amyloid A (SAA). In some diseases, patients do not have strong evidence for systemic inflammation but may have organ or tissue specific inflammation (e. g. autoinflammatory disorders with prominent cutaneous manifestations where inflammation can be confirmed by skin biopsy)
2. The disease is likely to be inherited as a monogenic trait (early-onset symptoms in sporadic patients or multiple affected siblings or dominantly inherited symptoms)
3. Plausible candidate causal gene (this can change with the rapid identification of new disease-associated genes (see Chap. 2)

12.2 Modes of Transmission

Key Points

- **Most hereditary autoinflammatory diseases have a classical autosomal recessive or dominant transmission**
- **Recent observations demonstrated patients who do not fulfil classic inheritance (e.g. dominant FMF)**
- **Oligogenism is newly recognized in autoinflammatory diseases and it needs to be further investigated**

All modes of transmission, except triplet expansions and X-linked diseases, have been described in autoinflammatory diseases [6].

12.2.1 Classic Modes of Inheritance of Autoinflammatory Diseases

Recessive disorders: FMF and mevalonate kinase deficiency (MKD) are classical examples of recessive hereditary recurrent fever syndromes. The causative genes are Mediterranean fever (*MEFV)* and mevalonate kinase (*MVK)*, respectively. Patients carry biallelic pathogenic

mutations in the same gene in the form of either homozygous (two identical) or compound heterozygous (two different) variants. In some cases (e.g. deficiency of adenosine deaminase 2; DADA2) the second cryptic (non-coding) mutation may not be found using sequencing technology and may require additional analyses that are likely beyond the expertise of standard testing laboratories. A clearly pathogenic mutation must be transmitted from each unaffected parent (*in trans*). Parental testing is highly recommended to confirm a clinical diagnosis of recessively inherited disease at the molecular level. This is particularly relevant when testing for mutations in *MEFV* and *ADA2* genes, which are highly polymorphic, and in some patients the gene variants might be inherited *in cis* (on the same allele).

Dominant disorders: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) and cryopyrin-associated periodic syndromes (CAPS) are two examples of dominant hereditary autoinflammatory disorders. The causative genes are *TNFRSF1A* and *NLRP3*, respectively. A single pathogenic (heterozygote) mutation is either inherited from a symptomatic parent or has arisen as a *de novo* event. Parental testing is necessary to confirm *de novo* mutations, also known as neomutations.

12.2.2 Unusual Modes of Inheritance

Mosaicism: Mosaicism is defined as the presence of two or more populations of cells with different genetic content in one individual (see Chap. 2). There are two types of mosaicism: gonadal (some gametes carry mutation and the rest are normal; this can cause a dominantly inherited disease) and somatic (tissue or cell-lineage restricted mutations). The first evidence of somatic mosaicism in autoinflammatory diseases was reported by Saito and colleagues in 2005 in a patient with neonatal onset multisystem inflammatory disease (NOMID) also known as chronic infantile neurologic, cutaneous, articular syndrome (CINCA) [7]. Subsequently, several patients with CAPS-like phenotype were demonstrated to carry myeloid lineage-restricted mutations in *NLRP3* [8–21]. Myeloid cell-specific mosaicism was also described in two patients with Schnitzler syndrome [14]. Search for mosaic mutations is done in specialized laboratories and research centres. Gonadal mosaicism has been reported in patients with TRAPS and Blau syndrome and in this situation, recurrence of a disease needs to be considered when individuals are counselled [22, 23].

Oligogenism: In some patients, the clinical symptoms of autoinflammation are associated with mutations in at least two (digenism) or more genes concomitantly; this is known as oligenic inheritance. Typically, pathogenic variants are identified in various genes that make up a multiprotein complex. This mechanism has been also postulated in some patients with uncharacterized hereditary recurrent fever syndromes (additive effect of mild variants such as p.R92Q in *TNFRSF1A* and p.V198M in *NLRP3*) [24]. Digenic inheritance has been confirmed in patients with proteasome-associated autoinflammatory syndromes (PRAAS) who have been shown to carry double mutations in any of four genes encoding for the subunits of constitutive proteasome or immunoproteasome [25] (see Chap. 24). Identification of two or more pathogenic variants in different genes in a single patient is not a confirmation of digenism *per se* because mutations can happen fortuitously. Only family-based segregation analysis showing a proper inheritance pattern can support digenism.

Multifactorial inheritance: This form of inheritance defines the additive involvement of common single nucleotide polymorphisms (SNPs) in multiple genes, which are found at the minor allele frequency (MAF) greater than 5% in the general population. These individual variants have a mild effect on the gene expression and/or protein function, but they may exert cumulative impact on the common pathway. The combined effect of common genetic risk variants is often exacerbated by environmental factors (e.g. diet). Examples of multifactorial autoinflammatory diseases are Behçet and Crohn disease [26, 27]. Such diseases are not eligible for genetic diagnosis, as by essence polymorphisms are also found in asymptomatic people and may be absent in patients.

12.3 Genes Currently Explored

The boundaries between autoinflammatory diseases and other inflammatory diseases have been blurred with the identification of new causal genes and pathways. Autoinflammatory diseases were historically defined as inflammatory diseases caused by dysregulation of the innate immune system. Prototypic hereditary recurrent fever syndromes lack the stigmata of classic autoimmune diseases such as high-titer autoantibodies or antigen-specific T cells. However, over time this definition has evolved to encompass conditions which present with features of autoinflammation, autoimmunity, and immunodeficiency (see Chaps. 28 and 38). Monogenic interferonopathies, DADA2, linear ubiquitin chain assembly complex (LUBAC) deficiencies, PLCG2-associated antibody deficiency and immune dysregulation (PLAID), autoinflammation, antibody deficiency, and immune dysregulation (APLAID), sideroblastic anemia with fever, immunodeficiency, fevers and developmental delay (SIFD), are the exemplary diseases [3, 4]. A more targeted definition of autoinflammatory diseases emerged recently following a DELPHI consensual approach (Ben Chetrit, in preparation, see Chap. 10). The constellation of clinical findings will guide the choice of genetic testing. Some diagnostic laboratories developed tools such as the Gaslini score [28] to propose the most effective explorations of single or combined genes.

12.4 Methods of Sequencing

Key Points

- **Most laboratories are progressively abandoning the classic Sanger sequencing method**
- **Next generation sequencing of known autoinflammatory gene panels is becoming the gold standard**
- **Whole exome sequencing as a systematic approach for genetic diagnosis is currently under evaluation and might be considered in the future**

Sequencing represents the gold standard for genetic confirmation of autoinflammatory diseases. The approach should be adapted depending on the features of the target gene, which is suspected from the phenotype of the patient. We will not discuss the best technique for each single gene test (see Chap. 2), but rather describe current sequencing methods.

12.4.1 Sanger

Sanger sequencing remains the conventional, rapid, inexpensive method to test for mutations in a single gene in a single patient. However, its performance is low (10–20% positive tests in all patient samples sent for a suspected autoinflammatory disorder) based on the reports from multiple laboratories. The indications for Sanger should be restricted to the following cases:

12.4.1.1 Frequent Mutations and/or Mutational Hot Spots in the Gene

Sanger is recommended for patients with unambiguous phenotypes, to explore for mutations in genes known to contain mutational hot spots, or to test for mutations in founder populations. The best example is sequencing of exon 10 of the *MEFV* gene in patients with FMF of Mediterranean ancestry. Genetic testing is not absolutely required; however, it might be important for genetic counselling in a family.

12.4.1.2 Confirmation of Biochemical Test

Sanger sequencing can confirm a biochemical diagnosis (in MKD or DADA2 for example). If Sanger method is not confirmatory, large scale sequencing approaches should be contemplated. Some laboratories use Sanger to confirm NGS results, however it is not required.

12.4.2 Next Generation Sequencing (NGS)

NGS is also called deep sequencing or multi-parallel sequencing. This naming is self-explanatory as it describes the principle of this revolutionary method. NGS allows investigations of multiple genes in multiple patients at a time. While it was initially a relatively expensive technology, this approach has become more and more time- and cost-effective and is currently practiced in many countries.

12.4.2.1 Targeted Gene Panels

Targeted NGS sequencing is today commonly used for genetic testing of autoinflammatory diseases [13, 29, 30]. It involves simultaneous analysis of the complete coding sequence of a selected panel of genes and it may investigate up to a few hundred candidate genes. The content of panels varies among laboratories, but typically includes the genes associated with classical hereditary recurrent fever syndromes (*MEFV, TNFRSF1A, MVK, NLRP3*), Blau syndrome, and deficiency of interleukin 1 receptor antagonist (DIRA). An initiative by a European consortium is in progress to define a minimum number of core genes to sequence in patients with a suspected autoinflammatory disorder. The performance of panels is currently being assessed and is probably around 20% based on current reports and studies [29, 31] (i.e. 20% of samples from patients with a suspected autoinflammatory disorder yield a clear result with a targeted gene panel). The low diagnostic yield can be attributed to inappropriate indication for testing of patients with inflammation and likely non-hereditary causes. The indications for panel testing are:

- Phenotypically undifferentiated autoinflammatory diseases: NGS-panels is the current method of choice when the patient fulfils criteria for several possible diseases.
- Genes known to harbour mosaic mutations: Patients with CAPS-like phenotype should be investigated by NGS sequencing of *NLRP3* as somatic mutations account for up to 20% of cases. Patients with somatic mutations may present with milder and late-onset disease manifestations. Apart from *NLRP3*, *NOD2*, *TNFRSF1A, TNFAIP3* responsible for haploinsufficiency of A20 (HA20), *TMEM173* responsible for STING-associated vasculopathy with onset in infancy (SAVI), other autoinflammatory disease-associated genes might also be affected by mosaicism.
- Oligogenism: Large exhaustive panels including all currently known autoinflammatory disease genes should detect double mutations in responsible genes in patients with PRAAS (and perhaps other) phenotypes. Interpretation of these data is more challenging and requires the expertise of research and/or reference centers.

12.4.2.2 Whole Exome Sequencing (WES)

WES involves complete sequencing of almost the entire (80–90%) coding region of expressed genes and it has revolutionized the discovery of novel autoinflammatory disease causal genes. Indeed, all recently identified genes in patients with autoinflammatory diseases were discovered by this comprehensive technology. WES is not yet used in routine diagnostics, however with decreasing costs and more effective bioinformatic pipelines, some labs are now considering implementing WES for genetic testing, for the reason that the diagnostic yield of targeted panel sequencing remains fairly low. While WES data promise more complete coverage, the data analysis still poses significant challenges as potentially hundreds of variants might be identified in a sporadic patient. Family-based (trio) WES analysis may help reduce the complexity of data. Proving the causality of a newly identified autoinflammatory disease candidate gene requires complex investigation in research labs. Currently, most general diagnostic labs report a 25–30% yield from WES analyses, which is a small increase from the targeted panel analysis. Patients and physicians should be properly counselled for the outcome of WES, in light of possible incidental findings (variants with known clinical significance and unrelated to the studied disease).

12.4.2.3 Whole Genome Sequencing (WGS)

This approach investigates the complete human sequence and is mainly used in a research setting [32, 33]. WGS is recommended for patients highly suspected to have a monogenic disorder and who remain undiagnosed after WES analysis. WGS would identify mutations in regulatory regions of known disease causal genes or putative disease-causing mutations in non-coding RNAs or copy number variations (i.e. deletion/duplication).

12.4.3 Other Approaches

Biochemical diagnosis can complement genetic diagnosis in patients in whom only a single mutation for the suspected recessive disease has been identified. These patients typically have a cryptic mutation (e.g. non-coding or genomic deletion) that is not easily identified (e.g. MKD, DADA2).

It is also sometimes useful to perform quantitative-PCR or use high-density SNP DNA arrays to search for a possible large deletion/duplication when there is a high suspicion for AR disease but only one mutation is found.

12.5 Interpretation

Decision trees for genetic diagnosis have been proposed. We suggest the new guidelines that have been revised to incorporate NGS data analysis (Fig. 12.1).

12.5.1 Variant Type

The Human Genome Variation Society (http://www.hgvs.org) has recommended avoiding the use of 'mutation' and 'polymorphism' as terms with opposite meanings (pathogenic vs. non-

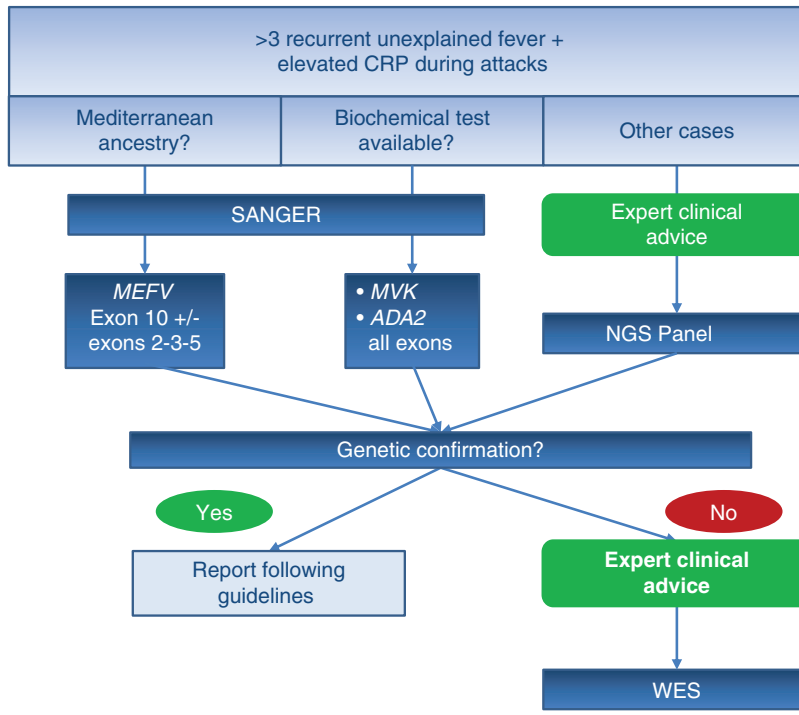

Fig. 12.1 Proposed decision tree for genetic diagnosis of hereditary periodic fever syndromes. *ADA2* adenosine deaminase 2; *CRP* C reactive protein; *MEFV* Mediterranean fever; *MVK* mevalonate kinase; *NGS* next generation sequencing; *WES* whole exome sequencing. Suggested guidelines [36]

pathogenic), since functional studies are scarce or unavailable to evaluate their pathogenicity. Sequence variants in autoinflammatory disease associated genes are linked to a broad range of phenotypes, but only a small proportion of them has been clearly shown to cause the disease.

The most common variants seen in the genes for hereditary recurrent fever syndromes are non-synonymous nucleotide changes and except for *MVK*, structural mutations (deletions, duplications, rearrangement) are infrequent. More recently, protein truncating mutations were reported in autoinflammatory diseases other than hereditary recurrent fever syndromes (https://fmf.igh.cnrs.fr/ISSAID/infevers), e.g. in *IL1RN*, *IL36RN* and *TNFAIP3*. Rare genomic deletions were identified in the *IL1RN* [34] and *ADA2* [35] genes. It has been suggested that biallelic deleterious variants would not be tolerated in some of the genes that regulate host innate immune defence pathways.

12.5.2 Variant Location

Mutational hot spots have been described in 3 of the 4 best characterized genes of the hereditary recurrent fever syndromes, *MEFV*, *NLRP3* and *TNFRSF1A*. Five clearly pathogenic mutations have been identified at the amino acid residue 694 of pyrin, encoded by *MEFV*: p.M694V, p.M694I, p.M694K, p.M694L and p.M694del (fmf.igh.cnrs.fr/ISSAID/infevers). Similarly, the majority of mutations associated with CAPS are reported in the NACHT ((NAIP (neuronal apoptosis inhibitory protein), CIITA (MHC class II transcription activator)) domain of cryopyrin/NLRP3, while mutations associated with TRAPS reside almost exclusively in the extracellular domain of TNFR1. Such observations suggest that the regions where pathogenic mutations cluster confer a crucial contribution to the protein function.

12.5.3 Variant Pathogenicity

Several generalist websites such as ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) report estimations of variant pathogenicity. Allele frequency of unreported variants can be checked in large databases such as ExAC and gnomAD (broadinstitute.org). Most classifications to date recognize 5 variant pathogenicity scores:

1. Clearly benign
2. Likely benign
3. Unknown significance (variant of unclear significance—VOUS/VUS)
4. Likely pathogenic
5. Clearly pathogenic

Criteria include *in silico* predictions (algorithms based on location and type of the variant, minor allele frequency in a population, and conservation across species), existence of already known mutations at the same codon, family segregation, and functional studies, when available. An initial classification of a limited number of variants in hereditary recurrent fever syndromes was proposed in 2012 by expert geneticists and clinicians [36]. A more extensive list including more than 800 variants has been recently established through a protocol adapted from a DELPHI approach [37].

12.5.4 Confirmation of the Diagnosis

As for any Mendelian conditions, the definitive genetic diagnosis of autoinflammatory diseases is based on the finding of unambiguous mutations in the causative genes. Theoretically, finding two biallelic clearly pathogenic mutations (assessed by studying the parental alleles) in recessive diseases, or one mutation in dominant diseases confirms the diagnosis. Heterozygous mutations must be either already known to cause the disease or if they are novel (unreported in databases) and/or *de novo* variants; laboratories should carefully evaluate the clinical relevance of these variants. In all other cases, patient care should be based on clinical grounds and should not prevent initiation of therapies.

12.6 Diagnostic Issues

Key Points

- **Interpretation of genetic tests should be done in the context of the sequencing approach**
- **Only NGS can detect somatic mutations and identify oligogenic inheritance**

12.6.1 Test Sensitivity

Interpretation of a result should always take into account the sensitivity of the molecular screening strategy, i.e. exhaustiveness of the mutation screening. A genetic test report should clearly state the scope of investigation and sensitivity of the described test.

Most FMF mutations are located in exon 10, which encodes the B30.2 domain of the pyrin protein, thus all testing laboratories analyse the exon 10 of *MEFV*. Absence of mutations in exon 10 should not definitively rule out the diagnosis of FMF, although it strongly decreases its probability, especially in patients of Mediterranean ancestry.

Mosaicism below 20% is likely missed by Sanger sequencing and easily detectable by NGS. NGS can potentially identify low level mosaic mutations at a frequency of mutant allele as low as 1–2%.

Deep intronic variants that affect protein translation and expression have been observed in other human diseases such as collagenopathies or cystic fibrosis. Up to date, none have been described in patients with autoinflammation.

12.6.2 Variable Modes of Inheritance/Phenotype of a Given Gene

Further challenging the interpretation of genetic testing is the observation of multiple modes of inheritance in a single gene that are linked to different phenotypes. The mutation location also influences the phenotype. The following examples illustrate the need to be aware of the genetic and phenotypic variability in a single gene.

12.6.2.1 MEFV

FMF has long been considered a recessive illness and the *MEFV* positional cloning studies were indeed based on the autosomal-recessive model of inheritance. This type of inheritance would favour disease-associated variants to behave as loss-of-function mutation. However, over time it has become apparent that most of the disease-causing variants are missense mutations and that there is paucity of protein truncating mutations, which suggested a gain-of-function mode of disease and a proinflammatory role of the pyrin protein (encoded by *MEFV)* (see Chap. 16). Consistent with these observation, a significant number of FMF patients are found to carry only one pathogenic *MEFV* mutation despite extensive search for a second disease mutation [38, 39]. In addition, it was shown that some carriers for *MEFV* mutations have elevated inflammatory biomarkers [40]. Thus, having a single pathogenic mutation in *MEFV* in the presence of other genetic or environmentally permissive factors might be sufficient to trigger the activation of pyrin.

Four mutations in exon 10 of the *MEFV* gene stemming from a founder effect account for the vast majority of the patients with typical FMF: p.M680I, p.M694V, p.M694I and p.V726A [41]. Mutations outside of the exon 10 of *MEFV* are typically associated with different clinical presentations [42]. Consanguinity is common in many countries, and pseudo-dominance is occasionally observed due to high carrier frequency of FMF mutations in respective populations. However, cases of true dominance for *MEFV* gene mutations have been reported:

1. Mutations located in the vicinity of amino acid position 242 in the exon 2 of *MEFV* are associated with a dominantly inherited neutrophilic dermatosis termed pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) (see Chap. 29) [43].

These mutations affect the phosphorylation of pyrin and cause constitutively active protein.

2. A severe autosomal-dominant periodic inflammatory disorder with renal AA amyloidosis and colchicine resistance was associated with the *MEFV* p.H478Y (c.1432C>T) variant in a Spanish kindred [39].
3. Four different mutations at amino acid position 577 of pyrin, p.T577N (c.1730C>A), p.T577S (c.1729A>T), p.T577S (c.1730C>G) and p.T577A (c.1729A>G) are associated with a colchicine-responsive dominantly inherited periodic fever syndrome [44].
4. Complex alleles (variants in *cis*) have also been identified in *MEFV* and in some cases appear to segregate in a dominant mode. Booth et al. suggested that both the single p.M694del mutation and the complex allele p.M694I;p.E148Q could behave this way [45]. In one Japanese family, a true dominant pattern was associated with the complex p.[L110P;E148Q;M694I] allele [46]. However, the clinical significance of p.E148Q and p.L110 variants is still debated. Similarly, the complex p.P369S;p.R408Q allele has been linked to non-specific inflammatory phenotypes [47]. The mechanism underlying the severity of complex alleles is unknown and may depend on differential transcription regulation or gene-gene interactions.

12.6.2.2 MVK

MKD is a classically recessively inherited disease and the rate of genetic confirmation nearly reaches 100% in patients with decreased mevalonate enzymatic activity. Unexpectedly, dominant mutations in this gene were associated with disseminated superficial actinic porokeratosis (DSAP) [48]. Although DSAP patients had no clinical features of MKD or abnormalities in their serum IgD concentrations, it remains unclear whether they had decreased enzymatic MVK activity. However, the mutations c.417_418insC and c.604G>A found in two patients with DSAP were also reported in two MKD patients in a compound heterozygous state, each in combination with another mutation.

12.6.3 Issues in Interpretations of Low Frequency Genetic Variants

One of the main challenges will be interpreting the clinical significance of low frequency genetic variants (1–5%) that will be ultimately identified in most genes as result of massive sequencing. In the field of autoinflammatory diseases, well known examples are p.E148Q in *MEFV*, p.R121Q (R92Q) or p.P75L (P46L) in *TNFRSF1A*, and p.V200M (V198M) or p.Q705K (Q703K) in *NLRP3* genes. Their allele frequency is not low enough (<0.001) to explain association with rare diseases and their impact on protein function is still unclear. These low frequency variants might act as susceptibility alleles to inflammation, however they are unlikely to be a cause of a disease in patients with early-onset symptoms. These patients are more likely to have high-penetrance rare disease-causing variants in the genome.

The accumulation of VOUS/VUS could either modify the severity of monogenic disorders or trigger multifactorial diseases in the appropriate genetic and environmental background. An increasing number of studies support the involvement of rare and common variants in autoinflammatory or autoimmune complex conditions. Low-penetrance FMF, TRAPS and CAPS associated variants were inconsistently linked to susceptibility to Crohn disease and multiple sclerosis [49–52], while high-penetrance FMF-causal variants have been strongly associated with Behçet disease in the Turkish population [53, 54]. Genetic diagnosis and counseling for complex disorders will require very large population data.

12.7 Conclusions

Emerging sequencing technologies and bioinformatics tools will lead to continuous discovery of new autoinflammatory genes in the coming years, while large accumulation of sequencing data will render genetic diagnosis and counseling increasingly difficult.

New concepts regarding modes of inheritance and interpretation of variant pathogenicity

will require a systematic review and update of genetic diagnostic strategies and new guidance on standardization and reporting of genetic tests. Together, these initiatives should help promote personalized patient care and provide opportunities for novel therapeutic approaches, which will prevent serious or life-threatening complications in these life-long conditions.

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Monitoring Disease Activity, Damage and Quality of Life

13

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Abstract

Systemic inflammation in autoinflammatory diseases can affect nearly all organ systems and hampers patients in their daily life. Ongoing inflammation can lead to irreversible organ damage, such as amyloidosis. Structural assessment of disease activity, damage, quality of life and adherence to therapy is therefore important in the follow-up of these patients. To guide physicians in the monitoring of patients and to unify outcome measures in therapeutic studies, scoring forms for disease activity (AutoInflammatory Disease Activity Index, AIDAI), disease damage (Autoinflammatory Disease Damage Index, ADDI) and patient reported outcomes (Juvenile Autoinflammatory Disease Multidimensional Assessment Report, JAIMAR) have been developed. In addition, an international group of experts developed recommendations for the management of autoinflammatory diseases. The aim of this chapter is to provide an overview of the important aspects of monitoring disease activity, to describe potential organ damage and to elaborate on the validated scoring systems, focusing on the four main monogenic diseases; familial Mediterranean fever (FMF), cryopyrin-associated periodic syndrome (CAPS), mevalonate kinase deficiency (MKD) and TNF-receptor associated periodic syndrome (TRAPS).

Keywords

Autoinflammatory diseases · Monitoring Treatment · Disease activity · Quality of life Damage

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Abbreviations

ADDI	Autoinflammatory Disease Damage Index
AIDAI	Autoinflammatory Disease Activity Index
CAPS	Cryopyrin-associated periodic syndrome
CHQ	Child Health Questionnaire
CNO	Chronic nonbacterial osteomyelitis

CRP	C-reactive protein
DADA2	Deficiency of adenosine deaminase 2
DIRA	Deficiency of the interleukin-1 receptor antagonist
EULAR	EUropean League Against Rheumatism
FACIT	Functional Assessment of Chronic Illness Therapy-Fatigue
FMF	Familial Mediterranean fever
JAIMAR	Juvenile Autoinflammatory Disease Multidimensional Assessment Report
MAS	Macrophage activation syndrome
MASIF	Medication Adherence Scale in Familial Mediterranean Fever
MKD	Mevalonate kinase deficiency
MWS	Muckle-Wells syndrome
MWS-DAS	Muckle-Wells Syndrome Disease Activity Score
NOMID	Neonatal onset multisystem inflammatory disease
PAPA	Pyogenic arthritis, pyoderma gangrenosum and acne
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis
PGA	Physician global assessment
QoL	Quality of life
SAA	Serum amyloid A
SF36	Short Form Health Survey 36
SHARE	Single Hub and Access point for paediatric Rheumatology in Europe
TRAPS	TNF-receptor associated periodic syndrome

Key Points

- **Monitoring of autoinflammatory diseases is aimed to decrease disease activity, improve patients' quality of life and to minimize secondary complications**
- **Patients with autoinflammatory diseases require expert monitoring in a multidisciplinary team, following the international Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) recommendations**
- **The use of appropriate tools, where available, is required for optimal adjustment of therapy**
- **Patients with autoinflammatory diseases are prone to develop organ damage; structural assessment of damage is important in the follow-up of patients**
- **There is a need for severity scores for all autoinflammatory diseases to improve the management of patients at the highest risk of severe complications**

13.1 Main Objectives and Principles in the Follow-Up of Patients with Autoinflammatory Diseases

Experience in chronic diseases such as rheumatoid arthritis, systemic lupus erythematosus and vasculitis has shown that monitoring of disease activity is helpful in guiding treatment decisions. The main objective when monitoring a patient with an autoinflammatory disease is to maintain a state of inactive disease, optimize the quality of life (QoL) and limit complications. To accomplish these goals, the patient needs to be guided during daily life, in order to recognize disease attacks as well as their triggers, presenting symptoms, and management. Patients wish to understand how treatments work for their disease and acquire information on potential side-effects. At each visit, physicians and other caregivers should evaluate the level of adherence to treatment. Patients may also need some adjustments in their school/professional lives and in some cases, other aids to compensate for their handicaps (e.g. hearing aids in cryopyrin associated periodic syndromes (CAPS) and psychosocial distress).

Optimal care should be delivered by a multidisciplinary team in a tertiary center with availability of genetic counseling. This principle has reached 100% consensus, with a low level of evidence (D), in the new European Single Hub and Access point for pediatric Rheumatology in Europe (SHARE) recommendations for management of autoinflammatory diseases [1]. In addition, the reference center should include a transition of care program from pediatric to adult clinic.

13.2 Initial Evaluation

The aim of the initial consultation is to reach a diagnosis, by obtaining a detailed history including family history, age at first symptoms, duration and rhythm of attacks, accompanying symptoms and triggering factors (see Chap. 11). A diary of clinical symptoms and pictures of any skin rash is very useful, because the clinical examination (as well as inflammatory markers) may be normal between attacks. A minimal set of laboratory investigations is essential. This should include a complete blood count, liver enzymes and function, renal function, C-reactive protein (CRP), and serum amyloid A (SAA) if available, both at the time of a clinical flare of disease and when the patient is clinically well. Both CRP and SAA are released by the liver during acute inflammation. Levels of CRP and SAA ≤6 mg/L are associated with a lower risk for the development of secondary amyloidosis [2]; however, their respective values do not strictly evolve in parallel. A recent study with 218 adult and pediatric patients including 6 with amyloidosis reported a good concordance of CRP and SAA in familial Mediterranean fever (FMF); a CRP threshold of <5 mg/L in children and 8.75 mg/L in adults was described as a convenient substitute for an SAA value of <10 mg/L [3]. Other investigations may be helpful in individual cases, to rule out other diagnoses.

The need for genetic testing depends on the presence of symptoms which make it sufficiently relevant and on its availability (see Chap. 12). The Gaslini score [4] (available on www.printo.it/periodicfever/index.asp) was endorsed by the SHARE consensus as a helpful tool to recommend genetic testing for FMF, TNF-receptor associated periodic syndrome (TRAPS) and mevalonate kinase deficiency (MKD) [1]. Classification criteria for FMF, TRAPS, CAPS, MKD and periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome have recently been established by the Eurofever international consortium (see Chap. 11 and specific disease chapters) [5]. Recommendations for genetic testing were published in 2012 [6], and are reviewed in Chap. 12. They highlight the difficulty of interpreting several variants in autoinflammatory genes with a lack of clear association to a specific disease phenotype, for example *V198M* and *Q703K* in CAPS [7], *R92Q* and *P46L* in TRAPS [8] and *E148Q* in FMF [9]. Other helpful clues are the response to colchicine in case of a high suspicion of FMF and urinary mevalonic acid levels during a febrile attack, when MKD is suspected.

13.3 Principles of Autoinflammatory Disease Follow-Up

The frequency of clinic visits is determined by the primary diagnosis and the patient's clinical state [1]. Patients with inactive disease and good QoL can be seen every 6–12 months (once a year is the minimum), but more frequent laboratory monitoring may be necessary. More frequent visits are needed in others situations, such as active disease, initiation of a new treatment, complications, etc. Patients with severe phenotypes and ultra-rare autoinflammatory diseases require follow-up in a certified reference center with multidisciplinary collaboration as exists in Europe or by a physician with expertise in the management of patients with autoinflammatory diseases [1]. Physicians should ask patients about their clinical symptoms, optimally documented in a diary, to assess the duration and frequency of attacks. QoL, tolerance and adherence to treatment needs to be recorded regularly. Laboratory tests are done to assess the presence or absence of systemic inflammation (complete blood count, CRP/SAA), to monitor drug toxicity (complete blood count, liver enzymes and function, muscle enzymes) and disease-related damage (e.g. serum creatinine and proteinuria).

13.4 Disease-Specific Disease Monitoring

13.4.1 Familial Mediterranean Fever (FMF)

In patients receiving colchicine, liver enzymes should be checked regularly [10]. If liver enzymes are elevated more than twofold the upper limit of normal, the dose

of colchicine should be reduced and the cause further investigated. In patients with decreased renal function, the risk of colchicine toxicity is very high, and therefore signs of colchicine toxicity, as well as creatinine kinase, should be carefully monitored and the colchicine dose reduced accordingly (see Chap. 40). In patients treated only with colchicine laboratory assessments should be performed every 6–12 months, while in those treated also with biologic therapy assessments should to be repeated every 3 months [10, 11].

13.4.2 Mevalonate Kinase Deficiency (MKD)

Patients with severe MKD require annual neurological evaluation, particularly to detect cognitive deficits and cerebellar function. A cerebral MRI should be performed to evaluate neurologic symptoms [1]. Furthermore, it is advised to refer MKD patients for an ophthalmologic evaluation once a year. As patients with MKD may have an increased risk of infections, vaccinations should be administered at least prior to initiation of biological agents (see Chap. 17 for further information on how to administer vaccinations in MKD patients), and severe bacterial infections may require hospital admission to monitor and treat. Besides infections, physicians should also be aware of a higher risk of macrophage activation syndrome (MAS) in patients with MKD.

13.4.3 Cryopyrin Associated Periodic Syndrome (CAPS)

Patients with the most severe phenotypes of CAPS (Muckle-Wells Syndrome—MWS and neonatal onset multisystem inflammatory disease—NOMID) need annual neurologic evaluation including cognitive testing, lumbar puncture if initially abnormal for signs of chronic meningitis (documentation of opening pressure, cell count, protein level), and brain MRI (including imaging of the inner ear) every 2–3 years [1]. In addition these patients require regular audiograms and ophthalmological examination (every 6–12 months if rapid worsening). Patients with NOMID may require skeletal radiographs and MRI investigation.

13.5 Monitoring Disease Activity

- **Patient (parent-proxy)-reported tools are the most appropriate to assess disease activity in autoinflammatory diseases**
- **Monitoring biological markers of inflammation, i.e. CRP (and SAA, if available) are helpful to detect patients feeling perfectly well but with subclinical inflammation**
- **The international SHARE consortium and European League Against Rheumatism (EULAR) have recommended the use of the AutoInflammatory Disease Activity Index (AIDAI) score to monitor disease activity in FMF, TRAPS, MKD and CAPS**

Monitoring disease activity is crucial to evaluate the impact of a disease on the general health and quality of life of patients, to evaluate the effectiveness of treatment, and thus to optimize disease follow-up and prevention of secondary complications. In the case of autoinflammatory diseases, inflammatory symptoms have a major impact on patients' QoL [12, 13] and can cause irreversible organ damage, such as amyloidosis, deafness and loss of visual acuity. Variables measuring disease activity may include one or more domains containing either objective or subjective (or both) clinical manifestations, biological measures and items relating to health status and QoL. These variables may be reported either by the patient or by proxy, including caregivers and healthcare providers. It is helpful to have a standardized tool to measure disease activity; however cross-sectional scoring of disease activity is difficult in autoinflammatory diseases, as symptoms are often not continuous and not all are always present at the same time. Conversely some patients may have clinical symptoms (like fatigue or exertion myalgia in FMF) and subclinical biological inflammation in between attacks [14]. The importance of measuring disease activity is highlighted in the international recommendations [1].

13.5.1 Autoinflammatory Disease Activity Index (AIDAI)

In 2009, an international group of experts designed and validated a standardized tool to measure disease activity in patients with FMF, CAPS, TRAPS and MKD, the Autoinflammatory Disease Activity Index (AIDAI) [15, 16]. The tool is common to all four diseases, and is a patient-reported diary containing 12 clinical symptoms, scored as 0 (absent) or 1 (present) (Table 13.1). The AIDAI score is a sum of the values of each symptom reported during a 1-month period. The clinically meaningful threshold for all four diseases indicating a state of active disease is an AIDAI value of at least 9. The AIDAI score is useful for patients, helping to recognize their clinical symptoms and to report them to their health-care provider. The use of the AIDAI in the management of patient follow-up is part of the SHARE recommendations with a high level of evidence and agreement [1]. The AIDAI score is the only standardized and validated patient-reported tool for measurement of disease activity available for clinical research, especially for therapeutic trials. In clinical trials, the use of the AIDAI is protected by a patent and researchers should refer to the exploiting society, Patent Valor. Recently, the AIDAI has been used as an exploratory outcome measure in the CLUSTER study, a phase III trial assessing the efficacy of canakinumab in colchicine-resistant FMF, and severe forms of TRAPS and MKD [17]. In this study the AIDAI score was sensitive to change and correlated with variables of QoL rather than inflammatory markers (i.e. CRP and SAA) [18]. Another validated disease activity score for patients with CAPS is the MWS Disease Activity Score (MWS-DAS) [19].

13.6 Monitoring Response to Treatment

- **Monitoring biological markers of inflammation, i.e. CRP (and SAA, if available) is required to evaluate the response to treatment**
- **Absence of full adherence to colchicine treatment is common in FMF patients and needs to be regularly evaluated**

Since the discovery of effective biologic therapies for autoinflammatory diseases, therapeutic trials have preceded the establishment of standardized outcome measures. In most thera-

Table 13.1 The autoinflammatory disease activity index (AIDAI) score [16]

ID:			Age:				Month:						Year:
Autoinflammatory diseases related symptoms today													
Days	Fever ≥38°C (100.4°F) (a)	Overall symptoms (b)	Abdominal pain (c)	Nausea/ vomiting (d)	Diarrhoea (e)	Head aches (f)	Chest pain (g)	Painful nodes (h)	Arthralgia or Myalgia (i)	Swelling of the joints (j)	Eyes manifestations (k)	Skin rash (l)	Pain relief drugs taken
Scored as:	0/1	0/1 Yes/No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	0/1 Yes/ No	
1													
2													
3													
...													
31													

Each line represents a day in a month, patients are asked to complete the diary during attacks and score symptoms (only if due to the autoinflammatory disease) as yes (1) or no (0). A different diary for each month is required; if patients do not have a flare, they are asked to return the diary empty. The AIDAI score is the sum of all symptoms present in 1 month

peutic studies on autoinflammatory diseases, the response to treatment has been assessed by the combination of the physician global assessment (PGA) value, individual symptoms, attack frequency and biological markers of acute inflammation; i.e. CRP and SAA [20].

13.6.1 FMF50

Ozen et al. developed outcome criteria that define response to treatment for patients with FMF [21]. A set of variables meaningful of response to treatment was obtained by Delphi survey followed by a consensus conference (Table 13.2). To obtain a response in patients with FMF, the final set requires 50% or more improvement in at least five of six criteria comprising the set, without worsening in any of them. The validation cohort included 289 patients with FMF, in whom 50% were children and 25% were resistant to colchicine treatment. However, the usefulness of this tool is questionable, as it was not found to be valid in the rilonacept FMF trial [22], and currently, a 50% improvement is not considered to be satisfactory.

13.6.2 Adherence to Treatment

Incomplete or lack of adherence to treatment (or to physician recommendations) has been estimated to occur in as many of 30–50% of patients, regardless of their age, the disease and the type of medication (included some of the injected biologics) [23]. The consequences include an increased risk of inappropriate medications prescribed by physicians and an increased burden of disease due to recurrent attacks and inflammatory complications. In FMF, various studies have assessed the level of adherence to colchicine treatment. In a recent survey conducted in French reference centers, 17 of 42 patients (40%) declared full adherence to colchicine treatment; adherence was greater in children (48%) than adults (22%). Thus, in a large proportion of patients, the inefficacy of colchicine might be explained by low adherence rather than true resistance [24]. To monitor therapy adherence the Medication Adherence Scale in Familial Mediterranean Fever (MASIF) was developed. The MASIF is an 18-item scale in which items are grouped into four categories: knowledge about the medication, adherence to treatment, barriers to drug use and factors that may increase compliance [25]. The participants answer each item on a five point Likert-type scale (1 = strongly agree, 2 = agree, 3 = no idea, 4 = disagree, 5 = strongly disagree) resulting in a total score range of 18–90. The MASIF instrument is short, simple, and easy to complete, taking only 3–4 min.

Table 13.2 List of items of the FMF50 score [21]

1. Percentage change in the frequency of attacks with treatment
2. Percentage change in the duration of attacks with treatment
3. Patients/parents' global assessment of disease severity (10 cm visual analogue scale (VAS))
4. Physicians' global assessment of disease severity (10 cm VAS)
5. Percentage change in arthritis attacks with treatment
6. Percentage change in C-reactive protein, erythrocyte sedimentation rate or serum amyloid A level with treatment

CRP C-reactive protein, *ESR* erythrocyte sedimentation rate, *FMF* familial Mediterranean fever, *SAA* serum amyloid A, *VAS* visual analogue scale

13.7 Monitoring Damage

- **Chronic inflammation can cause damage in nearly all organ systems**
- **Structural assessment of damage is important in monitoring individual patients and to determine outcomes in therapeutic studies**
- **The Autoinflammatory Disease Damage Index (ADDI) provides a tool for assessing disease-related damage**

Damage is defined as symptoms or complications caused by previously encountered inflammatory episodes that have persisted for at least 6 months, are irreversible and are not due to actual inflammation at the time of assessment [26]. Recurrent episodes of inflammation with possible subclinical inflammation between episodes render patients with autoinflammatory diseases susceptible to develop damage. However, besides FMF, there are very few prospective

cohorts with long-term follow-up. Therefore, little is known about the actual prevalence of damage in autoinflammatory diseases.

13.7.1 Occurrence of Damage

13.7.1.1 Amyloidosis and Renal Failure

As autoinflammatory diseases are characterized by a marked acute phase response, including large increases in SAA levels, patients are prone to developing AA amyloidosis, especially when the underlying inflammation is inadequately controlled (see Chap. 15) [2]. In the absence of treatment, it is estimated that amyloidosis occurs in approximately 50% of FMF patients, 10–20% of TRAPS patients, <10% of MKD patients and varying percentages of CAPS patients (<10% in familial cold autoinflammatory syndrome, approximately 25% in MWS, unknown for NOMID) [27]. The kidney is the most common organ affected by AA amyloidosis. Renal amyloidosis presents with proteinuria and ultimately results in end-stage renal failure, requiring dialysis or transplantation. Amyloid deposits can also be found in other organs, such as the spleen, heart and liver. In a cohort of 374 patients with systemic AA amyloidosis, including 32 patients with autoinflammatory diseases, the median survival after diagnosis was 133 months [2]. The SAA serum level during follow-up was strongly inversely associated with survival [2]. Hence, SAA serum levels and urinalysis are important during follow-up of patients with autoinflammatory diseases. As SAA measurements are not available in many countries, measuring CRP levels is a valid alternative [3]. Other renal complications like glomerulonephritis, nephropathy and renal angiomyolipoma are also described in autoinflammatory diseases, also stressing the importance of urinalyses during follow-up [28–30].

13.7.1.2 Infertility

Infertility, often defined as an inability to conceive after 1 year of unprotected intercourse [31], can have a huge impact on patients' lives. Besides FMF, not much is known about the prevalence of infertility in autoinflammatory diseases. Female fertility in FMF may be impaired by abdominal/pelvic adhesions due to attacks of peritonitis. Oligo- or amenorrhea or ovarian damage may be due to amyloid deposition; male fertility may be impaired by testicular amyloidosis or as a complication of acute orchitis [31]. Despite suggestions from *in vitro* and animal studies, colchicine does not cause azoospermia but rather protects from FMF-related infertility when given early and in an adequate dose (see Chap. 40) [31]. Patients with amyloidosis have a higher risk of infertility; hence semen analysis and the possibility of cryopreservation can be offered to young male patients with amyloidosis [31]. In both female and male patients with CAPS, subfertility and infertility are reported [32, 33]. Despite the lack of evidence for infertility in other autoinflammatory diseases, fertility deserve close attention due to the shared pathology, like amyloidosis and abdominal adhesions.

13.7.1.3 Growth and Development

Patients with autoinflammatory diseases can have growth failure, puberty delay and (secondary) amenorrhea, caused by either chronic inflammation or by long-term treatment with corticosteroids [34]. These complications can at least be partially reversed if adequate therapy is started early and long-term use of high doses corticosteroids avoided. Monitoring growth and development is therefore part of the follow-up of children with autoinflammatory diseases [1].

13.7.1.4 Serosal Scarring

Serosal scarring is a complication of recurrent serositis such as peritonitis and pleuritis. Another cause of serosal scarring is abdominal surgery, often performed in patients with FMF, as the presentation of abdominal FMF attacks mimics appendicitis [35]. Serosal scarring can lead to symptomatic intestinal obstruction. Abdominal adhesions/occlusions have been reported in 3–8% of MKD patients [28, 29, 36] and approximately 3% of FMF patients [37, 38]. Adhesions can also occur in other autoinflammatory diseases.

13.7.1.5 Neuropsychiatric Damage

Neurologic problems are described mainly in patients with CAPS, especially in those with the

more severe NOMID phenotype. The main neurologic complication is the development of chronic meningitis, which can lead to elevated intracranial pressure, chronic headache and mental retardation [39, 40]. As adequate blockade of interleukin-1 can prevent the progression of neurological damage; therapy must be started as early as possible in patients with CAPS [1]. Mevalonic aciduria, the severe phenotype of MKD, is also characterized by serious neurological manifestations such as ataxia, psychomotor retardation and hypotonia ([41]; see Chap. 17). Although rare, patients with FMF can also present with a variety of neurological complains including demyelinating lesions and cerebrovascular disease [42]. Ischemic and/or hemorrhagic strokes, polyneuropathy and other severe neurological manifestations are frequently reported in the deficiency of adenosine deaminase 2 (DADA2), a recently discovered, but relatively common, autoinflammatory disease ([43, 44]; see Chap. 23). Also patients with interferonopathies as Aicardi-Goutières syndrome can result in a severe neurologic phenotype ([45]; see Chaps. 10 and 24).

Fatigue, depression and anxiety are quite frequent in patients with chronic illness and are often found in patients with autoinflammatory diseases: Twenty percent of 35 patients with CAPS reported depression [13] and in a study of 90 patients with FMF, 30% scored positive for depression and 50% for anxiety [46]. Besides disease activity and the severity of organ damage, several factors such as pain and disfiguring skin lesions, as seen in pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome, might play a role in mood disturbances. Some studies showed that the frequency of fibromyalgia, a risk factor for depression, is relatively high in patients with rheumatic diseases such as FMF [47, 48]. No studies investigated the influence of disease activity and organ damage on depression and anxiety in autoinflammatory diseases. However, the high reported prevalence warrants attention for mood disorders during the follow-up of patients with autoinflammatory diseases.

13.7.1.6 Hearing Loss

Hearing loss is typically found in patients with CAPS. Audiometry shows a sensorineural hearing loss which is often bilateral and symmetric and specifically observed in the high frequencies, not always noticed by patients ([49]; see Chap. 19). Measuring hearing thresholds at the highest possible frequencies by audiometry is therefore recommended to detect hearing loss at an early stage. In other autoinflammatory diseases, hearing loss is infrequently described, and it is not known whether this is more often than found in the general population. Early treatment can prevent hearing loss, and might prevent further hearing loss in those who have already lost hearing.

13.7.1.7 Ocular Damage

In patients with CAPS, especially those with the more severe NOMID phenotype, chronic meningitis can cause papilledema and optic atrophy, which results in visual loss [50]. Another cause of visual loss in CAPS is uveitis, which is reported in approximately 15% of patients with CAPS [50]. Granulomatous uveitis is the hallmark of another autoinflammatory disease, Blau syndrome ([51]; see Chap. 20). Some patients with MKD developed uveitis, retinitis pigmentosa or cataracts [36, 41, 52]. Cataract and glaucoma can also be a complication of chronic corticosteroid use [53]. Ophthalmologic investigation is therefore advised in patients with CAPS, Blau syndrome and MKD and patients on long-term corticosteroid therapy.

13.7.1.8 Musculoskeletal Damage

Overgrowth arthropathy is a complication of the NOMID phenotype of CAPS, which seems to be caused by abnormal bone formation rather than synovitis [54]. Additionally, patients with autoinflammatory diseases can suffer from destructive arthritis leading to joint contractures. Furthermore, osteoporosis, which may be caused by the disease or by long-term corticosteroid use [53], is described in all four of the main monogenic fever syndromes. These complications can cause impairment in function and chronic musculoskeletal pain. Lastly, musculoskeletal damage secondary to severe arthritis is also reported in other autoinflammatory diseases, such as Blau and PAPA syndrome; osteomyelitis occurs in chronic nonbacterial osteomyelitis (CNO), deficiency of interleukin-1 receptor antagonist (DIRA) and Majeed syndrome.

13.7.2 Monitoring Damage: The Autoinflammatory Disease Damage Index (ADDI)

In 2018, a large group of European and North American specialists in pediatric and adult rheumatology and internal medicine published the Autoinflammatory Disease Damage Index (ADDI) [55]. The ADDI consists of 18 items, grouped in eight categories: reproductive, renal/amyloidosis, developmental, serosal, neurological, auditory, ocular and musculoskeletal dam-

Table 13.3 Definitive Autoinflammatory Disease Damage Index (ADDI) including glossary of terms [55]

Damage item	Grading*	Points**
Reproductive		***Max. 2***
Sub/infertility		2
Amenorrhea		1
Renal/amyloidosis		***Max. 6***
Amyloidosis	Limited/extensive	2/3
Proteinuria		1
Renal insufficiency	Moderate/severe	2/3
Developmental		***Max. 3***
Growth failure		2
Puberty delay		1
Serosal		***Max. 1***
Serosal scarring		1
Neurological		***Max. 6***
Developmental delay		2
Cognitive impairment		3
Elevated intracranial pressure		2
Central nervous system involvement		3
Ears		***Max. 2***
Hearing loss	Moderate/severe	1/2
Ocular		***Max. 3***
Ocular involvement	Mild/moderate/severe	1/2/3
Musculoskeletal		***Max. 4***
Joint restriction		2
Bone deformity		2
Osteoporosis		1
Musculoskeletal pain		1
The total ADDI score is the sum of the 8 categories (maximum 27 points)		
***Grading**: scoring depends on the severity of damage **Amyloidosis**: limited, affecting one organ; extensive, affecting more than one organ **Renal insufficiency**: moderate, GFR between 15 and 60 ml/min/1.73 m^2; severe, GFR<15 ml/min/1.73 m^2, dialysis or transplantation **Hearing loss**: moderate, hearing impairment without requirement of hearing aids or a cochlear implant; severe, hearing impairment requiring hearing aids or a cochlear implant **Ocular involvement**: mild, ocular damage without visual impairment; moderate, with visual impairment; severe, legal blindness **Points** are given when the item is present. For items with grading in severity, the lowest score is given for mild involvement and the highest for severe involvement. For each category, the score is limited to a specific maximum		
Glossary of terms		
Amenorrhea: Primary amenorrhea: absence of menarche at the age of 16 years or absence of menarche 5 years after thelarche in a female. Secondary amenorrhea: absence of the menses for six consecutive months or more, in a female who previously had menstrual cycles		
Amyloidosis Symptomatic amyloidosis confirmed by examination of tissue sections by Congo red dye or seru, amyloid P (SAP) scintigraphy		
Bone deformity Bone deformation or overgrowth on clinical examination and/or imaging studies		

(continued)

Table 13.3 (continued)

Damage item	Grading*	Points**
Central nervous system involvement Focal deficits (gross and/or fine sensorimotor), diffuse deficits (e.g. memory, behaviour), seizures, and spinal cord symptoms. Neuropsychiatric disorders unrelated to the disease should not be scored		
Cognitive impairment Requirement of special education because of cognitive impairment or IQ below 70 as defined by neuropsychological assessment (e.g. WISC) or other age-appropriate equivalents		
Developmental delay[a] Failure to reach age-appropriate developmental milestones, including language/speech, motor, social/emotional, and cognitive milestones		
Elevated intracranial pressure Signs and/or symptoms of elevated intracranial pressure supported by appropriate techniques[b]		
Growth failure Defined as the presence of at least two of the three features:		
– lower than the 3rd percentile or −2 standard deviations (SD) height for age		
– growth velocity over 6 months lower than the 3rd percentile or −2 SD for age		
– crossing at least 2 centiles (5%, 10%, 25%, 50%, 75%, 90%, 95%) on growth chart		
For patients older than 18 years: Pathological short stature (e.g. below 3rd percentile or −2 SD for normal ethnic population)		
Hearing loss Sensorineural hearing impairment of better ear, confirmed by audiometry or another age appropriate technique, or requirement of hearing aids or a cochlear implant		
Infertility A disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse, not due to known disorders in the unaffected partner		
Joint restriction Fixed limitation in the normal range of motion of joints affecting function, with or without destructive arthropathy or avascular necrosis		
Musculoskeletal pain Non-inflammatory musculoskeletal pain impairing activities of daily living		
Ocular involvement Ocular damage (e.g. optic nerve atrophy, elevated intraocular pressure or cataract) of better eye, documented by an ophthalmologist, with or without visual impairment		
Osteoporosis Reduced bone mineral density with vertebral collapse and/or pathological fractures confirmed with imaging, which may include bone densitometry. Requires both evidence of decreased bone density and fracture, 'low bone density' by itself is insufficient		
Proteinuria Persistent urinary protein to creatinine ratio of >20 mg/mmol in the first morning void; and/or a daily protein excretion of >0.3 g/24 h, or urine albumin to creatinine ratio of >15 mg/mmol		
Puberty delay A Tanner stage below −2 SD for age or below the 3rd percentile for age or any Tanner stage after pharmacological induction of puberty		
Renal insufficiency Glomerular filtration rate (GFR) of <60 ml/min/1.73 m^2, dialysis or transplantation		
Serosal scarring Symptomatic adhesions or fibrosis affecting pericardium, pleura, peritoneum and/or retroperitoneum, supported by imaging techniques, endoscopy or surgery		

[a]Only for pediatric patients
[b]Such as fundoscopy, neuroimaging or lumbar cerebrospinal fluid (CSF) pressure measurement

age (Table 13.3). The items were defined by Delphi consensus building, which included a literature search and surveys or interviews with patients and experts to retrieve all possible damage items, followed by surveys, a consensus meeting and decision-making software to select the most relevant damage items and define definitions and grading of all items. Patients and parents of patients were involved during all stages of the consensus process, in order to include items relevant for patients' daily lives. The ADDI has been validated using semifictional cases (not yet published). ADDI can be used to monitor structural damage in individual patients with FMF, TRAPS, CAPS and MKD and to assess (prevention of) damage in therapeutic studies.

13.8 Monitoring the Severity of Autoinflammatory Diseases

Assessing the risk of a severe disease course with secondary complications is a major issue in autoinflammatory diseases, which are not curable,

Table 13.4 The International Severity Scoring System for Familial Mediterranean Fever (ISSF) [58]

	Criteria	Points
1	Chronic sequela (including amyloidosis, growth retardation, anemia, splenomegaly)	1
2	Organ dysfunction (nephrotic range proteinuria, FMF-related)	1
3	Organ failure (heart, renal, etc., FMF-related)	1
4a[a]	Frequency of attacks (average number of attacks between 1 and 2 per month)	1
4b[a]	Frequency of attacks (average number of attacks >2 per month)	2
5	Increased acute-phase reactants (any of C-reactive protein, serum amyloid A, erythrocyte sedimentation rate, fibrinogen) during the attack-free period, ≥2 weeks after the last attack (at least two times 1 month apart)	1
6	Involvement of more than two sites during an individual acute attack (pericarditis, pleuritis, peritonitis, synovitis, ELE, testis involvement, myalgia, and so on)	1
7	More than two different types of attacks during the course of the disease (isolated fever, pericarditis, pleuritis, peritonitis, synovitis, ELE, testis involvement, myalgia, and so on)	1
8	Duration of attacks (more than 72 h in at least three attacks during a year)	1
9	Exertional leg pain (pain following prolonged standings and/or exercising, excluding other causes)	1
Total score		**10**

Severe disease ≥6, intermediate disease 3–5, mild disease ≤2
FMF familial Mediterranean fever, *ELE* erysipelas-like erythema
[a]Criterion 4a/4b can give 0 or 1 or 2 points altogether per the definition

even with pharmacologic treatments. In addition, available therapeutic solutions for patients with difficult to treat diseases include very expensive symptom-relieving orphan products or even high-risk therapy like bone marrow transplantation and gene therapy. Several severity scores have been developed and validated for adult patients with FMF, almost exclusively in the Israeli and Turkish populations [56, 57]. A first international effort has recently proposed new severity items for FMF valid for both pediatric and adult patients (Table 13.4) [58]. This score is not yet widely used and its reliability in patients with colchicine resistance and/or those treated with biologics has not been established. No severity scores are available for other autoinflammatory diseases; further international efforts are needed.

13.9 Monitoring Quality of Life (QoL)

The consequences of an autoinflammatory disease on daily activities and QoL are very high. QoL can be measured with generic questionnaires, such as the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT) questionnaire, the Child Health Questionnaire (CHQ) and Short Form Health Survey (SF36), a 36-item questionnaire that measures the impact of disease on the physical and psychosocial health-related QoL in adults. All studies that investigated QoL in autoinflammatory diseases found an impaired QoL, mainly in physical functioning, body pain and general health [12, 13, 28, 46, 59]. Effective therapy was shown to dramatically improve the QoL in all of the therapeutic studies [13, 60–62].

13.9.1 Juvenile Autoinflammatory Disease Multidimensional Assessment Report (JAIMAR)

Recently, a group of autoinflammatory disease experts have developed and validated a quality of life scale for four autoinflammatory disorders including FMF, MKD, TRAPS, and periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome [63]. This tool called Juvenile Autoinflammatory Disease Multidimensional Assessment Report (JAIMAR) comprises 16 parent- or patient-centered measures in four dimensions, which assess functional status, pain, therapeutic compliance, and health-related QoL (physical, social, school and emotional status). The JAIMAR was validated in 2 pediatric FMF trials, with 250 and 179 children, respectively, but

further validation is needed in adults and in patients with other autoinflammatory diseases.

13.10 Conclusion

The nature of systemic inflammation in autoinflammatory diseases requires a broad monitoring of patients, preferably in a reference center with a multidisciplinary team and access to genetic counseling. Patient-reported complaints, the presence of subclinical inflammation, therapy adherence and regular investigation of disease-specific symptoms and complications are important aspects of the management of these patients. Disease activity, damage, severity and quality of life are important to monitor and can best be assessed by standardized tools.

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14 The Role of International Registries for Rare Autoinflammatory Diseases

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Abstract

The main aims of international registries for rare diseases are to evaluate genetic and epidemiological features, clinical presentations, treatment, outcome and prognostic factors. In 2008, the Pediatric Rheumatology European Society (PReS) promoted the Eurofever Project, specifically aimed at the creation of a permanent network for the study of autoinflammatory diseases in childhood. The Eurofever Project involved more than 400 centers in 60 countries worldwide; enrollment started in November 2009. In its first version, Eurofever was established as a cross-sectional registry, collecting information of the patients from disease onset to disease diagnosis. In 2015, it was transformed to a longitudinal registry, collecting information on a yearly basis on the clinical evolution and the efficacy and safety of different treatments used in these rare conditions. The first aim of the Eurofever registry was to improve the knowledge about the presentation, disease course, complications, genotype-phenotype correlations and response to treatment of these rare disorders. Further purposes of the Eurofever registry were to generate evidence-based diagnostic and classification criteria and to elaborate on disease activity parameters. During the last 8 years, the international effort to build a common registry on autoinflammatory diseases led to considerable accumulation of new information enabling to fulfill most of the initial aims.

Keywords

Eurofever · Autoinflammation · Registries
Periodic fevers · Interleukin-1 blockade

Abbreviations

ADDI	Autoinflammatory Disease Damage Index
AIDAI	Autoinflammatory Disease Activity Index
CANDLE	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase activation and recruitment domain
CNO	Chronic non-bacterial osteomyelitis
CRF	Case report form
DADA2	Deficiency of adenosine deaminase 2

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DIRA	Deficiency of the interleukin-1 receptor antagonist
DITRA	Deficiency of the interleukin-36 receptor antagonist
ESID	European Society of Immune-Deficiencies
EULAR	European League Against Rheumatism
FCAS2	Familial cold autoinflammatory syndrome 2
FMF	Familial Mediterranean fever
HRF	Hereditary recurrent fever
IL	Interleukin
ISSAID	International Society of Systemic Autoinflammatory Diseases
MKD	Mevalonate kinase deficiency
NLRP	Nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain
NSAIDs	Nonsteroidal anti-inflammatory drugs
PAPA	Pyogenic arthritis, pyoderma gangrenosum, acne
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
PI	Principal investigator
PReS	Pediatric Rheumatology European Society
ROC	Receiver operating characteristic
SAVI	STING-associated vasculopathy with onset in infancy
SOBI	Swedish Orphan Biovitrum
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome

Key Points

- **An overview of the different international registries for autoinflammatory diseases developed prior to the Eurofever registry**
- **The methodological approach used for developing the Eurofever registry**
- **A summary of the main results obtained by the Eurofever registry to date and future perspectives**

14.1 Introduction: The Need for International Registries on Rare Conditions; Why Eurofever

The main aims of international registries for rare diseases are to evaluate genetic and epidemiological characteristics, clinical presentations, therapeutic strategies, outcome and prognostic factors, as well as the genotype-phenotype correlations of these diseases. In this chapter, we report the advances obtained with the institution of Eurofever, a large international registry on the autoinflammatory diseases and briefly, present other registries related to autoinflammatory diseases.

In 2008, the Pediatric Rheumatology European Society (PReS) promoted a project specifically aimed at the creation of a permanent network for the study of autoinflammatory diseases in childhood named Eurofever, supported by the European Executive Agency for Health and Consumers. The main aims of this project were to create an international web-based registry on autoinflammatory diseases that could help in early recognition, knowledge of clinical presentation, treatment, and outcome of these rare diseases. Additional aims of this project were to provide clinical classification criteria, to support development of indications for genetic analysis, to establish patient cohorts for future outcome studies and clinical trials, to identify informative families or clusters for genetic studies and to provide reliable information for affected families and physicians.

The Eurofever Project has created a single registry for the following diseases: familial Mediterranean fever (FMF), cryopyrin-associated periodic syndromes (CAPS), tumor necrosis factor receptor-associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD), Blau syndrome, chronic non-bacterial osteomyelitis (CNO), Behçet disease, pyogenic arthritis, pyoderma gangrenosum, acne (PAPA) syndrome, deficiency of the interleukin (IL)-1 receptor antagonist (DIRA), familial cold autoinflammatory syndrome 2 (FCAS2-

nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain (NLRP)-12-mediated), Schnitzler syndrome, Majeed syndrome, periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome and undefined periodic fever [1]. More recently, caspase activation and recruitment domain (CARD)14 mediated psoriasis (CAMPS), deficiency of the IL-36 receptor antagonist (DITRA), deficiency of adenosine deaminase (DADA2) and interferonopathies (chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature—CANDLE and STING-associated vasculopathy with onset in infancy—SAVI) have been added to the list of disorders that are included in the Eurofever registry.

14.1.1 Other Previous and Current Registries on Autoinflammatory Diseases

Before the development of Eurofever, separate registries for several of the "classic" recurrent fever syndromes were available. In 2009 Ben-Chetrit and Touitou described the epidemiologic, genetic and clinical characteristics of a large international cohort of patients with FMF [2]. The study provided an overview on the clinical features of FMF in different countries from the Mediterranean basin and Middle East region. The first formal international registry on an autoinflammatory disease was set up for MKD in the Netherlands [3]. This international database (www.hids.net) collected clinical and laboratory data of 244 patients with MKD enrolled until 2007. Clinical data from 103 patients out of the 126 validated patients enrolled from 18 countries were reported (see Chap. 17).

A multidisciplinary consortium named Eurotraps (http://fmf.igh.cnrs.fr/ISSAID/EUROTRAPS/) was developed in parallel to the Eurofever registry Data for this registry were obtained from six different countries (Austria, France, Germany, Israel, Italy, United Kingdom) with the aims to gain insights into the natural course and pathophysiology of TRAPS, particularly in children. Since the clinical data pertinent to TRAPS were collected with the same case report form (CRF) used for the Eurofever registry, Eurotraps was subsequently merged with Eurofever.

Hofer et al. in 2008 [4] established a web-based multi-center registry for PFAPA, as an international collaboration within the framework of the "periodic fever" study group of PReS. The registry resulted in a publication on 301 patients from 15 centers describing the clinical features, clinical course and long-term follow-up of this condition [5].

In 2011 Koné-Paut et al. [6] published a concise report about an international cohort of patients with suspected pediatric Behçet diseases (BD), collecting demographic and clinical information on 110 patients followed for 3 years. This study led to the development of evidence-based new classification criteria for pediatric BD [7]. In 2009 Rose and Wouters established an international registry on Blau syndrome [8] and early onset sarcoidosis, which has gathered data on more than 31 patients from 11 countries [9].

Among other national registries which are ongoing parallel to Eurofever is the German Registry for Autoinflammatory diseases (AID-net) [10].

Finally, a post-registration observational registry (Beta-confident) aimed to collect data on the safety and efficacy of the anti-interleukin (IL)-1β monoclonal antibody canakinumab, used in patients with CAPS, has also been developed [11].

14.2 The Advent of the Eurofever Project

In 2008, thanks to a grant from the European Agency for Health and Consumers, an international initiative related to the novel group of rare conditions included under the umbrella term of autoinflammatory diseases was started: the Eurofever Project. One of the main goals of the project was to establish an international registry on these rare conditions. At variance with the previous international initiatives which aimed to

collect data of patients affected only by a single condition (MKD registry, Eurotraps, PFAFA registry), the Eurofever registry was conceived as a single source of information for known and future autoinflammatory diseases. Indeed, the major advantage of a single registry for different conditions is related to the possibility to collect demographic, genetic and clinical information in a homogenous fashion facilitating the comparison among the various conditions. Thanks to collaboration with the Paediatric Rheumatology International Trials Organization (PRINTO, http://www.printo.it), the Eurofever registry was able to reach a large number of pediatric rheumatology centers already involved in the management of pediatric rheumatic conditions. Moreover, adult centers and members of the International Society of Systemic Autoinflammatory Diseases (ISSAID) and the European League Against Rheumatism (EULAR) were also engaged in the project in order to collect data also on adult patients. Indeed, the Eurofever Project survey involved all centers linked to PRINTO that currently includes more than 400 centers in 60 countries worldwide. Beside the registry, the Eurofever Project was an opportunity to bring together centers that care for patients with autoinflammatory diseases, establishing a network able to foster research in the field, including the organization of clinical trials. In this line, the development of new classification criteria and of new outcome measures for the autoinflammatory diseases were among the main aims of the project. The registry has elected an International Steering Committee (Marco Gattorno, Italy; Seza Ozen, Turkey; Joost Frenkel, The Netherlands; Helen Lachmann, United Kingdom and the secretary elect of the PReS Working Group for Autoinflammatory diseases), that coordinate the activities of the registry.

Following the European grant, Eurofever was supported by unrestricted grants from Novartis and Swedish Orphan Biovitrum (SOBI) and receives continuous technical and operational support by PRINTO.

14.2.1 How the Eurofever Registry Was Developed

The registry was created in 2008 with the active collaboration of experts involved in the management of autoinflammatory diseases [12]. The experts were asked to identify all significant variables for each disorder. The forms for data collection were divided into two parts: demographic and clinical data (Fig. 14.1). Demographic data included: subject ID (patients were identified by alphanumeric code), date of birth, sex, ethnicity, country of birth, onset age, date of first visit to the center, patient diagnosis and molecular analysis. Genetic testing was not mandatory, but if performed, details were requested. These included the gene screened and whether the complete gene was sequenced or the most relevant exons or just the most relevant point mutations and the laboratory where the test was performed. Finally, information on consanguinity and any relevant family history was collected. Clinical data included: (1) signs and symptoms, (2) laboratory examinations, (3) imaging and other diagnostic procedures and (4) response to treatment(s). Further revisions of the forms were subsequently evaluated by the experts and inclusion criteria for each disease were established with a final approval of the definitive version during a Consensus Meeting in March 2009. Continuous revisions of the form are performed to include newly described diseases with genetic information and associated clinical manifestations. Access to the database is available only for centers authorized by PRINTO, with a username and password on an https platform. For each disease one coordinator (Disease Principal Investigator-PI) is chosen among the associate and collaborating partners on the basis of their expertise in the specific diseases or participation in other ongoing initiatives in connection with the Eurofever Project. Data on single diseases are under the direct responsibility of the Disease-PIs and the Eurofever Steering Committee. Disease-PIs collect and analyze data from the Registry according to the aims for their specific disease and in agreement with the

Fig. 14.1 The Eurofever forms for data collection

Eurofever Steering Committee. General epidemiological data derived from the Registry are under the responsibility of the Steering Committee. All participants in Eurofever can propose new studies on a specific disease or particular issues/aspects involving various diseases. Criteria for the evaluation of secondary studies include the: (1) scientific relevance; (2) clinical/scientific experience of the sponsor in the disease/field; (3) number of patients enrolled in the Eurofever registry for a given disease. Ethical committee approval for patient enrollment in the registry was obtained by participating centers as required by local legal requirements [13]. Informed consent was signed by parents or legal representatives or by the patient of adequate age.

Enrollment started in November 2009. In its first version, Eurofever was established as a cross-sectional registry, collecting information of the patients from disease onset to disease diagnosis. Although Eurofever collects a large amount of clinical variables, the registry was designed to

prevent the prospect of missing data, through the development of web-based forms that do not allow to continue data entry in case of missing data in a required field. Despite this careful approach, the main limitation of the first version of the registry was the retrospective collection of data, possibly leading to inaccurate collection of all the clinical information pertinent to each patient. For this reason, in 2015, the registry was transformed into a longitudinal registry, collecting information on a yearly basis on the clinical evolution and the efficacy and safety of different treatments used in these rare conditions. In particular, the sections related to treatment, which in the original cross-sectional version of the registry were rather concise, were completely revised in order to enable entry of more detailed information on efficacy and safety of all treatments used to treat the different autoinflammatory conditions. The creation of this large cohort of patients affected by autoinflammatory diseases with an extended follow-up will be the base for better knowledge of these disorders, with a focus on genotype/phenotype correlation and long-term efficacy and safety of different treatments. Moreover, since approximately 75–80% of patients with clinical features consistent with autoinflammatory diseases have no recognized mutations in any of the known genes [14, 15], a large cohort of patients with undefined periodic fevers will allow characterization of novel genes in this current challenging disease group. As of April 2018, 4048 patients have been enrolled in the registry from 60 different countries, with longitudinal data available for 446 patients (Table 14.1).

Table 14.1 Number of patients enrolled in the Eurofever registry

Disease	Number of enrolled patients	Number of patients with longitudinal data
FMF	1147	103
PFAPA	702	15
CNO	585	111
Undefined fever	362	29
CAPS	348	38
TRAPS	302	32
Behçet disease	221	67
MKD	214	30
Blau syndrome	73	0
PAPA	42	9
DADA2	13	5
NLRP12/ FCAS2	13	0
Schnitzler syndrome	11	5
DIRA	4	1
Majeed syndrome	4	0
SAVI	3	1
CAMPS	2	0
CANDLE	1	0
DITRA	1	0
Total	4048	446

FMF familial Mediterranean fever; *PFAPA* periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis; *CNO* chronic non-bacterial osteomyelitis; *CAPS* cryopyrin-associated periodic syndromes; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *MKD* mevalonate kinase deficiency; *PAPA* pyogenic arthritis, pyoderma gangrenosum, acne; *DADA2* deficiency of adenosine deaminase 2; *NLRP12/FCAS2* nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain/familial cold autoinflammatory syndrome 2; *DIRA* deficiency of the interleukin-1 receptor antagonist; *SAVI* STING-associated vasculopathy with onset in infancy; *CAMPS* caspase activation and recruitment domain 14 mediated psoriasis; *CANDLE* chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; *DITRA* deficiency of the interleukin-36 receptor antagonist

14.3 What Did We Learn from the Registry?

During the last 9 years to date, the Eurofever registry provided 12 papers with 800 citations. The main new information coming from the registry can be summarized as follows

- **Insights on the clinical presentation, disease course, complications and response to treatment for the most common autoinflammatory diseases, providing the largest series available in the literature**
- **Detailed information on genotype-phenotype correlations in hereditary periodic fever syndromes**

- **Development and validation of novel instruments for the management of autoinflammatory diseases in daily practice**
- **Evaluation of the accuracy of previous diagnostic criteria and development of new evidence-based classification criteria**

The first aim of the Eurofever registry was to improve the knowledge about the presentation, disease course, complications, genotype-phenotype correlations and response to treatment of these rare disorders.

The first analysis of the demographic data coming from the Eurofever registry was performed by Toplak et al., in 2012 [5]. This report gave the first overview of the distribution of these rare monogenic conditions based on the collection of 1880 patients from 67 centers in 31 countries. Most of the patients (74%) lived in western Europe, 16% in the eastern and southern Mediterranean region (Turkey, Israel, and North Africa). Only 6% of the patients were from eastern European countries, highlighting the possibility of a lack of recognition of these diseases or lack of resources in some less economically developed countries. The study was able to analyze the impact of diagnostic delay in these conditions. The median diagnosis delay was 7.3 years (range 0.3–76), with an encouraging reduction in the time to diagnosis in patients born after the identification of the first gene associated with autoinflammatory diseases (*MEFV* for FMF) in 1997 [16].

14.3.1 Data from Common Autoinflammatory Conditions

14.3.1.1 Familial Mediterranean Fever (FMF)

The homogeneous collection of pediatric patients with FMF coming from different countries and ethnicities enabled for the first time an analysis of the impact of ethnic, environmental and genetic factors on the severity of disease presentation. For this aim, demographic, genetic and clinical data from pediatric patients with FMF enrolled in the Eurofever registry were analyzed. Patients were divided into three subgroups: (1) patients living in the eastern Mediterranean countries; (2) patients with an eastern Mediterranean ancestry living in western Europe; (3) Caucasian patients living in western European countries [17].

The study was conducted on 346 pediatric patients with FMF from 64 centers in 28 countries. European patients had a lower frequency of the high penetrance M694V mutations and a significant delay of diagnosis ($p < 0.002$). The study confirmed in the pediatric setting that patients living in the eastern Mediterranean display a more severe disease presentation with a higher frequency of fever episodes per year, more frequent arthritis, pericarditis, chest pain, abdominal pain and vomiting compared to the other two groups. A multivariate analysis was able to confirm that beside the presence of the M694V mutation and a positive family history, the country of residence (eastern Mediterranean countries) was a variable independently associated with severity of disease presentation.

14.3.1.2 Cryopyrin-Associated Periodic Syndromes (CAPS)

Levy et al. [18], evaluated genetic, demographic and clinical features in patients with CAPS from the Eurofever registry. This study was based on the collection of 136 patients with CAPS enrolled in the registry with a median onset at age 9 months, median diagnosis at age 15 years and median follow-up duration of 15 years. A heterozygous germline mutation was found in 133 patients, while no mutation was identified in three patients. Thirty-one different *NLRP3* mutations were recorded, 7 of which represented 78% of all the patients. More than half of the patients (57%) displayed a chronic disease course, while 43% of the patients were characterized by recurrent episodes. Fever, cutaneous and musculoskeletal involvement were the most prevalent manifestations. Neurological involvement was observed in 55 (40%) patients, with severe involvement in 16 (12%). Ophthalmological involvement was found in 71% of cases and sensorineural hearing loss in 42%. AA amyloidosis was detected in five patients (three carrying a R260W mutation, one V198M mutation and one A439 V mutation).

The authors also analyzed the correlation between genotype and phenotype in this cohort. They found that the T348M variant was associated with a more severe disease course, characterized by early onset, chronic course and neurological involvement. Patients with Q703K polymorphism had a milder disease. Results from this study led to a better understanding of risk factors, prognosis and genotype-phenotype associations in CAPS patients. These findings may have an important impact on therapeutic decisions and on the management of these patients.

14.3.1.3 Tumor Necrosis Factor Receptor-Associated Periodic Syndrome (TRAPS)

In 2013 Lachmann et al. [19], described genetic findings, demographic features and clinical presentation of TRAPS in patients from the Eurofever/Eurotraps international registry. This study, the largest series of patients with TRAPS, enrolled 158 patients with a median onset age of 4.3 years; median diagnosis age was 25.9 years and median follow-up duration was 15.6 years.

The most common *TNFRSF1A* variant found in 54 patients (34% of cases) was R92Q (a low-penetrant variant), followed by T50M (10%). Regarding clinical features, the disease course was of recurrent episodes in 139 (88%) patients, with episode duration of more than 14 days in 25% of cases. About half of the patients (43%) displayed attacks of 7–14 days. The most common symptoms besides fever were limb pain (85%), abdominal pain (74%), rash (63%) and eye manifestations (45%). AA amyloidosis was noted in 16 (10%) patients at a median age of 43 years. This large series of patients enabled the description of molecular and clinical features of TRAPS syndrome, with emphasis on different phenotype among low-penetrant and pathogenic variants.

14.3.1.4 Mevalonate Kinase Deficiency (MKD)

Ter Haar et al. [20], evaluated the phenotype, genotype and response to treatment of patients with MKD enrolled in the registry. Complete information on 114 patients with MKD from 31 centers in 12 countries were available. The median onset age was 0.5 years; median age at diagnosis 6.5 years and median follow-up period was 11.5 years. The disease was characterized by recurrent episodes in 99/114 (87%) patients, with most patients being well between attacks; however, 10–20% displayed constitutional symptoms between fever episodes such as malaise (61%), fatigue (61%) and weight loss (1%). Gastrointestinal symptoms were observed in almost all the patients (98%). Other main features of this series were mucocutaneous involvement in 87% of patients, lymphadenopathy in 89% and musculoskeletal symptoms in 78%.

Regarding neurological involvement, headache was reported as the most common symptom (38%). The main complication was AA amyloidosis, which developed in 5 (4%) patients, more often in those with p.V377I/p.I268T compound heterozygosity. The most common mutation emergent from the analysis was p.V377I; 84% of the patients had at least one p.V377I mutation.

Treatments used included nonsteroidal anti-inflammatory drugs (NSAIDs) given to 66 (58%) patients, which relieved symptoms in 48 (73%) of them. Corticosteroids given during attacks were completely effective in resolving inflammatory episodes in 19/49 (39%) patients with a partial response in 21/49 (43%). Biologic agents (anakinra, canakinumab and etanercept) were able to induce a complete response in many patients. Specifically, anakinra was administered to 8 patients only during attacks (5 with complete response, 3 with partial response) while 19 patients received anakinra as maintenance treatment; 13 patients obtained a complete remission, while 3 had a partial response.

14.3.1.5 Chronic Non-bacterial Osteomyelitis (CNO)

Recently, the first report on patients with CNO collected in the Eurofever registry has been reported [21]. Complete information on 486 patients was available, representing the largest series reported to date. The mean age at onset was 9.9 years (range 1–17.7 years). Adult onset was observed in 31 (6%) patients. The mean time from disease onset to final diagnosis was 1 year (range 0–15 years).

At baseline, all patients displayed musculoskeletal symptoms with 431 (89%) patients reporting bone pain, 302 (62%) arthralgia, 58 (12%) myalgia, 72 (15%) monoarthritis, 54 (11%) oligoarthritis and 10 (2%) polyarthritis. Nineteen percent of the patients had mucocutaneous manifestations (5% acne, 5% palmo-plantar pustulosis, 4% psoriasis, 3% papulo-pustular lesions, 2% urticarial rash), 8% displayed gastrointestinal symptoms. Among imaging techniques, MRI was performed at baseline in 426 (88%) patients, revealing a mean number of 4.1 lesions. Overall, 37% of patients displayed metaphyseal lesions, 23% epiphyseal, 15% diaphyseal, 25% pelvic, 23% vertebral, 19% clavicle, 15% tarsal, 10% thoracic, 3% carpal and 3% cranial. Bone biopsy was performed in 281 (58%) patients.

Three hundred and sixty-one (74%) patients were treated with NSAIDs, 112 (23%) with corticosteroids, 61 (13%) with bisphosphonates, 58 (12%) with methotrexate, 47 (10%) with sulfasalazine, 26 (5%) with anti-tumor necrosis factor and 4 (1%) with anakinra, with a variable response to all these treatments. However NSAIDs, bisphosphonates and sulfasalazine displayed the highest rate of complete or partial response.

The study showed that CNO often presents during early adolescence and the range of clinical manifestations and response to treatment is heterogeneous.

14.3.2 Genotype-Phenotype Correlations

The large number of polymorphisms and common variants in hereditary recurrent fever (HRF) genes makes it difficult to find associations between genotype and phenotype. The Infevers database collects all these variants and provides a brief description of clinical manifestations of the first patient reported for each mutation. With the aim of improving knowledge of genotype-phenotype correlations, Papa et al. [22], in a recent study, developed an open web-based registry of genotype-phenotype associations derived from all the patients with HRF enrolled and validated in the Eurofever registry.

In this study genotype-phenotype associations observed in all patients with HRF were retrospectively evaluated, analyzing all the mutations for CAPS and TRAPS (autosomal dominant diseases), while all homozygous and heterozygous combinations were described for FMF and MKD (autosomal recessive disorders).

The authors created tables for each variant/combination of mutations for all the HRF diseases with evaluation of mean age of onset, type of disease course (recurrent/episodic or chronic), prevalent clinical manifestations, less common clinical features, complications and response to treatment. A total of 751 patients (346 FMF, 158 TRAPS, 133 CAPS and 114 MKD) included in the Eurofever registry were enrolled in this study; 149 gene variants were reported: 46 of *TNFRSF1A* and 27 of *NLRP3*, 48 of *MVK* and 28 *MEFV*.

The objective of this study was to provide a practical tool for clinicians and geneticists with the aim to reach a correct interpretation of genetic analysis. This tool is complementary to the Infevers database and will be available at the Eurofever and Infevers websites.

14.3.3 Development of New Classification Criteria

As mentioned previously, one of the main purposes of the Eurofever registry was to generate evidence-based diagnostic and classification criteria. Formal diagnostic criteria have been developed for some inherited periodic fevers (FMF and CAPS), based on the main clinical manifestations associated with the specific disease within the context of limited populations (see Chap. 11). Thus, there is a question of their generalization to other populations [23, 24].

Therefore, the large Eurofever registry was used to test the accuracy of different diagnostic criteria currently in use for FMF and compared them with the performance of previous criteria for the diagnosis of FMF.

The performances of the Sohar Tel-Hashomer, Livneh Tel-Hashomer, and Yalcinkaya FMF criteria were assessed in pediatric patients with FMF compared to other periodic fevers, including

MKD, TRAPS, CAPS, PFAPA and undefined periodic fever from the same registry [25].

The FMF group included 339 patients whereas the control group consisted of 377 patients (53 TRAPS, 45 MKD, 32 CAPS, 160 PFAPA and 87 undefined periodic fevers). Patients with FMF were correctly diagnosed using the Yalcinkaya criteria with a sensitivity rate of 87.4% and a specificity rate of 40.7%. On the other hand, the Sohar Tel Hashomer and Livneh Tel-Hashomer criteria displayed a sensitivity of 45.0 and 77.3%, respectively. Both latter criteria displayed a better specificity than the Yalcinkaya criteria: 97.2 and 41.1% for the Sohar Tel Hashomer and Livneh Tel-Hashomer criteria, respectively. The overall accuracy for the Yalcinkaya criteria was 65 and 69.6% (using 2 and 3 criteria), respectively. Ethnicity and residence had no effect on the performance of the Yalcinkaya criteria.

Thus, the pediatric Yalcinkaya criteria yielded a better sensitivity than the other criteria in this international cohort of patients. However, the specificity was lower than the previously suggested adult criteria.

In 2015 Federici et al. [26], developed and validated a new set of clinical criteria for the classification of patients affected by the four main autoinflammatory recurrent fever syndromes. Patients with HRF diseases (FMF, MKD, TRAPS and CAPS) enrolled in the Eurofever Registry until March 2013 were evaluated. Patients with PFAPA syndrome were used as negative controls. The 'gold-standard' for diagnosis of the monogenic diseases was based on the presence of a confirmatory genetic analysis [27, 28]. Patients with non-confirmatory genetic analysis, such as low-penetrance mutations, were excluded from the study. Patients with PFAPA were classified according to current diagnostic criteria [29].

Twelve hundred and fifteen patients enrolled in the registry were analyzed: 518 were selected as the 'gold standard group' (291 FMF, 74 MKD, 86 TRAPS and 67 CAPS) and 119 patients with PFAPA were evaluated as the negative controls.

The authors randomly divided the 'gold standard group' into two subgroups. Univariate and multivariate analyses were performed in the first training set subgroup (412 patients) to identify clinical variables which strongly correlated with each disease. The second validation subgroup (305 patients) was used to assess the performance of the 4 scores originated from statistical analysis in an independent group of patients. All criteria displayed a high sensitivity and specificity (Table 14.2).

This study facilitated the development of a validated evidence-based tool that may be useful either as an indication for performing genetic testing or for clinical classification of patients with suspected autoinflammatory periodic fevers.

14.3.4 Validation of Disease Activity and Damage Scores

As previously stated, the main outcome measure in therapeutic trials is the disease activity. However, validated indices of disease activity were lacking before the creation of the registry. Development and validation of these activity parameters represented another aim of the Eurofever project.

The first index of activity (Autoinflammatory Diseases Activity Index, AIDAI) was proposed in 2013 for patients affected by the four major HRF diseases: FMF, MKD, TRAPS and CAPS (see Chap. 13) [30].

This study was initiated in November 2010 by an international collaboration of eight centers belonging to the PRINTO/Eurofever network. They established the content of a disease activity tool for HRFs and started with the enrollment of consecutive patients attending participating centers. Each patient had to complete a 1-month prospective diary before a scheduled clinical appointment during which the physician assessed the disease activity by a questionnaire. Data coming from the various centers were centrally collected in the Eurofever database and then eight international experts in autoinflammatory diseases evaluated the patients' disease activity by a blinded web evaluation. The second step of the score validation was a consensus conference where the experts evaluated the level of disease activity. The last step of the study was the calculation of the score to discriminate active from inactive disease by statistical analysis.

Table 14.2 The Eurofever clinical diagnostic/classification criteria [26]

FMF		MKD		CAPS		TRAPS	
Presence[a]	Score	Presence[a]	Score	Presence[a]	Score	Presence[a]	Score
Duration of episodes <2 days	9	Age at onset <2 years	10	Urticarial rash	25	Periorbital edema	21
Chest pain	13	Aphthous stomatitis	11	Neurosensorial hearing loss	25	Duration of episodes >6 days	19
Abdominal pain	9	Generalized enlargement of lymph nodes OR splenomegaly	8	Conjunctivitis	10	Migratory rash[c]	18
Eastern Mediterranean[b] ethnicity	22	Painful lymph nodes	13			Myalgia	6
North Mediterranean[b] ethnicity	7	Diarrhea (sometimes/ often)	20			Relatives affected	7
		Diarrhea (always)	37				
Absence		**Absence**		**Absence**		**Absence**	
Aphthous stomatitis	9	Chest pain	11	Exudative pharyngitis	25	Vomiting	14
Urticarial rash	15			Abdominal pain	15	Aphthous stomatitis	15
Enlarged cervical lymph nodes	10						
Duration of episodes >6 days	13						
Cut-off	**≥60**	**Cut-off**	**≥42**	**Cut-off**	**≥52**	**Cut-off**	**≥43**

FMF familial Mediterranean fever; *MKD* mevalonate kinase deficiency; *CAPS* cryopyrin-associated periodic syndrome; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome

[a]The clinical features should be related to the typical fever episodes (i.e. exclusion of intercurrent infection or other co-morbidities)

[b]Eastern Mediterranean: Turkish, Armenian, non-Ashkenazi Jewish, Arab; North Mediterranean: Italian, Spanish, Greek

[c]Centrifugal migratory, erythematous patches most typically overlying a local area of myalgia, usually at limbs or trunk

One hundred six patients were enrolled (42 FMF, 39 CAPS, 14 TRAPS and 11 MKD). During the second step of the validation process, consensus was achieved for 98/106 (92%) cases (39 FMF, 35 CAPS, 14 TRAPS and 10 MKD); 26 patients were declared to have inactive disease and 72 had active disease, with different grades of activity (low-mild-severe). Statistical analysis performed with receiver operating characteristic (ROC) curve revealed that an AIDAI cut-off score ≥ 9 discriminated active from inactive disease with the best accuracy (sensitivity of 89% and specificity of 92%).

After this first study, a damage index score (Autoinflammatory Disease Damage Index, ADDI) was developed in 2016 by Ter Haar et al. [31], for FMF, CAPS, TRAPS and MKD (see Chap. 13). The top 40 enrollers of patients in the Eurofever registry and 9 experts from the Americas participated in multiple rounds of online surveys to select items and definitions of damage. Also, 22 patients or parents of patients were invited to participate in an online survey. Authors used the 1000minds software to assess the scoring system of ADDI with the correct weight to each damage item. The online surveys were completed by >80% of experts, who suggested 16 new damage items. The next step of the index assessment was a consensus meeting, which was attended by 31 experts. During this meeting, items that didn't reach consensus in the online survey were discussed. At the end of this

process, the preliminary ADDI score contained 18 items, classified in the following categories: reproductive, renal/amyloidosis, developmental, serosal, neurological, ears, ocular and musculoskeletal damage, each with a different weight. The highest weight was attributed to the renal and neurological categories. Authors highlighted the strength of this index score, due to the number of experts that attended the survey and to the involvement of patients or parent of patients.

14.3.5 Response to Treatment

In 2013 the first report on the response to treatment of autoinflammatory diseases from the registry was reported [32]. The following diseases were included: FMF, CAPS, TRAPS, MKD and PFAPA. Cases were independently validated by experts for each disease. Response to treatment was considered as: (1) complete (absence of clinical manifestations and normalization of acute phase reactants; (2) incomplete (amelioration of the clinical picture but persistence of some clinical manifestation and/or elevation of acute phase reactants); (3) no response. Continuation and discontinuation of medications was also registered. The study was performed 22 months from the beginning of enrollment and complete information on 496 validated patients was available. Data from the registry confirmed that colchicine is the treatment of choice for FMF, although as many as 37% of enrolled patients with FMF displayed an incomplete response. As expected, IL-1 blockade was extremely effective in CAPS. Anakinra was used in 61 patients with a complete response in 39 (64%) and a partial response in 21 (34%). Canakinumab induced complete remission in 39 patients (75%) and partial remission in 13 (25%). On demand corticosteroids represented a valid therapeutic strategy to abort attacks of fever and was used in 90% of patients with PFAPA, 43% of patients with TRAPS and 24% of patients with MKD.

Biologics were used in patients with TRAPS and MKD not responding adequately to on demand corticosteroid approach. In TRAPS, etanercept was beneficial in 32 of the 37 (86%) treated patients, although only 11 (30%) experienced a complete response. Conversely, anakinra was associated with a complete response in 26 of 33 patients (79%) with a partial response in five others. This observation raised for the first time the possible primary role of IL-1 blockade in the management of this condition.

The pattern of response in MKD was much less homogeneous. Colchicine was used in 17 patients with a lack of response in the majority (65%). Anakinra was effective in 24 (89%) of 27 patients, inducing complete remission in 6 (22%). Three of four patients treated with canakinumab had a complete response. Etanercept was effective in 11 (65%) of 17 treated patients, but with only one complete response.

14.4 Conclusions

During the last 8 years, the international effort to build a common registry on autoinflammatory diseases led to a considerable accumulation of new information, concerning the modality of presentation, disease course, genotype-phenotype correlations and response to treatment in rare inflammatory conditions. New tools for every-day practice have been also developed with an evidence-based approach coming from real patients enrolled in the registry. Studies on other rare and newly recognized conditions (such as DADA2 and interferonopathies) are currently ongoing. New evidence-based classification criteria based on genetic and clinical data are currently under development. Long-term studies will help understand the efficacy and safety of different treatments used in these rare conditions.

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Systemic Amyloidosis

15

Tamer Rezk and Philip N. Hawkins

Abstract

Amyloidosis describes a group of rare diseases caused by abnormal fibrillar protein aggregation within the interstitium of tissues and organs throughout the body. This chapter focuses upon the pathogenesis, epidemiology, diagnosis and management of these heterogeneous disorders. AA amyloidosis is one of the most feared complications of autoinflammatory syndromes but it is becoming increasingly rare with the advent of effective anti-inflammatory therapy. The most common of the systemic amyloidoses are immunoglobulin light chain (AL) type and wildtype transthyretin (wtATTR) amyloidosis, the latter a probably much underdiagnosed cause of heart failure in the elderly. Precise diagnosis, confirmation of amyloid type, evaluation of amyloidotic organ involvement and associated underlying disorders are imperative for optimal patient care. Although histology has long been the diagnostic gold standard, new technologies including mass spectrometry of tiny tissue samples and highly specific imaging comprising; I^{123} labelled serum amyloid P (SAP) component scintigraphy, ^{99m}Tc-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy and cardiac MRI (CMR), have lately transformed the evaluation of patients. A multidisciplinary approach to management is key. Treatment comprises support of failing amyloidotic organs, measures to reduce production of the respective amyloid fibril protein such as suppression of serum amyloid A (SAA) in systemic AA amyloidosis, and recently, novel therapies aimed at enhancing clearing of existing amyloid deposits.

Keywords

Amyloidosis · Autoinflammatory · Systemic Suppression · Nephrotic syndrome · Heart failure · Chemotherapy · Neuropathy

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Abbreviations

ACE	Angiotensin-converting enzyme
AEF	Amyloid-enhancing factor
ARB	Angiotensin receptor blockers
ASCT	Autologous stem cell transplantation
BJP	Bence Jones proteinuria

P. J. Hashkes et al. (eds.), *Textbook of Autoinflammation*, https://doi.org/10.1007/978-3-319-98605-0_15

CAPS	Cryopyrin-associated autoinflammatory syndrome
CINCA	Chronic inflammatory neurological cutaneous articular
CKD	Chronic kidney disease
CMR	Cardiac MRI
CPHPC	((R)-1-(6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexa-noyl) pyrrolidine-2 carboxylic acid), a novel bis (D-proline)
DPD	^{99m}Tc-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD)
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
ESRF	End stage renal failure
FAP	Familial amyloid polyneuropathy
FCAS	Familial cold autoinflammatory syndrome
FLC	Free light chains
FMF	Familial Mediterranean fever
GAG	Glycosaminoglycans
IHC	Immunohistochemical
IVSD	Interventricular septal diameter
MGUS	Monoclonal gammopathy of unknown significance
MKD	Mevalonate kinase deficiency
MM	Multiple myeloma
MWS	Muckle-Wells syndrome
NAC	National Amyloidosis Centre
NOMID	Neonatal onset multisystem inflammatory disease
NT-proBNP	N terminal pro brain natriuretic peptide
SAA	Serum amyloid A
SAP	Serum amyloid P
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic syndrome
TTR	Transthyretin
UK	United Kingdom
USA	United States of America

Key Points

- **Systemic amyloidosis is a heterogeneous multisystem disease caused by extracellular deposition of protein in a specific highly ordered fibrillar form**
- **The natural history of systemic amyloidosis is invariably progressive, usually with a fatal outcome**
- **Optimal characterisation of systemic amyloidosis requires clinical assessment, serum and urine biochemistry and multidisciplinary laboratory investigations including histology, proteomics, genetic testing and various imaging modalities, notably including cardiac MRI (CMR) for assessment of cardiac amyloidosis**
- **The presence of a monoclonal gammopathy or a chronic inflammatory disorder may be gravely misleading in suggesting systemic amyloidosis is of AL or reactive AA type, respectively, since these conditions are common in the general population and may be incidental to the type of amyloid**
- **Treatment of systemic amyloidosis includes best supportive care, maximal possible reduction of the supply of the amyloid fibril precursor protein such as suppression of serum amyloid A (SAA) in AA amyloidosis; novel therapies aimed at removing existing amyloid deposits are in development**

15.1 Introduction

- **Amyloidosis is a heterogeneous disease caused by extracellular deposition of protein**
- **The most common circumstance in which amyloid deposition occurs is the presence of an abnormal protein such as monoclonal immunoglobulin light chains in AL amyloidosis**
- **Untreated systemic amyloidosis is invariably progressive, typically leading to organ failure and death**

Amyloidosis comprises a group of rare diseases caused by the extracellular accumulation of amyloid, a highly ordered, insoluble and remarkably stable fibrillar protein material. Amyloid fibrils are derived from a diverse collection of soluble precursor proteins that have a specific propensity to misfold and aggregate in a highly abnormal cross β sheet conformation [1]. Amyloid deposits cause disease by accumulating at a rate that exceeds the body's capacity to clear them, progressively disrupting the structure and function of tissues and organs throughout the body [2]. Amyloid type is classified according to the respective fibril protein, of which more than 30 have been identified in vivo (Table 15.1) [3]. Amyloid deposits have a pathognomonic histologic appearance comprising apple-green dichroism (birefringence) in tissue sections that have been stained with Congo red dye and visualized under cross-polarized light (Fig. 15.1). On electron microscopy, amyloid fibrils appear as rigid non-branching fibrils with a diameter of ~10 nm [4]. Amyloid deposition is highly heterogeneous, ranging from small incidental deposits, through localised accumulations that can cause disease through an infiltrative or mass effect, to systemic (generalized) forms of amyloidosis that can affect almost any organ in the body with fatal consequences. Precise diagnosis, typing and evaluation of the organ distribution, severity and adverse effects of amyloid are imperative to ensure best clinical care.

15.2 Fibril Formation and Amyloid Proteins

Amyloid fibrillogenesis remains poorly understood. Under certain laboratory conditions all sorts of proteins can be induced to misfold and aggregate, but relatively few have the specific properties to form genuine amyloid fibrils *in vivo*. Despite the heterogeneity of those proteins that form amyloid in vivo, the resultant fibrils have remarkably similar morphological ultrastructure and histological properties. The common core structure is one of anti-parallel β-strands that form sheets (Fig. 15.2) [5, 6]. These β-sheets run parallel to the axis of the protofilament with their component β strands perpendicular to the fibril axis, which appear on electron microscopy as non-branching structures 7–10 nm in diameter [7].

All amyloid deposits additionally contain certain non-fibrillary constituents including glycosaminoglycans (GAGs), sulphated proteoglycans, heparin sulphate, apolipoproteins E and A4, type IV collagen and serum amyloid P component (SAP) [8]. GAGs are located primarily on the cell surface in the extracellular matrix and although universal to all amyloid deposits, their role remains unclear. SAP is a normal plasma protein that binds in a reversible calcium dependant manner to a ligand present on all amyloid fibrils, its presence in vivo being an essential amyloid defining characteristic. It is a member of the pentraxin group of plasma proteins that is relatively resistant to proteolysis, and for which there is evidence it both promotes amyloid fibril formation and inhibits their degradation by phagocytic cells and proteolytic enzymes. In vivo, circulating SAP exists in a dynamic equilibrium with SAP bound to amyloid fibrils, forming the basis for diagnostic radiolabelled SAP scintigraphy. SAP knock-out mice are relatively resistant to induction of experimentally induced amyloidosis [9].

There are essentially three circumstances in which amyloid deposition occurs. The most common is the presence of an abnormal protein with pronounced amyloidogenic properties such as the monoclonal immunoglobulin light chains that form AL amyloid and genetic variants of transthyretin (TTR), fibrinogen A-a chain, apolipoprotein AI, apolipoprotein A2, apolipoprotein C3, apolipoprotein C2, gelsolin and lysozyme in hereditary amyloidosis. A second situation is the presence of an abnormally high concentration of a 'normal' protein, examples of which are elevated serum amyloid A protein (SAA) in chronic inflammatory disorders predisposing to AA amyloidosis, and elevated β2M microglobulin in dialysis-related amyloidosis. Third, amyloid deposition can occur in advanced age in the presence of a 'normal' protein that is present in normal abundance, such as the case with wildtype transthyretin in non-hereditary ATTR amyloidosis.

Table 15.1 Classification of systemic amyloidosis by precursor protein

Fibril protein	Precursor protein	Systemic (S) and/or localized (L)	Acquired (A) or hereditary (H)	Target organs
AL	Immunoglobulin light chain	S, L	A	All organs except CNS
AH	Immunoglobulin heavy chain	S, L	A	All organs except CNS
AA	(Apo) Serum amyloid A	S	A	All organs except CNS
ATTR	Transthyretin, wild type	S	A	Heart mainly in males, ligaments, tenosynovium
	Transthyretin, variants	S	H	PNS, ANS, heart, eye, leptomeningeal
Aβ2M	β2-Microglobulin, wild type	L	A	Musculoskeletal system
	β2-Microglobulin, variant	S	H	ANS
AApoAI	Apolipoprotein A I, variants	S	H	Heart, liver, kidney, PNS, testis, larynx (C-terminal variants), skin (C-terminal variants)
AApoAII	Apolipoprotein A II, variants	S	H	Kidney
AApoAIV	Apolipoprotein A IV, wild type	S	A	Kidney medulla and systemic
AGel	Gelsolin, variants	S	H	PNS, cornea
ALys	Lysozyme, variants	S	H	Kidney
ALECT2	Leukocyte chemotactic factor-2	S	A	Kidney, primarily
AFib	Fibrinogen α, variants	S	H	Kidney, primarily
ACys	Cystatin C, variants	S	H	PNS, skin
ABri	ABriPP, variants	S	H	CNS
ADan	ADanPP, variants	L	H	CNS
Aβ	Aβ protein precursor, wild type	L	A	CNS
	Aβ protein precursor, variant	L	H	CNS
APrP	Prion protein, wild type	L	A	CJD, fatal insomnia
	Prion protein variants	L	H	CJD, GSS syndrome, fatal insomnia
ACal	(Pro)calcitonin	L	A	C-cell thyroid tumors
AIAPP	Islet amyloid polypeptide	L	A	Islets of Langerhans, insulinomas
AANF	Atrial natriuretic factor	L	A	Cardiac atria
APro	Prolactin	L	A	Pituitary prolactinomas, aging pituitary
AIns	Insulin	L	A	Iatrogenic, local injection
ASPC‡	Lung surfactant protein C	L	A	Lung
AGal7	Galectin 7	L	A	Skin
ACor	Corneodesmosin	L	A	Cornified epithelia, hair follicles
AMed	Lactadherin	L	A	Senile, aortic media
Aker	Kerato-epithelin	L	A	Cornea, hereditary
ALac	Lactoferrin	L	A	Cornea
AOAAP	Odontogenic ameloblast-associated protein	L	A	Odontogenic tumors
ASem1	Semenogelin 1	L	A	Vesicula seminalis
AEnf	Enfuvirtide	L	A	Iatrogenic

CNS central nervous system, *PNS* peripheral nervous system, *ANS* autonomic nervous system, *CJD* Creutzfeldt-Jakob disease, *GSS* Gerstmann–Sträussler–Scheinker

‡Lung surfactant protein C

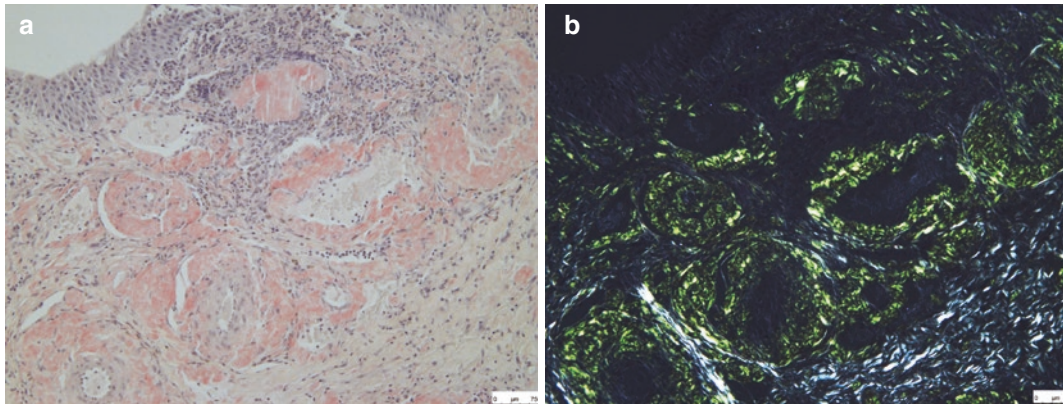

Fig. 15.1 (**a**) Congo red staining of AA amyloid deposition in a biopsy of the bladder. (**b**) Apple green birefringence when viewed under cross polarised light

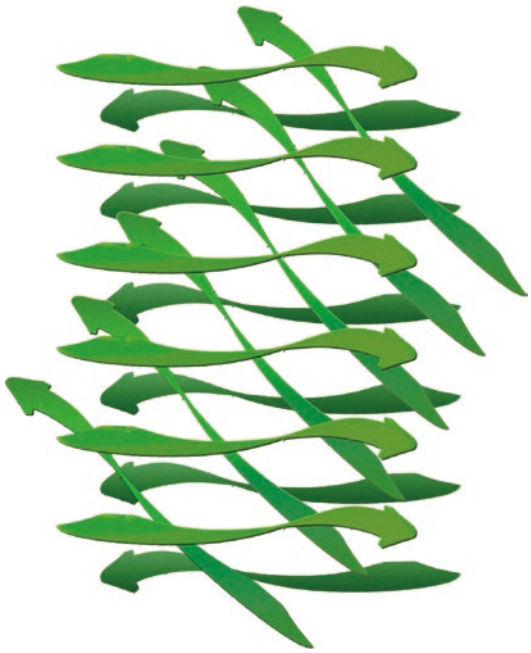

Fig. 15.2 Image depicting anti-parallel β-strands that form sheets

15.3 Pathogenesis of Amyloidosis Accumulation and Degradation

Amyloid deposition causes organ dysfunction due to physical replacement of parenchymal tissue and cellular injury. Pre-fibrillar oligomers may also exert direct toxicity; the strongest evidence for this is derived from observations in cardiac AL amyloid although it has also been postulated in ATTR amyloidosis [10]. Organ damage is ultimately due to a combination of mechanisms that depend on both the type of amyloidosis and the organ in question.

The factors that govern the pattern of organ involvement in different types of amyloidosis, or indeed different patients with the exact same type, remain poorly understood. For example, there can be major phenotypic differences in close family members with the same type of hereditary amyloidosis.

A potential amyloid precursor protein can be present for very prolonged periods without formation of amyloid but, once started, it will continue indefinitely as long as the supply of the precursor protein persists, and at a rate that depends on the abundance of the latter. The notion of 'amyloid-enhancing factor' (AEF) initiating amyloid formation was established many decades ago. In studies of experimentally induced AA amyloid in mice, microscopic amounts of ex vivo amyloidotic material parenterally administered into mice with elevated SAA concentrations were found to trigger substantial AA amyloid deposition within only a few hours [11]. It is now clear that this extraordinarily potent phenomenon is mirrored in patients. Once amyloid formation has begun, it will be forever propagated so long as there

remains a supply of the amyloid precursor protein in question. It is likely that the initiating event is a stochastic protein misfolding/aggregation phenomenon that may only occur after decades of an amyloidogenic protein having been present. The extremely rapid (within weeks) recurrence of nephrotic syndrome following relapse of inflammatory activity in patients with previous apparently resolved AA amyloidosis is an example underlining the rapidity with which amyloid can accumulate [12]. Another example is the rapid progression of hereditary cardiac ATTR amyloidosis following liver transplantation performed to remove the source of the genetic TTR variant, but which is now known to result in enhanced deposition of wildtype TTR on a template of amyloid derived from variant TTR [13].

Untreated systemic amyloidosis is almost always progressive, typically leading to organ failure and death within months to a few years. However, amyloid deposits are constantly being cleared away to a minor and variable extent, such that substantial suppression of the fibril precursor protein supply can gradually result in net regression of the amyloid burden [14]. This slow natural and very inefficient clearance of amyloid is thought to be mediated by macrophages, which can occasionally be identified in amyloidotic tissue, sometimes in sufficient numbers to form multinucleate giant cells that surround and engulf the amyloid material. Depletion of macrophages with liposomal clodronate inhibits amyloid regression in mice [15]. Serial SAP scintigraphy in patients whose amyloid precursor protein production has been halted indicates that the rate of amyloid clearance, i.e. regression, varies widely between individuals and between different organs; typically, clearance of amyloid is substantially slower in the kidneys than the liver, and very slow in the heart.

It is striking, given the highly abnormal and acquired nature of amyloid, that this material does not evoke a significant host response. Pepys hypothesised that the coating of amyloid by SAP, a normal plasma protein, may act as an anti-opsonin, which is supported by inhibited amyloidogenesis in SAP knockout mice. CPHPC, ((R)-1-(6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexa-noyl) pyrrolidine-2 carboxylic acid), a novel bis (D-proline) drug was developed with the aim of removing SAP from amyloid and thus potentially promote removal of amyloid via macrophage infiltration of amyloid deposits.

15.4 Epidemiology

Amyloidosis is a rare condition for which there is a paucity of epidemiological data. It has been estimated to cause 0.5–1.0 deaths per 1000 in the United Kingdom (UK) [16]. The most common type diagnosed in the UK is systemic AL amyloidosis, studies suggesting an incidence of 5.1–12.8 per million person-years. Currently about 800 new patients with various types of amyloidosis are evaluated each year at the National Amyloidosis Centre (NAC). Previous work in our centre in 2008 estimated a minimum incidence of systemic amyloidosis in England of 0.4/100,000 of the population. The incidence peaked at 60–79 years with systemic AL amyloidosis being the most common type, with a minimum incidence of 0.3/100,000 [17].

Non-hereditary, i.e. wildtype, transthyretin amyloidosis, which predominantly causes a cardiomyopathy in older individuals and was previously known as senile systemic/cardiac amyloidosis, is lately being diagnosed much more frequently than hitherto. This reflects the remarkable diagnostic value of cardiac MRI (CMR) and repurposing of bone scintigraphy for this indication. The true prevalence of cardiac ATTR amyloidosis remains unknown, but may be much higher than is currently apparent since post-mortem studies have long demonstrated that some ATTR deposits are present in the hearts of up to 20% of people over the age of 80 years [18]. Diagnosis of wildtype ATTR amyloidosis at the NAC has risen exponentially in recent years and currently exceeds 200 patients per year.

15.5 Types of Amyloidosis

- **AL amyloidosis is the most common form of systemic amyloidosis in the western world**
- **Wildtype transthyretin cardiac amyloidosis (previously known as senile systemic/cardiac amyloidosis) is an increasingly recognised cause of heart failure with preserved ejection fraction (HFPEF)**
- **AA amyloidosis, once the most common form of systemic amyloidosis, is now rare with the advent of improved treatments for chronic inflammatory disorders**
- **Systemic amyloidosis has a varying prognosis depending on both the type and pattern of organ involvement, with survival ranging from months to many years**

15.5.1 Systemic AA Amyloidosis

Reactive systemic (AA) amyloidosis, in which the fibrils are composed of AA protein derived from the acute phase protein SAA, occurs as a rare complication of many chronic inflammatory disorders. The AA amyloid precursor protein is the N terminal fragment of the acute phase reactant SAA, an apolipoprotein constituent of high-density lipoprotein. SAA is synthesized by hepatocytes and its concentration may rise 1000-fold from healthy values of less than 3 mg/L in response to inflammation. Gene transcription of SAA is regulated by cytokines, in particular interleukin (IL)-1 and IL-6.

The lifetime incidence of AA amyloidosis in patients with chronic inflammatory conditions is less than 1–5% [19]. In Western Europe and the United States of America (USA) the most frequent predisposing conditions are idiopathic rheumatic diseases, notably rheumatoid arthritis and juvenile idiopathic arthritis (Table 15.2). AA amyloidosis has become increasingly rare, reflecting improved treatment of chronic inflammatory disorders, and for reasons that are not clear, the incidence is lower in the United States than in Europe. Amyloidosis is exceptionally rare in systemic lupus erythematosus, related connective tissue diseases, and in ulcerative colitis in which there is a blunted acute phase response of SAA. Longstanding, though not necessarily constant elevation of SAA, is a prerequisite to the development of AA amyloidosis. Tuberculosis and leprosy are important causes of AA amyloidosis where these infections remain endemic. Chronic osteomyelitis, bronchiectasis, chronically infected burns, and decubitus ulcers are other well-recognized associations (Table 15.2). Hodgkin disease and renal cell carcinoma, which often cause an acute phase response, are the malignancies most commonly associated with systemic AA amyloidosis.

Intriguingly, at least 10% of patients with AA amyloidosis do not have a clinically obvious

Table 15.2 Conditions associated with reactive systemic amyloid AA amyloidosis

Chronic inflammatory disorders
Rheumatoid arthritis
Juvenile idiopathic arthritis
Ankylosing spondylitis
Psoriasis and psoriatic arthropathy
Reactive arthritis
Adult onset Still disease
Behçet syndrome
Crohn disease
Whipple disease
Hereditary autoinflammatory/periodic fever syndromes
Familial Mediterranean fever
Tumor necrosis factor receptor-associated periodic syndrome
Cryopyrin-associated periodic syndromes
Mevalonate kinase deficiency
Chronic microbial infections
Leprosy
Tuberculosis
Bronchiectasis
Decubitus ulcers
Chronic pyelonephritis in paraplegics
Chronic infected burns
Chronic osteomyelitis
Malignant neoplasms
Hodgkin disease
Renal cell carcinoma
Carcinomas of gut, lung, urogenital tract
Basal cell carcinoma
Hairy cell leukemia
Unknown etiology

chronic inflammatory disease, and may erroneously be assumed to have AL amyloidosis. The most common identifiable diseases found in our experience in such cases are inherited autoinflammatory syndromes and cytokine-secreting Castleman disease tumors of the solitary plasma cell type, located either in the mediastinum or the gut mesentery. However, in the majority of these challenging patients the precise nature of the causative inflammatory disorder cannot be determined.

15.5.1.1 Autoinflammatory Diseases and Amyloidosis

The hereditary periodic fever/autoinflammatory syndromes are a well-described cause of AA amyloidosis, among which four are most commonly implicated. These are familial Mediterranean fever (FMF), Tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), the cryopyrin-associated autoinflammatory syndrome (CAPS) and to a lesser extent mevalonate kinase deficiency (MKD) (see Chaps. 16–19). FMF is the most common of these diseases. It is characterised by recurrent self-limiting attacks of fever, serositis and sometimes arthritis or rash [20]. There is a clear ethnic preponderance, with FMF being prevalent in the Eastern Mediterranean, where it is the most common monogenic autoinflammatory disease. CAPS comprises a continuous spectrum of three disorders, familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease (NOMID), also known as chronic inflammatory neurological cutaneous articular syndrome (CINCA).

It is important to recognise that genetic associations with the development of amyloidosis are weak and not useful predictors of risk in individual patients. The remarkably high risk of AA amyloidosis in systemic autoinflammatory diseases reflects persistent and uncontrolled inflammation. Although small series have identified increased risks with specific genotypes in FMF and perhaps with mutations affecting cysteine residues in TRAPS (see below) there are no guaranteed 'safe' mutations and all patients should be treated to completely suppress chronic inflammatory disease and SAA production. The same is true for the data on SAA polymorphisms and risk of the development of AA amyloidosis.

Serum Amyloid A (SAA) Polymorphisms

While persistent and sustained inflammation is the key risk factor for the development of AA amyloidosis in autoinflammatory diseases, studies have shown (predominantly in FMF) the contribution of serum amyloid A1 (SAA1) polymorphisms, differing gene mutations and birthplace. Most of these studies are small and subject to confounding influences such as increased investigation of patients who present with AA amyloidosis and more recently, a significant impact from the availability of effective long-term prophylactic treatment.

SAA1 has 5 polymorphic coding alleles, SAA1.1, SAA1.2, SAA1.3, SAA1.4 and SAA1.5 [21]. The gene products of these coding alleles vary by only a few amino acids at different positions of the mature SAA1 protein. Despite these minor differences, the allelic variants of SAA1 have shown differences in both in vitro assays as well as correlation with various diseases [22]. The importance of polymorphisms in SAA1 differs between populations. In Japan homozygosity for SAA1.3 has been known to increase the risk of AA amyloid in rheumatoid arthritis for many years [23]. In other populations with a different distribution of polymorphism, homozygosity for SAA1.1 is a risk factor. This is the case in Turkish patients with FMF who have an increased incidence of SAA1.1 homozygosity in FMF patients with amyloidosis (56%) compared to FMF patients without amyloidosis (31%), a 2.5 fold increased risk [24].

Risk Factors for Amyloidosis in Familial Mediterranean Fever (FMF)

The *MEFV* M694V variant has been associated with the most severe form of FMF and a higher risk of AA amyloidosis [25]. In 170 Armenian patients with FMF; 60% had a concurrent diagnosis of AA amyloidosis. The most common genotype in this cohort was M694V/M694V homozygosity which was present in 36% of

patients and was associated with an increased risk of AA amyloidosis when compared to M694V heterozygosity [26].

While M694V was previously thought to be the key risk factor for AA amyloidosis; in a large online study of 35 centres in 14 countries, 2482 cases of FMF were identified of whom 260 developed renal amyloidosis. Interestingly, country of recruitment rather than the *MEFV* genotype was the leading risk factor for the manifestation of renal amyloidosis which may indicate a potential environmental origin of amyloidosis susceptibility [27].

Risk Factors for Amyloidosis in the Tumor Necrosis Factor Receptor-Associated Periodic Syndrome (TRAPS)

In TRAPS, the international Eurofever/Eurotraps registry identified 158 patients in whom the most common variant of *TNFRSF1SA* was R92Q (34% of cases) and T50M (10%) with disrupted cysteine residues in 27% of cases. AA amyloidosis developed in 16 (10%) patients at a median age of 43 years. This group included 7 patients with cysteine variants (44%), two with T50M (13%) and none with R92Q. Patients who developed AA amyloidosis had significantly longer disease duration than those who did not [28].

Risk Factors for Amyloidosis in the Cryopyrin-Associated Periodic Syndrome (CAPS)

Retrospective literature review of all cases of CAPS from the French network for rare diseases identified 67 patients diagnosed with CAPS who developed AA amyloidosis. While AA amyloidosis was seen in all CAPS phenotypes it appeared to be more common in MWS [29]. Due to the rarity and severity of disease with high mortality rates there is less data on amyloidosis in patients with NOMID.

15.5.1.2 Clinical Features

AA amyloid involves the viscera, but may be widely distributed without causing symptoms. It predominantly affects the kidneys with more than 95% of patients presenting with proteinuria and around 10% having already reached end-stage renal failure (ESRF) at diagnosis [12].

The predominant presentation is nephrotic syndrome with non-selective proteinuria from glomerular deposition of amyloid and or chronic kidney disease (CKD). Splenic involvement is evident on SAP scintigraphy in almost all cases and while deposits commonly occur in the adrenal gland and gastrointestinal tract, this is usually without associated organ dysfunction. Liver involvement in AA amyloidosis is a feature of advanced disease and confers a poor prognosis [30]. Cardiac amyloidosis and amyloid-related neuropathy are rare manifestations of AA amyloidosis and are seen only in advanced cases.

15.5.2 Systemic AL Amyloidosis

This is the most common form of amyloidosis in the developed world and is associated with dyscrasias of cells within the B-lymphocyte lineage, including multiple myeloma (MM), malignant lymphomas and macroglobulinemia. Most cases develop in the context of what would otherwise be a low grade monoclonal gammopathy of unknown significance (MGUS). The age adjusted incidence in the USA is 8.9 per million person-years [31]. Amyloidosis occurs in up to 10% of cases of MM and in a lower proportion of other malignant B-cell disorders. Approximately 2% of patients with an MGUS eventually develop AL amyloidosis [31]. The fibrils are formed from the N terminal domain of monoclonal lambda (more common) or kappa immunoglobulin light chains, and consist of the whole or part of the variable (VL) domain.

A monoclonal immunoglobulin can be detected in the serum or urine by immunofixation electrophoresis in 65% and 86% of patients, respectively. A monoclonal excess of free light chains (FLC) can be identified at baseline in 98% of patients with systemic AL amyloidosis. Subnormal levels of some or all serum immunoglobulins, or increased numbers of marrow plasma cells may provide less direct clues to the underlying etiology. Until recently, it has been the practice to consider apparent primary cases of

amyloidosis, with no previous predisposing inflammatory condition or family history of amyloidosis, as AL type. However, it has now been recognized that some patients with mutations associated with autosomal dominant hereditary non-neuropathic amyloidosis, particularly that caused by variant fibrinogen α-chain, do not develop fibrinogen α-chain amyloidosis. The coincident occurrence of a monoclonal gammopathy, which occurs in more than 10% of the healthy older population, may then be gravely misleading and it is essential to exclude other forms of amyloidosis by genotyping all known amyloidogenic mutations, and to seek definitive immunohistochemical or proteomic identification of the amyloid fibril protein in all cases.

15.5.2.1 Clinical Manifestations

Clinical suspicion of AL amyloidosis should be raised in any patient with unexplained nephropathy, cardiac failure, peripheral and/or autonomic neuropathy or any other multisystem disease. Potentially all organs can be directly affected by amyloid deposits in systemic AL amyloidosis except the central nervous system. Renal involvement is the most common manifestation with approximately 70% of patients presenting with either proteinuria or elevated serum creatinine. Cardiac amyloidosis is present in 50% of patients at baseline and is the key determinant of mortality. Cardiac amyloidosis typically manifests with a restrictive cardiomyopathy; concentric ventricular wall thickening resulting in diastolic dysfunction manifesting with congestive cardiac failure and, more often than not, hypotension. Autonomic nervous system involvement presents variably and is often challenging to diagnose. It can lead to orthostatic hypotension, erectile dysfunction, urinary retention and fecal incontinence. In patients in whom a peripheral neuropathy is present there is most commonly a distal sensory deficit which can be subclinical at presentation. Systemic chemotherapy aimed at suppressing the monoclonal light chain can cause worsening of peripheral and autonomic neuropathy depending upon the neurotoxicity of therapy. Liver involvement as a presenting feature is rare but is a quite common finding at post mortem examination and on SAP scintigraphy. Hepatomegaly and obstructed liver function tests are the most common clinical findings but can be absent in patients despite the presence of significant hepatic amyloid deposits [32].

There are a plethora of soft tissue features in AL amyloidosis with macroglossia and periorbital bruising thought to be pathognomonic for AL type. Gastrointestinal involvement can result in malabsorption, altered bowel habit and gastrointestinal hemorrhage [33].

15.5.3 Hereditary Systemic Amyloidosis

15.5.3.1 Familial Amyloid Polyneuropathy

Familial amyloid polyneuropathy (FAP) is associated with more than 100 mutations in the gene encoding TTR. TTR is predominantly synthesized in the liver and is a tetrameric protein which has a role in the transport of thyroxine and retinol binding protein. FAP is an autosomal dominant syndrome with onset of symptoms at any point from the second decade onwards. It was first described in 1952 in Portuguese kindreds [34]. It is characterised by progressive peripheral and autonomic neuropathy alongside varying involvement of visceral organs. Extra-neural manifestations predominantly include cardiomyopathy as well as more rarely vitreous amyloid, renal involvement and oculoleptomeningeal amyloid deposition leading to encephalopathy, seizures and dementia. The combination of neuropathy and cardiomyopathy leads to muscle wasting and malnutrition that usually results in death within 9–13 years [35]. The most common encoding mutation is a valine for methionine substitution at position 30 (V30M) and quite numerous cases are seen in Sweden, Japan and Portugal. The T60A variant is most common in the UK, and the low penetrance V122I variant associated with predominant cardiomyopathy occurs in 3–4% of black individuals. Proposed mechanisms of ATTR amyloidogenesis include dissociation of the TTR tetramer into monomers and mechano-enzymatic cleavage with resulting destabilising of the tetrameric TTR protein [36].

15.5.3.2 Non neuropathic Systemic Amyloidosis

The non-neuropathic forms of hereditary systemic amyloidosis were first described in 1932 and are derived from variants of apolipoprotein AI, apolipoprotein AII, lysozyme and fibrinogen A-α chain. Renal involvement is often the most common manifestation however the heart, spleen, liver and bowel may all also be involved. Presentation can vary both within and between kindreds. Clinical presentation is usually around the sixth decade although can occur in early adulthood or before. Following clinical presentation, there is an inexorable progression to organ failure requiring dialysis, organ (renal/cardiac) transplantation or death. In fibrinogen A-α chain amyloidosis the median time from presentation to end stage renal disease (ESRD) is approximately 5 years [37]. The progression of renal disease is much more gradual in apolipoprotein AI and lysozyme amyloidosis with a median time from presentation to ESRD of greater than 10 years.

15.5.3.3 Wildtype Transthyretin Amyloidosis (Previously Known as Senile Systemic Cardiac Amyloidosis)

Wild type transthyretin amyloidosis (ATTRwt) also known as senile systemic/cardiac amyloidosis is a disease of older people with a strong male preponderance. The amyloid deposits are composed of wildtype TTR [38]. The clinical phenotype comprises predominantly of cardiac amyloidosis manifesting as congestive cardiac failure. ATTR deposits are present in other sites including the lungs, gut and bladder, where they can occasionally cause symptoms [39]. Carpal tunnel syndrome is common and often precedes cardiac manifestations by up to a decade or more [40].

15.6 Diagnosis

- **Congo red staining and immunohistochemistry are the standard methods for identification and typing of amyloid deposits in clinical practice**
- **Due to the high prevalence (up to 10%) of monoclonal gammopathies of unknown significance (MGUS) in older populations, presence of a monoclonal immunoglobulin is not sufficient to infer that amyloid is of AL type**
- **Cardiac MRI (CMR) is a sensitive and very specific method for the diagnosis of cardiac amyloidosis**

15.6.1 Histology

The diagnosis of amyloidosis is frequently made late in its natural history, often months or years after the onset of first symptoms. Delays in diagnosis are due to a combination of the heterogeneous nature of the disease, its perceived rarity and the need for histological confirmation. The gold standard for diagnosis is staining of the deposits with Congo-red and pathognomonic apple green birefringence when the tissue sections are observed under cross polarised light microscopy [41] (Fig. 15.1). Congo red staining has a variable sensitivity depending on user experience with very high specificity when performed optimally [42, 43]. Nonetheless, our own experience of many thousands of cases has been at least 10% false positive and false negative rate when Congo red staining has been performed in non-specialist laboratories.

Target organ biopsies such as renal, cardiac and gastrointestinal tissue are usually diagnostic. Rectal biopsies have been used in the past as a screening tool for systemic amyloidosis with a published sensitivity of 75–94% [44]. Abdominal fat pad fine needle aspiration is a quick, simple, minimally invasive bedside test that has limited diagnostic sensitivity and experience is required in its interpretation. One recent study reported that abdominal fat aspiration is a useful test in cardiac amyloidosis often sparing the need for an endomyocardial biopsy [45]. In patients with cardiac AL amyloidosis, abdominal fat aspiration sensitivity correlated with whole-body amyloid burden as assessed by SAP component scintigraphy with a sensitivity of 100%, 97% and 78% of those with a large, moderate and small whole body amyloid load, respectively [45]. It was less useful in ATTR cardiac amyloidosis with a diagnostic sensitivity of 45% in variant ATTR and 15% in wildtype ATTR cardiac amyloidosis [45].

15.6.2 Determining the Fibril Precursor Protein

Immunohistochemical (IHC) staining of amyloidotic tissue is widely available for identifying the amyloid fibril protein, but requires specific expertise. It is relatively quick and is the preferred method in clinical practice [41]. An alternative method for diagnosis and typing of amyloid is immuno-electron microscopy [46] but this method is not routinely available, is expensive and performed only in a few centres.

IHC has variable sensitivity and specificity for typing amyloid. Deposits often fail to stain definitively with a panel of antibodies. This is especially problematic in AL amyloid, in which approximately 30% of samples fail to stain definitively with antibodies to kappa or lambda light chains [47].

15.6.3 Proteomics and Mass Spectrometry

Proteomic analyses comprising mass spectrometry on amyloid deposits cut from tissue sections using laser dissection is increasingly used to confirm the presence of amyloid and identify its type. Expert centres estimate between 98 to 100% specificity and sensitivity [48]. A key advantage over traditional IHC is that it can be performed on tiny amounts of formalin fixed tissue, such as a single glomerulus. Experience to date has mainly been with organ biopsies, but the role of proteomics in identifying amyloid in fat aspirates has lately been reported to have sensitivity for diagnosis and identifying the type of amyloid in up to 90% of cases [49].

A challenge in proteomics typing of amyloid is the detection of more than one potentially amyloidogenic protein in the sample, notably both immunoglobulin and TTR, which are abundant plasma proteins. One study has shown that in cases where more than one amyloidogenic protein is detected, decellularisation of amyloid tissue biopsies can increase the accuracy of proteomic typing and substantially enhance the specificity of detecting the culprit protein [50].

15.6.4 Genetic Sequencing

Five to ten percent of systemic amyloidosis is hereditary [51]. Genetic testing is often key to identifying the type of amyloid but the results need to be interpreted in clinical context and with due caution. The phenotype of hereditary forms of amyloid can vary a great deal within a single family, and novel mutations may be completely incidental (i.e. non-disease causing) (see Chap. 12). Conversely, the presence of a known pathogenic and amyloidogenic mutation may also be incidental; for example, the TTR T60A mutation was present in one study in 1 of 100 apparently healthy Irish individuals, and a case was reported in which the presence of this mutation resulted in delayed treatment of AL amyloidosis [52]. The prevalence of MGUS has been estimated at over 5% in patients aged greater than 70 years and 7.5% in those 85 years or older [53]. The frequency of incidental MGUS has been much higher among patients with ATTR amyloidosis referred to our centre, presumably due to referral bias, i.e. the presence of MGUS having erroneously increased the suspicion of amyloidosis.

15.7 Assessment of Organ Involvement and Function

15.7.1 Imaging

15.7.1.1 Serum Amyloid P (SAP) Component Scintigraphy

I^{123} labelled SAP scintigraphy is a specialised imaging technique developed at the NAC which is not generally available. It identifies amyloid deposits in visceral organs such as the spleen, liver, kidneys and adrenal glands [54]. Organ involvement by SAP scintigraphy can be pathognomonic of amyloid subtype. For example, bone uptake is almost always diagnostic of AL amyloidosis (Fig. 15.3). A major limitation of SAP scintigraphy is its inability to identify cardiac or pulmonary amyloid deposits due to movement and blood pool background in the heart and lungs. Also, there is insufficient resolution to identify deposits in hollow, diffuse or very small

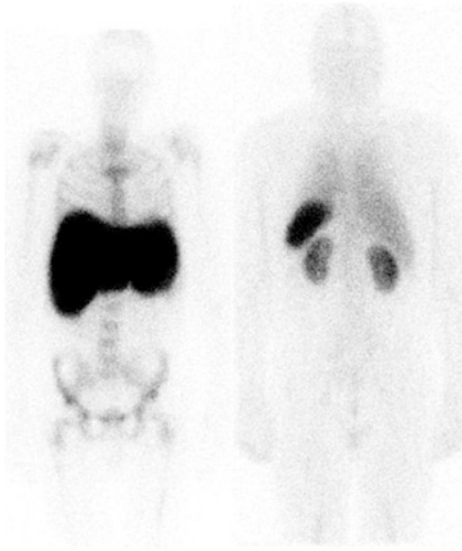

Fig. 15.3 (Left) Anterior whole body scintigraphic image following intravenous injection of ^{123}I-human serum amyloid P (SAP) in a patient with AL amyloidosis. Uptake is seen in the bones, a finding which is pathognomonic for AL amyloidosis and uptake is also present in the liver and spleen. (Right) Posterior whole body SAP scintigraphic image in a patient with hereditary fibrinogen amyloidosis. Uptake is seen in the spleen and kidneys

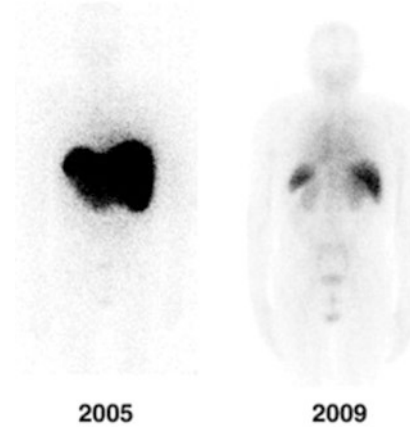

Fig. 15.4 Posterior whole body images of serum amyloid P (SAP) scintigraphy scans of a patient with systemic monoclonal immunoglobulin type (AL) amyloidosis who presented with major liver involvement and proteinuria in 2005. He responded well to chemotherapy with substantial regression of amyloid by 2009 when his liver and renal function had returned to normal

structures such as the gastrointestinal tract, skin and nerves. However, SAP scintigraphy is a powerful and uniquely informative tool that can determine organ distribution, progression and regression of visceral amyloid deposits. It reveals the dynamic nature of amyloid deposits and remains the only method for estimating organ and whole body load of amyloid as well as the response to treatment (Fig. 15.4) [55].

15.7.1.2 Cardiac Imaging

Cardiac involvement is the key predictor of mortality in systemic amyloidosis [56]. Assessment of cardiac amyloidosis has historically been based on transthoracic echocardiography. This classically demonstrates thickening of the left ventricular free wall and interventricular septal diameter (IVSD) along with restrictive diastolic physiology. A widely used definition of cardiac involvement in systemic AL amyloidosis has been a mean left ventricular wall thickness >12 mm in the absence of an alternative cause of left ventricular hypertrophy [57]. However, there are many limitations in the use of echocardiography for diagnosis and evaluation of cardiac amyloidosis with studies showing both poor sensitivity and specificity, particularly in differentiating cardiac amyloidosis from other causes of myocardial thickening, such as hypertrophic cardiomyopathy and hypertensive heart disease [58].

CMR in amyloidosis was first reported in 2005 by our group and is now increasingly used in clinical practice to diagnose infiltrative cardiomyopathies. CMR demonstrates the structure of the heart more accurately than echocardiography and contrast studies demonstrate highly characteristic patterns of late gadolinium enhancement, initially in the sub-endocardium and later more diffusely throughout the myocardium, i.e. in a transmural distribution [59]. The dogma that cardiac amyloid causes concentric symmetrical thickening has lately been refuted by CMR studies in ATTR amyloidosis, which demonstrated asymmetrical hypertrophy as the most common pattern of ventricular remodelling [60]. Recently, the role of CMR in systemic amyloidosis has evolved far beyond just diagnostic utility. Amyloid infiltration results in expansion of the extracellular space, which can be measured with

remarkable accuracy using T1 mapping technologies, providing a novel tool to monitor amyloid load in the heart and track response to treatment.

15.7.2 Cardiac Rhythm Analysis

Electrocardiographic changes are common in cardiac amyloidosis. The largest study comprised 127 patients with biopsy proven cardiac AL amyloidosis evaluated at the Mayo Clinic which revealed a characteristic appearance of low QRS voltage (limb leads <5 mm) with poor R wave progression in the chest leads in approximately 50% of patients [61]. Cardiac rhythm analysis is particularly pertinent in patients with cardiac AL amyloidosis where the early mortality rate is very high (30–40%), often within months of diagnosis, due to sudden cardiac death. The prevalence of serious tachyarrhythmias and bradyarrhythmias in patients with cardiac AL amyloidosis has been estimated at up to 30% [62]. A study from our centre using loop recorders in 20 patients with advanced cardiac AL amyloidosis (Mayo Stage 3b) showed that the most frequent pre-terminal dysrhythmias were bradycardias and complete heart block [63].

Cardiac ATTR amyloidosis is also associated with various electrocardiographic abnormalities, but low QRS voltages occur less often than in cardiac AL amyloidosis [64]. Conduction system disease is common in patients with cardiac ATTR amyloid and atrial fibrillation occurs in 40% of patients [65].

15.7.3 Biochemical Analysis

15.7.3.1 Investigations for Clonal Disease

AL amyloid fibrils are composed of fragments of monoclonal immunoglobulin light chains produced by a clonal B-cell dyscrasia that is often very subtle, but can represent overt MM or lymphoplasmacytoid lymphoma in a small proportion of cases. Characterisation and qualification of the clonal cell dyscrasia requires use of an array of sensitive assays, including serum and urine electrophoresis and immunofixation and serum free light chain assay. Use of the latter technique has greatly improved the sensitivity of detection of an underlying clone [66] but no systemic clone can be identified in approximately 1–2% of patients with systemic AL amyloidosis [67], making both diagnosis and monitoring chemotherapy extremely challenging. All patients should undergo a bone marrow biopsy with aspiration and trephine to assess and further characterize the clonal cell infiltrate. Cytogenetic studies may help predict response to treatment and patient outcome [68]. The translocation t11:14 is present in up to half of patients with systemic AL amyloidosis but fewer than 15% of patients with MM [69]. Traditional skeletal radiographic surveys for bone lesions of MM are now being superseded by more sensitive MRI and low-dose whole-body CT studies [70]. Accurate characterization of the underlying plasma cell disease and determination of where it lies on the spectrum between MGUS and MM is important for both prognosis and treatment options.

15.7.3.2 Cardiac Biomarkers

The two biomarkers used routinely in the diagnosis and monitoring of patients with cardiac amyloidosis are N terminal pro brain natriuretic peptide (NT-proBNP) and high sensitivity cardiac troponin T. Both are part of the widely used Mayo staging system for cardiac AL amyloidosis (Table 15.3) [71]. The Mayo staging provides powerful prognostic information on overall survival in patients with cardiac AL amyloidosis. Median survival is 27 months, 11 months and 4 months in Mayo Stage I, II and III disease, respectively. Further sub-classification of Mayo

Table 15.3 Mayo staging in cardiac AL amyloidosis

Mayo stage	Cardiac biomarkers
Stage 1	NT proBNP <332 ng/L and cardiac troponin T <0.035mcg/L
Stage 2	NT proBNP ≥332 ng/L or cardiac troponin T ≥0.035mcg/L
Stage 3	NT proBNP ≥332 ng/L and cardiac troponin T ≥0.035mcg/L

NT proBNP N terminal pro brain natriuretic peptide

Stage III disease into IIIa and IIIb is used to identify patients at very high risk of early mortality based upon the presence of systolic dysfunction defined as either systolic blood pressure <100 mm/Hg and/or NT-proBNP >8500 ng/L [56]. Both NT-proBNP and cardiac troponin T can be elevated due to other factors including atrial fibrillation, pneumonia and renal failure [72]. The limitations of NT-proBNP in patients with systemic AL amyloidosis and advanced renal excretory impairment have been noted [73]. NT-proBNP may increase substantially during chemotherapy and in response to fluid retention. A greater than 30% fall in NT-proBNP is a key determinant of a cardiac response to treatment in systemic AL amyloidosis, per current consensus criteria [57].

Cardiac ATTR amyloidosis is an increasingly frequently diagnosed progressive cardiomyopathy, the natural history of which can vary significantly. A new staging system for ATTR cardiac amyloidosis based upon a combination of baseline NT-proBNP and estimated glomerular filtration rate (eGFR) identified three disease stages; Stage 1 was defined as NT-proBNP ≤3000 ng/L and eGFR ≥45 mL/min, Stage 3 as an NT-proBNP of ≥3000 ng/L and eGFR ≤45 mL/min and the remainder are stage 2. Median survival in Stage 1, Stage 2 and Stage 3 disease was 69, 46 and 24 months, respectively [65].

15.7.3.3 Renal Biomarkers

Renal involvement in amyloidosis typically manifests with proteinuric CKD, often associated with nephrotic syndrome. Nonetheless the degree of proteinuria varies both between and within different types of renal amyloidosis as well as stage of CKD and/or urinary output. The three key biomarkers for the diagnosis and prognostication of patients with renal amyloidosis are serum albumin, degree of proteinuria and serum creatinine/eGFR. Consensus criteria define renal involvement in systemic AL amyloidosis as non-Bence Jones proteinuria (BJP) of >0.5 g/24 h. Both renal progression and renal response to treatment are dependent upon improvement or worsening of proteinuria in the context of a change in the eGFR.

The monitoring of renal amyloidosis based on proteinuria has its limitations in light of alternative pathologies that can drive urinary protein leak, including diabetes and hypertension. Novel urinary biomarkers have been used in monoclonal gammopathies of renal significance to detect renal insult and may offer improved methods of both diagnosis and monitoring of renal amyloidosis [74].

15.7.3.4 Liver Function Tests

Liver function is often remarkably well preserved despite massive hepatic amyloid infiltration and enlargement. Alkaline phosphatase and γ-glutamyl transferase may rise very substantially before synthetic function is affected, but studies in systemic AL amyloidosis have shown that even a modestly raised bilirubin concentration is associated with a high risk of early death [75].

15.8 Treatment

- **Best supportive care is crucial in the management of systemic amyloidosis**
- **Suppression, or ideally complete elimination, of the supply of the respective amyloid fibril precursor protein is the cornerstone of treatment in amyloidosis**
- **Numerous novel therapies aimed at removing existing amyloid deposits are now in late stage development**

15.8.1 General Management Principles

There are three key principles in the management of systemic amyloidosis. Supportive care to preserve organ function, reduction, or ideally elimination, of the ongoing supply of the respective amyloid fibril precursor protein, and relatively novel therapies aimed at inhibiting the formation of amyloid fibrils or removing existing amyloid deposits.

15.8.1.1 Supportive Care

Best supportive care is vital for patients with all forms of systemic amyloidosis. The aim is to support failing amyloidotic organ function and reduce the risk of complications in vulnerable organs.

Kidneys extensively infiltrated by amyloid are exquisitely vulnerable to intercurrent insults such as hypo/hyper perfusion and nephrotoxic drugs which should be avoided as much as possible. The management of nephrotic syndrome includes meticulous fluid balance encouraging patients to pursue a low salt diet in combination with a total fluid restriction of 1.5 L per day. Diuretic therapy is the mainstay of medical management and loop diuretics are often required at high doses and/or in combination with either thiazide or potassium sparing diuretics [76]. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) have been shown to reduce proteinuria and reduce the risk of progression to ESRD in patients with nephrotic syndrome [77]. Nonetheless due to their risk of acute kidney injury they are often not used in the initial setting. One study of 44 patients with systemic AA amyloidosis showed a reduction in proteinuria in patients treated with ARB although there was no clear long-term benefit in reducing the risk of progression to ESRD [78].

Anticoagulation in nephrotic syndrome remains a controversial issue with no clear consensus. Patients with systemic amyloidosis have an increased risk of bleeding due to amyloidotic vascular fragility while patients with heavy proteinuria and associated hypoalbuminemia are at an increased risk of venous thromboembolic disease. Decisions regarding anticoagulation should be made on an individual basis and in high risk patients low molecular weight heparin treatment can offer a suitable option with a short half-life and easy reversal compared to warfarin therapy.

Management of cardiac amyloidosis is challenging. Patients with cardiac amyloidosis do not tolerate hypotension well due to the low cardiac output state and while there are no clear guidelines on the use of traditional heart failure medication, ACE inhibitors and β-blockers are generally best avoided. Arrhythmias are common in AL amyloidosis. The mainstay of therapy is with oral anti-arrhythmias, most commonly amiodarone. There are limited data on the role of implantable intracardiac defibrillators in patients with cardiac amyloidosis. Appropriate device therapy has been reported in a significant proportion of patients and while it has been shown to be lifesaving in the short-term, long-term survival benefit remains unclear [79].

Gastrointestinal involvement can present with chronic diarrhea, malabsorption and cachexia with symptoms often becoming debilitating. Gastrointestinal bleeding can occur manifesting with melena and anemia. Somatostatin analogues have provided relief in some case studies, but if symptoms lead to malnutrition total parenteral nutrition may be needed to support the patient until bowel function improves.

Adrenal involvement in AA amyloidosis is common but frank adrenal insufficiency is rare. Patients are often receiving corticosteroid therapy for their underlying inflammatory condition. Addisonian symptoms can be difficult to identify, particularly orthostatic hypotension which can be explained by alternative pathologies such as nephrotic syndrome and concurrent diuretic use.

Amyloid related autonomic nerve dysfunction is predominantly seen in AL amyloidosis and hereditary ATTR amyloidosis. Autonomic failure often manifests predominantly with postural hypotension but other symptoms include altered bowel habit, incontinence and erectile dysfunction. Anecdotal evidence supports the use of oral inotropes such as midodrine for the treatment of postural hypotension.

15.8.1.2 Organ Transplantation in Hereditary Amyloidosis

Disease modifying treatment for hereditary systemic amyloidosis remains limited. The mainstay of treatment is supportive therapy including organ transplantation for the failing amyloidotic organ. When the liver is the main source of production of variant precursor protein, liver transplantation can be performed to replace the variant protein with the wildtype non amyloidogenic protein.

Fibrinogen, TTR and ApoA1 are predominantly synthesised in the liver. While liver transplantation can be lifesaving in selected patients with hereditary amyloidosis, careful consideration needs to be taken due to the considerable peri-operative risk, long term immunosuppression, renal toxicity and development of secondary malignancies.

Hereditary AFib amyloidosis is a predominantly renal disease leading to ESRD within 5–10 years. Kidney transplantation has been performed in many cases, but recurrence of renal amyloidosis within 7–10 years commonly causes graft failure. While combined liver-kidney transplantation eliminates the source of the amyloidogenic AFib variant, with potential to prevent further amyloid deposition, the procedure is associated with some mortality and is best reserved for younger patients [80].

15.8.2 Treatment of AA Amyloidosis

Treatment depends on the nature of the underlying chronic inflammatory disorder and ranges from potent anti-inflammatory and immunosuppressive biological drugs in patients with rheumatoid arthritis, to lifelong prophylactic colchicine in FMF and surgery in conditions such as refractory osteomyelitis and the cytokine secreting tumors of Castleman disease.

Most patients with AA amyloidosis complicating inflammatory arthritis can now be treated effectively with one or other of the many biological agents now available, i.e. anti-cytokine (TNF, IL-1, IL-6) and anti-CD20 antibodies. Nonetheless, while there have been advances in the use of biologic therapies, progressive renal dysfunction remains common in AA amyloidosis and the need for renal replacement therapy occurs in up to a 40% of patients with a median time to dialysis from diagnosis of 6.5 years. Mortality, amyloid burden and renal prognosis are all significantly correlated with SAA concentration during follow up. In a study of 374 patients with systemic AA amyloidosis, the risk of death was 17.7 times higher in patients with SAA concentrations ≥155 mg/L compared to <4 mg/L. In fact, even in patients with AA amyloidosis and modestly elevated SAA levels (4–9 mg/L), the risk of death was fourfold higher compared to those with SAA <4 mg/L. [12]. Complete suppression of inflammation (SAA concentration persistently <4 mg/L) is frequently associated with gradual regression of amyloid and preservation of renal function [12].

Colchicine (in FMF) at the maximum tolerated dose and IL-1 inhibition with biological agents has revolutionised the management and prognosis of many patients with inherited autoinflammatory/periodic fever syndromes. Work from the NAC in AA amyloidosis complicating hereditary autoinflammatory/periodic fever syndromes has shown that that this diagnosis was not considered in half of patients prior to presentation with AA amyloidosis, almost 25% had evidence of ESRD at presentation and a further 28% developed ESRD over the course of follow-up with a median time of 3.3 years. Of the 46 patients assessed, 24 had FMF (6 were asymptomatic, i.e. phenotype 2-see Chap. 16), 12 TRAPS and 6 CAPS. The majority of patients with FMF (22/24) were treated with high dose colchicine with complete remission in 19 patients and partial remission in one. Of the 12 patients with TRAPS, 6 patients were initially treated with anti-TNF therapy with a transient response seen in 4; all switched to IL-1 blockade. Four patients were treated upfront with IL-1 blockade. Of the 6 patients with CAPS, 4 were treated with IL-1 blockade with dramatic clinical and laboratory improvement and 2 died before the role of IL-1 therapy in CAPS was recognised. Of the total 37 patients from the cohort who were treated successfully, or in whom at least partial remission of the underlying autoinflammatory condition was achieved, 17 (46%) showed amyloid regression, 14 (38%) had a stable amyloid load, and the amyloid deposition increased in 2 (5%) [81].

A Turkish case series of 29 patients with FMF-related amyloidosis receiving IL-1 blockade revealed that in patients with relatively preserved renal excretory function (n = 13) (serum creatinine <130 mmol/L) proteinuria improved dramatically from a median of 3.7 g/24 h to 1.3 g/24 h, while in patients with more advanced CKD at presentation the role of IL-1 blockade was less pronounced [82].

The successful use of IL-6 blockade has been reported in colchicine-resistant FMF and in systemic AA amyloidosis complicating autoinflammatory diseases. IL-6 is a key driver of SAA production, and inhibition of the latter through IL-6 blockade is associated with stabilisation or gradual regression of AA amyloid deposits [83]. In a Turkish series of 12 patients with AA amyloidosis complicating FMF, five of whom had coexisting autoimmune disease, IL-6 blockade reduced the frequency of FMF attacks, suppressed the acute phase response and stabilised renal function [84].

The preferred form of renal replacement therapy in AA amyloidosis remains renal transplantation and suppression of the underlying inflammatory disorder is imperative prior to transplantation and during follow up to prevent recurrence of amyloidosis and graft failure. In a study looking at renal transplantation in 128 patients with AA amyloidosis and ESRF, 43 underwent renal transplantation with a median time from ESRF to transplantation of 1.5 years. The median estimated graft survival noncensored for death was 10.3 years; with 5 and 10-year graft survival of 86% and 59% respectively. Sixteen (37%) patients died, most commonly from infection. Median SAA levels were higher in patients with recurrent amyloid in the graft compared to those in whom amyloid did not recur [85]. In FMF the combination of transplant immunosuppression and prophylactic colchicine remains the mainstay of treatment to prevent systemic inflammation and recurrence of AA amyloidosis. However, there have been case reports of the effective use of IL-1 blockade in patients with colchicine-resistant FMF who underwent renal transplant for AA amyloidosis [86].

No effective specific therapy for AA amyloidosis has yet been developed. A promising agent that ultimately failed to translate into clinical benefit was eprodisate. This is a negatively charged, highly sulphonated molecule that is thought to interfere with the association of AA amyloid fibrils and GAGs, which was shown to inhibit amyloid formation in an experimental murine model of AA amyloidosis [87]. Although a multicentre, international randomised controlled clinical trial of patients with renal impairment due to AA amyloidosis suggested the possibility of clinical benefit [88], a subsequent study designed to confirm and extend these findings found absolutely no benefit, and further development was ceased (results never published).

15.8.3 Treatment of AL Amyloidosis

The current management of AL amyloidosis is aimed at suppressing the underlying B-cell clone as quickly and completely as possible with chemotherapy and novel agents. This in turn halts the production of amyloidogenic light chains. Remission of the underlying clonal disease, i.e. hematologic response, may be associated with preservation of organ function and in some cases improvement in organ function, i.e. organ response, especially when hematologic remission has been sufficient to facilitate some gradual regression of the amyloid deposits.

Consensus criteria to define hematologic and organ response in AL amyloidosis have been devised [89]. Patients who achieve a complete hematologic response have the best clinical outcomes [90]. This is defined by no detectable monoclonal immunoglobulin [M] band in serum or urine by immunofixation and normal free light chains, or a very good partial response, defined as the difference between the involved and uninvolved free light chains (dFLC) <40 mg/L.

Although chemotherapy for AL amyloidosis has very largely been adapted from substantial experience in MM, adverse effects of treatment in patients with amyloidosis are much more frequent and serious, due to the reduced functional reserve of amyloidotic organs and poor performance status of many patients. This has led to risk adapted chemotherapy protocols, with most AL amyloidosis patients being classed as intermediate risk and best suited to cyclic combination chemotherapy regimens. These have historically included oral melphalan with dexamethasone as well as a combination of cyclophosphamide, thalidomide and dexamethasone. More lately, proteasome inhibitors, initially

bortezomib and now others, have become the cornerstone of treatment [91]. First line combination therapy with bortezomib, cyclophosphamide and dexamethasone has been shown to deliver high overall response rates [92]. Ixazomib is the first oral proteasome inhibitor and is available in combination with lenolidamide and dexamethasone after at least one prior line of therapy. Carfilzomib is a novel irreversible proteasome inhibitor approved for relapsed/refractory MM. While Phase I/II studies of its use to treat systemic AL amyloidosis have shown promising hematologic response rates, cardiac, renal and pulmonary toxicity have been noted, warranting close monitoring of side effects and dose reduction.

Autologous stem cell transplantation (ASCT), both in the initial and relapsed disease settings, is an effective treatment for AL amyloidosis leading to deep and durable clonal responses with an excellent median overall survival of over 5 years. However, this high intensity treatment is suitable for only a minority of patients due to significant procedure related morbidity and mortality [93]. Stringent risk stratification has helped improve outcomes, and use of Mayo cardiac staging criteria has resulted recently in procedural mortality rates of 7% or less [93].

Median survival in AL amyloidosis has improved a great deal over the past decade with a current estimated 4-year survival rate of 50%. Sadly, nearly 25% of patients still die from disease related complications within the first few months of treatment and this is primarily due to the presence and severity of cardiac involvement [94].

15.8.4 Novel Therapeutic Approaches in Clinical Trials

A number of different therapies aimed specifically at inhibiting the formation of amyloid fibrils or promoting fibril regression are currently under development, and some have already been clinically evaluated.

In-vitro studies have shown that amyloidogenic misfolding of TTR may be inhibited by compounds that bind TTR in the plasma. Tafamidis, which is a TTR stabiliser, has been developed specifically to treat ATTR amyloidosis and to slow neuropathic disease in patients with V30M familial amyloid polyneuropathy [95]. Diflunisal, a non-steroidal anti-inflammatory drug, has lately been repurposed as an amyloid treatment, unrelated to its anti-inflammatory properties; it also binds to and stabilises TTR in vitro [96]. A randomized controlled trial confirmed that it slows neurological progression in hereditary ATTR amyloidosis [97]. TTR is almost exclusively synthesized by the liver which presents a target for state of the art RNA-inhibiting therapies. Anti-sense oligonucleotide and small interfering RNA therapies have been shown to reduce circulating TTR by 70–85% respectively [98], and phase 3 studies have lately been completed of both class of agents with great success, showing substantial inhibition and even reversal of neuropathic features in FAP [99].

15.8.4.1 Anti-amyloid Antibodies

The role of therapeutic antibodies to directly target existing amyloid deposits is being investigated with vigor. There are currently two monoclonal antibodies that are undergoing testing.

The first antibody approach focuses on the murine monoclonal 11-1F4 antibody prepared against human light chain related fibrils which are recognised as an amyloid-associated conformational epitope [100]. In animal models of mice bearing human amyloidomas, defined as a solitary localised deposit of amyloid, rapid and complete elimination of the masses without toxicity was demonstrated. In an open-label, dose escalation phase I clinical trial, the drug was tolerated well by participants with no grade 4 or 5 adverse events reported. Organ responses were seen in 60% of evaluable patients with a median time to response of only 2 weeks after the start of treatment [101].

The second antibody approach, potentially applicable to all types of amyloidosis, targeted SAP. SAP binds to and is present in all amyloid deposits, which is believed to protect them from degradation by phagocytic cells and proteolytic enzymes [102]. CPHPC, a drug that cross-links pairs of circulating SAP molecules in vivo trigger-

ing their removal by the liver, rapidly and almost completely eliminates SAP from the bloodstream. By contrast, even long term treatment with CPHPC only modestly depletes SAP from amyloid deposits. Subsequent work showed that antibodies to SAP can then target the remaining SAP present in all amyloid deposits, resulting in their rapid clearance by macrophage and complement mediated mechanisms. In an open-label Phase I dose escalation study of 16 patients, a combination of CPHPC and anti-SAP antibody has been shown by ^{123}I-SAP scintigraphy to result in swift and marked removal of liver amyloid deposits with associated improvement in liver function tests [55]. This therapy is currently being tested in a phase 2 trial of patients with cardiac ATTR and cardiac AL amyloidosis.

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Part IV

Monogenic Autoinflammatory Diseases

Familial Mediterranean Fever

16

Shai Padeh, Yelda Bilginer, and Seza Ozen

Abstract

Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease characterized by recurrent, sporadic, self-limited episodes of fever accompanied by peritonitis, pleuritis, arthritis, and/or erysipelas-like erythema. The disease is prevalent among populations surrounding the Mediterranean Sea, however, in recent years, more cases have been reported in countries not related or close to this region. The frequency of episodes varies from once every week to several times a year, and unlike the term "periodic disease", the attacks are at random and not cyclic. A typical attack of FMF lasts 0.5–3 days, and between attacks patients are mostly asymptomatic. One of the devastating outcomes of FMF is the development of AA amyloidosis, which mostly affects the kidneys but may involve other organs. Since 1972, life-long prophylactic colchicine has been the treatment of choice for FMF, which reduces the number of acute attacks and prevents the development of amyloidosis. Several sets of diagnostic criteria and several severity scores have been proposed. The disease is caused by gain-of-function mutations in the *MEFV* gene, encoding pyrin, a protein which by binding to additional proteins form the pyrin inflammasome. Mutated pyrin is associated with activation of caspase-1 and the release of interleukin (IL)-1ß, resulting in inflammation. Unraveling the molecular mechanisms of FMF led to the understanding of the potential of IL1ß blockade as a novel treatment in patients with colchicine-resistant FMF. However, colchicine remains the main treatment of FMF.

Keywords

Familial Mediterranean fever · Pyrin · Colchicine · Interleukin 1 · Treatment · Canakinumab, anakinra, rilonacept · Autoinflammatory disease

Abbreviations

ADDI	Autoinflammatory Disease Damage Index
AIDAI	Autoinflammatory Disease Activity Index
ANCA	Antineutrophil cytoplasmic antibodies
ASC	Apoptotic speck protein containing a caspase recruitment domain

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CARD	Caspase recruitment domain
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FAVOR	FMF Arthritis Vasculitis and Orphan Disease Research group
FMF	Familial Mediterranean fever
IL	Interleukin
ISSF	International Severity Score for FMF
JIA	Juvenile idiopathic arthritis
LPS	Lipopolysaccharide
MEFV	MEditerranean FeVer gene
NET	Neutrophil extracellular traps
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
PFMS	Protracted febrile myalgia syndrome
PKN	Protein kinase N
PRINTO	Pediatric Rheumatology International Trials Organization
PYD	Pyrin domain
SAA	Serum amyloid A
SHARE	Single Hub and Access point for paediatric Rheumatology in Europe
TNF	Tumor necrosis factor
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome
TRIM	Tripartite motif

Key Points

- **Familial Mediterranean fever (FMF) was the first described autoinflammatory disease. It is also the most frequent monogenic periodic fever/autoinflammatory disease around the world**
- **Mutations in the *MEFV* gene encoding the pyrin protein are responsible for FMF**
- **Typical attacks include fever of short duration and serositis**
- **Treatment with colchicine is beneficial in both reducing the frequency of attacks and preventing the potentially fatal complication of amyloidosis**

16.1 Introduction and History

For many years, familial Mediterranean fever (FMF) has been considered the most common of the "periodic fever syndromes". Various syndromes including recurrent attacks of fever, abdominal, and chest pain associated with joint symptoms were described as early as by Osler [1]. A more likely description of FMF was published in 1945 by Siegal, reporting 10 cases of 'benign paroxysmal peritonitis' [2]. Later descriptions of a periodic syndrome, possibly FMF, appeared under several other names [3]. An extensive description of different periodic fevers was published by Reimann in 1948, hence the name La Maladie périodique de Reimann [4]. In 1951, Cattan and Mamou were the first to notice the association of these periodic diseases with renal disease [5]. It was only in 1958 that "familial Mediterranean fever" was coined as a separate nosologic entity by Heller et al., emphasizing the genetic nature of the disease [6]. The increased frequency of FMF among Arabs, Armenians, Jews, and Turks, and the possible hereditary nature was noticed earlier [7, 8]. The common denominator of these ethnicities, residing in the eastern Mediterranean basin raised the hypothesis that the origin of the founder mutations first appeared around 2500 BC in Mesopotamia.

Colchicine, known for hundreds of years in an herbal form (Colchicum autumnale) for the treatment of joint pains and gout (see Chap. 40), was first shown in 1972 by Goldfinger to prevent FMF attacks [9], and at the same time in Turkey by Özkan [10]. The introduction of prophylactic treatment with colchicine in FMF has dramatically reduced the frequency of attacks as well as the incidence of amyloidosis [9–12].

The *MEFV* gene, identified by positional cloning by 2 separate groups in 1997, was mapped to chromosome 16 [13, 14]. The *MEFV* gene product, a 781 amino acid protein, was named pyrin by the American/International group and marenostrin (Mare Nostrum: Latin for the Mediterranean Sea, meaning our sea) by the French group [15].

Following the discoveries of pyrin as the protein responsible for controlling inflammation in FMF and in 1999 the gene responsible for the tumor necrosis factor receptor-associated periodic syndrome (TRAPS) (see Chap. 18), a new concept was coined, the hereditary autoinflammatory diseases.

The discovery of a caspase-activating complex by Tschopp's group in 2002 [16], which was named "inflammasome", led to unraveling the molecular mechanisms of many of these diseases. In 2007, Papin et al. showed that pyrin binds components of the inflammasome, particularly caspase-1 and interleukin (IL)-1β [17]. The pyrin inflammasome was only recently identified [18, 19]. These findings led to the understanding of the importance of IL-1β as the major cytokine in the inflammatory process of FMF.

16.2 Definition/Classification

- **FMF is an autosomal recessive disease due to mutations in the *MEFV* gene encoding the pyrin protein**
- **Historically, FMF was categorized in three phenotypes depending on the presence of clinical features**

FMF is an autosomal recessive disease characterized by short febrile episodes accompanied by inflammation in one of the serous membranes, resulting in peritonitis, pleuritis or synovitis. During attack-free periods, patients are mostly asymptomatic. Secondary amyloidosis in FMF is the product of tissue deposition of AA amyloid, which is a proteolytic cleavage product of the acute-phase reactant serum amyloid A (SAA) [8].

Initially, FMF was categorized into two phenotypes [20]:

- Phenotype I: FMF with overt clinical symptoms and signs.
- Phenotype II: FMF with amyloidosis as the only clinical manifestation in an otherwise asymptomatic individual.

Following the identification of the *MEFV* gene responsible for FMF, a third phenotype was suggested:

- Phenotype III. Individuals carrying biallelic *MEFV* pathogenic variants on molecular genetic testing without any clinical symptoms or signs [21], or elevated acute phase reactants [22, 23].

In recent years, there have been only very rare reports of phenotype II [24]. In fact, most investigators have not encountered any patients with a phenotype II presentation, raising the question as to whether this entity is only a consequence of previously undiagnosed disease, or unnoticed by the patients, or possibly obtaining an incomplete history [8, 25–27]. Data from a large study of 260 patients with FMF-related amyloidosis challenged the rationale of initiating prophylactic treatment with colchicine to asymptomatic individuals who are incidentally discovered to have homozygous M694V mutations in areas with a low risk of renal amyloidosis, particularly western countries [26]. A preventive approach (e.g., performing urinalysis and checking inflammatory markers every 6 months) may be more justified [26]. Nevertheless, since there are no long-term follow-up studies in asymptomatic individuals with two mutations, or of monitoring elevation of acute-phase reactants in these individuals, this issue remains controversial.

16.3 Epidemiology

- **FMF is frequent among ethnic groups of the eastern Mediterranean basin, including Jews, Turks, Armenians and Levantine Arabs**
- **FMF exists, albeit as a rare disease, among other populations, as well. Cases have recently been defined from European and Japanese ancestry**

FMF is the most common hereditary autoinflammatory disease worldwide and affects more

than 120,000 people [28, 29]. FMF occurs primarily among ethnic groups of Mediterranean ancestry, most commonly Jews, Turks, Armenians and Arabs, in as many as 1/500 persons [30, 31]. The carrier frequencies among non-Ashkenazi Jews is 1:5, Arabs 1:16, Turks 1:5, and Armenians 1:7 [28]. Turkey is probably the country with the highest number of patients with FMF, with an estimated prevalence of about 1:400 to 1:1000 (highest in the areas of Anatolia). With a population of approximately 75 million, it is estimated there are more than 100,000 patients with FMF in Turkey [31–34]. Armenia probably has the second highest prevalence, estimated at 1:500 in a population of three million, totaling approximately 6000 patients [35]. In Israel, the prevalence is slightly more than 1:1000 (depending on the ethnic group, with a mixed population of Ashkenazi and Sephardic Jews, and Arabs); with a population of approximately 8.8 million, there are an estimated 20,000 patients with FMF. Estimated carrier rates vary from 1/6–1/7 in North African Jews, 1/13 in Iraqi Jews to 1/135 in Ashkenazi Jews [31, 36]. More than 90% of Jewish patients with FMF are of Sephardic or Middle Eastern origin. The descendants of the Jews expelled from Spain in the fifteenth century and dispersed through various North African and Mediterranean countries are the Sephardic Jews, while Middle Eastern Jews (mainly Iraqi) are descendants of Jews exiled to Mesopotamia by the Babylonians more than 2500 years ago. The Ashkenazi Jews are primarily from eastern and western Europe, and their origin is combined from Jews exiled from Judea by the Romans 2000 years ago and through later persecutions and conversions. A cluster of patients with FMF with several variants of *MEFV* has been identified among the "Chuetas" (descendants of converted Jews) in Palma on the Spanish Mediterranean island of Mallorca [37], implying they are descendants of Jews from various origins.

Following identification of the *MEFV* gene, FMF has been increasingly reported from all around the globe [33]. The spread of FMF from the Mediterranean basin is presumably due to the extensive population movements of the twentieth century. FMF has been reported in European countries such as France, Germany, Italy, Greece, Crete, and Spain as well as the United States, Japan, North African countries, and Australia [24, 32, 38–40]. Carriers of *MEFV* variants were found at various rates in subjects from different central and southeastern European countries such as Macedonia (16%), Serbia (11%), Bosnia and Herzegovina (8%), Slovenia (6%), and Hungary (5%). A high prevalence of FMF among patients with unexplained fever was recently reported in Japan [41]. However, there are countries where FMF has not been found or reported. These include sub-Saharan African countries, Ethiopia, Yemen, and Scandinavian states, as well as South Asian and Far Eastern countries such as Thailand [29]. A publication from India stated that very few genuine cases of FMF have been reported from India and China, despite being vast countries, constituting approximately 40% of the worlds' population [42].

16.4 Etiology

- **Mutations of the *MEFV* gene, which includes 10 exons and encodes the pyrin protein, causes FMF**
- **The M694V mutation is associated with the most severe disease and is the most frequent mutation among patients with FMF from Turkey and Israel**

FMF is mostly an autosomal recessive disease caused by mutations the *MEFV* gene, a 10 exon gene located on the short arm of chromosome 16 encoding the pyrin protein. Pyrin is a 781 amino acid protein also denoted as marenostrin (pyrin from the Greek for fever, marenostrin—Latin for Mare Nostrum, our sea) or tripartite motif (TRIM)20 [13, 14]. Pyrin is expressed in neutrophils, monocytes, and dendritic cells, and in peritoneal, pleural, synovial, and dermal fibroblasts. Mutations in pyrin cause an exaggerated inflammatory response as a result of the uncontrolled production of IL-1β [43]. The N-terminal ~90 amino acids of pyrin are the prototype for a motif (the pyrin domain-PYD) that mediates protein-

protein interactions and is found in more than 20 different human proteins that regulate inflammation and apoptosis. Many of the FMF-associated variants in pyrin are located at the C-terminal B30.2 domain, encoded by exon 10 of *MEFV*. More than 333 variants are listed in the INFEVERS online database (http://fmf.igh.cnrs.fr/ISSAID/infevers, see Chap. 12) [15], nearly all of which are missense substitutions. Many variants are considered to be disease causing and associated with the FMF phenotype [44], but the majority of cases of FMF are caused by only four founder mutations clustered on exon 10: p.M694V, p.V726A, p.M680I and p.M694I, the prevalence of which varies according to the population studied. In Turks, the largest ethnic group with FMF, p.M694V is the leading *MEFV* mutation (51% according to the Turkish FMF Study group) followed by p.M680I (14%) and p.V726A (9%). p.M694del is associated with a more severe form of the disease [45]. The meta-analysis of population genetics in FMF suggests that the mutations are not uniformly distributed in various communities and differ in phenotypes, and that western populations might present with other autoinflammatory syndromes that mimic FMF but are not *MEFV*-related [46]. Jews were proposed as the candidate population for founder effects in *MEFV* mutations due to genetic isolation and genetic drift.

MEFV variants affecting the p.M680I and p.M694V amino acid residues are associated with a more severe phenotype, with early onset of FMF, more frequent attacks, a higher prevalence of arthralgia and arthritis and an increased risk of AA amyloidosis [33].

16.4.1 Other Genetic Variants

The E148Q variant, of exon 2, is defined as a variant of unknown significance. It is the most common variant among healthy carriers, present in up to 3–18% of the major ethnicities at risk for FMF [47–49]. Additionally, E148Q homozygotes are rarely found in the FMF population [47]. This non-founder variant is found more frequently in patients with FMF in countries where FMF is distinctly rare, such as the Japanese, Chinese, Punjabi Indians and Philippines [41, 50, 51], thus indicating that this variant is not pathogenic. Cis P369S/R408Q substitutions have been reported with a highly variable phenotype, infrequently associated with typical FMF symptoms and a trial of colchicine was suggested, but often not beneficial [52].

Most of the pathogenic mutations are in exon 10, however, pathogenic mutations have also been described in other exons as well. In populations where FMF is a rare disease (outside the eastern Mediterranean basin), unusual mutations outside exon 10 are frequently encountered.

On the other hand, Masters and his group have recently identified an autosomal dominant severe mutation in position 242 on exon 2 of the MEFV gene leading to a completely different phenotype called pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) [53] (see Chap. 29).

16.5 Pathogenesis

- **Increased activation of the pyrin inflammasome leads to a series of events with the activation of the innate immune response with fever, an influx of neutrophils to serosal sites and increase synthesis of various inflammatory proteins from the liver including the acute phase reactants**
- **Interleukin (IL)-1 is the main pro-inflammatory cytokine in FMF**
- ***MEFV* mutations are gain-of-function mutations resulting in activation of the pyrin inflammasome**
- **Various infectious agents inactivate RhoGTPase. Pyrin senses the inactivation of RhoGTPases and thus acts as a pathogen recognition receptor**

The hallmark of FMF is an acute inflammatory reaction affecting serosal tissues such as the pleura, peritoneum, and/or synovia accompanied by fever. The inflammatory response is a self-amplifying process that, if unregulated, can result in repeated attacks or persistence of inflammation.

Acute attacks of FMF develop following activation of neutrophils at the serosal and synovial surfaces. The key role of neutrophils in FMF is supported by their massive presence in the serosal fluid during an attack, by the prominent expression of pyrin in neutrophils, and by the favorable effect of colchicine in preventing attacks [54]. During attacks, there is a massive influx of neutrophils to the affected tissues [55]. The half-life of mature neutrophils in the circulation is about 7 h when they irreversibly traverse the vascular endothelium into the tissues, where they die after 1 or 2 days. Once provoked, neutrophils induce inflammation, which lasts 1–2 days, the duration of an average FMF attack. The short half-life of neutrophils can also explain the clinical experience showing that missing 1 day of colchicine treatment, may render the neutrophils susceptible to subclinical stimulation, culminating in an attack of FMF. More support for this observation are studies showing that an attack of FMF is characterized by the release of neutrophil extracellular traps (NET), including active IL-1β, during the first hours of FMF attacks, and subsiding with its resolution [56]. NETs restrict their generation through negative feedback mechanisms, which may explain the self-limiting nature of FMF attacks. Another study showed that in response to inducing agents (such as tumor necrosis factor (TNF)-α, lipopolysaccharide (LPS) and others), neutrophils from patients with FMF display significantly elevated apoptotic rates essential for the successful resolution of inflammation and the prevention of tissue damage [57].

16.5.1 Cytokine Profile During FMF Attacks

Although suppression of IL-1β secretion has become a major target for the treatment of FMF, evidence for increased secretion of IL-1β in patients with FMF during an attack is inconclusive. LPS-stimulated monocytes from patients with FMF display enhanced IL-1β secretion, which correlates with the number and penetrance of *MEFV* mutations [58]. In another study, attacks of FMF were associated with significantly higher mean levels of plasma IL-6 but not IL-1β [59]. Others have shown that serum levels of IL-1β in attack-free patients with FMF were considerably higher than in healthy controls [60]. In vitro, unstimulated neutrophils from M694V positive patients spontaneously secreted more S100A12, IL-18, and caspase-1 compared to neutrophils from healthy controls [61].

Kogan et al. showed that the serum level of eight cytokines were significantly increased during an attack of FMF compared to a healthy control group (IL-4, IL-6, IL-7, IL-17, IL-18, G-CSF, sCD54, and CXCL10), with IL-6 being the most significant for distinguishing flares of FMF from remission or healthy controls [62].

16.5.2 Pathogenesis at the Molecular Level

16.5.2.1 Inflammasome Assembly

Pyrin, which forms an inflammasome when mutated or in response to bacterial toxins, results in an increase in IL-1β mediated inflammation [19].

Pyrin belongs to a large family of proteins sharing a conserved domain structure with TRIM consisting of an N-terminal RING domain, B-box domain(s) and a C-terminal coiled-coil domain [63]. In TRIM20 the RING domain is replaced by the PYD, which belongs to the death domain superfamily [64–66]. The PYD interacts with an adapter protein denoted apoptotic speck protein containing a caspase recruitment domain (CARD) (ASC), a pro-apoptotic protein that induces the formation of large cytosolic "specks" in transfected cells, participating in the regulation of apoptosis, inflammation, and IL-1β processing [65, 67]. Thus, through the N-terminal PYD, pyrin was first considered to modulate caspase-1 and IL-1β activation exerting proinflammatory [68–71] or anti-inflammatory regulatory effects [17, 72, 73], depending on the experimental system employed. Pyrin also carries an additional ~200-amino acid C-terminal B30.2/rfp/PRY/SPRY domain, where the majority of the disease-associated *MEFV* variants are located.

In some early studies, the nucleotide binding and oligomerization domain, leucine rich repeat, pyrin 3 (NLRP3) inflammasome complex was implicated in the pathogenesis of FMF [17, 58]. More recently, homozygous knock-in mice harboring the mouse pyrin protein fused to the human B30.2 domain containing FMF-associated mutations has been shown to secrete large amounts of IL-1β in an NLRP3-independent manner, suggesting the formation of an inflammasome that does not include NLRP3 [74]. Recently the RhoGTPase was shown to be crucial in the pathogenesis of FMF (see below).

16.5.2.2 The Pyrin Inflammasome

The existence of a pyrin inflammasome has recently been proposed. Three proteins participate in the regulation and formation of this inflammasome: the RhoGTPases, 14-3-3 protein, and serine/threonine-protein kinase N (PKN) [18, 19].

The Rho family of GTPases is a family of small signaling G proteins, which have been shown to regulate many aspects of intracellular actin dynamics. The functions of RhoA in the cell are primarily related to cytoskeletal regulation, myosin phosphorylation and cellular responses to stress, such as the formation of focal adhesions and actin stress fibers. It has also been shown to be directly related to myosin chain elongation, actin filament rearrangement, gene expression, cell shape determination and cell proliferation.

Various bacterial toxins or effectors trigger an inactivation of Rho GTPases, resulting in formation of the pyrin inflammasome [18, 19]. Pyrin also acts as a pattern recognition receptor (see Chap. 4), sensing pathogen modification and inactivated Rho GTPases [19]. This response is mediated by dephosphorylation of 2 serines residues on pyrin, Ser-208 and Ser-242, both of which are required for 14-3-3 binding and which undergo dephosphorylation upon toxin stimulation.

PKN, is a protein belonging to the protein kinase C superfamily. This kinase is activated by the Rho family of small G proteins and mediates the Rho-dependent signalling pathway. It has been shown that the RhoA effector kinases of the PKN family suppress pyrin inflammasome activation, and PKNs bind and phosphorylate pyrin [75]. Similarly, pyrin binds to the 14-3-3 proteins, a family of conserved regulatory molecules, which have the ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and transmembrane receptors. In a similar way, the pyrin inflammasome is inhibited by phosphorylation and subsequent 14-3-3 protein binding [75]. In short, active RhoA signals PKN pyrin phosphorylation (Ser208 and Ser242 units), which binds to the 14-3-3 proteins, inhibiting the activation of pyrin inflammasome. Pyrin is activated when it is dephosphorylated at Ser208/Ser242. For illustration, when certain toxins enter the body, they "paralyze" RhoA, which prevents the entire chain of inhibitory events and the body responds with over-production of IL-1β [75].

Various bacterial toxins or effectors, such as *Clostridium difficile*, *Vibrio parahaemolyticus*, *Histophilus somni*, *Clostridium botulinum*, and *Burkholderia cenocepacia*, which modify and inactivate Rho, can activate the pyrin inflammasome [19, 76]. Thus, pyrin senses bacterial virulence rather than directly recognizing a microbial molecule. Cytosolic inflammasome complexes, mediated by a pattern recognition receptor, defend against pathogen infection by activating caspase 1 [19].

16.5.2.3 Pyrin and Cytoskeleton Microtubules

In early studies, pyrin was demonstrated to be located in the nucleus. Subsequent studies have shown the cytosolic localization of the full-length pyrin, and the interaction of the N-terminal part of pyrin with microtubules and co-localization of pyrin with actin [77], suggesting that pyrin is a part of novel cytoskeleton-signaling pathway [78]. Thus, pyrin may function to monitor the proper dynamics of the actin cytoskeleton and particularly pathological disruptions of the actin dynamics.

16.5.2.4 A Gain-of-Function Model

Early publications of genetic studies in patients with severe FMF suggested that a recessive mode

of inheritance and loss-of-function mutations cause the disease [8, 13, 14]. Papin et al. showed an increase in caspase-1 activation and IL-1β secretion as a result of pyrin knockdown [17]. Hesker et al. demonstrated enhanced IL-1β release by macrophages in response to inflammatory stimuli in a mouse line lacking the *MEFV* gene [73]. Further studies, however, showed that as many as 30% of patients with clinical FMF have only a single demonstrable mutation in *MEFV* [79–81], even after complete sequencing.

Several studies have tried to address the mechanism for the clinical expression of FMF in patients with heterozygous mutations for *MEFV* [50, 82, 83]. In a series of 20 patients with FMF carrying only one *MEFV* mutation, a full sequencing of complementary DNA (cDNA) samples and multiplex ligation-dependent probe amplification analysis showed 18 patients with no identifiable additional mutations, large genomic deletions, or duplications. Analysis of single-nucleotide polymorphisms along the cDNA ruled out a lack of expression of one of the alleles. Partial penetrance and variable expression in heterozygous subjects were suggested to explain the lack of a second mutation in many of these patients. It could also explain vertical transmission in some families and mild FMF-like symptoms in seemingly unaffected family members [80].

Additional reports even demonstrated cases of apparently dominantly inherited FMF [82–84]. The T577 variant located before the C-terminal B30.2/SPRY domain is crucial for pyrin function [85], and various mutations at this site have been shown to cause autosomal dominant FMF. In another report, p.M694del was shown to cause dominant FMF in association with variable penetrance and a tendency to begin later in life than the common disease phenotype. However, p.M694Vdel is associated with considerable morbidity and three patients (14%) in one series have developed AA amyloidosis [84].

Chae et al. [74] have demonstrated an inflammasome in which gain-of-function pyrin mutations cause autoinflammatory disease. They generated pyrin-deficient mice and "knockin" mice harboring mutant human B30.2 domains. The homozygous knockin mice exhibited spontaneous inflammation similar to human FMF. When stimulated with LPS, caspase-1 was constitutively activated in knockin macrophages and active IL-1β secreted. This was completely ablated by IL-1 receptor blockage. These data support a gain-of-function model, where the mutant pyrin induces an inflammasome causing IL-1β–mediated systemic inflammation in a "dose" dependent manner [74].

16.5.3 Potential Population Advantage Among Carriers of MEFV Mutation

The combined frequencies of FMF mutations among several Mediterranean potential population advantage are extraordinarily high (up to 1:3), suggesting the possibility of a heterozygote advantage. A selective advantage against pathogenic microbes of heterozygous FMF mutations carriers has long been proposed. A recent hypothesis suggests that pyrin can guard against a broad spectrum of potential pathogenic infections by nucleating an inflammasome to defend against bacteria, such as *Clostridium difficile*, *Burkholderia cenocepacia* and *Vibrio cholerae*, which utilize toxins to inactivate RhoGTPase. Other bacterias such as *Bordetella pertussis*, *Bacillus anthracis*, *Pseudomonas aeruginosa* and *Yersinia pestis* secrete adenylate cyclase toxins, potentiating pyrin inflammasome activation [75]. Studies have failed to show protection against tuberculosis [86]. Also, the *MEFV* variant carrier state was suggested to be associated with protection against allergy [87].

16.6 Clinical Findings

- **The typical clinical features are self-limited attacks of fever and serositis**

- **Several associations of FMF with other diseases have been identified, such as sacroiliitis and some forms of vasculitis**

Symptoms of FMF usually start in childhood and only 5% develop the disease after the age of 30 years [8, 88]. A typical attack of FMF consists of fever accompanied by signs of peritonitis, pleuritis or acute synovitis lasting 0.5–3 days. Patients with FMF often appear well and free of symptoms between attacks. The frequency of attacks varies from once per week to once in several months. Fatigue, surgery, menstruation, cold exposure and vigorous exercise may trigger an attack [89]. In general, there are no major clinical differences between children and adults except that especially in very young children fever may be the only manifestation of an attack [90]. The temperature may rise to 40 °C [88]. Abdominal pain is present in almost all patients [33, 89, 91]. A typical clinical episode is fever accompanied with acute peritonitis resembling acute appendicitis. Sometimes constipation accompanies the clinical picture whereas in children diarrhea (even with peritonitis) may occur [88].

Chest pain is usually unilateral. Pleuritis rarely lasts longer than 3 days and be the presenting manifestation in 5% of patients [88]. Recurrent pericarditis was reported in 0.5% of patients [88].

16.6.1 Musculoskeletal Manifestations

Arthritis is a common and important feature of FMF. Monoarthritis affecting the large joints (especially the ankles, knee or hip) is seen in 25–30% of patients with FMF [91]. During the acute attacks of FMF, there may be large sterile effusions with neutrophil counts of the magnitude seen in septic arthritis. The arthritis usually resolves spontaneously and is non-destructive. However chronic arthritis has been defined in some patients, especially those with severe mutations. There is debate whether chronic arthritis is a feature of FMF per se or an association. Patients with FMF may also be at increased risk of sacroiliitis, irrespective of HLA-B27 status or colchicine prophylaxis. Langevitz et al. found a 0.4% prevalence of sacroiliitis in patients with FMF [92]; however increased frequencies up to 7% and enthesitis has been reported [93]. Sacroiliitis should be considered in patients with FMF with low back pain [92].

Myalgia, mostly of the lower extremities, usually develops after strenuous exercise or prolonged standing. Protracted febrile myalgia syndrome (PFMS) is defined as severe disabling myalgia of at least 5 days' duration in a patient with FMF, usually associated with fever and elevated levels of inflammatory markers. This feature is more frequent among patients with at least one M694V mutation and is usually responsive to corticosteroid therapy [94, 95]. A high prevalence of fibromyalgia has also been reported in some case series [96].

16.6.2 Less Common Clinical Manifestations

Erysipelas-like skin lesion appears unilaterally on the extensor surfaces of the leg, over the ankle joint or dorsum of the foot, and occasionally even on the face, and is usually not associated with an attack. The lesion resembles erysipelas or cellulitis and is seen in 7–40% of patients with FMF. It resolves spontaneously within 2–3 days [23, 88]. The frequency has been decreasing both in adults and children.

Ongoing inflammation can put the patient at risk for various complications such as anemia and splenomegaly [97]. Splenomegaly has been described in older series with rather high frequencies [88]. If the diagnosis has been missed or there is colchicine resistance, growth retardation may be expected due to ongoing inflammation. A low bone density due to subclinical inflammation

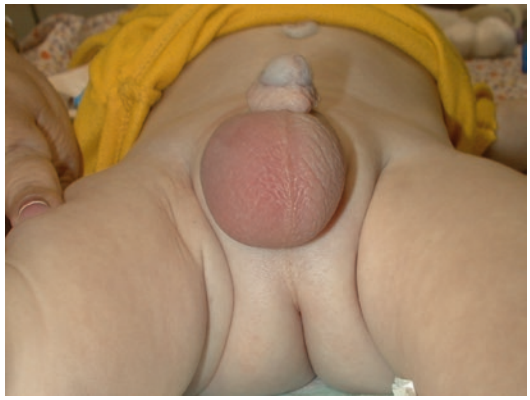

Fig. 16.1 Acute scrotal pain and swelling resulting from inflammation of the tunica vaginalis in a patient with familial Mediterranean fever (FMF)

has also been reported [98]. With better management the complications of chronic inflammation is rapidly decreasing.

A small percentage of males with FMF develop acute scrotal pain resulting from inflammation of the tunica vaginalis (Fig. 16.1) [99]. There have also been isolated reports of aseptic meningitis in FMF [100]. Microscopic hematuria can be seen in patients with FMF. Various types of glomerulonephritis such as IgA nephropathy and post-streptococcal glomerulonephritis have also been reported in patients with FMF [101].

16.6.3 Diseases Associated with FMF or *MEFV* Mutations

Vasculitis is one of the most common associated diseases in patients with FMF. It has been proposed that certain vasculitides are more common because of the enhanced innate immune response. IgA vasculitis (Henoch-Schonlein purpura) and polyarteritis nodosa (the latter usually more mild than idiopathic disease) tend to be more common among patients with FMF than in the general population [102, 103]. On the other hand, an increased association with other vasculitidies such as antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis has not been reported. Many, but not all, investigators found an increased frequency of *MEFV* mutations in cohorts of patients with Behçet disease [104]. Juvenile idiopathic arthritis (JIA) and inflammatory bowel disease was also found to be increased among patients with FMF (3.5%) when compared to the general population [30]. Also, it has been reported that patients with systemic JIA had a significantly higher frequency of *MEFV* mutations, however, this association is still not clear [105]. Recent studies have reported the presence of *MEFV* heterozygous variants in a substantial proportion of patients with periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome [106].

16.7 Amyloidosis

Before the colchicine era, amyloidosis was reported to occur in a large proportion of untreated patients with FMF. Amyloidosis in FMF is the result of tissue deposition of AA amyloid, which is a proteolytic cleavage product of the acute phase reactant serum amyloid A (SAA) [28].

The overproduction of SAA leads to the extracellular accumulation of fibrillar protein and the development of amyloidosis [107] (see Chap. 15). The clinical presentation of amyloidosis in FMF is mostly renal, progressing from proteinuria and nephrotic syndrome to kidney failure and end-stage renal disease.

In a study encompassing 2482 cases of FMF collected from 35 centers in 14 countries including 260 patients with renal amyloidosis, amyloid nephropathy was present in 11.4% of the cases. The country of residence (rather than country of origin) was the leading risk factor for renal amyloidosis in FMF, followed by M694V homozygosity, a family member with

amyloidosis (especially if amyloidosis occurred in the initial case of FMF in a family), and disease duration [26]. The risk for amyloidosis was in parallel to infant mortality rates in these countries, suggesting a strong epigenetic-environmental effect related to economic development and quality of environment overcoming the effect of the *MEFV* genotype. These authors suggested that prophylactic colchicine treatment for asymptomatic M694V homozygotes (phenotype III) living in countries with a low risk of renal amyloidosis is unwarranted [26]. SAA polymorphisms have been shown to contribute to the severity of FMF phenotype but have no effect on the clinical features of FMF. SAA1-α allele was strongly associated with amyloidosis in patients with FMF [108]. During systemic inflammation, SAA may promote the production of IL-1β in tissues. SAA-induced secretion of active cathepsin B may lead to extracellular processing of SAA, potentiating the development of AA amyloidosis [109, 110].

16.8 Laboratory Investigations

- **Acute phase proteins are elevated during an FMF attack and may remain above normal also between attacks**

Acute phase reactants such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and SAA increase during attacks. CRP and SAA levels may remain above normal during attack free periods in untreated patients and symptomatic heterozygotes [111]. SAA is a sensitive marker of chronic inflammation that helps guide treatment and monitors the treatment response [112]. It has been recently reported that CRP <5 mg/L in children and <8.75 mg/L in adults appear to be in good agreement with SAA <10 mg/L in attack free patients with FMF, especially if patients have an *MEFV* mutation other than homogenous M694V or are currently taking colchicine [113]. Leukocytosis may be present during attacks. A complete blood count, liver function tests, acute phase reactant and urinalysis should be checked every 6–12 months to monitor disease activity and damage. S100 protein levels have also been reported to increase in patients with FMF, however the commercial use is limited (see Chap. 9) [114].

A renal biopsy should be suggested in patients with FMF who develop proteinuria. The literature reports that secondary amyloidosis may also be diagnosed with rectal biopsy.

16.9 Diagnosis

- **Several classification/diagnostic criteria have been suggested to aid the physician in diagnosing FMF**
- **Genetic results should be interpreted carefully**

The diagnosis of FMF is based on clinical grounds and is supported by genetic testing. Ethnicity and family history are important in the diagnostic workup.

Since early diagnosis is important in preventing complications, a set of diagnostic and classification criteria have been proposed (see Chap. 11). The Tel-Hashomer criteria were the first to be proposed for adult patients [8]. There were later revised by Livneh et al. [115]. The four major criteria are typical attacks (lasting 12–72 h, >3 attacks of the same type with rectal temperature >38 °C) with any one of pleuritis, monoarthritis, peritonitis or fever alone. The minor criteria were defined as incomplete attacks, exertional leg pain and favorable response to colchicine. One major or 2 minor criteria, or 1 minor plus 5 supportive criteria should be satisfied to establish a diagnosis [115].

A set of pediatric criteria (Turkish pediatric criteria) were proposed in 2009 [116]. According to these criteria, the presence of at least two of the following is required: fever (lasting 6–72 h,

Table 16.1 Major genetic variants associated with familial Mediterranean fever (adapted from [7])

	Gene	Pathogen variants	Variants of unknown significance	Variants without clinical significance
FMF	*MEFV*	M694V, M694I, M680I, V726A, R761H, A744S, I692del, E167D, T267I	E148Q, P369S, F479L, I591T, K695R	R202Q, R408Q

Table 16.2 Diagnosis and treatment decisions regarding the analysis of the *MEFV* genotype (adapted from [8])

Homozygote for pathogenic mutations	FMF confirmed Start colchicine[a]
Compound heterozygote for two pathogenic mutations	Confirm that the mutations are on separate alleles Start colchicine[a]
One pathogenic and one uncertain mutation	Confirm that the mutations are on separate alleles Confirm elevated CRP or SAA levels Start colchicine
Two uncertain mutations or one clearly pathogenic mutation	Check again for other autoinflammatory diseases or other conditions Check for high CRP levels during attack and/or high SAA levels in between attacks Start colchicine
One uncertain mutation or none	FMF unlikely

FMF familial Mediterranean fever; *CRP* C-reactive protein; *SAA* serum amyloid A
[a]See Sect. 16.2, 16.7 and 16.10 for asymptomatic patients

≥3 attacks), abdominal pain (lasting 6–72 h, ≥3 attacks), chest pain (lasting 6–72 h, ≥3 attacks, unilateral), arthritis (lasting 6–72 h, ≥3 attacks, monoarthritis), exertional leg pain and family history of FMF [116]. The Eurofever group compared the performance of the existing criteria in an international cohort and found that the Turkish pediatric criteria had a better sensitivity (87.4%) but lower specificity (40.7%) than the Tel Hashomer criteria [117].

Consensus-based guidelines have been proposed for the genetic testing of hereditary recurrent fevers, including FMF [118] (see Chap. 12). According to these guidelines, it is recommended to test for 9 pathogenic variants and 5 variants of unknown significance (Table 16.1). Since most disease-causing mutations are located on exon 10, many FMF centers routinely sequence the entire exon 10.

The variants of unknown significance should be assessed carefully. If only one such variant is present in a patient with periodic fever, a careful work-up for other periodic fever diseases is mandatory. Ozen and Bilginer have suggested an algorithm based on the genetic test result to guide the diagnosis and treatment of these patients (Table 16.2) [119].

We and others have shown that heterozygote patients may display a typical FMF phenotype. Treatment with colchicine and evaluation at follow-up would be recommended in such patients, especially if there are elevated acute phase reactants. Care should be taken to distinguish these patients from the association of PFAPA (see Chap. 30 and Sect. 16.6.3).

Evidence based recommendations have been recently developed by the Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) initiative for the genetic diagnosis of FMF [120]. Patients homozygous for M694V mutation are at risk of early onset disease and severe phenotype. Also, patients carrying two of the common mutated alleles for mutations at position 680–694 on exon 10 must be considered at increased risk for having a more severe disease [120]. The E148Q variant is of unknown pathogenic significance and when found as the only *MEFV* variant does not support the diagnosis of FMF [120].

16.10 Treatment

- **The main treatment of FMF is with colchicine**
- **IL-1 inhibition should be reserved for patients who are resistant or intolerant to colchicine**

The aims of treating patients with FMF are to stop or reduce the frequency and severity of attacks, to suppress both chronic and subclinical inflammation and to provide a good quality of life. The need

for treatment of asymptomatic individuals with pathogenic mutations remains unclear. Physicians may treat these individuals with colchicine in families with a history of secondary amyloidosis or those living in the eastern Mediterranean basin.

16.10.1 Colchicine

Colchicine is the drug of choice for the treatment of patients with FMF, both decreasing the frequency and severity of attacks and preventing the development of amyloidosis [27, 121].

16.10.1.1 Colchicine Mechanisms of Activity

The prophylactic effect of colchicine in preventing FMF attacks can be explained by its capacity to inhibit the increased chemotactic activity during FMF attacks and its elevated concentration, mainly in neutrophils [55], with a mean neutrophil colchicine concentration twofold higher than in mononuclear cells [122] (see Chap. 40). The high concentration of colchicine in neutrophils, which exclusively express pyrin, may be due to the absence of the P-glycoprotein efflux pumps on their membranes [123, 124]. It is accepted that the therapeutic action of colchicine in FMF is caused mainly by reduction of leukocyte migration, but blocking signal transduction and gene expression also play a role [43, 125, 126].

Colchicine exerts its therapeutic effects by binding to beta tubulin thus inhibiting self-assembly and polymerization of microtubules and interfering with several cellular functions [127]. A number of mechanisms have been suggested for the mechanism of action of colchicine in FMF [128]. Microtubules are necessary for activation of the NLRP3 inflammasome [129]. It has been shown that microtubule depolymerizing drugs prevents pyrin-mediated caspase-1 activation and secretion of IL-1β and IL-18 from mouse macrophages and human peripheral blood mononuclear cells [130]. Regulation of the actin cytoskeleton by microtubules is mediated by the Rho family GTPases [131]. Colchicine also blocks activation of the pyrin inflammasome by blocking the oligomerization of pyrin with ASC, not by the upstream pyrin-dephosphorylation and 14-3-3 dissociation. Colchicine exerts various anti-inflammatory effects mostly related to microtubule disruption and depolymerization in a dose-dependent manner. In a recent study, the reorganization of the actin cytoskeleton in THP-1 cells by colchicine has been described. Additionally, colchicine has no consistent dose-dependent inhibitory effect on the activation of other inflammasomes.

16.10.1.2 Colchicine Treatment Recommendations

According to the recent European League Against Rheumatism (EULAR) recommendations treatment with colchicine should start as soon as a clinical diagnosis is made [95]. The recommended starting dose of colchicine in FMF is <0.5–0.6 mg/day for children less than 4 years of age (dependent on the type of colchicine tablet used); 0.9–1 mg/day for children 4–12 years of age; and 1–1.2 mg/day in children >12 years of age and in adults [95, 132]. The ideal dose should be determined according to the clinical response, type of mutations and tolerance of the patient. Higher starting doses should be considered in patients with high disease activity and/or amyloidosis [95]. Both CRP and SAA (if available) levels should be measured every 3–6 months while adjusting the colchicine dose in patients with active disease [95]. Subclinical inflammation and the attack frequency are important factors affecting the decision of dose increment. Colchicine can be given in single or divided doses, depending on the tolerance and compliance of patients [95, 133]. Colchicine treatment should be lifelong in definite FMF with two pathogenic mutations.

Recently a study has suggested that colchicine can be discontinued in children who carry only one mutation in the MEFV gene who are asymptomatic for a prolonged period with very close monitoring [134].

Adherence with the maximum dose of colchicine and other medications is essential for being able to evaluate whether the patient is resistant to colchicine. The Medication Adherence Scale in FMF patients (MASIF) has been recommended to assess and follow up the adherence to treatment in pediatric patients with FMF, especially of colchicine [135].

16.10.1.3 Colchicine Safety

Colchicine is a safe and generally well tolerated drug. At maximal suggested oral doses of colchicine (up to 1.8–2 mg/day in children and 3 mg/day in adults), serious or fatal adverse events are rare [23]. Gastrointestinal adverse effects are the most prevalent, especially abdominal pain, diarrhea, nausea and vomiting. Dose reduction, lactose free diet (and/or use of lactase before dairy products) and dividing the daily dose may relieve gastrointestinal symptoms [136]. Colchicine can cause mild and transitory increases in transaminase levels [137]. Thus, liver enzymes should be monitored regularly and if they are greater than twofold the upper limit of normal dose reduction and further investigations for the cause should be performed [95]. Vitamin B12 deficiency, reversible peripheral neuritis, myopathy and bone marrow suppression are rare adverse effects of colchicine, especially in patients with concomitant renal disease [138, 139]. Colchicine is also a safe drug in both pregnancy and lactation and should not be discontinued during conception. Current evidence does not support amniocentesis for detecting teratogenicity associated with colchicine [95].

Colchicine toxicity is a serious complication. It should be used cautiously in patients with impaired hepatic or renal function [95, 140]. Colchicine is a substrate for the P-glycoprotein 1 efflux transporter and the cytochrome P450 3A4 isoenzyme. Concomitant administration of drugs that inhibit these enzymes such as cyclosporine, ketoconazole, ritonavir, clarithromycin (but not azithromycin), verapamil and diltiazem may increase plasma colchicine levels [141], requiring a reduction in the dose of colchicine [142].

16.10.2 Interleukin (IL)-1 Inhibition in Colchicine-Resistant FMF

Unfortunately, approximately 5% of patients do not respond to the highest tolerable dose of colchicine [23, 143]. Although there is no consensus on the definition of colchicine resistance, in the EULAR recommendations FMF experts define resistance as patients with one or more attacks per month who adhere to treatment with colchicine at the maximally tolerated dose for at least 6 months [95]. Persistence of high levels of acute phase reactants is also important. One needs to assess adherence to treatment when defining colchicine resistance. In patients with FMF resistant to colchicine, IL-1 blockade has been the focus of therapy since its role in the pathogenesis of FMF is evident. Several case studies and controlled studies have shown that anakinra and canakinumab may be effective in patients with FMF resistant to colchicine [144–148]. A recent review of the literature has reported a 76.5% complete response in patients treated with anakinra and 65.7% on canakinumab treatment, where complete response is defined as cessation of attacks and normal acute phase reactants [149]. In a recently published randomized controlled trial, anakinra appears to be an effective and safe treatment for patients with FMF resistant to colchicine [113, 150, 151]. In another study, rilonacept reduced the frequency of attacks of FMF in a randomized, double blind study [152]. Case series and two phase II studies in FMF have shown that canakinumab is an effective and a safe treatment in colchicine-resistant patients [153–157]. A recent phase 3 double-blind controlled trial has confirmed the efficacy and safety of canakinumab in patients with hereditary periodic fevers, including colchicine-resistant FMF [158]. In the first 16 weeks of the trial study patients were randomized to receive 150 mg canakinumab (2 mg/kg in children <40 kg) or placebo every 4 weeks and significantly more patients (61% vs. 6%) achieved complete remission, defined as resolution of the index attack at randomization by day 15 and the lack of development of attacks during these 16 weeks. A further 10% of patients who developed an attack on the initial dose of canakinumab and whose dose was therefore increased blindly to 300 mg every 4 weeks (4 mg/kg in patients <40 kg) also achieved complete remission (overall 71%). CRP and SAA levels decreased significantly compared to placebo and there was a significant improvement in the physician global

disease assessment. In the subsequent phase of the trial it was demonstrated that almost half of the patients were able to maintain excellent disease control on an 8-week canakinumab dosing interval. Canakinumab is labeled by the United States Food and Drug Administration and European Medicines Agency for the treatment of colchicine-resistant FMF. Colchicine should be continued while receiving these biological therapies, since it is not known whether IL-1 blockade prevents amyloidosis [95].

16.10.3 Other Treatment Issues

16.10.3.1 Treatment of Acute Attacks

Treatment of an acute attack of FMF is aimed at relief of pain with rest, analgesics and nonsteroidal anti-inflammatory drugs [95]. Increasing the dose of colchicine is not recommended during an attack. Fluid administration may decrease the intensity of attacks. However, there is no clear evidence on the efficacy of short-term administration of narcotics during attacks.

16.10.3.2 Treatment of Chronic Arthritis

Disease modifying anti-rheumatic drugs or anti-TNF agents have been found to be effective in FMF patients with chronic arthritis and/or sacroiliitis [159].

16.10.3.3 Treatment of Protracted Febrile Myalgia Syndrome (PFMS)

Corticosteroid treatment is required and effective in suppressing the symptoms of PFMS [94, 160, 161]. Anakinra has also been found to be beneficial in two patients with PFMS [162].

16.10.3.4 Biologic Treatment of Amyloidosis

In patients with established amyloidosis anti-IL-1 treatment can reverse proteinuria [149]. Also, tocilizumab has been found to improve the acute phase response and the renal function in patients with FMF complicated with AA amyloidosis [163].

16.11 Outcome

- **An activity index and several severity scores are available to assess outcome in FMF**
- **Most of the patients adherent to treatment will have a good quality of life**

16.11.1 Outcome Measurement Tools in FMF

Several activity, severity, damage and outcome scores have been developed to improve our knowledge about autoinflammatory diseases in general and FMF in particular. Many still need to be validated.

The Autoinflammatory Disease Activity Index (AIDAI) has been created and validated by the Eurofever, Eurotraps and Pediatric Rheumatology International Trials Organization (PRINTO) groups [164, 165] (see Chap. 13). A number of severity scores have been developed in FMF, especially for adults [166, 167]. A new severity score, the International Severity Score for FMF (ISSF) has been developed by the FMF Arthritis Vasculitis and Orphan Disease Research in Pediatric Rheumatology (FAVOR) group [168]. Recently, the Autoinflammatory Disease Damage Index (ADDI) has been established, however this index still needs further validation to measure applicability [169]. The FMF50 is a score for assessing outcome in FMF [170]. In the CLUSTER study the primary outcome was resolution of the index attack and no attacks up to Week 16. Secondary outcomes were physician global assessment <2 (out of 5), CRP ≤10 mg/L, and SAA levels ≤10 mg/L [158].

16.11.2 Outcome of Patients with FMF

Early diagnosis and effective treatment can prevent irreversible organ damage. The most significant complication of FMF is secondary amyloidosis. There has been a significant decrease in the rate of secondary amyloidosis in Turkey [171]. The main reason for this decrease

is better medical care with increased awareness and treatment of disease. Furthermore, the accumulating data indicates that anti-IL-1 drugs are effective in treating patients with colchicine-resistant patients with FMF and improving their quality of life.

16.11.3 Future Challenges

Presently, there are no definitive diagnostic biochemical markers for defining an attack, as opposed to the non-specific increases in acute phase reactants and S100 proteins. The diagnosis of FMF remains a challenge since many variants have reduced penetrance and cannot always be identified on both alleles. Although it has been more than 20 years since the gene and the protein associated with the disease has been defined, more data are still needed to better understand the pathogenesis, to diagnose and to manage patients with FMF.

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Mevalonate Kinase Deficiency

17

Joost Frenkel and Anna Simon

Abstract

Mevalonate kinase deficiency (MKD) is a rare autoinflammatory disease caused by loss of function mutations in both alleles of *MVK*, the gene encoding the enzyme mevalonate kinase. Deficiency of this enzyme results in impaired isoprenoid biosynthesis. The inflammatory attacks in MKD are characterized by fever, lymphadenopathy, gastrointestinal symptoms, aphthous ulcers, rash, arthralgias and/or arthritis. Severely affected patients may in addition have neurological involvement, cataract, uveitis, and failure to thrive, often dying in early childhood. This severe end of the phenotypic spectrum is called mevalonic aciduria (MA) as opposed to the milder phenotype also known as hyperimmunoglobulinemia D periodic fever syndrome (HIDS). In this chapter, we detail clinical phenotype and pathophysiological background as well as treatment options.

Keywords

Mevalonate kinase deficiency · Mevalonic aciduria · Periodic fever
Hyperimmunoglobulinemia D syndrome
Autoinflammatory · Isoprenoid metabolism

Abbreviations

CAPS	Cryopyrin-associated periodic syndrome
CRP	C-reactive protein
DSAP	Disseminated superficial actinic porokeratosis
EMA	European Medicines Agency
FDA	Food and Drug Administration (FDA)
FDPS	Farnesyl diphosphate synthase
FMF	Familial Mediterranean fever
GC-MS	Gas chromatography-mass spectrometry
HIDS	Hyperimmunoglobulinemia D syndrome
HMG-CoA	Hydroxymethylglutaryl-coenzyme A
IgD	Immunoglobulin D
IL-1	Interleukin-1
MA	Mevalonic aciduria
MK	Mevalonate kinase

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MKD	Mevalonate kinase deficiency
MVD	Mevalonate decarboxylase
MVK	Mevalonate kinase gene
NSAIDs	Non-steroidal anti-inflammatory drugs
PBMC	Peripheral blood mononuclear cells
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, adenitis
PMVK	Phosphomevalonate kinase
SAA	Serum amyloid A
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic syndrome

Key Points

- **Mevalonate kinase deficiency is an autosomal recessive disorder caused by mutations in the gene for mevalonate kinase**
- **The clinical spectrum varies from a phenotype known as hyperimmunoglobulinemia D syndrome (HIDS) to mevalonic aciduria (MA)**
- **Inflammatory episodes are characterized by fever, lymphadenopathy, aphthous ulcers, skin rash, abdominal pain, myalgia and arthralgia**
- **Other symptoms, especially in patients of the MA phenotype, include progressive cerebellar ataxia, psychomotor retardation, dysmorphic facies, liver dysfunction, hematological abnormalities and early death**
- **Treatment of inflammatory symptoms currently focusses on interleukin (IL)-1 inhibition; for severe abnormalities, stem cell transplantation may be warranted**

17.1 Introduction

Mevalonate kinase deficiency (MKD) is an autosomal recessive disorder linked to two clinical phenotypes, mevalonic aciduria (MA, MIM610377) and hyperimmunoglobulinemia D syndrome (HIDS, MIM 260920), as the ends of a spectrum of disease, where many patients present with an overlap of symptoms. It is in some ways a unique autoinflammatory syndrome, since it combines the inflammatory features with those of an inborn error of isoprenoid metabolism.

17.2 Epidemiology

MKD is very rare. International series of patients, including the Eurofever registry (see Chap. 14) generally contain less than 200 patients [1, 2]. A German pediatric surveillance study involving 370 children's hospitals identified 16 cases of MKD in a 3-year period, resulting in an estimated incidence of MKD at 0.39 per 1 million person-years in Germany [3]. Approximately 75% of patients with MKD in the registries are from Western Europe, and 50% are from the Netherlands and France [2] or from Italy and the Netherlands [1]. Most MKD patients are of Caucasian origin. These observations can be explained partly by a founder effect [4]. In the Netherlands, the carrier frequency of the most common mevalonate kinase mutation is 1:153 [5]. There is also likely to be a reporting or recognition bias [1]. Males and females are affected equally [1, 2, 6].

17.3 Etiology and Pathogenesis

Key Points

- **Mevalonate kinase deficiency (MKD) is caused by loss of function mutations in both alleles of *MVK***
- **MKD impairs isoprenoid biosynthesis**
- **Shortage of the isoprenoid geranylgeranylpyrophosphate affects small GTPases**
- **Impaired function of the small GTPase RhoA activates the pyrin inflammasome**
- **Interleukin (IL)-1β is an important mediator of inflammation in MKD**

17.3.1 Etiology

MKD is an autosomal recessive inborn error of metabolism. Loss-of function mutations in *MVK* give rise to a reduced function of the biosynthetic enzyme mevalonate kinase [7, 8]. Over 200 sequence variants have been reported in *MVK*, of which 194 are possibly or certainly disease associated. Mutations occur in all 11

exons of *MVK* and range from single amino acid substitutions to frame shift mutations and premature termination [9]. Some mutations are incompatible with the production of any enzymatically active protein. However, such null mutations are never observed in a homozygous state, but only in compound heterozygosity with milder mutations. This indicates that some residual enzyme activity, however small, is required for intrauterine survival. Residual enzyme activity in these cases may be below the lower limit of detection. Such severely deficient individuals often present with the severe (MA) phenotype. Combinations of milder mutations that result in a residual MKD between 1% and 15% commonly give rise to a pure autoinflammatory phenotype, known as HIDS [10, 11]. The most common of these is the c.1129G > A (p.V377I) variant. The change of valine to isoleucine allows for considerable residual enzyme activity. The phenotype in individuals homozygous for this mutation ranges from clinically unaffected (personal observations) to frequent severe inflammatory attacks. Heterozygous carriers are clinically unaffected.

Mevalonate kinase enzyme is present in the cytoplasm of all nucleated cells. Its physiological function is to catalyze the conversion of mevalonate to phosphomevalonate, a crucial step in the isoprenoid biosynthesis pathway. The isoprenoid pathway yields many lipid products. These include cholesterol and all sterols derived from it, as well as the non-sterol isoprenoids. The latter are linear polyunsaturated hydrophobic molecules of varying length that can be covalently attached to target proteins (Fig. 17.1). The transfer of such hydrophobic groups to specific proteins is a post-translational modification known as protein isoprenylation. Isoprenyl groups are essential for the subcellular localization and function of proteins like the small GTPases Ras, Rac, and RhoA [12].

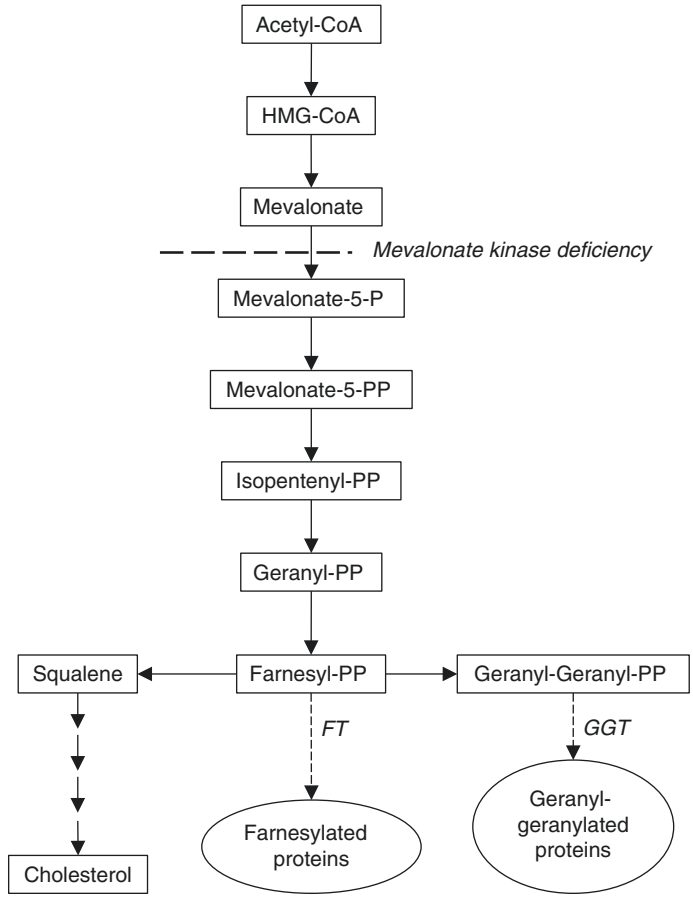

Fig. 17.1 Isoprenoid biosynthesis pathway. Acetyl-CoA is converted to hydroxy-methyl-glutaryl (HMG)-CoA and subsequently to mevalonate, the substrate of mevalonate kinase. The block in mevalonate kinase deficiency is indicated by the horizontal dashed line (------). Mevalonate kinase deficiency (MKD) leads to accumulation of its substrate, mevalonate and shortage of end-products, notably geranylgeranyl pyrophosphate. Geranylgeranyl transferase (GGT) and farnesyltransferase (FT) covalently link the respective isoprenoid groups to target proteins

17.3.2 Pathogenesis

MKD leads to the accumulation of the mevalonate kinase substrate, mevalonate, as well as reduced production of isoprenoid end-products and hence a shortage of isoprenylated proteins [13, 14]. The accumulated mevalonic acid is excreted in the urine. Its presence in urine during attacks supports the diagnosis of MKD [15].

The clinical phenotype is highly variable and is loosely related to the severity of enzyme deficiency [6, 11]. The mechanism by which the metabolic defect gives rise to the clinical and immunological features of the disease is only partly understood.

Interleukin (IL)-1β is an important mediator of inflammation in mevalonate kinase deficiency as reflected by the favorable effect of treatments which cause IL-1 blockade [16]. However, there are likely to be other mechanisms involved, since IL-1 blockade is less successful in preventing symptoms than in purely IL-1 mediated disease such as the cryopyrin-associated periodic syndrome (CAPS). Serum γ-interferon rises during attacks [17], as do tumor necrosis factor (TNF)-α and IL-6 [18]. In addition, patient mononuclear cells stimulated ex vivo produce not only excess IL-1β, but also TNF-α, especially when studied during inflammatory attacks [18, 19].

There is currently no satisfactory explanation for the pathogenesis of the neurologic, ocular and renal problems that may occur in severely affected individuals. There are two possible mechanisms by which MKD could promote inflammation: excess of the accumulating substrate mevalonate and shortage of end-products of the isoprenoid biosynthesis pathway.

Several lines of evidence suggest that it is the shortage of one specific non-steroid isoprenoid, geranylgeranyl-pyrophosphate, which is responsible for the inflammatory features of the disease. The hyper-secretion of IL-1β by patient cells can be abolished by exogenous geranylgeranyl pyrophosphate. Conversely, mononuclear cells from healthy controls produce excess IL-1β when protein geranylgeranylation is blocked [20, 21].

Recently, it was shown that geranyl-geranylated RhoA was required to silence pyrin, the protein affected in familial Mediterranean fever. RhoA acts via protein kinase N which phosporylates pyrin to allow binding of inhibitory 14-3-3ε proteins. Shortage of geranyl-geranylated RhoA results in the assembly of pyrin-inflammasomes and hence the proteolytic activation of IL-1β. In cells from individuals deficient in mevalonate kinase, either exogenous geranylgeranyl pyrophosphate or bypassing RhoA by direct activation of protein kinase N restores the binding of inhibitory 14-3-3ε proteins to pyrin and reduces IL-1β secretion to normal [22].

Other mechanisms by which mevalonate kinase deficiency may contribute to inflammation include defective isoprenylation of the small GTPase Kras, which in turn leads to a PI3-kinase-AKT mediated activation of the NFκB transcription pathway [23]. Finally, it has been suggested that shortage of a leukocyte specific steroid end-product of the isoprenoid biosynthesis pathway, 25-hydroxy cholesterol, could lead to enhanced transcription and proteolytic activation of IL-1β [24, 25].

Excess mevalonate within mononuclear phagocytes might also contribute to the inflammatory phenotype of MKD. Mevalonate was shown to induce trained immunity in murine monocytes, i.e. an epigenetically altered state leading to increased production of pro-inflammatory cytokines TNFα, IL-6 and IL-1β [26].

As for the role of immunoglobulins in MKD, serum IgD as well as IgA may be highly elevated in many patients with MKD. It is unknown how the metabolic defect gives rise to this. The physiological role of serum IgD is poorly understood as is its role, if any, in the pathogenesis of inflammation in MKD remains unknown.

17.4 Clinical Manifestations

Key Points

- **Inflammatory episodes in MKD are characterized by fever, lymphadenopathy, abdominal pain, arthralgia, myalgia, aphthous ulcers and rash**
- **In the MA phenotype, patients also suffer from progressive cerebellar ataxia, psycho-**

motor retardation, dysmorphic features, liver dysfunction and hematological abnormalities

- **Some familial forms of porokeratosis have also been associated with mutations in the mevalonate kinase gene**

MKD is classically described as consisting of two different phenotypes, the more severe MA and the less severe HIDS. It is important to realize that in clinical practice it is rather a continuous spectrum of disease severity. However, for the sake of clarity we will describe the two phenotypes at the extreme ends of the spectrum.

17.4.1 Hyperimmunoglobulinemia-D Syndrome (HIDS) Phenotype

HIDS is a disease characterized by exacerbations, commonly described as 'fever/inflammatory attacks', and symptom-free periods of remission. A typical inflammatory attack of HIDS lasts about 4–6 days, although shorter and longer attacks occur. The usual age of onset of the inflammatory attacks is before the end of the first year of life, although in rare cases and milder phenotypes, a later age of onset has been reported [1, 2, 27].

Sometimes, patients recognize prodromes like general malaise and fatigue. Chills are often an early sign of an attack, followed by a rapid rise in temperature. Accompanying signs and symptoms of an attack (Table 17.1) include tender cervical lymphadenopathy and abdominal pain with vomiting and diarrhea [1, 2, 6, 27].

Table 17.1 Accompanying signs and symptoms during an inflammatory attack of mevalonate kinase deficiency (MKD)

• Lymphadenopathy, splenomegaly, hepatomegaly
• Abdominal pain, diarrhea, vomiting
• Arthralgia, arthritis, myalgia
• Erythematous skin lesions
• Aphthous ulcers
• Headache
• Pharyngitis
• Conjunctivitis

Many patients have large joint arthralgia or arthritis. Oral or genital aphthous ulcers can occur. Skin lesions are common, and may include erythema, papules, purpura or urticaria-like rash (Fig. 17.2).

Rare accompanying signs or sequela of an inflammatory attack include pericarditis, macrophage activation syndrome [1, 6], erythema nodosum, uveitis or protracted fever and inflammation. In some patients, a severe inflammatory attack may be accompanied by frank colitis, which in rare cases has even been described as the presenting feature [28].

Hepatosplenomegaly may be present, especially prominent during an inflammatory attack; on imaging, hypodense lesions may be seen in either, which disappear during remission [29].

Ophthalmic features include retinitis pigmentosa and cataract [30], though rare (e.g. 2–3% reported in the Eurofever series [1]) and less severe than in the MA phenotype.

The series by Bader-Meunier et al. [6] reported recurrent infections in 13 of 49 patients with MKD (which included both MA and HIDS phenotypes), including otitis media, sinusitis and pneumonitis. Infections can be hard to distinguish from inflammatory attacks.

The frequency of attacks varies within and between patients. On average attacks occur every 4–6 weeks. The frequency tends to be highest in early childhood, and attacks may decrease later in life although this is not always the case. Factors that can trigger an attack include vaccination, infection, trauma and both physical and emotional stress, though often there is no clear trigger [1, 2, 6, 27]. It is characteristic in HIDS that the first attack is triggered by a childhood vaccination, with an age of onset in the first year of life. Growth and development in children with HIDS is typically normal.

Not much is known about fertility in men or women with HIDS. During pregnancy, women usually experience a significant reduction in disease activity. Childbirth can be a trigger for an attack of HIDS in the mother (personal observations).

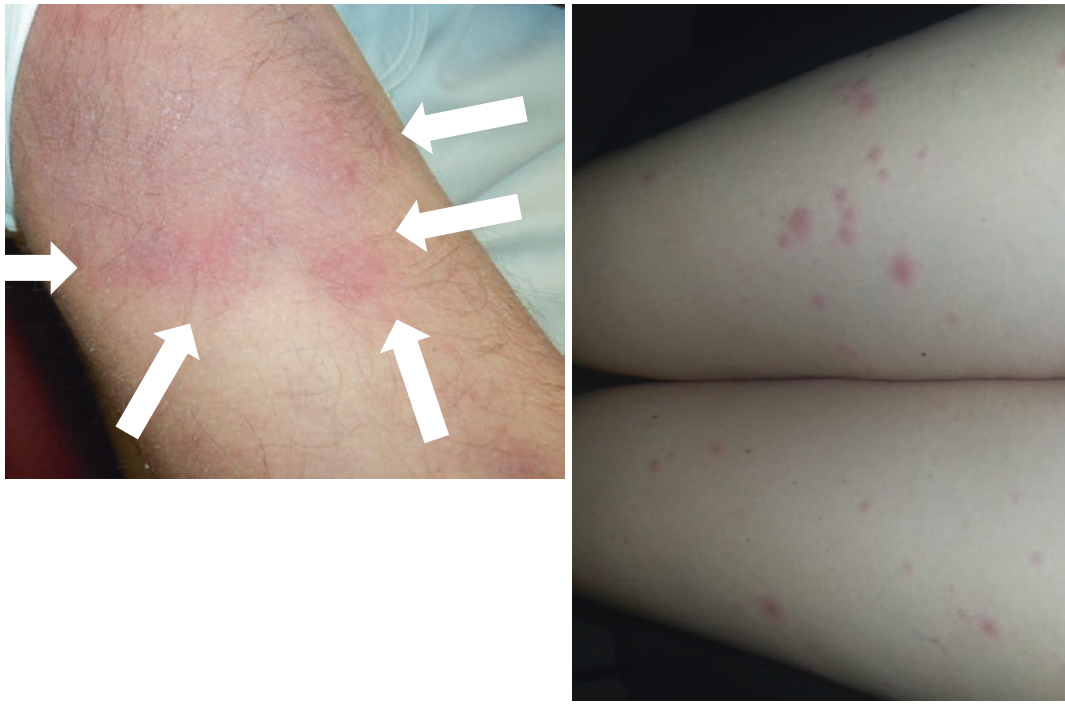

Fig. 17.2 Skin lesions as seen in inflammatory attacks of mevalonate kinase deficiency (MKD)

17.4.2 Mevalonic Aciduria (MA) Phenotype

Severe inflammatory attacks, as seen in HIDS, are also experienced by patients with severe MA. In addition, these patients suffer from continuous symptoms and signs from birth, which are often very severe [31]. These symptoms include psychomotor retardation (varying from mild to severe), progressive ataxia and dysarthria with cerebellar atrophy (developing after infancy), muscular hypotonia, failure to thrive, and cataracts. Patients with MA can have facial dysmorphic features including dolichocephaly, frontal bossing, posteriorly rotated, low-set ears and down-slanted eyes [31]. Hepatosplenomegaly and lymphadenopathy are common, becoming more prominent during inflammatory attacks. Cholestatic liver disease may be present [32, 33]. Sometimes, severe hematological abnormalities are prominent, including anemia and thrombocytopenia [33]. These may indicate the development of macrophage activation syndrome.

Many patients with MA die in early childhood; in Hoffmann's series of 11 children with MA, 4 patients died within the first 4 years of life [31].

In patients with MA, a progressive cerebellar atrophy, which develops after infancy, can be seen on neuroimaging [31]. Neurological follow-up is recommended in these cases. Retinitis pigmentosa may develop and warrants regular ophthalmologic assessment in MA.

17.4.3 Other Clinical Phenotypes Associated with Mutations in Mevalonate Kinase

Rare cases of seemingly isolated retinitis pigmentosa linked to MKD have been described [34], although detailed medical history revealed mild episodes of inflammation in childhood in some of those patients, or signs of ataxia.

Apart from these systemic syndromes, the skin disorder porokeratosis has been linked with heterozygous mutations in the mevalonate kinase gene, inherited in an autosomal dominant manner

[35–38]. Porokeratosis is a disorder of keratinization, characterized by atrophic macules or patches. Other patients with this skin disorder have been found to carry mutations in other genes in the mevalonate pathway, including those for phosphomevalonate kinase (PMVK), mevalonate decarboxylase (MVD) and farnesyl diphosphate synthase (FDPS) [36]. There are several clinical types of porokeratosis 3 (MIM175900), including disseminated superficial actinic porokeratosis (DSAP) and porokeratosis of Mibelli. These patients do not have fever or other extracutaneous symptoms. The mutations seem to lead to reduced expression of the mutant alleles, so presumably reduced activity of the isoprenoid pathway.

17.5 Laboratory Investigations

The inflammatory attacks in MKD are characterized by a high acute phase response, with very high concentrations of C-reactive protein (CRP), serum amyloid A (SAA), and elevated erythrocyte sedimentation rate (ESR). Leukocytosis with distinct neutrophilia is seen. These typically return to normal between attacks, but subclinical or minimally symptomatic inflammatory episodes can occur.

In the MA phenotype, severe hematological abnormalities and liver function abnormalities can also occur, which are usually persistent [32, 33].

Many patients with MKD, but by no means all, have high serum concentration of IgD and/or IgA [39–41]. This does not increase further during inflammatory attacks [42], and serum IgD or IgA concentrations are not correlated to disease severity. A French study included 50 patients referred to one center because of symptoms resembling the HIDS phenotype of MKD, of whom 24 did have MKD mutations, and 26 did not have MKD [41]. They found 19 out of 24 patients with MKD had high serum IgD (sensitivity 79%), while 19 out of 26 patients without MKD also had high serum IgD (specificity 27%) [41]. There is no role for repeat measurement of these immunoglobulins in the routine follow-up of patients.

AA amyloidosis is a rare but severe complication of MKD (see Sect. 17.8, and Chap. 15). For early detection of the first signs of AA amyloidosis it is recommended to check for proteinuria on an annual basis.

17.6 Diagnosis

The first steps in the diagnostic process are taking a detailed medical history, and preferably seeing a patient during an inflammatory attack. During an inflammatory attack, an acute phase response should be detectable; if there is no raised CRP, the diagnosis of MKD can be excluded. Serum IgD or IgA concentration can be determined as an intermediate step in diagnosis, but sensitivity and specificity, as previously noted (Sect. 17.5) are low [41].

An evidence-based clinical criterion to safely exclude the diagnosis of MKD and prevent unnecessary further diagnostic procedures for MKD was developed in a cohort of 149 French patients (among whom 35 had MKD), and validated on 93 Dutch patients (of whom 28 had MKD) [43]. This study showed that the diagnosis of MKD can be excluded in patients who had their first fever attack at age of 5 years or higher in combination with an attack duration of more than 14 days or absence of joint pain [43].

Patient data from the Eurofever database was used to generate evidence-based clinical classification criteria within a group of autoinflammatory disorders, including MKD [44] (see Chaps. 11 and 14). This is a statistical scoring system based on clinical features observed during typical inflammatory attacks (with exclusion of intercurrent infections or other comorbidities). For MKD, the criteria were age at onset <2 year (10 points), aphthous stomatitis (11 points), generalized enlargement of lymph nodes or splenomegaly (8 points), painful lymph nodes (13 points), diarrhea sometimes/often (20 points), persistent diarrhea (37 points) and absence of chest pain (11 points). At a cut-off sum of 42 points or more, this set of criteria gave a sensitivity of 93% and specificity

of 89% in the validation cohort [44]. These criteria should only be used after careful exclusion of other causes of fever and inflammation, such as infections, immunodeficiency, malignancy or other immunologic conditions. These criteria can help to direct further diagnostic testing in patients with a suspected autoinflammatory disease.

To confirm a diagnosis of MKD, it is necessary to either find evidence for decreased MK enzyme activity, or to detect two pathogenic mutations in the gene for the enzyme. The preference for either method depends largely on availability of diagnostic tests. The genetic test is the most readily available.

There are two main methods to examine MK enzyme activity. The first is to determine the amount of mevalonic acid, the substrate for MK, in the urine. In MA, the urinary mevalonic acid concentration is usually persistently very high, and easily detected. However, in patients with the HIDS phenotype, the mevalonic acid spike in the urine will only be detectable during an inflammatory episode [45–47], and requires a sensitive method such as gas chromatography-mass spectrometry (GC-MS) [15]. One retrospective study in a single expertise center found a sensitivity of 92% and a specificity of 90% for detection of increased urinary mevalonic acid, when compared to the gold standard of genetic mutations in the *MVK* gene [15]. The range of mevalonic acid excretion in the urine in patients with MKD (with HIDS phenotype) in this study was from 1 to 5000 mmol/mol creatinine [15].

The second method is to determine the enzyme activity directly in patient-derived cell lines, such as Epstein Barr virus-converted lymphoblast cell lines or immortalized fibroblast cell lines. This is commonly only done in research setting, but can be diagnostic.

A recent study showed that defective protein prenylation, as demonstrated with a prenylation assay on lysates from peripheral blood mononuclear cells (PBMCs) from patients, could be a diagnostic marker as well [13]. This needs further study, and is currently available only in a research setting.

The differential diagnosis of the HIDS phenotype in MKD in young children includes periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) syndrome (see Chap. 30), other (hereditary) autoinflammatory disorders or recurrent (viral) infections. For the MA phenotype, the differential diagnosis includes other metabolic disorders.

17.7 Treatment

Key Points

- **IL-1 inhibition is currently the most effective treatment for inflammatory symptoms of MKD**
- **In severe cases, especially with neurological and/or metabolic abnormalities in young children, stem cell transplantation may be warranted**

Management of patients with MKD warrants a multidisciplinary approach (see Chap. 13) [48]. Aims of treatment include control of disease activity, prevention of disease-related damage and improvement of health-related quality of life.

17.7.1 Control of Inflammatory Episodes: Anti-inflammatory Treatment

Non-steroidal anti-inflammatory drugs (NSAIDs) may provide symptom relief during inflammatory episodes, but do not shorten the episode [48]. Especially in children, short-term corticosteroids taken as soon as the first symptoms of an inflammatory attack manifest itself, may be effective in alleviating symptoms [48], however in case of frequent episodes this may result in a high cumulative dose with detrimental effects.

IL-1 inhibition is currently the most effective treatment for the inflammatory symptoms in MKD. This has been shown in case reports and case series for continuous treatment with the short-acting IL-1 inhibitor anakinra (dose in adults 100 mg/day by subcutaneous injection; in children starting dose of 2 mg/kg/day, may increase to 5–8 mg/kg/day) [48, 49]. It has also been shown

that it is possible to treat inflammatory episodes with anakinra "on-demand", started at the first prodrome of an episode [50]. In this study, when anakinra was started as soon as the fever occurred, the episode could be shortened to 2–3 days duration [46]. In clinical practice, if patients start anakinra at the first sign of prodrome it is even more effective. "On demand" use of anakinra should only be advocated in patients with relatively infrequent episodes. At a frequency of episodes of more than 1 episode per 4–6 weeks, it is recommended to switch to continuous treatment.

The long-acting anti-IL1β antibody canakinumab has been shown to be effective in HIDS to reduce the number and severity of inflammatory episodes [49, 51–53]. An open-label phase II study assessing the efficacy and safety of canakinumab was performed in nine patients (six pediatric patients and three adults) with the HIDS phenotype of MKD [53]. Patients had to have active disease, defined as at least 3 inflammatory episodes in a 6-month period. During a 6-month treatment period with 300 mg canakinumab (or 4 mg/kg for those weighing ≤40 kg once per month), the number of inflammatory episodes per patient decreased significantly (median number of attacks 0, range 0–2), with 7 of 9 patients experiencing no inflammatory episodes for the whole period [53]. In the 24-month extension treatment period that was part of this study, in which 8 of 9 patients participated, only 8 inflammatory episodes occurred (median number of attacks per patient 0, range 0–4).

Results from a double-blind placebo controlled study indicate that canakinumab (150 mg/4 weeks) is superior to placebo with 35% of patients attaining complete disease remission within 2 weeks and persisting without attacks for 16 weeks (versus 6% on placebo). When the dose was raised to 300 mg/4 weeks the percentage of patients with complete remission increased to 57% (versus 71% of patients with colchicine-resistant familial Mediterranean fever (FMF) and 73% of patients with TNF receptor-associated periodic syndrome (TRAPS) in the same study) [16]. In a subsequent phase of the trial, patients with response to canakinumab were re-randomized to receive either canakinumab (in the dose of 150 or 300 mg depending on the previous response) or placebo, once every 8 weeks. In that phase, only 23% of patients with MKD maintained disease control (versus 46% of patients with colchicine-resistant FMF and 53% of patients with TRAPS) upon this lower dosing frequency [16]. This study resulted in an Food and Drug Administration (FDA) and European Medicines Agency (EMA) registration for canakinumab in MKD in patients ≥2 years of age, at a recommended starting dose of 150 mg once every 4 weeks for patients with a body weight >40 kg (can be increased to 300 mg once every 4 weeks if clinical response is inadequate); or 2 mg/kg once every 4 weeks in patients with body weight between 7.5 and 40 kg (can be increased to 4 mg/kg every 4 weeks if clinical response is inadequate).

In patients with MKD who do not respond to IL-1 inhibitors, inhibition of TNF (etanercept, adalimumab) or IL-6 (tocilizumab) can be tried. Efficacy of these inhibitors has been shown in several case reports and in the Eurofever data [50, 54–57].

As for oral options of treatment, colchicine and thalidomide are ineffective in MKD [48, 58]. Following the good effect of simvastatin, an inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme one step prior to mevalonate kinase (Fig. 17.1), in a patient with HIDS phenotype a placebo-controlled trial was performed in six additional patients. This trial showed a small decrease in the number of days of illness per 6 months [46], however, in clinical practice, patients do not notice a significant benefit of statins. In one patient with the MA phenotype, treatment with a statin appeared to induce a severe attack [31]. Treatment with statins is therefore not recommended in MKD. One case report showed a good effect of weekly oral alendronate in a 14-year old patient with MKD (HIDS phenotype) [59]. Bisphosphonates such as alendronate are inhibitors in the isoprenoid metabolism downstream from mevalonate kinase, which have an effect on isoprenylation; this observation needs further study.

Macrophage activation syndrome (see Chap. 33) can occur as a rare but serious complication of an inflammatory attack in MKD, especially in

Table 17.2 Reports of stem cell and/or organ transplantation in early childhood in severe cases of mevalonate kinase deficiency (MKD) with mevalonic aciduria (MA) phenotype

Symptoms prompting transplantation	Sex	Age at transplantation	Details on transplantation	Outcome	Reference [number]
Severe episodes of inflammation every 2 weeks, severe hepatosplenomegaly	M	3 years	Stem cell from HLA-identical sister, heterozygous for *MVK* mutation	Follow-up 15 months No fever episodes, size of liver and spleen reduced to normal; no further progression of neurological symptoms	Neven et al. (2007), [62]
Severe episodes of inflammation	M	8 years	Stem cell from sibling	Follow-up 16 months, no more episodes of inflammation; chronic mild graft-versus-host disease (after acute onset)	Arkwright et al. (2007), [61]
Severe liver disease, severe episodes of inflammation	F	2.5 years	Liver from deceased donor	Improvement of liver function, some neurological improvement, no effect on episodes of inflammation	Chaudhury et al. (2012), [63]
Severe episodes of inflammation (same girl as above)	F	6.5 years	Stem cell, HLA identical unrelated donor	No further inflammatory episodes	Chaudhury et al. (2012), [63]
Severe episodes of inflammation and mild psychomotor delay	M	2 years, 10 months	Cord blood, unrelated	Follow-up 5 years. No more episodes of inflammation, normal psychomotor and neurological development, normal growth	Giardino et al. (2015), [64]
Severe hepatosplenomegaly and ascites	M	6 months	Bone marrow from HLA identical sister	Died of sepsis 3.5 months after transplant	Erdol et al. (2016), [66]

HLA human leukocyte antigen, *MVK* mevalonate kinase

children and in patients with severe phenotype (towards the MA end of the spectrum) [60].

17.7.2 Stem Cell Transplantation

Successful treatment with stem cell transplantation in early childhood has been described in several cases of severe MKD at the MA end of the spectrum [61–65] (Table 17.2).

Stem cell transplantation is an option for treatment in patients with MA, with prevention of attacks of fever and inflammation, resolution of hepatosplenomegaly and hematological abnormalities, and there are also indications that it can prevent further neurological deterioration. The selection of patients and the timing of the procedure should be done in consultation with physicians experienced in the disorder.

17.7.3 Treatment of Other Symptoms, Especially in MA

Little is known about treatment options for the symptoms and signs in MA not directly related to inflammation, such as progressive cerebellar atrophy. Conflicting results are available, and only from case reports. These include experience with supplementation with several end-products or by-products of isoprenoid metabolism, such as cholesterol, ubiquinone-10, ursodeoxycholic acid and vitamin E [31].

17.8 Outcome/Prognosis

Prognosis in MA is poor with early mortality and severe developmental delay. Early stem cell transplantation seems a promising option.

However, some patients with MA survive into adulthood with limited disabilities. Patients with the HIDS phenotype have a better prognosis. In many patients, the frequency and severity of inflammatory episodes decrease in adulthood. However, a substantial proportion of patients still suffer from regular attacks of fever in adulthood.

AA amyloidosis can occur as a complication of long-term inflammation (see Chap. 15) and has been described in several patients with MKD [1, 66–69]. The percentage of AA amyloidosis in MKD is estimated to be about 4–5% (in the Eurofever series of 114 patients, 5 patients had AA amyloidosis), which is lower than in some of the other hereditary autoinflammatory syndromes [1, 67]. The reason for this lower incidence (even when only compared to patients with other autoinflammatory disorders from developed countries) is unclear. There is no genotype correlation with mutations in *MVK* or SAA genotype, and patients with MKD can also have increased inflammation markers while symptoms are in remission [70].

Progressive retinitis pigmentosa can occur. A survey of 50 patients with MKD revealed three patients who developed renal angiomyolipoma [6]. Flexion contractures and abdominal adhesions can occur as rare long-term complications of the recurring inflammatory attacks [1].

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18

Tumor Necrosis Factor (TNF) Receptor-Associated Periodic Syndrome (TRAPS)

Sinisa Savic and Michael F. McDermott

Abstract

Tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) is an autosomal dominant hereditary disease, caused by heterozygous mutations in *TNFRSF1A,* which encodes for TNF-receptor 1 (TNFR1). Most of the pathogenic mutations are single-nucleotide missense variants localized in extracellular, cysteine rich domains of the receptor. The pathogenesis of TRAPS is complex and likely involves several mutually non-exclusive molecular mechanisms, however, co-expression of the mutated and wild type of the receptor is required in all cases. The proposed mechanisms include abnormal TNFR1 cleavage; increased activation of nuclear factor kappa B (NF-κB)/mitogen-activated protein kinase; ligand-independent activation of mutant TNFR1; generation of mitochondrial reactive oxygen species (ROS) leading to enhanced activation of the NLRP3 inflammasome; TNFR1 misfolding and retention within the endoplasmic reticulum (ER) leading to activation of ER-associated endonuclease, inositol-requiring enzyme 1 (IRE-1) and resulting in hyper-responsiveness to lipopolysaccharide via selective degradation of microRNAs (miRs).

The majority of patients with TRAPS are symptomatic from childhood, with the median age of symptom onset reported to be about 4 years. Most patients report episodic attacks of fever, with serositis manifesting as abdominal and/or chest pain, myalgia with or without typical overlying migratory rash, arthralgia and arthritis. The minority of patients will have continuous symptoms, and many will have biochemical evidence of systemic inflammatory response even in the absence of symptoms. Prior to effective therapies, systemic amyloidosis was found in up to 15% of patients. The diagnosis of TRAPS still depends on molecular genetic analysis for conformation since formal diagnostic criteria have yet to be developed. Anti-interleukin (IL)-1 biological agents are currently the first choice of treatment for patients who require ongoing therapy.

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Keywords

TNF-receptor 1 (TNFR1) · Endoplasmic reticulum (ER) stress · Mitochondrial reactive oxygen species (ROS) · Inositol-requiring enzyme 1 (IRE1) · Anakinra, canakinumab

Abbreviations

3-MA	3-Methyladenine
ADAM	A disintegrin and metalloproteinase
CAPS	Cryopyrin-associated periodic syndrome
CRD	Cysteine-rich domains
CRP	C-reactive protein.
DF	Dermal fibroblasts
DMARDs	Disease-modifying anti-rheumatic drugs
ER	Endoplasmic reticulum
FHF	Familial Hibernian fever
FMF	Familial Mediterranean fever
IκB	I kappa beta
IKK	I kappa B kinase
IL	Interleukin
IRE1	Inositol-requiring enzyme 1
JNK	c-Jun N-terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
miR	MicroRNA
MKD	Mevalonate kinase deficiency
mROS	Mitochondrial ROS
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor-κB
NLRP3	NACHT, LRR and PYD domains-containing protein 3
NOX	NADPH oxidases
NSAIDs	Nonsteroidal anti-inflammatory drugs
OXPHOS	Oxidative phosphorylation
PCR	Polymerase chain reaction
PERK	Protein kinase (PKR)-like endoplasmic reticulum kinase
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
PGA	Physician global assessment
RIP	Receptor-interacting protein
ROS	Reactive oxygen species
SAA	Serum amyloid A
TACE	TNF-alpha converting enzyme
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNFR1	TNF receptor 1
UPR	Unfolded protein response
XBP1	X-box binding protein 1

Key Points

- **Tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) is a rare autosomal dominant hereditary disease with an estimated incidence of 1/100,000 in populations of Northern-European ancestry**
- **Almost all pathogenic mutations are located in exons 2–6 of *TNFRSF1A*, which code for the extracellular domains of the receptor**
- **The pathogenesis is complex and might be mutations specific**
- **Targeted inhibition of interleukin (IL)-1β is the mainstay of therapy**

18.1 Introduction and Epidemiology

Tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) is an autosomal dominant hereditary disease, which was originally termed familial Hibernian fever (FHF), following the first published clinical description of the condition in a Scottish-Irish family in 1982 [1]. Subsequently, additional patients of Irish, Scottish, and mixed Scottish/Irish heritage were identified, and genome wide linkage analysis of these families lead to the identification of chromosome 12p13 as a likely location for the TRAPS gene [2, 3]. The gene was identified in 1999 as *TNFRSF1A,* encoding TNF receptor 1 (TNFR1) [4]. The term FHF was dropped in favor of TRAPS, which better reflects the molecular basis of this disorder and the fact that TRAPS is not

confined to one ethnic group, but is, in fact, found at similar frequencies in several other ethnicities [5]. Nevertheless, this is a rare condition with an estimated incidence of 1/100,000 in populations of Northern-European ancestry.

18.2 Etiology: The Genetic Basis of TRAPS

- **The most damaging mutations disrupt the cysteine-rich domains of the extracellular part of TNF receptor 1 (TNFR1)**
- **The more common variants such as R92Q and P46L are typically associated with milder clinical phenotypes, and can also be found in asymptomatic individuals**

TNF receptor 1 (TNFR1), a 55 kDa molecule (p55), is one of two receptors for the proinflammatory cytokine TNF, and is encoded by *TNFRSF1A* on chromosome 12p13.2. The other receptor is TNFR2 or p75, encoded by *TNFRSF1B* on chromosome 1p. Although both receptors have similar structures and belong to a family of membrane proteins, with repeating cysteine-rich extracellular motifs, to date only mutations affecting the TNFR1 receptor have been reported to cause TRAPS. In addition, these pathogenic mutations almost exclusively affect the extracellular domains of the receptor, and no mutation that affects the intracellular domains of the protein, or resulting in null mutations (in which the protein is not expressed) have been clearly associated with the TRAPS clinical phenotype. Another clue to the potential pathological mechanisms underpinning TRAPS is the observation that the most severe mutations seem to disrupt the cysteine-rich domains (CRD) of the extracellular portion of TNFR1 by interfering with one of the disulfide bonds, such as C88Y, or hydrogen-bond stabilization, for example T50M, that keep these structures in place. According to the new nomenclature these mutations are now designated C117Y and T79M. On the other hand, R92Q (new nomenclature R121Q) and P46L (new nomenclature P75L) variants are not only found at a much higher frequency in certain populations, 1–4% of Caucasian control chromosomes for R92Q [6], and approximately 2% of African-American and Arab control chromosomes for P46L [7], the clinical phenotypes associated with these variants are much broader and often milder than classic TRAPS [8]. Furthermore, these variants are not always associated with a disease phenotype and can also be found in asymptomatic individuals. To date there are over 150 variants in *TNFRSF1A* gene catalogued on the INFEVERS Website (http://fmf.igh.cnrs.fr/infevers/) (accessed April 2018) and the vast majority are confined to exons 2–6 (Fig. 18.1).

18.3 Pathogenesis/ Immunopathology

- **The pathogenesis of TRAPS is complex and involves multiple but non-mutually exclusive mechanisms**
- **Expression of the mutated TNFR1 alongside the wild type variant is essential for the disease pathogenesis**
- **Intracellular retention of the mutated receptor is associated with several pathological responses including increased endoplasmic reticulum (ER) stress, excessive mitochondrial reactive oxygen species (mROS) and enhanced nuclear factor κB (NF-κB) activation**

Unlike the pathophysiology of other hereditary fevers, for example cryopyrin-associated periodic syndrome (CAPS), where there is one predominant mechanism, in the case of TRAPS several alternative, but mutually non-exclusive, mechanisms have been proposed over the years. This, in part, might be due to the complexity of TNF signalling pathways, which simultaneously govern multiple, and often opposing, cellular functions; for example, cell survival and apoptotic cell death, which have several levels of control [9, 10]. One way of controlling the TNF signalling pathways is to generate soluble forms of TNF, TNFR1 and TNFR2 [11]. TNF is produced mainly by epithelial cells, the cells of the

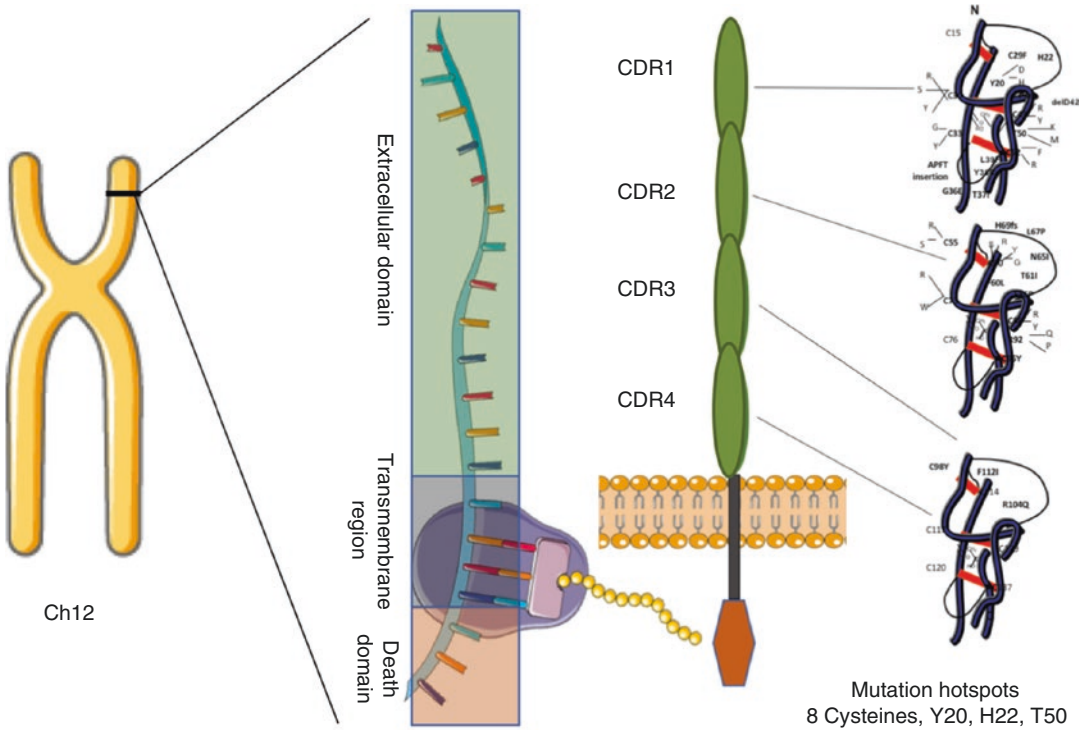

Fig. 18.1 Schematic representation of the *TNFRS1* gene, tumor necrosis factor (TNF) receptor 1 (TNFR1) protein and mutations associated with the TNF receptor-associated periodic syndrome (TRAPS)

innate immune system and some T-cell subsets. It is expressed as a homotrimer on the cell surface and engages the similar membrane-bound homotrimers of TNFR1 and TNFR2 [12]. Both the receptors and the ligand are cleaved off the cell surface by the TNF-α converting enzyme (TACE/a disintegrin and metalloprotease domain (ADAM)17) [13]. The soluble forms of the receptors are thought to have a neutralizing effect on the TNF in the circulation. Early studies into the pathogenesis of TRAPS suggested that it is precisely this lack of the soluble TNFR1 which might be responsible for the hyperinflammatory state associated with TRAPS. The extracellular location of the pathogenic mutations was thought to interfere with proteolytic cleavage of TNFR by TACE/ADAM17, and, although the 'shedding' defect was observed in some patients, this was not universally present [14]. Furthermore, etanercept, a fusion molecule of TNFR2/FcIg, designed to neutralize soluble TNF, is only partially effective in patients with TRAPS [15, 16] and has been superseded by the use of anti-interleukin (IL)-1β therapies (see below Sect. 18.7). Therefore, additional immunopathological mechanisms, other than the 'shedding defect', must be operative in TRAPS. An important clue as to what these mechanisms might be comes from animal models of the disease. Thus, in the murine model of TRAPS, expression of the disease phenotype is dependent on expression of the mutated receptor alongside the wild type. The mice which were homozygous for TRAPS-associated mutations were resistant to lipopolysaccharide (LPS)-induced toxic shock, rather than exhibiting any tendency towards spontaneous inflammation, which is typical of TRAPS [17]. It seems that it is necessary to have a combination of TNF signalling via wild type receptor, associated with the presence of the mutated form, to result in dysregulated inflammatory response(s) and the disease phenotype.

Several studies have examined the specific role of the mutated TNFR1 receptor in this

situation. Early studies have shown that the mutated receptor does not get expressed at the cell surface, but instead remains in an intracellular location [18]. This could have several consequences, including the possibility that the mutant intracellular TNFR1 may retain partial function and exert a role independent of activation of receptor-ligand binding [19]. This is supported by the fact that the intracellular death domains of TNFR1 are rarely mutated in TRAPS; however, direct evidence for such a mechanism is lacking.

Another, and possibly more important consequence of the receptor retention, is the causation of endoplasmic reticulum (ER) stress and an unfolded protein response (UPR) in affected cells. In cell lines transfected with mutated TNFR1, the receptor tends to accumulate within the ER [20]. The physiological response of the cell to intracellular accumulation of the misfolded protein is to increase the production of chaperone proteins, in order to help with transit of the mutated protein from the ER, and also to reduce protein translation and, therefore, the burden on the ER. Ultimately, if these measures are inadequate to deal with the ER stress, the cell may die by apoptosis. These are all traditional elements of the UPR, but there are also other physiological effects triggered by this process. Upregulation of UPR response genes has been reported in TRAPS patients [21]. In a study of 16 patients with TRAPS with different mutations, 22 healthy controls and HEK293 wild type and mutant cellular transfectants, increased splicing of X-box binding protein 1 (sXBP1), a key UPR transcription factor, was detected, alongside increased protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) phosphorylation. These increased responses were seen in monocytes from patients with TRAPS and in HEK293 mutants compared with healthy controls and wild type HEK293 cells.

Interestingly, six other UPR-related genes which were also tested, were not differentially upregulated, suggesting that the UPR in TRAPS was limited in scope or atypical in nature. This seemingly selective or non-canonical activation of XBP1 is thought to have a proinflammatory effect in TRAPS and, in turn, explains the propensity of monocytes derived from TRAPS patients to respond excessively to LPS stimulation. Intriguingly, XBP1 splicing, which is dependent on activation of inositol-requiring enzyme 1 (IRE1), was previously described in the context of LPS activation of toll-like receptor (TLR) 4 [22]. The same study also showed that sXBP1 binds to the promoters of both TNF and IL-6 genes. Therefore, in cells from patients with TRAPS, pre-existing sXBP1 could potentiate LPS-induced signalling via TLR4 and, at the same time, prime the cells towards a higher production of proinflammatory cytokines. A more recent study has suggested another mechanism to explain LPS hyper-responsiveness, whereby activated IRE1, which is one of the three ER stress sensors, exerts its endonuclease function and targets a variety of mRNA and miR species, and, in this way, limits protein production and helps to resolve ER stress [23, 24]. It seems that other specific targets of IRE1 also include miR155 and miR146a, two miRNA species which have previously been identified as regulating the magnitude of cellular response to LPS [25]. Using a graded challenge, it was established that miR-146a was necessary for prevention of TLR4 responses, at sub-inflammatory doses of LPS, which may be relevant to maintaining tolerance to the host's own microbiome. On the other hand, miR-155 was found to limit TLR4 responses following exposure to higher proinflammatory doses of LPS, and thus, failure to upregulate these miRs may lead to chronic hyper-responsiveness of the TLR4 pathway. Using dermal fibroblasts (DF) from patients with TRAPS, it was shown that both miR155 and miR146a are downregulated in TRAPS prior to LPS stimulation, and that levels of these miRs fail to increase following LPS challenge [26]. This situation was corrected by using a specific IRE1 inhibitor, showing that miR155 and 146a were both targeted by activated IRE1. Furthermore, pre-treatment of healthy donor DFs with IL-1β resulted in the same abnormal miRs response which was seen in TRAPS DF, and, again, was dependent on IRE1. Altogether, these findings suggest a model whereby mutated TNFR1 protein misfolding activates the IRE1

branch of the UPR. IRE1 activation leads to cleavage of miR-146a and miR-155, and consequently results in hyper-responsiveness to LPS and increased proinflammatory cytokine production, such as IL-1β and IL-6. Subsequently, the paracrine effects of these cytokines, as well as the proinflammatory local environment, observed in TRAPS, maintain the UPR activation state with eventual degradation of miR-146a and miR-155.

A shift in the cell's metabolic state has recently emerged as an important physiological mechanism whereby inflammatory responses are regulated. For example, inflammatory macrophages have been shown to have enhanced glycolytic metabolism and impaired mitochondrial oxidative phosphorylation (OXPHOS), similar to activated dendritic cells [27–30]. This might be particularly relevant to TRAPS since increased reactive oxygen species (ROS) has been observed in cells from patients with TRAPS and in cells transfected with TRAPS-associated TNFR1, both at baseline and after stimulation with LPS [31]. Furthermore, increased IL-6 production in response to LPS, seen in cells harboring TRAPS-related TNFR1 mutants, could be reduced by using anti-oxidant treatment [21]. This effect seems to be related specifically to mROS, since nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) did not have any effect, but the antioxidants targeting mROS specifically did [31]. The enhanced inflammatory cytokine production is mediated by sustained phosphorylation of c-Jun N-terminal kinase (JNK) kinase and p38, and maintained by ROS from dysfunctional mitochondria [31]. Indeed, ROS may inactivate mitogen-activated protein kinase (MAPK) phosphatases and perpetuate MAPK activation [32]. Increased JNK and p38 in cells from patients with TRAPS are associated with enhanced activation of nuclear factor κB (NF-κB), resulting in translocation of this transcription factor to the nucleus and augmented transcription of the proinflammatory cytokines IL-1β, IL-6 and TNF [32]. The importance of these cytokines in the pathogenesis of TRAPS is supported by the therapeutic success of the biological drugs that, in particular, block IL-1β, and to a lesser extent TNF.

Lastly, impaired autophagy has also been reported in monocytes from patients with TRAPS. The main impact of this seems to be on clearance of the mutated receptor and restoration of the wild type TNFR1 to the cell membrane. It appears that inhibition of autophagy rather than the proteasome degradation pathway leads to intracellular accumulation of mutated and wild type receptors [33]. Furthermore, using geldanamycin to promote autophagy or, alternatively, the autophagy inhibitor 3-methyladenine (3-MA), it was demonstrated that the normal ultra-structural appearance in TRAPS monocytes and the membrane localisation of TNFR1 could be restored, following stimulation of autophagy [33, 34]. Another consequence of impaired apoptosis is increased levels of p62 protein which have been shown to be increased in some TRAPS mutations. p62 is typically degraded by autophagy, and, normally, has a role in various processes including ubiquitination and intracellular aggregate formation. More specifically, it is involved in caspase 8 and receptor-interacting protein (RIP) activation, the latter leading to RIP-dependent I kappa B kinase (IKK), I kappa beta (IκB) and ultimately NF-κB activation [35]. However, p62 also promotes NLRP3 degradation, which should balance out the proinflammatory effects of increased p62 levels. In the context of TRAPS, it is possible that the activating effects of p62 on RIP outweighs the autoinflammatory role of this protein on NLRP3, resulting in an overall inflammatory phenotype [34]; however, this still needs to be experimentally validated.

In summary, there appears to be several pathogenic mechanisms, including non-canonical UPR, dysregulated ROS and impaired autophagy, all operating synergistically to enhance proinflammatory cytokine production (Fig. 18.2). It is possible that some of these mechanisms are mutation specific, which explains the heterogeneity of pathological processes and clinical manifestations associated with TRAPS.

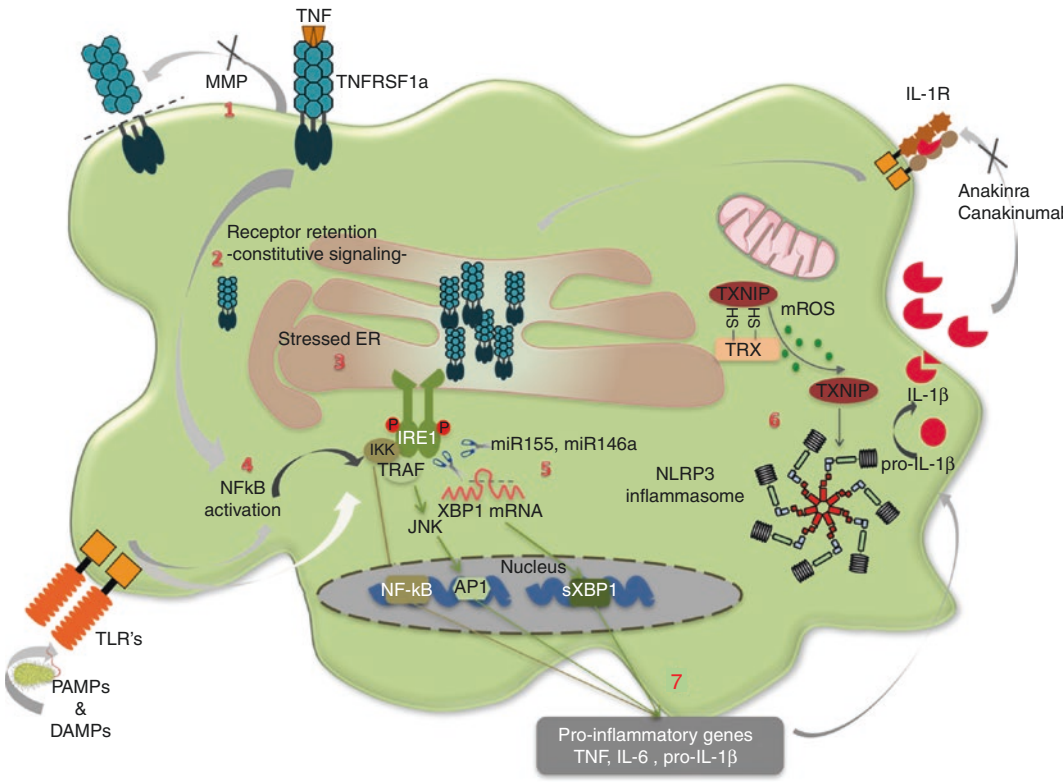

Fig. 18.2 Various molecular mechanisms involved in the pathogenesis of tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS). *1* Impaired cleavage of the mutated receptor from cell surface resulting in reduced negative regulation of the TNF signalling pathway; *2* intracellular retention and ligand-independent activation of mutant TNF receptor 1 (TNFR1); *3* endoplasmic reticulum (ER) stress because of accumulation of the mutant receptor leading to inositol-requiring enzyme 1 (IRE1) activation; *4* increased nuclear factor-κB (NF-κB) activation mediated by multiple mechanisms including the retained receptor (2), toll-like receptor (TLR) activation and IRE1 endonuclease-mediated destruction of miR155 and miR146a as illustrated in *5*; *6* reactive oxygen species (mROS) contributes to enhanced NLRP3 activation, with interleukin (IL)-1β and IL-18 processing; *7* increased production of proinflammatory cytokines

18.4 Clinical Presentation and Features

- **Most patients will first develop symptoms during childhood. However, late presentation in adulthood has been described**
- **The course of the disease varies from episodic flares (the majority), continuous symptoms to clinically asymptomatic individuals but with biochemical evidence of systemic inflammatory response (raised C-reactive protein and/or serum amyloid A)**
- **Fever and serositis presenting as chest and abdominal pain, arthralgia, migratory skin rash and myalgia, are the most common clinical features**
- **A more distinct and milder clinical phenotype is associated with low penetrance variants (R92Q and P46L)**

18.4.1 Overview of Clinical Manifestations

Early clinical reports of patients with TRAPS (FHF) described individuals suffering from febrile attacks, typically accompanied by severe abdominal pain and localized myalgia associated with

Table 18.1 Clinical features reported in the tumor necrosis factor receptor-associated periodic syndrome (TRAPS) and features highly predictive of the disease

General and organ specific manifestations	Clinical features previously reported in TRAPS	Clinical features highly predictive of TRAPS[a]
Family history	Sporadic or autosomal dominant	Family history (score 7)
Duration of fevers/episode	1–3 weeks, continuous	>6 days (score 19)
Cutaneous	Centrifugal migratory erythema, edematous plaques, annular and serpiginous patches, urticaria-like lesions	Typical migratory rash (score 18)
Musculoskeletal	Muscle cramps, migratory myalgia, fasciitis, arthralgia, oligo- or monoarthritis, sacroileiitis	Myalgia (score 6)
Gastrointestinal	Abdominal pain, peritonitis-like tenderness, vomiting	Absence of vomiting [14], absence of aphthous stomatitis [15]
Ocular	Periorbital edema, conjunctivitis, ocular pain, uveitis	Periorbital edema (score 21)
Respiratory	Chest pain, pleurisy	Nil
Central nervous	Headache, aseptic meningitis, optic neuritis, behavioral abnormalities	Nil
Urogenital	Urethral strictures, scrotal pain	Nil
Cardiovascular	Pericarditis, myocarditis, ventricular tachycardia, restrictive cardiomyopathy, risk of myocardial infarction and arterial thrombosis	Nil
Lymphatic	Swollen and painful lymph nodes	Nil
Renal	Amyloidosis-related nephrotic syndrome	Nil
Autonomic nervous system	Orthostatic hypotension, bowel disturbance	Nil

[a] Several of clinical characteristics were shown to be highly associated with TRAPS. Those features were given a score depending on the strength of the association [54]. A score ≥43 is highly suggestive of TRAPS

migratory erythematous patchy rashes. Conjunctivitis, unilateral periorbital edema, arthralgia and pleuritic pain were also frequently seen and, typically, these patients responded to moderate or high dose corticosteroids. Prior to the genetic identification of TRAPS, other kindreds, with similar clinical features, had been described, and were usually labelled as autosomal dominant familial periodic fevers, clinically different from familial Mediterranean fever (FMF), not only in their mode of inheritance, but also by the fact that these patients did not respond to colchicine [3]. This distinction from FMF was important in identifying these patients with a novel clinical syndrome, which was later confirmed to be TRAPS. Unlike FMF, which in most patients is characterized by distinct attacks of fever and inflammation, patients with TRAPS have attacks that may either be discrete, lasting anywhere between 1 and 3 weeks, and/or almost continuous. Furthermore, the incidence of amyloidosis was historically much lower than in FMF [5].

As more cases were identified the wider spectrum of clinical manifestations associated with TRAPS became more obvious (Table 18.1), Various triggers have been associated with the onset of attacks such as minor infection, injury, variable stress, exercise and hormonal changes. However, attacks are often spontaneous; the initial stages of an attack frequently manifest as muscle cramps or centrifugally migratory myalgia that precedes the onset of fevers, and others symptoms.

18.4.2 Dermatologic Manifestations

Skin rashes are frequent and TRAPS may be associated with variety of cutaneous manifestations; such as erysipelas-like erythema, edematous plaques and urticaria which have all been described in individual patients with TRAPS [36, 37]. Both dermal perivascular lymphocytic and monocytic infiltrates are seen on skin

biopsies [36]. The classic erythematous migratory patch skin lesions are both warm to the touch and painful and their appearance can evolve during the course of an attack. At onset, erythematous macules or papules can be found, either isolated or in groups, but as the attack progresses, these tend to migrate in a centrifugal direction to form larger plaques at the limb extremities (Fig. 18.3), or on the torso [38]. The associated myalgia is also migratory and although MRI studies have demonstrated underlying muscle edema, subsequent histological findings revealed this to be mainly due to monocytic fasciitis [39, 40].

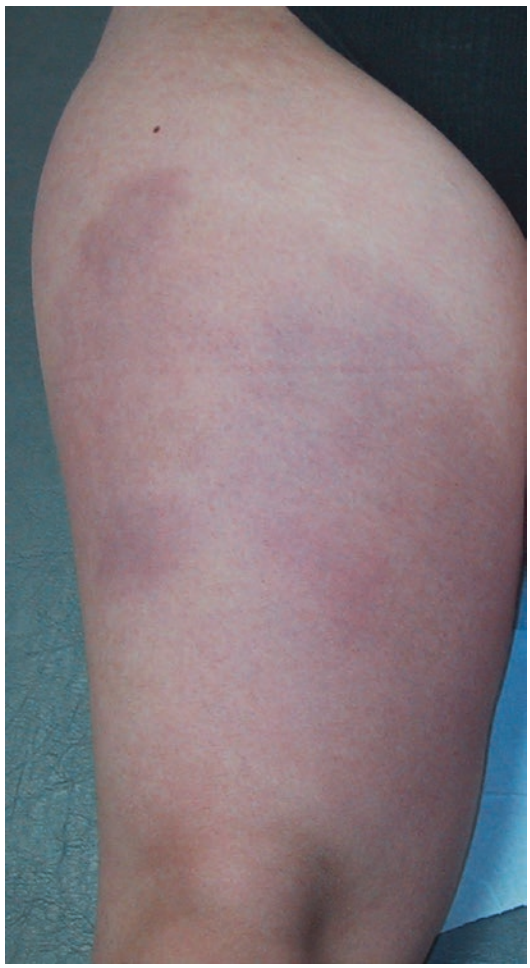

Fig. 18.3 Characteristic painful migratory erythematous migratory patch in a patient with the tumor necrosis factor receptor-associated periodic syndrome (TRAPS). Courtesy of Dr. Anna Simon, Radboud University Medical Center, Nijmegen, Netherlands

18.4.3 Other Manifestations

Serositis is frequent and is associated with chest and abdominal pains. Abdominal pain can also originate from abdominal wall muscle involvement [41]. Interestingly, isolated pericarditis, often with a chronic course, can be associated with low-penetrance *TNFRSF1A* variants [42]. Arthralgia is far more common than frank arthritis, and when present, usually is non-erosive, asymmetrical oligo- or monoarthritis with a predilection for large joints [43]. Lastly, as far as the more common manifestations are concerned, periorbital edema, when it occurs, is pathognomonic of TRAPS, and can be associated with eye pain, uveitis or conjunctivitis (Fig. 18.4). More rare clinical manifestations include scrotal swelling and pain, aseptic meningitis and behavioral changes [44, 45].

18.4.4 Clinical Data from Registries

The Eurofever/Eurotraps registry has produced the most detailed and comprehensive data on the broad range of clinical features encountered in patients with TRAPS ([46]; see Chap. 14). A report from this registry describes the largest single cohort of adult (n = 105) and pediatric patients (n = 53). The median age of symptom onset for the whole group was 4.3 years, with some patients having symptoms almost from birth (age 2 months), and some reported to have developed symptoms for the first time much later in life, up to the age of 63 years. The onset of TRAPS in adulthood was seen in 22% of patients. At the onset of disease, before commencing regular therapy, most patients (88%) reported an episodic course of their symptoms, 7% described their symptoms to be continuous and 5% had a continuous disease pattern with episodic flares. Although the median attack duration for the whole cohort was reported to be 10.8 days, there was significant variation between patients. The majority (43%) reported attacks lasting between 7 and 14 days, and, for the rest, roughly similar proportions of patients (around 28% each) experienced attacks lasting >14 days, or had shorter

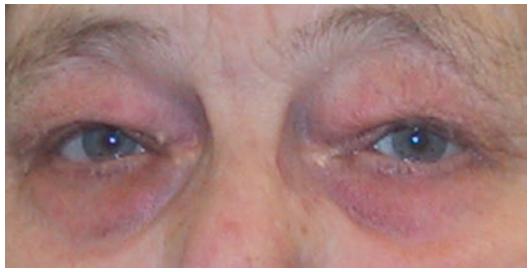

Fig. 18.4 Characteristic periorbital edema in a patient with the tumor necrosis factor receptor-associated periodic syndrome (TRAPS). Courtesy of Dr. Anna Simon, Radboud University Medical Center, Nijmegen, Netherlands

attacks lasting <7 days. In the majority of cases, attacks had an irregular pattern, not occurring at regular intervals (58%). In only 25% of all cases, specific triggers were attributable to attacks, including emotional stress (21 patients), menstrual cycle (17), fatigue (10), infection (9), exercise (7) and vaccination (6). The most common symptoms reported by all groups included fever (88%), followed by limb pain (85%), abdominal pain (74%) and rash (63%). Interestingly, periorbital edema, which was thought to be a cardinal feature of TRAPS, was reported by only 20% of all patients, and was more common in patients with childhood onset of TRAPS.

Patients carrying what are considered to be low penetrance variants, R92Q and P46L, comprise almost a third of the TRAPS cohort registered with the Eurofever database. When analysed separately this group of patients shows a distinct disease phenotype, which was milder overall compared to patients carrying mutations involving cysteine residues (see below), and also, for example, the T50M variant, which is associated with more severe disease and amyloidosis. The disease phenotype was also somewhat different in patients having a lower incidence of familial disease, as in general, they experienced more headaches with fewer rashes and eye manifestations. These findings were broadly replicated by a number of other studies, which also analyzed both clinical features and long-term outcomes, including the incidence of systemic amyloidosis, which, so far, has not been seen in this group of patients [8, 47–49]. Furthermore, in some cases, patients with the low penetrance variants can present with a clinical picture resembling the periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome [50]. Interestingly, R92Q has been found at a higher frequency in other inflammatory disorders, including patients with early arthritis [51] and Behçet disease [52]; however, the precise contribution of R92Q variant to these phenotypes is still unknown.

18.5 Laboratory Investigations

The clinical investigations and diagnosis of patients with TRAPS include many of the same general principles applied to other autoinflammatory diseases. In most cases, when faced with a new patient presentation, where there is no known family history, the exclusion of more common conditions, such as infections, autoimmune diseases, and, particularly in adults, malignancy is mandatory (see Chaps. 11 and 38). It is also often helpful to ask patients to record their symptoms, as well as measuring their body temperature and to have some of the routine laboratory investigations performed during acute episodes of the illness. Although routine laboratory tests are usually not diagnostic, these should show some evidence of elevated acute phase response proteins during acute attacks. The attacks are typically associated with the presence of neutrophilia and thrombocytosis (particularly in children), in addition to elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin and fibrinogen levels. Although these variables may fluctuate considerably with attacks, in some patients they remain elevated, even between attacks. In untreated cases, there is often evidence of anemia of chronic disease with polyclonal hypergammaglobulinemia [53]. Previous work in this disease had already shown that soluble TNFR1 levels tend to be subnormal between attacks and, in contrast to other inflammatory disorders, increase only modestly during active

disease [4]. However, this test has not been adopted widely in routine clinical practice.

As part of routine follow up and as a measure of disease control, patients should have regular laboratory tests including complete blood counts and inflammatory parameters, such as CRP and SAA, if available. In addition, regular testing for proteinuria will help to screen for development of complications such as amyloidosis.

18.6 Diagnosis

- **There are no agreed clinical diagnostic criteria for TRAPS; genetic analysis of *TNFRSF1A* gene is required to confirm the diagnosis**
- **The presence of periorbital edema, duration of episodes lasting more than 6 days, typical migratory rash, and absence of aphthous stomatitis and vomiting are suggestive of TRAPS**

The diagnosis of TRAPS is largely dependent on molecular genetic analysis for confirmation, unlike some other hereditary fevers such as FMF and CAPS, for which formal diagnostic criteria have been developed. In an attempt to generate supporting evidence based clinical classification criteria for TRAPS and related hereditary fevers syndromes, a group of experts had analyzed clinical information from well-defined groups of patients with FMF, mevalonate kinase deficiency (MKD), CAPS and TRAPS [54]. For each hereditary fever syndrome, patients were considered to be the 'gold standard' based on the presence of a confirmatory genetic test. In the case of TRAPS, only patients who had confirmed heterozygous *TNFRSF1A* mutations, with established pathogenicity, were included; patients with R92Q genotype, or other low penetrance or uncertain variants were excluded. The clinical features most associated with TRAPS are shown in Table 18.1 as well as the score assigned to these features. A score ≥43 was highly suggestive of TRAPS. The overall sensitivity of these classification criteria was calculated to be 56%, with a specificity of 84%. Although helpful, the low sensitivity of these classification criteria means that clinicians still rely heavily on genetic investigations to confirm the diagnosis of TRAPS. In this respect, there were also attempts, in the past, to develop clinical criteria to help in the selection of patients in whom genetic testing was indicated. Based on a study involving 228 pediatric patients who were investigated for FMF, MKD and TRAPS and also underwent genetic testing, it was found that clinical features such as young age of onset, positive family history, thoracic pain, abdominal pain, diarrhea and oral aphthosis, were highly associated with the finding of a pathogenic mutation in one of the three hereditary fever syndrome genes, *MEFV*, *TNFRSF1A* or *MVK* [55]. With wider access to massive parallel sequencing technology, there has been an increasing tendency towards panel-based testing, which allows for simultaneous analysis of multiple genes, not restricted to specific 'hot spots' where most or the pathogenic mutations have been detected in the past, but also covering the whole range of known susceptibility genes (see Chaps. 2 and 12). This has resulted in an improved diagnostic yield on one hand, but also has led to other difficulties, for example finding of rare genetic variants of uncertain significance. One distinct advantage of massive parallel sequencing technology over standard Sanger sequencing-based analysis is that cases of somatic mosaicism can be identified, which, in the past, would have been missed. The contribution of somatic mosaicism and, more recently, acquired myeloid-restricted mutations in *NLRP3,* in the pathogenesis of CAPS, is now well established [56–59]. There is a recent report of this phenomenon in a patient who was eventually diagnosed with TRAPS [60], where a novel in-frame deletion of 24 nucleotides (c.255_278del) in the *TNFRSF1A* gene, was identified. Gonosomal transmission (see Chap. 2 for definition) was eventually confirmed, and the presence of the mutation in hematopoietic cells was thought to be critical to the development of clinical symptoms in this patient.

18.7 Treatment

- **Corticosteroids are effective in TRAPS, however its use is limited due to long term toxicity**
- **Patients with low penetrance mutations might respond to colchicine and generally have a more favourable response to TNF blockade**
- **IL-1 blocking therapies are now treatment of choice for the majority of patients with high penetrance mutations and active disease**
- **New small molecule inhibitors of NACHT, LRR and PYD domains-containing protein 3 (NLRP3), or drugs that selectively target the inositol-requiring enzyme/X-box binding protein 1 (IRE1/XBP1) axis might be options for future treatment of TRAPS**

The general principles of treating patients with TRAPS have been set out in a document, written by a group of experts in the field of autoinflammatory diseases, and provides recommendations for management of CAPS, TRAPS and MKD [61]. The main goals of treatment include early and rapid control of disease activity, prevention of disease and treatment-related damage, enabling participation in daily activities and improvement of health-related quality of life. Specific recommendations regarding TRAPS reflect some of the unique features of this condition. For example, subclinical inflammation might often be present, as reflected by elevated CRP and SAA levels; however, this is not always accompanied by obvious symptoms. Furthermore, disease activity and the course and pattern of attacks may vary widely between individuals and over the lifetime of an individual patient. Therefore, the treatment strategy is divided into two main groups: therapeutic measures to deal with acute attacks and therapies which are used on a regular basis to control either subacute inflammation, symptomatic flares and, in the long run, prevent complications developing, such as amyloidosis.

It is worthwhile noting that several therapies are not effective in TRAPS and should be avoided. These include colchicine, hydroxychloroquine and traditional disease-modifying antirheumatic drugs (DMARDs), such as azathioprine and methotrexate. Furthermore, the treatment approach to patients with high penetrance mutations and those carrying R92Q, of P46L variants may differ. First, patients with the low penetrance mutations are at much lower risk of developing amyloidosis, so the benefits of aggressive therapy need to be weighed carefully against the potential toxicity of such therapies. Also, patients with low penetrance mutations may, in some circumstances, respond to colchicine [62] and their response to biological therapies might be different, favoring TNF to IL-1 blockade [50].

Corticosteroids are very effective for the treatment of TRAPS, but their long-term use is limited by their toxicity and diminishing efficacy with chronic use. However, corticosteroids can be often used with or without nonsteroidal anti-inflammatory drugs (NSAIDs) to provide symptomatic relief and to terminate acute attacks. A short course of prednisolone (0.5–1 mg 1 mg/kg/d) given for 5–10 days is often sufficient in acute attacks. This might be the preferred strategy for patients with few flares and no evidence of subacute inflammation between attacks, and, therefore, do not require ongoing treatment (see Chap. 42).

18.7.1 Tumor Necrosis Factor (TNF) Inhibitors

Selective therapies targeting the TNF signaling pathway were the first biologic to be used in TRAPS. Etanercept proved to have limited efficacy despite biological plausibility. A small prospective study of 15 patients showed that treatment with etanercept reduced, but did not completely normalize the frequency and severity of the inflammatory episodes and inflammatory markers [63]. There are also conflicting accounts regarding the effectiveness of etanercept in arresting or reversing the onset of renal amyloidosis, with some reports describing both the slowing and reversal of amyloidosis, but also the subsequent onset of proteinuria, with amyloid present on renal biopsy, while taking

etanercept [64]. A retrospective study that looked at treatment outcomes of 134 patients with hereditary fevers syndromes (FMF, MKD and TRAPS), included 47 patients with TRAPS. Out of 41 patients who received biologics, 54% received etanercept as a first agent whilst the rest received either anakinra (41%) or canakinumab (5%) [65]. Patients who were treated with anakinra were statistically more likely to achieve complete clinical and biochemical remission compared to patients who received etanercept. In total, 54% of patients discontinued etanercept, all due to lack of efficacy, while 35% patients stopped anakinra, either due to adverse effects (most commonly injection site reactions), or patients being enrolled in a clinical trial.

Historically, other anti-TNF agents have fared far less well than etanercept, with some patients reported to have had severe flares of the disease after commencing infliximab [66, 67]. The underlying mechanisms leading to this complication are not entirely understood, but it is possible that the modes of action of etanercept are different than antibody based anti-TNF therapies, which may activate TNFR1 signalling by binding directly to its ligand, TNF, on the cell surface [68]. Therefore, current recommendations do not advise the use of anti-TNF therapies, other than etanercept.

18.7.2 Interleukin (IL)-1 and IL-6 Inhibitors

Over the years IL-1 blocking agents have become a favored treatment modality in TRAPS. There are number of case reports and case series demonstrating excellent and sustained response to anakinra [69–72]. A prospective study from 2008 showed sustained efficacy of anakinra in 5 patients over periods of between 4 and 13 years [71]. More recently, there have been several studies looking at the efficacy of the anti-IL-1β monoclonal antibody, canakinumab in patients with TRAPS requiring long-term therapy. An open label phase II study, which included 19 patients, showed that the response to canakinumab was rapid, with the median time to clinical remission being only 4 days [73]. All patients had a relapse of symptoms after the drug was withdrawn, with the median time to relapse of 91.5 days (95% CI 65–117 days). The clinical response was associated with sustained improvement in health-related quality of life and the medication was generally well tolerated. These results were replicated in a much larger, phase III randomized controlled trial, which looked at the efficacy of canakinumab in patients with colchicine-resistant FMF, MKD and TRAPS. The results of this trial were just reported [74]. The primary objective of the trial was to demonstrate that canakinumab 150 mg (or 2 mg/kg for patients ≤40 kg) given by subcutaneous injection every 4 weeks, is superior to placebo in achieving a clinically meaningful response, defined as resolution of the index flare at Day 15 and no new disease flares over 16 weeks of treatment. In the case of TRAPS this was achieved by 45.5% of patients treated with canakinumab compared to 8.3% who received placebo (OR = 9.17, $p = 0.005$). Similar results were also seen for secondary objectives, which looked for the proportion of patients who could achieve a physician global assessment (PGA) of disease activity <2 (minimal/none), a CRP ≤10 mg/L and an SAA level ≤10 mg/L at week 16. Compared to patients on placebo, canakinumab treated patients achieved statistically superior results in all three measures: PGA (OR = 23.79, $p = 0.0028$), CRP (OR = 6.64, $p = 0.0149$) and SAA (OR = 16.69, $p = 0.0235$).

Considering the biological effects of *TNFRSF1A* mutations, it is plausible that biological therapies targeting IL-6 might also be effective. The use of tocilizumab in TRAPS has been limited, but early reports suggest that this indeed is effective for some patients [75–78].

18.7.3 Potential Novel Treatment Pathways

New small-molecule inhibitors of NLRP3 are currently in development and some have entered early clinical trials (see Chap. 42) [79, 80]. If indeed dysregulated and NLRP3 activation is

crucial to the pathogenesis of TRAPS, such medications could prove to be equally successful to currently used biologics for the treatment of TRAPS. Another potential novel therapeutic approach is to target the molecules implicated in ER stress and associated hyperinflammatory response. These biological processes are also implicated in the pathogenesis of rheumatoid arthritis. Thus, there are both animal models and trials in humans demonstrating the potential therapeutic benefits using this approach. For example, the infusion of recombinant human GRP78/BiP, which as a molecular chaperone has a central role in modulating ER stress, has been shown, in phase I/II clinical trials, to have a positive effect on patients with rheumatoid arthritis [81, 82]. Similarly, the inhibition of IER1 in animal models of rheumatoid arthritis has resulted in suppression of TLR-induced pro-inflammatory cytokine production [83], while targeted inhibition of IRE1, in ex-vivo synovial fibroblasts from patients with rheumatoid arthritis, resulted in reduced viability of these cells [84]. Lastly, inhibition of XBP1, which provides a convergence point for both ER-stress and TLR-mediated inflammation, might prove to be a suitable target in the future [85].

18.8 Outcome

Prior to the introduction of modern biologics for TRAPS, most patients were heavily reliant on the use of corticosteroids for their symptom control. We do not have reliable epidemiological data to determine how many patients developed complications associated with the chronic corticosteroid treatment, but it is likely that this was significant. In addition the initial favorable response to corticosteroids tend to decrease over time [86]. Furthermore, historically 25% of patients carrying high- penetrance *TNFRSF1A* variants were thought to be at risk of developing amyloidosis [87]. A more recent report from the Eurofever/Eurotraps registry found that only 10% of such patients developed amyloidosis, and we do not know how many of these were on the optimal biological therapy [46].

18.9 Concluding Remarks

The pathogenesis of TRAPS remains to be fully elucidated. However, considering the ongoing success of IL-1β targeting biologics in the treatment of TRAPS, it is very likely that many of the molecular processes, which have been found to be disturbed in patients with TRAPS, converge to cause dysregulation of IL-1β release. For example, enhanced NLRP3 activation can be driven by excessive generation of ROS, whilst at the same time increased NF-κB activity will result in enhanced transcription of pro-IL-1β. How other cellular pathways feed into this pathological response remains to be fully explained. These pathways may lead to new treatment approaches (see above Sect. 18.7.3).

More detailed characterization of the molecular pathways involved in the pathogenesis of TRAPS, combined with greater use of modern genetic techniques, such as whole exome and genome sequencing, will most likely help to identify novel genetic causes in patients with diseases resembling TRAPS, but who do not have identifiable *TNFRSF1A* mutations.

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Cryopyrin-Associated Periodic Syndromes (CAPS)

19

Hal M. Hoffman, Jasmin B. Kuemmerle-Deschner, and Raphaela Goldbach-Mansky

Abstract

The cryopyrin-associated periodic syndromes (CAPS) include familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease (NOMID) with shared and unique clinical features. Most patients possess heterozygous *NLRP3* mutations leading to a hyperactive inflammasome, subsequent overproduction of interleukin (IL)-1β and inflammatory symptoms. Diagnostic challenges include a heterogeneous multi-systemic clinical presentation, somatic mosaicism, and low penetrance mutations. IL-1 targeted therapy has become the standard of care for CAPS based on its clinical efficacy and safety.

Abbreviations

AIDAI	Autoinflammatory Disease Activity Index
ASC	Apoptosis-associated speck like protein containing a caspase recruitment domain
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase activating and recruitment domain
CINCA	Chronic infantile neurologic cutaneous and articular
CNS	Central nervous system
CRP	C-reactive protein
DAS	Disease activity scale
ESR	Erythrocyte sedimentation rate
FCAS	Familial cold autoinflammatory syndrome
FMF	Familial Mediterranean fever
IL	Interleukin
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
MKD	Mevalonate kinase deficiency
MWS	Muckle-Wells syndrome
NLR	NOD-like receptor
NLRP3	Nucleotide binding and oligomerization domain, leucine rich repeat, pyrin 3
NOD	Nucleotide-binding and oligomerization domain

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NOMID	Neonatal-onset multisystem inflammatory disease
NSAID	Non-steroidal anti-inflammatory drug
PGE	Prostaglandin E
PKA	Protein kinase A
PYD	Pyrin domain
SAA	Serum amyloid A
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome
VAS	Visual analog scale

Key Points

- **Cryopyrin-associated periodic syndrome (CAPS) is a disease spectrum caused by heterozygous gain-of-function mutations in the *NLRP3* gene leading to an overactive inflammasome**
- **CAPS is characterized by skin, musculoskeletal, ocular, otologic, and neurologic symptoms and chronic systemic inflammation that may lead to organ damage and/or amyloidosis**
- **Diagnosis is made clinically or by genetic testing, but can be challenging**
- **Interleukin (IL)-1 targeted therapy is the standard of care for CAPS resulting in excellent control of symptoms and chronic inflammation**

19.1 Introduction

The cryopyrin-associated periodic syndromes (CAPS or cryopyrinopathies) are a clinical continuum of autoinflammatory conditions that are defined by the presence of mutations in the *NLRP3* gene that codes for the protein cryopyrin. The disease spectrum consists of three previously described distinct syndromes: familial cold autoinflammatory syndrome (FCAS; MIM #120100), Muckle-Wells syndrome (MWS; MIM #191900) and neonatal-onset multisystem inflammatory disease (NOMID) also known mostly in Europe as chronic infantile neurologic cutaneous and articular (CINCA) syndrome (MIM #607115) [1, 2].

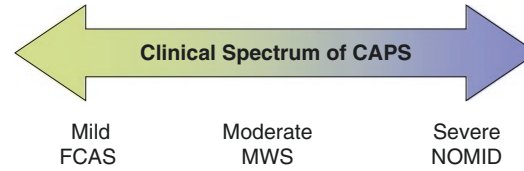

Fig. 19.1 Cryopyrin-associated periodic syndromes (CAPS) is understood as a continuous spectrum of disease ranging from mild to severe

FCAS, at the milder end of the CAPS spectrum, was first described in 1940 as an inherited disorder with the predominant defining clinical feature of cold induced skin and musculoskeletal symptoms [3]. In 1962, Muckle and Wells described a familial syndrome characterized by urticarial rash, neurosensory hearing loss and amyloidosis [4]. NOMID, at the more severe end of the CAPS continuum, characterized by its significant bony malformations and neurologic sequelae from aseptic meningitis, was first described in 1981 [5] (Fig. 19.1).

19.2 Epidemiology

- **CAPS is rare and presents as a disease severity spectrum including FCAS, MWS and NOMID (from mild to most severe)**
- **CAPS patients are distributed worldwide, but there is some geographical variation in the prevalence of CAPS spectrum subtypes**

As with many rare inherited diseases, there are no accurate figures for the true incidence of CAPS due to underdiagnosis, underreporting, and selection bias. However, prevalence estimates based primarily on the European and North American experience range from 1:300,000 to 1:1,000,000. NOMID is the least common since the severe phenotype may affect reproduction thereby limiting disease transmission. Conversely, very large families with multiple family members affected by FCAS and MWS have been reported, which might bias towards a higher reported disease incidence. MWS is the most common sub-phenotype reported in European centers, whereas a founder mutation associated with FCAS patients

in North America is observed in up to 75% of cases of CAPS in the United States of America (U.S.A). CAPS has been reported on every continent which is consistent with sporadic cases (the rule in NOMID) and the classic dominant inheritance pattern often observed in families with FCAS and MWS. Geographical distribution of patients with CAPS may be influenced somewhat by external factors as many patients with FCAS in the U.S.A. avoid living in locations with colder weather or widespread use of air conditioning.

19.3 Etiology (Genetics)

- **CAPS is caused by heterozygous germline or somatic gain-of-function mutations in *NLRP3***
- **CAPS can be sporadic or inherited in an autosomal dominant fashion**

Heterozygous gain-of-function mutations in *NLRP3 (also known as CIAS1, NALP3 or PYPAF1)* were first identified in patients with FCAS and MWS in 2001, and later in patients with NOMID [6–8]. Nucleotide binding and oligomerizaton domain, leucine-rich repeat, pyrin 3 (NLRP3) is expressed in detectable levels in monocytes, granulocytes, T-cells, chondrocytes, and keratinocytes [7, 8]. Little is known about the factors influencing expression of NLRP3. More than 90 confirmed disease-causing mutations have been reported in the INFEVERS database (http://fmf.igh.cnrs.fr/infevers/ accessed 2018) with almost all being single base pair substitutions. More than 90% are located in exon 3, which encodes the central regulatory domain of the protein. There are a few mutation hot spots with certain affected amino acid residues associated with the full range of clinical severity depending on the amino acid substituted. There is no association of specific domain sub-regions with sub-phenotypes, but there is good genotype-phenotype correlation in that each mutation is likely to be associated with only one of the clinical phenotypes along the disease continuum.

Up to 50% of patients with NOMID, 25% of patients with MWS, and 10% of patients with FCAS do not possess germline *NLRP3* mutations [1]. The majority of these patients have somatic mosaicism in *NLRP3,* which has been reported in 70% of Sanger sequencing "mutation negative" patients with NOMID. It is often tissue or cell restricted in which mutant cells are present at levels of less than 10% of total accessible cells. This can make mutation detection a challenge for Sanger sequencing and even for many standard next generation sequencing protocols, so that identification may require sub-cloning and/or deep sequencing ([9, 10]; see Chap. 2). Patients with adult-onset disease who present with systemic features, including rash, fever, conjunctivitis and arthralgia, had somatic mutations in hematopoietic precursors [11]. Some of these patients have clinical features consistent with Schnitzler syndrome (see Chap. 37). Most of these patients do not develop organ-specific features of the disease such as hearing loss, aseptic meningitis and bony deformities.

Similar to observations in other inherited autoinflammatory disorders, low penetrance mutations have also been described in CAPS. Specifically, V198M, R488K, and Q703K have been identified in up to 10% of normal control populations. While there is some controversy concerning their functional significance, these variants have also been observed in patients with classic CAPS symptoms, atypical CAPS symptoms, or other inflammatory disorders [12].

19.4 Pathogenesis

- **Disease-associated *NLRP3* mutations lead to increased inflammasome activation and IL-1 production**
- **CAPS disease mechanisms have been elucidated using cell lines, primary cells, and mouse lines**

The identification of NLRP3 followed by *in vitro, ex vivo,* and *in vivo* functional studies in cultured cells, mice, and humans has led to a better understanding of the underlying mechanisms of disease in CAPS. *NLRP3* encodes the protein cryopyrin which is part of the NOD like receptor (NLR) family of proteins that are characterized

by the presence of a central nucleotide-binding and oligomerization domain (NOD), a C-terminal leucine-rich repeat (LRR) domain, and either a pyrin domain (PYD) present in cryopyrin, or an N-terminal caspase activating and recruitment domain (CARD) observed in some other NLRs. Cryopyrin nucleates an intracellular multimolecular complex, the NLRP3 inflammasome (see figure in Chap. 5), that consists of specific adaptor proteins such as apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) and several chaperone proteins [13, 14]. Formation of this complex enables activation of the proinflammatory protease, caspase-1 [14], which like other caspases can regulate cell death in a unique inflammatory process known as pyroptosis. Caspase-1 can also cleave pro-interleukin (IL)-1β and pro-IL-18 to their biologically active and pro-inflammatory forms, IL-1β and IL-18. IL-1β and to a lesser extent IL-18 can elicit neutrophilic inflammation that is often observed in CAPS. Evidence suggests that CAPS mutations are gain-of-function, leading to increased NLRP3 inflammasome activation and subsequent inflammation, although the specific molecular mechanisms driven by disease-associated mutations remain unknown.

Cell lines with native or recombinant expression of NLRP3 and other inflammasome components were used initially to elucidate the pathways described above by demonstrating oligomerization of inflammasome components, cleavage of caspase-1 and pro-cytokines, release of mature IL-1β and IL-18, and pyroptosis. In these models, CAPS associated mutations were shown to result in constitutive NLRP3 inflammasome activation [15].

Similarly, leukocytes isolated from CAPS patients consistently show a lower threshold for NLPR3 inflammasome activation as measured by IL-1β release following lipopolysaccharide (LPS) stimulation in the absence of classic NLRP3 triggers (second signals) such as ATP, nigericin, or crystals. In some reports, CAPS leukocytes demonstrate significant NLRP3 inflammasome activation in the absence of stimulation, consistent with constitutive activation. Evidence suggests that this *ex vivo* phenotype is due to CAPS cells having increased levels of reactive oxygen species due to increased redox stress, resulting in over-activation or ineffective anti-inflammatory mechanisms [16]. A unique feature of monocytes isolated from patients with FCAS is inflammasome activation when cultured at a slightly cooler temperature of 32 °C instead of the traditional 37 °C. Again, the molecular mechanisms for this *ex vivo* phenotype have not been elucidated [17].

In order to further understand disease pathogenesis, several knockin mice have been developed with different CAPS-associated mutations. Cells isolated from these mice demonstrate a similar *ex vivo* phenotype to human leukocytes including a lower threshold of inflammasome activation in all mutants, and cold-induced activation in FCAS-associated mutations. The mice also have some similar clinical features to CAPS patients including neutrophilic inflammation in skin, conjunctiva, joints, blood, and the cerebrospinal fluid. Breeding these mice to inflammasome pathway knockout mice have demonstrated a disease phenotype that is dependent on ASC and caspase-1, partially dependent on IL-1β, IL-18, and tumor nexrosis factor (TNF), and independent of IL-6 and IL-17. The stronger contribution of non-IL-1 activating pathways to the contribution of murine CAPS contrasts clinical findings of the profound response of human disease to IL-1 blocking agents and points to differences between inflammasome activation in murine models and in human disease [18].

In fact, the best evidence supporting the CAPS mutation-dependent mechanisms is the remarkable efficacy of IL-1 targeted therapies in patients with CAPS. Early reports showed complete abrogation of daily inflammatory symptoms in patients with MWS or cold-induced symptoms in patients with FCAS following experimental cold challenge. Treatment was also associated with reduction in acute elevation in IL-6 and blood neutrophilia after cold exposure, reduction of markers of systemic inflammation, (C-reactive protein (CRP) and serum amyloid A (SAA) and over time, organ specific inflammation [19–21].

19.5 Clinical Manifestations

- **The clinical subtypes of CAPS define conditions with unique symptoms, different organ damage and long-term morbidity**
- **Laboratory parameters of systemic inflammation and MRI imaging are useful tools for assessing the extent of systemic and organ-specific inflammation, and in monitoring disease activity**

19.5.1 Three Distinct Diseases Versus One Continuous Spectrum of Disease

Historically, FCAS, MWS and NOMID have been described as three distinct diseases, but a common causative gene, overlapping signs and symptoms, and patients with clinical features characteristic of more than one of the syndromes has firmly established that this is a single disease spectrum. However, there is clinical utility in distinguishing sub-phenotypes due to the significant differences in disease severity resulting in diverse short-term and long-term organ damage and morbidity, and therefore the need for intensified therapy in patients with more severe disease (Fig. 19.1). There is reasonable genotype-phenotype correlation in CAPS in that patients with the same mutation are likely to fall nearby on the disease spectrum. However, in the same family, carriers of the identical mutations display phenotypic heterogeneity which indicates that other factors can modify disease severity. For example, in families with MWS carrying the E311K mutation, rash was observed in just 54% of patients, and febrile episodes in only 31%, including variability in members of the same family [22].

19.5.2 CAPS Signs and Symptoms

Similar to other autoinflammatory disorders, CAPS is a multi-system inflammatory disease, and therefore it may affect skin, muscles, joints, bones, eyes, ears, and the central nervous system. Age of onset ranges from birth or infancy, as observed in most patients with FCAS and NOMID, to adulthood as described in some patients with MWS, particularly those with somatic mosaicism. (Table 19.1). Symptoms of CAPS can be caused by acute inflammation but can also be due to organ damage following chronic inflammation. If untreated, most patients with CAPS usually have some daily symptoms in addition to intermittent symptom flares that can last hours to days. Triggers for flares may include stress, alcohol, sleep deprivation or infections, and cold is a common potent trigger in patients with FCAS.

Some symptoms in all subgroups of the CAPS spectrum are associated with systemic inflammation such as fatigue, headache, and influenza-like muscle aches. Although difficult to quantify, fatigue is a major component of CAPS and is associated with severely compromised quality of life [23]. Clinical signs observed in some patients with CAPS associated with systemic inflammation include fever, lymphadenopathy, hepatosplenomegaly, and growth retardation. While CAPS is classified as a hereditary fever disorder, fever is not a common presenting complaint and often, objective measurement of body temperature in patients with CAPS does not meet standard criteria for fever. For example, in patients with FCAS undergoing cold challenge, body temperature increases only slightly (less than 1 °C) beginning at 1–2 h, peaks at approximately 6–8 h, and returns to baseline usually within 12–24 h after cold exposure. There are also some systemic symptoms that are more commonly associated with distinct sub-phenotypes such as chills or shivers, sweating, excessive thirst, and dizziness, described by many patients with FCAS [8, 24].

19.5.2.1 Cutaneous Involvement

The characteristic dermatological manifestation of CAPS is a neutrophilic dermatitis that presents clinically with “urticaria-like” lesions. Skin lesions start as small erythematous and edematous papules and progress to wheals (Fig. 19.2a). The rash is rarely itchy, but it is often painful and sensitive to touch and typically occurs on the trunk and limbs. It can also be seen on the face,

Table 19.1 Clinical manifestations and characteristics of cryopyrin-associated periodic syndromes (CAPS)

	Mild		Moderate		Severe	
	CAPS-FCAS		CAPS-MWS		CAPS- NOMID	
Age at onset	Infancy—adult		Early childhood—adult		Perinatal	
Typical attack duration	30 min – 72 h		1–2 days or continuous with flares		Continuous with exacerbations	
	Acute inflammation	***Chronic damage***	***Acute inflammation***	***Chronic damage***	***Acute inflammation***	***Chronic damage***
Systemic inflammation	Fever, increased acute phase reactants	Amyloidosis uncommon (~2%)	Fever, increased acute phase reactants	Amyloidosis in up to 25% of cases	Fever, lymphadenopathy, hepato-splenomegaly, increased acute phase reactants	Amyloidosis observed in untreated patients
Dermatological manifestations	Cold-induced neutrophilic urticaria	None	Neutrophilic urticaria	None	Neutrophilic urticaria	None
Ocular manifestations	Conjunctivitis	None	Conjunctivitis, episcleritis, optic disk edema/papilledema	Corneal opacification	Conjunctivitis, uveitis, optic disk edema/papilledema	Chronic papilledema, optic atrophy resulting in progressive amaurosis, corneal opacification
Inner ear manifestation	None	None	Cochlear edema	Progressive sensorineural hearing loss	Cochlear edema	Progressive sensorineural hearing loss
Central nervous system	Headache	None	Headache, intermittent meningitis	None	Headache, chronic aseptic meningitis	Increased intracranial pressure resulting in encephalomegaly, brain atrophy, and cognitive impairment
Musculoskeletal	Myalgia, arthralgia	None	Myalgia, arthralgia, oligoarthritis	None	Myalgia, arthralgia, arthritis	Chronic arthritis, epiphyseal bony overgrowth, limb-length discrepancies and contractures

FCAS familial cold autoinflammatory syndrome, *MWS* Muckle-Wells syndrome, *NOMID* Neonatal-onset multisystem inflammatory disease

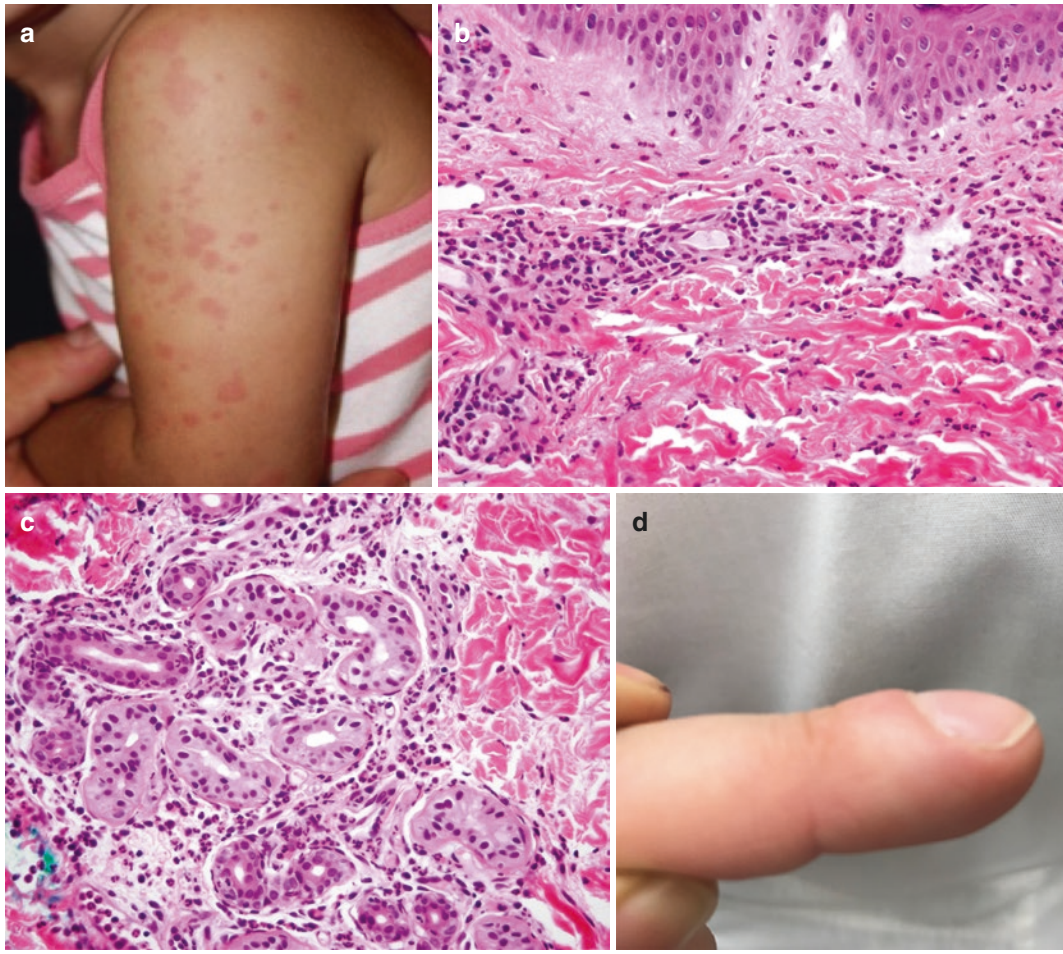

Fig. 19.2 Skin manifestations (**a**–**c**) of cryopyrin-associated periodic syndromes (CAPS). (**a**) Urticaria-like rash (**b**, **c**) Skin-biopsy section from a patient with neonatal-onset multisystem inflammatory disease (NOMID) showing neutrophilic infiltrates in the dermis and near sweat glands respectively (**d**) Clubbing

but spares the palms and soles. In some patients, there is predisposition to the skin overlying fatty areas including upper arms, thighs, and abdomen; however, the subdermal fat is not infiltrated by inflammatory cells. If untreated, most patients have at least limited rash on a daily basis with more generalized distribution during flares. Unlike classic fleeting urticaria associated with allergies, specific lesions usually persist for several hours. Some patients may also experience painful extremity swelling similar to angioedema. In FCAS, rashes are not induced by direct immediate contact with cold objects or water (negative ice cube test), but often appear 1–4 h after cold exposure in areas not necessarily subjected directly to cold air. Skin biopsies show histological features consistent with dermal edema, and a neutrophilic infiltrate consisting of segmented neutrophils in the dermis and often near sweat glands (Fig. 19.2b, c). Lymphocyte and eosinophilic infiltrations are not prominent features of the skin pathology of CAPS [25].

19.5.2.2 Musculoskeletal Involvement

Muscle, joint, and bone symptoms in CAPS vary by phenotype and disease severity. In FCAS and mild MWS, myalgia and arthralgia are often

limited to acute flares. Patients with FCAS may develop painful periarticular swelling [24]. Patients suffering from MWS can have frank arthritis with wrists, knees and ankles most commonly affected. Chronic polyarticular arthritis is seen in severe forms of MWS and NOMID. (Table 19.1).

The characteristic arthropathy with bone and joint deformation occurring in 30–40% of patients with NOMID is caused by overgrowth and asymmetry of the cartilage, excessive uncontrolled growth of the patella and the epiphysis of the long bones, and abnormal epiphyseal calcification [26]. Osseous lesions often affect growth plates asymmetrically with unilateral reduced longitudinal growth of affected bones causing asymmetric severe limb length discrepancies (Fig. 19.3). This may result in limited mobility and, in extreme cases, inability to ambulate that may require corrective surgery [26, 27].

In contrast to most other symptoms, the tumor-like lesions of the long bones that start in the growth plate (Fig. 19.3c) do not respond to IL-1 blockade once they are formed. In some patients the development of these lesions may even occur in utero or very early in life. The process appears to be independent of inflammatory cells as a biopsy of the growth plate from a NOMID patient was devoid of inflammatory cells [28]. The lesions derive from osteoblast progenitor cells that form fibroblastoid tumors. Similar to patients with genetic defects that lead to increased cAMP-dependent protein kinase A (PKA) signaling, the NOMID osteoblast progenitors cells have increased PKA and prostaglandin E2 (PGE2) activity which leads to cAMP dependent activation of Wnt signaling. The IL-1 independent, but caspase-1 and cAMP dependent growth of the osteoblasts from patients with NOMID patients', is a likely reason for the unresponsiveness to IL-1 inhibition.

Other common bony features include clubbing (Fig. 19.2d), premature patellar ossification [29] as well as joint contractures, arthritis, osteitis, osteopenia, and small size. Patients with hydrocephalus often present with a "typical facies" with frontal bossing, large cephalic perimeter and flattening of the nasal dorsum with the appearance of a "saddleback nose" [19, 29].

19.5.2.3 Ocular Involvement

A variety of inflammatory eye diseases have been reported in patients with CAPS including conjunctivitis, keratitis, episcleritis, and anterior or posterior uveitis. The most common inflammatory eye manifestation is conjunctivitis occurring during flares in many patients with CAPS. However, conjunctivitis can also be chronic and associated with perilimbal erythema in many patients with NOMID. Patients often complain of burning pain and redness without significant discharge. The cornea is involved in 40% of patients with NOMID, including interstitial keratitis with clouding of corneal stroma requiring corneal transplants. Band keratopathy and corneal neovascularizations are seen as consequences of recurrent anterior uveitis. In fact, mild to moderate non-granulomatous anterior uveitis is seen in 50% of patients with NOMID. Inflammation of the posterior eye segments is less frequent, but can present as vitritis, retinal vasculitis, and focal chorioretinitis. Elevated intracranial pressure associated with chronic meningitis is observed in many patients with NOMID leading to bilateral optic nerve swelling (papilledema) and subsequent optic disc atrophy (Fig. 19.4). Papilledema is present in more than 80% of patients with NOMID and chronically elevated intracranial pressure leads to progressive optic nerve atrophy and vision loss, starting with progressive loss of peripheral vision, worsening tunnel vision and eventually blindness. Anterior and rarely posterior uveitis, contribute to this progressive vision loss [19]. Reversible "reduction of visual acuity" is seen with corneal manifestations that improve with treatment if scarring has not yet occurred [30, 31]. Corneal transplants have improved vision in patients who had corneal clouding due to scarring (Table 19.1).

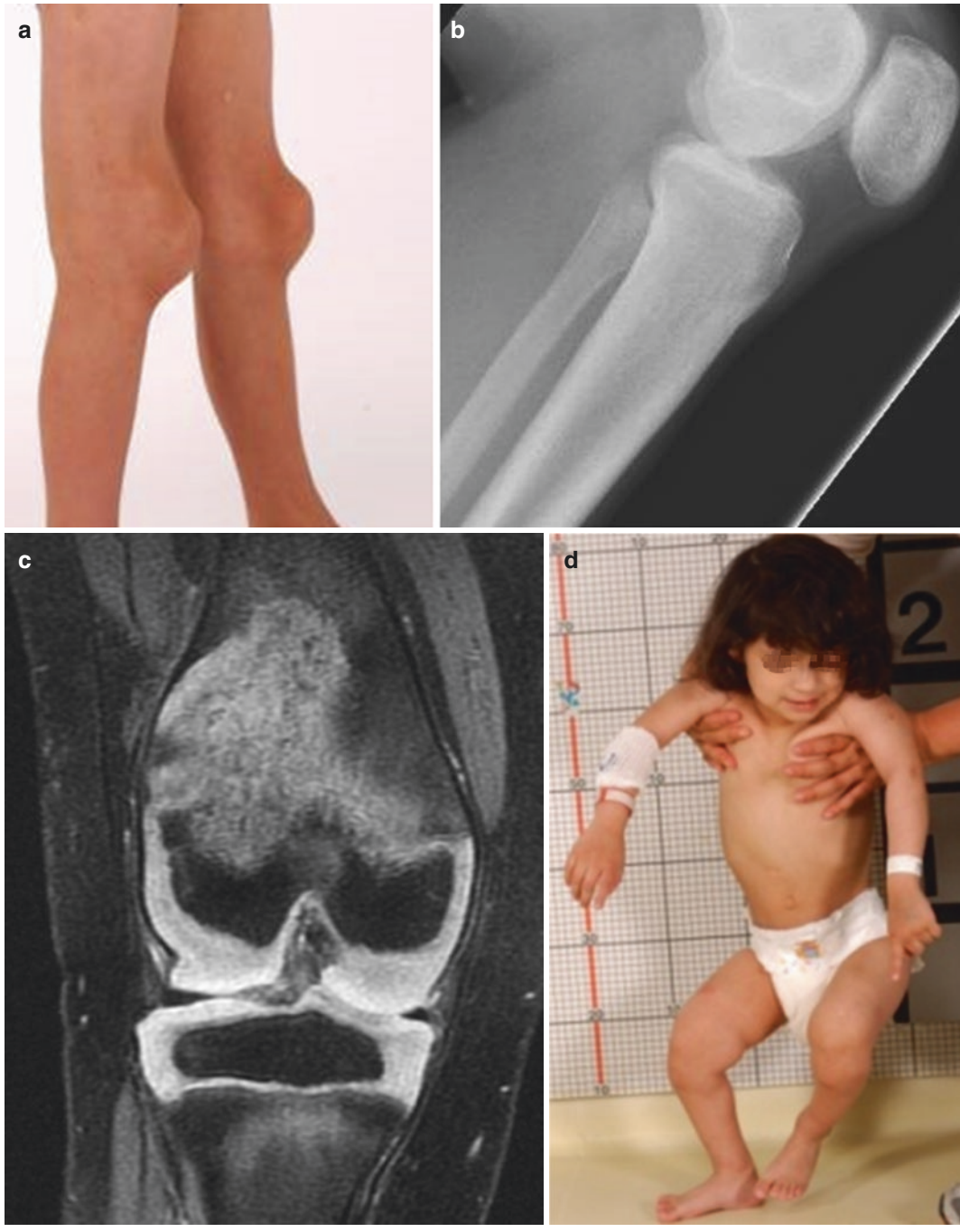

Fig. 19.3 Musculoskeletal manifestations of cryopyrin-associated periodic syndrome (CAPS). (**a**) Patellar overgrowth in a patient with neonatal-onset multisystem inflammatory disease (NOMID) (**b**) Radiograph of the knee showing a large patella (**c**) MRI of the distal femur shows epiphyseal lesions and rearrangement resembling a chondroma. These lesions result in early and partial closure of the growth plate resulting in bowing and deformities of the long bones and limb-length discrepancies (**d**) Severe involvement of distal and proximal tibia epiphyses as well as femurs, wrists, elbows and shoulders

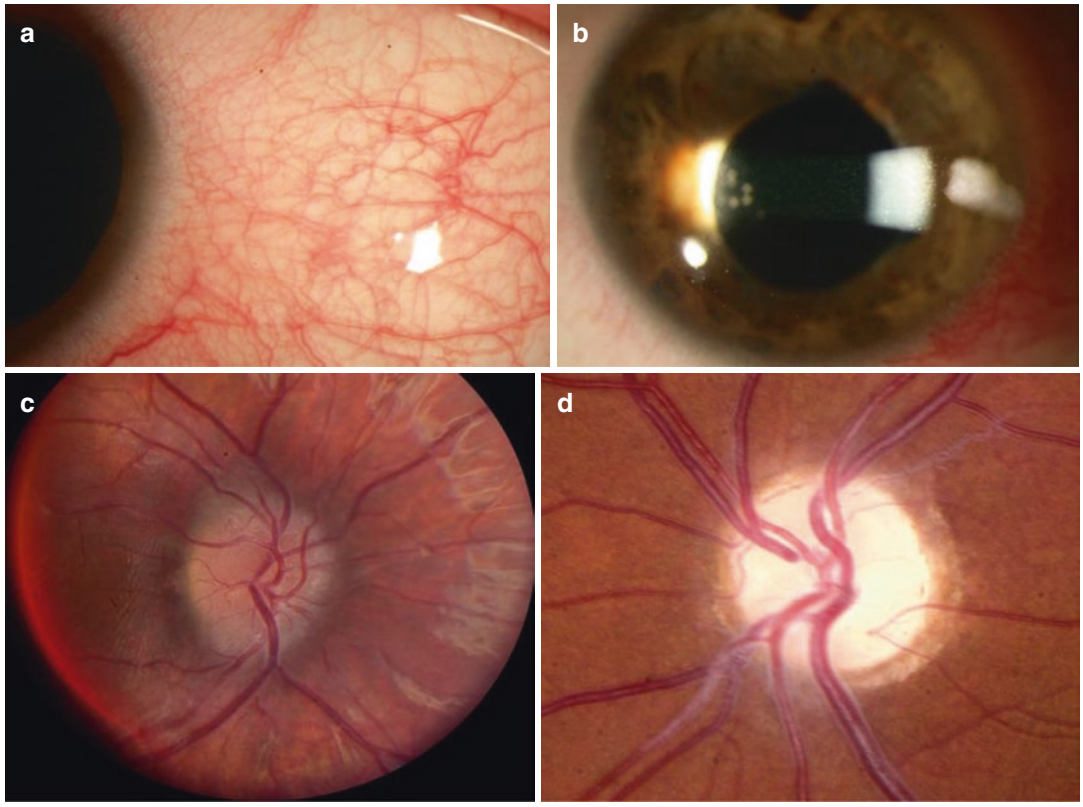

Fig. 19.4 Ocular manifestations in cryopyrin-associated periodic syndrome (CAPS). (**a**) Conjunctivitis, (**b**) uveitis or episcleritis in the milder forms and (**c**, **d**) optic disk edema/papilledema in the more severe forms leading to progressive amaurosis in patients with untreated neonatal-onset multisystem inflammatory disease (NOMID) *courtesy of R Bishop and C Deuter, NEI, NIH*

19.5.2.4 Audiologic Involvement

Progressive sensorineural hearing loss is a major symptom in patients with MWS and NOMID. Onset can be early in life in severely affected patients and as late as adulthood in some patients. Primarily high frequencies (4–10 kHz) are affected early [32], but lower frequencies become affected and progressive worsening occurs throughout the course of the disease and with age [33] (Fig. 19.5). Unlike more common diseases associated with hearing loss, the pathogenesis is due to ongoing cochlear inflammation leading to degeneration of sensory structures in the organ of Corti. Therefore, a reversal or halt in the progression of hearing loss may be achieved by timely introduction of targeted anti-inflammatory treatment. While not commonly described in untreated CAPS patients, vertigo has also been reported in some patients on specific IL-1 targeted therapy, but the etiology is currently unknown [34].

19.5.2.5 Neurologic Involvement

Central nervous system (CNS) impairment is the most devastating feature of CAPS. While patients with MWS may rarely have intermittent attacks of aseptic meningitis, patients with NOMID have chronic aseptic neutrophilic meningitis leading to the development of persistently elevated intracranial pressure resulting in hydrocephalus, enlarged ventricles, and brain atrophy (Fig. 19.6). Patients may present with irritability, severe headache, nausea or vomiting predominantly in the morning, and sometimes

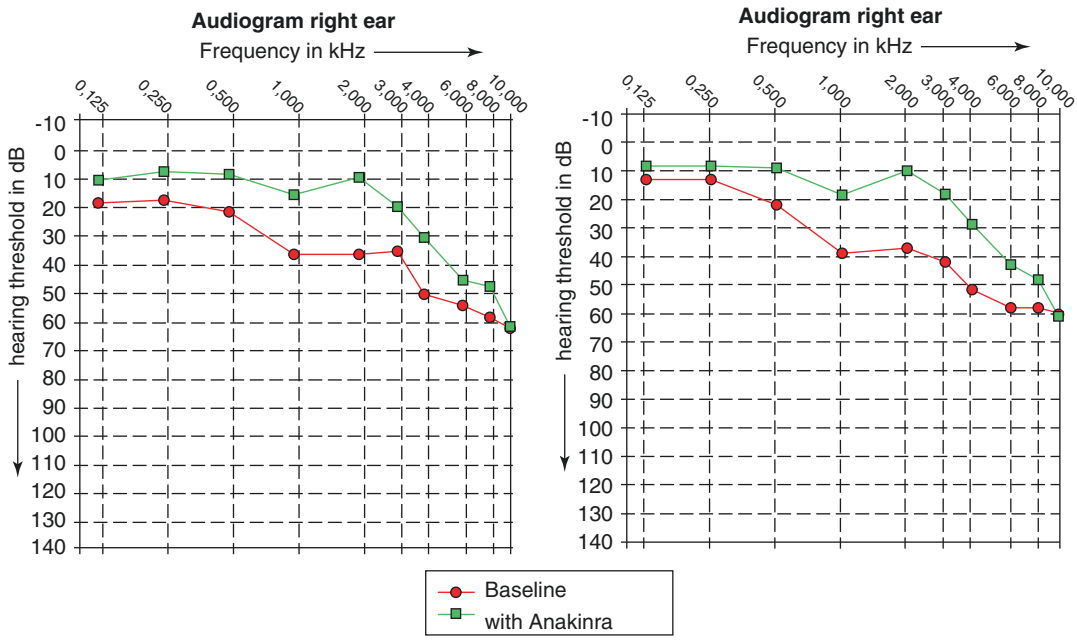

Fig. 19.5 Audiogram of a patient with Muckle-Wells syndrome (MWS). Hearing loss becomes apparent with the characteristic decrease in thresholds in the high frequencies. Treatment with anakinra improved hearing in the most relevant frequencies for speech discrimination

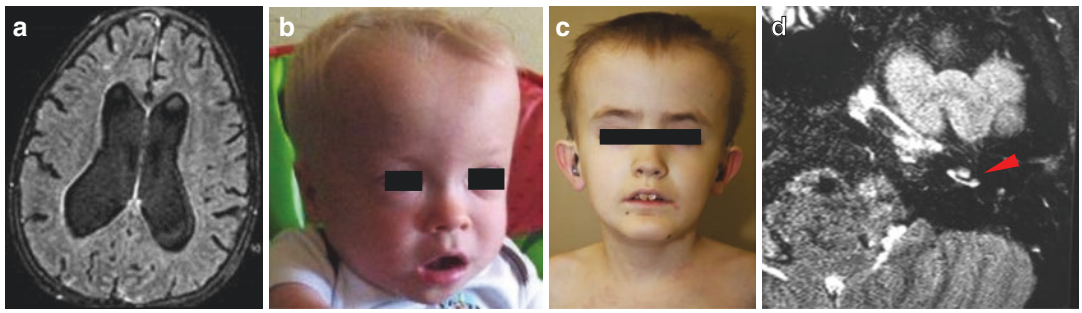

Fig. 19.6 Central nervous system (CNS) manifestation in patients with neonatal-onset multisystem inflammatory disease (NOMID). (**a**) Severe hydrocephalus and brain atrophy (**b**, **c**) Facial signs of hydrocephalus including frontal bossing in NOMID (**d**) Post-gadolinium enhancement FLAIR image of the left cochlea shows abnormal enhancement of the basal and one additional turn of the cochlea (arrow)

seizures. As described previously, increased intracranial pressure also results in chronic papilledema that leads to optic nerve atrophy and progressive vision loss. Chronic leptomeningeal inflammation also causes arachnoid adhesions. Chronic CNS inflammation and brain atrophy can also lead to cognitive delay. Less commonly reported CNS-symptoms are stroke and vascular occlusions [19, 27] (Table 19.1).

19.6 Laboratory Testing

Abnormal laboratory findings include marked blood leukocytosis with neutrophilia, thrombocytosis and anemia. The neutrophil count may be elevated between flares, but usually increases significantly during a flare. For example, in patients with FCAS, blood neutrophil counts increase noticeably within 2 h after a cold challenge and

peak at approximately 8 h before returning to baseline by 16 h. The generalized inflammation in CAPS is also apparent in elevated inflammatory markers such as erythrocyte sedimentation rate (ESR), CRP [35] and SAA. Most untreated patients with CAPS have elevated inflammatory markers at baseline. Levels of SAA and CRP may increase within 6–12 h after start of a flare. Levels stay elevated during a flare and then decrease, with a half-life of 12 h. SAA, traditionally the most dynamic marker of inflammation, is a precursor of amyloid A, and therefore, an important biomarker for the development of AA amyloidosis ([36], see Chap. 15). Consequently, reduction of baseline SAA (or CRP if not clinically available) is one goal of effective therapy. The phagocytic-specific S100 proteins S100 A12 and MRP 8/14 may be used as surrogate parameters for inflammation (see Chap. 9). Increased serum levels in several inflammatory conditions and a good correlation to response to therapy has been demonstrated for these markers. Potentially subclinical disease activity might be detected with measurement of these markers [37, 38]. As described above, proteinuria is usually the first sign of systemic amyloidosis. In patients with signs of aseptic meningitis, opening pressure, cell count and protein levels may be elevated in cerebrospinal fluid analysis.

19.7 Imaging

Imaging plays a particular role in NOMID to evaluate bone and central nervous system manifestations. Bone radiographs may reveal patellar hypertrophy/overgrowth, epiphyseal overgrowth, and complications of arthritis [26] (Fig. 19.3). Brain imaging may show ventricular enlargement and/or brain atrophy due to increased intracranial pressure (Fig. 19.5). In addition, brain MRI may reveal leptomeningeal and cochlear enhancement as signs of central nervous system inflammation. Inner ear FLAIR MRI may demonstrate inner ear inflammation as a cause for sensorineural hearing loss in patients with CAPS [27] (Fig. 19.5). In patients with NOMID, sequential MRI imaging to assess resolution of inner ear enhancement can be used to tailor treatment. If enhancement persists treatment may be adjusted, with repeat MRI performance every 6 months to 1 year until the enhancement resolves [39]. In patients with concern for adequate control and or ongoing hearing loss, MRI may be useful in monitoring ongoing inner ear inflammation.

19.8 Diagnosis

- **A genetic diagnosis may be delayed, particularly in patients with somatic mutations**
- **Validated diagnostic criteria aid in early clinical diagnosis and early initiation of IL-1 blocking therapy**
- **Diagnostic challenges include early diagnosis of NOMID before central nervous system and bony damage occur**

19.8.1 CAPS Diagnosis Is Often Delayed

Primary care and specialty physician awareness of CAPS is low due to disease rarity and the fact that patients may present initially with a variety of non-specific symptoms or signs described above. Therefore, there are many different specialists that may encounter a patient with CAPS including dermatologists, allergists, rheumatologists, neurologists, ophthalmologists, otolaryngologists, and nephrologists. Due to the severity of the disease, NOMID is often diagnosed quite early, while in FCAS the diagnosis is often significantly delayed. In one study, 44% of patients with CAPS carried another diagnosis before the correct diagnosis was made [40], resulting in inappropriate treatment and mistrust of the health care system by patients with CAPS and their families. In the past, the median delay between disease onset and diagnosis was reported to be 40 years, but increased distribution of knowledge regarding CAPS by patient or physician groups and drug manufacturers has reduced the median delay to approximately one year. Further reduction of that delay is likely to occur as genetic testing becomes more widely available.

19.8.2 CAPS Is Diagnosed Clinically and Genetically

In most cases CAPS becomes apparent during early childhood. Early onset of disease is therefore a strong indicator for CAPS. But because the disease is rare and some patients present with only mild symptoms, the diagnosis of CAPS also must be considered in adults [41]. The suspected diagnosis is frequently supported by the patient's history, family history, physical examination, and additional specific examinations such as audiograms or MRI (see Chap. 11). At the initial presentation, standardized questionnaires and examinations may be applied, which can help to identify CAPS and also uncover hidden organ manifestations [42]. Patient disease diaries and in some cases "diagnostic" therapeutic trials can also be used to assist in reaching the correct diagnosis.

If the diagnosis of CAPS appears likely, molecular genetic testing for mutations in the *NLRP3* gene may help confirm the diagnosis. In France, a twofold increase in requests for molecular genetic examinations was registered between 2005 and 2009, but an *NLRP3* mutation was confirmed only in 16% of 821 cases [43]. Therefore, screening criteria that may be applied to reduce unnecessary genetic testing have been suggested, such as ≥3 recurrent disease episodes, age at disease onset <20 years, elevated CRP level, urticarial rash, and fever [43]. Although most patients with CAPS carry germline mutations in *NLRP3*, the diagnosis can often be made on clinical grounds alone as there are patients with clinical features that are clearly consistent with CAPS who do not have readily identifiable mutations.

Therefore, the presence of *NLRP3* mutations can be confirmatory, but is not necessary to make the diagnosis, and a genetic diagnosis should not be required prior to initiation of appropriate therapy, as "mutation positive and negative patients" respond equally to IL-1 blocking therapy.

19.8.3 Differential Diagnosis

The differential diagnosis includes other periodic inflammatory disorders, such as tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD), familial Mediterranean fever (FMF), Behçet disease, Blau syndrome, Schnitzler syndrome, and other more common rheumatologic disorders such as systemic juvenile idiopathic arthritis.

19.8.4 Diagnostic Criteria

Diagnostic criteria for CAPS have been proposed following consensus building and validation of the criteria using different cohorts of patients with CAPS and with other autoinflammatory and autoimmune diseases. The following criteria recognize that all but a few patients with CAPS have detectable systemic inflammation and uses uniquely CAPS-specific clinical features along the disease continuum to achieve reasonable specificity and sensitivity to aid clinicians in making the diagnosis [44]. These criteria do not include genetic confirmation and therefore can be applied in places where genetic testing is not available.

Box 19.1

If genetic testing is not available or negative, the following criteria can be used to diagnose cryopyrin-associated periodic syndrome (*CAPS*) [44]:

Raised inflammatory markers (C-reactive protein/Serum amyloid A) (Mandatory criterion) **plus**

≥ 2 of 6 **CAPS typical signs/symptoms**:

- Urticaria-like rash
- Cold/stress triggered episodes
- Sensorineural hearing loss
- Musculoskeletal symptoms (arthralgia/arthritis/myalgia)
- Chronic aseptic meningitis
- Skeletal abnormalities (epiphyseal overgrowth/frontal bossing)

19.8.5 Diagnostic Challenges

Patients with late onset or atypical disease manifestations present the greatest challenge for the

clinician. As described previously, patients with somatic mosaicism may develop symptoms later in life and frequently have negative genetic tests. Low-penetrance *NLRP3* variants are also frequently not reported by genetic testing labs since some of these variants can be found in apparently healthy controls in the general population. However, functional studies indicate that these variants (V198M, R488K, Q703K) may have an intermediate biologic phenotype. Patients with low penetrance *NLRP3* variants also have a unique clinical phenotype since as a group they were shown to have significantly more fever and gastrointestinal symptoms but less eye disease, hearing loss, and renal involvement [12]. Additionally, these patients are often more resistant to IL-1 targeted therapy.

For patients with *NLRP3* variants of unknown significance the criteria below have been used to make a diagnosis of CAPS:

Box 19.2
Presence of a genetic variant of *NLRP3* of unknown significance and at least 2 among:

- Urticaria like rash
- Red eye (conjunctivitis, episcleritis, uveitis)
- Sensorineural hearing loss

(These criteria have not yet been published)

19.9 Treatment

- **There are three IL-1 blockade therapies currently available for the treatment of CAPS**
- **Dosing modification may be required in order to adequately control symptoms or prevent organ damage from chronic inflammation resulting from CAPS**
- **Drugs directly targeting the NLRP3 inflammasome are in preclinical screening**

CAPS is a translational success story as gene identification and elucidation of the molecular basis of disease pointed to IL-1 as a therapeutic target. Despite clinical heterogeneity, all patients with CAPS respond to IL-1 blockade. There are three IL-1 blockade therapies approved by the Food and Drug Administration (FDA) or European Medicines Agency (EMA) for the treatment of CAPS (see Chap. 41). Anakinra (Kineret® Sobi, Sweden), a recombinant IL-1 receptor antagonist, is approved for NOMID in the U.S.A. and for all patients with CAPS in Europe. Rilonacept (Arcalyst® Regeneron, U.S.A.), an IL-1 Trap, is a fusion protein of the IL-1 receptor and the Fc portion of IgG, and is approved for the treatment of FCAS and MWS only in the U.S.A. Canakinumab (Ilaris® Novartis, Switzerland), an IL-1β blocking antibody, is approved for the treatment of FCAS and MWS in the U.S.A. and for all patients with CAPS in Europe [19, 34, 45].

All three drugs are injectable biologics, but the pharmacokinetics of each is very different resulting in varying dosing recommendations (see Chap. 41). Anakinra has a short half-life and therefore is usually dosed daily. Rilonacept has a longer half-life resulting in the opportunity for once-weekly dosing. Canakinumab has a long half-life and unique pharmacokinetic profile in patients with CAPS allowing for dosing every 2 months in milder patients. Some patients with CAPS require dosage schedule adjustment in that patients with mild FCAS are able to maintain symptom control with decreased dosing frequency while patients with more severe disease require more frequent dosing. Dose requirements are often higher in younger and more severely affected patients, and CNS inflammation can be particularly difficult to control [39].

For anakinra, the typical dosing regimen varies from 1 to 2 mg/kg/day for patients with FCAS and up to 10 mg/kg/day for severe patients with NOMID to achieve optimal clinical responses. The dose of rilonacept for adults is 160 mg/week and varies from 2.2 mg/kg/week to 4.4 mg/kg/week in children. Canakinumab is administered at 150 mg for patients with MWS and FCAS with

Table 19.2 Interleukin (IL)-1 targeted therapies used in cryopyrin-associated periodic syndromes (CAPS)

Drug	Disease severity	Adult dose	Pediatric dose
Anakinra	Mild Severe	100 mg/1–2 days ≥100 mg/day	1–2 mg/kg/day up to 10 mg/kg/day
Rilonacept	Mild Severe	160 mg/week 320 mg/week	2.2 mg/kg/week 4.4 mg/kg/week
Canakinumab	Mild Severe	150 mg/8 weeks 300 mg/4 weeks	2 mg/kg/8 weeks 3–4 mg/kg/4 weeks

body weights greater than 40 kg, and at 2 mg/kg for patients with CAPS with body weight greater than or equal to 15 kg and less than or equal to 40 kg every 8 to 4 weeks (the latter in severe cases). For children 15–40 kg with an inadequate response, the dose can be increased to 3–4 mg/kg (Table 19.2). In NOMID, doses up to 10 mg/kg/dose every 4 weeks have been administered [46]. However, in patients with NOMID with severe CNS disease, inflammatory control of aseptic meningitis and increased intracranial pressures (assessed by opening pressures at rest) should be monitored with lumbar punctures. Data from patients with severe CNS disease who have been treated sequentially with anakinra and canakinumab suggest that anakinra may be superior in controlling aseptic meningitis in patients with NOMID, likely due to better CNS penetrance [47].

Successful treatment with IL-1 blocking agents leads to complete resolution of symptoms in most cases and is therefore the treatment of choice [45, 48]. Therapeutic success can be monitored by systemic assessment of symptoms using longitudinal daily diary entries, which have been used to develop disease activity scores. These scoring systems include a mean diary score such as the Auto inflammatory Disease Activity Index (AIDAI) and MWS-Disease Activity Score (DAS), which were developed for clinical trials but can also be used in a clinical setting to monitor disease activity on therapy (see Chap. 13). An AIDAI cut-off score of nine accurately differentiated patients with active versus inactive disease. The MWS-DAS determines disease activity in nine MWS domains attributing 0, 1, or 2 points to each level of disease activity. MWS-DAS captures fever, headache, eye disease, hearing impairment, oral ulcers, abdominal pain, renal disease, musculoskeletal disease, and rash. The tenth domain is the Patient Global Assessment score measured on a visual analogue scale (VAS) and categorized into 0 points for ≤1 cm, 1 point for >1 to 5 cm, and 2 points for >5 to 10 cm. A MWS-DAS of <10 points was considered mild disease activity. Scores ≥10 points indicated severe MWS disease activity, as previously described [42, 49].

While there are patients with FCAS who choose to avoid therapy as some patients can limit symptoms by controlling their environment, patients with more severe forms of MWS and NOMID or patients with FCAS or mild MWS with a family history of amyloidosis or hearing loss are likely to develop organ damage from untreated disease. Therefore, optimal IL-1 targeted therapy should be initiated early to prevent the development and progression of organ damage [27]. Disease monitoring for organ damage in all patients with CAPS includes regular CRP or SAA measurements for baseline systemic inflammation and urinalysis screening for proteinuria. Other organ specific monitoring tests include audiology exams, ophthalmologic exams, cerebrospinal fluid measurements, radiographs and MRI [39].

Cohort studies of patients with sustained clinical responses to anakinra, [27, 50], rilonacept [24, 51] and canakinumab [52] have been published. While symptom control is usually achieved, growth retardation, CNS inflammation and hearing loss may only partially improve in some patients [27, 33]. Since treatment can have a significant effect on systemic and organ inflammation, it is critical to diagnose patients with severe disease early in life to prevent permanent and irreparable organ damage. Bony overgrowth

progresses on IL-1 blocking treatment, suggesting that once the lesion is established, continuation of bone growth is likely independent of IL-1. All three IL-1 targeted drugs appear to control symptoms, hearing loss and vision loss in a similar fashion if dosed appropriately. However, there may be some advantage of anakinra in patients with NOMID, as anakinra may provide better control of CNS disease in severe cases [46, 47]. Adjunctive therapies with efficacy in some patients include antihistamines for pruritus, non-steroidal anti-inflammatory drugs (NSAIDs) for fever, joint /muscle pain, and headache, and ophthalmic corticosteroids for inflammatory eye disease. Warming therapies including hot baths and liquids can be useful for patients with FCAS.

There are little data to suggest that IL-1 blocking treatment increases the risk for opportunistic infections in CAPS, however a specific increase in Group A *Streptococcus* (GAS) infection is seen with IL-1 blocking therapies. GAS, independent of inflammasome regulation, activates IL-1 through a cysteine protease SpeB, which leads to IL-1 dependent restriction of the invasiveness of GAS, which is specifically inhibited in the context of IL-1 blockade. Therefore, all infections including skin abscesses and respiratory infections should be carefully monitored and aggressively treated including appropriate antibiotic coverage [53]. IL-1 blocking treatment may blunt some of the clinical signs of infection including fever and leukocytosis, therefore, early and aggressive use of antimicrobial agents in patients with CAPS on therapy may be warranted. Other adverse effects include local injection site reactions. Vertigo has also been observed in some patients with CAPS on canakinumab. While vaccines are usually well tolerated by patients with CAPS, pneumococcal vaccines have triggered mild to severe disease flares (including the need for hospitalizations due to cellulitis and meningitis) in about 70% of patients with CAPS who received pneumococcal vaccines compared to just 7% of patients receiving influenza and 17% receiving tetanus/diphtheria vaccines. Patients with the more severe CAPS phenotype (NOMID) appeared to have more frequent and more severe events than patients with CAPS with the less severe phenotype (FCAS) [54].

Additional IL-1 blocking agents are currently under development, which could be used to treat CAPS in the future. However, biologic therapies have some disadvantages including high cost, requirement for injection, and storage restrictions. There are also small molecule inhibitors under development that target NLRP3 directly and may be useful for the treatment of CAPS in the future [55].

19.10 Outcomes

Clinical outcomes and quality of life for patients with CAPS varies by disease subtype. In the past, many patients with NOMID and MWS had increased morbidity and mortality due to organ damage or adverse effects from ineffective therapies. The prognosis of patients with CAPS has changed considerably since the use of IL-1 targeted therapy. However, early and aggressive treatment is crucial to improve quality of life and to avoid end organ damage. Consistent with other autoinflammatory disorders, patients with CAPS are at risk for the development of secondary AA amyloidosis due to chronic inflammation (see Chap. 15). The most common affected organ is the kidney often leading to end stage renal disease. In fact, proteinuria is often the first indicator of systemic amyloidosis. While disease severity and persistently elevated serum SAA levels appear to be important predictors for the development of amyloidosis, the most important risk factor is a family history of amyloidosis, suggesting heritable components other than specific disease gene mutations. Estimated prevalence for amyloidosis in FCAS in the U.S.A. is 2% [24], while 25–33% of patients with MWS living in Europe were affected prior to the availability of effective targeted therapy [56]. Many patients with NOMID died before adulthood so the prevalence of amyloidosis is difficult to estimate [57].

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Autoinflammatory Granulomatous Disease: Blau Syndrome

20

Carlos D. Rose and Carine H. Wouters

Abstract

Blau syndrome (BS) is a monogenic granulomatous polyarthritis associated with rather severe uveitis resulting from mutations at or near the nucleotide-binding oligomerization domain (NOD) domain of NOD2. It encompasses early-onset sarcoidosis (EOS), a form of granulomatous arthritis affecting children before the age of 5 years and known since the mid-1970s, and a familial form described separately by Blau and Jabs in 1985. Once the mutation was identified it was clear that BS and EOS were the same disease and both are now covered under the eponym Blau syndrome. This chapter covers the spectrum of clinical manifestations of and diagnostic strategies for the investigation of patients with Blau syndrome, provides an update on pathogenesis and highlights several management recommendations. These developed as knowledge of the natural history improved with the creation of multicenter cross-sectional studies and an ongoing multicenter cohort study.

Keywords

NOD2 · Sarcoidosis · Blau syndrome Granulomatous diseases

Abbreviations

ACE	Angiotensin converting enzyme
BS	Blau Syndrome
CARD	Caspase recruitment domain
CD	Crohn disease
CRP	C-reactive protein
EOS	Early-onset sarcoidosis
IL	Interleukin
JIA	Juvenile idiopathic arthritis
LRRs	Leucine-rich-repeats
MAP	Mitogen-activated protein
MDP	Muramyl dipeptide
MGC	Multinucleated giant cell
NF-κB	Nuclear factor-κB
NOD2/CARD15	Nucleotide-binding oligomerization domain 2/caspase activation recruitment domain 15
PBMC	Peripheral blood mononuclear cells

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PIP	Proximal interphalangeal
RIP2	Receptor-interacting protein kinase 2
TAK	Transforming growth factor β-activated kinase
TB	Tuberculosis

Key Points

- **Blau syndrome (BS) is the only monogenic granulomatous autoinflammatory condition, and is caused by autosomal dominant mutations in *NOD2***
- **BS is characterized by a clinical triad of dermatitis, polyarthritis and uveitis, and can affect internal organs in almost half of the patients**
- **Morbidity in BS is mostly related to recalcitrant eye involvement**
- **Advanced insights in disease pathogenesis are essential to the development of effective targeted therapies**

20.1 Introduction

Blau syndrome (BS) is a granulomatous inflammatory disorder considered part of the spectrum of pediatric sarcoidosis and as such primarily characterized by the presence of non-caseating epithelioid giant cell granulomas in a variety of tissues and organs. The clinical phenotype associated with an autosomal dominant inheritance pattern was described in 1985 [1]. The finding of a mutation in the nucleotide-binding oligomerization domain 2/caspase activation recruitment domain 15 (*NOD2/CARD15*) among patients with BS in 2001 allowed for the first monogenic autoinflammatory granulomatous disease to be described [2]. BS and early-onset sarcoidosis (EOS) constitute the familial and sporadic forms, respectively, of a pediatric disease characterized by a triad of polyarthritis, uveitis and rash; and a unique association with mutations in or near the central NOD/NACHT domain of the *NOD2* gene. Visceral and vascular manifestations beyond the classic triad have since been documented in 29–48% of patients with BS, ratifying its true systemic nature [3, 4].

Granulomatous inflammation can be seen in primary immunodeficiencies, and has been reported in patients with various genetic defects. Cutaneous and subcutaneous lesions in patients with primary immunodeficiencies are most frequently found, but liver, spleen, lymph node, lung, gastrointestinal tract and brain involvement have also been reported [4]. However, this chapter focuses only on BS, the autoinflammatory form of granulomatous diseases.

20.2 Epidemiology

BS is a rare disease and epidemiologic studies focusing specifically on its prevalence have not been done. An approximation can be gathered by looking at sarcoidosis registries. The Danish National Registry included 48 children within a cohort of 5536 patients with sarcoidosis, resulting in a calculated overall incidence for childhood sarcoidosis of 0.29/100,000/year. The incidence ranged from 0.06/100,000/year for children below 5 years old to 1.02/100,000/year for children 14–15 years old [5]. It is in the younger age group where most cases with BS are likely to be found.

An earlier international registry of patients with pediatric sarcoidosis reported on 53 pediatric patients of whom 14 had a family history yielding a ratio of 1:5 for familial to sporadic forms [6]. The International Registry of Pediatric Sarcoidosis established in 2005 shows no gender difference or geographic predominance; the majority of patients exhibiting the classic triad of arthritis, uveitis, and rash have disease onset before reaching the age of 5 years [7].

20.3 Etiology

Blau syndrome features an autosomal dominant transmission pattern. Using linkage analysis of the original pedigree, the susceptibility locus for BS was mapped to a region of chromosome 16 found to contain a gene associated with Crohn disease (CD) called *IBD1* [8, 9]. The *IBD1* gene was later found to be *NOD2* [10]. In 2001, Miceli and colleagues identified mutations within the central NOD/NACHT domain of the *NOD2* gene in four French families with the Blau phenotype [2]. This seminal work revealed that NOD2 substitutions associated

with BS were located in a different domain of the protein than those associated with CD, which are predominantly found in the leucine-rich-repeats (LRRs) carboxy-terminal end. Wang and colleagues reported *NOD2* mutations in 50% of 10 pedigrees with the BS phenotype [11]. Later identical mutations were reported among patients with EOS, a sporadic disease with the same phenotype of granulomatous arthritis uveitis, and rash described years before [12]. Currently, BS and EOS are considered the same disease [13, 14]. Over the years, an expanding number of genetic mutations of *NOD2* have been published and reported within the Infevers Registry [15]. Substitutions R334W (arginine to glutamine in position 334) and R334Q (arginine to tryptophan) are by far the most common. More recently, using targeted deep *NOD2* sequencing, genetic mosaicism and transmission by somatic mutations have been reported in a few patients [16]. *NOD2* mutations can be seen in patients with the complete clinical triad but also in both incomplete and expanded phenotypes. Incomplete penetrance in asymptomatic carrier status individuals has rarely been reported [17, 18]. It is not clear whether there is a true relation between specific *NOD2* mutations and severity of eye involvement [19, 20].

20.4 Pathogenesis

- **Blau syndrome *NOD2* mutations are considered gain of function variants causing downstream receptor-interacting protein kinase 2 (RIP2) kinase phosphorylation and nuclear factor-κB (NF-κB) activation**
- **Additional NOD2 binding partners are probably involved in the mechanism of cell fusion leading to giant cell and granuloma formation**

The *NOD2* gene encodes a 1040 amino-acid protein composed of three main functional domains. These include two amino-terminal caspase recruitment domains caspase recruitment domains (CARDs), a central nucleotide binding oligomerization domain (NOD/NACHT), and carboxyterminal LRRs domain. The NOD2 protein is a member of the family of NOD-like receptor cytosolic proteins (NLRs) involved in pathways of inflammation, apoptosis and phagocytosis. The two amino-terminal CARD domains of NOD2 have an important role in the mediation of nuclear factor-κB (NFκB) activation and secretion of pro-inflammatory cytokines, resulting from CARD-CARD interactions between NOD2 and a pivotal downstream kinase protein called receptor-interacting protein kinase 2 (RIP2). The centrally located NOD domain mediates self-oligomerization of NOD2 followed by downstream activation of effector molecules. The LRR region is structurally related to the LRR regions of the toll-like receptors which are pattern recognition molecules of the innate immune system, (sensing and binding molecular motifs specific to pathogens; see Chap. 4). NOD2 recognizes muramyl dipeptide (MDP), a building block of peptidoglycan of both Gram-positive and Gram-negative bacterial cell walls. Monocytes, granulocytes, dendritic cells and Paneth cells in the villous crypts of the small intestine constitutively express NOD2 [21, 22].

NOD2, like other NLR proteins, occurs in two states: a tense comma shaped auto-inhibited state, and a relaxed NOD domain exposure state after ligand engagement. NOD domain exposure is a pre-requisite for NOD2 oligomerization and downstream pathway activation. Hydrophobic forces and salt bond interactions within the four subdomains of the NOD domain as well as ADP binding maintain the "tight" inactive state [23]. The stabilizing role of a folded LRR domain over the central NOD domain may not be as important as it is for other NLR proteins. There is evidence that engagement with the ligand could allosterically affect the NOD domain yet the mechanism by which ligand engagement translates into a relaxed state and NOD domain exposure remains unknown [23]. NOD2 oligomerization renders a CARD domain scaffold allowing for interaction with a CARD-containing RIP2 kinase and downstream pathway activation (Fig. 20.1).

Substitutions R334W (arginine to glutamine in position 334) and R334Q (arginine to tryptophan) are by far the most common *NOD2* mutations in BS. In a recently published crystal structure of NOD2 using an 85% homolog rabbit NOD2, these mutations would disrupt a large hydrophobic area that maintains NOD subdomains in the tense autoinhibited state described above [23].

The downstream effects of *NOD2* auto-activating mutations associated with BS, and their relationship

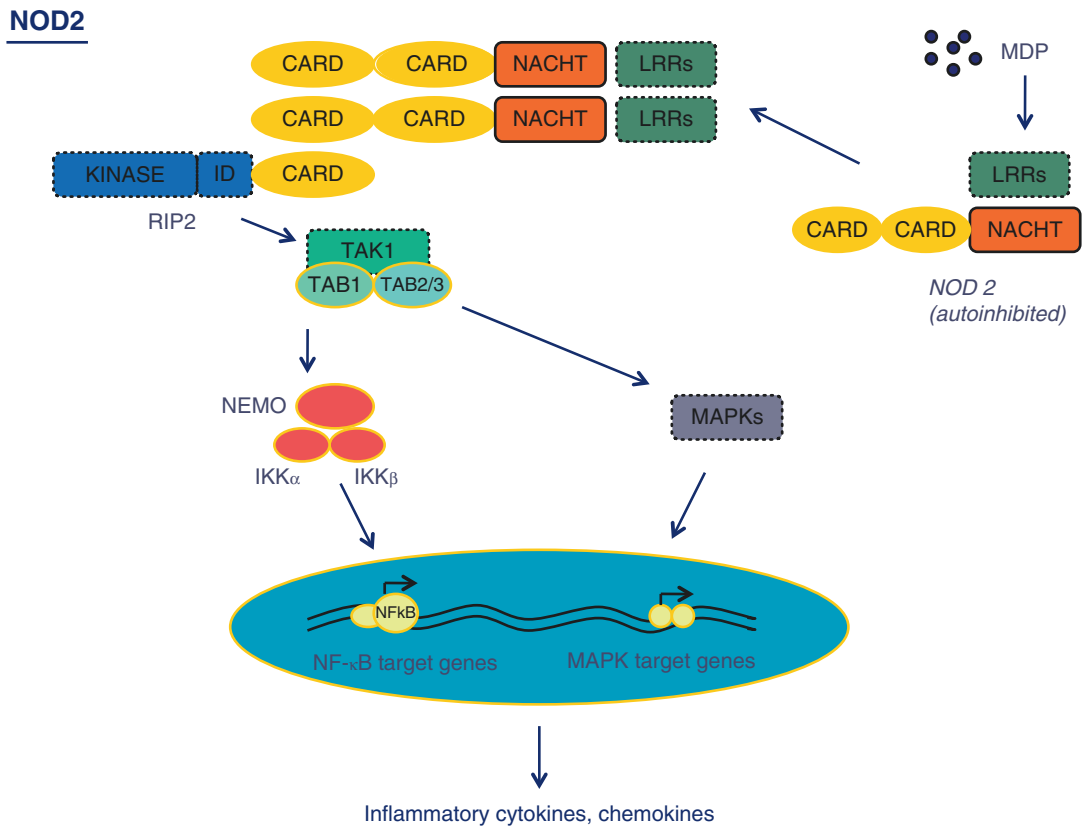

Fig. 20.1 Nucleotide-binding oligomerization domain 2 (NOD2) is expressed inside epithelial cells, granulocytes, monocytes, macrophages and dendritic cells in a monomeric and autoinhibited state. Upon recognition of muramyl dipeptide (MDP), NOD2 unfolds, oligomerizes through its NOD domain, recruits receptor-interacting protein kinase 2 (RIP2) kinase through caspase recruitment domain (CARD)-CARD interaction, causing its autophosphorylation. Activated RIP2 recruits transforming growth factor β-activated kinase 1 (TAK1) complex, allowing nuclear factor-κB (NF-κB) and mitogen-activated protein (MAP) kinase activation and production of pro-inflammatory cytokines. Reproduced with permission from Rose CD, Wouters CH (2016). Pediatric Sarcoidosis In Petty R, Laxer RM, Lindsley CB, Wedderburn LR (Ed.), Textbook of Pediatric Rheumatology 7th Ed. Elsevier, Philadelphia PA: pp 517–525

with both granuloma formation and clinical phenotype are not yet understood. Consistent with the autosomal-dominant inheritance pattern and according to early experimental work, *NOD2* mutations associated with BS are gain-of-function variants. Transient transfection assays performed *in vitro* using plasmids with powerful promoters that overexpress *NOD2* have found that mutations associated with BS cause excessive NF-κB and mitogen-activated protein kinase (MAPK) activation compared to the wild-type form of *NOD2* [24]. This gain-of-function concept has been endorsed *ex vivo* by an experiment with BS peripheral blood mononuclear cells (PBMCs) showing RIP 2 phosphorylation of serine-176 in the absence of MDP stimulation [25]. Conversely, experiments using patients' circulating mononuclear cells could not confirm upregulation and release of interleukin (IL-1) and other NFκB dependent cytokines, an ex-vivo phenomenon seemingly contradictory with the notion of gain of function and activation of NFκB [26]. Furthermore, a *NOD2* knock-in mouse carrying the R314Q mutation, the most common in BS (corresponding to R334Q in humans) showed reduced serum IL-6 and keratinocyte-derived chemokine (a murine IL-8 homologue) levels in response to intraperitoneally injected MDP. R314Q-knock-in mice macrophages showed a reduction in

full length NOD2 protein levels, as well as reduced cytokine responses and activation of NF-κB and p38 MAPK in response to MDP. The same research team confirmed that human monocytes from patients with BS showed attenuated cytokine production in response to MDP [27]. To reconcile these apparently contradictory data, one could conceive that the gain of function effect is not demonstrable in human PBMCs due to a phenomenon of attenuation and/or unknown modulating factors. Conversely, at the tissue level there is granulomatous inflammation with well-documented robust pro-inflammatory cytokine expression (see below). Recent ex vivo experiments using BS-specific induced pluripotent stem cell lines showed that interferon (IFN)γ acts as a priming signal through upregulation of NOD2. This effect translated into NFκB transnucleation and activation with pro-inflammatory cytokine production, supporting the significance of ligand-independent autoinflammation in the pathogenesis of BS [28].

Although RIP2 activation following NOD2 oligomerization in BS is well-documented, one should bear in mind that there are more than 30 proteins binding NOD2 with different degrees of affinity. Several of the NOD2 binding partners directly influence or regulate its functional activity. Some interactions promote autophagy and some elicit a negative impact on NFκB signaling, illustrating the complexity of NOD2 regulation and signaling [29]. The role of NOD2 in autophagy is of great interest since many of the interacting proteins bind NOD2 at the cytoskeleton and cell membrane where the cell fusion machinery involved in multi-nucleation and granuloma formation resides. The link between NOD2 activating mutations and granuloma formation is fascinating, yet there is still a significant gap in our understanding of the pathogenesis of BS.

20.5 Pathology

The pathological hallmark common to BS and sarcoidosis is the presence of non-caseating epithelioid granulomas thought to result from an exaggerated immune-inflammatory response to a persistent unidentified antigen. Granulomas consist of a central cluster of monocytes/macrophages in various stages of activation, epithelioid cells aligned in a way reminiscent of epithelial cells, and multinucleated giant cells. Blau granulomas display a distinct morphology characterized by large polycyclic granulomas with dense lymphocytic coronas (Fig. 20.2). They reflect an exuberant inflammatory response, which is in line with a gain-of-function mutation in *NOD2*. Using immunohistochemistry, a predominance of CD68+ macrophages and CD4+T lymphocytes and an abundant inflammatory cytokine expression *in situ* is typically observed. A prominent expression of IFNγ is in accordance with an important role for Th1 lymphocytes in granulomatous inflammation. This is seen in association with a very high expression of IL-6, transforming growth factor (TGF)-β and IL-17 as well as an increased expression of IL-23 receptor on granuloma cells (Fig. 20.3). These findings are suggestive of activation of the Th17 lymphocyte axis in BS granulomas [30]. In BS granulomas, widespread extensive emperipolesis (cell-in-cell phenomenon) of lymphocytes within multinucleated giant cells, associated with multinucleated giant cell death was seen as well, a finding of interest in view of the role of NOD2 in autophagy via both RIP2 dependent and RIP2 independent pathways [31].

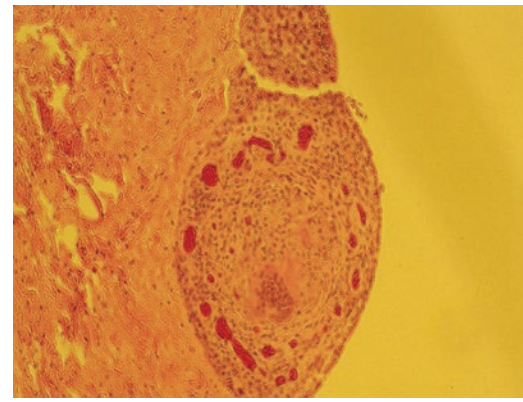

Fig. 20.2 Synovial biopsy showing a typical non-caseating epithelioid cell granuloma with multinucleated giant cells. Reproduced with permission from Rose CD, Wouters CH (2016). Pediatric Sarcoidosis In Petty R, Laxer RM, Lindsley CB, Wedderburn LR (Ed.), Textbook of Pediatric Rheumatology 7th Ed. Elsevier, Philadelphia PA: pp 517–525

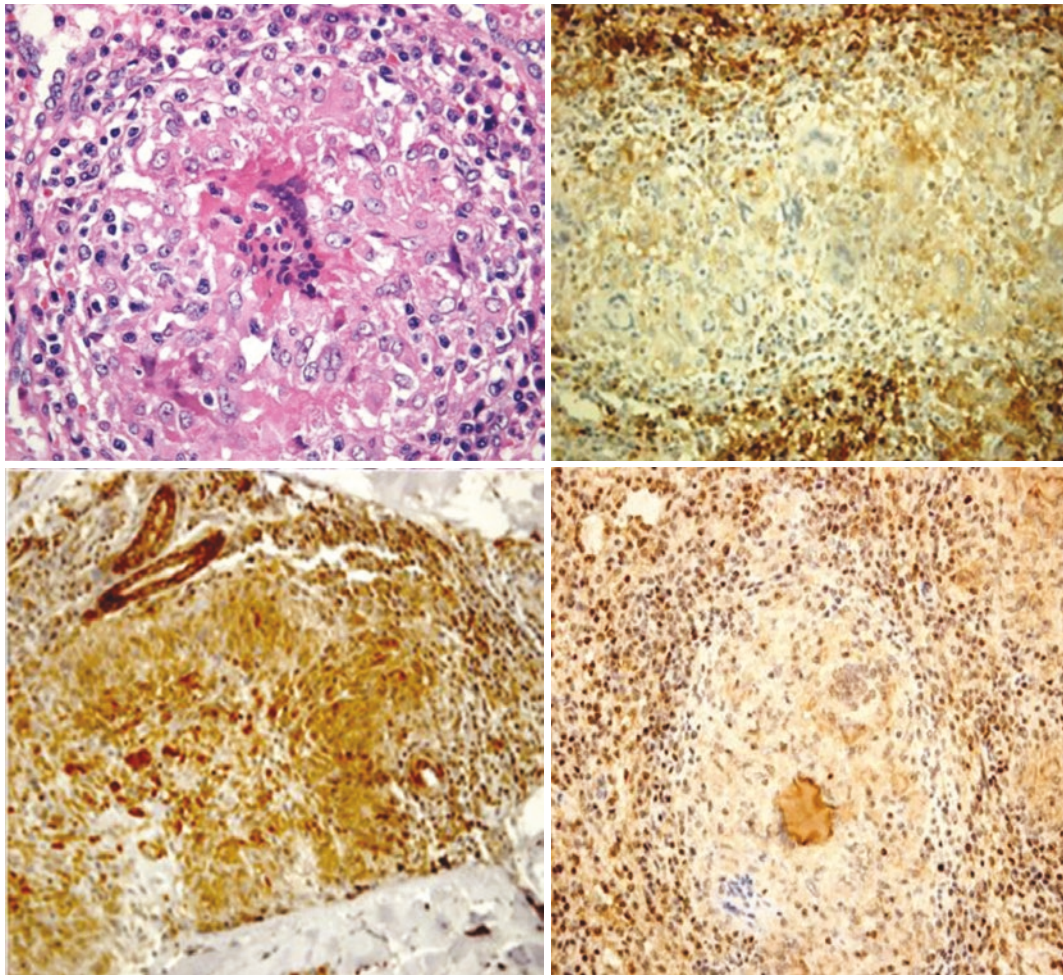

Fig. 20.3 Morphological and immunohistochemical characteristics of Blau granulomas. In clockwise appearance (top left). H&E staining showing prominent lymphocyte corona, emperipoletic lymphocytes, and multinuclear giant cell (MGC) death with fragmented cytoplasm and pycnotic nuclei. Using immunohistochemistry, dense staining was observed for interferon γ (top right), interleukin 6 (bottom left) and interleukin 17 (bottom right). Reproduced with permission from Rose CD, Wouters CH (2016). Pediatric Sarcoidosis In Petty R, Laxer RM, Lindsley CB, Wedderburn LR (Ed.), Textbook of Pediatric Rheumatology 7th Ed. Elsevier, Philadelphia PA: pp 517–525

20.6 Clinical Manifestations

- **A monomorphic micropapular rash during infancy is the initial manifestation of BS in most patients**
- **Arthritis in BS shows a symmetric polyarticular exuberant synovial and tenosynovial inflammation. Erosive disease is rare**
- **Ocular involvement is frequent and severe, involving both anterior and posterior eye segments**
- **BS affects internal organs and large vessels, with variable severity, in almost half of the patients**

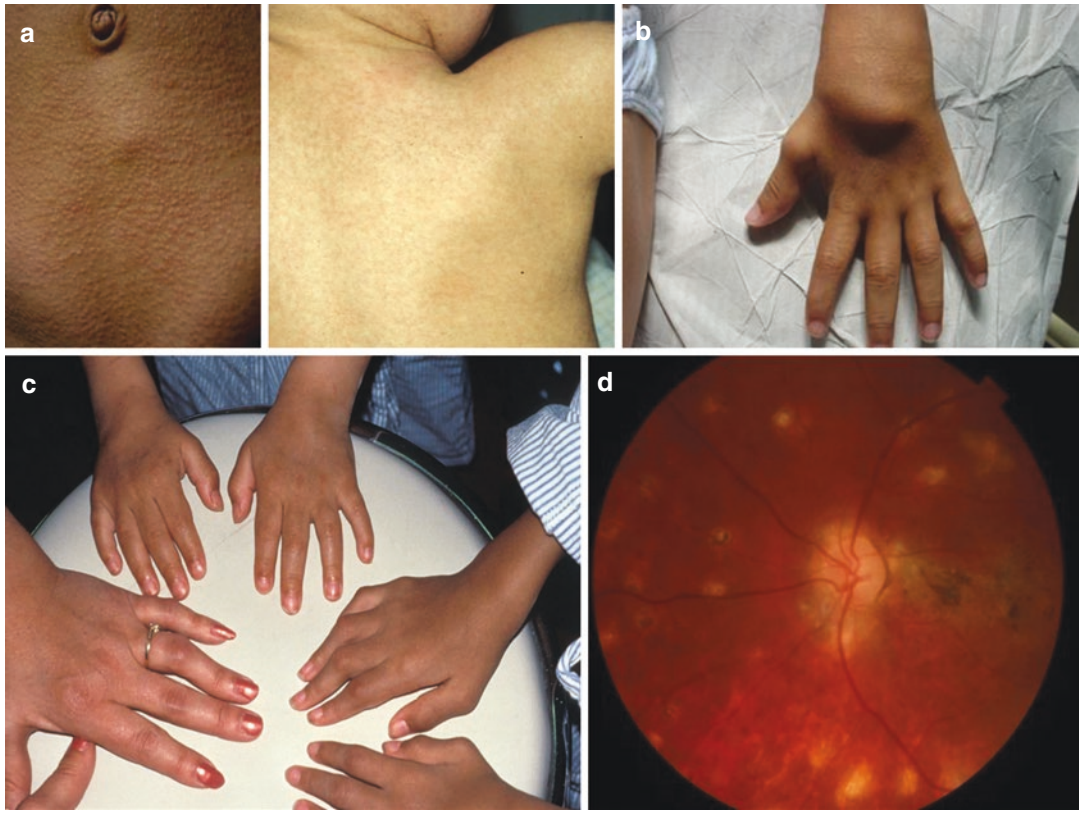

Fig. 20.4 Clinical triad typical of Blau syndrome. *Upper left, middle* (**a**): cutaneous features with fine maculopapular erythematous/tan eruption with ichthyosiform appearance (*right*). *Upper right* (**b**): typical "boggy" synovitis with preserved range of motion and cyst-like synovial swelling. *Below left* (**c**): a family with *NOD2*-associated polyarthritis and proximal interphalangeal contractures causing camptodactyly. *Below right* (**d**): multifocal choroiditis characteristic of granulomatous panuveitis. Reproduced with permission from Rose CD, Wouters CH (2016). Pediatric Sarcoidosis In Petty R, Laxer RM, Lindsley CB, Wedderburn LR (Ed.), Textbook of Pediatric Rheumatology 7th Ed. Elsevier, Philadelphia PA: pp 517–525

BS features a clinical phenotype of polyarthritis, dermatitis and uveitis (Fig. 20.4). In recent years, because of the availability of genetic testing, a more protean clinical picture than initially conceived is being unveiled.

The initial manifestations include the typical exanthema followed within months by a symmetrical polyarthritis. Ocular involvement tends to occur later in the disease course. The median age at onset in the International Blau Registry was 26 months with a range of onset between the ages of 2-months and 14-years [7].

20.6.1 Cutaneous Involvement

The rash varies in color from pale pink with varied degrees of tan to intense erythema. The lesions appear on the trunk mainly dorsally and later extend to the face and limbs with accentuation of the tan color on extensor surfaces, where it may become scaly brownish over time (Fig. 20.4a). The lesions are tiny (5–7 mm), round, and barely palpable. At onset, the rash often shows a very fine desquamation, which may lead to confusion with atopic dermatitis. Over the course of years the rash waxes and wanes. With time, the

desquamation predominates, and, in adolescence, it may mimic ichthyosis vulgaris.

Subcutaneous nodules, often located in the lower limbs, are the second most common dermatological manifestation and may be clinically indistinguishable from erythema nodosum [7]. The nodules are mildly tender and resolve without atrophy or pigmentation, even in patients with recurrent episodes. Erysipelas-like lesions have been observed, and in one case an urticarial rash showed typical histological features of leukocytoclastic vasculitis [32].

20.6.2 Articular Disease

The majority of patients present with a polyarticular symmetrical or additive arthritis, affecting large and small peripheral joints and tendon sheaths. The most frequently involved joints comprise wrists, knees, ankles and proximal interphalangeal (PIP) joints. A characteristic feature of both the synovitis and tenosynovitis is the exuberance of the swelling. The flexor tendons of the digits, the extensor and peroneal compartments of the ankle, and the flexor groups of the carpus can reach significant size. The synovial outpouching can acquire a cystic appearance in the dorsum of carpus and tarsus. Despite the prominent "boggy" synovitis, pain and morning stiffness appear to be mild to moderate and are overall well tolerated. Except for the PIP joints, where a characteristic flexion contracture described as "camptodactyly" can be seen, the range of motion is relatively well preserved, at least in childhood (Fig. 20.4b). The course of the arthritis is variable, and erosive changes are mostly rare and modest. However, limited joint mobility and joint contractures may develop with time; ulnar deviations, wrist subluxations, and joint space narrowing have been described [3, 33, 34]. In addition, the abnormalities on radiographs include a number of characteristic dysplasia-like bone changes (see below) (Fig. 20.5).There are limited data on the functional outcomes of the arthritis of BS over time. In a recent study, it was noted that 28% of patients reported moderate to severe functional impairment after a median disease duration of 12.8 years. Almost half of patients mentioned moderate to severe impact on their global well-being, and 43% experienced moderate to severe pain [3].

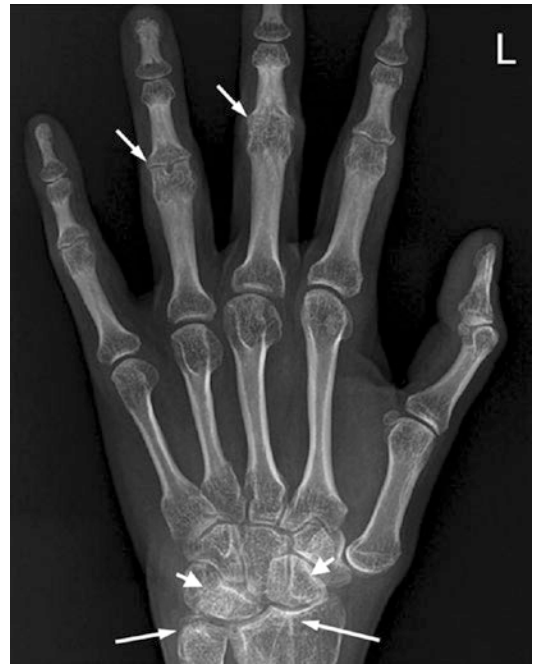

Fig. 20.5 Hand radiograph of a 37 year old patient with Blau syndrome showing diffuse peri-articular osteopenia and joint space narrowing in the proximal interphalangeal and wrist joints (short arrows). Dysplasia of the scaphoid and lunate bones are present (arrowhead). Associated biconcave radial epiphysis and abnormal plump distal ulna (long arrows), typical of the Blau hand are also demonstrated. Reproduced with permission from reference [3], Rose CD, et al. Rheumatology (Oxford) 2015;54:1008–16

20.6.3 Ocular Disease

An insidious granulomatous iridocyclitis and posterior uveitis can evolve into a severe destructive panuveitis. Of the clinical triad components, the ocular disease exhibits the

most somber functional prognosis. It tends to start within the first 2 years of disease as an asymptomatic uveitis. Over time characteristic iris nodules, focal synechiae and clumpy keratic precipitates at the limbus appear, and cataract and increased intraocular pressure ensue. Nodules may also occur in the conjunctivae and, in this location, offer an early biopsy site and diagnostic clue. A description of the slit lamp appearance of sarcoid uveitis compared to juvenile idiopathic arthritis (JIA)-associated uveitis was published by Lindsley and Godfrey [35]. Posterior involvement includes vitritis, multifocal choroiditis, retinal vasculopathy, and optic nerve edema (Fig. 20.4c). Ocular disease can be difficult to control. A prospective study on the natural course of the disease showed that uveitis duration was significantly associated with progression to severe disease and posterior involvement. After a median ocular disease duration of 12.1 years and despite prolonged topical and systemic treatment, persistent active anterior uveitis and vitreous inflammation were seen in in 40% and 42.7% of patients, respectively. Persistent ocular inflammation and cumulative complications including cataract, posterior synechiae, increased intra-ocular pressure, chorioretinal changes and macular edema were associated with moderate to severe visual impairment in 42% of patients [3, 20].

20.6.4 Visceral and Systemic Involvement

It has become apparent that the clinical phenotype of BS is not restricted to the classic triad. According to an ongoing prospective cohort study, systemic and visceral involvement affects 48% of patients with BS [3]. A myriad of clinical manifestations including granulomatous and interstitial nephritis, chronic renal insufficiency, small vessel vasculitis, interstitial pneumonitis, peripheral and mediastinal (excluding hilar) lymphadenitis, pericarditis, cranial neuropathy (VII cranial nerve), and parotitis have been documented in recent studies [36–40], although they were known before the association with the *NOD*2 mutation was discovered [39, 41].

Systemic symptoms, including prolonged fever, can be a presenting manifestation and may recur during first few years of the disease as described in a Spanish series, and later confirmed by the authors of this chapter [4, 40].

Occurrence of large vessel vasculopathy in BS has been known from old studies [42, 43] and from one NOD2 mutated family, as reported by Wang [11]. One case of Takayasu-like arteritis was reported in a girl with BS [44]. Severe arterial hypertension was described in a family with granulomatous arteritis and polyarthritis of juvenile onset, before the definition of BS as a clinical entity [45]. Arterial hypertension with normal digital vascular imaging was observed in 25% of patients from an international registry and was also frequently found in a Spanish cohort of adult patients with BS [40]. The mechanism is unknown, but renal vasculopathy has been suggested. In a single case report a sinus of Valsalva aneurysm in a patient with BS was described [46].

Two separate reports of families with BS with symptomatic and asymptomatic members carrying mutation E383K have been published [17, 18].

20.7 Laboratory Testing

Peripheral blood cell counts are usually within normal limits, although mild anemia, leukopenia, or lymphopenia can be seen. Patients with BS do not typically show increased levels of acute phase reactants, thus they are not reliable markers of disease activity. In a BS cohort study, levels of C-reactive protein (CRP) were only mildly elevated compared to healthy controls, and did not correlate with articular disease activity [3]. Conversely, S100A9 and A12 protein plasma levels are increased, and were

shown to correlate significantly with the active joint count. S100 proteins are secreted by neutrophils and macrophages, and may reflect the burden of granulomatous inflammation in BS [47] (see Chap. 9). Serum IgG levels may be elevated, but antinuclear autoantibodies are mostly absent. Elevation of angiotensin converting enzyme (ACE) is not consistent, and the value of serum ACE levels in diagnosing and managing BS remains unclear. ACE levels are influenced by ACE-gene polymorphisms, and physiological values vary according to age with a higher normal range of serum values in children. Hypercalciuria and hypercalcemia result from overproduction of 25-hydroxyvitamin D-1 α-hydroxylase resulting in the conversion of 25-hydroxyvitamin D to 1,25 dihydroxyvitamin D by macrophages in granulomas. Hypercalciuria can lead to nephrocalcinosis and nephrolithiasis. Both complications have been documented in patients with BS [3].

20.8 Imaging

The increasing understanding of the spectrum and natural history of BS allows for ascertainment of the usefulness of different imaging techniques in patient evaluation and follow-up.

Hand radiographs show a symmetrical, nonerosive arthropathy, with a number of characteristic dysplasia-like bone changes. Some of the most frequently observed deformities are characteristic, including a biconcave radius, carpal crowding, a short plump distal ulna and a thin second metacarpal diaphysis. These findings may allow recognition of BS from a single wrist radiograph [34] (Fig. 20.5). The radiographic image, which we dubbed the "Blau hand", is very different from the "rheumatoid hand" [34] (Table 20.1).

Interstitial lung involvement is rare, yet prolonged periods of unabated constitutional symptoms may warrant performing a chest radiograph or CT. Similarly, an echocardiogram may detect

Table 20.1 Main phenotypic differences between Blau syndrome (BS), polyarticular juvenile idiopathic arthritis (JIA) and adult rheumatoid arthritis (RA)

	BS	Poly-JIA (RF−/RF+)	RA
Age at onset	Before age 4 years	Any pediatric age	16 years or older
Parents and grandparents with polyarthritis	50%	Rare	Rare
Extra-articular disease	Granulomatous dermatitis, erythema nodosum, visceral involvement, large vessel or leukocytoclastic vasculitis	Rheumatoid nodules in RF positive poly-JIA	Rheumatoid nodules, interstitial lung disease
Uveitis	Uveitis in 75% (55% panuveitis)	Anterior uveitis in 10–20%[a]	Very rare
Articular findings	Boggy synovitis and tenosynovitis with camptodactyly	Symmetrical polyarthritis with contractures and local growth abnormalities. Early erosions in RF+ JIA.	Symmetrical destructive polyarthritis
Spinal involvement	None	Cervical	Mostly cervical
Hip involvement	Rare	Common	Common
Hand radiographs	Non erosive, dysplastic changes ("Blau hand"); sparing of MCPs; PIP contractures	Cartilage loss, erosive changes, growth abnormalities, PIP > MCP involvement	Cartilage loss, erosive disease, ulnar deviation, MCP and PIP involvement
ANA/RF	Negative/negative	Commonly positive/positive in 5–10%	Rarely positive/ commonly positive

[a]Posterior involvement can be seen in severe forms; *RF* rheumatoid factor, *MCP* metacarpophalangeal, *PIP* proximal interphalangeal, *ANA* antinuclear antibody

pulmonary hypertension, a rare, yet lethal complication observed in one young man in the retrospective Blau registry (CDR personal communication). Renal sonography is required to assess for nephrocalcinosis in patients with BS with hypercalcemia and/or abnormalities in urinalysis.

20.9 Diagnosis

- **The demonstration of *NOD2* mutations in the context of a compatible phenotype is required for a definite diagnosis of BS**
- **BS is a great imitator of many pediatric immune and inflammatory diseases**

The definite diagnosis of BS rests on genetic confirmation of a *NOD2* mutation in the context of either a typical clinical phenotype and/or the demonstration of characteristic non-caseating granulomatous inflammation.

20.9.1 Pathology

Typical non-caseating epithelioid and multinucleated giant cell granulomas can be documented in biopsies of skin, synovium, lymph node, kidney or liver. Skin biopsy has shown the best yield among patients with classical granulomatous dermatitis. A synovial biopsy can offer a good alternative, particularly in patients whose rash has resolved or appears inactive. Granulomatous inflammation is a common finding when tissue biopsies are performed in patients with BS [7].

20.9.2 Genetic Testing

In early publications the frequencies of *NOD2* mutation among patients exhibiting the clinical phenotype compatible with BS varied between 50% in familial forms and 90% in sporadic forms [11, 24]. We found *NOD2* mutations in 98% of the patients of the International Pediatric Granulomatous Arthritis Registry exhibiting the classic triad phenotype with either a sporadic or a familial form [7]. The recent discovery of *NOD2* mutations in a few asymptomatic individuals of a large family, and the finding of extended clinical manifestations, suggest the interaction with as yet unidentified supplementary modulating genes in the clinical phenotype. Of note, patients with the classical clinical phenotype occasionally test negative for *NOD 2* mutation. Recent work by Arostegui et al, has shed light on this intriguing finding, by finding somatic and gonosomal *NOD2* mosaicism among patients with BS phenotype, who apparently had a milder phenotype with oligoarticular involvement, but without tenosynovitis or camptodactyly [16, 48].

20.10 Differential Diagnosis

The diagnosis of BS in a child with granulomatous inflammation requires a concerted effort to exclude chronic infections, notably mycobacterial and fungal, by appropriate staining and cultures.

In geographic areas endemic for tuberculosis (TB), granulomatous arthritis should raise the suspicion of TB. Even in the absence of a positive purified protein derivative (PPD) test, mycobacterium cultures and stains, the finding of any level of caseation in the granulomas requires treatment for TB. In addition, a reactive non-granulomatous polyarthritis (Poncet's disease) may be seen in patients with active TB. This polyarthritis responds to anti-tuberculous therapy, with complete resolution.

Monoarticular granulomatous synovitis can be seen in patients with foreign body arthritis. Penetration of thorns from Yucca plants, sea urchin spines and other inert foreign bodies are not that unusual in exposed children and should be suspected in the appropriate clinical scenario.

A challenging differential diagnosis for BS polyarthritis with uveitis is polyarthritis JIA. In addition, adults with BS may be incorrectly diagnosed with rheumatoid arthritis or spondyloarthropathy. Table 20.1 summarizes the main clinical differences among these three conditions.

Occasionally BS presents with arthritis and systemic features reminiscent of systemic JIA [40].

BS needs to be differentiated from other systemic inflammatory disorders associated with granulomatous inflammation in children, such as CD. Although BS does not affect the gastrointestinal tract some extra-intestinal manifestations can be confusing including arthritis, uveitis, hepatitis, cutaneous vasculitis and erythema nodosum. Different from BS granulomas, CD granulomas are isolated, ill-defined and without lymphocytic coronas [30]. Although both diseases show *NOD2* mutations, these typically occur at different domains of the NOD2 protein (see above). Patients with granulomatosis with polyangiitis often have granulomatous inflammation of the upper respiratory tract.

Adult sarcoidosis may present in adolescence and exhibit a similar visceral organ involvement as seen in BS. The pattern of joint and eye involvement is different, whereas hilar adenopathy has not been observed in BS. *NOD2* mutation analysis is normal in adult sarcoidosis.

Large vessel vasculitis can be a presentation of BS. Therefore, BS needs to be considered among patients with abdominal aortitis, renal artery stenosis, Takayasu-like syndrome and aortic root disease.

Various primary immunodeficiency disorders can present with granulomatous inflammation without an identifiable infectious cause, and should be excluded by evaluation of neutrophil function, analysis of circulating lymphocyte subsets and serum levels of immunoglobulins.

Granulomas have been described in association with common variable immunodeficiency, a number of combined immune deficiencies with various monogenic defects (mainly hypomorphic recombination-activating genes (RAG) mutation syndrome, hypomorphic Artemis deficiency, hypomorphic Janus kinase (JAK)3 deficiency, CD40L deficiency, ataxia telangiectasia, Nijmegen breakage syndrome, lipopolysaccharide-responsive and beige-like anchor (LRBA) deficiency, cartilage hair hypoplasia) and chronic granulomatous disease (reviewed in [49]). The mechanism is unknown, and the extent of organ involvement varies, although lymph nodes, skin, lung, liver, and spleen are the main sites involved. Histopathologic features of granulomatous lesions are heterogeneous; there could be (1) sarcoidosis-like granulomas, mainly composed of epithelioid cells associated with few lymphocytes and giant cells arranged in well-circumscribed nodules, (2) poorly defined tuberculoid granulomas with numerous giant multinucleated cells associated with some lymphocytes and few epithelioid cells and (3) histiocytic palisading granulomas with a central necrobiotic area [49]. Immunohistochemical studies have documented a predominance of CD8+ T cells and a lower CD4+/CD8+ ratio in these granulomas as compared to the findings in sarcoidosis [49].

20.11 Treatment

Evidence-based data on the optimal treatment for BS are nonexistent. Moderate- to low-dose daily corticosteroid therapy is effective to control uveitis and joint disease, but the side effects of prolonged use may become unacceptable. In a prospective BS cohort study, more than two-thirds of patients with BS received medical therapy for several years, often combining systemic corticosteroids, immunosuppressive and/or biologic drugs to control both uveitis and arthritis [3]. Methotrexate at a dosage of 10–15 mg/m^2 once weekly reportedly was effective in suppressing articular disease activity and may be steroid sparing. Anti-tumor necrosis factor (TNF) monoclonal antibodies are the most used biologic therapy. Infliximab and adalimumab were found to control chronic arthritis and visceral manifestations in a number of patients; however, the effect on uveitis activity is less convincing [3, 7, 50, 51]. Anti-TNF therapy was effective in controlling one case of glomerulonephritis related to BS [4]. The observation of persistently active disease in a majority of patients with BS in the cohort study underlines the need for development of effective targeted therapies. A good response to IL-1 inhibition with anakinra was reported in a single case [40] and the clinical benefit of canakinumab on refractory uveitis in a 4-year old boy has also been reported [52]. Tocilizumab has been used in isolated cases, yet at present its efficacy remains unknown.

Of note, in the few patients with renal, pulmonary, salivary and/or hepatic involvement seen by the authors, these manifestations were mild and easily reversible with treatment. The few cases of hypercalcemia observed in the cohort were managed with increased corticosteroid dose for a few weeks without recurrence.

Anti-hypertensive medication may be required in patients who developed arterial hypertension with or without obvious renal involvement. ACE inhibitors have been effective in the few documented cases [3].

20.12 Prognosis

Until recently there were very limited data on the outcome of BS, yet early observations already suggested that the disease is not always benign [38]. In the prospective BS cohort study, articular and ocular disease were still active after more than 10 years of systemic therapy in 70% and 78% of patients respectively. At baseline evaluation, active ocular inflammation was seen in more than one third of patients and was associated with moderate to severe visual impairment in 27% and 15% of patients, respectively. There was no decrease in inflammatory activity and a progressive worsening of visual acuity was seen during 3 years of follow-up [3]. The arthritis appears to be nondestructive, especially during the first years, but as the disease progresses, flexion deformities, camptodactyly, and less frequently, erosions can be observed. Persistent joint swelling is common, with active arthritis (median joint count of 15) seen in 70% of patients with more than 10 years of disease. Twenty-eight percent of patients graded their functional disability as moderate or severe [3]. Severe hypertension and visceral involvement, including glomerulonephritis with renal failure and interstitial pneumonitis, have been observed in patients from the international retrospective registry, highlighting the need for careful surveillance throughout the disease course [4]. Pulmonary arterial hypertension was the cause of death in one patient at the age of 23 years (CDR, personal observation).

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Very Early Onset Inflammatory Bowel Disease (VEOIBD)

21

Aleixo M. Muise

Abstract

Inflammatory bowel disease (IBD) is a chronic gastrointestinal tract disorder with many clinical presentations. The most common forms of IBD are ulcerative colitis, Crohn disease, and overlapping disease termed IBD unclassified (IBDU). In general, IBD is considered a complex disease with contributions from genetics (polygenic), an abnormal immune response and the microbiome, and unknown environmental factors. Recently, there has been a world-wide increase in the incidence of IBD including in developing countries. In developed nations, the biggest increase is observed in children, especially very young children who develop the disease before 6 years of age (very early onset IBD—VEOIBD). Recent genetic studies have shown that some VEOIBD patients will have monogenic forms of IBD and these are described in this chapter.

Keywords

Very early onset inflammatory bowel disease · Interleukin (IL)-10R · XIAP · FOXP3 · TTC7A

Abbreviations

ARPC	Actin-related protein complex
CD	Crohn disease
CGD	Chronic granulomatous disease
CMPI	Cow's milk protein intolerance
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DUOX	Dual oxidase
GWAS	Genome-wide association studies
HLH	Hemophagocytic lymphohistiocytosis
IBD	Inflammatory bowel disease
IBDU	IBD undetermined
IPEX	Imunodysregulation polyendocrinopathy, enteropathy X-linked
LRBA	Lipopolysaccharide-responsive and beige-like anchor
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa B
NLRC4	NOD-like receptors caspase containing 4
NO	Nitric oxide
NOD2	Nucleotide-binding oligomerization domain-containing protein 2
NOS	Nitric oxide synthase

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NOX	NADPH oxidase
PID	Primary immunodeficiency
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
TRIM22	Tripartite motif-containing 22
TTC7A	Tetratricopeptide repeat domain 7
UC	Ulcerative colitis
VEOIBD	Very early onset inflammatory bowel disease
XIAP	X-linked inhibitor of apoptosis

Key Points

- **Inflammatory bowel disease (IBD) is a severe intestinal disease that can be multi-systemic**
- **IBD has become a common pediatric intestinal disease with a rapid increase in the diagnosis of pediatric patients**
- **IBD is most often caused by complex risk factors including genetic; however, there are monogenic causes that may be indistinguishable from polygenic forms of IBD**
- **It is now common for children to be diagnosed at a very young age with typical polygenic IBD**
- **There are a growing number of monogenic causes of IBD that have been identified in patients with very early onset inflammatory bowel disease (VEOIBD)**

21.1 Introduction

Inflammatory bowel disease has traditionally been classified based on its clinical, endoscopic and histologic features as either Crohn disease (CD), ulcerative colitis (UC) or unclassified (IBDU). However, it is now recognized that IBD encompasses a spectrum of chronic gastrointestinal tract disorders with many clinical presentations and variable genetic risk and monogenic causes [1, 2]. Typical symptoms of CD include abdominal pain, diarrhea and weight loss; whereas, the cardinal symptoms of UC are rectal bleeding and urgency. CD can affect any part of the digestive tract and manifests as transmural inflammation, leading to serious complications, such as intestinal stricture formation, fistulization and development of abscesses [3]. The inflammatory process in UC is confined to the superficial mucosa of the colon only and occurs in a continuous fashion from the anus proximally [4]. This chapter will review the most common monogenic forms of IBD often found in very young child with severe disease.

21.2 Definition and Classification

Children diagnosed with IBD at less than 10 years of age develop a disease that is significantly different than the disease in older children and adults [5–8]. Unique features of pediatric IBD include the relative rarity of ileal inflammation, the predominance of colonic inflammation, and the predilection for perianal disease. This has led to the recent Pediatric Paris modification [9] of the Montreal classification [10] and the designation of IBD onset before age 10 years as "very early onset IBD" (VEOIBD). However, the definition of VEOIBD is now generally considered to be children diagnosed with IBD less than 6 years of age [11]. VEOIBD patients are more likely to have familial disease [5–8], making it a fruitful population to identify risk genes. Infantile IBD constitutes an important subset of VEOIBD patients (approximately 1–3% of pediatric IBD) [5, 8]. In contrast to the etiology of IBD in older children and adults, which may be complex and include environmental and epigenetic factors, mounting evidence suggests that determinants of inflammation in infantile IBD patients are largely genetic, often with a singular genetic defect [12].

21.3 Epidemiology

- **The prevalence of inflammatory bowel disease (IBD) in western countries is now 0.3%**
- **There has been a dramatic increase in the incidence of IBD in developing countries**
- **Pediatric IBD is the fastest increasing group of patients with IBD**
- **Very early onset IBD (VEOIBD) is defined as children diagnosed with IBD less than 6 years of age**

Recent epidemiological studies show a remarkable 0.3% prevalence of IBD in western countries and a concurrent dramatic increase in developing countries making IBD a truly global disease (Fig. 21.1) [13]. In most western countries, the rates of pediatric IBD are increasing rapidly [14], with international incidence rates for IBD ranging from 0.47/100,000 in Saudi Arabia to 15.9/100,000 in the South Asian population in British Columbia, Canada [15, 16]. Studies also suggest that immigrants to western countries have a lower incidence of IBD than non-immigrants; however, their children born in Canada have a similar high IBD incidence as the children of non-immigrants [17, 18]. Canadian studies showed that children with VEOIBD now make up approximately 25% of all pediatric onset IBD, and the incidence has increased by 65% over the past decade in Ontario, Canada [14]. VEOIBD now represents the fastest growing group of newly diagnosed patients of any age including adults (Fig. 21.2) [14, 19], with similar trends seen in Scotland [20] and Ireland [21]. Together, these epidemiological studies support the important role of local environmental factors as driving the rapid increase of IBD incidence. Therefore, the increasing global burden of inflammatory bowel disease will pose significant challenges to health care systems around the world, especially in pediatrics.

21.4 Etiology and Pathogenesis

- **IBD in older children is considered a complex disease with genetic and significant but as yet unidentified environmental risk factors including the diet and microbiome**
- **VEOBID is most often also a complex disease similar to adult disease**
- **Growing evidence suggests that a significant proportion of patients with VEOIBD have monogenic mutations as a cause of their disease**
- **A number of genes involved in the innate immune system and autoinflammation are associated with IBD risk and monogenic causes of IBD**

The etiology of IBD reflects a dysregulated intestinal immune response in a genetically susceptible individual following exposure to an environmental trigger. Bacteria, including members of the commensal gut microflora as well as invasive pathogens, are thought to be important factors related to disease onset and course [22]. Genome-wide association studies (GWAS) of IBD and a subsequent meta-analysis [23] have identified over 200 variants with an estimated 23–35% of the susceptibility determinants for CD [24] and 17–25% for UC [25]. Furthermore, recent studies have begun to determine key genetic network drivers and tissue specific expression profiles of these GWAS variants [26, 27]. Pediatric GWAS studies based mainly on children over 10 years of age at diagnosis have identified overlapping variants with adult onset disease [28, 29]. Overall, genetic studies, have identified critical components of the pathways involved in the development of IBD [1, 23, 30–39]. Although IBD is one of the best-studied models of complex disease, it remains largely unknown how genetic variations affect cellular pathways and contribute to IBD [40].

21.4.1 Nucleotide-Binding Oligomerization Domain-Containing Protein 2 (NOD2)

NOD2 was the first gene identified as the most important Crohn disease risk gene and plays a critical role in its underlying pathogenesis [31, 35]. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) regulates innate immunity through nuclear factor kappa B (NF-κB)-induced pro-inflammatory responses triggered by peptidoglycan [41]. Genetic and functional studies have shown that *NOD2* loss of function variants are associated with Crohn disease and result in the loss of NF-κB-induced pro-inflammatory cytokine response to muramyl dipeptide [42, 43]. Recent studies have shown that genes that cause VEOIBD such as *XIAP* and *TRIM22* (see below) also result in loss of NF-κB-induced pro-inflammatory cytokine response to muramyl dipeptide. Therefore,

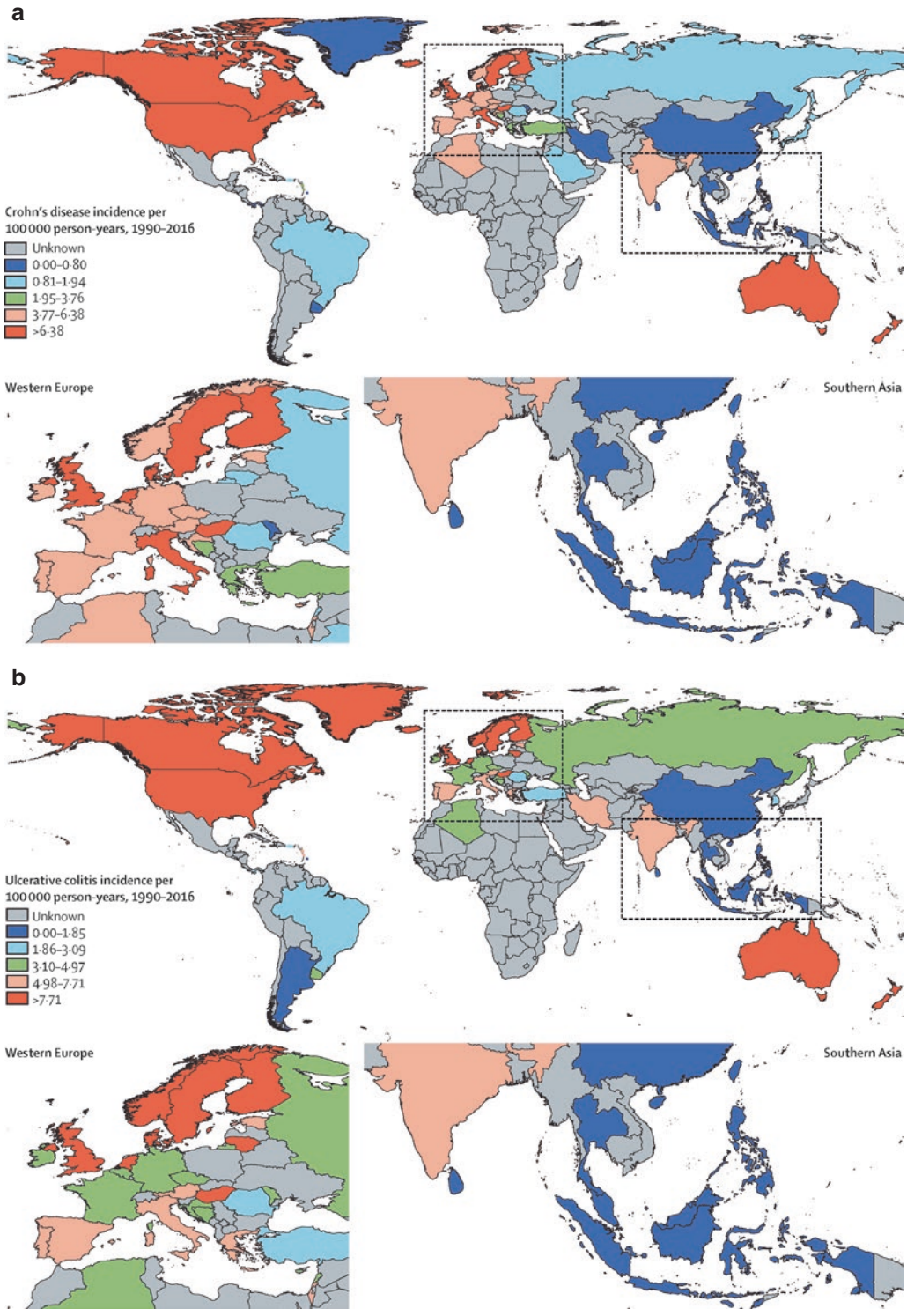

Fig. 21.1 Adapted from **Ng Lancet 2018. Map of worldwide incidence in quintiles for (a) Crohn's disease and (b) ulcerative colitis**

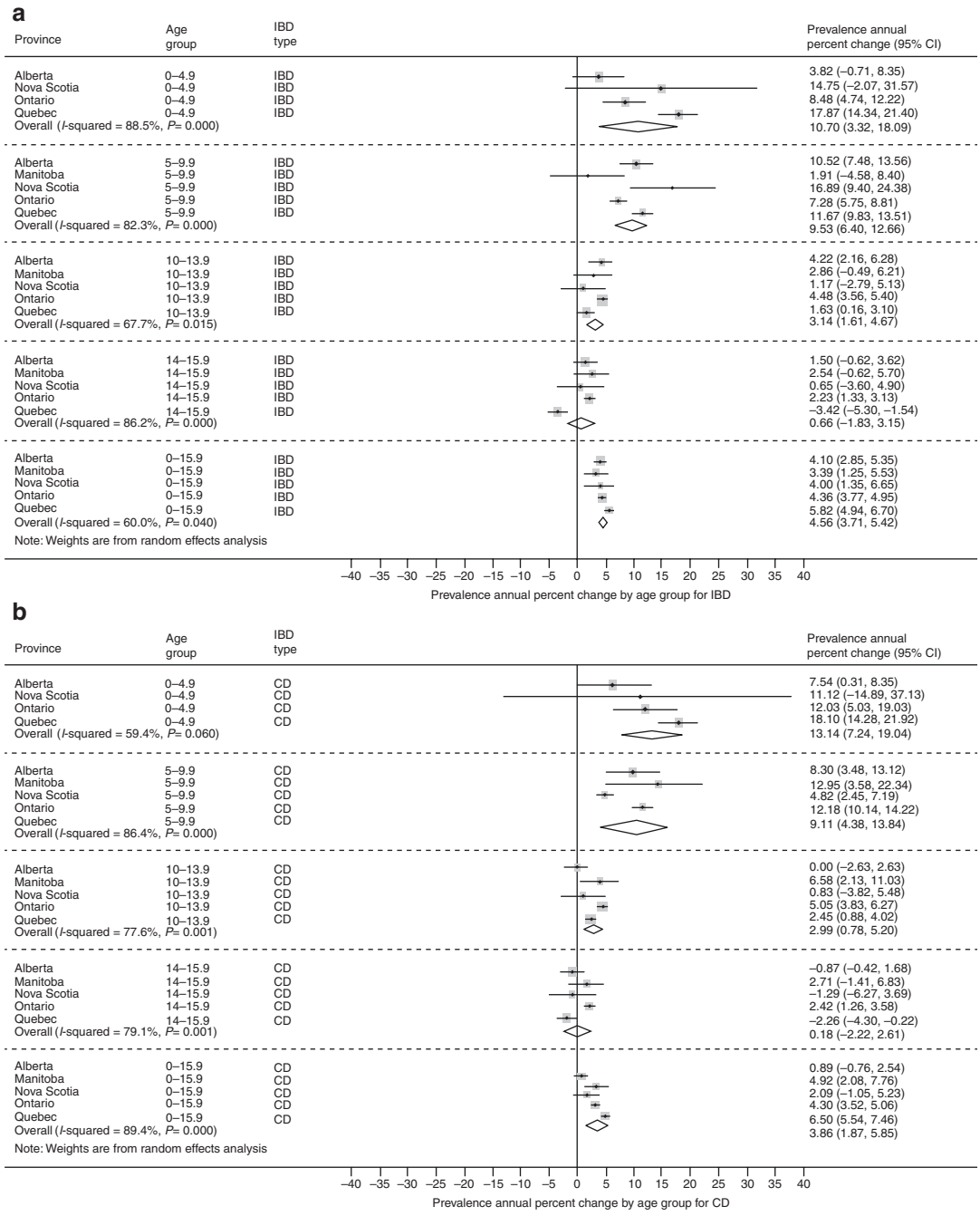

Fig. 21.2 Adapted from **Benchimol Am J Gastro 2017.** Annual percentage change in prevalence of (**a**) IBD, (**b**) CD, (**c**) UC by age group. Meta-analysis was conducted to combine provincial estimates. P was calculated to determine heterogeneity in provincial results

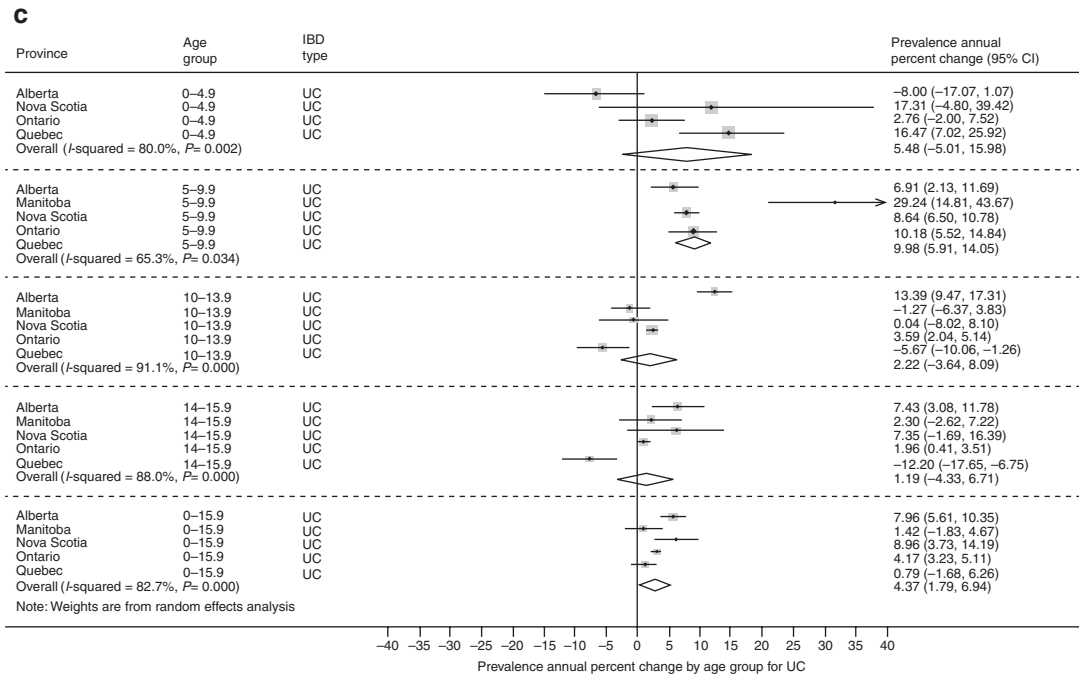

Fig. 21.2 (continued)

NF-κB regulation appears to be a key driver in monogenic forms of VEOIBD. Other monogenic autoinflammatory VEOIBD genes are outlined below.

21.4.2 Environmental Factors

As shown by Benchimol, there has been a rapid increase in the incidence of pediatric IBD and this is especially true in Ontario, Canada [44]. The rapid increase in incidence indicates that environmental factors must play a major role in the development of pediatric IBD. Despite numerous studies, only a handful of environmental risk factors have been reproducibly confirmed in any age group. The only reproducible environmental factor in any age group is smoking with a meta-analysis concluding that active smokers, followed by ex-smokers, were at increased risk for developing CD [45], although the risk of smoking and second hand smoke in children is unknown. Breastfeeding has also shown to reduce the risk of developing CD [46]. The environmental factors associated with the dramatic increase in the incidence of VEOIBD are currently unknown.

21.4.3 Clinical Manifestations

- **Patients with VEOIBD often present with pancolitis**
- **Patients with VEOIBD may have very mild or very severe phenotypes**
- **Patients with severe phenotypes often do not respond to therapies including biologic agents**
- **Patients with monogenic disease may be challenging to diagnose and treat, and often have multisystemic disease**

There is a distinct difference in the presentation of early onset versus later onset IBD. Children with UC often present with pancolitis compared to adults who often only have left-sided disease [47–49]. Children with CD often present with ileocolonic disease and only

rarely present with ileal disease in isolation that is commonly observed in adults [47–49]. Of note, children with CD diagnosed under the age of 8 years often have isolated colonic inflammation which may lead to a misclassification of UC or IBDU during the initial evaluation [50, 51]. However, at presentation, there does not appear to be a difference regarding the rates of complicated forms of CD including stricturing or penetrating disease [51].

Patients with VEOIBD are often phenotypically and genotypically distinct from older children. There is limited published evidence on those aged <2 years of age. Case series suggests that young children often have a more severe presentation and serious infections may complicate their disease course, raising concerns of immunodeficiency [5]. Of concern in those presenting at an earlier age are the effects of chronic inflammation on growth and global development [52]. Patients with VEOIBD are believed to have a more aggressive disease course; however, with the recent rapid increase in the incidence of polygenic IBD in very young children, VEOIBD is now described as a heterogeneous group with variable disease severity, with some children having very mild disease with polygenic risk genes and others with complicated disease that may be monogenic [53].

21.4.4 Genetics in VEOIBD

Recent genetic discoveries that demonstrated that primary immunodeficiencies can present with isolated clinical manifestations such as colitis, enterocolitis, or perianal disease, without other major features of immunodeficiency, have dramatically changed our approach to diagnosing and treating these patients. Most excitingly, many of these VEOIBD patients can be cured through allogenic bone marrow transplantation. Therefore, screening VEOIBD patients for known causal genetic variants is now standard of care in many academic centres. Here we will review the recent studies focusing on both polygenic and monogenic genes involved in VEOIBD.

21.5 Polygenic Forms of VEOIBD

- **Little is known regarding the genetic risk factors involved in VEOIBD**
- **Limited studies have shown that VEOIBD risk genes are mostly involved in microbial regulation**
- **Large scales genetic studies are needed to determine genetic risk in VEOIBD compared to older children and adults**

Most forms of IBD (including VEOIBD) are not caused by single gene mutations but are multifactorial with genetics appearing to play a greater role in very young children with IBD. A number of genetic studies have focused on VEOIBD susceptibility. The identified VEOIBD associated variants do not cause disease but may place a young child at greater risk of developing disease at an early age.

21.5.1 Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase

It is well recognized that chronic granulomatous disease (CGD; caused by defects in the genes that encode components of the NADPH oxidase complex; see below) is commonly associated with the development of a chronic colitis resembling CD. Studies examining the NADPH oxidase complex demonstrated that one-third of patient cohorts with VEOIBD carried heterozygous functional hypomorphic variants in NADPH oxidase complex components [54], including a variant in Neutrophil Cytosolic Factor 2 (NCF2) [55]. These NADPH oxidase complex component variants do not cause overt immunodeficiency, but instead influence susceptibility to VEOIBD.

21.5.2 Nitric Oxide Synthase 2 (NOS2)

The *NOS2* gene encodes for the inducible nitric oxide synthase (iNOS), responsible for nitric oxide (NO) production. NO contributes to

anti-microbial and anti-pathogenic activity in the intestine. A *NOS2* single nucleotide polymorphism (SNP) was found to be associated with VEOIBD in two independent cohorts demonstrating the importance of iNOS in genetic susceptibility to younger IBD presentation due to higher NO production [56].

21.5.3 NADPH Oxidase-1 (NOX2) and Dual Oxidase-2 (DUOX2)

Reactive oxygen species (ROS) production by epithelial cells attenuates the pathogenicity of intestinal pathogens. A recent study identified novel missense variants in each of the epithelial NADPH oxidases *NOX1* and *DUOX2* in a number of VEOIBD patients with pancolitis. Despite appropriate cellular localization of mutant protein, cells harboring these mutations had defective host resistance to infection with the enteric pathogen *Campylobacter jejuni.* Therefore, defective ROS production from intestinal epithelial cells is also a risk factor for developing inflammatory bowel disease [57].

21.6 Monogenic Forms of VEOIBD

- **Recently a number of monogenic causes of VEOIBD have been identified**
- **Monogenic VEOIBD can be caused by primary immunodeficiency (PID) or by combined PID and epithelial defects or only epithelial defects**
- **Monogenic VEOIBD caused by PID can be cured with allogenic stem cell transplant**
- **Monogenic VEOIBD caused by combined PID and epithelial defects or only epithelial defects may not respond to stem cell transplant and novel therapies are required**

Young children and infants with IBD may have one of the 67 identified monogenic defects that have been recently been shown cause forms of VEOIBD. These genes can be generally grouped into defects in epithelial barrier and immunodeficiencies (both innate and adaptive immunity). Knowledge of the gene expression and protein function are often critical in clinical decision-making for patients with VEOIBD. VEOIBD causal genes that are expressed primarily in the immune system can be treated and cured with allogenic stem cell transplant (*IL-10RA/B, XIAP*, and *ARPC1B*), while genes primarily expressed in enterocyte or co-expressed in enterocyte and immune cells may not be amenable to transplant (*TTC7A*).

The following sections provide information on some of the recently described monogenic forms of IBD with more information regarding VEOIBD causal genes found in Fig. 21.3 and Table 21.1.

21.6.1 Immunodysregulation, Polyendocrinopathy, Enteropathy X-Linked (IPEX) Syndrome

Mutations in the *FOXP3* gene are known to cause *I*munodysregulation *P*olyendocrinopathy, *E*nteropathy *X*-linked (IPEX) syndrome. The gene is located on the X chromosome and therefore males are affected [108]. Typically, patients with IPEX present before 6 months of age with severe watery diarrhea that does not respond to dietary changes. The intestinal pathological features vary from complete villus atrophy with apoptosis in a graft-versus-host appearance with loss of goblet and Paneth cells, to mild intestinal inflammation. Patients may also have anti-enterocyte antibodies. *FOXP3* mutations should be considered in all male patients with persistent diarrhea especially those with diabetes and/or thyroiditis. Recently, patients with *FOXP3* mutations have been shown to present with a milder phenotype with predominant IBD features without systemic disease [163]. Patients with *FOXP3* mutations usually do not respond to conventional IBD therapy and require allogenic stem cell transplant.

21.6.2 X-Linked Inhibitor of Apoptosis (XIAP)

X-linked inhibitor of apoptosis (XIAP), encoded by the *BIRC4* gene is another X-linked disease

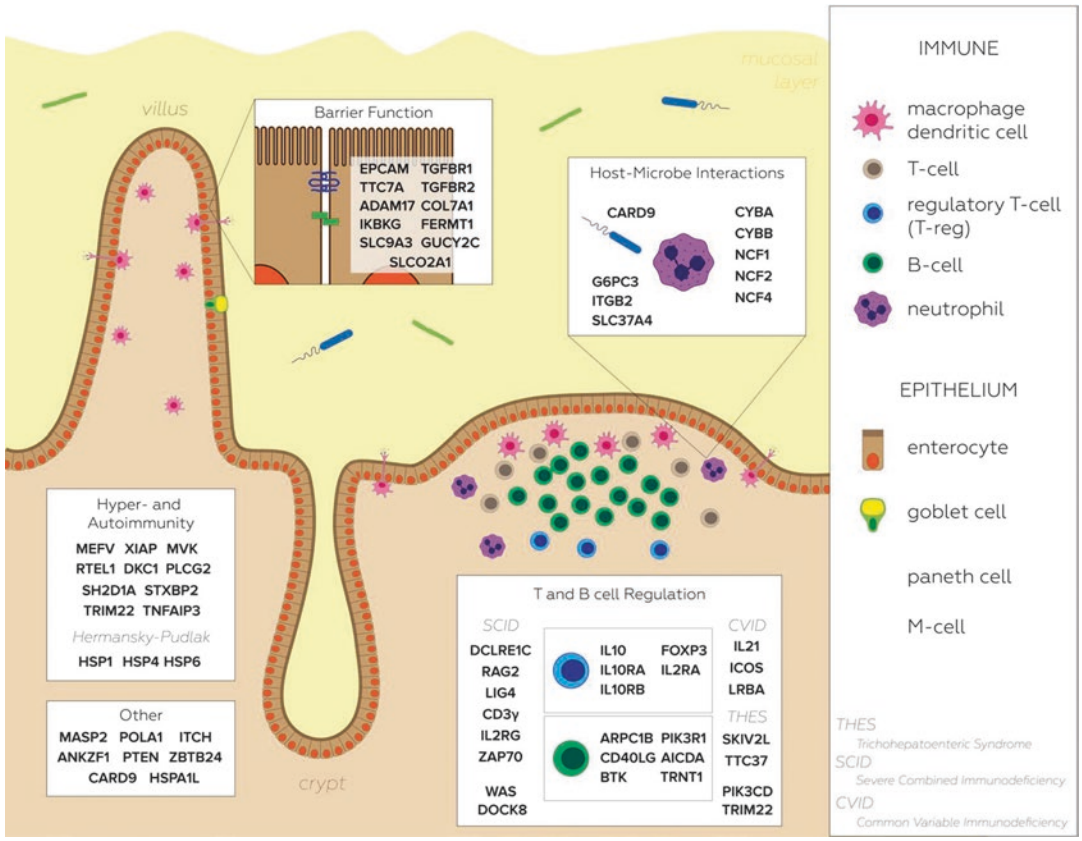

Fig. 21.3 Genes associated with very early onset inflammatory bowel disease (VEOIBD) genes

Table 21.1 VEOIBD Genes

Intestinal barrier function defects		
Gene	Condition	Clinical manifestations
ADAM17	ADAM17 deficiency [58]	Neonatal diarrhea—watery, progressing to bloody. Nail and hair abnormalities
COL7A1	Dystrophic epidermolysis bullosa [59–61]	Bloody diarrhea
EPCAM	Tufting enteropathy [62–64]	Congenital diarrhea, variable intestinal inflammation
FERMT1	Kindler syndrome [59, 65–67]	Skin trauma induced blistering, esophageal and anal stenosis, hemorrhagic colitis
IKBKG (NEMO)	X-linked ectodermal dysplasia and immunodeficiency [68–71]	Diarrhea, failure to thrive, susceptibility to infection, vasculitis and arthritis
SLC9A3	Congenital sodium diarrhea [72, 73]	Congenital secretory diarrhea, severe metabolic acidosis, hyponatraemia—late onset inflammatory bowel disease (IBD)
TTC7A	VEOIBD, intestinal atresia, immunodeficiency [74]	Apoptotic enterocolitis, immunodeficiency, recurrent intestinal atresia, severe combined immunodeficiency (SCID)
GUCY2C	Familial diarrhea [75, 76]	Neonatal onset of watery diarrhea—late onset IBD

(continued)

Table 21.1 (continued)

Gene	Condition	Clinical manifestations
Adaptive immune system defects		
ADA	SCID [77]	Intestinal inflammation, skin involvement and autoimmune hemolytic anemia.
AICDA	Hyperimmunoglobulinemia IgM syndrome [78]	Recurrent and severe infections, lymphoid hyperplasia, predisposition to autoimmune or inflammatory conditions
ARPC1B	Wiskott-Aldrich syndrome (WAS)—like phenotype and intestinal inflammation [79]	Microthrombocytopenia, defects in platelet function, invasive infections, IBD, vasculitis, eosinophilia, autoimmunity, infections
BTK	Agammaglobulinaemia [80, 81]	Crohn disease type inflammation, autoimmune hemolytic anemia
CARD9	Familial candidiasis, IBD phenotype [82]	Candida meningoencephalitis, intestinal infection, classic IBD
CD3γ	SCID [83]	Crohn disease type inflammation, skin involvement
CD40LG	Hyperimmunoglobulinemia M [84]	Oral inflammation perianal disease, autoimmune hemolytic anemia
DCLRE1C	Omenn syndrome [85]	Crohn disease like inflammation, immunodeficiency
DKC1	Hoyeraal-Hreidarsson syndrome, early onset colitis [86–88]	Progressive bone marrow failure, pancytopenia, concomitant immunodeficiency, ataxia, microcephaly, developmental delay
DOCK8	Hyperimmunoglobulinemia M, Hyperimmunoglobulinemia E syndrome [76, 89]	Susceptibility to infection (staphylococcal) and intestinal inflammation
IL-21	Common variable immunodeficiency (CVID), very early onset IBD (VEOIBD) [90]	Early onset colitis and immunodeficiency
IL-2RA	Imunodysregulation polyendocrinopathy, enteropathy X-linked (IPEX)—like syndrome [91]	Enteropathy, endocrinopathy, eczema, hemolytic anemia, hepatosplenomegaly, lymphadenopathy
IL-2RG	Atypical SCID [92, 93]	Increased susceptibility to infections, severe diarrhea and failure to thrive
LRBA/CTLA4	Lipopolysaccharide-responsive and beige-like anchor (LRBA)-deficiency [94, 95] cytotoxic T-lymphocyte-associated protein 4 (CTLA)4-deficiency [96]	Hypogammaglobinemia and intestinal inflammation, autoimmunity
LIG4	SCID [77]	Early onset colitis, skin involvement, autoimmune neutropenia
PIK3R1	Agammaglobulinemia, IBD phenotype [97]	Colitis, erythema nodosum, autoimmunity
PIK3CD	P13K delta syndrome [98]	Intestinal inflammation, recurrent respiratory infections. B-cell lymphoma
PTEN	PTEN syndrome [99, 100]	Inflammatory polyps, multiple tumours, immune dysregulation, autoimmunity
RAG1 or RAG2	Omenn syndrome [101]	Diffuse erythroderma, hepatosplenomegaly, lymphadenopathy and intestinal disease
TGFBR1, TGFBR2	Loeys-Dietz syndrome [102, 103]	Ulcerative colitis type inflammation, skeletal involvement, aortic and arterial aneurysms and craniofacial abnormalities. Eosinophilic gut disorders
RTEL1	Hoyeraal-Hreidarsson syndrome [104, 105]	Stricturing disease, skin involvement (may also be considered an epithelial defect)

Table 21.1 (continued)

Gene	Condition	Clinical manifestations
WASP	Wiskott-Aldrich syndrome [106]	Ulcerative colitis like inflammation with skin and joint involvement, autoimmune hemolytic anemia, other autoimmunity, microthrombocytopenia, defects in platelet function, infections
ZAP70	SCID [107]	Ulcerative colitis like inflammation, eczema
Regulatory T-cell defects		
FOXP3 *STAT1*	IPEX syndrome [101, 108–110] IPEX-like [111]	Colonic disease and enteropathy ± neonatal diarrhea, failure to thrive, infection, skin rash, diabetes, thyroiditis, cytopenias, autoimmunity
ICOS	IBD phenotype [112]	Rheumatoid arthritis, interstitial pneumonitis, psoriasis
Interleukin 10/Interleukin 10R pathway defects		
IL-10RA/B and *IL-10*	Neonatal or infantile VEOIBD [113, 114]	Severe enterocolitis and perianal disease (± arthritis, folliculitis, lymphoma)
Bacterial Recognition and Clearance Defects		
CYBB, *CYBA*, *NCF1*, *NCF2*, *NCF4*	Chronic granulomatous disease (CGD) [115–121]	Intestinal inflammation and autoimmune disease; association with eczema and perianal disease, infections
G6PC3	Congenital neutropenia [122–124]	Crohn disease—like inflammation, perianal disease, folliculitis
ITGB2	IBD phenotype [125, 126]	Bacterial infections, laboratory studies notable for peripheral granulocytes
SLC37A4	IBD phenotype [127–129]	Crohn disease—like inflammation with granuloma, perianal disease with skin involvement
Innate defence defects		
XIAP	X linked lymphoproliferative syndrome (XLP1/2) [115, 130–135]	Severe colonic and perianal fistulising disease, Epstein-Barr virus (EBV) can results in fatal hemophagocytic lymphohistiocytosis (HLH)
SH2D1A	X linked lymphoproliferative syndrome 1 (XLP1) [136]	Intestinal inflammation, HLH/macrophage activation syndrome (MAS), neoplasia
TRIM22	IBD phenotype [137]	Granulomatous colitis and perianal disease
Mitochondrial stress defects		
ANKZF1	Early onset colitis [138]	Perioral and oral inflammation, perianal involvement, ulcerative skin involvement
TRNT1	Infant onset colitis [139, 140]	Aseptic febrile episodes, sideroblastic anemia, retinitis pigmentosa, hepatosplenomegaly
Hyperinflammatory and autoinflammatory disorders		
HPS1, HPS4, HPS 6	Hermansky-Pudlak Syndrome [141–145]	Crohn disease—like inflammation, some perianal and skin involvement; oculocutaneous albinism, coagulopathy, pulmonary fibrosis
MEFV	Mediterranean fever, IBD phenotype [146–148]	Intestinal inflammation, arthritis and vasculitis
MVK	Mevalonate kinase deficiency [149, 150]	Enterocolitis, stricturing disease, skin disease, anemia
PLCG2	Phospholipase C-y2 defects [151]	Ulcerative colitis like inflammation, eczema, non-specific interstitial pneumonitis
STXBP2	Familial hemophagocytic lymphohistiocytosis type 5 [152]	Intestinal inflammation, HLH/MAS
TNFAIP3	Behçet disease—like disorder [153]	Ulceration of mucosal surfaces, oral and genital areas, skin rash, uveitis, polyarthritis

(continued)

Table 21.1 (continued)

Gene	Condition	Clinical manifestations
Protein structure and transport		
HSPA1L	IBD phenotype [154]	Both Crohn disease and ulcerative colitis phenotype characteristics described
SLCO2A1	Primary hypertrophic osteoarthropathy [155, 156]	Enterocolitis, digital clubbing, painful joint enlargement, thickened facial skin and scalp
SKIV2L	Trichohepatoenteric syndrome [157, 158]	Intestinal inflammation with skin and hair involvement
TTC37	Trichohepatoenteric syndrome [157]	Intestinal inflammation with skin and hair involvement
ZBTB24	IBD phenotype [159]	Recurrent infection, growth failure, facial abnormalities
Complement system deficiencies		
MASP2	IBD phenotype [160]	Ulcerative colitis type inflammation, skin involvement, arthritis
DNA replication		
POLA1	Pigmentary disorder and infantile colitis [161, 162]	Enterocolitis, diffuse skin hyperpigmentation, distinctive reticulate pattern, recurrent urethral strictures

that was initially described in patients with X-linked hemophagocytic lymphohistiocytosis (HLH) syndrome [130]. However, it is now known that XIAP patient may present with intestinal disease that varies from villous atrophy to severe enterocolitis with perianal disease [131]. Unlike other causes of VEOIBD, the onset of symptoms can occur in male patients from months of age to up to 40 years of age [115, 132, 164], and heterozygous females carriers may present with XIAP deficiency due to selective X-inactivation [115]. A high degree of suspicion is needed for male patients with diarrhea and biochemical features of HLH. Patients with *XIAP* mutations have very high morbidity and mortality and usually do not respond to conventional IBD therapy and require allogenic stem cell transplant.

21.6.3 Lipopolysaccharide-Responsive and Beige-Like Anchor (LRBA)/Cytotoxic T Lymphocyte-Associated Protein 4 (CTLA)

Recently a new multi-systemic disease with autoimmunity, primary immune deficiency, and autoimmune enteropathy was determined to be caused by two distinct genes. *CTLA4* deficiency is caused by autosomal dominant mutations with variable penetrance [96], while *LRBA* deficiency [94, 95], a phenocopy *CTLA4* deficiency, is caused by an autosomal recessive mutations. CTLA4 functions as an early checkpoint controlling T cell response to antigens through co-stimulation. Lipopolysaccharide-responsive and beige-like anchor (LRBA) is expressed on Rab11 positive recycling endosomes and appears to function in CTLA4 recycling [165]. Patients with *CTLA4* and *LRBA* mutations have a variable age of presentation from perinatal to adulthood. These patients may have severe villous atrophy with IPEX-like autoimmune features including autoimmune enteropathy and intestinal inflammation, similar to IBD. These patients may be treated with abatacept, a drug containing the extracellular domain of CTLA4 [165]; however, the colitis associated with *LRBA/CTLA* deficiency may be challenging to treat and allogenic stem cell transplant may ultimately be required for effective long term treatment.

21.6.4 Tripartite Motif-Containing 22 (TRIM22)

Recently, a novel form of VEOIBD termed tripartite motif-containing 22 (TRIM22)-deficiency was identified in 3 patients with granulomatous

colitis and severe perianal disease with recurrent bacterial and viral infections that was refractory to medical and surgical therapies [137]. TRIM22, a RING finger E3 Ub ligase [166], is expressed in the intestine [167], macrophages [168], and lymphocytes [169]. TRIM22 is an interferon inducible protein that possesses antiviral activity [170–172], and activates NF-κB signalling [168]. Functional studies showed that TRIM22 interacts with and ubiquitinates NOD2 and the disease-causing variants disrupted the ability of TRIM22 to enhance NOD2-dependent activation of interferon-β and NF-κB signalling [137]. Because of the expression of TRIM22 in both immune cells and enterocytes it is unknown if stem cell transplant is a possible therapeutic option.

21.6.5 Interleukin-10 Receptor A/B (IL-10RA/B)

In 2009 Klein and colleagues identified loss-of-function mutations in *IL-10RA/B* [113] (and later *IL-10* [114]) that presented as granulomatous colitis with perianal disease, folliculitis and arthritis. Including the original description of causative *IL-10R* mutations, many young children with severe intestinal inflammation and perianal disease have successfully received curative allogenic stem cell transplantation [173–175]. Importantly, *IL-10R* mutations have also been identified in a number of older children who developed lymphoma and had a previous diagnosis of IBD since infancy [176]. In some cases, the identification of *IL-10R* deficiency has resulted in a change from a planned autologous stem cell transplant as lymphoma management to a more appropriate allogeneic stem cell transplantation that not only cures both the lymphoma and the underlying PID but also prevents disease recurrence [177]. Other studies have demonstrated that *IL-10RA* variants are also associated with VEOIBD without evidence of primary immunodeficiency suggesting that targeting IL-10 in patients with hypomorphic IL-10-pathway variants may be effective in a subset of IBD patients [178]. *IL-10R* mutations are the most common cause of monogenic VEOIBD and the clinical triad of granulomatous colitis, perianal disease and folliculitis (and often arthritis) developing before 3 months of age should not be missed (Fig. 21.4). The only effective treatment option is allogenic stem cell transplant.

21.6.6 Chronic Granulomatous Disease (CGD)

CGD is a rare genetic disorder with a prevalence of 1/200,000 to 1/250,000 caused by X-linked (*CYBB*) and autosomal recessive (*CYBA, NCF1,2,4*) mutations in genes encoding components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex (also referred to as NOX2 NADPH oxidase or phagocyte oxidase) [179]. Patients with CGD have severe and recurrent infections as a result of the inability of phagocytes to mount sufficient respiratory burst to kill invading pathogens and may present from infancy to adulthood [180]. Interestingly, up to 40% of CGD patients develop a form of colitis that is endoscopically and pathologically very similar to the colitis observed in CD and this may be the only feature of their disease [116, 117, 181]. These patients may have significant left-sided colitis and perianal disease that does not respond to conventional treatments and allogenic stem cell transplant is now standard of care.

21.6.7 Tetratricopeptide Repeat Domain 7 (TTC7A)

Biallelic mutations in the tetratricopeptide repeat domain 7 (*TTC7A*) gene were found in a Canadian infant with severe infantile enterocolitis who died prior to 1 year of age and in two unrelated families (with VEOIBD and intestinal atresia), both with two affected siblings (five patients in total) [74]. TTC7A-deficiency results in an enterocyte defect leading to inflammatory intestinal atresia and severe IBD. Subsequently it has been shown that TTC7A-deficiency also results in combined recurrent intestinal inflammation (enterocyte) and primary immunodeficiency [74, 182–187], and treatment through stem cell transplant does not cure/treat the bowel disease [188]. Unfortunately, there is no viable treatment for these children and conservative and/or palliative care is often recommended.

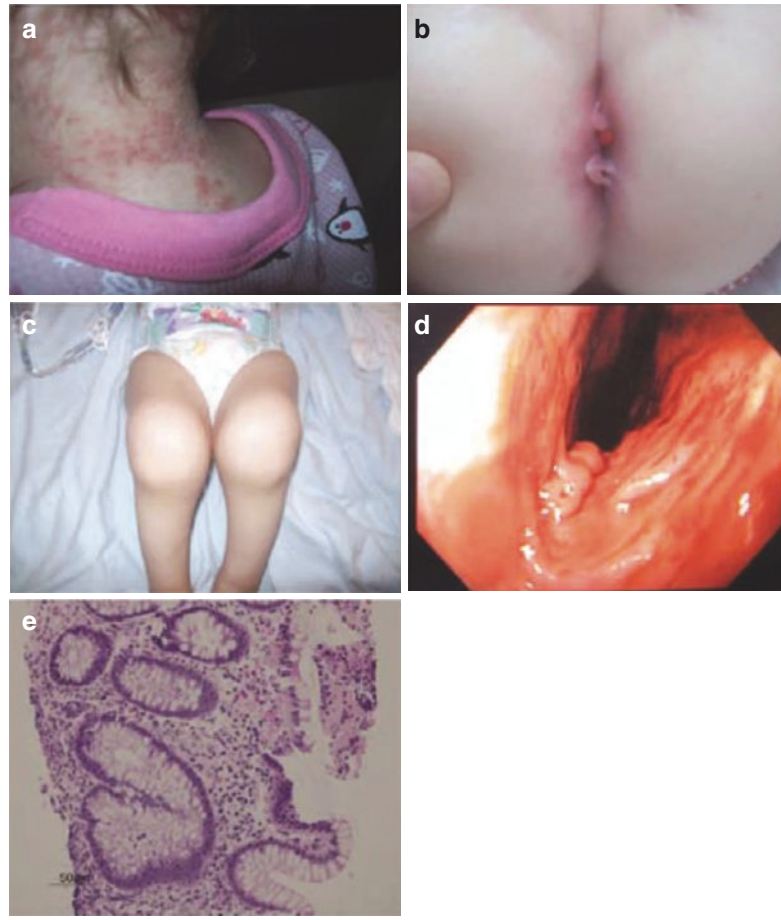

Fig. 21.4 From **Moran et al. IBDJ 2014: Classic presentation of IL-10R-deficiency. (a) Folliculitis, (b) perianal disease, (c) joint disease, (d) endoscopic colitis, (e) pathologic granuloma**

21.6.8 Actin-Related Protein Complex 1B (ARPC1B)

Whole exome sequencing of a patient (and consanguineous parents) suffering from microthrombocytopenia, eczema, vasculitis and IBD identified a homozygous frameshift mutation in *ARPC1B* which was validated in two siblings from an independent, non-consanguineous family with similar disease [79]. Actin-related protein complex 1B (ARPC1B) is a component of the actin-related protein 2/3 (Arp2/3) complex that is required for the formation of branched actin filaments essential for a variety of cellular processes [189–191]. The clinical phenotype of ARPC1B deficiency strongly resembles Wiskott-Aldrich syndrome and is also curable through allogenic stem cell transplant (unpublished data).

21.6.9 Hyperinflammatory and Autoinflammatory Disorders

VEOIBD has been described in a number of hyperinflammatory and autoinflammatory disorders. As described in Table 21.1, mevalonate kinase deficiency best exemplifies an autoinflammatory disorder that can present as VEOIBD (see Chap. 17). Mevalonate kinase deficiency is characterized by activation of caspase-1 with subsequent activation of IL-1β [149, 150]. Other disorders include phospholipase C-γ2 defects (see Chap. 28) [151], familial Mediterranean fever (see Chap. 16) [146–148] and Hermansky–Pudlak syndrome (type 1, 4, and 6) [141–145]. Recently, two independent groups [192, 193] identified *de novo* gain-of-function mutation in the NOD-like receptor caspase containing

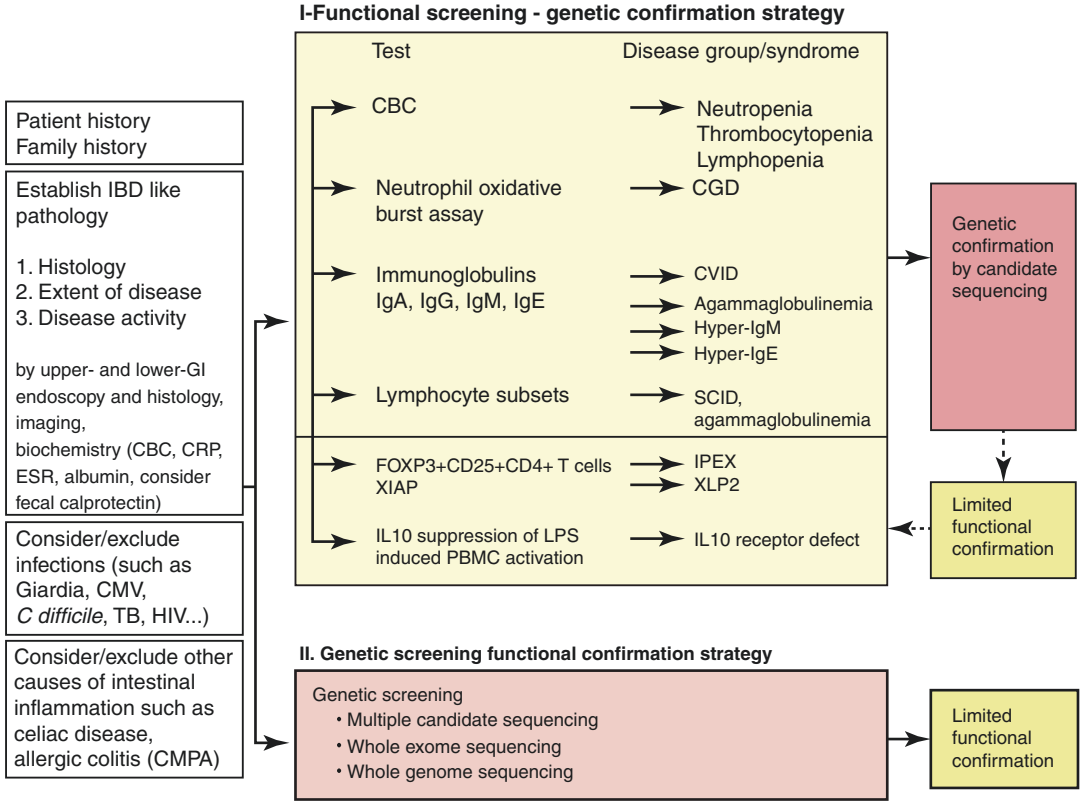

Fig. 21.5 From **Holm et Gastro 2014: Clinical work-up for very early onset inflammatory bowel disease (VEOIBD)**

4 (*NLRC4*) gene in neonatal-onset enterocolitis, periodic fever, and fatal/near-fatal episodes of macrophage activation syndrome (see Chaps. 10 and 29). This disease results in increased and constitutive inflammasome activation and increased secretion of IL-1β and IL-18.

21.7 Diagnosis of VEOIBD

The diagnosis of VEOIBD may be challenging, especially in children who present with only diarrhea or bloody stool. Most infants and young children who present with diarrhea and/or blood in the stool will have cow's milk protein intolerance (CMPI) that may be severe. The diagnosis of CMPI is most often made by gradual clinical improvement with the exclusion of all cow's milk in the mother's diet (breast feeding infant) or switching to exclusive elemental formula. Both CMPI and infections (that are often not identified) may have severe biochemical, endoscopic, and histologic features that mimic more severe monogenic forms of VEOIBD. Persistent diarrhea or systemic disease in this age group may be due to congenital diarrhea (non-bloody diarrhea usually an enterocyte defect), autoimmune enteropathy (diarrhea with villous blunting and enterocyte apoptosis on histology), and VEOIBD (often bloody diarrhea) [11, 194].

To aid in the diagnosis of VEOIBD, we developed the following **mnemonic** "YOUNG AGE MATTERS MOST" (**YOUNG AGE** onset, **M**ultiple family members and consanguinity, **A**utoimmunity/Autoinflammation, **T**hriving failure, **T**reatment with conventional medication fails, **E**ndocrine concerns, **R**ecurrent infections or unexplained fever, **S**evere perianal disease, **M**acrophage activation syndrome and hemophagocytic lymphohistiocytosis, **O**bstruction and atresia of intestine, **S**kin lesions, dental and hair abnormalities, and **T**umors) [11]. Figure 21.5 outlines an approach to investigate patients with suspected monogenic forms of IBD [11].

21.8 Treatment and Outcomes of Monogenic VEOIBD

Recent advances in genetic diagnosis of VEOIBD have revolutionized the diagnosis and treatment of patients with VEOIBD. The understanding that primary immunodeficiencies can present with predominant intestinal disease including colitis, enteropathy, and perianal disease has allowed for curative allogenic stem cell transplantation for a number of patients that previously suffered from intractable disease. However, the relative infrequency of these monogenic VEOIBD patients has made outcome data scarce with the exception of IL10R deficiency [173–175] and CGD [195], where a large number of patients have been successfully treated with allogenic stem cell transplantation. Outcomes for other forms of VEOIBD with a predominant intestinal phenotype such as FOXP3, LRBA and IPEX, are scarce but these disorders are considered amenable to allogenic stem cell transplantation. Recent advances in low intensity stem cell transplant have greatly reduced morbidity and mortality and length of stay for VEOIBD patients with primary immunodeficiency [196].

However, the identification of enterocyte defects as the primary driver of VEOIBD has allowed for the avoidance of futile treatments with high morbidity and thereby preventing considerable suffering to the patient and family. This includes TTC7A-deficiency where stem cell transplantation results in transient improvement followed by significant recurrence of the intestinal disease with high morbidity and eventual mortality. Unfortunately, for the majority of these epithelial driven diseases there are no adequate therapies; however, work is currently underway to develop novel therapies for TTC7A-deficiency.

Overall, largescale VEOIBD studies, such as the SickKids National Early Onset Pediatric IBD Cohort Study (www.NEOPICS.org), have shown that between 10 and 20% of patients with severe VEOIBD can be diagnosed with the genes outlined here (unpublished data). We anticipate that as the number of VEOIBD causal genes increases and are grouped into common pathways, this will allow for greater understanding of the disease pathogenesis and eventually lead to improved diagnosis and novel therapeutic options for these young patients.

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22

Pyogenic Arthritis Pyoderma Gangrenosum and Acne (PAPA) Syndrome

Marilynn G. Punaro and Carol A. Wise

Abstract

PAPA is a rare autosomal dominant disorder classically characterized by early onset destructive ***P***yogenic ***A***rthritis, ***P***yoderma gangrenosum, and severe nodulocystic ***A***cne (PAPA). It is caused by missense mutations in the *PSTPIP1* gene. Proline-Serine-Threonine Phosphatase-Interacting Protein 1 (PSTPIP1) is a multifunctional adaptor protein that is expressed predominantly in hematopoietic cells. Its adaptor function with pyrin originally linked it to the inflammasome and related it to familial Mediterranean fever and other autoinflammatory diseases.

Although medications targeting interleukin (IL)-1 and tumor necrosis factor (TNF) have been effective for certain disease manifestations in some PAPA patients, a consistently effective treatment remains elusive. PSTPIP1 has been linked more recently to Wiskott-Aldrich syndrome protein (WASP)-mediated macrophage podosome function, and to SHIP1/2-mediated osteoclast functions. These pathways are likely involved in invasive skin and bone destruction, respectively, and are attractive candidates for therapeutic targeting. Thus, although PAPA is a "simple" Mendelian disease, its pathogenesis is complicated by the effects of mutations on the diverse roles of the PSTPIP1 adaptor protein. Current outlook suggests that combined therapies targeting each PSTPIP1-mediated pathway may prove the most beneficial to individual PAPA patients.

It is now appreciated that PAPA syndrome represents a single clinical entity within a spectrum of PSTPIP1 associated inflammatory diseases (PAIDs) that have been linked to specific mutations. In addition, reports of PAPA cases negative for *PSTPIP1* mutations raise the possibility of additional disease genes. Further research to define molecular mechanisms and to develop specific molecular therapeutics is warranted and will likely provide insights into other related autoinflammatory diseases and disorders marked by invasive tissue destruction.

Keywords

Pyogenic arthritis · Pyoderma gangrenosum Acne · Pathergy · PSTPIP1 · Autoinflammatory disease · Rare Mendelian Inflammasome Podosome biogenesis

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Abbreviations

CD2BP1	CD2-binding protein 1
CNO	Chronic non-bacterial osteomyelitis
FasL	Fas ligand
F-BAR	Fer-CIP4 homology—Bin/Amphiphysin/Rvs
IL	Interleukin
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis syndrome
PAID	PSTPIP1 associated inflammatory diseases
PAMI	PSTPIP1-associated myeloid-related proteinemia inflammatory syndrome
PAPA	Pyogenic arthritis pyoderma gangrenosum acne
PSTPIP1	Proline-Serine-Threonine Phosphatase-Interacting Protein 1
PTP-PESTs	Protein tyrosine phosphatases-rich in proline (P), glutamic acid (E), serine (S), and threonine (T) residues
SAPHO	Synovitis acne palmoplantar pustulosis hyperostosis osteitis
SH3	Src homology 3
SHIP1/2	SH-2 containing inositol 5′ polyphosphatases 1 and 2
TNF	Tumor necrosis factor
WASP	Wiskott-Aldrich Syndrome Protein

Key Points

- **Pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome is characterized by recurrent destructive episodes of sterile pyogenic arthritis, pyoderma gangrenosum and cystic acne**
- **PAPA is a rare disease with autosomal dominant inheritance**
- **It is marked by activation of the pyrin inflammasome and subsequent overproduction of interleukin (IL)-1β**
- **IL-1 or tumor necrosis factor (TNF) blockade may be helpful**

22.1 Introduction

Pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome is a rare autosomal dominant autoinflammatory disorder classically characterized by the triad of ***P***yogenic sterile ***A***rthritis, ***P***yoderma gangrenosum and ***A***cne (Fig. 22.1). The first case was described as "Streaking Leucocyte Factor" in 1975 because a partially purified, ~160 kd component of this patient's serum enhanced the random migration of normal mononuclear cells and neutrophils when mixed *in vitro* [1]. More than 20 years later, in 1997, Lindor reported the first multigenerational family and in recognition of the key symptoms coined the acronym, PAPA syndrome. (OMIM no. 604416) [2]. Subsequently, a second extended family was reported as "recurrent familial arthritis" and in 2000, localization of the associated gene to 15q22–24 for these two families was reported [3, 4]. Further research identified mutations (p.A230T and p.E250Q) in Proline-Serine-Threonine Phosphatase-Interacting Protein 1 (*PSTPIP1*), also known as *CDBP1* gene, as the cause of PAPA syndrome [5]. An immediate observation in these families was lack of genotype/phenotype correlation, i.e. clinical variation between affected family members with the same mutation was as extensive as the variation between families. This suggested that other modifiers are involved in the triad of PAPA symptoms predisposed by *PSTPIP1* mutations.

Diagnostic sequencing for *PSTPIP1* mutations is now commercially available. Aggregated sequencing results are freely available through the ClinVar database [6, 7]. (https://www.ncbi.nlm.nih.gov/clinvar/) It is interesting that as of June 2017 the only mutations clearly denoted as pathogenic for PAPA symptoms were A230T, E250K, and E250Q. There are also reports of PAPA cases that are negative for coding mutations in PSTPIP1, suggesting that the full spectrum of PAPA mutations, whether due to other mechanisms such as splicing alterations, genetic heterogeneity, mosaicism, etc. is yet to be defined [8, 9].

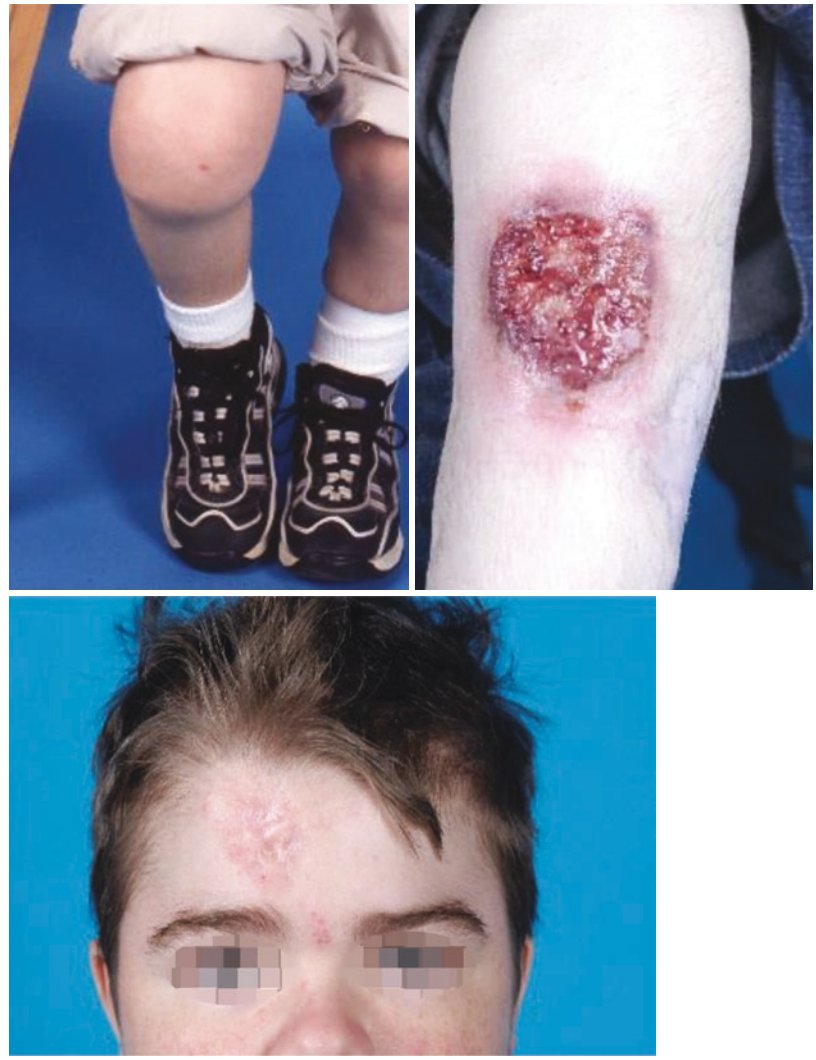

Fig. 22.1 Triad of pyogenic arthritis, pyoderma gangrenosum, and acne. Top left: pyogenic arthritis; Top right: pyoderma gangrenosum; Lower picture: nodulocystic acne

22.2 Epidemiology

PAPA syndrome is rare. By 2016, 52 patients with PAPA syndrome from multiple ethnic groups had been reported [10]. Males and females were equally affected. Onset of disease is usually in early childhood. Typically, the joint disease precedes the skin inflammation which often starts around puberty.

22.3 Etiology and Pathogenesis

- Mutations in the PSTPIP1 adaptor protein (A230T, E250K, E250Q) alter its interaction with PEST-type PTPs
- One consequence of disease mutations is PSTPIP1 hyperphosphorylation, constitutive activation of the pyrin inflammasome, and resulting overproduction of interleukin (IL)-1β
- A distinct PSTPIP1 R405C mutation is associated with pyoderma gangrenosum/acne but not pyogenic arthritis. *In vitro* studies support a Wiskott-Aldrich Syndrome Protein (WASP)-mediated mechanism of macrophage activation, suggesting that distinct mechanisms are responsible for the features of PAPA syndrome

22.3.1 PSTPIP1-Mediated Molecular Mechanisms

PSTPIP1, originally identified in the mouse through its interaction with PEST (rich in proline (P), glutamic acid (E), serine (S), and threonine (T) residues)-type protein tyrosine phosphatases (PTP-PESTs), is a cytoskeletal adaptor protein that is highly expressed in hematopoietic cells [11, 12]. The human homolog has been called CD2BP1 denoting its interaction with the T cell surface protein CD2 [12], but is now more generally cited as "PSTPIP1". The PSTPIP1 protein contains two clearly recognizable domains, a Fer-CIP4 homology—Bin/Amphiphysin/Rvs (F-BAR) region and a Src homology 3 (SH3) region at its N- and C-termini, respectively (Fig. 22.2). The F-BAR domain binds PTP-PEST, where PAPA syndrome mutations primarily cluster. Its adaptor activity is mediated by the SH3 domain that interacts with various immune/inflammatory proteins including CD2, WASP, c-Abl kinase, Fas ligand (FasL), pyrin, and SH-2 containing inositol 5′ polyphosphatases 1 and 2 (SHIP1/2) [13–18]. Each of these proteins is a target for PTP-PEST-mediated dephosphorylation, as is PSTPIP1 itself [13, 18–21] (Fig. 22.2). F-BAR domains directly associate with the surface of lipid bilayer membranes and subsequently re-assemble into spiral filaments that deform the membrane into tubules [22]. Indeed biophysical studies have shown that PSTPIP1 induces the formation of tubules through the interaction of its F-BAR domain and the liposomal surface, and moreover that increasing doses of PSTPIP1 can transform entire liposomes into long tubules [23]. This inherent property of PSTPIP1 appears to be essential to its role in linking the cytoskeleton to the plasma membrane (described in more detail below).

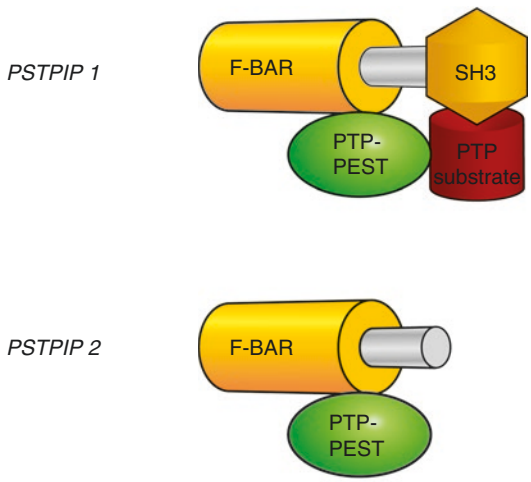

Fig. 22.2 PSTPIP1/2 protein domain structures. PSTPIP1 and 2 share similar structural features with the exception of a C-terminal SH3 domain in PSTPIP1 (see text). Both proteins harbor N-terminal F-BAR domains that share 47% amino acid identity, as well as binding sites for PEST-type protein tyrosine phosphatases

The PSTPIP1 interaction with pyrin in monocytes prompted the classification of PAPA as an autoinflammatory disorder, a group of diseases whose hallmark is overproduction of IL-1β [16, 24]. In PAPA, mutations in the F-BAR domain disrupt PTP-PEST binding with subsequent potentiation of pyrin-mediated IL-1β production [5, 16]. Specifically how IL-1β is overproduced is unclear, but has been explained by at least two proposed mechanisms: the first, by which a hyperphosphorylated PSTPIP1/pyrin interaction triggers activation of the inflammasome, and the other in which the same interaction triggers activation of the so-called "pyroptosome" leading to cell death and cytokine release [25, 26]. Either way, the overproduction of IL-1β does not fully explain the PAPA phenotype given its distinction from other autoinflammatory diseases and the fact that IL-1β blockade does not always resolve its symptoms, particularly the cutaneous features of pyoderma gangrenosum and acne.

New insight in this regard came more recently with the identification of a patient and his father with a missense mutation (R405C) in the PSTPIP1 SH3 domain, unlike "classic" PAPA syndrome mutations that occur in the F-BAR domain [27]. The patient presented with recurrent, episodic pyoderma gangrenosum without arthritis and his father had a history of severe acne. Studies of patient macrophages compared to controls found fewer podosomes, the actin-rich structures that mediate adhesion, but many more filopodia-like structures that co-localized with strong matrix degradation activity. This is in contrast to macrophages from PAPA patients with the "classical"

A230T mutation that also form fewer podosomes that are decreased in invasion and matrix degradation compared to controls but do not form filopodia. These and other data suggested that PSTPIP1 regulates podosome biogenesis, possibly mediated by its binding partner WASP (Fig. 22.3). WASP is a key regulator of macrophage podosome formation. Accordingly, cells treated with the WASP inhibitor wiskostatin showed reversal of the podosome/filopodia phenotype as well as reversal of the increased matrix degradation phenotype in the presence of the PSTPIP1 R405C mutation. The fact that the R405C patients did not have pyogenic arthritis could suggest that the PSTPIP1-mediated mechanisms underlying skin and joint manifestations in PAPA are distinct. It is interesting that an analogous scenario has been described for pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), a disorder described in an extended family with clinical cutaneous manifestations resembling PAPA but lacking pyogenic arthritis (see Chap. 29). This disease is caused by a pyrin mutation S242R, outside the WT-B30.2 domain where "classic" FMF mutations primarily occur [28]. The similarity between PAPA syndrome and PAAND raises the interesting possibility that they share a distinct disease pathway.

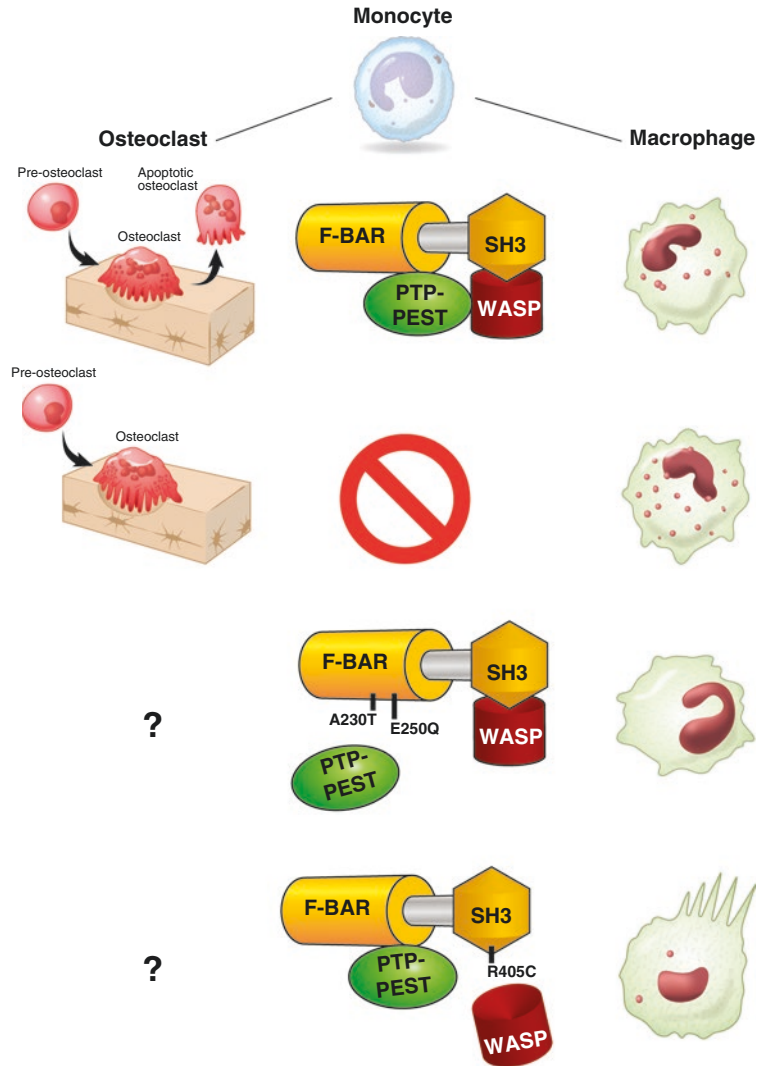

Fig. 22.3 PSTPIP1 and podosome functions. PSTPIP1 is expressed in monocyte lineages and participates in podosome biogenesis. In osteoclasts, knockdown or loss of PSTPIP1 inhibits podosome sealing zone disassembly and consequently increases osteoclast activity (left side) [17]. In macrophages, an increase in podosomes is observed with depletion of PSTPIP1. In the presence of classic PAPA mutations (PSTPIP1 A230T and E250Q) podosome numbers dramatically decrease and macrophages show defects in migration and extracellular matrix degradation. However the novel R405C mutation in PSTPIP1 is associated with a disruption in podosomes and concomitant formation of prominent filopodia, increased macrophage activity, and loss of WASP/PSTPIP1 interaction (figure adapted from [49])

Large-scale proteomic approaches have recently yielded new insight into the role of PSTPIP1 and its close homolog PSTPIP2 in osteoclasts. PSTPIP1 and PSTPIP2 proteins share approximately 60% amino acid identity, but PSTPIP2 lacks the terminal SH3 domain found in PSTPIP1 (Fig. 22.2) [29]. Both proteins possess F-BAR domains involved in binding with PEST-type phosphatases and membrane tubulation as described above [30]. A spontaneous L98P mutation in mouse PSTPIP2 produces a phenotype most closely resembling human chronic non-bacterial osteomyelitis (CNO), an autoinflammatory disease of bone that may also involve skin (see Chap. 31). In humans, a rare haplotype in a region of chromosome 18q21.3–22 encoding *PSTPIP2* has been associated with CNO, but its role in the disease is unclear [31]. Both PSTPIP1 and PSTPIP2 localize to osteoclast podosomes, structures that create "sealing zones" that are important in attaching the cell to bone. *In vitro* siRNA-mediated knockdown in Raw264.7 osteoclasts showed that PSTPIP2 participates in podosome assembly, while PSTPIP1 is required for podosome disassembly. Furthermore, mouse osteoclasts specifically lacking endogenous PSTPIP1 (tamoxifen-treated Cre-ERT2+/+, PSTPIP1+/+) show intact podosome formation and digested three-fold more osteologic material than wild type, consistent with prolonged podosome activity/decreased podosome disassembly. In osteoclasts, podosomes are critical in the remodeling process, forming resorption lacuna that deliver lysosomal hydrolases to digest bone. To define the PSTPIP1-mediated mechanism of podosome biogenesis in osteoclasts Sztacho et al. [17] identified PSTPIP1,2 substrates using mass spectrometry-based proteomics. While this identified many interactions, PSTPIP1 formed a specific complex with PTPN6 that de-phosphorylated SH-2 containing inositol 5′ polyphosphatase 1/2 (SHIP1/2) bound to its SH3 domain (Fig. 22.3). Loss of any part of this complex increased osteoclast activity by decreasing podosome disassembly. Thus PSTPIP1 may regulate SHIP1/2-dependent PIP [3–5] turnover and the balance between protein kinase and phosphatase signaling pathways.

In T cells, PSTPIP1 is proposed to participate in the WASP-dependent formation of the immunological synapse, and it also interacts with the cluster of differentiation protein 2 (CD2) [20]. However it is not apparent that T cell functions contribute to the PAPA phenotype, which lacks the features of lymphocytic autoimmunity.

22.4 Clinical Manifestations

- **Pain is a major symptom**
- **Joint disease typically occurs in early childhood followed by cutaneous inflammation in puberty**
- **Pathergy is common and may be a presenting symptom**

22.4.1 Constitutional Symptoms

PAPA syndrome is a pleotropic disease with variable expression characterized by early onset of recurrent destructive episodes of inflammation of joints and skin. Pain is the predominant feature of this condition. Surprisingly, fever is not typical during disease flares although it has been occasionally reported. In contrast fever is reported in PAAND (see Chap. 29) [28, 32, 33].

22.4.2 Musculoskeletal Manifestations

The onset of arthritis in PAPA syndrome usually occurs in early childhood, but can appear anytime between infancy and adolescence. The arthritis is typically oligoarticular in nature with 1–3 joints involved at any one time. Knees, elbows, and ankles are commonly involved joints [3]. Involvement of hips, wrists, hands, feet, and shoulders has also been reported [1, 34]. One family with an E250Q mutation was notable for temporo-mandibular joint (TMJ) arthritis with micrognathia, and cervical spine ankylosis in multiple family members [34].

Joint flares can occur spontaneously or after mild physical trauma. Although the patient usually remains afebrile, the flare often resembles an

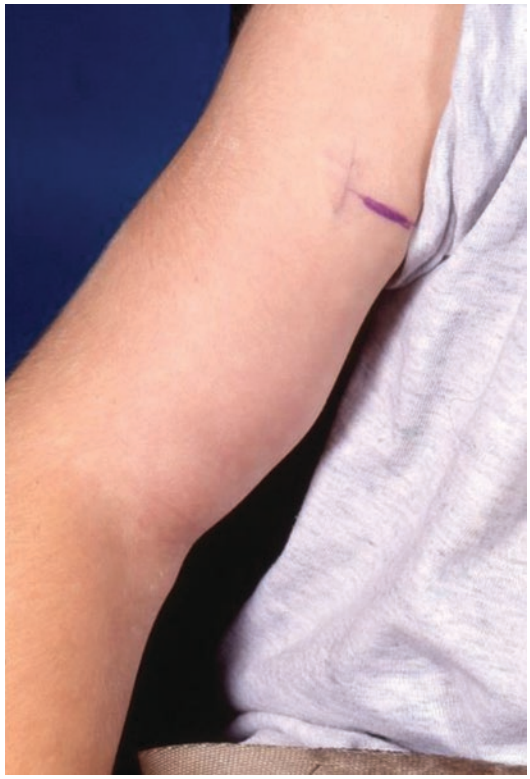

Fig. 22.4 Periarticular pseudo-abscess in PAPA. Inflammation extending beyond the joint into the muscle is evident

acute infection with sudden onset, severe pain, swelling and erythema of the affected area. Periarticular areas including muscle, skin and subcutaneous tissue may also be involved in this pseudo abscess formation (Fig. 22.4).

Multifocal sterile osteomyelitis with lytic bone lesions has been reported in several PAPA syndrome patients [33, 35]. Biopsy reveals chronic osteomyelitis without evidence of infection.

22.4.3 Dermatologic Manifestations

Pathergy is common and may be the presenting symptom of PAPA syndrome [35]. Early in life, pustule formation or sterile abscess may follow parenteral immunization or minor trauma [2]. As the patient approaches adolescence, cutaneous manifestations often predominate.

Recurrent episodes of pyoderma gangrenosum may occur. Pyoderma gangrenosum is a neutrophilic dermatosis characterized by painful ulcers with violaceous, raised and indeterminate borders most typically on the legs [36]. Nodules, papules and /or pustules rapidly evolve into extremely painful large lesions reaching diameters of 5–10 cm within a few weeks. In highly aggressive cases, the PG can involve fascia, muscle and tendons [37]. Initially, the lesions are sterile but superinfection by staphylococci and streptococci can occur.

Cystic acne is the third component of the classic triad. Typically, severe nodulocystic acne starts in early adolescence and persists into adulthood. Persistence of acne as late as the seventh decade has been reported [2].

Other cutaneous manifestations reported in association with PAPA syndrome include psoriasis, rosacea and hidradenitis.

22.5 Laboratory Testing and Imaging

- **Laboratory testing and histology reveal non-specific signs of acute and chronic inflammation**
- **Serologic testing is negative**

During episodes of flare, the laboratory tests may reveal non-specific signs of acute and chronic inflammation with anemia, elevated aldolase and high ESR. The white blood cell count and differential are usually normal [3]. Autoantibodies are absent and complement levels are normal [2, 3]. Synovial fluid aspiration can yield cloudy, seropurulent or purulent fluid with a high neutrophil count. Microorganisms are never visualized and cultures are invariably sterile [2].

Synovial tissue biopsy reveals a polymorphonuclear infiltrate without evidence of immunoglobulin or complement deposition [3].

The histology of the pyoderma gangrenosum is non-specific demonstrating pan-dermal neutrophilic infiltrates, abscess formation, matrix degeneration and necrotic areas. Leucocytoclastic vasculitis is present in about 40% of cases [37].

The histopathology of the cystic acne demonstrates distended follicles with cystic spaces and follicular openings filled with

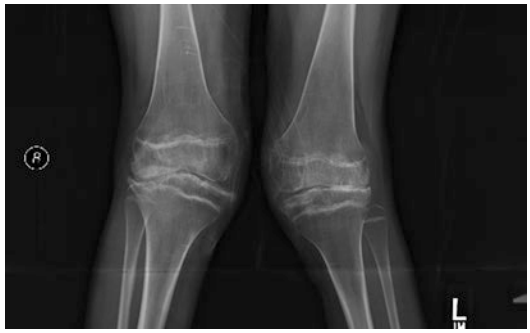

Fig. 22.5 Radiographic evidence of arthritic changes

keratinaceous debris and abundant bacteria. Ruptured cystic contents lead to a robust neutrophilic inflammatory infiltrate surrounding expanded follicles [38].

Radiographs may reveal periosteal proliferation of bone. Later in the disease course, advanced arthritic changes including ankylosis may be evident on plain films [2] (Fig. 22.5).

22.6 Diagnosis

Pathergy, pyoderma gangrenosum, and severe acne associated with pyogenic arthritis or a family history suggestive of PAPA syndrome should trigger consideration of this diagnosis. Gene testing is commercially available and can confirm the diagnosis.

The differential diagnosis of the arthritis includes monoarticular septic arthritis and if osteolytic lesions are present in a child, CNO [33, 38]. Synovitis, acne, palmoplantar pustulosis, hyperostosis and osteitis (SAPHO) syndrome and the pyoderma gangrenosum of inflammatory bowel disease also may need to be considered in the differential diagnosis. The recently described PAAND syndrome shares similar cutaneous features with PAPA syndrome i.e. severe acne, sterile skin abscesses, and pyoderma gangrenosum, but lacks the characteristic pyogenic arthritis of PAPA [28].

22.7 Treatment

- **Intra-articular steroids administered at onset of arthritis symptoms may ameliorate flare**
- **IL-1 and TNF blockade may be helpful**

Prompt therapy at the onset of flare is essential. If given at the time of earliest symptoms, the arthritis may respond to local steroid injection and adequate drainage. Historically patients with PAPA were treated with minimal success with a myriad of immune modulating agents as well as corticosteroids. As noted above, corticosteroids still play a role in ameliorating the arthritis when used as an intra-articular injection into a newly flaring joint. However, the use of systemic corticosteroids should be minimized due to the significant long-term toxicities associated with this treatment.

For the severe cystic acne, topical and systemic retinoid therapy may be helpful as adjunctive therapy [38].

The PSTPIP1 adaptor function originally linked it to the inflammasome via its interaction with pyrin, thereby defining PAPA as an autoinflammatory disease. PAPA patients with active disease have increased NLRP-3 mediated IL-1β production as well as TNF-α in peripheral blood leukocytes supporting a rationale for the use of medications targeting IL-1 and TNF-α [16, 32, 39]. There are case reports of successful treatment with anakinra, canakinumab, etanercept, infliximab, and adalimumab [32, 35, 40–43], as well as recent reports of success with golimumab (Hashkes, personal communication). Some authors have suggested that anakinra may be more effective for joint manifestations and TNF blockade for pyoderma gangrenosum [40, 44]. Nevertheless, these biologics have proven to be inconsistently effective for managing various disease manifestations. This may not be surprising given the multiple biochemical pathways regulated by PSTPIP1 adaptor activity. Aggressive pyoderma gangrenosum and acne are particularly troublesome in PAPA and often not effectively controlled by immunosuppressive therapies. The work of Starnes et al. suggests WASP overactivation in the presence of PSTPIP1 mutations may be a major cause of the invasive dermatologic findings in PAPA, perhaps mediated by macrophages [27]. Although more research is needed to define the precise mechanisms of PSTPIP1/WASP-mediated skin destruction in PAPA, current evidence suggests that novel therapies targeting WASP signaling could be effec-

tive. Drugs aimed at activated WASP itself (e.g. wiskostatin) are likely too broad-acting to be safe [45]. Likewise, significant and aggressive joint destruction in PAPA might be at least in part a consequence of heightened kinase signaling in osteoclasts. A number of tyrosine kinase inhibitors that target src are available, as are other general osteoclast inhibitors [46], but their utility in the context of PAPA is unknown.

22.8 Outcome/Prognosis

- **Damage is cumulative with repetitive episodes of joint and skin inflammation**
- **Severe skin disease may be challenging to treat**
- **Pain, scarring, and physical disability may lead to psychosocial impairment**

PAPA syndrome is characterized by recurrent flares of destructive inflammation in joints and skin. After a single episode of arthritis, the patient may recover much of the function of a joint, but can be left with contractures and reduced mobility. During the course of the disease, multiple joints may become involved albeit in an episodic and migratory fashion with only one site actively involved at any one time. Across time, ankylosis and significant joint destruction can occur [2, 34].

In some patients, cutaneous manifestations may prove even more challenging than the arthritis to treat successfully. The extremely invasive nature of the pyoderma gangrenosum may necessitate hospitalizations. The deep and intense inflammation associated with both the pyoderma gangrenosum and the cystic acne also can result in significant scarring [40].

It is important to note that the considerable pain induced by the joint and skin manifestations, the side effects including growth retardation from systemic corticosteroid therapy, the widespread permanent cutaneous scarring, and substantial physical disability associated with PAPA syndrome may lead to significant psychosocial impairment. Anecdotally, these patients may experience serious depression, school absenteeism/avoidance, and drug- seeking behaviors [41].

The outcome of PAPA disease varies. It depends both on the expression of the disease, particularly whether the cutaneous manifestations are severe, and on the success of the treatment in the individual patient. It is hoped that prompt diagnosis and the eventual development of consistently effective therapy will lead to an improved prognosis for patients with PAPA syndrome.

22.9 PSTPIP1 Associated Inflammatory Diseases (PAID)

In recent years, as new mutations with distinct phenotypes have been described, the spectrum of autoinflammatory diseases associated with mutations in *PSTPIP1* has expanded. Please see the INFEVERS database of systemic autoinflammatory diseases (http://fmf.igh.cnrs.fr/ISSAID/infevers/) for an updated summary of *PSTPIP1* mutations [8]. PAPA syndrome now represents a single clinical entity within the spectrum of PSTPIP1 associated inflammatory diseases (PAID). In particular, a number of patients with novel E250K mutations, and a single patient with E257K mutation, have been reported [10, 35, 47]. In addition to the classic PAPA triad of symptoms, these patients had some unique features including very early onset of severe chronic systemic inflammation, lymphadenopathy, hepatosplenomegaly, pancytopenia, and failure to thrive. Although most of these patients have arthritis, unlike in PAPA syndrome, it is rarely pyogenic. A hallmark of this disease is the very highly elevated levels of the pro-inflammatory alarmins myeloid-related protein MRP 8/14 with concomitant increased zinc levels (see Chap. 9). The mutations p.E250K and p.E257K cause a charge reversal in PSTPIP1 resulting in increased avidity for pyrin binding compared to E250Q mutants [10]. Recently, a patient with a E257K mutation of the PSTPIP1 gene was reported who developed a cerebral artery vasculopathy/vasculitis that resulted in subarachnoid hemorrhage from a ruptured dissecting posterior cerebral artery aneurysm [48]. The term PSTPIP1-associated myeloid-related proteinemia Inflammatory (PAMI) syndrome has been proposed for this entity (Fig. 22.2).

Other rare PAPA-like disorders with associated PSTPIP1 mutations are described, but whether these are causal or coincidental awaits further genetic or functional evidence. For a more comprehensive review of the clinical and genetic features of PAID please see reference [10].

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Deficiency of Adenosine Deaminase 2 (DADA2)

23

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Abstract

Deficiency of adenosine deaminase 2 (DADA2) is an autosomal recessive autoinflammatory disease resulting from mutations in *ADA2* (formerly named *CECR1*). Initially described by two groups in 2014, additional reports have documented that the phenotypic expression is quite broad. Although commonly presenting in childhood, patients can present throughout their lives with varying forms of inflammatory, neurologic, hematologic, and immunologic phenotypes. Frequently reported disease manifestations include lacunar strokes, non-cirrhotic portal hypertension, immunodeficiencies, and bone marrow involvement that can include an overt pancytopenia ranging to cell-specific immune destruction. The role of adenosine deaminase 2 (ADA2) remains incompletely understood. The lack of ADA2 in patients with DADA2 results in endothelial cell fragility and a skewing of macrophage development toward the inflammatory, M1 macrophage. Current treatment options are tailored toward the individual clinical presentations but the utilization of anti-tumor necrosis factor (TNF) medications has been highly effective at reducing the risk for stroke. Hematopoietic stem cell transplant offers a potential cure for the disease.

Keywords

Adenosine deaminase 2 · DADA2 · *ADA2*
Autoinflammation · Polyarteritis nodosa
Stroke · Cytopenia · Immunodeficiency
Hematopoietic stem cell transplantation
Anti-tumor necrosis factor drugs

Abbreviations

ADA1	Adenosine deaminase 1
ADA2	Adenosine deaminase 2
ADGF	ADA-related growth factor
CERC1	Cat eye syndrome chromosome region, candidate 1
DADA2	Deficiency of adenosine deaminase 2
FFP	Fresh frozen plasma
GvHD	Graft versus host disease
HSCT	Hematopoietic stem cell transplant
IL	Interleukin
MPO	Myeloperoxidase
MRA	Magnetic resonance angiography
NIH	National Institutes of Health
NRH	Nodular regenerative hyperplasia

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PAN	Polyarteritis nodosa
PHA	Phytohemagglutinin
PMA	Phorbol myristate acetate
SCID	Severe combined immunodeficiency
shRNA	Short hairpin RNA
TNF	Tumor necrosis factor

Key Points

- **Deficiency of adenosine deaminase (DADA2) is caused by recessively inherited mutations in *ADA2***
- **DADA2 has vast phenotypic presentation with common involvement of the hematologic, immunologic, neurologic, vascular, and hepatologic systems**
- **Adenosine deaminase (ADA2) is important in endothelial cell development as well as the anti-inflammatory, M1 macrophage**
- **Hematopoietic stem cell transplant (HSCT) can be a potential cure and anti-tumor necrosis factor (TNF) agents strongly reduce the risk for stroke**

23.1 Introduction

- **Deficiency of adenosine deaminase (DADA2) was described in 2014 by two different groups who identified mutations in *ADA2* as being causative of disease**

Autoinflammatory diseases are a relatively new group of disorders characterized by early onset of recurrent or persistent inflammation, seemingly unprovoked and appears in the absence of infection or any other apparent cause [1]. Currently, more than 25 monogenic disorders are known to cause autoinflammatory diseases, which typically lack specific biomarkers. Deficiency of adenosine deaminase 2 (DADA2) is an autoinflammatory disorders described at 2014 by two independent groups [2, 3].

During the last two decades, Israeli rheumatologists followed a group of children of Georgian Jewish origin with familial polyarteritis nodosa (PAN) vasculitis. Since PAN is usually not a familial disorder, nor common in childhood, and all patients were of the same ethnic isolate, a genetic basis was suspected. Using whole exome sequencing in a cohort of 24 patients, an autosomal recessive loss-of-function mutation in the cat eye syndrome chromosome region, candidate 1 (*CECR1*, now referred to as the *ADA2*) gene, encoding adenosine deaminase 2 (ADA2) was associated with this PAN-like vasculitis [2]. All 19 Georgian Jewish patients were homozygous for a mutation encoding a Gly47Arg substitution. Their unaffected relatives were either heterozygous for the mutation or did not carry it. Of 246 random unrelated controls of Georgian Jewish ancestry, 25 were heterozygous and none were homozygous for this variant, yielding a carrier frequency of 10% in this community. At the same time, a group from the National Institutes of Health (NIH), led by Dan Kastner and Ivona Aksentijevich, identified a syndrome of intermittent fevers, early-onset lacunar strokes and other neurovascular manifestations, livedoid rash, hepatosplenomegaly, and systemic vasculopathy in three unrelated patients. They suspected a genetic cause because the disorder presented in early childhood. Using whole-exome sequencing in the initial three patients and their unaffected parents and candidate-gene sequencing in three patients with a similar phenotype, they also found recessively inherited mutations in *ADA2*, encoding ADA2, that were predicted to be deleterious; these mutations were rare or absent in healthy controls [3].

Both groups published their findings in the same issue of the New England Journal of Medicine, and the name DADA2 was given to this autoinflammatory disorder. Since the first publications, numerous additional mutations in this gene have been described, and the clinical spectrum has expanded greatly. In this chapter we will discuss the genetic basis of the disease, its etiology and pathogenesis, clinical manifestations and laboratory workup of this disorder, diagnosis and treatment.

23.2 Epidemiology

- **Although initially described in a founder population, the diagnosis of DADA2 has now been made in ethnic groups all over the world**

DADA2 has been described in many ethnic groups. This is a rare disorder, with yet unknown prevalence. A founder mutation is known in the Georgian Jewish community, with a carrier frequency of 10%. Interestingly, the same mutation was found in patients from non-Jewish Turkish origin [4].

23.3 Etiology and Pathogenesis

- **New pathogenic mutations continue to be discovered, including deletions**
- **Adenosine deaminase 2 (ADA2) is expressed in myeloid cells and secreted by monocytes, macrophages, and dendritic cells**
- **ADA1 and ADA2 have partial structural homology and are involved in purine metabolism but have differing roles in different tissues**
- **Computer modeling and 3D modeling predict mutations to be loss-of-function**
- **Patients with deficiency of ADA2 differ from those with ADA1 deficiency in that there is not an accumulation of toxic deoxyadenosine nucleotides nor severe combined immunodeficiency (SCID)**
- **ADA2 acts as a growth factor for endothelial cells, thus, patients with DADA2 have vascular fragility**
- **ADA2 promotes development of the anti-inflammatory, M2 macrophage. Deficiency results in the skewing of macrophage development**
- **The zebrafish model demonstrates hemorrhagic stroke and neutropenia which are two of the clinical findings in patients with DADA2**

23.3.1 Adenosine Deaminase 2 (ADA2) Gene and Pathogenic Variants

The *ADA2* gene, mapped to chromosome 22q11.1, is a 28 Kb gene, which constitutes 10 exons [2]. It encodes the enzyme ADA2, a protein composed of four domains: the signal sequence, the dimerization domain, the putative receptor-binding domain and the catalytic domain (Fig. 23.1). Thus far, nineteen mutations have been reported in *ADA2* [4]. The most prevalent mutations are the G47R mutation, detected in homozygous state in almost all patients of Georgian Jewish and Turkish origin, and the R169Q mutation, more frequently detected in the European Caucasian population [4]. The mutations detected so far affect the signal peptide (n = 2), the 5′ untranslated region (n = 1), the dimerization domain (n = 4), the putative receptor binding (n = 1) and the catalytic domain (n = 11) (Table 23.1) [4]. In addition, two patients with a homozygous deletion on chromosome 22q.11.1 (which encompasses the *ADA2* gene) were recently described [5].

23.3.2 Normal Gene Product

ADA2 is a 57 kDa 511-amino-acid secreted homodimer protein highly expressed in plasma. It is expressed mainly in myeloid cells and is secreted by monocytes, macrophages and dendritic cells. It has two known functions: ADA2 is responsible for extracellular degradation of adenosine and is also a growth factor for endothelial and leukocyte development and differentiation.

23.3.3 Normal ADA2 Activity

ADA2 is a major extracellular adenosine deaminase. ADA2 has partial structural homology with human adenosine deaminase 1 (ADA1). Both proteins play a role in purine metabolism, con-

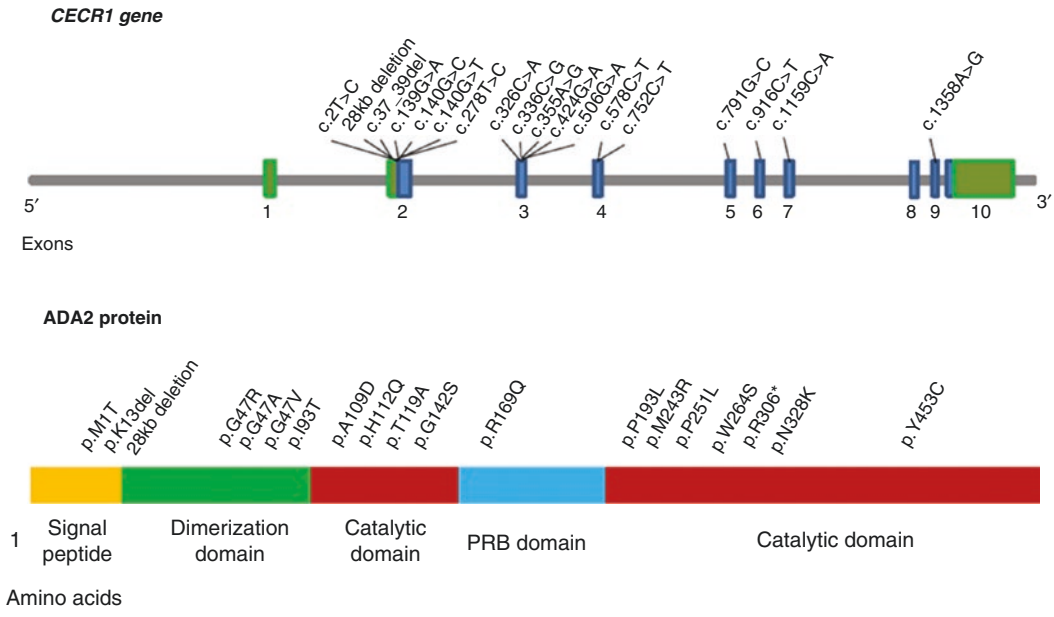

Fig. 23.1 *ADA2* gene mutations and adenosine deaminase 2 (ADA2) protein defects described to date. From Caorsi et al., reproduced with permission (4)

verting adenosine to inosine and 2′-deoxyadenosine to 2′-deoxyinosine. However, the affinity of ADA2 for adenosine is 100-fold less than that of ADA1. In addition, ADA1 is monomeric and largely intracellular, whereas ADA2 is dimeric and is secreted to the extracellular environment. ADA2 is more stable than ADA1 in high temperatures and its optimal pH activity is acidic. These two features suggest a role for ADA2 in hypoxia and inflammation [6].

In humans, irreversible degradation of adenosine to inosine and deoxyadenosine to deoxyinosine is catalyzed by intracellular ADA1 and by extracellular ADA2. Patients who have an inherited ADA1 deficiency present with profound lymphopenia and severe combined immunodeficiency disease (SCID), which is associated with the toxic intracellular accumulation of deoxyadenosine nucleotides. ADA2 deficiency manifests as vascular inflammation with milder forms of immune deficiency. Additionally, in contrast to patients with deficiency of ADA1, deoxyadenosine nucleotides do not accumulate in DADA2. While adenosine signaling dampens the inflammatory response in acute disease states, especially in ischemia and hypoxia, chronically elevated adenosine may promote tissue injury and fibrosis by prolonging inflammation. This reflects the complexity of the role of adenosine in the inflammatory response.

23.3.4 ADA2 as a Growth Factor (ADA-Related Growth Factor, ADGF)

ADA2 is primarily expressed by monocytes and cells of the myeloid lineage [6]. In the immune system, it has autocrine activity; when released by activated monocytes, it induces monocyte proliferation and macrophage defferentiation [7]. This is mediated by direct binding to cellular receptors, thus co-stimulating monocyte-induced CD4+ T cell proliferation. This role is unique to ADA2, and is independent of ADA2 catalytic deaminase activity.

ADA2 is also involved in the balance between pro-inflammatory (M1) and anti-inflammatory (M2) monocytes. When ADA2 is deficient or absent, macrophage differentiation is skewed leading to a prevalence of pro-inflammatory M1 macrophages [3].

Table 23.1 *ADA2* mutations described to date

Mutation	Exon	HGVS sequence name	Amino acid substitution	No of patients	Enzymatic domain
M1T	2	c.2 T > C	Met 1 Thr	1 in compound heterozygosis	Signal peptide
K13del	2	c.37_39del	37_39del	2 in compound heterozygosis	Signal peptide (?)
28-kb-deletion	2	deletion	deletion	1 in compound heterozygosis	Untranslated region (5'UTR)
G47R	2	c.139G > A	Gly47Arg	27 in homozygosis	Dimerization
				1 in compound heterozygosis	
G47A	2	c.140G > C	Gly47Ala	2 in compound heterozygosis	Dimerization
G47V	2	c.140G > T	Gly47Val	1 in compound heterozygosis	Dimerization
l93T	2	c.278 T > C	lle93Thr	1 in compound heterozygosis	Dimerization
A109D	3	c.326C > A	Ala109Asp	1 in compound heterozygosis	Catalytic
H112Q	3	c.336C > G	His 112Gln	1 in compound heterozygosis	Catalytic
T119A	3	c.355A > G	Thr119Ala	4 in compound heterozygosis	Catalytic
G142S	3	c.424G > A	Gly142Ser	4 in compound heterozygosis	Catalytic
R169Q	3	c.506G > A	Arg 169Gln	15 in homozygosis	Putative receptor-binding (PRB)
				9 in compound heterozygosis	
P193L	4	c.578C > T	Pro193Leu	1 in compound heterozygosis	Catalytic (?)
M243R	4	NA	Met243Arg	2 in compound heterozygosis	Catalytic
P251L	4	c.752C > T	Pro251 Leu	4 in compound heterozygosis	Catalytic
W264S	5	c.791G > C	Trp264Ser	1 in compound heterozygosis	Catalytic
R306*	6	c.916C > T	p.Arg306*	1 in compound heterozygosis	Catalytic (?)
N328K	7	c.1159C > A	Cys1159Arg	2 in compound heterozygosis	Catalytic
Y453C	9	c.1358A > G	Tyr453Cys	3 in compound heterozygosis	Catalytic

HGVS Human Genome Variation Society, *NA* not available
From: Caorsi et al. reproduced with permission [4]

23.3.5 Abnormal Gene Product

DADA2 results from loss-of-function variants of *ADA2*. This is supported by computer modeling of the missense mutations presented by Zhou Q et al. [3], based on the crystal structure of human ADA2. They showed that these mutations affect the catalytic and dimerization domains and protein stability, therefore are probably loss-of-function mutations. In addition, analysis of 3D protein structures of ADA2 reported by Navon et al. [2], suggests that Gly47Arg and Gly47Val could affect the stability of homodimers and/or their individual subunits, such that Arg169Gln

could alter the receptor binding domain, and Pro251Leu and Trp264His are likely to affect the active site of the enzyme.

23.3.6 Functional Studies of Mutant ADA2

ADA2 activity in plasma and serum samples from patients with two ADA2 mutations, their heterozygote relatives, and healthy adult and pediatric controls, showed that patients had significantly diminished ADA2 activity in plasma [2, 3]. Using Western blot analysis to analyze the ADA2 expression, the ADA2 protein was reduced in cell lysates and absent from the supernatants of macrophages collected from patients with DADA2.

In assays measuring both ADA1 and ADA2 activity in monocytes and plasma from the patients with DADA2, ADA1-specific activity was not distinguishable from healthy control patients whereas there was significant reduction in ADA2-specific activity. Additionally, erythrocyte hemolysates had normal thin layer chromatographic assays for ADA1 activity and there was not an accumulation of deoxyadenosine nucleotides (ADA1 deficiency's defining biomarker) in the erythrocytes from patients with DADA2 [3].

23.3.7 ADA2 Deficiency in the Immune System

As compared with patients with ADA1 deficiency, those with ADA2 deficiency have only a mild immunodeficiency, most evident in B cells. As mentioned previously, ADA2 associated vasculopathy may be related to impairment of ADA2's growth factor activity, which is important both in the immune system and in early development of macrophages.

Previous studies have shown a role for human ADA2 in the differentiation of monocytes to macrophages. Using short hairpin RNA (shRNA) constructs to silence the expression of ADA2 in myeloid U937 cells, Zhou et al. observed marked impairment of macrophage differentiation induced by phorbol myristate acetate (PMA) with significant M1 predominance. *ADA2* silencing in myeloid cells was associated with reduced differentiation of monocytes to macrophages [3].

Neither serum cytokine analysis nor the production of cytokines produced by cultured peripheral-blood mononuclear cells showed any convincing differences between patients with DADA2 and healthy controls. As patients with deficient ADA1 have marked T-cell defects, Zhou et al. investigated T-cell function and thymic output in patients with DADA2. Results showed normal naïve T cells and recent thymic emigrants as well as normal short-term T-cell activation and proliferative responses to anti-CD3 antibodies. There was a slight increase in the proliferation of CD4+ and CD8+ subsets in response to phytohemagglutinin (PHA) stimulation; however, there was normal production of effector cytokines [3].

Due to the rarity of the disease, all available data on the pathogenic consequences of ADA2 defect in humans come from only a few patients; further studies are therefore needed in order to better understand the activity of ADA2 in the innate and adaptive immune response and its role in endothelial homeostasis.

23.3.8 Effect of ADA2 Deficiency on Endothelial Development

Although ADA2 is not expressed in endothelial cells, there is a defect in endothelial integrity in the small vessels of patients with ADA2 mutations. The reason why the endothelium represents the main target of inflammation in DADA2 is still largely unknown. *ADA2* is not expressed, nor is the ADA2 protein detectable, in cultured human endothelial cells. However, ADA2 acts as a growth-factor for endothelial cells, and the deficiency of ADA2 is associated with damage of the vascular endothelium and over-expression of activation markers.

Zhou et al. showed that there was substantial endothelial damage in patients with DADA2 from biopsy specimens from both the brain and the skin. Endothelial cell activation was observed

in biopsies from both locations. Biopsies also showed increased staining for interleukin (IL)-1β, inducible nitric oxide synthase, and tumor necrosis factor (TNF) which indicates an inflammatory component to the disease.

23.3.9 Animal Model

Although there is no murine orthologue of *ADA2*, there are two paralogs in zebrafish: *cecr1a* (chromosome 25) and *cecr1b* (chromosome 4). Zhou et al. generated a *cecr1a* hypomorphic line, which had no overt phenotype. When they developed a transient knockdown strategy for *cecr1b*, they observed intracranial bleeding with *cecr1b* morpholinos in a dose dependent manner, suggesting the presence of defects in vessel development or integrity (Fig. 23.2). The morphologic features of the blood vessels appeared to be normal, despite evidence of hemorrhage and ischemia in embryos injected with *cecr1b*-specific morpholino oligonucleotides targeting the translation initiation site. Intracranial hemorrhage was blocked by co-injection with non-mutant human *ADA2* messenger RNA (mRNA); conversely, the development of intracranial hemorrhage was not blocked when injected with mutant transcripts [3].

An additional finding in the zebrafish was the profound neutropenia observed in the embryos that had *cecr1b* expression disrupted. These experiments show that *cecr1b* is essential for both vascular integrity and neutrophil development in the zebrafish embryo and both phenotypes are prevented by non-mutant, but not by mutant, human *ADA2* mRNA. When taken together, the zebrafish data support the role for both vasculopathy and inflammation in DADA2.

23.4 Clinical Manifestations

- **Multi-system involvement is common**
- **Expanding phenotypic presentation can make DADA2 difficult to diagnose**
- **The majority of cases present in childhood, but there are some patients who have delayed presentation until adulthood**

Clinical manifestations of DADA2 are broad and can be divided up into various phenotypes

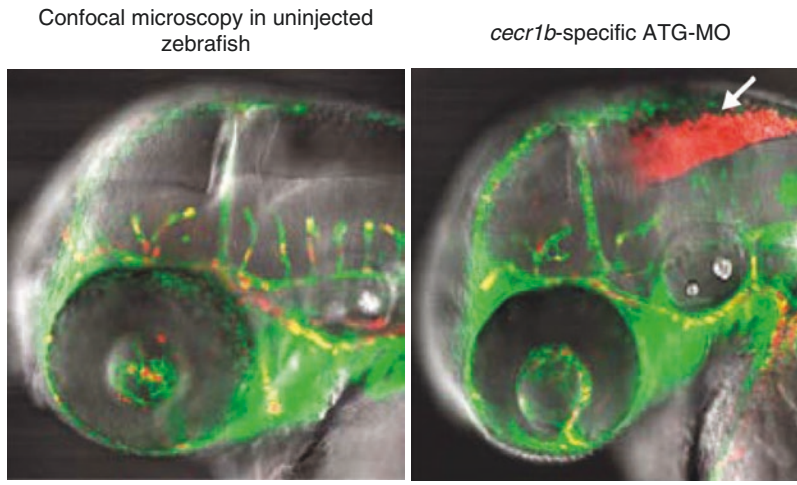

Fig. 23.2 Confocal microscopy in zebrafish highlighting intracranial bleeding (arrow). Intracranial bleeding is observed in embryos injected with *cecr1b*-specific morpholino oligonucleotides. ATG-MO: morpholino-modified antisense oligonucleotides that bind close to the ATG start site of the mRNA transcript thereby blocking translation and preventing protein synthesis. From: Zhou Q, Yang D, Ombrello AK, et al. Early-onset stroke and vasculopathy associated with mutations in ADA2. N Engl J Med 2014;370:916. Copyright © 2014, Massachusetts Medical Society. Reprinted with permission

based upon the systems involved. What was initially described as a constellation of symptoms including recurrent fevers, a livedo racemosa rash, recurrent ischemic strokes and a mild form of immunodeficiency has expanded significantly. Neurologic, vascular, inflammatory, immunologic, and hematologic phenotypes have all been observed with significant overlapping of each phenotype.

Although most patients present in childhood [2, 3], there are also patients that remain asymptomatic or undiagnosed until the fifth decade of life due to low disease activity or symptoms that could be attributed to another diagnosis such as polyarteritis nodosa and/or common variable immune deficiency [8–10]. The disease pattern varies from patient to patient; it is not uncommon for patients to present with marked inflammatory episodes that may persist for extended periods followed by prolonged quiescent periods. To date, there is not a clear genotype/phenotype correlation which makes it virtually impossible, at this time, to predict outcomes and, therefore, make targeted treatment recommendations.

Although patients may seemingly have a certain phenotype (inflammatory vs. hematologic vs. immunologic) during one point in the life, extension of the phenotypic presentation of disease is unpredictable and requires an astute physician monitoring for new clinical manifestations of disease.

Patients with DADA2 can have periods of intense inflammation that are interspersed throughout their life spans. Patients can present with fever and marked elevation of their acute phase reactants. Often, these inflammatory episodes will accompany symptoms of vasculitis. Patients can have concomitant strokes, worsening cutaneous disease, systemic features of PAN such as ischemic bowel, mesenteric aneurysmal disease, renal involvement and muscle inflammation.

23.4.1 Neurologic Manifestations

Patients with DADA2 have a propensity for strokes occurring in the small, terminal vessels of the brain (Fig. 23.3). These are also termed lacu-

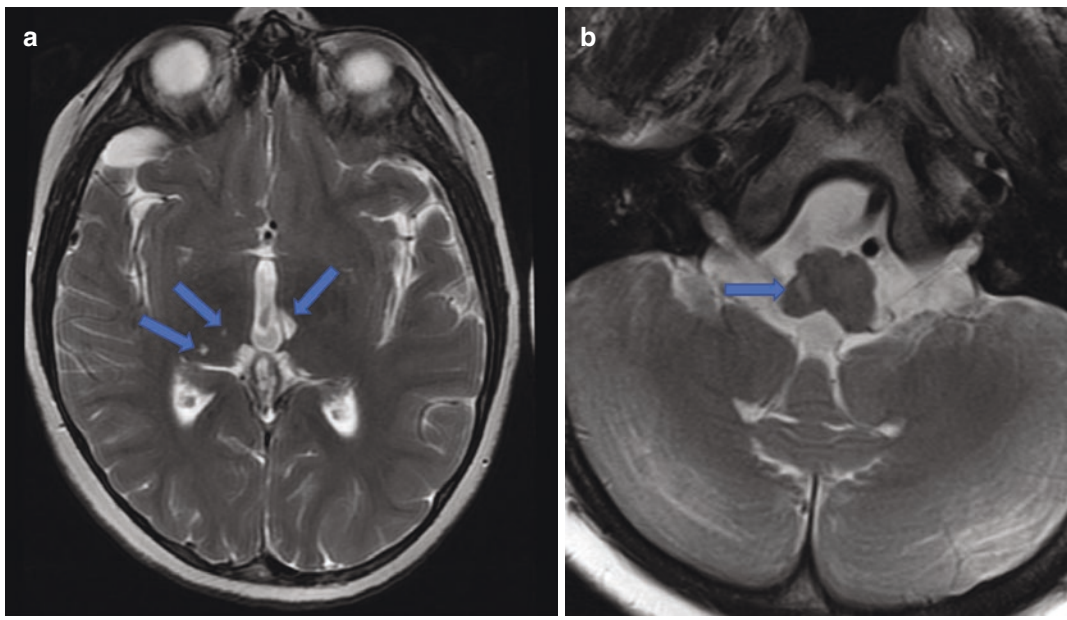

Fig. 23.3 Ischemic strokes in patients with deficiency of adenosine deaminase 2 (DADA2): The strokes tend to occur in small terminal vessels and are termed lacunar strokes. (**a**) This patient had a history of multiple lacunar strokes. The blue arrows highlight bilateral thalamic strokes with the two strokes on the right occurring in the pulvinar of the thalamus (**b**) The blue arrow highlights a right upper medulla paramedian stroke

nar strokes. Commonly affected areas include the brainstem, internal capsule, basal ganglia, thalamus, corpus callosum, and cerebellum. These areas are all sites of very small, terminal vessels. Therefore, magnetic resonance angiography (MRA) imaging is typically unremarkable as it is not sensitive enough to identify the affected vessels. There can also be spinal cord ischemia resulting in bilateral extremity involvement and bowel/bladder difficulties. In addition to ischemic strokes, there are patients who have large hemorrhagic strokes [3, 11]. Hemorrhagic strokes have occurred at the sites of previous lacunar infarct but they also can occur in other areas such as the subdural and subarachnoid space. There is evidence that supports that patients with DADA2 have an increased risk for hemorrhagic strokes when treated with some form of anti-thrombotic therapy but, conversely, there are cases of spontaneous cerebral hemorrhage without any inciting trauma or anti-thrombotic therapy (personal communication). Clinical manifestations involving the peripheral nervous system have included carpal tunnel syndrome and mononeuritis multiplex.

23.4.2 Dermatologic Manifestations

Patients with DADA2 commonly present with clinically apparent livedo racemosa [12]. Unlike the finer pattern of livedo reticularis, patients with livedo racemosa have broader patterns that are often interrupted. Although commonly observed in the extremities, there are patients who have livedo racemosa extending to their abdomen and even to their face (Fig. 23.4). Additional dermatologic findings can be associated with the inflammatory/vasculitic processes such as subcutaneous nodules, urticarial rash, erythema nodosum, cutaneous vasculitis, as well as those that resemble more of an ischemic process such as Raynaud phenomenon, cutaneous ulcers, and digital necrosis. Autoamputation of the digits may occur as can osteomyelitis (Fig. 23.5). Skin biopsies can show histologic evidence of vasculopathy without direct involvement of the blood vessels (Fig. 23.6), extending to small/medium vessel vasculitis.

23.4.3 Gastrointestinal Manifestations

There are several forms of gastrointestinal and hepatic presentations associated with DADA2. Liver biopsies from patients with DADA2 have shown nodular regenerative hyperplasia and hepatoportal sclerosis. Patients can have varying degrees of non-cirrhotic portal hypertension which can result in splenomegaly and esophageal varices [3]. Hepatomegaly is common. Many patients with DADA2 complain of nonspecific abdominal pain but there have also been cases of bowel perforation, with areas of ischemia and active vasculitis [13]. Patients have also presented with features of ischemic bowel as well as ulcerative bowel disease [14].

23.4.4 Immunologic Manifestations

Patients with DADA2 can present with a broad spectrum of disorders. Many patients have an IgM deficiency but others may have a more pronounced hypogammaglobulinemia [3, 10]. Patients may have previously been diagnosed with common variable immunodeficiency or selective antibody deficiency [10]. Zhou et al. observed decreased transitional B cells in multiple patients. CD4 lymphopenia and neutropenia have been reported in multiple cases as has decreased memory B cells [10]. Specific infections that are reported in multiple patients include herpes and verrucous-associated infections.

23.4.5 Hematologic Manifestations

Patients with DADA2 can have varying forms of anemia such as an anemia of chronic inflammation or pure red cell aplasia. Pancytopenia, which can present with fatigue, pallor, easy bruising and infection, has also been observed in multiple cases. Bone marrow biopsies can demonstrate both hypo- and hypercellularity and there have been multiple reports of large granular lymphocytes which may indicate an immune mediated

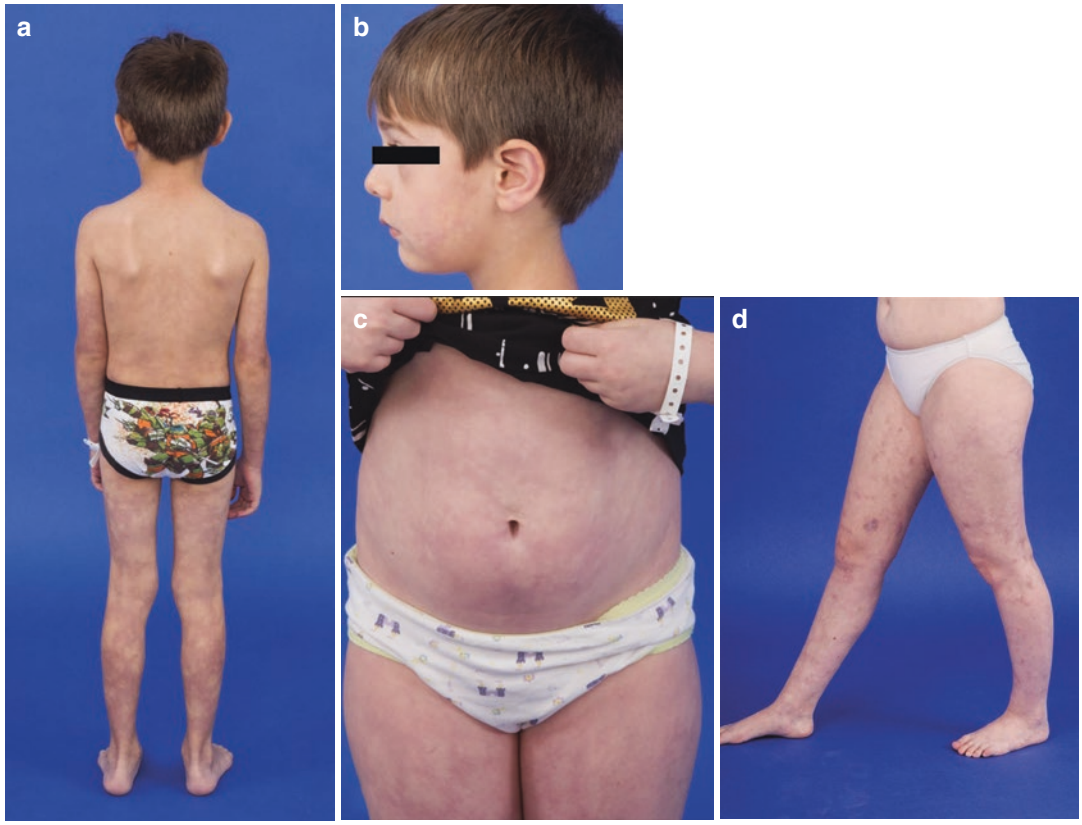

Fig. 23.4 Dermatologic findings in deficiency of adenosine deaminase 2 (DADA2). The livedo pattern can be very mild and virtually undetectable in some patients but, in others, there can be significant livedo racemosa. The presence or relative absence of livedo does not necessarily correlate with the severity of disease. (**a** and **b**) This patient presented with extensive livedo racemosa extending proximally on all extremities, including the abdomen, back and face. This patient did not have neurologic events or features of polyarteritis nodosa (PAN) (**c**) This patient has extension of her livedo onto the abdomen. She had a history of 2 ischemic strokes (**d**) This patient has livedo with nodular lesions consistent with cutaneous PAN

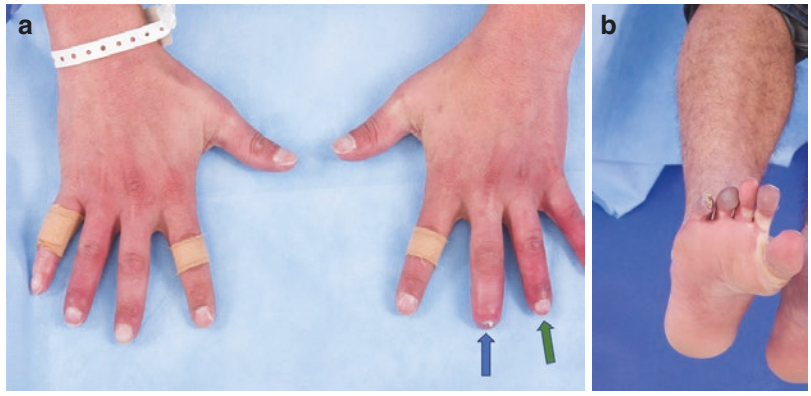

Fig. 23.5 Peripheral vascular disease in deficiency of adenosine deaminase 2 (DADA2). (**a**) This patient has pronounced peripheral vascular disease that has resulted in resorption of some of the upper extremity distal phalanges (blue arrow) as well as a pernio-like appearance to some of his digits (green arrow) (**b**) This patient has a pronounced cyanotic appearance to three of the toes on his right foot with subsequent disordered nail growth of the fifth digit

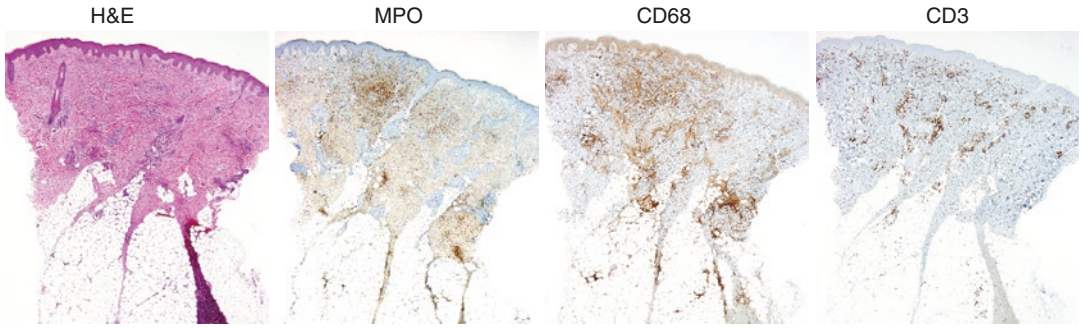

Fig. 23.6 Skin biopsy findings in deficiency of adenosine deaminase 2 (DADA2). This biopsy was taken from a patient with extensive livedo racemosa. Histologically, an interstitial inflammatory infiltrate is observed comprised of myeloperoxidase positive (MPO) neutrophils and $CD68^+$ macrophages. There is a $CD3^+$ lymphocyte predominant perivascular infiltrate. From: Zhou Q, Yang D, Ombrello AK, et al. Early-onset stroke and vasculopathy associated with mutations in ADA2. N Engl J Med 2014;370:S23. Copyright © 2014, Massachusetts Medical Society. Reprinted with permission

Table 23.2 Recommended baseline evaluation in DADA2 patients

Laboratory evaluation	Imaging/testing	Optional tests	Possible consultants
Complete blood count with differential	MRI brain	Echocardiogram	Neurology
Serum chemistries	Abdominal ultrasound with Doppler	MRI/MRA abdomen	Hepatology
Hepatic panel	Electrocardiogram	Renal ultrasound	Dermatology
Erythrocyte sedimentation rate		Nerve conduction study/ electromyelogram	Ophthalmology
C-reactive protein		MRA extremities	Immunology
Immunoglobulin levels		Extremity angiography	Hematology
Lymphocyte phenotype panel			Nephrology
Urinalysis			Cardiology
Creatine kinase			
Lactate dehydrogenase			
Aldolase			

effect on the marrow [8, 15]. It is important to be aware of the hematologic manifestations as they can be the initial presenting symptom of DADA2.

23.4.6 Other Manifestations

Renal manifestations include hematuria and hypertension. Patients can have arthralgias, myalgias as well as frank synovitis and myositis [3, 10]. Ocular manifestations include strabismus and ptosis due to muscular weakness after strokes. There was a reported case of panuveitis as well as optic neuritis [13, 14] and episcleritis [10]. Patients can have neurosensory hearing loss [2].

23.5 Laboratory and Ancillary Testing

- **Once a diagnosis is made, it is important to conduct a comprehensive baseline evaluation as the phenotypic presentation can be extremely broad**
- **Even when clinical symptoms are minimal, annual laboratory studies should be performed to assess for potential new disease-related complications**

Once a diagnosis is made, it is recommended that an expansive baseline evaluation is conducted (Table 23.2). Keeping in mind that the phenotypic

presentation continues to expand and evolve, clinicians should be attuned to patient symptoms and clinical findings, thereby ordering tests as indicated. Each patient should undergo a complete blood count with differential to evaluate for any significant cytopenias or evidence of inflammation such as leukocytosis and thrombocytosis. Acute phase reactants should be checked, assessing for inflammatory burden. To evaluate renal status, serum chemistries and a urinalysis should be tested. Hepatic involvement can be assessed by checking hepatic transaminases and coagulation studies.

Immunologic laboratory tests should include serum immunoglobulins as well lymphocyte phenotyping to evaluate for the various lymphocyte subsets. Patients with a history of significant muscle pain should have their muscle enzymes evaluated.

Any patients with history of hypertension or cardiac arrhythmias should have a baseline electrocardiogram and echocardiogram.

Additionally, patients who present with paresthesias/dysesthesias or signs of a motor neuropathy should have electromyogram/nerve conduction studies of the extremities to evaluate for polyneuropathy and mononeuritis multiplex.

Once a set of baseline labs have been assessed, annual laboratory testing should include a complete blood count, serum chemistry, hepatic transaminases, acute phase reactants and urinalysis. Clinical symptoms can direct the need to order other, organ-specific tests such as esophagogastroduodenoscopy and colonoscopy, as well as other forms of imaging (see next section).

23.6 Imaging

- **Once a diagnosis is made, a baseline MRI of the brain is recommended as patients may have a history of "silent" strokes**
- **Abdominal ultrasound is strongly recommended to assess the liver and spleen**
- **Additional imaging can be performed based on clinical presentation**

Once a diagnosis is confirmed, each patient should undergo a baseline MRI of the brain to assess for lacunar infarcts (Fig. 23.2). Patients can have "silent" strokes apparent on MRI of the brain making it important to perform this assessment so that if symptoms change, going forth, the baseline MRI is available for comparison. It is not necessary to have patients undergo MRA of the brain as the sensitivity of MRA is subpar for the detection of small vessel infarcts [16]. In patients who have neurologic symptoms and examination (e.g. bilateral limb involvement and neurogenic bladder) consistent with spinal cord involvement, MRI of the spine should be considered.

In order to assess hepatic disease, all patients should undergo abdominal ultrasound to assess for hepatomegaly and/or splenomegaly. Doppler examination should also be performed to assess for portal blood flow as some patients with significant portal hypertension can have recanalization of the umbilical vein [3]. Esophagogastroduodenoscopy to evaluate for gastric varices should be considered in patients with suspected portal hypertension. Fibroscan of the liver is recommended to assess for fibrosis and can be routinely performed without placing patients at significant risk of a liver biopsy. Performance of a liver biopsy may be considered on a case by case basis.

If patients have a history of abdominal pain that resembles claudication, MRI/MRA of the abdomen is recommended. There have been multiple cases of intestinal wall perforation so, if presenting with signs of an acute abdomen, radiographs and/or CT scan should be done to assess for free air in the abdomen. Abnormal MRA findings are often seen in those patients with an inflammatory presentation; therefore, these patients may benefit from a baseline abdominal MRA.

Patients with significant acrocyanosis of the extremities should have an MRA of the extremities or conventional angiography to evaluate the extent of vascular involvement. Other imaging should be ordered as directed by clinical presentation.

23.7 Diagnosis

- **Diagnosis can be made by:**
  - **Demonstration of two mutations in *ADA2***
  - **Marked reduction in serum ADA2 level**

There are two important pieces that can act either in synergy or independently toward making the diagnosis of DADA2: genetic analysis and measurement of serum ADA2 levels. Biallelic mutations of *ADA2* will confirm the diagnosis but, there are a subset of patients in which the identification of a second mutation can prove elusive. These are patients in who a second mutation may be a genetic deletion. However, if a patient has a phenotype suspicious for DADA2 but only one detectable mutation, or if genetic testing is difficult to obtain, the diagnosis can be established by assessing serum ADA2 levels. Although not commercially available, there are multiple assays available for assessing ADA2 activity with varying reference ranges. Therefore, each result needs to be compared to the wild type, heterozygote, and affected ranges for that specific assay [3, 8, 17]. Additionally, there should be careful attention paid to the reference ranges in relation to the age of the patient as, in general, children tend to have higher ADA2 levels.

Once a patient has been diagnosed with DADA2, genetic testing should be offered to all siblings within the family. There have been multiple cases of DADA2 made in siblings who were thought to be unaffected. As DADA2 has been observed to present in adulthood, early diagnosis could be critical to preventing some catastrophic sequelae of disease [8–10].

23.8 Treatment

- **The use of anti-tumor necrosis factor (TNF) agents has resulted in a dramatic reduction in strokes**
- **Hematopoietic stem cell transplant (HSCT) is a potentially curative treatment for patients with severe hematologic and/or immunologic phenotypes**
- **Fresh frozen plasma infusions are not an effective approach to replace ADA2**
- **Anti-thrombotic/anti-platelet/anti-coagulation drugs should be implemented with caution and only after strong consideration of the risk: benefit ratio due to the vascular fragility and potential of spontaneous bleeding of patients with DADA2**

Table 23.3 Immunomodulatory treatments in the deficiency of adenosine deaminase 2 (DADA2) cohort taken prior to the initiation of anti-tumor necrosis factor (TNF) agents (N = 15)

Medication	Number of patients
Prednisone	13
Cyclophosphamide	6
Intravenous immunoglobulin	5
Anakinra	3
Azathioprine	3
Mycophenolate	3
Methotrexate	2
Canakinumab	1
Cyclosporine	1
Rituximab	1

The primary focus of the treatment of DADA2 can be separated into two areas: enzyme replacement and treatment of the disease manifestations.

23.8.1 Immunosuppressive Therapy

Many immunosuppressants have been utilized to treat the inflammatory symptoms with varying success in treating symptoms such as skin nodules, fever, myalgia and arthralgia. However, aside from anti-TNF drugs, other medications have not been able to significantly reduce the risk for stroke (Table 23.3) [18]. Additionally, many patients with a significant inflammatory component to their disease are unable to wean corticosteroids.

In an attempt to reduce corticosteroid use and based upon the reported success in several Georgian-Jewish patients [2], the NIH initiated a trial of anti-TNF treatment as adjunctive therapy in 15 consecutive patients from their DADA2 cohort, with a cumulative history of 55 strokes [18]. There was a total of 2077 patient-months before anti-TNF initiation (using patient date of birth as time zero) with 55 strokes and 733 patient-months post-anti-TNF initiation with no strokes ($P = 1.42 \times 10^{-9}$). Matched follow-up time analysis using time zero as time of anti-TNF initiation revealed 37 strokes in 733 patient months pre-anti-TNF and 0 strokes in the 733 months post-anti-TNF. ($P = 1.45 \times 10^{-11}$) [18].

The use of anti-TNF agents for other clinical aspects of DADA2 remains unclear. While effective at reducing the inflammatory burden of disease, the effect on patients with bone marrow involvement is not as evident. There are reports of anti-TNF medications having no effect on the bone marrow phenotypes but there are cases that have demonstrated clear improvement [9]. Additional studies are needed to better understand the potential effects of anti-TNF agents on differing phenotypes. Regarding specific anti-TNF agents, the use of monoclonal antibodies may theoretically provide enhanced anti-inflammatory coverage compared to the soluble p75 TNFR:Fc fusion protein (etanercept); however, there is not adequate data to support this at the current time. The use of concurrent methotrexate should be considered if using the mouse-human chimeric anti-TNF antibody, infliximab, to reduce the development of human anti-chimeric antibodies, but the effect of concurrent methotrexate use has not been widely studied in DADA2. Since the ramifications of stroke can be immediate and life-altering, strong consideration for the initiation of anti-TNF treatment should be given even to those patients with immunodeficiencies and varying hematologic presentations.

23.8.2 Enzyme Replacement Strategies

Regarding enzyme replacement, there have been several reports of patients with DADA2 who underwent hematopoietic stem cell transplants (HSCT). Hashem et al. reported the results of 14 patients with DADA2 who underwent HSCT. The clinical indication for HSCT was bone marrow dysfunction manifesting as pure red cell aplasia, thrombocytopenia, or neutropenia and/or immunodeficiency [15]. Twenty transplants were performed on the 14 patients. Two patients needed a second transplant due to the initial donor being an undiagnosed sibling also with DADA2. Conditioning regimens varied from patient to patient. Post-transplant complications included acute graft versus host disease (GvHD) in 6 (43%), chronic GvHD in 1 (7%), veno-occlusive disease in 2 (14%), and viral reactivation in 10 (71%). Autoimmune complications included idiopathic thrombocytopenic purpura in 2 (14%), autoimmune hemolytic anemia in one patient (7%) and neutropenia with immune mediated pure red cell aplasia in one patient (7%).

A Phase I trial was conducted at the NIH utilizing fresh frozen plasma (FFP) as a vehicle for enzyme replacement. After ADA2 was determined to be present in donor FFP, three patients with DADA2 underwent a two-step trial assessing for safety and pharmacokinetics. Although the infusions of FFP were well-tolerated, the volume of distribution was low and the half-life was short (range: 5–9 h) [18].

Treatment with anti-thrombotic/anti-platelet/anti-coagulation agents is generally discouraged due to the underlying endothelial cell defect in DADA2. This is especially pertinent as many institutions have a protocol for the treatment of acute stroke that would involve these agents. The need for these drugs for comorbid reasons should be made on a case by case basis.

Gene therapy and other forms of enzyme replacement are in varying stages of development but are not yet ready for clinical trials.

23.9 Outcome/Prognosis

- **DADA2 can have tremendous phenotypic variability making it difficult to determine the prognosis**

As DADA2 was not described until 2014, the data on medium and longer-term outcome are minimal. There can be serious sequelae that result from some of the known disease manifestations. Patients can have profound neurologic deficits due to stroke (both ischemic and/or hemorrhagic), patients can develop hepatic failure due to DADA2-related liver disease, perforation of the bowel requiring surgical intervention, digital amputation due to the peripheral vascular disease (Fig. 23.5), transfusion-dependency due to failing bone marrow and the ramifications of repeated infections, often related to treatment. The utilization of anti-TNF agents is a step

toward reducing some of these complications as is the implementation of HSCT. However, even the treatments themselves can result in substantial co-morbidity such as the development of autoimmune disease post-transplant, GvHD, veno-occlusive syndrome, and immunosuppression. Each patient with DADA2 should undergo a thorough, systematic evaluation and treatment recommendations should be made after careful analysis of the clinical phenotype and careful consideration of the health of the affected individual.

Prognostically, it is unpredictable as to who will have mild versus severe disease. There are not enough cases yet to be able to fully assess for possible genotype/phenotype correlation. Furthermore, even within families sharing the same mutation/s, there can be tremendous phenotypic variability which promotes the need for exhaustive evaluation as more subtle findings (such as emerging neutropenia or peripheral vascular disease) may be overshadowed by some of the dramatic presentations (stroke, transfusion-dependent anemia). In the current literature, the vast majority of patients survive. As more cases are identified, the most common causes of death are due to gastrointestinal complications such as perforation or ischemia, sequelae relating to cerebral hemorrhage, and infectious complications such as pulmonary aspergillosis, necrotizing pneumonia, and septic shock [2, 3, 4, 5, 10, 17].

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Genetic Interferonopathies

24

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Abstract

The interferonopathies comprise an expanding group of monogenic diseases characterised by disturbance of the homeostatic control of interferon (IFN)-mediated immune responses. Although differing in the degree of phenotypic expression and severity, the clinical presentation of these diseases shows a considerable degree of overlap, reflecting their common pathogenesis. Increased understanding of the molecular basis of these Mendelian disorders has led to the identification of targeted therapies for these diseases, which could also be of potential relevance for non-genetic IFN-mediated diseases such as systemic lupus erythematosus (SLE) and juvenile dermatomyositis. In this chapter we summarise the current knowledge of the molecular basis, clinical features, and treatment of monogenic interferonopathies.

Keywords

Interferonopathies · Aicardi Goutières syndrome · Proteasome · CANDLE · SAVI

Abbreviations

AGS	Aicardi-Goutières syndrome
ALDD	Autoinflammation lipodystrophy and dermatitis
ANA	Antinuclear antibody
APECED	Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy
APLAID	Autoinflammation and PLCG2-associated Antibody Deficiency and Immune Dysregulation
CANDLE	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
CK	Creatine kinase
COPA	Coatomer protein complex subunit alpha
CRP	C-Reactive protein
CRV	Cerebroretinal vasculopathy
CSF	Cerebrospinal fluid
DADA2	Deficiency of adenosine deaminase 2
ESR	Erythrocyte sedimentation rate

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HVR	Hereditary vascular retinopathy
HERNS	Hereditary endotheliopathy with retinopathy, nephropathy and stroke
IFN	Interferon
ISG	Interferon stimulated genes
JAK	Janus kinase
NSAID	Nonsteroidal anti-inflammatory drug
PLCG2	Phospholipase C gamma 2
PRAAS	Proteasome associated autoinflammatory syndrome
PSM	Proteasome subunit
RVCL	Retinal vasculopathy with cerebral leukodystrophy
SAVI	STING associated vasculitis with onset in infancy
SGMRT	Singleton-Merten syndrome
SLE	Systemic lupus erythematosus
SPENCDI	Spondyloenchondrodysplasia with immunodysregulation
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
THES	Trichohepatoenteric syndrome
TLR	Toll-like receptor
TORCH	Toxoplasmosis, others, rubella, syphilis, herpes
USP	Ubiquitin-specific protease

Key Points

- **Interferonopathies are characterised by disturbance of the homeostatic control of IFN mediated immune responses, particularly type 1 interferons**
- **The monogenic interferonopathies are inflammatory diseases that present early in life, and may mimic congenital infection or be mistaken for sporadic autoimmune diseases**
- **There is an ever growing number of monogenic interferonopathies, discovered through more widespread use of next generation genetic sequencing. The identification of additional diseases in this class seems likely**
- **Targeted treatment such as Janus kinase (JAK) inhibitors that downregulate JAK/STAT signalling may be effective in treating the monogenic interferonopathies, although data are limited**

24.1 Introduction

The interferons (IFN) are signalling proteins synthetized and released by immune host cells in response to the presence of several pathogens such as viruses, bacteria, parasites and tumour cells [1–4] (see Chap. 6). The induction, transmission, and resolution of the IFN-mediated immune response is tightly regulated, and finely-tuned by opposing augmenting and suppressive signals induced by host factors [1–5]. These signals rapidly mobilize an effective antimicrobial response against the invading pathogen, while restraining the magnitude of the response to avoid excessive inflammatory responses, thus limiting host injury. The interferonopathies are an expanding group of complex genetic disorders characterised by disturbance of the homeostatic control of these IFN mediated immune responses (Fig. 24.1), with upregulated interferon gene expression a near ubiquitous feature ("positive interferon signature"), considered in more detail below. Although differing in the degree of phenotypic expression and severity, the clinical presentation of these diseases shows a considerable degree of overlap reflecting their common pathogenesis. This chapter summarises the current knowledge of the molecular basis, clinical features and treatments available for the monogenic interferonopathies (Table 24.1). Other monogenic disorders that may also involve aberrant IFN responses such as deficiency of adenosine deaminase type 2 (DADA2) and monogenic lupus. DADA2 is discussed in Chap. 23.

24.2 CANDLE (PRAAS) Syndrome

- **Mutations in genes encoding proteasome subunits are responsible for CANDLE/PRAAS syndrome either as bi-allelic recessive monogenic diseases, or as a digenic disease model**
- **Skin manifestations are pathognomonic, and include annular, purpuric plaques, violaceous periorbital and perioral edema, and lipodystrophy**

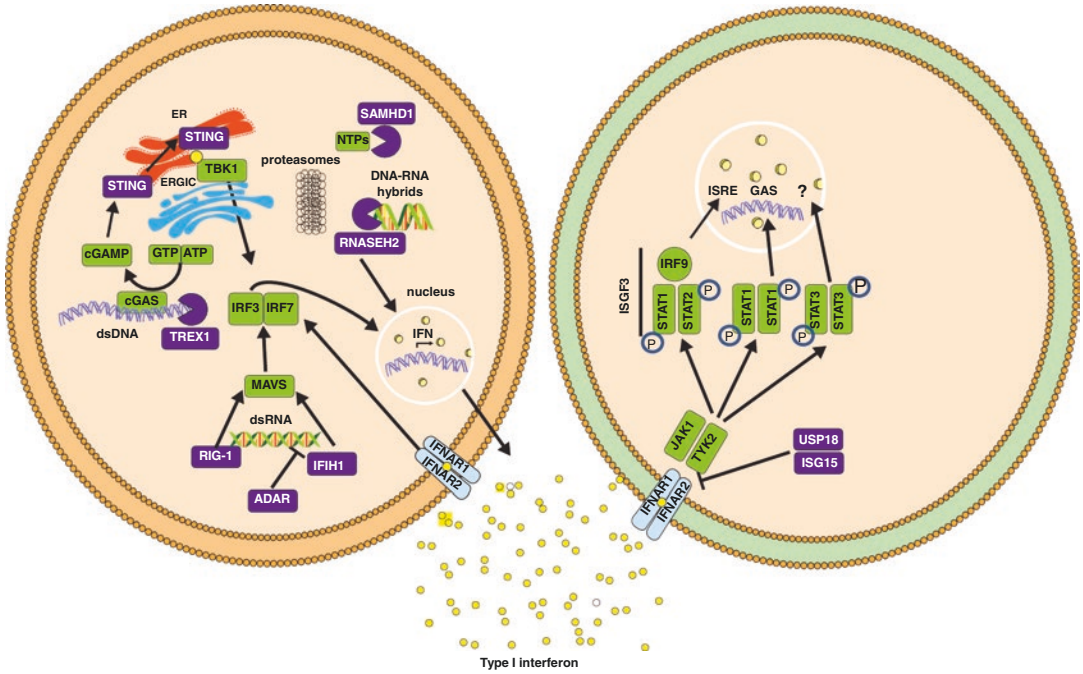

Fig. 24.1 Schematic representation of pathways affected in genetic interferonopathies. Coloured in purple are some of the proteins mutated in type I interferonopathies. See Table 24.1 for the linked disorders. *STING* stimulator of interferon genes, *SAMHD1* deoxynucleoside triphosphate triphosphohydrolase SAM domain and HD domain 1, *TREX1* DNA 3′ repair exonuclease 1, *ISG15* interferon-stimulated gene 15, *MAVS* mitochondrial antiviral-signalling protein, *RIG-I* retinoic acid-inducible gene 1, *TBK1* TANK-binding kinase 1, *USP18* ubiquitin-specific protease 18, *RNASHEH2* Ribonuclease H domain 2, *IFIH1* IFN-induced helicase C domain-containing protein 1, *IRF3* interferon regulatory factor 3, *IRF7* Interferon regulatory factor 7, *IRF9* interferon regulatory factor 9, *cGAMP* cyclic di-GMP-AMP, *cGAS* cyclic GMP-AMP synthase, *ER* endothelial reticulum, *ERGIC* endothelial reticulum-Golgi intermediate compartment, *IFNAR* interferon-α receptor, *ISGF3* the transcriptional activator induced by interferon alpha, *ISRE* interferon-sensitive response element, *GASs* candidate interferon activated sites, *JAK1* Janus kinase 1, *TYK2* tyrosine kinase 2. P indicates phosphorylation

- **A dermal-hypodermal infiltrate of mononuclear cells with irregularities in their nuclei are the hallmark findings in skin biopsies**
- **JAK inhibitors seem to be helpful for CANDLE/PRAAS syndrome**

CANDLE (chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature) syndrome [6, 7] is an autosomal recessive genetic autoinflammatory disease showing characteristic skin lesions, lipodystrophy and multisystem inflammation. CANDLE is clinically identical to two other recessive genetic diseases, JMP (joint contractures, muscle atrophy, microcytic anemia and panniculitis-induced lipodystrophy syndrome) [8] and 'Nakajo-Nishimura syndrome' in Japan [9]. Several alternative terms have been proposed, such as PRAAS (proteasome-associated autoinflammatory syndrome) [10] and ALDD (autoinflammation, lipodystrophy and dermatitis) [11] to describe this autoinflammatory syndrome. There is no international consensus on nomenclature, and it is sometimes referred to in the literature as CANDLE/PRAAS syndrome. Some authors prefer to name it CANDLE syndrome. Although it was initially thought that CANDLE syndrome was due to biallelic mutations in the gene *PSMB8* encoding the subunit β5i of the immunoproteasome [6, 12–14], it was recently demonstrated in patients with CANDLE syndrome that monogenic or digenic mutations in genes encoding other proteasome or immunoproteasome subunits (for example the combination of loss of function mutations

Table 24.1 The genetic interferonopathies

Disease	Gene (s)	Protein function/pathway
CANDLE (Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature) syndrome; also referred to as Proteasome associated autoinflammatory syndromes (PRAAS)	*PSMA3, PSMB4, PSMB8, PSMB9, POMP*	Proteasome pathway: responsible for regulating proteolysis in eukaryotic cells
STING associated vasculitis with onset in infancy (SAVI)	*TMEM173*	Adapter molecule involved in IFN production
Aicardi-Goutières syndromes (types 1–7)	*TREX1, RNASEH2A/B/C, SAMHD1, ADAR, IFIH1*	Regulation of cytoplasmic DNA/RNA
Retinal vasculopathy with cerebral leukodystrophy (RVCL)	*TREX1*	Regulation of cytoplasmic DNA/RNA
Spondyloenchondrodysplasia (SPENCD)	*ACP5*	Lysosomal acid phosphatase activity/osteoclastic dysfunction
Singleton-Merten Syndrome	*IFIH1/DDX58*	Cytosolic sensor of ds-RNA
ISG15 deficiency	*ISG15*	Negative regulator of type I IFN by stabilisation of USP18
USP18 deficiency (pseudo-TORCH syndrome)	*USP18*	Negative feedback regulator of type I IFN signalling
Trichohepatoenteric syndrome 2	*SKIV2L*	RNA helicase

PSMB proteasome subunit B, *PSMBA* proteasome subunit A, *POMP* proteasome maturation protein, *TMEM173* Transmembrane Protein 173, *RNASHE* Ribonuclease H2 Subunit A, *SAMHD1* SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1, *ADAR* adenosine deaminase RNA specific, *TREX1* three prime repair exonuclease 1, *ACP5* acid phosphatase 5, tartrate resistant, *IFIH1* interferon induced with helicase C domain 1, *DDX58* DExD/H-Box Helicase 58, *ISG15* interferon stimulated protein 15, *USP18* ubiquitin specific protease 18, *SKIV2L* superkiller viralicidic activity 2-like

Table 24.2 Summary of mutations found in patients with CANDLE syndrome

PSMB8	PSMB9	PSMB4	PSMA3	POMP
T75M	G165D	5′UTR: c.-9G > A	H111Ffs*10	E115Dfs*20
A92T		P16Sfs*45	R233del	
A94P		D212_V214del		
K105Q		Y222X		
M117V				
C135X				
G197V				
G201V				
Y222X				

All these mutations result in loss of function of protein. *UTR* untranslated region, *fs* frameshift, *del* deletion

in *PSMB4/PSMB9*) or the proteasome maturation protein (*POMP*) were responsible for the syndrome [15]. It seems that the most accepted nomenclature for all these disorders is CANDLE syndrome, because of the wider spectrum of gene mutations and to emphasize the general appearance of consumption of the patients, resembling a burnt-out candle.

CANDLE/PRAAS is not a primary interferonopathy (i.e., a primary defect in IFN synthesis or release), but the result of proteasome dysfunction, that eventually leads to an enhanced IFN signalling and release.

24.2.1 Epidemiology

CANDLE/PRAAS syndrome is very rare, with fewer than 40 cases described to date. There is a clustering of cases in Japan, possibly as the result

of a founder effect of *PSMB8* mutations, and cases have been reported in different parts of the world. A Spanish, Portuguese, Brazilian or Mexican origin is known for at least ten cases reported [7, 8]. Other isolated cases from USA [15] Turkey, China, Israel, Ireland and UK have also been reported [10].

24.2.2 Etiology

CANDLE/PRAAS syndrome is due to mutations in protein subunits of the proteasome or immunoproteasome, or in the proteasome maturation protein (*POMP)* gene [6, 12–14]. CANDLE/PRAAS syndrome is the first primary disease of proteasome—immunoproteasome dysfunction to be identified.

The most common mutations in CANDLE/PRAAS syndrome occur in the gene *PSMB8* (proteasome subunit, beta-type, 8), encoding for the β5i subunit of the immunoproteasome, which has chymotrypsin-like activity. Less common mutations impair immunoproteasome assembly or maturation or lead to complete absence of the subunit.

The gene *PSMB4* (proteasome subunit, beta-type, 4) encodes for the β7 subunit of the proteasome, which is important for proteasome assembly and stabilization. Different mutations in *PSMB4* lead to deficient proteasome assembly or failure of the β7 subunit to incorporate into the proteasome.

Rare mutations in the gene *PSMA3* (proteasome subunit, alpha-type, 3), encoding for the α7 subunit of the proteasome have been reported. They can impair incorporation of the subunit to the proteasomes or lead to an unstable transcript.

A single mutation has been reported in the gene *PSMB9* (proteasome subunit, beta-type, 3), encoding for the β1i subunit of the immunoproteasome. This subunit has caspase-like proteolytic activity, and the mutation causes an impaired proteolytic activity.

Finally, the gene *POMP* (proteasome maturation protein) encodes for the proteasome maturation protein, a key protein for the maturation and assembly of the proteasome subunits. A single patient with CANDLE has been described with a heterozygous *POMP* mutation leading to a truncated protein and POMP insufficiency, thus causing reduced proteasome formation and overall proteasome activity [15].

Most patients are homozygous or compound heterozygous for *PSMB8* mutations. However, combinations such as compound heterozygous for *PSMA4*, or heterozygous for *PSMA3/PSMB8, PSMB9/PSMB4* or *PSMB8/PSMB4* have been reported [15]. As a result of digenic mutations, different combinations of defects cause variable degrees of proteasome dysfunction.

24.2.3 Pathogenesis

The proteasome system has different actions in eukaryotic cells (see Chap. 7). The proteasome and the immunoproteasome are involved in proteolysis of waste proteins. Thus, proteasome dysfunction leads, primarily, to impaired clearance of intracellular waste proteins that accumulate within the cell, leading to cellular stress.

Viral infections or other causes of cellular stress induce transcription of type I IFN genes and release of IFNs [16, 17]. Recognition of IFNs by IFN receptors leads to activation of the JAK/STAT signalling pathway (Fig. 24.1). Dimerized STAT proteins enter the nucleus and cause transcription of type I IFN genes. The processing of excessive waste proteins within the cell requires intact proteolytic degradation machinery, i.e., the proteasome/immunoproteasome system, for their proper degradation [18]. In CANDLE syndrome, deficient protein degradation and removal leads to accumulation of waste proteins that become polyubiquitinated and cause further cellular stress and further type I IFN release [6, 12–14].

This constitutive situation leads to a sustained state of pro-inflammation, but when triggers such as cold, viral infections or physical stress occur, the proteasome/immunoproteasome system deficiency becomes more critical, resulting in episodic inflammation. Type I IFNs increase the release of pro-inflammatory substances and recruit inflammatory cells [19]. Sustained type I

IFN release causes rapid mobilization of myeloid cells from the bone marrow. Under situations of increased type IFN release, 'atypical', immature myeloid cells reach the organs, which explains the 'atypical' skin infiltrate [20]. Since the proteasome is an important link between innate and adaptive immunity, it is not surprising that some patients with CANDLE/PRAAS develop autoantibodies [15], but these are often transient and likely represent an epi-phenomenon rather than being major drivers of disease pathogenesis.

24.2.4 Clinical Manifestations

The skin manifestations of CANDLE [7] are the first to appear, usually in the first months of life, but may be present as early as the neonatal period (Fig. 24.2). Newborns and infants often present with acral, perniotic lesions, consisting of red or purplish, edematous plaques on the nose, ears, fingers or toes; they may be triggered by cold. As the child grows, such perniotic lesions become less frequent, and infants develop annular, erythematous or purpuric edematous lesions with annular shape and raised borders. These resolve spontaneously after a few weeks leaving a purpuric macule, but new lesions will reappear throughout life, though less intensely in adulthood. A persistent perioral and periocular violaceous edema develops in infancy or childhood; it may be less conspicuous in adults although the reasons for this are uncertain.

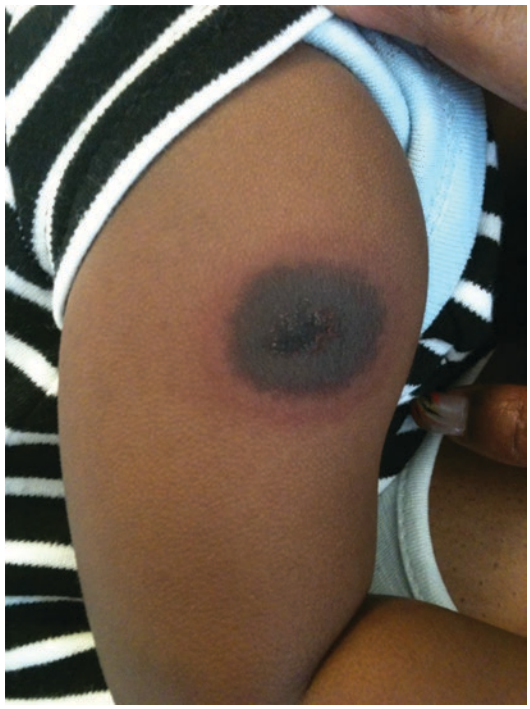

Fig. 24.2 Typical cutaneous eruption in the chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome (PSMB4/PSMB9 digenic form)

Skin lesions are usually accompanied by fever that also appear very early in life. They may appear daily or almost daily, and can be triggered by cold. However, patients can look relatively well, even with fevers.

Lipodystrophy (Fig. 24.3) is a key manifestation of CANDLE that is usually recognizable as early as in infancy, but it is progressive and devastating [6, 7, 12–14]. It usually starts on the face and then progresses to the trunk, upper limbs and to a much lesser extent to the lower limbs. Loss of fat may be related to chronic panniculitis, although this is usually not overt on histopathology; alternatively, the prominent interferon signature in CANDLE may play a role in the pathogenesis of lipodystrophy.

Mild to moderate growth delay is evident from early childhood, alongside muscle wasting, related to myositis [6, 7, 12–14]. Developmental delay is not a usual feature of the syndrome. Most patients develop hepatomegaly, which could be due to extensive metabolic disturbance in fat processing. The spleen may be massively enlarged, and generalized lymphadenopathy is common.

Besides the constitutive signs and symptoms of CANDLE, other manifestations can be a consequence of acute attacks of inflammation that can involve virtually every organ. They may be apparently spontaneous or provoked by common triggers such exposure to cold ambient temperature, intercurrent viral infection or physical or mental stress. Aseptic meningitis and meningoencephalitis can eventually cause basal ganglia calcifications [7]. Acute sterile epididymitis, conjunctivitis and nodular episcleritis, parotitis, pneumonitis, nephritis, carditis, arthritis, joint contractures and otitis media have been reported [6–8].

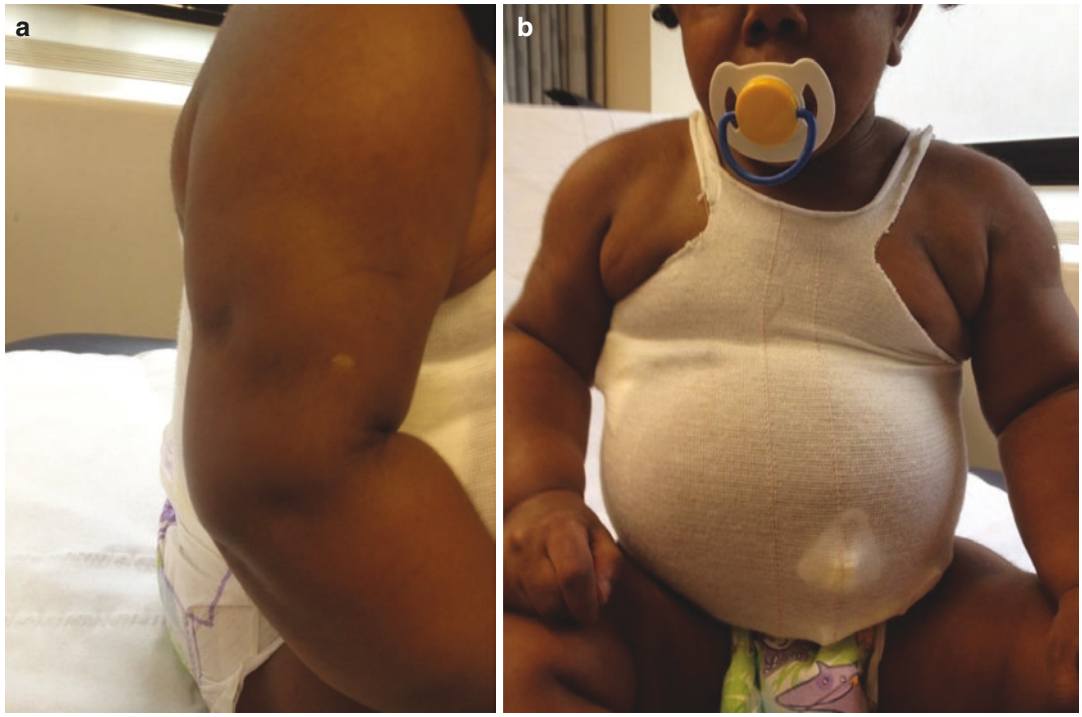

Fig. 24.3 (**a**) Lipodystrophy (right arm); (**b**) same child, demonstrating abdominal adiposity

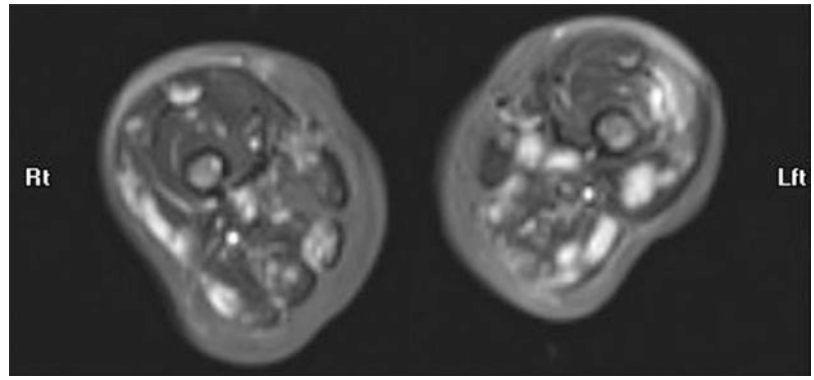

Fig. 24.4 Magnetic resonance imaging of lower limbs demonstrating myositis in a patient with chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome

24.2.5 Laboratory Testing

Laboratory findings relate mainly to chronic inflammation and are thus usually diagnostically non-specific, but of relevance when monitoring disease activity and therapeutic response [6, 7, 12–14]. Elevation of acute phase reactants (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], thrombocytosis) and chronic anemia, sometimes severe, are the most common findings. Hepatic transaminase levels can be moderately elevated, as well as serum level of muscle enzymes (creatine kinase [CK], aldolase) due to myositis (Fig. 24.4) [7]. Antinuclear antibodies (ANA) are found in some patients, but specific autoantibodies are typically absent.

Skin biopsy findings in patients with CANDLE syndrome are very characteristic, and may allow for an early diagnosis [7, 20]. Dermal, perivascular and interstitial infiltrates of mononuclear cells, many of

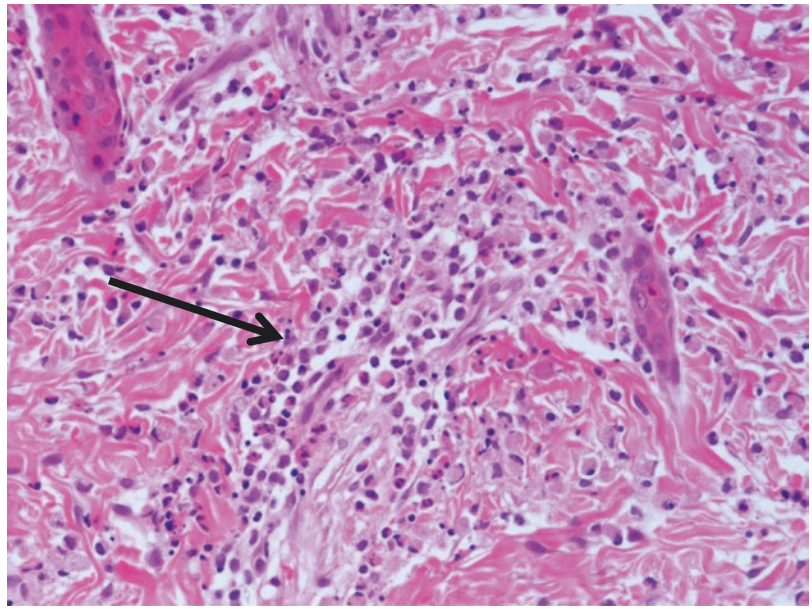

Fig. 24.5 Skin biopsy from a patient with chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome: a mixed dermal inflammatory infiltrate, with the subcutis and fat involved to a lesser extent; leukocytoclasia with prominent vessel wall endothelial cells but no fibrinoid necrosis

which have large, irregularly shaped nuclei, are seen; often, the infiltrate extends partially into the subcutaneous tissue (Fig. 24.5). The atypical appearance of the infiltrate is striking, and may be misdiagnosed as leukemia cutis. Often there is karyorrhexis and presence of mature neutrophils, sometimes with leukocytoclastic vasculitis (Fig. 24.5). The skin infiltrate is mixed, containing myeloid cells (positive for myeloperoxidase), macrophages (positive for CD163 and CD68/PMG1), and CD123-positive plasmacytoid dendritic cells [7]; lymphocytes are usually not in abundance.

24.2.6 Imaging

Radiographs, CT scans and MRI are usually not necessary for the diagnosis of CANDLE syndrome. There is no specific manifestation of CANDLE on imaging, and most of the imaging manifestations are reflections of the organ involvement by acute and chronic inflammation. Brain CT scan and MRI may show basal ganglia calcification, a common feature with Aicardi-Goutiéres syndrome. Hand joint radiographs do not show typical manifestations of arthritis, but bone resorption can be seen in long-standing disease. Ultrasound may reveal hepatosplenomegaly. Occasionally, muscle MRI may reveal acute inflammation (Fig. 24.4).

24.2.7 Diagnosis

The diagnosis of CANDLE syndrome is suspected by the combination of fever, skin clinical features and the histopathology findings of the skin biopsy. It should be confirmed by gene testing for *PSMB8* and if negative, for other genes encoding proteasome subunits (Table 24.1).

The differential diagnosis includes Sweet syndrome and other autoinflammatory diseases featuring atypical and/or neutrophilic infiltrates in the skin biopsies, including autoinflammation and phospholipase Cγ2-associated antibody deficiency and immune dysregulation (APLAID); (see Chap. 28) and gain-of-function mutations in *SAMD9L*. Immunodeficiency is not a feature of CANDLE syndrome, although some patients carry a significant burden of immunosuppression from treatment of the disease (see below). Lipodystrophy can be a prominent feature in other genetic syndromes, including generalized congenital lipodystrophy, partial familial lipodystrophy, leprechaunism, or acquired partial lipodystrophy of Barraquer-

Simons. Recurrent lipoatrophic panniculitis of children [21] features signs of lipodystrophy and autoinflammation. Finally, lipodystrophy is a prominent feature of otulipenia [22] (see Chap. 29).

24.2.8 Treatment

No treatment is consistently effective in CANDLE syndrome. The following have been tried with minimal or moderate success: corticosteroids, methotrexate, non-steroidal anti-inflammatory drugs (NSAIDs), dapsone, colchicine, cyclosporine, azathioprine, intravenous immunoglobulins, etanercept, and tofacitinib [7]. In acute attacks of inflammation, systemic corticosteroids can be used, along with organ-specific therapy. The selective JAK1/2 kinase inhibitor baricitinib has been used in eight patients (https://clinicaltrials.gov/ct2/show/NCT01724580), achieving clinical and laboratory improvement, and allowing corticosteroid sparing [23]; however, these results need to be validated in long term studies.

24.2.9 Prognosis

The clinical manifestations are related to the organs involved. Some of these complications and the acute attacks of inflammation have been reported to be fatal [7]. The outcome is variable; while some patients have had a lethal course, in other patients, a long survival is possible, with variable degrees of disability [6, 7, 12–14].

24.3 Stimulator of Interferon Genes (STING)–Associated Vasculitis with Onset in Infancy (SAVI)

- **STING-associated vasculopathy with onset in infancy (SAVI) is characterized by neonatal-onset systemic inflammation with a severe cutaneous vasculopathy, extensive tissue loss, and interstitial lung disease**
- **SAVI is caused by gain-of-function mutations in *TMEM173* which encodes the stimulator of interferon genes (STING)**
- **Immune cells and fibroblasts derived from SAVI patients show constitutive, i.e., ligand-independent activation of the STING-IFNβ pathway**
- **There may be a therapeutic benefit from JAK 1/2 inhibition for patients with SAVI, but data are limited**

STING-associated vasculopathy with onset in infancy (SAVI) is an autosomal dominant autoinflammatory disease characterized by neonatal-onset systemic inflammation with a severe cutaneous vasculopathy leading to extensive tissue loss and interstitial lung disease [24–26].

24.3.1 Etiology

SAVI is caused by gain-of-function mutations in *TMEM173* which encodes the stimulator of interferon genes (STING), an adaptor molecule linking sensing of foreign (viral and bacterial) DNA to the production of type I IFNs as part of the innate immune response [24–26]. These gain-of-function mutations lead to constitutive activation of STING and upregulated type I IFN production [24–26]. STING is widely expressed in alveolar macrophages, bronchial epithelial cells, and alveolar pneumocytes, which may explain the extensive lung pathology seen in SAVI [24–26]. In the initial description of the disease, Liu et al. performed whole exome sequencing of a single patient with early onset symptoms of systemic inflammation, cutaneous rash, and pulmonary manifestations and her unaffected parents and identify a *de novo* mutation, p.N154S, in the *TMEM173* gene [24]. Further candidate gene screening in other patients with similar features identified mutations in five additional sporadic cases [24]. In total, three *de novo* deleterious missense mutations, (p.V147L, p.N154S, p.V155M,) were described in six patients with this severe vasculopathy [24]. Subsequent studies suggest that the *TMEM173* p.V155M mutation has highly variable clinical expression, and that it can be associated also with

a phenotypically distinct lupus-like phenotype [27]. The p.V147L mutation, was suspected to be mosaic with a variable prevalence in different cell types, suggesting that SAVI joins the list of other autosomal dominant autoinflammatory diseases that can have disease caused by somatic mosaicism, with potentially important implications for methodologies used for genetic screening [24] (see Chaps. 2 and 12). Pathogenic variants are clustered in exon 5 of *TMEM173*, and they reside close to the STING dimerization site. Of note, the patients reported in the study by Liu et al., are of diverse ancestry, which is consistent with *de novo* origin of their causal variants [24]. The study by Jeremiah et al. also importantly highlights the possibility of reduced penetrance in families with dominantly inherited traits [27]. It is likely that germline and somatic mutations in *TMEM173* may also be of relevance in other types of late onset idiopathic cutaneous vasculopathies and lupus-like phenotypes. Other *TMEM173* mutations recently identified include p.A284G, p.C206T and p.A281G [28].

24.3.2 Pathogenesis

In vitro experiments suggest that gain of function *TMEM173* missense mutations in HEK293T cells result in increased IFNβ activity [24]. Immune cells and fibroblasts derived from SAVI patients show constitutive, i.e., ligand-independent activation of the STING-IFNβ pathway [24]. Patients also exhibit a strong IFN response-gene expression signature in peripheral blood, elevated circulating levels of IFN-induced cytokines, and have constitutive phosphorylation of STAT1 in mutant cells [24]. In addition, skin biopsy samples obtained from patients with SAVI show widespread small-vessel vasculopathic changes, occlusions, and lymphocytic inflammation [24–27]. STING is also expressed in endothelial cells and bronchial epithelium; thus, the pathological changes in the vascular wall and lung are considered also to be secondary to intrinsic defects in these cells, as well as up-regulation of type I interferons in immune cells [24, 26, 27]. Stimulation of human primary endothelial cells with the STING ligand cGAMP results in increased expression of many genes that mediate inflammation and apoptosis [24]. As a result, activated endothelial cells are more susceptible to apoptosis [24]. Thus, these *TMEM173* mutations are postulated to mediate chronic vascular inflammation, leading to the vasculitic rash and vaso-occlusive processes seen in SAVI [24]. Lastly, higher rates of spontaneous cell death were also observed in patients' monocytes and T cells, perhaps contributing further to the inflammatory phenotype [24, 26, 27].

24.3.3 Clinical Manifestations

Patients with SAVI have neonatal-onset systemic inflammation with elevations in ESR and CRP, a severe cutaneous vasculopathy leading to extensive tissue loss, and major interstitial lung disease [24–27]. Liu et al. reported six unrelated children with SAVI. Four patients presented within the first 8 weeks of life with skin lesions on the extremities (Fig. 24.6), including telangiectatic, pustular, or blistering rashes on the cheeks, nose, fingers, toes, and soles; two patients presented with tachypnea in the perinatal period [24]. All eventually developed severe skin lesions that extended to the pinna of the ears and sites on the limbs [24]. The acral skin lesions, which worsened in the winter, developed into painful, ulcerative lesions with eschar formation and tissue infarction, necessitating amputation of digits and causing scarring of the ear cartilage and perforation of the nasal septum [24]. Other features included livedo reticularis, Raynaud phenomenon, nail bed capillary tortuosity, failure to thrive and recurrent low-grade fevers [24–27]. Patients may also develop myositis and arthritis [24–27]. Other symptoms reported in subsequent reports of SAVI include necrotising fasciitis, significant pulmonary arterial hypertension, and polyarthritis with antinuclear antibodies and rheumatoid factor [29, 30]. Interestingly, and in contrast to other interferonopathies, brain involvement has not been reported to date in patients with SAVI, and cognition is normal among survivors [31].

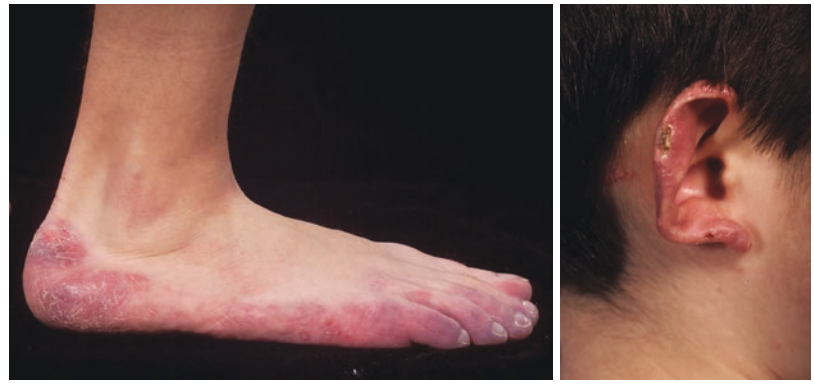

Fig. 24.6 Cutaneous lesions in stimulator of interferon genes (STING) associated vasculitis with onset in infancy (SAVI)

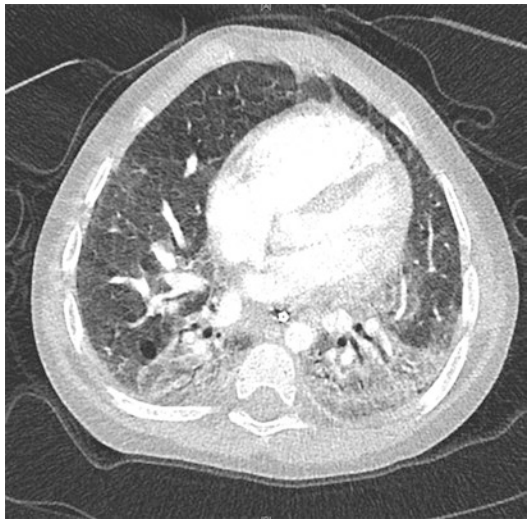

Fig. 24.7 CT of thorax showing bilateral lower lobe fibrosis in an 18 month old infant with stimulator of interferon genes (STING) associated vasculitis with onset in infancy (SAVI)

24.3.4 Imaging/Laboratory Findings

Patients in the initial and subsequent reports had radiographic evidence of interstitial lung disease and adenopathy with varying degrees of pulmonary fibrosis [24–27] (Fig. 24.7). Biopsies of skin lesions show features of a dense neutrophilic inflammatory infiltrate with blood-vessel damage, and lung biopsies reveal a lymphocytic inflammatory infiltrate resulting in interstitial fibrosis and emphysematous changes [24–27]. ESR and CRP are commonly elevated; serum levels of muscle enzymes may be elevated in patients who develop myositis.

24.3.5 Diagnosis

The diagnosis of SAVI should be considered in patients typically presenting with severe interstitial lung disease and cutaneous vasculopathy, with disease onset usually beginning in infancy. Molecular confirmation is required to establish a diagnosis. The differential diagnosis includes other monogenic interferonopathies (Table 24.1), SLE (including monogenic lupus-like diseases such as C1q deficiency) and coatomer protein complex subunit alpha (COPA) syndrome, a novel autosomal dominant immune dysregulatory disease with severe interstitial lung disease [31, 32].

24.3.6 Treatment

Treatment options in SAVI remain limited [24–27, 31]. At best, there is only a partial response to corticosteroids and other disease-modifying antirheumatic drugs, including biological therapies [24–27, 31]. Liu et al. showed that incubation of lymphocytes from patients with SAVI with a JAK inhibitor resulted in reduced levels of STAT1 phosphorylation and a reduction of IFNβ production in fibroblasts activated by cGAMP [24]. These results suggest a possible avenue for treatment in patients with SAVI. In line with these observations, recent reports have suggested that there may be a therapeutic benefit from JAK 1/2 inhibition (ruxolitinib or baricitinib) to block type I IFN signalling, despite constitutively activated STING [33, 34]. Treatment of patients with

SAVI with JAK inhibitors has resulted in normalization of inflammatory markers, resolution of cutaneous symptoms and gradual improvement of the lung disease in some, although data are scarce and so far largely anecdotal [33, 34].

24.3.7 Prognosis

Despite these encouraging advances, the outcome of the disease still remains overall poor with death occurring due to respiratory or cardiac failure or infectious complications [24–27, 31]. In that context, opportunistic viral infection (such as BK virus) is likely to be an increasing concern with more widespread use of JAK inhibitors for the treatment of interferonopathies.

24.4 Aicardi–Goutières Syndrome (AGS)

- **Aicardi-Goutières syndrome (AGS) was originally defined as an early onset progressive brain disease mimicking the sequelae of in utero viral infection**
- **The phenotype of AGS has expanded to include chilblains, other lupus like symptoms, and glaucoma**
- **Mutations in the genes encoding any of seven different proteins cause AGS**

Aicardi-Goutières syndrome (AGS) was originally defined as an early onset progressive brain disease mimicking the sequelae of in utero viral infection [2, 5, 35, 36]. Over time, as more genetic variants were identified associated with AGS, other features of the disease were also recognized including: chilblains, raised intraocular pressure (glaucoma) and, in some cases, an overlap with the clinical manifestations of SLE, thus expanding the disease phenotype [2, 5, 35, 36].

24.4.1 Etiology

Mutations in the genes encoding any of following proteins cause AGS: DNA 3′ repair exonuclease 1 (*TREX1*), the three subunits of the ribonuclease H2 (RNase H2) endonuclease complex (*RNASEH2A, RNASEH2B* and *RNASEH2C*), the deoxynucleoside triphosphate triphosphohydrolase and ribonuclease SAM domain and HD domain 1 (*SAMHD1*), adenosine deaminase acting on RNA (*ADAR1*; also known as *DRADA*) or the double-stranded RNA (dsRNA) cytosolic sensor IFN-induced helicase C domain-containing protein 1 (*IFIH1*; which encodes for the melanoma-differentiation-associated protein 5 (MDA5) [2, 5, 35–45]. Table 24.3 summarises the spectrum of disease associated with mutations in AGS-related genes. The actual frequency of AGS is unknown. Pathogenic variants have been found in affected individuals of all ethnic origins, and have been

Table 24.3 Range of diseases caused by mutations in Aicardi Goutières Syndrome (AGS)-related genes

Gene	Inheritance	Phenotype
TREX1 (AGS type 1)	Autosomal recessive or autosomal dominant	AGS Familial chilblain lupus, systemic lupus erythematosus, retinal vasculopathy with cerebral leukodystrophy
RNASEH2B (AGS type 2)	Autosomal recessive	AGS and spastic paraparesis
RNASEH2C (AGS type 3)	Autosomal recessive	AGS
RNASEH2A (AGS type 4)	Autosomal recessive	AGS
SAMHD1 (AGS type 5)	Autosomal recessive	AGS Familial chilblain lupus chronic lymphocytic leukemia
ADAR1 (AGS type 6)	Autosomal recessive or autosomal dominant	AGS dyschromatosis symmetrica hereditaria, bilateral striatal necrosis and spastic paraparesis, complete non penetrance
IFIH1 (AGS type 7)	Autosomal dominant	Various neuroimmunologic and non-neurologic phenotypes, including AGS, spastic paraparesis, complete non penetrance and Singleton Merten syndrome

highlighted in Ashkenazi Jewish [46] and Amish patients in some reports [47], but detailed epidemiological data in different populations are not available.

24.4.2 Pathogenesis

The identification of the genetic basis of AGS has highlighted the fundamental role of nucleic acid signalling in the induction of type I IFNs [2, 5, 35–45]. For patients with *TREX1* or *RNASEH2* gene mutations, the hypothesis is that dysfunction of the proteins encoded by these genes might result in the accumulation of endogenous nucleic acid products, which are then sensed as by the innate immune machinery [2, 5, 36]. In *TREX1*-deficient mice, activation of a TLR-independent cytosolic pathway by DNA has been to shown to lead to a type I IFN response as a consequence of signalling through cGAS, STING, IFN regulatory factor 3 (IRF3) and serine/threonine-protein kinase TBK1 [48, 49]. By contrast, knock-out of the *RNASEH2B* gene in mice is embryonically lethal, but is not associated with type I IFN induction [50, 51]. Instead, this mouse model has demonstrated an essential role of the RNaseH2 complex in removing the ribonucleotides that are incorporated into DNA during DNA replication [50, 51]. More recent reports have established an additional knock-in mouse model with an *RNASEH2* AGS mutation in a highly conserved residue of the catalytic subunit, $Rnaseh2a^{G37S/G37S}$ (G37S) [52]. Importantly p.G37S homozygotes are perinatal lethal, in contrast to the early embryonic lethality previously reported for *RNASEH2b* or *RNASEH2c* -null mice described above [52]. Pokatayev et al. showed that the p.G37S mutation led to increased expression of IFN stimulated genes dependent on the cGAS–STING signaling pathway [52]. Overall, these studies suggest that by-products of defective DNA replication trigger an IFN-mediated immune response. It is possible, however, that the RNaseH2 complex may have additional completely distinct activity that remains to be established.

The subsequent identification of AGS -associated mutations in *SAMHD1, ADAR* and *IFIH1* added weight to the hypothesis that disturbance of endogenous nucleic acid pathways triggers an innate immune response normally induced by exogenous nucleic acids [43–45, 53–55]. *SAMHD1* has been shown to have a role in regulating the dNTP pool, and this is necessary for DNA synthesis in the context of HIV-1 infection [55]. Notably, *SAMHD1*-deficient mice and *ADAR* deficient mice also have an upregulated IFN signature [56, 57]. Finally, mice with an N-ethyl-N-nitrosourea (ENU)-induced missense mutation in *IFIH1* have upregulation of IFN-induced signalling and develop an autoimmune phenotype [58].

24.4.3 Clinical Features

An estimated 20% of patients with AGS develop severe neurological dysfunction diagnosed soon after birth, manifesting as spasticity, dystonia, seizures, cortical blindness and progressive microcephaly [2, 5, 35–45]. In general, this early-onset neonatal form of AGS is most frequently seen in association with biallelic pathogenic variants in *RNASEH2A, RNASEH2C,* or *TREX1* [5, 35–37, 59]. Patients may develop fevers with no clear causal infection and severe irritability in the first few months of the disease process [5, 35–37, 59]. Most children deteriorate neurologically and end up with no purposeful gross motor, hand or communication function [5, 35–37, 59]. Some patients with *TREX1*-related AGS may also develop other symptoms mimicking congenital infection such as thrombocytopenia, hepatosplenomegaly and liver enzyme abnormalities [37]. Chilblain-like lesions are observed in 1/3 of *TREX1* patients (Fig. 24.8) while some may have more severe skin disease [37, 60]. Some cases have been described with prominent lupus-like features and a range of autoantibodies detected [37]. A full blown picture of SLE is very unusual, but has been reported [37].

Late-onset presentation of AGS, sometimes occurring after some months of apparently normal child development, is also described [2, 5,

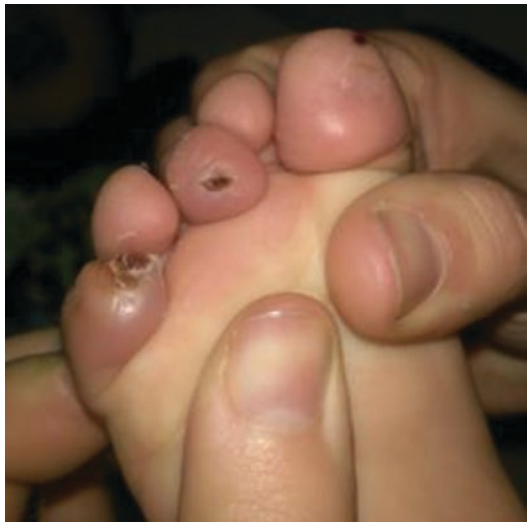

Fig. 24.8 Typical chilblain lesions of Aicardi Goutières syndrome (heterozygous *TREX1* mutation)

35–45]. The first symptoms can be very non-specific such as extreme irritability, disturbed sleep, feeding difficulties, and low grade pyrexia which may be the first signs of the onset of subacute encephalopathy [2, 5, 35–45]. This may be followed by psychomotor delay and/or loss of acquired skills and poor head growth. This encephalopathic phase usually lasts a few months, beyond which time the clinical picture typically stabilizes [2, 5, 35–45]. A later-onset presentation, occasionally associated with remarkably preserved neurologic function, is most frequently seen in association with biallelic pathogenic variants in *RNASEH2B, SAMHD1, or ADAR1;* but may also be seen in individuals who have an autosomal dominant heterozygous pathogenic variants in *ADAR1 or IFIH1* [2, 5, 35–45]. Of note, some individuals with biallelic pathogenic variants in *RNASEH2B* have relatively preserved intellectual function, with a few having completely normal intellectual development and head circumference [37].

Familial chilblain lupus, often associated with dominant Asp18Asn mutation in *TREX1,* may be the only manifestation in some patients [60]. Lesions are characterized by cold-induced, bluish-red lesions on the hands, feet and ears that may ulcerate, occasionally leading to significant tissue loss [60, 61]. The skin lesions are similar to those seen in patients with AGS with neurological involvement, and in some patients with non-genetic forms of SLE.

Mutations in *ADAR1* have been associated with acute bilateral striatal necrosis [43, 44]. Such patients present with encephalopathy with changes on brain imaging (Fig. 24.9) which include symmetric signal changes in the caudate and putamen, often associated with swelling and later shrinkage in the context of an acute or subacute onset of refractory four-limb dystonia [43, 44]. These symptoms may develop in early childhood on the background of a completely normal development, and might be initially confused with a viral etiology [43, 44].

Intracerebral vasculopathy, including intracranial stenosis and aneurysms, is observed more frequently in individuals who have biallelic pathogenic variants in *SAMHD1* often with late onset [53, 54]. These patients may also develop chilblains and glaucoma while arthropathy and lipodystrophy have also been reported [53, 54]**.**

24.4.4 Laboratory/Imaging Findings

Typical neuroimaging features of AGS include intracranial calcification, leukodystrophy, and cerebral atrophy [2, 5, 35–45] (Figs. 24.9 and 24.10). The distribution and extent of the calcification can be variable, with periventricular distribution in some cases [2, 5, 35–45]. It is important to emphasise that intracranial calcification is not always recognized on MRI. Thus AGS should be considered in the differential diagnosis of any unexplained leukoencephalopathy, and CT scanning is often required to establish the presence of calcification, particularly early in the disease process. Some patients have frontotemporal white-matter involvement with cyst formation [2, 5, 35–45]. Cerebrospinal fluid (CSF) analysis shows chronic leukocytosis, predominantly of lymphocytes (>5–100 cells/mm^3); elevated neopterin levels (often highest in the early stages of the disease) that may normalize over time; and elevated IFNα activity, which again, declines with increasing age [2, 5, 35–45].

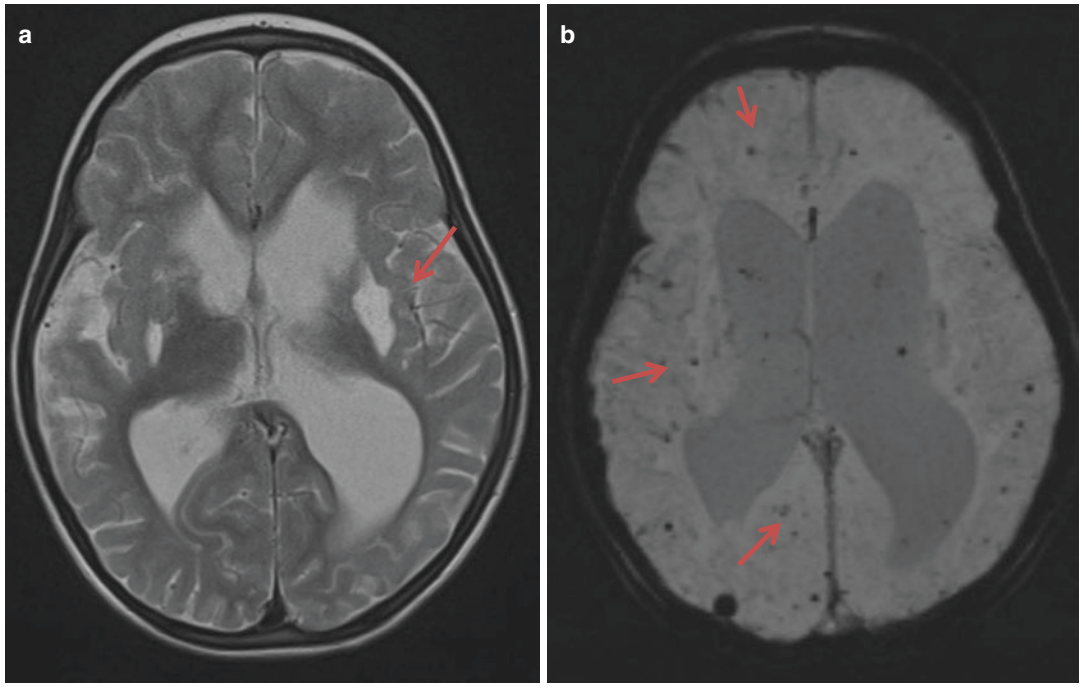

Fig. 24.9 Magnetic resonance imaging brain from a child with Aicardi Goutières syndrome caused by homozygous mutations in *ADAR1*. (**a**) Shows bilateral basal ganglia changes; (**b**) shows widespread small areas of calcification, indicated as multiple areas of low signal intensity

In addition to elevated IFNα that has been long known to be detected in the CSF of patients with AGS, an increased level of expression of IFN-stimulated genes (ISGs) in peripheral blood — an 'IFN signature' — has been reported to be present at any age in almost 100% of patients with mutations in *TREX1, RNASEH2A, RNASEH2C, SAMHD1, ADAR1 or IFIH*1 [62]. Approximately 30% of patients with *RNASEH2B* mutations had no such upregulation of ISGs, however [62]. More recently Rodero et al. reported their experience of using high sensitivity single-molecule array (Simoa) digital ELISA technology coupled with a very high affinity antibody against IFNα derived from patients with genetically confirmed autoimmune polyendocrinopathy (APECED), that enabled detection of differences in IFNα at the attomolar level in healthy donors, viral infection, and complex and monogenic interferonopathies [16]. These IFNα levels correlated well with interferon gene expression scores. Thus, the ability to detect very low concentrations of IFNα concentrations by digital ELISA and high affinity antibodies will undoubtedly enhance our understanding of IFN biology in health and disease, and could lead to improvement in diagnostic strategies, and also to track therapeutic efficacy (for example with JAK inhibitors or other targeted treatments) in individual patients with AGS, and other interferonopathies.

24.4.5 Diagnosis

The diagnosis of AGS should be considered in the context of an early-onset encephalopathy with basal ganglia calcification (Fig. 24.10) and cerebral white matter abnormalities [2, 5, 35–45]. CSF analysis, peripheral blood and CSF IFNα levels and peripheral blood IFN transcriptomic signature (Fig. 24.11) may provide laboratory clues to the diagnosis of AGS that requires molecular confirmation [2, 5, 35–45]. Other inherited white matter diseases, monogenic interferonopathies (Table 24.1), and genetic

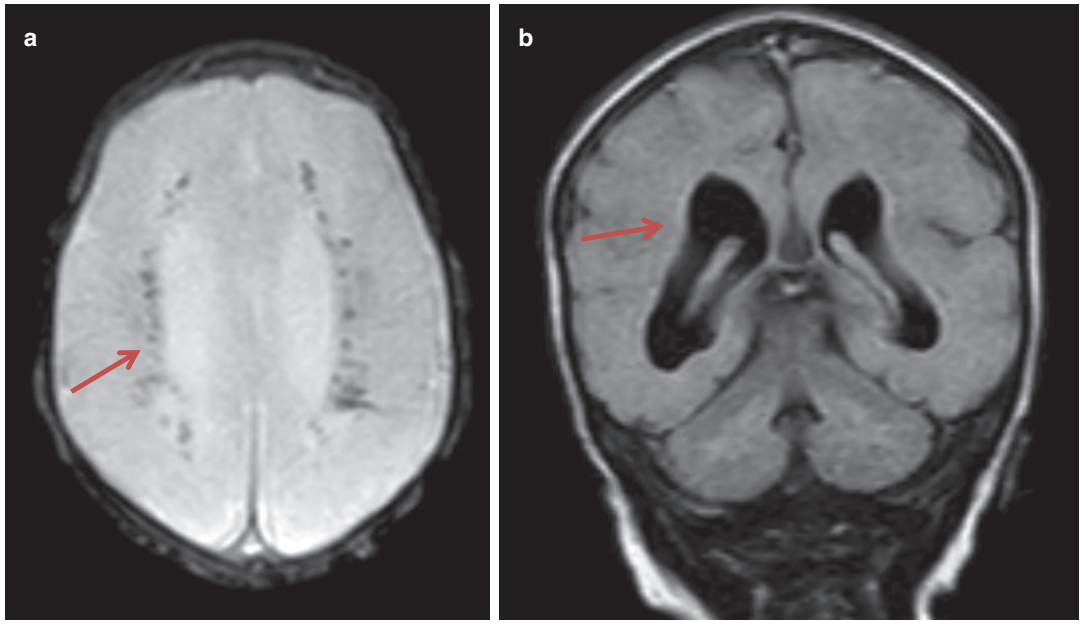

Fig. 24.10 Magnetic resonance imaging of brain from a patient with Aicardi Goutières syndrome (homozygous *TREX1* mutation). (**a**) Shows widespread intracerebral calcification (which appears as areas of low intensity on this T2 weighted image); (**b**) shows reduced white matter bulk and dilatation of the ventricles in the same patient

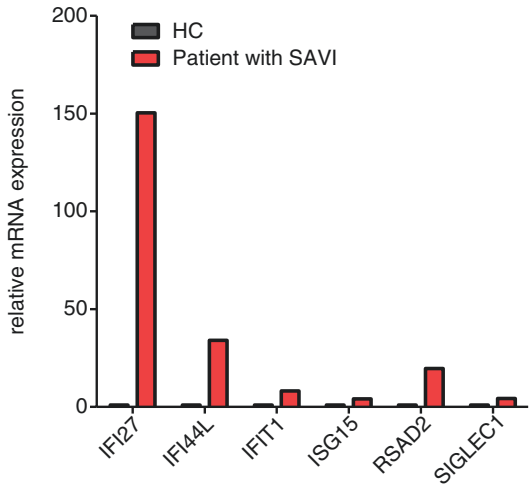

Fig. 24.11 Type I interferon-stimulated genes (ISGs) transcript levels in a patient with STING associated vasculitis with onset in infancy (SAVI). Quantitative reverse transcription polymerase chain reaction of a panel of six ISGs in whole blood measured in this patient compared to healthy controls (HC) is shown

conditions characterised by intracerebral calcification such as cerebroretinal microangiopathy with calcifications and cysts 1 due to homozygous mutations in *CRMCC1,* or mutations in *SNORD118,* and USP18 deficiency (discussed below in more detail) should also be considered in the differential diagnosis [2].

24.4.6 Treatment

Generally, the management of AGS is supportive, with no cure available. Anti-convulsive treatments may be required for those with seizures [2, 5, 35–45]. Some patients may derive relief from dystonia from botulinum toxin injections, or levodopa [2, 5, 35–45]. Corticosteroids have been shown to lower the CSF concentration of IFNα, and may be beneficial for the cutaneous manifestations of AGS (chilblains or pernio lesions), but do not reverse the neurological phenotype [2, 5, 35–45]. The occlusive and aneurysmal arteriopathies described in association with patients with *SAMHD1* mutations could be amenable to revascularization procedures, but this procedure is limited to centres with particular expertise. Novel strategies to block IFN-signalling through use of JAK inhibition are also emerging, and may be beneficial in AGS as well as the other interferonopathies discussed above

(https://clinicaltrials.gov/ct2/show/NCT01724580). In addition, given the possible role of endogenous retroviruses in the activation of nucleic acid receptors in AGS, a phase 2 trial with reverse transcriptase inhibitors has been developed (https://clinicaltrials.gov/ct2/show/NCT3304717). Some patients have been treated empirically with reverse transcriptase inhibitors although preliminary results have not yet been reported. A single case of cerebral vasculopathy caused by *SAMHD1* mutation treated successfully with tocilizumab has been reported, but it's too early to draw any firm conclusions from that limited experience [63].

Other than the role of corticosteroids and immunomodulators mentioned above, there is no specific treatment necessarily proven to be effective for pernio in AGS. Anecdotally, anti-inflammatory treatments such as nonsteroidal anti-inflammatory drugs may provide some symptomatic relief; vasodilators such as calcium channel blockers may have a role if vasospasm is suspected clinically; antiplatelet therapy with low dose aspirin is unproven but anecdotally has been tried in a few patients with the intention of reducing any possible thrombotic component to peripheral skin lesions (PB, personal unpublished observation); hydroxychloroquine has been tried in a single patient, with unconvincing results (PB, personal unpublished observation). Thus in the absence of any definitive pharmacological interventions, it is logical to advise patients to maintain peripheral warmth and avoid cold/wet exposure of the peripheries through behavioural means where possible, similar to that for patients with Raynaud phenomenon and related vasospastic conditions.

24.4.7 Prognosis

Mortality rates are overall high for patients with AGS [37, 64]. Rice et al. reported on a large cohort of patients with AGS, and demonstrated that mortality was correlated with genotype: 34% of individuals with *RNASEH2A, RNASEH2C, and TREX1* pathogenic variants were known to have died, the majority (81%) by age 10 years old, compared to only 8% with *RNASEH2B* pathogenic variants ($p = 0.001$) [64].

24.5 Other Interferonopathies

24.5.1 Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL)

Retinal vasculopathy with cerebral leukodystrophy (RVCL) is usually an adult-onset autosomal dominant disorder involving the microvessels of the brain, with CNS degeneration, progressive loss of vision, stroke, motor impairment, and cognitive decline [65, 66]. Recent genetic analyses have demonstrated that RVCL is caused by heterozygous frameshift mutations in *TREX1* in the C-terminus required for ER localisation [65, 66]. RVCL encompasses three conditions: cerebroretinal vasculopathy (CRV), hereditary vascular retinopathy (HRV); and hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS), which have previously been regarded as distinct clinical entities, but are now known to be caused by mutations in the same gene [65, 66]. Death occurs in most patients 5–10 years after onset. A subset of affected individuals have other symptoms such as Raynaud phenomenon, micronodular cirrhosis, or glomerular dysfunction [65, 66]. Major cutaneous symptoms that are seen in *TREX-1* mutation associated AGS are not usually present in this condition; however whether or not *RVCL* represents *a forme fruste* of *TREX-1* mutation associated AGS, or is a truly separate clinical entity remains unclear.

24.5.2 Spondyloenchondrodysplasia with Immune Dysregulation (SPENCDI)

Spondyloenchondrodysplasia with immune dysregulation (SPENCDI) is an immuno-osseous dysplasia combining the typical metaphyseal and vertebral bone lesions of spondyloenchondrodysplasia (SPENCD) with immune dysfunction and neurologic involvement. SPENCDI is a recessive genetic disease caused by homozygous or compound heterozygous mutation in the *ACP5* gene on chromosome 19p13 [67–69]. The skeletal dysplasia is characterized by radiolucent and irregular spondylar and metaphyseal lesions that

represent islands of chondroid tissue within bone [67–69]. The vertebral bodies show dorsally accentuated platyspondyly with disturbance of ossification [67–69]. Clinical abnormalities such as short stature, rhizomelic micromelia, increased lumbar lordosis, barrel chest, facial anomalies, and clumsy movements may be present [67–69]. Central nervous system involvement includes spasticity, mental retardation, and cerebral calcifications. Immune dysregulation ranges from autoimmunity to immunodeficiency [67–69]. Neurologic and autoimmune manifestations have been observed in different combinations [67–69]. Briggs et al. also noted variability in skeletal, neurologic, and immune phenotypes, which was sometimes marked even between members of the same family [67, 69].

24.5.3 Singleton–Merten (SGMRT) Syndrome

Singleton-Merten syndrome (SGMRT) is a rare autosomal dominant disorder caused by heterozygous mutation in the *IFIH1* gene [70]. The disease is characterized by abnormalities of blood vessels, teeth, and bone [70]. Calcifications of the aorta and aortic and mitral valves occur in childhood or puberty and can lead to early death [70]. Dental findings include delayed primary tooth exfoliation and permanent tooth eruption, truncated tooth root formation, early-onset periodontal disease, and severe root and alveolar bone resorption associated with dysregulated mineralization, leading to tooth loss [70]. Osseous features consist of osteoporosis, either generalized or limited to distal extremities, distal limb osteolysis, widened medullary cavities, and easy tearing of tendons from bone [70]. Less common features are mild facial dysmorphism (high anterior hair line, broad forehead, smooth philtrum, thin upper vermilion border), generalized muscle weakness, psoriasis, early-onset glaucoma, and recurrent infections [70]. An atypical form of SGMRT syndrome (SGMRT2) characterised by glaucoma, aortic calcification, and skeletal anomalies is caused by mutations in the *DDX58* gene [71].

24.5.4 USP18 Deficiency (Pseudo–TORCH Syndrome)

Loss-of-function recessive mutations of ubiquitin-specific peptidase 18 (*USP18)*, a key negative regulator of type I IFN signalling were recently identified as the cause of a type I interferonopathy leading to severe pseudo-TORCH (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex) syndrome (PTS), characterized by microcephaly, enlarged cerebral ventricles, cerebral calcification, and, occasionally, by systemic features at birth resembling the sequelae of congenital infection but in the absence of an infectious agent [72].

24.5.5 ISG15 Deficiency

A less severe phenotype than that associated with AGS has been described in patients presenting with idiopathic basal ganglia calcification, seizures and autoantibodies, and harbouring mutations in the *ISG15* gene [73]. Intracellular ISG15 is an IFN-α/β-inducible ubiquitin-like modifier which can covalently bind other proteins [73]. Absence of intracellular ISG15 prevents the accumulation of USP18, a potent negative regulator of IFNα/β signalling, resulting in the enhancement and amplification of IFNα/β responses [73]. Patients with *ISG15* deficiency are prone to mycobacterial disease and also display cellular, immunological and clinical signs of enhanced IFNα/β immunity, similar to other interferonopathies [73].

24.5.6 Trichohepatoenteric Syndrome 2

Trichohepatoenteric syndrome 2 (THES) is caused by mutation in the *SKIV2L* gene [74]. Typical characteristic features of THES include intrauterine growth retardation, woolly hair, facial dysmorphism, intractable diarrhea in infancy requiring total parenteral nutrition, and immunodeficiency [74]. Hepatic involvement contributes to the poor prognosis of affected patients [74].

24.6 Summary and Conclusions

The interferonopathies are a relatively new class of inherited disorders associated with an inborn elevated IFN response leading to overlapping disease phenotypes [1–5]. The study of patients with these rare genetic diseases has revealed a central role of abnormal nucleic acid recognition and type I IFN pathway activation in human diseases characterized by autoinflammation and autoimmunity [1–5]. These conditions often present as complex clinical cases and remain undiagnosed for years. Considering the complexity of the IFN response and given the advent of next generation genetic sequencing techniques, the identification of further monogenic diseases belonging to this disease grouping seems likely. Development of biomarkers such as the transcriptomic signature of IFN-stimulated genes and IFNα measurement by digital ELISA even at very low concentrations will undoubtedly improve the diagnosis and stratification of diseases associated with IFN dysregulation [16, 62]. Lastly, several non-Mendelian disorders, particularly SLE and dermatomyositis, are also characterized by an up-regulation of type I IFN signalling [75, 76]. Therefore, the insights derived from these monogenic diseases with respect to novel targets for therapy could have relevance for the management of these sporadic conditions.

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Genetic Causes of Inflammatory Bone Disease

25

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Abstract

This chapter focuses on monogenic autoinflammatory disorders that affect bone. The presence of sterile bone inflammation may be accompanied by inflammation of the skin and intestinal tract. The pathophysiology varies by syndrome and includes dysregulation of the IL-1 pathway or aberrant intracellular signaling defects leading to activation of innate immune cells including osteoclasts. These are rare disorders with variable outcomes. IL-1 inhibitors have been used successfully to decrease inflammation in Majeed syndrome, deficiency of the interleukin receptor antagonist and for non-osseous manifestations of neonatal onset multisystem inflammatory disease. For other disorders such as cherubism, treatment remains challenging. Recognition of additional monogenic autoinflammatory is likely as this is a very new field of investigation.

Keywords

Majeed syndrome · Deficiency of the interleukin-1 receptor antagonist (DIRA) · Interleukin-1 Neonatal onset multisystem inflammatory disease (NOMID) · Cherubism · Schnitzler syndrome · Primary hypertrophic osteoarthropathy · LPIN2 · NLRP3 · Pstpip2 · SH3BP2

Abbreviations

ADP	Adenosine diphosphate
ASC	Apoptosis-associated speck-like protein containing a carboxy-terminal CARD
c-ABL	Abelson murine leukemia viral oncogene homolog 1
CDA	Congenital dyserythropoietic anemia (CDA)
CNO	Chronic non-bacterial osteomyelitis
CRMO	Chronic recurrent multifocal osteomyelitis
CRP	C-reactive protein
Csk	C-terminal Src kinase
DIRA	Deficiency of the interleukin-1 receptor antagonist
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate
FBLIM1	Filamin binding LIM protein 1 gene
FBLP1	Filamin binding LIM protein 1
HPGD	15-hydroxyprostaglandin dehydrogenase

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IL	Interleukin
IL-1AcP	IL-1 receptor accessory protein
IL-1RI	IL-1 receptor I
IL1RN	Interleukin-1 receptor antagonist gene symbol
IRAK4	Interleukin 1 receptor associated kinase 4
LPIN2	Lipin2 gene symbol
MAPK	MAP kinase
M-CSF	Macrophage colony-stimulating factor
MRI	Magnetic resonance imaging
MyD88	Myeloid differentiation primary response 88
NFAT	Nuclear factor of activated T-cells
NLRP3	NLR family pyrin domain containing 3
NOMID	Neonatal onset multisystem inflammatory disease
NR4A2	Nuclear receptor subfamily 4 group A member 2
NSAID	Non-steroidal anti-inflammatory drugs
P2X7R	Purinergic receptor P2X 7
PAP	Phosphatidate phosphatase
PHO	Primary hypertrophic osteoarthro pathy
Pstpip2	Proline-serine-threonine phosphatase interacting protein 2
PTP PEST	Protein-tyrosine phosphatase with proline (P), glutamic acid (E), serine (S), and threonine (T) motif
RANKL	Receptor activator of nuclear factor kappa-B ligand
SH3BP2	SH3 binding protein 2
SHIP1	Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1
STAT	Signal transducer and activator of transcription
STIR	Short tau inversion recovery
Syk	Spleen tyrosine kinase
TNF	Tumor necrosis factor

25.1 Introduction

Autoinflammatory disorders result in innate immune system mediated inflammation of tissues including bone. In fact, sterile bone inflammation may be the only manifestation of autoinflammation. More often, bone inflammation is seen in conjunction with inflammation of the skin or intestinal tract. Chronic recurrent multifocal osteomyelitis (CRMO)/chronic non-bacterial osteomyelitis (CNO) is the most common autoinflammatory bone disorder. This disease predominantly affects children and may co-occur with a form of psoriasis or with inflammatory bowel disease. CNO appears to have a genetic component to disease as there are reports of multiple affected first degree relatives. However, for most CNO cases, the genetics remains uncertain (Chap. 31).

This chapter will focus on autoinflammatory disorders involving bone in which the genetic basis is known. These include Majeed syndrome (mutations in *LPIN2*), CNO associated with mutations in *FBLIM1*, deficiency of the interleukin-1 receptor antagonist, also known as DIRA (mutations in *IL1RN*), cherubism (mutations in *SH3BP2*) and murine models of CNO (mutations in *Pstpip2*). There are also monogenic autoinflammatory disorders that can affect the bone, but in which the osseous manifestations are not among the most common features of the phenotype including neonatal onset multisystem inflammatory disease (NOMID; mutations in *NLRP3*) (see Chap. 19), pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPA; mutations in *PSTPIP1,* see Chap. 22) and some cases of Schnitzler syndrome (in a few cases due to mosaicism in *NLRP3*) (see Chap. 37). Lastly, primary hypertrophic osteoarthropathy (mutations in *HPGD*) will be discussed in context of its bone involvement.

25.2 Majeed Syndrome

Key Points

- **The main features of Majeed syndrome include CNO, dyserythropoietic anemia, and neutrophilic dermatosis**
- **Majeed syndrome is caused by mutations in *LPIN2* and the bone inflammation is IL-1 mediated**

25.2.1 Clinical Features of Majeed Syndrome

Majeed syndrome was recognized as a distinct clinical entity by Dr. Hasan Majeed in 1989 [1]. Early reports documented a classic triad of clinical findings including severe early onset CNO, microcytic congenital dyserythropoietic anemia and the neutrophilic dermatosis Sweet syndrome [1–3]. The disease has been reported in families from the Middle East, India and Spain [1–8]; in most cases consanguinity was reported [4–6, 9]. CNO is the prominent clinical phenotype of this syndrome but is more severe and less likely to remit (Table 25.1). The CNO in Majeed syndrome typically presents prior to 2 years of age (range of 1 month to 8 years; mean of 22.9 months; median of 10.5 months) and is characterized by frequent exacerbations of bone pain often associated with recurrent fever [4, 5, 10, 11]. The disease presents with bone pain, usually worse at night and may be associated with swelling and/or warmth overlying the affected bones [1–5, 7]. This may mimic arthritis as the epiphyses of the long bones are most commonly affected; therefore, the swelling is typically localized to the periarticular regions of the joints of the long bones [1, 3–7]. Imaging of the bone reveals osteolytic lesions with surrounding sclerosis that are most commonly located abutting the growth plates of the long bones but may occur elsewhere. Technicium^{99} bone scan reveals multifocal tracer uptake predominantly in the metaphyseal regions, but also in the epiphyseal regions of the long bones. Other bones may be affected but to date the vertebrae have not been reported to be affected [1, 3–7]. Magnetic resonance imaging (MRI) is the preferred imaging modality and shows low signal intensity on T1 weighted images and increased signal intensity on T2, fat suppressed and short tau inversion recovery (STIR) sequences [4–6] (Fig. 25.1a). Bone biopsy, reported in six patients, documented sterile osteomyelitis in five patients and osteonecrosis in one patient [1, 2, 5]. These imaging and histopathologic abnormalities mimic what is seen in non-syndromic CNO [12–14].

Congenital dyserythropoietic anemia (CDA) has been reported in 11 of 11 bone marrow biopsies performed (Fig. 25.1c). The CDA of Majeed syndrome is atypical in that the majority of patients have microcytic anemia. Nearly 50% of patients required at least one red blood cell transfusion and one child had transfusion dependent CDA [1, 3]. A neutrophilic dermatosis was reported in 50% of the six affected individuals described by Dr. Majeed [1–3, 10]. However, the subsequent eight reported patients with Majeed syndrome did not have dermatologic manifestations at the time their cases were published [4, 5, 7]. Other clinical manifestations include hepatosplenomegaly and poor growth that occurred in the majority of patients. Laboratory abnormalities include marked elevation of acute phase reactants including erythrocyte sedimentation rate and C-reactive protein [1, 3–8].

25.2.2 Genetic Basis and Pathophysiology of Majeed Syndrome

Majeed syndrome is an autosomal recessive disorder due to mutations in *LPIN2* that encodes the protein LIPIN2 [9]. Five different mutations were discovered in the seven unrelated families. Two families share a p.Ser743Leu missense mutation [7, 9] and two share a mutation in the donor splice site of exon 17 [4, 8]. The other four mutations are distinct frameshift mutations that result in premature stop codons [4–7, 9]. LIPIN2 is a member of the LIPIN family of proteins that are key enzymes in adipogenesis by acting as phosphatidate phosphatases (PAP) for the Mg^{++}-dependent conversion of phosphatidic acid to diacylglycerol [15–17]. The p.Ser743Leu mutation disrupts PAP activity [18]. However, the role of LPIN2 in inflammation remained unclear. Data from the clinical treatment of patients with Majeed syndrome point to IL-1 dysregulation as a key mediator of disease as treatment with recombinant IL-1 receptor antagonist or IL-1β blocking agents resulting in marked clinical improvement [5]. Lorden et al. used an *in vitro* model of LIPIN2 deficiency (human and murine) and demonstrated

Table 25.1 Clinical Features in Majeed Syndrome

	A1	A2	A3	A4	B1	B2	C1	D1	D2	E1	E2	F1	F2	G1
Recurrent fever	+	+	+	+	+++	+++	+	−	+	−	−	NR	NR	−
Failure to thrive	+	+	+	+	+	+	−	NR	NR	+	−	NR	NR	+
CNO	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Age at onset (months)[a]	12	19	9	1	0.75	9	15	6	3	24	96	6	48	72
LE long bones	+	+	+	+	+	+	+	+	+	+	+	+	+	+
UE long bones	+	+	+	+	+	+	+	+	+	+	+	−	−	NR
Feet	−	−	−	−	−	+	+	−	−	+	−	+	−	+
Spine[b]	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Other			hands			hands		ribs	hands					
Bone biopsy	osteo	osteo	osteo	NR	NR	NR	ND	osteo[c]	ND	ND	ND	ND	necrosis	osteo
Objective joint swelling	+	+	+	NR	+	+	+	−	+	+	+	+	+	+
Hepatosplenomegaly	+	+	+	+	+	NR	+	−	−	+	−	NR	NR	NR
↑ ESR/CRP	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	++	+++	++	+++
Microcytic anemia	+	+	+	+	+	+	+	+[d]	+[d]	+	+[e]	+	+	NR
Transfusion Rx	+	+	+	+	+	+++	−	−	−	−	−	−	−	NR
Dyserythropoiesis on BM	+	+	+	+	+	+	+	+	+	+	ND	ND	+	NR
Neutrophilic dermatosis	+	+	−	−	−	−	−	−	−	−	−	−	−	NR

Reference for family A & B [1]; family C [4], family D [5], family E [6], two patients reported by Moussa F [7], patient G [8]
Modified from Ferguson PJ; Elsevier, Edited by Cimaz and Lehman, Pediatric in Systemic Autoimmune Diseases, Volume 11, Chap. 15, Pages 315–339, 2016
BM = bone marrow, CNO = chronic non-bacterial osteomyelitis, CRP = C-Reactive Protein, ESR = erythrocyte sedimentation rate, LE = lower extremity, UE = upper extremity, Osteo = osteomyelitis, Rx = treatment
ND = not done; NR = not reported
− = not present; + = present or mildly elevated; ++ = moderately elevated; +++ = markedly elevated
[a]Months to year conversion: 24 months = 2 years; 48 months = 4 years; 72 months = 6 years; 96 months = 8 years
[b]Whole body MRI not done in most
[c]Granulomatous inflammation
[d]Anemic but MCV not reported
[e]Mild anemia MCV 86.8

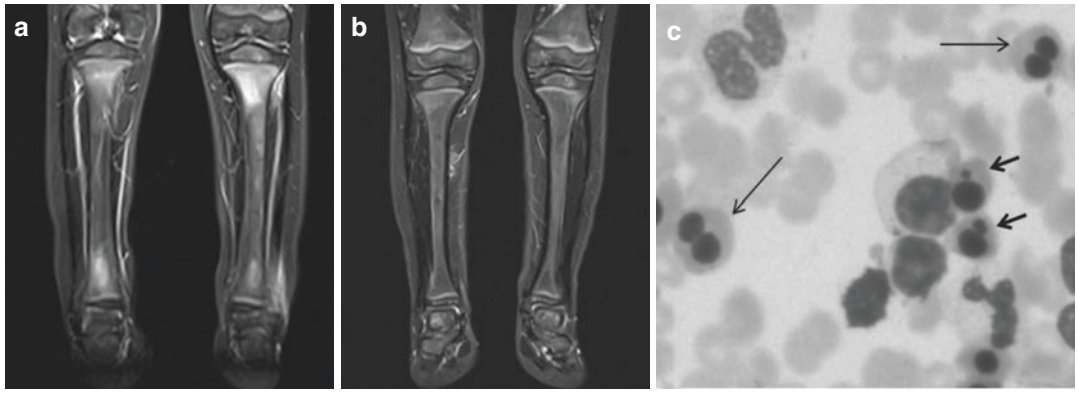

Fig. 25.1 Clinical findings in Majeed syndrome. (**a**) Increased signal intensity on MRI STIR sequences primarily located in the metaphyseal regions of the long bones in a child with Majeed syndrome. (**b**) Resolution of abnormal STIR signal 3 months following initiation of an IL-1 blocking agent. (**c**) Bone marrow from a patient with Majeed syndrome shows erythroblasts with binucleated (long thin arrows) and nuclear budding (short thick arrows). Used with permission from Herlin et al., Ann Rheum Dis 2013; 72:41–413

that LIPIN2 is a negative regulator of the NLRP3 inflammasome [19]. They showed that LIPIN2 regulates MAP kinase (MAPK) activation which is important for synthesis of pro-IL-1β during NLRP3 inflammasome priming [19]. It also alters the activation and sensitization of the $P2X_7$ receptor and subsequent K^+ efflux, ASC oligomerization and caspase-1 processing [19]. Fat metabolism appears to be important in disease pathogenesis as under-expression of LIPIN2 in macrophages leads to reduced intracellular cholesterol levels; further, reversing the cholesterol deficit can improve the defect in $P2X_7R$ function [19]. Thus, the data support the theory that Majeed syndrome is an NLRP3 inflammasomopathy [19].

25.2.3 Treatment of Majeed Syndrome

Treatment for Majeed syndrome is empiric. There are only case reports or case series describing treatment approaches and perceived efficacy of those treatments. Less severe disease (one patient) has been treated with non-steroidal anti-inflammatory drugs (NSAIDs) alone [7]. However, most patients have required additional immunomodulatory agents to control disease. Non-biologic options have included corticosteroids, colchicine, methotrexate, and pamidronate either alone or in combination with variable success. Biologic agents have been used in more severe or treatment refractory cases. Herlin et al. reported the failure of two brothers to improve when treated with a tumor necrosis factor inhibitor, but treatment with the recombinant IL-1 receptor antagonist anakinra resulted in marked sustained improvement of bone inflammation, inflammatory markers and anemia [5] (Fig. 25.1a, b). Subsequently, due to impaired quality of life issues that resulted from daily injections, treatment was switched to the IL-1β blocking antibody canakinumab with continued control of all disease manifestations [5]. Since then, two additional cases of treatment refractory Majeed syndrome received anakinra [8] or canakinumab [7], both reporting significant and sustained improvement in disease control. This suggested that Majeed syndrome is an IL-1β mediated disease and is consistent with the basic science that LIPIN2 is a negative regulator of the NLRP3 inflammasome.

25.3 Role of *FBLIM1* in CNO

- **CNO typically presents around 9 years of age, usually with multifocal bone lesions**
- **The genetic basis of CNO is not yet known, except in rare cases**

For the majority of cases of CNO the genetic basis is not known yet. However, data support a genetic component to the disease. Using a whole exome sequencing approach, Cox et al. identified a rare homozygous mutation in *FBLIM1* in one consanguineous family with CNO. The affected child presented with CNO and psoriasis [20]. Whole exome sequencing revealed multiple homozygous mutations in the child as expected given that her parents were first cousins. One of the homozygous mutations was in the filamin binding domain of *FBLIM1*. *FBLIM1* encodes FBLP1 (filamin binding LIM protein 1) [21] that stood out as the most likely candidate gene based on multiple lines of evidence. First, it is the most differentially expressed gene in bone marrow macrophages in the murine model of CNO due to mutations in *Pstpip2* (discussed below in Sect. 25.4) [20]. Second, there are data that support FBLP-1 as a STAT3 regulated molecule with anti-inflammatory properties mediated via IL-10 effects. This is clinically relevant as Hedrich et al. have reported abnormalities in IL-10 regulation in patients with CNO [22, 23]. Third, FBLP-1 is important in bone remodeling. It has a role in ERK1/2 signaling and downstream regulation of RANKL activation, and knockout mice have severe osteopenia [21, 24]. Finally, FBLP-1 is a key regulator of the cytoskeleton. It binds filamin, acting as an anchor for filamin-containing actin filaments and cell-extracellular matrix adhesion proteins [25, 26] and is involved in integrin activation [27, 28].

Because of this information supporting the role of *FBLIM1* as a CNO susceptibility gene, Cox et al. sequenced a cohort of 96 individuals (mostly children) with CNO. One additional patient was identified with compound heterozygous mutations with a novel frameshift mutation in exon 6 of *FBLIM1* and a mutation in an enhancer in the third intron that contains binding sites for STAT3 and NR4A2 (present in trans, i.e., on the other allele) [20]. Both STAT3 and NR4A2 are transcription factors that are involved in the regulation of inflammation. Cumulatively, these data support the assertion that *FBLIM1* is a CNO susceptibility gene.

25.4 Deficiency of the IL-1 Receptor Antagonist (DIRA)

- **DIRA present early in life with pustular rash, systemic inflammation, osteopenia and lytic bone lesions**
- **Treatment with IL-1 receptor antagonist is highly effective at resolving these symptoms**
- **These findings confirm the importance of IL-1 in osteoclast activation**

Patients born with a deficiency of the IL-1 receptor antagonist (DIRA) present shortly after birth with a severe pustular, erythematous rash and systemic inflammation (Fig. 25.2) [29, 30]. All of these infants with DIRA were born early (<39 weeks) and half developed fetal distress, suggesting a role for IL-1 receptor antagonist *in utero*. Joint effusions, stomatitis, hepatosplenomegaly, failure to thrive and respiratory distress may occur. White blood cell counts and inflammatory markers are typically elevated, and anemia is prominent, while fevers are not common. Patients with DIRA exhibit a variety of bone abnormalities from birth, including diffuse osteopenia, rib and clavicular flaring, heterotopic ossifications, and multiple lytic lesions with sclerosis (Fig. 25.2) [29–31]. In rare instances there may be epiphyseal overgrowth similar to what is seen in NOMID (see below) [30]. Skin biopsies demonstrate subcorneal pustulosis with neutrophilic infiltrates, and bone marrow biopsies demonstrate neutrophilia and bone scalloping [29]. Prior to the discovery of the cause and effective treatment for this disorder, it was fatal in approximately one third of cases [30].

Genetic analysis of affected patients demonstrated autosomal recessively inherited mutations in the *IL1RN* gene encoding the IL-1 receptor antagonist. These mutations included stop codons, frameshift mutations, and deletions [29, 30, 32–35]. There appears to be a founder effect in the Puerto Rican population due to a 175 KB deletion on chromosome 2q13 encompassing six IL-1 gene family members; *IL1RN* (IL-1 receptor antagonist), *IL1F10*, *IL36RN* (IL-36 receptor antagonist), *IL36A*

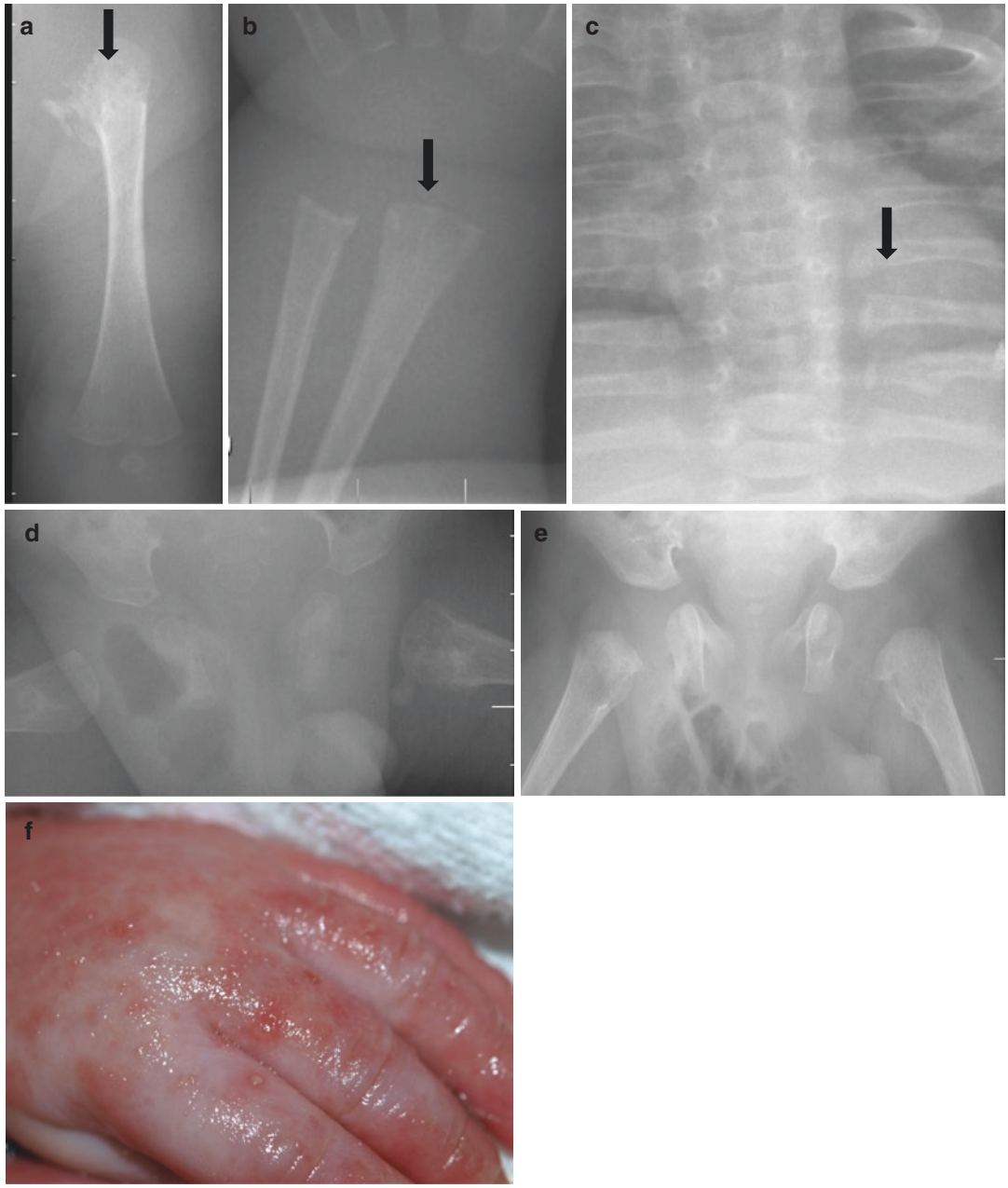

Fig. 25.2 Bone and skin abnormalities in DIRA. Representative lytic lesions of the proximal femur (**a**), and the distal radius and ulna (**b**). Widening of anterior ribs with lytic changes and osteopenia (**c**). Osteopenia of pelvic bones and lytic changes to the left proximal femur (**d**), and resolution of these findings after 5 months of treatment with the IL-1 receptor antagonist (anakinra) (**e**). (**f**) Erythematous, pustular rash seen in DIRA

(IL-36α), *IL36B* (IL-36β), and *IL36G* (IL-36γ) [29, 30, 36]. Interestingly, deficiency in *IL-36RN* has been associated with pustular psoriasis (see Chap. 26) [37, 38].

Corticosteroids and NSAIDs have been used to treat DIRA with partial efficacy, although these treatments did not fully suppress the disease or prevent complications [39]. Not surprisingly

treatment with recombinant IL-1 receptor antagonist (i.e., anakinra) results in a rapid and sustained response with correction of laboratory abnormalities, resolution of rash, and healing of bone lesions [29, 30]. IL-1 is a potent mediator of osteoclast activation and bone resorption [40–44]. IL-1α and IL-1β bind the type 1 IL-1 receptor (IL-1RI) and IL-1 receptor accessory protein (IL-1AcP) to activate MyD88/IRAK4 and initiate downstream signaling events. The IL-1 receptor antagonist binds to IL-1RI but not IL-1AcP thereby blocking activation. The inability to block IL-1 signaling is central to the pathophysiology of DIRA. This is in contrast to NOMID, a disorder of enhanced IL-1β production, where the bone manifestations do not response to IL-1 blockade while the systemic symptoms typically respond (see below).

25.5 Cherubism

- **Cherubism presents with jaw swelling due to osteoclast activation in the mandible**
- **This disorder typically begins in childhood, then regresses after puberty**
- **Treatment is largely surgical**

Cherubism was first described in 1950 by Jones et al. as a condition starting in childhood with swelling of the mandible and maxilla [45]. In severe cases the swelling can affect the floor of the orbit causing the eyes to turn upward. Because of the apparent chubbiness of the cheeks and the eyes "looking up to heaven", this condition was named cherubism after the "cherubs" of the Renaissance painters [45]. This disorder typically presents between 2 and 7 years of age with painless jaw swelling that progresses through puberty when it typically stabilizes and regresses over time [46, 47]. However, there are severe cases where it may continue to progress after puberty and growth cessation. Affected patients exhibit prominent dental anomalies, including irregularly placed or absent deciduous teeth and malocclusion [47]. Radiographs demonstrate multilocular radiolucencies (i.e. osteolysis) with sclerotic borders in the affected areas (Fig. 25.3)

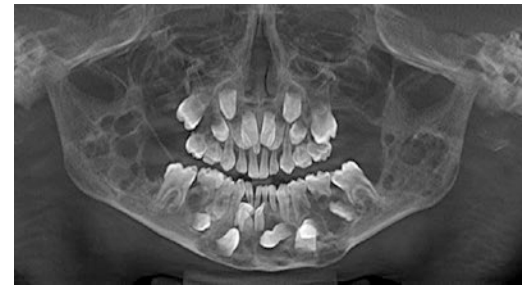

Fig. 25.3 Bone abnormalities in cherubism. Panorex view of a five year old male with cherubism demonstrating bilateral multilocular cyst-like radiolucencies (i.e. osteolysis) with sclerotic borders in the mandible and to a lesser extent the maxilla, causing significant alteration of the permanent dentition

[48]. Multiple family members may be affected since this disorder is inherited in an autosomal dominant manner with variable penetrance and expressivity [49]. For severe lesions, surgical curettage and reconstruction can be beneficial and may arrest the growth of the lesions and stimulate bone regeneration [47]. Pathologic analysis of these lesions demonstrates fibrous lesions with multinucleated giant cells which have been shown to be osteoclasts based on the expression of osteoclast specific proteins such as tartrate-resistant acid phosphatase, the vitronectin receptor, and the ability to resorb bone [50].

The gene for cherubism was mapped to chromosome 4p16, and later found to be caused by mutations in the SH3 binding protein domain 2 (*SH3BP2*) gene [51–54]. SH3BP2 binds to the oncogene c-Abl, as well as well as a variety of signaling molecules including Syk, a tyrosine kinase involved in lymphocyte receptor signaling [55]. All of the disease-causing mutations occur in a region of exon 9, with the proline 418 most commonly affected. This region of the SH3BP2 protein interacts with tankyrase, a member of the poly (ADP-ribose) polymerase family, which leads to ADP-ribosylation, ubiquitylation, and destruction of SH3BP2 [56]. How this leads to osteoclast activation was unknown until knock-in mice were generated that harbored the P418R mutation. These mice developed spontaneous osteoporosis, mandibular bone loss, and increased number of osteoclasts [57]. Monocytes from

these mice demonstrated increased sensitivity to RANKL and M-CSF, and generated large osteoclasts with spontaneous TNF-α expression. Homozygous P418R mice exhibited widespread macrophage infiltration that was dependent on TNF-α [57]. These mice also exhibited enhanced responsiveness to toll like receptor agonists, such as LPS, resulting in enhanced TNF-α expression, raising the possibility that oral microbes may drive the inflammation in the jaw particularly during growth and oral remodeling. Administration of etanercept to these mice prevented the inflammatory manifestations and bone loss, however pilot studies in humans with TNF-α blocking agents have been disappointing [58, 59]. Interestingly, osteoclastogenesis is dependent on the transcription factor NFATc1, and deficiency of this transcription factor can rescue the bone phenotype in mice with the P418R mutation [60]. Since NFATc1 activation is dependent on calcineurin, a trial of the calcineurin inhibitor tacrolimus was instituted in a single patient and showed clinical benefit [61].

25.6 Pstpip2 Mutations in Murine Chronic Multifocal Osteomyelitis

Byrd et al. described a spontaneous mouse model of CNO which they called the cmo mouse [62]. These mice develop tail kinks, deformity of the hind- and sometime forepaws, and inflammation of the skin and cartilage of the ear [62, 63]. The mice are active, lead a normal life span and breed normally [62, 63]. These features are the result of spontaneous recessive mutations in *Pstpip2* [63]. Pstpip2 is an adaptor protein involved in cytoskeletal function. Pstpip2 is known to interact with PTP PEST proteins, actin, Csk and SHIP1 [64, 65]. Under-expression of Pstpip2 disrupts podosome assembly in osteoclasts [66] which can alter their function toward a more tissue destructive phenotype [67]. Since the identification of *Pstpip2* as the causative gene in the cmo mice, there have been other *Pstpip2* mutant murine models of CNO made by various strategies including ENU mutagenesis, conventional knockout or conditional knockout; all have had a similar phenotype of sterile bone inflammation [68–70]. Bone inflammation in the cmo mouse is hematopoietically driven and occurs independent of the adaptive immune system [71]. The disease is IL-1 dependent yet is an Nlrp3 inflammasome independent process [72, 73]. In this model, neutrophils secrete excessive IL-1β in response to innate immune system triggers such as silica [72]. The phenotype in the cmo mouse model is typically highly penetrant when fed standard rodent chow [63, 74]. However, Lukens et al. demonstrated that dietary induced changes in the microbiome are associated with changing severity of sterile osteomyelitis in the cmo mouse model [74]. They showed that a high fat diet could protect cmo mice from the development of bone inflammation and that the protection could be reproduced with fecal transplants [74]. Additional studies confirm the critical role of neutrophils in the aberrant secretion of IL-1β in the cmo mouse [74].

25.7 Schnitzler Syndrome

The clinical features, genetics, pathomechanisms and treatment of Schnitzler syndrome are presented in detail in Chap. 37. In this chapter, we focus on osseous manifestations of the disease.

25.7.1 Bone Involvement in Schnitzler Syndrome

In brief, Schnitzler syndrome is an autoinflammatory disorder that typically presents with a chronic urticarial-like rash (sparing the palms and soles) with or without fever, arthralgias and bone pain [75]. While chronic urticaria-like lesions are present in 100% of all patients, arthralgia occurs in 66%, and 55% report bone pain. Objective arthritis is rare. Typically, the bone pain is localized to the shins and other long bones, the hips and the back. Other inflammatory manifestations may also be present. Laboratory studies reveal monoclonal IgM (or IgG) gammopathy in addition to markers of inflammation

[76]. In a large literature review of 281 individuals with Schnitzler syndrome, plain radiographs were reported to be abnormal in 43% of patients, most often showing hyperostosis (39%), osteolysis (1%), and periosteal reaction (<1%) [75]. Bone scintigraphy showed increased uptake in 85%, infarction in 1% and osteomyelitis in 1% [75]. Bone biopsies were reported in only 14 cases and described as normal in 43%, while demonstrating sclerosis in 29%, increased osteoblast and osteoclast activity in 14%, and inflammation in 7% [75]. Lesions in the long bones (proximal tibia and distal femur) were most common but other bones were affected [75]. Evidence points to dysregulation of IL-1 in the pathogenesis of Schnitzler syndrome and are suggestive of the disease being an inflammasomopathy. Supportive of this is the discovery of myeloid lineage restricted somatic mosaicism of *NLRP3* mutations in patients with Schnitzler syndrome [77]. Various treatments have been utilized in Schnitzler syndrome but IL-1 blockade appears to be the most effective [75]. In-depth studies of bone involvement in Schnitzler syndrome are necessary to better define the histologic process and how it evolves over time. This is yet another example of bone disease in an IL-1 mediated disorder.

25.8 Primary Hypertrophic Osteoarthropathy

- **Primary hypertrophic osteoarthropathy (PHO) is a genetic disorder affecting bone and skin due to inappropriate degradation of prostaglandins, an inflammatory mediator**
- **PHO occurs due to mutations in HPDG, an enzyme important in the degradation of prostaglandins that are known to affect bone turnover**

Although not a systemic autoinflammatory disorder, PHO presents with periostitis and osteolysis of bone due to excessive levels of prostaglandin, important inflammatory molecules involved in osteoclast activation. PHO is characterized by digital clubbing due to periostitis of the diaphysis and metaphysis of the distal extremities [78, 79].This condition has been recognized for centuries, first described by Hippocrates in a patient with a chronic empyema, and thus has been referred to as the "Hippocratic finger". Secondary hypertrophic osteoarthropathy occurs in pulmonary disorders such as cystic fibrosis, gastrointestinal disorders, and neoplasms and in cyanotic heart disease. Primary hypertrophic osteoarthropathy (PHO) occurs in families and is usually inherited in an autosomal recessive manner, although families with autosomal dominant inheritance have been described. Features associate with PHO may include pachydermia, or the excessive proliferation and thickening of skin, delayed closure of the fontanels, congenital heart disease (i.e., patent ductus arteriosus), seborrhea, hyperhidrosis, and arthritis [78, 80–82]. Bone manifestations include periostitis and periosteal thickening of the digits and long bones, and in some cases osteolysis of the distal phalanges (acroosteolysis) [79]. Markers of systemic inflammation are typically normal.

PHO is caused by mutations 15-hydroxy prostaglandin dehydrogenase (HPDG), an enzyme important in the degradation of prostaglandins [83, 84]. Mutations in the prostaglandin transporter gene *SLCO2A1* may also cause PHO [85–87]. Prostaglandin E2 levels are elevated in these disorders, as are markers of bone turnover [84]. Prostaglandin E2 has multiple effects on bone formation, and can promote bone formation or promote absorption depending on the experimental conditions [88–92]. Limited information is available regarding treatment, but case reports have shown some benefit to NSAIDs and prednisone [80].

25.9 Neonatal Onset Multisystem Inflammatory Disorder (NOMID)

NOMID is the severe form of a spectrum of conditions caused by mutations in cryopyrin, a protein important in activation of the inflammasome resulting in the conversion of pro-IL-1β to

biologically active IL-1β. NOMID and the other cryopyrinopathies are discussed in Chap. 19. We will focus on the osseous abnormalities in NOMID.

25.9.1 Osseous Abnormalities in NOMID

- **Patients with NOMID develop an arthropathy characterized by fibrous masses of disorganized cartilate but without inflammatory changes or arthritis**
- **These changes are not reversed by IL-1 blockage, suggesting a different pathophysiologic mechanism**

Patients with NOMID can develop an arthropathy, particularly of the knees and ankles, however these changes are not thought to be the result of bone inflammation. MRI studies of 20 patients with NOMID showed enlarged and deformed femora, patella, and tibiae resulting in valgus or varus knee deformities [93] (Fig. 25.4). There were heterogenous calcified masses that appeared to arise from the physis [93, 94]. Biopsy of these masses showed relatively acellular disorganized cartilage with areas of fibrosis and calcification, but without evidence of inflammatory changes [93, 95].

While the cause of this osseous overgrowth is unclear, it doesn't appear to be caused by excessive IL-1β production. Treatment with the IL-1 receptor antagonist anakinra does not reverse the bone abnormalities (and may not prevent them either), while this treatment is very effective at ameliorating the other manifestations of the disease [96–98]. In addition, patients with DIRA do not typically have the same physeal changes seen in NOMID, and the bone abnormalities in most patients with DIRA reversed with IL-1 receptor antagonist treatment [29, 30]. It is likely that NLRP3 has other roles in cells outside of its role in producing IL-1β. Cryopyrin appears to be involved in preventing apoptosis [99, 100], as shown by the ability of LPS to induce apoptosis of monocytes in a patient with NOMID but not in a patient with DIRA [29]. It is possible that the anti-apoptotic effect of cryopyrin results in chondrocyte death and the knee changes noted above. Recently, mice genetically engineered to express gain of function mutations in cryopyrin (i.e., D301N) exhibited diffuse osteopenia and joint manifestations, and targeting this mutation to osteoclasts resulted in osteoporosis without other systemic manifestations [101]. Interestingly, osteoclasts from these mice downregulated poly(ADP-ribose)polymerase 1, possibly linking the pathophysiology of bone changes in NOMID to those in cherubism (see above). Another possible explanation comes from the observations that humans and mice with defects in regulation of protein kinase A have tumors histologically similar to the lesions in NOMID and in similar

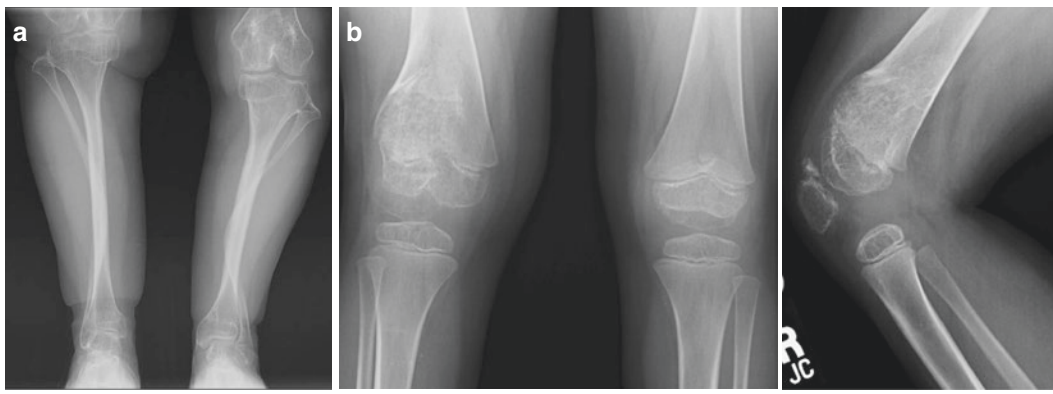

Fig. 25.4 Bone abnormalities in NOMID. (**a**) AP view of distal femur and tibia/fibula demonstrating physeal overgrowth with ossification, as well as fibular tortuosity. (**b**) AP and lateral views of the knees of a second patient with NOMID. Images courtesy of Dr. Raphaela Goldbach-Mansky from the National Institutes of Health

locations [102]. The authors demonstrate that PKA activates the transcription factor Ets-1 in these tumors, which drives caspase-1 activation, IL1b production and subsequent PGE2 production that further activates PKA through cAMP. Thus inappropriate caspase-1 activation could lead to a similar feedback loop in NOMID, resulting in mesenchymal stem cell proliferation and the formation of fibroblastoid tumors.

25.10 Summary

Sterile bone inflammation occurs in a variety of autoinflammatory disorders. Several genetic defects have been described with predominant bone inflammation. The pathophysiology of these disorders varies; some are associated with inappropriate activation or regulation of the inflammasome and IL-1, while others affect intracellular signaling pathways of innate immune cells and osteoclasts. Treatment of many of these disorders has improved with the development of IL-1 blocking agents. With the advent of advance sequencing and other genetic technologies, more autoinflammatory disorders with bone involvement will likely be characterized.

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Pustular Forms of Psoriasis Related to Autoinflammation

26

Satveer K. Mahil, Jonathan N. Barker, and Francesca Capon

Abstract

Psoriasis is a common inflammatory skin disorder that is classified into multiple disease subtypes. The pustular variants, which are characterized by neutrophilic infiltrates, present with acute, potentially-life threatening episodes of pustulation and systemic upset (generalised pustular psoriasis, GPP) or with chronic and disabling pustulation of the hands and feet (palmar plantar pustulosis, PPP and acrodermatitis continua of Hallopeau, ACH). These are very severe conditions that are extremely difficult to treat.

In the last few years, genetic studies have identified three disease genes (*IL36RN, AP1S3* and *CARD14*) that are mutated in one or more forms of pustular psoriasis. This has demonstrated a shared genetic basis for these conditions and highlighted an autoinflammatory pathogenesis, driven by abnormal IL-36 signalling. In this context, the ongoing development of IL-36 antagonists holds the promise of delivering efficient therapeutics for a group of diseases that have a profound impact on quality of life.

Keywords

Pustular psoriasis · Interleukin-36 · IL-36 *IL36RN* · *AP1S3* · *CARD14* · Skin autoinflammation · DITRA

Abbreviations

ACH	Acrodermatitis continua of Hallopeau
CAMPS	*CARD14* mediated psoriasis
DITRA	Deficiency of the interleukin 36 receptor antagonist
GPP	Generalised pustular psoriasis
IL-36Ra	Interleukin-36 receptor antagonist
PPP	Palmar plantar pustulosis.

Key Points

- **The term pustular psoriasis refers to a group of severe neutrophilic skin disorders characterised by painful pustular eruptions**
- **These conditions are incompletely understood at the molecular level and notoriously difficult to treat in the clinic**

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- **Genetic studies have identified disease alleles within genes linked to IL-36 signalling**
- **The development of IL-36 blockers holds the promise to deliver health benefits to patients**

26.1 Introduction

Psoriasis is a common skin disorder that globally affects 2–3% of the population. The most frequently observed clinical variant is psoriasis vulgaris (Ps), which presents with well-demarcated, scaly erythematous plaques and accounts for approximately 90% of disease cases [1]. Rarer variants include guttate and erythrodermic psoriasis, as well as pustular psoriasis. The latter term refers to a heterogeneous group of diseases characterised by painful eruptions of neutrophil-filled pustules. These can manifest as acute episodes of widespread pustulation (generalised pustular psoriasis—GPP) or as chronic pustular eruptions that affect the palms and soles (palmar plantar pustulosis—PPP) or fingers and toes (acrodermatitis continua of Hallopeau—ACH). All conditions have a profound impact on quality of life, but are extremely difficult to treat, as the biologics that are used to good effect in psoriasis vulgaris show limited efficiency in the pustular forms of the disease [1].

While the rarity of pustular psoriasis has long hindered the study of pathogenic pathways, the advent of next-generation sequencing and the definition of consensus diagnostic criteria have recently enabled the identification of three disease genes. These advances have shed some light on pathogenic mechanisms, highlighting the involvement of keratinocyte innate immune responses and identifying a key role for abnormal IL-36 signalling.

26.2 Epidemiology

Key Concepts

- **GPP is a very rare disease that can be triggered by pregnancy, drug withdrawal or respiratory tract infections**
- **PPP is the most common pustular form of psoriasis. It mostly affects middle-aged female smokers**
- **Owing to the extreme rarity of the disease, very little is known about the epidemiology of ACH**

It has been estimated that only 2–5% of psoriatic patients are affected by a pustular variant of the disease [1]. As a result, epidemiological findings are scarce, especially in relation to generalised pustular psoriasis and acrodermatitis continua of Hallopeau. Nonetheless, sufficient evidence is available to demonstrate that each form of the disease has a distinct epidemiology.

26.2.1 Generalised Pustular Psoriasis

GPP is extremely rare in individuals of European descent and the only study that attempted to estimate disease frequency in this ethnic group reported an incidence of 0.64 cases per million per year and a prevalence of 1.76 per million [2]. This would correspond to 0.01% of psoriasis cases. The disease appears to be more common in Asian populations, with 16.5 new cases per year reported in Japan and an estimated 0.9% of psoriatic patients affected by GPP [3].

The age of onset of GPP is variable; while the majority of individuals show the first symptoms in adulthood, paediatric case series have also been described [4, 5]. A sex bias has been repeatedly reported with male to female ratios ranging from 0.71 to 0.77 [2, 4].

Many triggering factors have been described, most notably pregnancy, upper-respiratory tract infections and systemic steroid treatment withdrawal and metabolic status [1, 4].

26.2.2 Palmar Plantar Pustulosis

PPP is the most common form of pustular psoriasis, with prevalence estimates ranging from 1:10,000 to 1:2000 in Europeans [6] to 1:800 in the Japanese population [7].

PPP is considered a pustular disease of adults, with the median age of onset ranging from 45 to 65 years [6].

There is a very marked sex bias as 67–92% of affected individuals are female [6]. This corresponds to male to female ratios ranging from to 0.08 to 0.49.

PPP is strongly associated with cigarette smoking, with 54–92% of patients reported to smoke at the time of diagnosis [1, 6]. Of note, smoking cessation does not necessarily result in an improvement of symptoms [1].

26.2.3 Acrodermatitis Continua of Hallopeau

ACH is the rarest form of pustular psoriasis. It is only described in case reports (~100 have been published to date) or small case series. Thus, the prevalence and incidence of the disease are unknown.

Age of onset is variable; the disease is thought to be rare in young adults and not infrequently diagnosed in old age [1]. In line with other forms of pustular psoriasis, an increased prevalence among females has been reported [1].

Symptoms are typically precipitated by minor trauma or infections at the tips of the digits [1].

26.3 Etiology

Key Concepts

- **There is a shared genetic basis between the various forms of pustular psoriasis**
- **The *IL36RN* and *AP1S3* genes are mutated in a proportion of GPP and ACH patients**
- ***CARD14* mutations are observed in a small fraction of GPP cases and in very rare familial forms of psoriasis vulgaris**
- **The genetic basis of PPP is poorly understood, as the studies carried out to date have generated conflicting results**

While the causal role of certain external triggers (e.g. pregnancy in GPP or smoking in PPP) has long been recognised, it has now become apparent that genetic determinants also play a critical role in the aetiology of pustular psoriasis (see below). In fact, a substantial number of GPP and ACH cases are caused by disease alleles that have a considerable effect on protein function. Thus, the aetiology of GPP and ACH can be described as nearly monogenic, which is in contrast with the multifactorial inheritance of psoriasis vulgaris. The genetic basis of PPP is at present less clear, as the studies carried out so far have generated conflicting results.

26.3.1 Generalised Pustular Psoriasis

Approximately 20% of GPP patients carry recessive mutations of the *IL36RN* gene, which encodes the interleukin-36 receptor antagonist (Table 26.1) [8]. These defects were originally identified in large consanguineous pedigrees of North-African descent (p.Leu27Pro mutation) and in a small sample of British unrelated individuals (p.Ser113Leu) [9, 10]. Subsequent *IL36RN* screens have identified a variety of

Table 26.1 *IL36RN* mutations associated with pustular psoriasis

IL36RN mutation	Proband country of origin	Phenotype
R10X, c.28C>T	Japan	GPP
L21P, c.62C>T	Pakistan	GPP
L27P, c.80T>C	Tunisia	GPP
H32R, c.95A>G	Iraq	GPP
K35R, c.104A>G	UK	GPP
c.115+6T>C	Japan	GPP, ACH
I42N, c.125T>A	Japan	GPP
N47S, c.140A>G	China	GPP
R48W, c.142C>T	UK	GPP
P76L, c.227C>T	Germany	GPP
E94X, c.280G>T	Germany	GPP
R102W, c.304C>T	UK	GPP, ACH
R102Q, c.305G>A	China	GPP
E112K, c.334G>A	Japan	GPP
S113L, c.338C>T	UK	GPP, ACH, PPP
T123R, c.368C>G	Japan	GPP
T123M, c.368C>T	Japan	GPP

Mutations listed in the Infevers registry (http://fmf.igh.cnrs.fr/ISSAID/infevers/) in May 2017

missense, splicing and nonsense mutations, with the p.Ser113Leu, p.Pro27Leu and c.115T+6C alleles accounting for the majority of European, North-African and Asian cases, respectively [8].

Genotype-phenotype correlations show that recessive *IL36RN* alleles are most prevalent among patients who suffer from early-onset (<18 years) disease that is accompanied by systemic inflammation [8]. The acronym DITRA (deficiency of the interleukin 36 receptor antagonist) has been proposed to describe this severe condition [9].

Surprisingly, early mutation screens identified a number of GPP cases who harboured a single heterozygous *IL36RN* mutation. While disease onset appears to be delayed in these individuals, the clinical presentation remains severe, with a risk of systemic inflammation that is comparable to that observed in DITRA (see also below) [8].

Subsequent gene identification studies uncovered two founder mutations of the *AP1S3* gene (p.Phe4Cys and p.Arg33Trp) in a small number of European cases [11]. While these changes were also detected in a follow-up cohort, no Asian patients bearing *AP1S3* mutation could be identified [12].

Of interest, one of the affected individuals harbouring the *AP1S3*p.Phe4Cys allele also carried a heterozygous *IL36RN* mutation. The patient suffered from early-onset, poorly controlled disease, but her sister, who had only inherited the *IL36RN* change, showed a less severe clinical presentation [12]. This suggests that the effect of *IL36RN* mutations may be modified by *AP1S3* alleles.

Finally, candidate gene studies identified an association between GPP and the p.Asp176His allele of the *CARD14* gene. As this variant is mostly found in Asian populations, the association is restricted to this ethnic group [13].

Of interest, *CARD14* mutations have also been observed in familial psoriasis. Jordan et al described two large-pedigrees of European and Asian descent where psoriasis vulgaris segregates as an autosomal dominant trait. Linkage studies and mutational analysis identified two deleterious variants that disrupt the splicing of *CARD14* and segregate with psoriasis vulgaris [14]. Hence, the acronym CAMPS (*CARD14*-mediated psoriasis) has been proposed to describe this condition. This is a rare disease, which has only been described in a very small number of families, despite the screening of extended datasets [13, 15].

Taken together, *IL36RN, AP1S3* and *CARD14* mutations only account for a minority of GPP cases (<30%), so that additional genetic determinants of the disease remain to be identified. Of note, the observation of digenic mutations, the well-established role of environmental triggers and the description of asymptomatic individuals carrying homozygous *IL36RN* alleles [16] cannot be reconciled with a simple model of monogenic inheritance. Somatic mosaicism, which contributes to the phenotypic variability of other autoinflammatory conditions (see Chap. 3) but has yet to be investigated in pustular psoriasis, may prove to be another modifier of disease severe severity. Thus, there is accumulating evidence for elements of complexity in the genetics of GPP.

26.3.2 Palmar Plantar Pustulosis

The role of *IL36RN* mutations in PPP is controversial. While Setta-Kaffetzi et al. reported the presence of mono- and bi-allelic mutations in various British patients, this finding could not be replicated in an independent study [17]. Likewise, the presence of *CARD14* mutations was observed in German and Estonian patients [17], but not British cases [13]. So far, only *AP1S3* mutations have been described in two independent datasets [12], but those were in large part ascertained by the same group.

It is possible that the conflicting results of genetic studies reflect the use of different diagnostic criteria or the presence of an ascertainment bias in some studies. Until these issues are addressed in large multi-centre collaborations, PPP will remain the least understood form of pustular psoriasis

26.3.3 Acrodermatitis Continua of Hallopeau

Recessive *IL36RN* mutations have been described in various ACH patients, demonstrating a shared genetic aetiology with GPP. In fact, the same disease alleles (most notably the p.Ser113Leu mutation) have been observed in GPP and ACH, suggesting the existence of modifier loci that determine the exact nature of a pustular phenotype [18].

While *IL36RN* mutations appear to account for the majority of Asian ACH cases [19], this is not the case in European datasets where *AP1S3* mutations are also found [18]. Again, the *AP1S3* disease alleles observed in ACH are the same that had been previously documented in GPP (p.Phe4Cys and p.Arg33Trp) [11].

No *CARD14* mutations have yet been described in ACH, and a sizeable number of European patients do not carry mutations at any known locus.

26.4 Pathogenesis

Key Concepts

- **Genetic studies have highlighted a key role for innate immune dysregulation in the pathogenesis of GPP and ACH**
- **The *IL36RN* and *AP1S3* mutations observed in GPP and ACH result in abnormal IL-36 signalling, leading to the up-regulation of cytokines that mediate epithelial (IL-1α) and systemic (IL-1β, IL-6) inflammation**
- ***CARD14* mutations cause abnormal NF-κB activation in keratinocytes**
- **The pathogenesis of PPP remains poorly understood**

26.4.1 Generalised Pustular Psoriasis and Acrodermatitis Continua of Hallopeau

The genetic studies carried out in the last few years have uncovered a key pathogenic role for the dysregulation of IL-36 and NF-κB signalling.

IL-36α, -β and -γ (hence IL-36) are three related epithelial cytokines that signal through a common receptor, composed of the IL-36R and IL-1RaCP subunits [20]. The activation of this complex triggers downstream signal transduction through the NF-κB and mitogen activated protein kinase (MAPK) pathways, leading to the up-regulation of innate cytokines such as IL-1α/β, IL-6 and IL-8 [20]. Importantly, the pro-inflammatory activity of IL-36 cytokines is modulated by the IL-36 receptor antagonist (IL-36Ra), which is encoded by *IL36RN*. Under homeostatic conditions, IL-36Ra competes with IL-36 cytokines for binding to IL-36R and blocks downstream signal transduction, once it has engaged with the receptor. The *IL36RN* mutations associated with GPP and ACH abolish this immune-modulatory activity and result in excessive IL-36 signalling [9, 10].

Given that IL-36 up-regulates the expression of numerous chemokines and innate cytokines, excessive IL-36 signalling is thought to cause skin inflammation (through the induction of IL-1α and IL-17), peripheral neutrophilia (through the up-regulation of IL-8) and systemic inflammation (through the up-regulation of IL-1β and IL-6).

Of note, *AP1S3* mutations have also been linked to abnormal IL-36 production [12]. *AP1S3* encodes a subunit of the AP-1 complex, a conserved cytosolic tetramer that contributes to the formation of autophagosomes. These are the specialised vesicles that mediate autophagy (see Chap. 8), the catabolic process ensuring the degradation of numerous immune signalling intermediates [12].

Mahil et al. showed that *AP1S3* deficiency impairs keratinocyte autophagy, resulting in the abnormal accumulation of p62 (a well-known mediator of NF-κB signal transduction) and IL-36α [12]. Thus, defects in different GPP/ACH loci seem to converge on the dysregulation of IL-36 signalling (Fig. 26.1). This notion is consistent with the over-expression of IL-36 dependent genes in GPP patients who do not carry *IL36RN* mutations [21].

The observation that IL-36 is produced following keratinocyte stimulation with viral mimics [22]

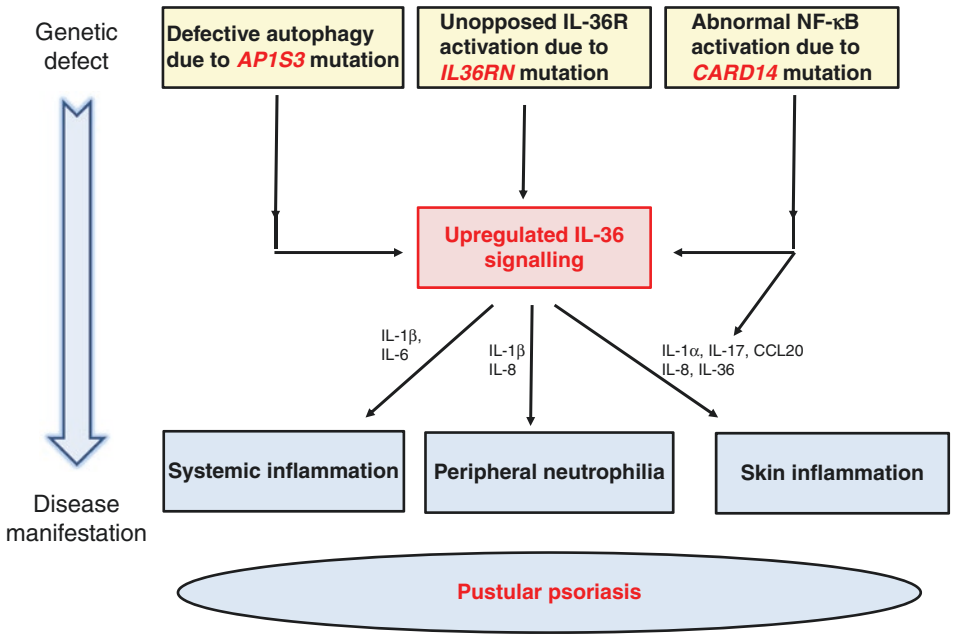

Fig. 26.1 Current understanding of disease pathogenesis. Mutations in *AP1S3, IL36RN* and *CARD14* all cause an up-regulation of IL-36 signaling. This results in the excessive production of innate cytokines (IL-1, IL-6, IL-36) and chemokines (IL8, CCL20), leading to neutrophilic skin infiltration and systemic inflammation

also links these cytokines to a well-established trigger of disease exacerbation and further supports their role in GPP pathogenesis.

A small proportion of GPP cases (and individuals affected by rare, autosomal dominant forms of psoriasis) carry heterozygous mutations in *CARD14*. This encodes an adaptor protein that mediates TRAF2-dependent NF-κB signalling in keratinocytes. The disease alleles observed in GPP and CAMPS are gain-of-function mutations which result in abnormal NF-κB activation [13]. Of note, IL-36 is one of the NF-κB dependent genes that is up-regulated by *CARD14* mutations [14].

26.4.2 Palmar Plantar Pustulosis

Given that the results of genetic studies have been conflicting, the pathogenesis of PPP remains poorly understood. While the observation of *AP1S*3 mutations seems to indicate an involvement of IL-36 signalling [11, 12], *IL36RN* disease alleles are rare among PPP patients [17, 18].

As smoking is the risk factor that is most robustly associated with PPP and the expression of nicotinic acetylcholine receptors is altered in patient skin [23], it has been suggested that nicotine could contribute to disease pathogenesis, possibly through an effect on neutrophil chemotaxis and keratinocyte differentiation. This, however, needs to be confirmed at the experimental level.

26.5 Clinical Manifestations

Key Concepts

- **Pustular psoriasis is a severe skin disorder characterised by the presence of sterile, neutrophil filled pustules**
- **Patients often suffer from concurrent psoriasis vulgaris**
- **Disease subtypes include generalised pustular psoriasis, in which acute flares of wide-**

spread pustulation can be accompanied by systemic illness, and palmar plantar pustulosis and acrodermatitis continua of Hallopeau, where the pustular eruptions are chronic and localised

Patients with pustular psoriasis present with non-infectious pustules on variably erythematous skin. Generalised and localised forms may be distinguished clinically, as detailed below.

26.5.1 Generalised Pustular Psoriasis

Generalised pustular psoriasis (GPP) was first described by Leopold von Zumbusch in 1910 as a dramatic relapsing-remitting condition [24]. Flares typically manifest with fever (>38°C) and leukocytosis (white cell count >12 × 10^9/L) for 1–2 days, followed by skin edema and erythema. Dense crops of pustules then appear within the erythematous skin, where they can rapidly evolve into lakes of pus (Figure 26.2a). Erythroderma may ensue and patients suffer from tender, pruritic skin and systemic illness (fatigue, malaise and anorexia). Importantly, although GPP often presents with concomitant psoriasis vulgaris, the pustulation is not restricted to plaques [25].

When occurring during pregnancy, GPP is often referred to as impetigo herpetiformis [1]. This condition begins in the flexures (axillae and groin) during the third trimester. Although spontaneous resolution post-partum is common, symptoms may recur in subsequent pregnancies.

Diseases that associate with GPP include psoriasis vulgaris, which affects more than 50% of patients, and arthritis, which occurs in approximately one third of GPP sufferers and often affects the distal interphalangeal joints [26]. Patients also commonly develop classical psoriatic nail changes, which may manifest as nail pitting, onycholysis and subungual hyperkeratosis. Co-morbid mucous membrane abnormalities include arcuate and circinate plaques on the tongue (known as 'geographic tongue') and buccal mucosa [27]. Purulent, sterile conjunctivitis may also be present, in addition to rarer complications such as corneal ulceration.

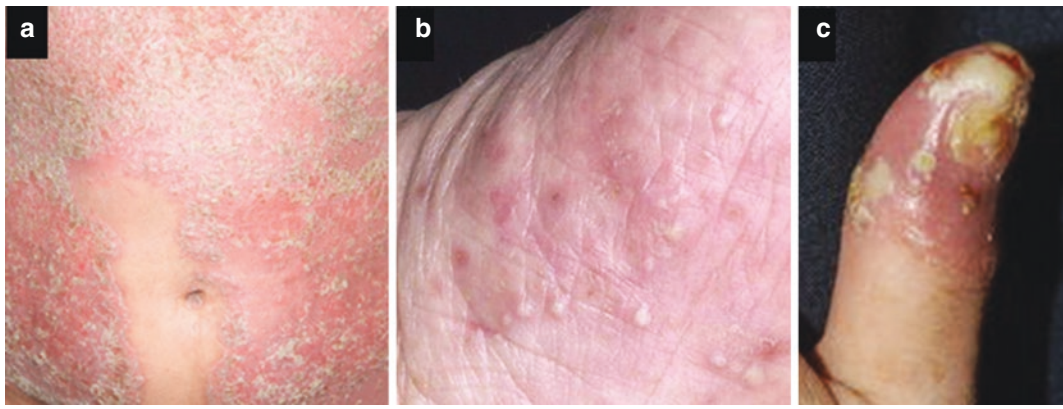

Fig. 26.2 Clinical features of pustular psoriasis. (**a**) Lakes of pustules and background erythema on the trunk of a patient with generalised pustular psoriasis. *(Source: Shu et al, Eur J Dermatol 2014, https://doi.org/10.1684/ejd.2014.2382; Fig 1D).* (**b**) Pustules on the palm of a patient with palmar plantar pustulosis. *(Source: Mossner et al, Arch Dermatol Res 2008, https://doi.org/10.1007/s00403-008-0831-8; Fig 1B*). (**c**) Pustules and onychodystrophy on the distal thumb and nail apparatus of a patient with acrodermatitis continua of Hallopeau. *(Source Wang et al, Arch Dermatol Res 2016, https://doi.org/10.1007/s00403-015-1611-x; Fig 1B)*

26.5.2 Palmar Plantar Pustulosis and Acrodermatitis Continua of Hallopeau

Localised pustular psoriasis encompasses palmar plantar pustulosis (PPP) and acrodermatitis continua of Hallopeau (ACH). In contrast to GPP, these disease subtypes are not associated with systemic symptoms.

PPP presents with crops of persistent (>3 months) pustules localised to the palms and soles, on a background of normal or inflamed skin (Fig. 26.2b). Secondary complications include painful fissures, pruritus and a burning sensation of the skin. Patients may also develop arthralgias and arthritis. PPP can manifest with inflammatory synovitis, acne, hyperostosis and aseptic osteitis in the context of SAPHO syndrome and chronic non-bacterial osteomyelitis [28]. There is also an increased prevalence of psoriasis vulgaris and autoimmune thyroid disease [29].

First described in 1890 by Francois Henri Hallopeau [30], ACH is characterised by the gradual onset of persistent (>3 months) pustules affecting the nail bed or matrix, leading to onychodystrophy, destruction of the nail plate and anonychia (Fig. 26.2c) [31]. Although the distal aspects of digits are initially affected, proximal progression may occur, with subsequent involvement of the hands, forearms and feet. Secondary complications include skin atrophy, ulceration and dermal sclerosis. Bone abnormalities such as osteolysis of the distal phalanges and interphalangeal joint arthropathy may arise in severe, refractory cases. Progression to GPP has also been described in long-standing cases.

26.6 Investigations

Key Concepts

- **Histology staining will reveal intra-epidermal collection of neutrophils in all forms of pustular psoriasis**
- **In GPP, blood tests will show leukocytosis and an elevation of acute phase reactants**
- **DNA testing can be undertaken to identify mutations in disease associated genes**

The pathognomonic histological hallmarks of pustular psoriasis are prominent intra-epidermal collections of neutrophils [32] (Fig. 26.3). These may be located in the upper epidermis (Kogoj spongiform pustules), spinous zone (Munro's microabscesses) and within or beneath the cornified layer (intra or sub-corneal pustules). Other characteristic findings are diffuse dermal neutrophilic infiltration, tortuous dilated blood vessels,

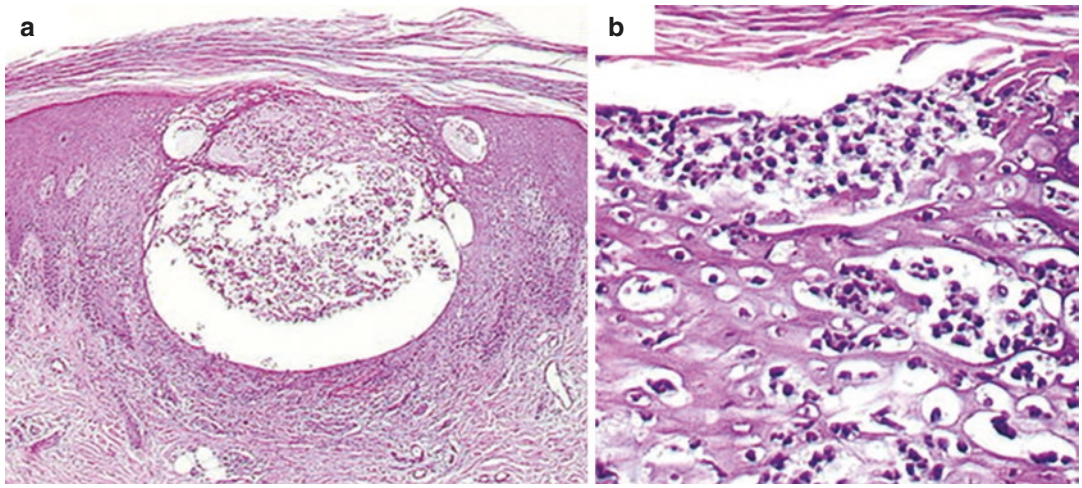

Fig. 26.3 Histological features of pustular psoriasis. (**a**, **b**) Intra-epidermal accumulation of neutrophils and subcorneal pustule formation in a patient with palmar plantar pustulosis (haematoxylin and eosin staining of a biopsy from the sole) (*Source: Mossner et al, Arch Dermatol Res 2008, https://doi.org/10.1007/s00403-008-0831-8; Fig 1C-1D)*

acanthosis with elongation of rete ridges and parakeratosis of the stratum corneum. The latter two psoriasiform features are most prominent in chronic lesions.

Skin cultures are negative, as the pustules are sterile.

In patients with GPP who are experiencing a systemic flare, blood tests show prominent leukocytosis, with neutrophilia and lymphopenia evident on differential white cell counts. Serum inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate are elevated and the serum albumin is reduced.

Liver function tests may be elevated and percutaneous fluid losses may cause pre-renal impairment (indicated by raised serum urea and creatinine). Serum IgG or IgA are increased.

In recent years, the results of the above laboratory tests have often been complemented by mutation screening of known pustular psoriasis genes (*IL36RN, AP1S3* and *CARD14*).

26.7 Diagnosis

Key Concepts

- **Consensus diagnostic criteria, based on the observation of sterile, macroscopically visible pustules, have recently been formalised**
- **The most important differential diagnosis is acute generalised exanthematous pustulosis**

GPP, ACH and PPP are diagnosed based on clinical presentation, with supportive evidence from histological staining, blood tests and genetic investigations. Consensus diagnostic criteria have been recently formalised by the European Rare And Severe Psoriasis Expert Network (ERASPEN) [25], as reported in Table 26.2.

It is important to exclude acute generalised exanthematous pustulosis (AGEP) by taking a detailed medication history and examining lesional skin biopsies for features suggestive of an underlying drug trigger (eosinophils within pustules and the dermis, necrotic keratinocytes and the absence of tortuous dilated blood vessels; [32]). A diagnosis of AGEP is also supported by blood eosinophilia. Infective causes of pustular rashes should also be excluded using microbial cultures of skin and blood samples, and histopathological stains such as Periodic-acid Schiff.

Table 26.2 Pustular psoriasis diagnostic criteria

	Clinical appearance	Course
Generalised pustular psoriasis	Primary, sterile, macroscopically visible pustules on non-acral skin (excluding cases where pustulation is restricted to psoriatic plaques) With/without systemic inflammation With/without psoriasis vulgaris	Relapsing (>1 episode) or persistent (>3 months)
Palmar plantar pustulosis	Primary, sterile, macroscopically visible pustules on palms and/or soles With/without psoriasis vulgaris	Persistent (>3 months)
Acrodermatitis continua of Hallopeau	Primary, sterile, macroscopically visible pustules affecting the nail apparatus With/without psoriasis vulgaris	Persistent (>3 months)

Adapted from [25]

26.8 Treatment

Key Concepts

- **Treatment options are limited as pustular psoriasis is often refractory to therapy**
- **Owing to the rarity of the disease, there is a paucity of trial data examining drug efficacy**
- **Current recommended therapies include acitretin, cyclosporine, methotrexate and TNF antagonists**
- **Emerging biologic agents targeting IL-17A and IL-1 cytokines may hold promise for the future**

Table 26.3 Systemic treatments for pustular forms of psoriasis [33]

Drug	Mechanism	Dose range	Onset of action
Acitretin	Retinoid; decreases activity of Th1 and T17 cells and normalises keratinocyte differentiation	0.125–1 mg/kg/day	Days to weeks
Cyclosporine	Calcineurin inhibitor; inhibits T cell activity	2.5–5 mg/kg/day	~2 weeks
Methotrexate	Reduces NF-κB mediated cytokine and chemokine transcription	5–25 mg/week	Several weeks
Infliximab	TNF antagonist	5 mg/kg every 8 weeks	Days to weeks
Etanercept	TNF antagonist	50 mg every week	Weeks
Adalimumab	TNF antagonist	40 mg every 2 weeks	Weeks

Since the prevalence of pustular psoriasis is low, there is a paucity of clinical trial data evaluating the efficacy of therapeutics. Current treatment guidelines, which are based predominantly on evidence from case reports, recommend tailoring therapy according to the disease severity and co-morbidities observed in each patient [33].

26.8.1 Generalised Pustular Psoriasis

First-line therapies for GPP include acitretin, cyclosporine and methotrexate, with TNF antagonists recommended as second-line agents [33] (Table 26.3). Although recent research has uncovered specific genetic causes of pustular psoriasis, there have been no studies of correlation with responses to particular treatments.

Acitretin is an oral retinoid that decreases the activity of Th1 and T17 cells and normalises keratinocyte differentiation. Response may be seen within several days to weeks. Due to the associated risks of hyperlipidaemia and liver toxicity, regular monitoring of blood parameters is required. Other dose-limiting side effects include cheilitis, hair loss and xerosis [34]. Importantly, acitretin must be avoided in women of childbearing age due to its teratogenicity, which lasts for years since the drug is stored in adipose tissue. Isotretinoin may be used as an alternative due to its short half-life, however it may be less efficacious.

Cyclosporine is a calcineurin inhibitor that inhibits T cell activity [35]. Although it is fast acting (response is typically observed within 2 weeks) and not contra-indicated in pregnancy, the use of this medication is limited by side effects including nephrotoxicity, hypertension and numerous drug-drug interactions. Long-term treatment is also associated with increased risk of malignancies such as lymphoma and squamous cell carcinoma.

Methotrexate reduces levels of NF-κB mediated cytokine and chemokine transcription through inhibition of the enzyme 5-aminoimidazole-4-caboxamide ribonucleotide transformylase [36]. It is slower acting than cyclosporine, requiring several weeks to reach a therapeutic dose, and is contra-indicated in pregnancy. Regular blood monitoring is required during therapy due to the risks of bone marrow toxicity (particularly in individuals with renal impairment, concurrent infections and the elderly) and cirrhosis. Risk factors for methotrexate-induced hepatic toxicity include diabetes, hyperlipidaemia, excessive alcohol intake and previous hepatitis B or C infections. Concurrent oral folate supplementation is recommended in order to reduce the risk of other side effects such as nausea and macrocytic anemia.

Oral corticosteroids are not recommended in GPP due to the risk of disease rebound upon withdrawal [33]. Topical corticosteroids may, however, be useful adjuncts to systemic therapies in GPP.

In patients with severe, acute GPP, cyclosporine and TNF antagonists, in particular infliximab, are recommended due to their rapid onset of action. Infliximab is an intravenous chimeric monoclonal antibody that binds TNF and several case reports and case series document its efficacy in GPP [37]. In contrast to traditional systemic

agents, biologics such as infliximab have less risk of liver, renal or bone marrow toxicity. TNF blockade has, however, been associated with increased rates of infections (most notably tuberculosis), demyelination, worsening heart failure and malignancies such as squamous cell carcinoma. The use of other TNF antagonists such as etanercept (a TNF receptor fusion protein) and adalimumab (a human monoclonal antibody targeted to TNF) are supported by case reports [38].

The more recently developed IL-12/IL-23 (ustekinumab) and IL-17A antagonists (secukinumab) have superior safety profiles to TNF blockers and reports are emerging of rapid and sustained responses in GPP [39, 40]. The efficacy of secukinumab was recently supported by a Japanese phase III open-label, single arm study (n = 12) [41] and dual therapy with ustekinumab and low dose acitretin has also been reported to be effective [42].

26.8.2 Palmar Plantar Pustulosis and Acrodermatitis Continua of Hallopeau

Localised forms of pustular psoriasis may be treated with topical corticosteroids, often administered in combination with salicylic acid or calcipotriol, and under occlusion, so as to improve penetration through the thick stratum corneum of the palms and soles. However, if multiple digits/nails are involved or if the disease is recurrent or resistant to topical treatment, systemic agents may be required. First line systemic therapies are acitretin, cyclosporine and psoralen combined with ultraviolet A irradiation (PUVA) [33].

PUVA photo-chemotherapy, which may be used in combination with acitretin, has anti-inflammatory effects by suppressing T17 lymphocytes, promoting Th1 class switching and activating Treg and Th2 cells [43]. However, long-term use of PUVA is precluded by its carcinogenic potential, since there is an association with increased risk of cutaneous squamous cell carcinoma and malignant melanoma.

Despite the potential for paradoxical induction or exacerbation of PPP during TNF antagonist therapy [44], there are numerous reports of effective treatment of localised pustular psoriasis with these agents [45]. However, a small (n = 15) placebo controlled trial of etanercept in PPP showed no benefit [46], so further systematic investigation in larger patient cohorts is required. There are also conflicting data regarding the effect of ustekinumab. Successful use has recently been documented in cases of refractory PPP [47], however a randomised controlled trial (n = 10 patients) showed no statistically significant difference between the drug and placebo at 16 weeks [48].

Recent research suggesting a pathogenic role for IL-1 up-regulation downstream of IL-36 [10, 12] has supported the use of IL-1 blockers as a novel therapeutic strategy. Indeed, case reports have documented the efficacy of the IL-1 receptor antagonist anakinra in all forms of pustular psoriasis [49] and a randomised controlled trial in patients with PPP is now underway (http://apricot-trial.com/). Monoclonal antibodies specifically targeting the IL-36 receptor have also been developed and testing of these agents in early phase clinical trials is eagerly anticipated [50].

26.9 Outcome/Prognosis

Key Concepts

- **ACH and PPP present as chronic diseases that are mostly refractory to treatment**
- **Acute GPP flares are life-threatening and require immediate hospitalisation**

The new consensus phenotypic criteria defined by the ERASPEN consortium emphasise that GPP may display either a relapsing (>1 episode) or a persistent (>3 months) course, whereas ACH and PPP follow a chronic pattern (>3 months), with intermittent relapses [25]. Although symptoms may spontaneously remit, this is a rare occurrence and the disease is often refractory to treatment. Further, only limited follow up is documented in the current literature, so that the consequences of stopping a successful therapy once the patient has achieved a period of remission are not known. Likewise, the long-term effects of the newer biologic agents have yet to be investigated.

Importantly, acute flares of GPP are life-threatening, since skin lesions can progress rapidly to cover a large body surface area, leading to hypovolemia, hypothermia and sepsis. During pregnancy, GPP can additionally result in an increased rate of fetal mortality, which is attributable to placental insufficiency. The disease can also cause symptomatic maternal hypocalcaemia, leading to tetany and convulsions [51]. Early admission of patients with acute onset GPP and commencement of therapy is thus essential.

Taken together, there is substantial unmet need in the management of all forms of pustular psoriasis and no curative therapies exist. Current treatments often lead to an incomplete resolution of disease or are not suitable for prolonged use due to the risks of organ toxicity or eventual loss of efficacy (e.g. formation of anti-drug antibodies to biologics).

Future investigation into the pathogenic mechanisms underlying pustular psoriasis will inform the design of new treatments. These will then need to be tested in multi-centre clinical trials in order to recruit sufficient numbers of patients with this rare yet debilitating disease.

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Hydatidiform Moles

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Abstract

Hydatidiform mole (HM) is a form of human pregnancy loss that is characterized by the absence of, or abnormal, embryonic development and hyperproliferation of the trophoblast. The common form is sporadic and has a multifactorial etiology. Recurrent HM has a Mendelian etiology and segregates according to an autosomal recessive mode of transmission. To date, two genes, *NLRP7* and *KHDC3L*, responsible for recurrent HM have been identified. *NLRP7* is the major gene for this condition and underlies the etiology of recurrent HM in 55% of patients with at least two occurrences of HM. Here, we review the current knowledge about this condition and focus on the known roles of *NLRP7* in the pathogenesis of recurrent HM.

Keywords

Hydatidiform mole · *NLRP7* · Imprinting defect · Subcortical maternal complex (SCMC) Early embryonic arrest · Decreased inflammation · Delayed rejection

Abbreviations

ASC	Apoptosis-associated speck-like protein containing a CARD
ATP	Adenosine triphosphate
CARD	Caspase activation and recruitment domain
CDKN1C	Cyclin dependent kinase inhibitor 1C
CHM	Complete hydatidiform mole
FIGO	International Federation of Gynecology and Obstetrics
GTN	Gestational trophoblastic neoplasia
hCG	Human chorionic gonadotropin
HEK293	Human embryonic kidney cells 293
HM	Hydatidiform mole
IL1B	Interleukin 1 beta
KHDC3	KH domain-containing protein 3
KHDC3L	KH domain containing 3 like, subcortical maternal complex member
LPS	Lipopolysacharide
LRR	Leucine-rich domain
NACHT	NAIP, CIITA, HET-E, and TP1 domain
NLR	NOD-like receptor
NLRP2	NLR family pyrin domain containing 2

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NLRP3	NLR family pyrin domain containing 3
NLRP5	NLR family pyrin domain containing 5
NLRP7	NLR family pyrin domain containing 7
NOD	Nucleotide-binding oligomerization domain
OOEP	Oocyte expressed protein
$p57^{KIP2}$	The protein coded by *CDKN1C*
PADI6	Peptidyl arginine deiminase 6
PHM	Partial hydatidiform mole
POC	Product of conception
PYD	Pyrin domain
RHM	Recurrent hydatidiform mole
SCMC	Subcortical maternal complex
THP1	Transfected monocytic cell line
TLE6	Transducin like enhancer of split 6
ZBED3	Zinc finger BED-type containing 3

Key Points

- **Two genes, *NLRP7* and *KHDC3L*, have been found to be responsible for recurrent diploid biparental hydatidiform mole**
- ***NLRP7* is the major gene and has roles in female reproduction and inflammation**
- **Ovum donation is recommended for patients with biallelic mutations in *NLRP7* or *KHDC3L***

27.1 Introduction

In his description of the first case of hydatidiform mole (HM) from New England in 1638, Governor Winthrop wrote, "If you consider each of them according to the representation of the whole, they were altogether without form; but if they were considered in respect of the parts of each lump of flesh, then there was a representation of innumerable distinct bodies in the form of a globe" [1]. The "innumerable distinct bodies in the form of a globe" is the appearance of hydropic chorionic villi observed in these pregnancies (Fig. 27.1a). This unfortunate event of pregnancy considered in the past as "monstrous birth" is now called HM, which comes from the Greek word "hydatidisia" (a drop of water) and "mola" (millstone). In this condition, the pregnancy arrests very early, most likely during early preimplantation development, and is characterized by the absence of, or abnormal, embryonic development and excessive proliferation of the trophoblast. HM can be sporadic, when it occurs once in the patient's reproductive life, or recurrent (RHM), defined by the occurrence of at least two HMs. HM can also happen in more than one family member and such cases are referred to as famil-

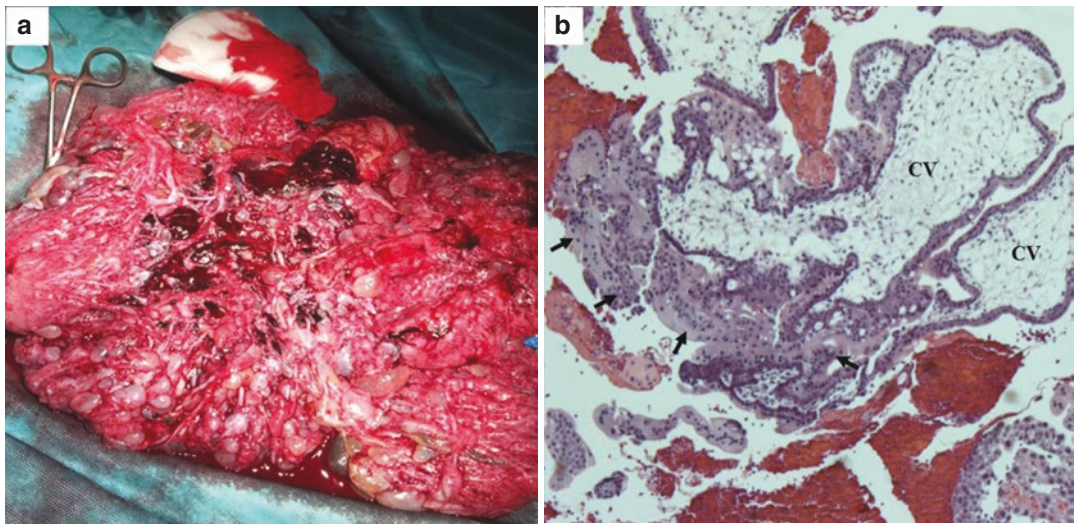

Fig. 27.1 (**a**) Gross morphology of a hydatidiform mole (HM). (**b**) Histopathological cross-section of a HM showing circumferential trophoblastic proliferation (arrows) around a chorionic villous (CV)

ial cases. In this chapter, we review the pathology of HM and describe the roles of the two known genes responsible for a Mendelian form of this condition.

27.2 Epidemiology

The incidence of HM is 1 in 600–1000 pregnancies in Western countries, [2, 3] but 2–10 times higher in Asian, African, and Latin American countries, with the highest frequency being 12 in 1000 pregnancies in Southeastern Asia [3–5]. The incidence of HM is higher in women at the extremes of reproductive age, slightly higher in women aged 15–20 and dramatically higher in women over 40 [2, 6]. Recurrence of a second HM affects 1–9% of women with a prior HM depending on populations and studies [7–12]. A study from the United Kingdom found that 1 in 76 women with HM will develop a second HM, and 13 out of 100 women with 2 prior HM will experience a third HM [6].

27.3 Classification Based on Histopathology

At the histopathological level, HM are classified into two types, complete HM (CHM) and partial HM (PHM), based on several features and most importantly on the degree of the trophoblastic proliferation and embryonic tissue differentiation. CHM usually have marked circumferential trophoblastic proliferation (Fig. 27.1b) with absence of embryonic tissues and extraembryonic membranes, while PHM have moderate focal trophoblastic proliferation and may contain embryonic tissues and extraembryonic membranes. HM is the most common of gestational trophoblastic diseases that include other clinical entities, invasive moles, choriocarcinomas, placental site trophoblastic tumors and epithelioid trophoblastic tumors. The latter four are considered as gestational trophoblastic neoplasia (GTN) due to their invasive and metastatic potential. Invasive moles show the same pathological features of CHM, but they invade the myometrium or the uterine veins. Choriocarcinomas, placental site trophoblastic tumors and epithelioid trophoblastic tumors are solid trophoblastic tumors that express immunohistochemical markers of villous and extra-villous trophoblasts [13].

27.4 Sporadic Hydatidiform Mole

27.4.1 Etiology

The fact that sporadic HM occur only once in the reproductive life of the patients and may follow or precede normal pregnancies suggest that sporadic HM do not have a strong inherited genetic etiology at their origin. To better understand what could underlie their occurrence, epidemiological studies have looked at various risk factors that could predispose to sporadic HM such as maternal age, reproductive history, ethnicity, and various environmental factors such as diet, oral contraception, herbicides, and ionizing radiation. Of all these factors, maternal age [2], history of miscarriages [14], maternal ethnicity [15], and the use of oral contraceptive drugs [16] are well-established risk factors for HM that were replicated in several studies and populations. Advanced maternal age is the strongest and most consistent risk factor for sporadic CHM [12, 17].

27.4.2 Genotype

There are three main genotypic types of sporadic HMs (Figs. 27.2 and 27.3). CHMs are usually diploid androgenetic with two copies of the paternal genome and no maternal genome, and may originate from monospermic (two identical copies of a haploid paternal genome) or dispermic (two different haploid paternal genomes) fertilization. PHMs are usually triploid dispermic with two copies of the paternal genome and one copy of the maternal genome.

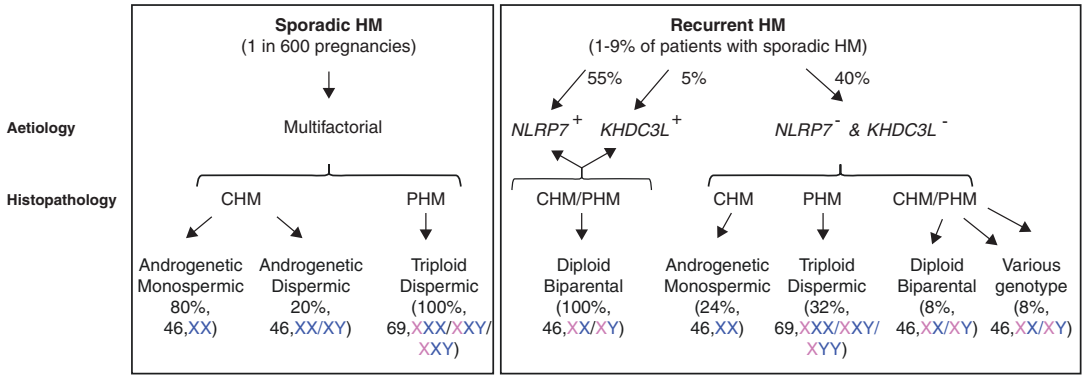

Fig. 27.2 Frequencies and classification of sporadic and recurrent hydatidiform mole (HM) by etiology, histopathology and genotype [18, 19]. The pink and blue colors refer to the presence of the maternal and paternal genomes, respectively, in the HM. In patients with RHM, the frequencies of the three types of genotypes are shown. The remaining patients were either misdiagnosed with RHM or had only one molar tissue available for genotype analysis [18]. CHM stands for complete hydatidiform mole; PHM, for partial hydatidiform mole; POC, for product of conception

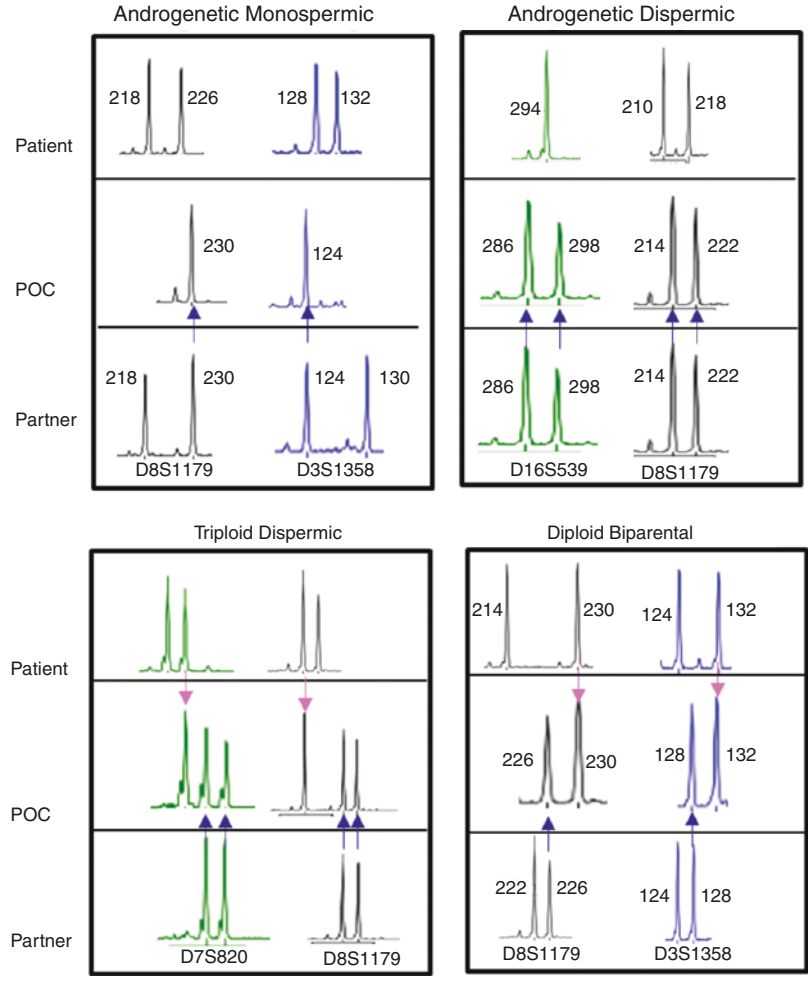

Fig. 27.3 The genotypes of hydatidiform mole (HM) can be determined by performing microsatellite genotyping at different markers and comparing the alleles in the product of conception with those of the parents. An example of each genotypic type of HM at two informative markers is shown. The number represents the size of the alleles in the sample. Allele sizes in triploid dispermic partial hydatidiform mole were omitted for simplification. The names of the markers are mentioned below the chromatograms. POC stands for product of conception

27.5 Recurrent Hydatidiform Moles

- **Patients with at least two episodes of HM should be tested for mutations in *NLRP7* and *KHDC3L***
- **Patients with biallelic mutations can be counselled that their chances of having a live birth from their own oocytes is 1.7% of all their pregnancies and this occurs in 7% of patients**
- **Ovum donation is recommended for such patients**

27.5.1 Etiology

Recessive mutations in two genes have been described to underlie the causation of RHM. *NLRP7* is the major gene associated with RHM and is mutated in 48–80% of patients, depending on patients' ascertainment criteria and populations [20–24]. The lowest frequency of patients with biallelic *NLRP7* mutations was found in China [21] (48%) and the highest in India [22], Pakistan [23], and Mexico [24], where it reaches approximately 80%. In a recent study [18], we found that among 113 unrelated patients referred to our laboratory from all populations, 55% had biallelic mutations in *NLRP7* (Fig. 27.2) and 5% had biallelic mutations in *KHDC3L,* another gene responsible for RHM [25].

27.5.2 Genotype of RHM

RHM from patients with biallelic mutations in *NLRP7* or *KHDC3L* are all diploid biparental (Figs. 27.2 and 27.3). In a recent study, we found that among patients with no mutations in either *NLRP7* or *KHDC3L*, only a minority of them (8%) have diploid biparental HM, while 24% have diploid androgenetic monospermic CHM and 32% have triploid dispermic PHM (Figs. 27.2 and 27.3) [18]. The remaining patients were excluded as they had been misdiagnosed with RHM. Of note, triploid dispermic PHM are not highly recurrent and usually recur only twice in each patient [18, 26]. Androgenetic monospermic mole can recur more than two times in the same patient, but less than diploid biparental HM caused by mutations in *NLRP7* or *KHDC3L*, which may recur more than ten times in the same patient.

27.5.3 Roles of NOD-Like Receptor (NLR) Family Pyrin Domain Containing 7 (NLRP7)

27.5.3.1 NLRP7 and Imprinting

The fact that recurrent diploid biparental and diploid androgenetic moles share the same histopathological features has led to the question about the role of NOD-like receptor (NLR) family pyrin domain containing 7 (NLRP7) in imprinting. Genomic imprinting is a process that leads to the expression of only one of the two parental copies of a gene. Several studies have investigated DNA methylation in the molar conceptions of patients with biallelic *NLRP7* or *KHDC3L* mutations either at specific imprinted genes [23, 27–29] or at the whole genome level [30]. These studies consistently demonstrated that there is a lack of DNA methylation marks on the majority of maternally methylated regions of imprinted genes, most likely due to an oocyte defect that started before the time of the acquisition of the methylation marks [30].

Consistent with the abnormal imprinting in molar tissues from patients with biallelic *NLRP7* mutations is the lack of expression of $p57^{KIP2}$ in most diploid biparental HMs, similar to androgenetic CHM. $p57^{KIP2}$ is coded by *CDKN1C* gene, which is expressed only from the maternal genome in the cytotrophoblast of normal first trimester pregnancies. In a comprehensive study by our group, we found a significant genotype-phenotype correlation between the severity of *NLRP7* mutations in the patients and the severity of the molar features. Severe *NLRP7* mutations were associated with the absence of $p57^{KIP2}$ expression, the absence of embryonic tissues, and excessive trophoblastic proliferation. However, less severe

NLRP7 mutations in the patients were associated with positive $p57^{KIP2}$ expression, presence of some embryonic tissues, and mild trophoblastic proliferation. This suggested that the NLRP7 defect acts upstream of $p57^{KIP2}$, and impairs, directly or indirectly, the balance between cellular proliferation and differentiation, according to the severity of its mutations [31]. The role of NLRP7 in trophoblast differentiation was also demonstrated in another in-vitro cellular model where *NLRP7* knockdown in human pluripotent cells accelerated the expression of some trophoblastic markers [32]. In conclusion, data from these studies suggest that biallelic *NLRP7* or *KHDC3L* mutations impair the establishment of maternal imprints during oocyte development, which is the earliest defect identified so far.

27.5.3.2 NLRP7 and the Subcortical Maternal Complex

In humans, *NLRP7* is expressed in many tissues with the highest level of its transcripts found in testis [33, 34]. However, among individual human cells, the highest level of *NLRP7* transcripts is found in oocytes at the germinal vesicle stage [35]. *NLRP7* transcripts are also present in different stages of oocyte development and in preimplantation embryos [35].

NLRP7 protein localizes mainly to the cortical region in all stages of human oocytes. After the first zygotic division, NLRP7 localization becomes restricted to the outer cortical region and absent from the cell-to-cell contact [36], which is identical to the localisation of other proteins of the subcortical maternal complex (SCMC). The SCMC is a multiprotein complex expressed only in oocytes and preimplantation embryos at the subcortical region. Recessive mutations in genes coding for the SCMC proteins lead to defective oocytes and consequently early embryonic arrest during preimplantation stages. In mice, four members of the SCMC were identified (NLRP5, OOEP, TLE6 and KHDC3) [37]. NLRP5, OOEP, and TLE6 were shown to interact directly with each other while KHDC3 interacts in oocytes only with NLRP5. Genetic ablation of *Ooep* or *Nlrp5* in mice result in the destabilization of the SCMC and the diffuse localization of its proteins in the cytoplasm. Other maternal-effect genes such as *Padi6*, *Nlrp2*, *Zbed3* were also suggested to be part of the mouse SCMC [38–40].

In humans, four members of the SCMC (KHDC3L, OOEP, NLRP5, and TLE6) were shown to interact in transfected cells. Of these, *KHDC3L* and *TLE6*, play causal roles in recurrent diploid biparental HM and female infertility, respectively [25, 41]. In addition, another maternal-effect gene suggested to participate in the SCMC in mice, *PADI6*, was shown to be responsible for female infertility [42, 43] and recently for recurrent miscarriages and a HM in one patient [44].

27.5.3.3 NLRP7 and Early Embryonic Development

Despite the fact that in vitro fertilization was introduced into medical practice 40 years ago, it is not known how a conception that leads to a HM develops during preimplantation stages. The first description of how HM develops was reported by Edwards in 1990 and 1992 [45, 46] in a patient with RHM and this was followed by two other case reports [47, 48]. However, unfortunately, the causative genes responsible for RHM in these three patients are not known nor are the genotypes of their moles. Recently, Sills et al. reported a patient with 5 RHMs and biallelic *NLRP7* mutations, recorded the early development of her embryos after intra-cytoplamic sperm injection, and provided photographs of two embryos [49]. In this patient, 15 oocytes were retrieved and 10 were fertilized. All embryos had diploid biparental genome, which is in line with the diploid biparental genomes of HM from patients with *NLRP7* mutations. Of the 10 fertilized oocytes, none was morphologically normal and suitable for transfer to the patient. This case is the first detailed account of how a molar pregnancy develop during preimplantation development and suggests that abnormalities in the conceptions of these patients start very early during preimplantation development. In addition, this case tells us that, perhaps, what leads to HM may not be a healthy, normal growing early cleavage embryo.

27.5.3.4 NLRP7 and Inflammation

NLRP7 is a member of the nucleotide-binding oligomerization domain (NOD)-like receptor

(NLR) family of proteins, a type of cytoplasmic pattern recognition receptor that senses intracellular danger signals and orchestrates inflammatory responses to fight them. The NLRP subfamily is characterized by the presence of an N-terminal pyrin domain (PYD) in addition to the two other domains, NAIP, CIITA, HET-E, and TP1 domain (NACHT) and leucine rich domain (LRR), found in NLR proteins. The PYD is required to recruit the adaptor protein apoptosis-associated speck-like protein containing a caspase-recruitment domain (CARD) (ASC), which in turn recruits pro-caspase-1 and forms an inflammasome, a cytosolic multi-protein complex that activates pro-caspase-1 and generates mature caspase-1. The latter cleaves pro-IL1β and pro-IL18 into their mature forms for secretion. The NACHT domain contains an ATPase domain and is required for self-oligomerization [50] and ATP hydrolysis. The LRR sense intra-cellular danger signals and bind to their ligands (see Chap. 5).

Among NLRPs, the NLRP3 inflammasome is the most studied. For NLRP7, we know that its inflammasome assembles in response to several stimuli such as some bacteria (gram-negative *Legionella pneumophili*, *Archoleplasma laidlawii*, *Staphylococcus aureus*), Mycoplasma species, *Mycobacterium bovis* (Beijing strain), and their derived products (some heat-killed bacteria, synthetic acylated lipoproteins, and lipopolysaccharides) [51–53]. Within these inflammasomes, overexpressed normal NLRP7 interacts with ASC, caspase-1, and IL-1β and downregulates IL-1β production. However, there are contradictory results about how overexpressed NLRP7 downregulates IL-1β production in transiently transfected HEK293 cells. While Kinoshita et al. found that NLRP7 inhibits pro-caspase 1 and pro-IL1β processing [33], Messaed et al., found that it reduces the amount of intracellular pro-IL-1β [51]. Despite the ubiquitous expression of NLRP7, its level of expression in hematopoietic cells is relatively low. Consequently, model systems overexpressing NLRP7 are unlikely to accurately reflect its physiological role.

At the physiological level, peripheral blood cells from patients with biallelic *NLRP7* mutations were shown to secrete lower amounts of IL-1β in the extracellular milieu as compared to cells from control subjects [51, 54]. This finding was replicated in a stably transfected monocytic cell line (THP1) where knocking down *NLRP7* with small interfering RNA decreased IL-1β secretion [52]. These data suggest that wildtype NLRP7 is required for normal IL-1β secretion and inflammation, but its biallelic mutations, or loss of function, downregulate IL-1β secretion and consequently inflammation. This remains to be dissected and elucidated in future studies. This suggestion is in agreement with the fact that patients with RHM, or HM in general, do not manifest signs of inflammation as do patients with mutations in *NLRP3* [55, 56] or *NLRP12* [57].

At the protein level, in THP1 cells stimulated with lipopolysaccharide (LPS), NLRP7 was shown to co-localize with giantin and γ-tubulin markers of cis-Golgi and microtubule organizing center, respectively [51]. This led to the suggestion that biallelic *NLRP7* mutations impair IL-1β secretion by affecting microtubule structures and consequently intra-cellular transport and trafficking of IL-1β containing vesicles to the extra-cellular milieu.

27.5.3.5 Synopsis of the Pathogenesis of RHM Due to Biallelic *NLRP7* Mutations

- **Impaired establishment of methylation marks at imprinted genes during oogenesis**
- **NLRP7 is a structural protein and part of the oocyte cytoskeleton**
- **Impaired differentiation and increased proliferation of the trophoblast**
- **Downregulated inflammatory response and delayed rejection of arrested pregnancies**

NLRP7 does not have a mouse orthologue; consequently, elucidating its functional roles and the impacts of its mutations has been addressed by studying patients' cells and tissues and using various cellular models, peripheral blood mononuclear cells, monocytic cell lines, and human embryonic stem cells. None of the results obtained on these cellular models alone may explain the different aspects of this

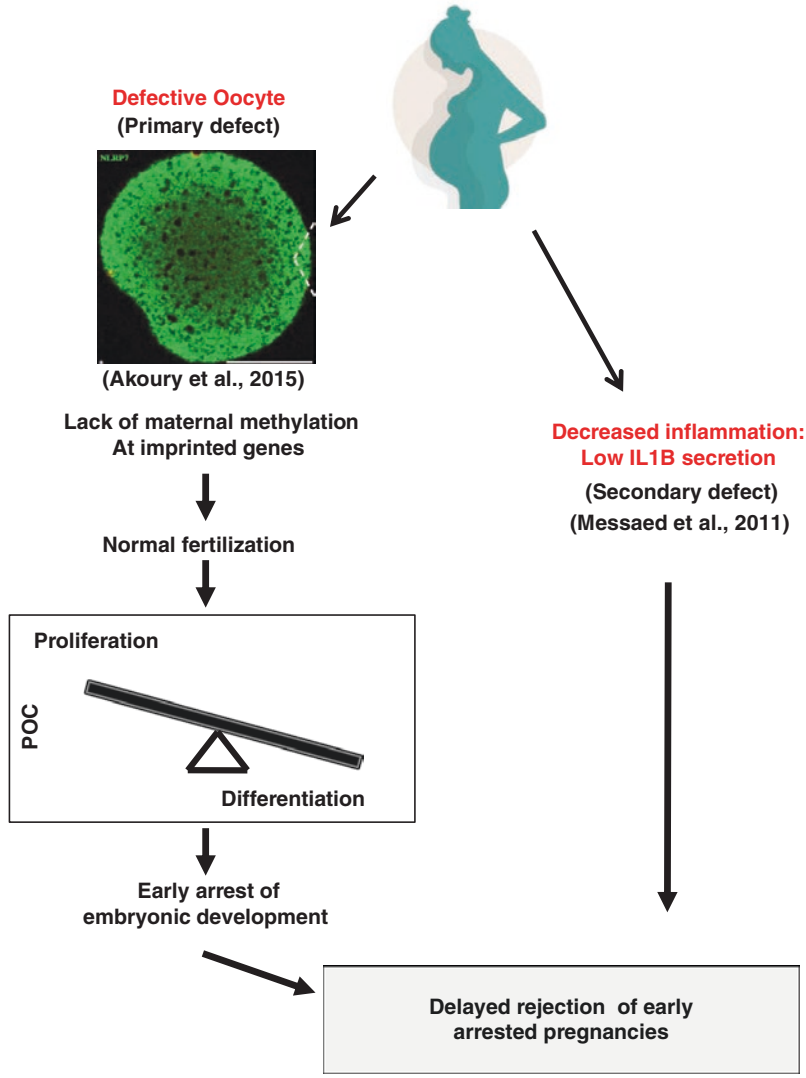

Fig. 27.4 A suggested model that recapitulates the various roles of NLRP7 in the pathology of recurrent hydatidiform mole (RHM). The primary defect is in the oocyte that fails to acquire maternal methylation marks at imprinted genes. This defect does not affect fertilization, which appears to be normal. However, early preimplantation development is impaired and the molar tissues display a shift from normal tissue differentiation toward excessive proliferation. Because blood mononuclear cells from the patients fail to secrete normal amounts of interleukin (IL)-1β, patients, perhaps, fail to mount an appropriate inflammatory response to reject their arrested pregnancies; it is the delayed rejection of these earlier arrested pregnancies that contribute, partly, to the molar phenotype. POC stands for product of conception

condition because this pathology involves several tissues and cannot be studied in one cellular type. We believe that combining the data and knowledge obtained in different cellular types and tissues may better recapitulate the various aspects of the pathogenesis of RHM (Fig. 27.4).

Patients with biallelic *NLRP7* mutations produce defective oocytes that lack maternal methylation marks on differentially methylated regions of imprinted, paternally expressed genes. This defect is the earliest detected and is the primary defect in this disease. Oocytes from such patients appear to fertilize normally, each by a single

spermatozoid, but their early embryonic development is severely impaired. Despite this, such embryos are able to form trophectoderm, implant, and their trophectoderm differentiates into its three main cellular types, cytotrophoblast, syncythiotrophoblast, and extravillous trophoblast. However, the cytotrophoblast does not complete its differentiation and exit the cell cycle; rather it keeps proliferating. Because blood mononuclear cells from patients with biallelic *NLRP7* mutations have decreased IL1β secretion, the patients may not be able to mount a normal and appropriate inflammatory response to reject these early arrested pregnancies. Consequently, the delayed rejection of these conceptions in which embryonic tissue differentiation is severely impaired, fetal circulation does not start, and the cytotrophoblast keeps proliferating, leading to the molar phenotype (Fig. 27.4).

27.6 Clinical Manifestations

- **Vaginal bleeding in the first trimester is the most common clinical manifestation**
- **Cystic changes in the placenta are detected on ultrasound**
- **There are higher human chorionic gonadotropin (hCG) levels than in normal pregnancies of matching gestational stage**

The clinical manifestations of HM have changed over time with the advances of medicine and the availability of ultrasonography. Consequently, HM are detected earlier than in the past and before the full manifestations of all their clinical and histopathological signs. In current medical practice, HM may be asymptomatic and discovered only after ultrasonography at the first obstetric visit or manifest one of the following symptoms, most commonly, vaginal bleeding in the first trimester of pregnancy and uterine size greater than expected for the time from conception. Less frequently, patients with HM may present with anemia, preeclampsia, hyperemesis, hyperthyroidism, respiratory distress, and ovarian theca lutein cysts [58].

27.7 Laboratory/Imaging/Pathology

27.7.1 Human Chorionic Gonadotropin (hCG)

Human chorionic gonadotropin (hCG) is one of the pregnancy hormones that is secreted by trophoblastic cells [59] when they differentiate from cytotrophoblast to syncytiotrophoblast. The increased proliferation of the trophoblast in HM is associated with an increased hCG secretion, frequently over 100,000 IU/L in CHM and usually below 100,000 IU/L in PHM [60]. After surgical evacuation of the HM, the patients are monitored weekly for hCG in order to detect trophoblast retention or post-molar malignant transformation into gestational trophoblastic neoplasia (GTN) [61, 62]. Remission after HM is defined by normalization of hCG level (or non-pregnant level). A plateau or increase of hCG titers during follow-up should be interpreted as trophoblast retention of post molar GTN.

27.7.2 Imaging

Ultrasound manifestations of HM include cystic changes in the placenta (Fig. 27.5), and usually the absence of fetus and membranes or the presence of non-viable fetus. Pelvic endovaginal

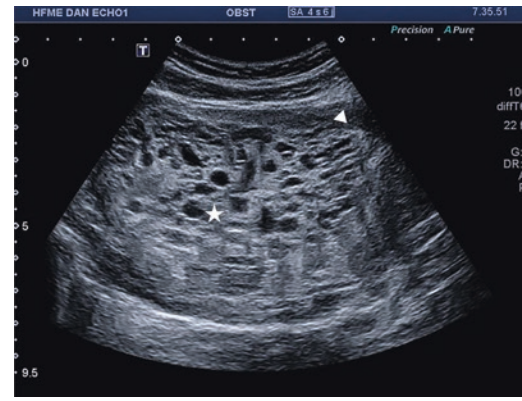

Fig. 27.5 Pelvic ultrasound of a patient with an intrauterine complete hydatidiform mole at 4 weeks of gestation +6 days. Triangle indicates the healthy myometrium and the star indicates some echogenic structure of the molar tissue

ultrasound is the only recommended imaging modality for the diagnosis of HM. Although the distinction between PHM and CHM may sometimes be difficult by imaging and the gold standard is histopathology. Pelvic ultrasound aims at ruling out the presence of an associated viable pregnancy. A mole coexisting with a living fetus is a rare phenomenon; it affects 0.005–0.01% of pregnancies and is more frequent with CHM than with PHM [63]. In patients with a diagnosis of post-molar GTN, imaging aims at assessing the extension of the disease beyond the uterus, i.e. the presence of vaginal, lung, liver or brain metastases, to adjust the protocol for chemotherapy using the International Federation of Gynecology and Obstetrics (FIGO) score [64, 65]. Extensive imaging workup includes brain and pelvic MRI, chest, abdomen and pelvis computed tomography (CT) and chest X-ray in case of lung metastases. Figure 27.6 shows liver and chest CT performed in a patient with multi-metastatic gestational choriocarcinoma.

27.8 Treatment

According to international guidelines [64], the treatment of hydatidiform moles is surgical and depends on the wish for subsequent pregnancies. Patients who are planning to have more children must have a dilation and curettage, irrespective of uterine size. A hysterectomy can be discussed if no further pregnancies are planned but does not exempt post-operative monitoring of serum hCG. The follow-up duration after hCG normalization varies among trophoblastic centers, usually 6 months after a CHM and 0–6 months after a PHM. It is also appropriate to avoid the occurrence of a new pregnancy during the surveillance period due to the interference of the increasing rate of hCG associated with the start of a new pregnancy with the hCG level linked to the previous mole.

27.9 Outcome

In current medical practice, HM is a curable disease, but death from malignant HM complications still occurs very rarely in western countries and more frequently in underdeveloped and developing countries. In such cases the death of the patients are mostly due to late diagnosis of metastases, mismanagement, or poor compliance to follow-up surveillance and therapy [66]. After treatment, up to 20% and 5% of CHM and PHM will evolve to post-molar GTN [60], respectively. The treatment of GTN is based on systemic chemotherapy. Clinicians use the FIGO scoring system to choose the best chemotherapy regimen to treat GTN [62, 64, 65]. A score ≤6 predicts a low risk of resistance to single agent chemotherapy, whereas a score ≥7 is associated with a high risk of resistance to mono-chemotherapy and requires the use of combination chemotherapy. Several single-agent regimens have been reported, of

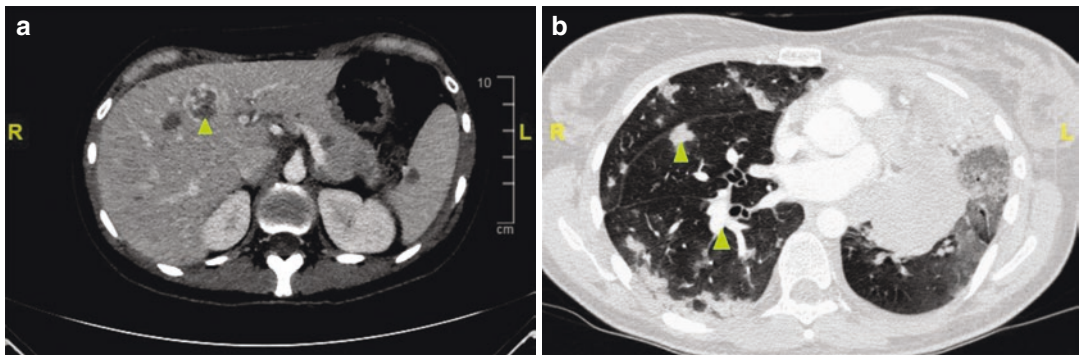

Fig. 27.6 Liver (**a**) and chest (**b**) computed tomography of a patient with metastatic gestational choriocarcinoma. Green arrowheads indicate liver and lung metastases

which methotrexate or dactinomycin is the most widely used today while EMA-CO (etoposide, methotrexate, dactinomycin alternated weekly with cyclophosphamide and vincristine) is the most commonly used combination chemotherapy [67]. Gestational trophoblastic neoplasia is overall highly sensitive to chemotherapy and the prognosis is excellent, particularly, in low-risk disease with an overall survival of almost 100% [68]. Surgery is the treatment of chemotherapy resistant diseases [64, 67].

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28

Monogenic Autoinflammatory Diseases Associated with Immunodeficiency

Michael J. Ombrello

Abstract

Despite the presence of accentuated inflammation, a subset of monogenic autoinflammatory disorders is also associated with increased susceptibility to infection. Mutations in *PLCG2* cause autoinflammation in the context of immune dysregulation with antibody deficiency. Deficiency of components of the linear ubiquitin chain assembly complex (LUBAC) leads to severe autoinflammation with immunodeficiency in association with polyglucosan myopathy. Mutations in *TRNT1* produce a syndrome of siderblastic anemia with B cell immunodeficiency, periodic fevers and developmental delay. Finally, patients with deficiency in *WDR1* manifest autoinflammatory periodic fever, immunodeficiency and thrombocytopenia.

Keywords

Immune deficiency · Immune dysregulation
PLCG2-associated antibody deficiency and immune dysregulation (PLAID)
Autoinflammatory PLAID (APLAID)
Deficiency of linear ubiquitination assembly complex (LUBAC) · Sideroblastic anemia with B cell immunodeficiency, periodic fevers and developmental delay *WDR1* deficiency

Abbreviations

AIP	Actin interacting protein
ANA	Anti-nuclear antibody
APLAID	Autoinflammatory PLAID
CCA	Cytosine-cytosine-adenosine
CRP	C-reactive protein
cSH2	Carboxy-terminal Src homology 2
DAG	Diacylglycerol
ESR	Erythrocyte sedimentation rate
FAMIN	Fatty acid metabolic-immune nexus
HOIL-1	Heme-oxidized IRP2 ubiquitin ligase 1
HOIP	HOIL-1 interacting protein
Ig	Immunoglobulin
IL	Interleukin
IP3	Inositol triphosphate
LPS	Lipopolysaccharide
LUBAC	Linear ubiquitination chain assembly complex
MAPK	Mitogen-activated protein kinase
MYD88	Myeloid differentiation primary response 88
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
NLRP3	Nucleotide binding and oligomerization domain, leucine rich repeat, pyrin 3

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PBMC	Peripheral blood mononuclear cells
PFIT	Periodic fever, immunodeficiency and thrombocytopenia
PIP2	Phosphatidylinositol bisphosphate
PLAID	PLCγ2-associated antibody deficiency and immune dysregulation
PLCγ2	Phospholipase Cγ2
RIP1	Receptor interacting protein 1
ROS	Reactive oxygen species
RSC	Receptor signaling complexes
SHARPIN	SHANK-associated RH domain-interacting protein like 1
SIFD	Sideroblastic anemia with B cell immunodeficiency, periodic fevers and developmental delay
TNF	Tumor necrosis factor
TRNT	tRNA nucleotidyltransferase
WDR1	WD domain repeat containing protein 1

Key Points

- **Despite producing excessive inflammatory responses, some autoinflammatory disease-causing mutations also lead to defective immune responses and predispose to infection**
- **Mutations in *PLCG2* lead to autoinflammation in the context of humoral immunodeficiency**
- **Mutations in genes encoding the linear ubiquitination assembly complex lead to a life threatening autoinflammatory disease with combined immunodeficiency**

28.1 Introduction

For the first decade following the birth of autoinflammation, it was widely believed that subjects bearing exaggerated innate immunity and excessive inflammation did not manifest increased susceptibility to infection. Moreover, the identification of autoinflammation in the context of immunodeficiency is challenging, given the potential that an unrecognized infectious process may underlie inflammation. Despite these confounders, the first monogenic syndromes manifesting concomitant autoinflammation and immunodeficiency were described in 2012. Since that time, this group of autoinflammatory diseases has continued to expand, and are the focus of this chapter.

28.2 Mechanisms of Coexistent Autoinflammation and Immunodeficiency

Autoinflammation results from abnormal activation of innate immunity, whereas immunodeficiency is the result of pathologic reduction in immune function that may involve innate and/or adaptive immunity. Conceptually, there are several ways that autoimmunity and immunodeficiency can coexist in monogenic conditions. One potential explanation is that cell-type specific or pleiotropic effects of mutated proteins lead directly to the combination of reduced immunity and enhanced inflammation. For example, differences between lymphocytes and monocytes seem to explain how the deficiency of the linear ubiquitin chain assembly complex (LUBAC) leads to both autoinflammation and immunodeficiency [1–3]. LUBAC deficient lymphocytes (and fibroblasts) demonstrate reduced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation in response to stimulation with interleukin (IL)-1β. In contrast, LUBAC deficient monocytes produce excessive amounts of inflammatory cytokines in response to IL-1β. A similar dichotomy exists in autoinflammatory *PLCG2*-associated antibody deficiency and immune dysregulation (APLAID). The hypermorphic S707Y mutation of *PLCG2* leads to enhanced nucleotide binding and oligomerization domain, leucine rich repeat, pyrin 3 (NLRP3) inflammasome activation and IL-1β cleavage in peripheral leukocytes in response to stimulation with lipopolysaccharide (LPS) [4]. This mutation also leads to a failure of B cell differentiation and antibody deficiency [5]. Another potential explanation for coexistent autoinflammation and immunodeficiency is that mutations that cause immune deficiency may result in reduced clear-

ance of pathogenic microbes and their by-products. The accumulation of microbial products in turn leads to exaggerated inflammatory responses. This model has been proposed for an autosomal recessive autoinflammatory syndrome caused by mutations in *LACC1*, encoding the fatty acid metabolic-immune nexus (FAMIN) protein [6].

28.3 PLCγ2-Associated Antibody Deficiency and Immune Dysregulation (PLAID

- **PLAID is an autosomal dominant syndrome that is characterized by cold urticaria and immune dysregulation**
- **PLAID is caused genomic deletions of *PLCG2* that alter its regulatory domain**

Phospholipase C-gamma 2 (PLCγ2)-associated antibody deficiency and immune dysregulation (PLAID) is an autosomal dominant condition with a wide array of clinical and laboratory features [7]. It is characterized universally by cold-induced urticaria, together with antibody deficiency and immune dysregulation that manifests as increased susceptibility to recurrent infection, atopic disease, autoimmunity and cutaneous granulomatosis.

28.3.1 Epidemiology

PLAID has been identified in nearly 50 members of three independent, multi-generational, non-consanguineous families of self-reported Northern European ancestry from the United States [7].

28.3.2 Genetics and Etiology

PLAID is an autosomal dominant syndrome caused by germline heterozygous in-frame deletions of *PLCG2*. Distinct *PLCG2* deletions are observed in each affected family, including two unique deletions of exon 19 and a deletion that spanned exons 20–22 (Fig. 28.1a). Within these three families, the phenotype was fully penetrant. Importantly, there are patients and families with a phenotype clinically indistinguishable from PLAID, in whom germline deletions/mutations of *PLCG2* have not been identified [8, 9].

28.3.3 Pathogenesis

PLCγ2 is a phospholipase enzyme that is predominantly expressed in lymphoid and myeloid cells, where it regulates cellular responses to activating signals. PLCγ2 catalyzes the hydrolysis of phosphatidylinositol bisphosphate (PIP2) to inositol triphosphate (IP3) and diacylglycerol (DAG), leading to mobilization of calcium stores from the endoplasmic reticulum and facilitating downstream cellular activation. In the resting state, the enzymatic site of PLCγ2 is obscured by its own autoinhibitory domain, rendering the enzyme inactive. Following an activating stimulus, serial phosphorylation events produce an allosteric change that unveils the catalytic site and unleashes its phospholipase activity [10].

PLAID-causing deletions of PLCγ2 alter the carboxy-terminal Src homology 2 domain (cSH2), a critical component of the PLCγ2 autoinhibitory domain (Fig. 28.1a). These mutations decouple PLCγ2 enzymatic activity from the upstream phosphorylation events, leading to constitutive enzymatic activity, *in vitro* [7, 8]. Paradoxically, B cells, natural killer (NK) cells and mast cells bearing these mutations demonstrate hyporesponsiveness or anergy in response to conventional stimulation at physiologic temperatures, which likely contributes to immune dysregulation [7, 8]. When exposed to subphysiologic temperatures (20 °C), cells bearing *PLCG2* deletions demonstrate spontaneous activation, recapitulating the universal feature of PLAID, cold urticaria [8].

28.3.4 Clinical Manifestations

28.3.4.1 Cold-Induced Urticaria

The primary autoinflammatory feature of PLAID is cold-induced urticaria that consists

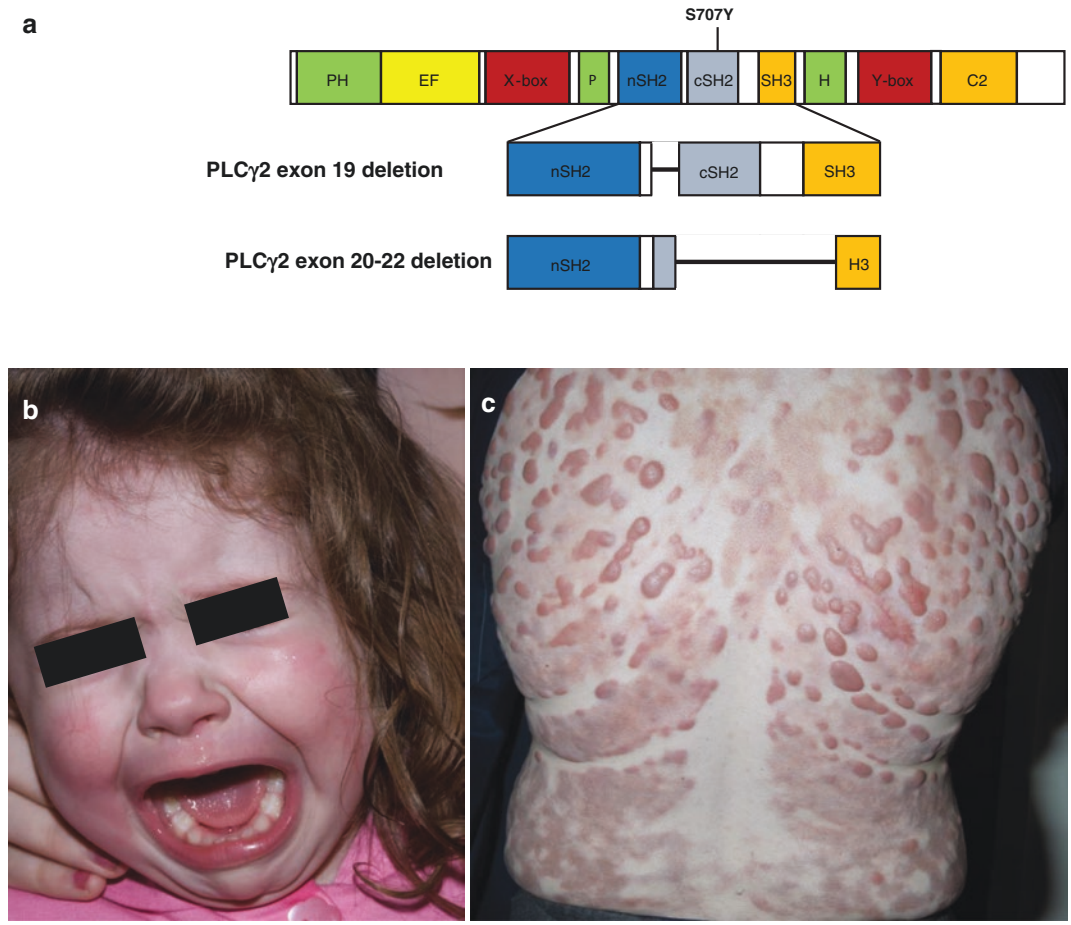

Fig. 28.1 Genomic deletions involving the carboxy-terminal SRC homology 2 (cSH2) domain of *PLCG2* cause *PLCG2*-associated antibody deficiency and immune dysregulation (PLAID). A hypermorphic missense mutation (S707Y) within the cSH2 domain causes autoinflammatory PLAID (**a**). Cold-induced urticaria in PLAID results from evaporative cooling, as was the case with this child's tears. (**b**). Some patients with PLAID developed severe, diffuse granulomata (**c**). Photographs courtesy of Dr. Joshua Milner, M.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

of pruritic, localized erythematous skin lesions. These lesions are truly urticarial and result from mast cell degranulation, in contrast to the neutrophilic cold-induced skin lesions observed in the cryopyrinopathies. The urticaria of PLAID are provoked by evaporative cooling and last from minutes to hours before completely resolving (Fig. 28.1b). Episodes frequently develop within the first months of life and occur during or following swimming or bathing, or upon exposure to cool air while perspiring. The urticaria of PLAID are not produced by direct cold stimulation or ice cube testing. Some patients report that the consumption of frozen foods produces a burning sensation in the throat or chest.

28.3.4.2 Immunodeficiency

Increased susceptibility to infection is commonly observed in PLAID. Most patients with PLAID have antibody deficiency and almost half develop recurrent sinopulmonary infections. A subset of these have severe, recurrent pneumoniae with bronchiectasis. Moreover, recurrent viral upper respiratory infections, recurrent viral pharyngitis, early onset herpes zoster (shingles) and severe

Table 28.1 Monogenic autoinflammatory diseases with concomitant immunodeficiency

Disease	Gene	Autoinflammatory features	Immunodeficiency features
PLCG2-associated antibody deficiency and immune dysregulation (PLAID)	*PLCG2*	Cold-induced urticaria, cutaneous granulomatosis	Antibody deficiency, recurrent sinopulmonary infections, recurrent viral infections
Autoinflammatory PLAID (APLAID)	*PLCG2*	Early-onset of colitis, uveitis and blistering skin lesions	Antibody deficiency, recurrent viral infections
Linear ubiquitination assembly complex (LUBAC) deficiency	*RBK1* *RNF31*	Periodic fever, persistent acute phase activation, polyglucosan myopathy	Specific antibody deficiency, recurrent pyogenic infections, abnormal anti-viral responses
Sideroblastic anemia with immunodeficiency, fevers and developmental delay (SIFD)	*TRNT1*	Periodic fever, persistent acute phase activation	Antibody deficiency, reduced peripheral B and natural killer cells
Periodic fever, immunodeficiency and thrombocytopenia (PFIT)	*WDR1*	Periodic fever, scarring oral inflammation	Multiple pyogenic infections, pneumocystis pneumonia, severe response to viral infections

onychomycosis are also commonly observed in patients with PLAID (Table 28.1).

28.3.4.3 Autoimmunity and Atopy

Patients with PLAID also have increased risk of developing autoimmunity and atopy. A quarter of patients with PLAID have symptomatic autoimmunity, most commonly vitiligo and autoimmune thyroid disease. Moreover, antinuclear antibody (ANA) testing is positive in over two-thirds of patients. Similarly, symptomatic allergic disease (i.e. asthma, eczema, allergic rhinitis and/or food allergies) is present in almost every patient.

28.3.4.4 Granulomatous Dermatitis

Patients with PLAID may also develop non-infectious granulomatous dermatitis. In some subjects, blistering skin lesion(s) develop shortly after birth on the tip of the nose, fingers, and/or ears,—areas most exposed to cold temperature. In most cases, these lesions are mild and self-limited, however severe non-caseating granulomatous lesions resulting in soft-tissue damage and destruction of nasal and auricular cartilage have also been observed. In the most severe cases, disseminated, destructive cutaneous granulomata may develop (Fig. 28.1c).

28.3.5 Laboratory Testing

Immunohistochemical examination of biopsied cold-induced urticarial plaques from patients with PLAID reveals the presence of mast cells and positive staining for tryptase, which together implicate mast cell degranulation. The urticaria of PLAID is not typically accompanied by systemic inflammation or the elevation of acute phase reactants. Antibody deficiency is almost universally observed in PLAID. Quantitative serum immunoglobulin screening shows reductions in serum IgG, IgM and/or IgA levels in most patients, ranging from subtle changes in some subjects to profound reductions in others [7]. Reduced antibody responses to specific stimuli, such as pneumococcal antigens, is also often observed. Almost all patients have low numbers of circulating class-switched memory B cells and many have reduced numbers of circulating NK cells.

28.3.6 Diagnosis

There are no formal diagnostic criteria for PLAID. The diagnosis is made molecularly by identification of pathogenic mutations of the autoinhibitory domain of *PLCG2*. Clinically, PLAID is defined by the presence of evaporative cold-induced urticaria with low peripheral $CD27^+$ class switched memory B cells and evidence of immune dysregulation. In addition to PLAID, the differential diagnosis for cold-induced skin lesions includes the cryopyrinopathies, which are caused by mutations in *NLRP3,* and familial cold autoinflammatory syndrome 2, which is caused by mutations in *NLRP12*.

28.3.7 Treatment

There is no effective targeted pharmacotherapy for PLAID. Therefore, the approach to its management is symptom-directed. Immunoglobulin replacement may be used to treat immune deficiency in patients with antibody deficiency and recurrent infection. Lifestyle modification with cold avoidance is the mainstay of treatment for the cold-associated symptoms of PLAID. Similarly, first and second generation antihistamines, including high doses of the second generation family, as well as leukotriene receptor antagonists are reported to minimize or prevent the effects of the atopic features in some patients [9].

28.3.8 Outcome/Prognosis

The cold-induced urticaria in PLAID is chronic and affected patients frequently adopt lifestyle modifications to avoid the triggers. Some patients experience progression of their immune dysregulation, developing recurrent infections or autoimmunity later in life.

28.4 Autoinflammatory PLCγ2-Associated Antibody Deficiency and Immune Dysregulation (APLAID)

- **APLAID is an autosomal dominant syndrome characterized by systemic and organ-specific inflammation and immunodeficiency**
- **APLAID is caused by a hypermorphic missense mutation in the regulatory domain of *PLCG2***

Autoinflammatory PLAID (APLAID) is an autosomal dominant condition of immune dysregulation that is closely related to PLAID [5]. It is characterized by antibody deficiency and immune dysregulation with early-onset blistering skin eruptions, early-onset colitis, early-onset ocular inflammation and recurrent sinopulmonary infections. Although some of the features of immune dysregulation observed in APLAID overlap with to those seen in PLAID, patients with APLAID do not develop cold-induced urticaria, which is universally present in subjects with PLAID.

28.4.1 Epidemiology

To date, the only description of APLAID was in two members of a non-consanguineous family of Ashkenazi Jewish descent [5].

28.4.2 Genetics and Etiology

APLAID is an autosomal dominant syndrome caused by the S707Y mutation of *PLCG2*. It was identified by exome sequencing of an affected father and daughter.

28.4.3 Pathogenesis

Like the deletions that cause PLAID, the S707Y mutation is located within the cSH2 domain of *PLCγ2* (Fig. 28.1a). However functionally, S707Y *PLCγ2* is quite different from the PLAID mutants. First, it produces only a small amount of constitutive enzymatic activity and its activation is dependent on upstream signaling events [5]. Second, upon activation S707Y, *PLCγ2* is hypermorphic *in vitro*. Similarly, cells bearing the S707Y mutation display hyper-responsiveness to stimulation *in vitro* and *ex vivo*. Finally, unlike PLAID mutants, cells bearing S707Y are not activated by exposure to cold environments [5]. Additionally, peripheral blood mononuclear cells (PBMC) from patients with APLAID were found to secrete IL-1β in response to LPS priming (alone), which could be attenuated by PLC inhibition and intracellular calcium blockade [4]. It is hypothesized that this phenomenon is responsible for the persistent autoinflammation seen in APLAID.

28.4.4 Clinical Manifestations

28.4.4.1 Early-Onset of Cutaneous and Ocular Inflammation

Both affected subjects developed an epidermolysis bullosa-like eruption in early infancy. In both cases, the eruptions evolved over time into recurrent erythematous plaques and vesiculopustular lesions which were exacerbated by heat, pressure and sun exposure [5, 8]. During infancy, one subject also developed several corneal blisters, which progressed into corneal erosions and ulcers. She has also developed severe posterior uveitis, intraocular hypertension and cataracts. Unlike PLAID, the 2 patients with APLAID have not developed cold urticaria.

28.4.4.2 Immunodeficiency

Antibody deficiency with recurrent infections is another characteristic of APLAID. Both subjects experienced a range of recurrent bacterial and viral sinopulmonary infections. Additionally, one of the two patients also had pulmonary aspergillus infection which developed in the context of chronic immunosuppression with corticosteroids and mycophenolate mofetil [11].

28.4.4.3 Other Inflammatory Features

Additional features observed in both of the patients with APLAID included nonspecific interstitial pneumonitis with respiratory bronchiolitis, an early-onset form of ulcerative colitis and arthralgia.

28.4.5 Laboratory Testing

Both patients with APLAID developed episodes of inflammation marked by elevations of the C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR).

They also had antibody deficiency with reduced levels of IgM and IgA, however their degree of deficiency varied over time. Moreover, they had reduced antibody responses to specific stimuli (i.e. pneumococcal antigens), and not unexpectedly, striking reductions in circulating class-switched $CD27^+$ memory B lymphocytes. They also had reduced circulating levels of NK-T cells. Examination of the skin lesions of APLAID revealed a mixed infiltration of neutrophils, eosinophils, histiocytes and lymphocytes.

28.4.6 Diagnosis

There are no diagnostic criteria for APLAID. The diagnosis is made molecularly by detection of the pathogenic S707Y mutation of PLCγ2. Clinically, the differential diagnosis of APLAID may include common variable immune deficiency and inflammatory bowel disease.

28.4.7 Treatment

There is no effective targeted pharmacotherapy for APLAID, and therefore its treatment is largely symptomatic. Immunoglobulin replacement has reduced the frequency of infection in both subjects, however they both continue to develop new inflammatory features despite this treatment. Moreover, the inflammatory features of the eye and skin are not responsive to either tumor necrosis factor (TNF)-α blockade or IL-1 directed treatment, and ultimately require chronic corticosteroid therapy, both systemically and topically.

28.4.8 Outcome/Prognosis

It is clear that the ocular disease of APLAID is very severe and requires aggressive treatment. Apart from this, it is difficult to predict the prognosis of APLAID based on the observation of 2 affected subjects. To date, APLAID has not been noted to cause premature death.

28.5 Deficiency of Components of the Linear Ubiquitination Chain Assembly Complex (LUBAC)

- **Deficiency of components of the LUBAC leads to a life-threatening autosomal recessive syndrome of severe immunodeficiency and autoinflammation**
- **LUBAC deficiency is caused by homozygous loss of function mutations of the LUBAC constituents, *RBCK1* and *RNF31***
- **Subjects with LUBAC deficiency also develop polyglucosan myopathy, which is marked by the accumulation of insoluble carbohydrate aggregates in skeletal and smooth muscle cells**

Ubiquitination is a post-translational modification whereby chains of ubiquitin proteins (polyubiquitin chains) are attached to target proteins. At least eight different ubiquitin resides may be linked to other proteins, including seven distinct lysine residues and its amino-terminal methionine residue. Through these different linkages, ubiquitination performs diverse regulatory functions [12]. Amino-terminal methionine (Met-1) linked ubiquitination, also known as linear ubiquitination, is centrally involved in innate and adaptive immune responses and is a critical regulator of cell death [13–15]. The attachment of Met-1 linked polyubiquitin chains is accomplished by LUBAC, an E3 ubiquitin ligase complex. The inherited deficiency of LUBAC components leads to a failure of linear ubiquitination and a severe, autosomal recessive syndrome of autoinflammation, immunodeficiency and polyglucosan myopathy.

28.5.1 Epidemiology

LUBAC deficiency has been identified in 4 subjects from three independent families [2, 3]. The affected families include a non-consanguineous French family, a consanguineous Italian family and a consanguineous Kuwaiti family.

28.5.2 Genetics and Etiology

LUBAC deficiency is an autosomal recessive condition that is caused by bi-allelic mutations of the LUBAC constituents, HOIL-1 (heme-oxidized IRP2 ubiquitin ligase 1; *RBCK1*) and HOIP (HOIL-1 interacting protein; *RNF31*). The French patients have a nonsense *RBCK1* variant that truncates the protein at residue 185 and a genomic deletion that encompasses the first four exons of *RBCK1*. The Italian patient has a homozygous 2 base pair deletion of *RBCK1* that creates a frameshift with a premature stop codon at residue 48. The Kuwaiti patient has a homozygous missense *RNF31* mutation, L72P. To date, mutations of the third LUBAC component, SHARPIN (SHANK-associated RH domain-interacting protein like 1; *SIPL1*), have not been shown to cause LUBAC deficiency in humans. Importantly, bi-allelic *RBCK1* (HOIL-1) mutations have also been identified in patients with polyglucosan myopathy who were not noted to have autoinflammation or immunodeficiency [16, 17].

28.5.3 Pathogenesis

LUBAC is an E3 ubiquitin ligase complex that attaches linear polyubiquitin chains to proteins via methionine 1 (Met-1) linkage [13, 14]. This complex is composed of three subunits, which include two accessory subunits, HOIL-1 and SHARPIN, and a catalytic subunit, HOIP. In immune cells, stimulation of pro-inflammatory receptors triggers LUBAC assembly and activation. The range of receptors regulated by linear ubiquitination is quite broad and includes CD40 ligand (CD40L), TNF receptor-1, IL-1 receptor 1, toll-like receptors and nod-like receptors [15]. Upon activation, LUBAC ubiquitinates important members of the receptor signaling complexes (RSCs), such as NF-κB, essential modulator (NEMO), [18, 19], receptor interacting protein 1 (RIP1) [13], myeloid differentiation primary response 88 (MYD88) [20] and others [15]. The Met-1 ubiquitinated signaling molecules are then recruited into the RSCs, where they act to stabilize and maintain the RSC

and ultimately lead to activation of NF-κB and mitogen-activated protein kinase (MAPK) signaling [15, 18–20].

The mutations that cause LUBAC deficiency are loss-of-function mutations that result in an absence (or near absence) of the mutated protein, which precludes assembly of functional LUBAC [2, 3]. Fibroblasts and B lymphocytes from patients with LUBAC deficiency display reduced NF-κB and MAPK activation in response to stimulation with IL-1β and CD40L, respectively. In contrast, monocytes from these patients are hyper-responsive to stimulation with IL-1β. Murine models of LUBAC deficiency recapitulate the hypo-responsiveness of fibroblasts and B cells and hyper-responsiveness of monocytes that is observed in humans with LUBAC deficiency [19, 21]. Together these observations demonstrate the context-dependent responses of LUBAC signaling pathways.

28.5.4 Clinical Manifestations

28.5.4.1 Recurrent Inflammatory Episodes

All affected subjects present within the first months of life with recurrent episodes of fever, systemic inflammation with profound elevation of acute-phase markers, hepatosplenomegaly and lymphadenopathy, which develop in the absence of a discernable trigger. Bowel inflammation with eosinophilic infiltrates were observed in 2 patients. Subjects also have abnormal persistence of inflammation following infections.

28.5.4.2 Immunodeficiency

Patients affected by LUBAC deficiency have a severe form of combined immunodeficiency. The disease course is marked by recurrent life-threatening pyogenic infections and abnormal anti-viral responses. In one patient, a T cell immunodeficiency was also present, presumably in relation to severe intestinal lymphangiectasia.

28.5.4.3 Polyglucosan Myopathy

Another common feature of LUBAC deficiency is the intracellular deposition of insoluble, poorly branched polysaccharide chains in cardiac, smooth and skeletal myocytes. These deposits, described as amylopectin-like, are also known as polyglucosan bodies and are observed in polyglucosan body disease, an adult-onset glycogen storage disease. In both HOIL-1 and HOIP deficiency, amylopectin-like deposits are observed in the skeletal, smooth and cardiac muscle of affected patients. In patients with HOIL-1 mutations, myopathy becomes clinically apparent at an early age with severe cardiomyopathy and heart failure. In the patient with a HOIP mutation, there were no clinical or electrophysiological evidence of cardiomyopathy. The only feature of myopathy was weakness of the proximal lower extremities with corresponding muscle atrophy and fatty replacement. Interestingly, some patients with homozygous or compound heterozygous mutations of HOIL-1 develop severe polyglucosan myopathy in the absence of immunodeficiency [16, 17].

28.5.4.4 Intestinal Lymphangiectasia

The single patient with the homozygous HOIP mutation had severe intestinal lymphangiectasia that resulted in T cell immunodeficiency. To date, this has not been observed with HOIL-1 mutations.

28.5.5 Laboratory Testing

The acute phase markers, CRP and ESR, are elevated during inflammatory episodes of LUBAC deficiency, but notably they do not return to normal levels between episodes. Patients have markedly reduced peripheral memory B lymphocytes. They also have qualitative and quantitative antibody defects. Immunohistochemical examination of skeletal, cardiac and smooth muscle identifies intramuscular deposition of amylopectin-like material.

28.5.6 Diagnosis

There are no diagnostic criteria for LUBAC deficiency, and its severe inflammatory presentation may be confused with overwhelming sepsis. The

diagnosis is made molecularly by detecting homozygous loss of function mutations in *RBCK1* or *RNF31*.

28.5.7 Treatment

Treatments for LUBAC deficiency focus on bolstering host immunity to treat or prevent infections and reducing uncontrolled inflammation. Treatments for immunodeficiency have included immunoglobulin replacement and antibiotic prophylaxis with trimethoprim/sulfamethoxazole. Treatments for excessive inflammation have included anakinra, TNF blockade and corticosteroids. Although TNF blockade was transiently effective in one patient, corticosteroids were the most effective at combatting inflammation. One patient underwent allogeneic hematopoietic stem cell transplant at age 13 months. Despite experiencing an apparent remission from recurrent infections and episodes of inflammation, the amylopectinosis continued to progress.

28.5.8 Outcome/Prognosis

LUBAC deficiency is a rare, severe congenital immunodeficiency for which there are no effective treatments. The prognosis for this condition is extremely poor and most affected subjects succumb to disease-related complications during early childhood.

28.6 Sideroblastic Anemia with B Cell Immunodeficiency, Periodic Fevers and Developmental Delay (SIFD)

- **SIFD is an autosomal recessive multisystem inflammatory disorder with wide phenotypic heterogeneity that is caused by mutation of *TRNT1***
- **Subjects with SIFD manifest periodic fever and B cell immunodeficiency, together with a wide range of protean features**

Sideroblastic anemia with B cell immunodeficiency, periodic fevers and developmental delay (SIFD) is an autosomal recessive syndrome with pleiotropic features that affect the hematologic, neurologic, immunologic, integumentary and musculoskeletal systems. There is a high degree of phenotypic variability among people affected by SIFD.

28.6.1 Epidemiology

SIFD has been described in 33 subjects from 27 families, including six consanguineous families [22–29]. It has been identified in many people of European ancestry, as well as in several Pakistani families, a Saudi Arabian family and an individual of Afro-Caribbean descent.

28.6.2 Genetics and Etiology

SIFD is an autosomal recessive syndrome that is caused by bi-allelic, loss of function mutations in *TRNT1,* a nuclear gene encoding the CCA-adding tRNA nucleotidyltransferase 1 (TRNT1) enzyme [22–29]. Most SIFD causing mutations are missense variants at evolutionarily conserved positions, however insertion/deletion mutants have also been observed. In contrast to the loss of function *TRNT1* mutations that cause SIFD, bi-allelic hyopmorphic *TRNT1* mutations have been reported to cause a milder phenotype that almost exclusively affects the retina [30, 31].

28.6.3 Pathogenesis

Although mutations in *TRNT1* have been genetically linked with SIFD, the exact mechanism(s) through which they act is unknown. TRNT1 is an essential enzyme involved in tRNA aminoacylation, that is, it catalyzes the addition of cytosine-cytosine-adenosine (CCA) trinucleotides to the 3′ end of all tRNA molecules. This is a critical step in the maturation of both cytosolic and mitochondrial tRNAs, and in humans it is exclusively performed by TRNT1. In addition to adding CCA

to all tRNAs, the TRNT1 enzyme also participates in the tRNA quality control process and the cellular stress response.

The mutations that lead to SIFD are largely loss-of-function mutations; patients bearing mutations that reduce but don't eliminate the enzymatic activity were noted to have a less severe phenotype [22]. The loss-of-function mutations that cause SIFD have significant detrimental effects on maturation of mitochondrial tRNAs [24] and lead to reductions in expression of mature cytosolic tRNAs [29].

Microscopic studies of fibroblasts derived from patients with SIFD showed that although SIFD-causing *TRNT1* mutations led to reduced expression of TRNT1, the sub-cellular localization of TRNT1 was not altered [32]. Moreover, the number and shape of mitochondria was not altered in the fibroblasts from patients with SIFD. Nonetheless, reductions in mitochondrial respiration have been observed in cells from patients with SIFD [32], as have reductions in translation of many oxidative phosphorylation complex subunits [24]. Consistent with these mitochondrial findings, cultured fibroblasts from patients spontaneously produce higher levels of reactive oxygen species (ROS) than do fibroblasts from healthy subjects [29]. Moreover, macrophages from patients produce more IL-1β than do those of healthy subjects [16]. Finally, cells deficient in TRNT1 have deficits in upregulating protein clearance mechanisms [24], which may have cell type- and context-specific consequences.

28.6.4 Clinical Manifestations

28.6.4.1 Recurrent Inflammatory Episodes

Severe episodes of fever and inflammation develop within the first weeks to months of life [16, 23, 25]. Inflammatory episodes generally last for 5–7 days, recur every 1 and 6 weeks and in some cases, worsen with age. They are often associated with cutaneous inflammation, mucosal ulceration, gastrointestinal complaints, metabolic acidosis and arthralgia/arthritis [23, 25, 29]. In addition, some patients exhibit features indicative of macrophage activation syndrome/hemophagocytic lymphohistiocytosis [29].

28.6.4.2 Immunodeficiency

Most patients with SIFD have a B-cell immunodeficiency that manifests with B lymphopenia and hypogammaglobulinemia. As a result, many patients develop recurrent sinopulmonary infections. Subtle NK and T cell abnormalities also occur, [23, 29].

28.6.4.3 Sideroblastic Anemia

A central feature of SIFD is congenital sideroblastic anemia [23, 25, 29]. Sideroblastic anemia is often discovered during the investigation of unexplained inflammatory episodes [25]. Moreover, many patients experience severe exacerbation of anemia during inflammatory episodes [29].

28.6.4.4 Developmental Delay and Neurologic Features

Because neurons are critically dependent on ATP and consume high amounts of oxygen, they are particularly susceptible to the detrimental effects of mitochondrial abnormalities [33], such as those present in SIFD. Developmental delay is observed in most patients with SIFD, and patients are almost always globally delayed. Some children display profound impairment of receptive and expressive communication. Sensorineural hearing loss, seizures, hypotonia and ataxia are also observed.

28.6.4.5 Retinitis Pigmentosa

A subset of patients with SIFD develops retinitis pigmentosa, which is characterized by retinal degeneration and a progressive loss of vision [23, 25, 29]. Moreover, a group of patients with isolated retinitis pigmentosa have been shown to have hypomorphic mutations of *TRNT1* [30, 31].

28.6.4.6 Other Features

Metabolic abnormalities observed in patients with SIFD may include aminoaciduria, metabolic (lactic) acidosis, hypokalemia, hypophosphatemia, hypocalcemia and hyperglycemia [23]. Metabolic abnormalities,

including metabolic acidosis, may be exacerbated during inflammatory episodes. Renal tubular dysfunction and nephrocalcinosis have been observed in a few patients with SIFD [23, 25]. Cardiac abnormalities have also been observed in SIFD. Dilated cardiomyopathy has been observed in 2 children with SIFD [23], while hypertrophic cardiomyopathy was observed in another patient [28]. Moreover, the original report of SIFD noted that cardiac failure was a factor contributing to death in at least 5 of 7 deceased patients [23].

28.6.5 Laboratory Testing

As in LUBAC deficiency, patients with SIFD can exhibit profound elevation of ESR and CRP during fever episodes, but in many patients, the acute phase reactants remain elevated between episodes. Patients also experience hyperferritinemia, however it is unclear whether this is related to chronic inflammation, iron overload, or both. Typical findings on peripheral blood smears include hypochromic microcytosis, basophilic stippling and an increased number of nucleated erythrocytes. The examination of bone marrow biopsy specimens almost universally reveals many ringed sideroblasts; rarely, evidence of hemophagocytosis may also be observed. Almost all patients with SIFD have hypogammaglobulinemia and B lymphopenia, and over time they also experience declining numbers of peripheral NK and T cells. Metabolic abnormalities observed in patients with SIFD may include aminoaciduria, metabolic (lactic) acidosis, hypokalemia, hypophosphatemia, hypocalcemia and hyperglycemia [23]. These metabolic abnormalities may be exacerbated during inflammatory episodes.

28.6.6 Imaging

Intracranial imaging may reveal a variety of abnormalities, including cerebral atrophy, delayed myelination of cortical white matter, abnormal thalamic and external capsular enhancement, communicating hydrocephalus and cerebellar abnormalities including decreased perfusion.

28.6.7 Diagnosis

There are no diagnostic criteria for SIFD. The diagnosis is made molecularly, by detecting homozygous loss of function mutations in *TRNT1*.

28.6.8 Treatment

Acute inflammatory episodes are managed with rehydration, electrolyte replacement and red blood cell transfusions for anemia. Myeloablative bone marrow transplantation has been successfully undertaken in 2 patients [23]. TNF blockade has also shown promise in remarkably reducing inflammatory episodes and ameliorating background inflammation in a set of patients with SIFD [29]. Immunoglobulin replacement should be instituted at the time of diagnosis in patients with hypogammaglobulinemia and/or B cell lymphopenia. Given the link between retinitis pigmentosa and mutations in *TRNT1* [23, 25, 29–31], it is critical that all patients with SIFD be screened by an ophthalmologist experienced in the diagnosis and care or retinitis pigmentosa.

28.6.9 Outcome/Prognosis

SIFD is typically a severe syndrome with life-threatening complications, and patients with SIFD often die in the first decade of life [23, 29]. Recent reports of patients bearing hypomorphic mutations of *TRNT1* suggest that the spectrum of *TRNT1*-related disease may also include less severe phenotypes [30, 31]. While less severe than SIFD, retinitis pigmentosa is a progressive, degenerative retinal disease that ultimately results in loss of vision.

28.7 Autoinflammatory Periodic Fever, Immunodeficiency, and Thrombocytopenia (PFIT) Caused by WDR1 Deficiency

- **PFIT is an autosomal recessive syndrome of immunodeficiency and autoinflammation that is caused by deficiency of WDR1**
- **Subjects with PFIT experience recurrent infections and severe, life-threatening infection-related inflammation**

The deficiency of *WDR1* (also called autoinflammatory periodic fever, immunodeficiency and thrombocytopenia or PFIT) is an autosomal recessive syndrome that is characterized by recurrent episodes of cutaneous and systemic inflammation, recurrent infections and recurrent stomatitis that results in peri-oral scarring and severe reductions in oral aperture.

28.7.1 Epidemiology

WD40 domain repeat containing protein 1 *(WDR1)* deficiency has been identified in 7 patients from four independent families [34, 35]. The affected families include a consanguineous Qatari family, a consanguineous Pakistani family and two non-consanguineous families of mixed European ancestry.

28.7.2 Genetics and Etiology

The deficiency of WDR1 is an autosomal recessive syndrome that is caused by bi-allelic, loss of function mutations in *WDR1*, which encodes WDR1 (also known as actin interacting protein 1 or AIP1). Among the 4 reported families with WDR1 deficiency, each family had distinct mutations. In total, 6 disease-causing mutations have been identified, including 5 missense variants and one in-frame deletion [34, 35]. All 6 mutations were located in an invariant regions of the protein and are predicted to disrupt protein function [34, 35].

28.7.3 Pathogenesis

Cytoskeletal actin filaments are essential for the structural integrity of all cells, and their balanced regulation is necessary for proper immune function. Certain cell types, such as neutrophils, require the ability to rapidly disassemble and specifically and directionally reassemble actin filaments to perform their normal functions. In situations requiring rapid actin disassembly, cofilin is the primary enzyme that cleaves actin filaments, in concert with a variety of other proteins. WDR1 works together with cofilin to accelerate actin de-polymerization by promoting the preferential cleavage of actin to its monomeric form and preventing subsequent polymerization [36, 37].

The complete deficiency of *Wdr1*in mice leads to embryonic lethality [38], however the *Wdr1*-mutated Wdr1^{rede} strain of mice, which has greatly reduced, but not absent expression of *Wdr1*, shows normal viability. The Wdr1^{rede} mice develop spontaneous inflammation of the ears, feet and tail, and demonstrate abnormalities of neutrophils and megakaryocytes [38]. The inflammatory lesions observed in this model are dependent on IL-18, but not IL-1β, that is produced by monocytes in a pyrin inflammasome-dependent fashion [39]. Neutrophils from Wdr1^{rede} mice show decreased actin depolymerization following chemokine stimulation and increased filamentous actin in their resting state [38]. Megakaryocytes from Wdr1^{rede} mice fail to undergo normal cytoplasmic maturation, which results in a failure of platelet shedding, leading to macrothrombocytopenia [38]. Importantly, the inflammatory lesions persisted when this model was crossed onto a *RAG*-deficient background [38]. Moreover, it was reversible with bone marrow transplantation from *Wdr1* wild type mice [38]. Taken together, these facts strongly suggest that the inciting cells are the bone marrow-derived myeloid cells.

The mutations of *WDR1* that cause WDR1 deficiency in humans are all situated at locations critical to its proper structure and/or function [34, 35]. Similar to the Wdr1^{rede} mice,

cells from patients with WDR1 deficiency demonstrate increased quantities of F-actin and evidence of diminished F-actin depolymerization [34, 35], as well as intense aggregates of WDR1 [35]. Some patients with WDR1 deficiency were also observed to have elevations of serum IL-18, similar to that observed in Wdr1^{rede} mouse [35]. Neutrophils from one patient were shown to produce increased IL-18 in response to LPS, relative to neutrophils from a control subject [35]. Adding to the similarities, monocytes from patients showed increased caspase-1 cleavage, relative to control cells, which is also consistent with the increased pyrin inflammasome activation observed in the Wdr1^{rede} mouse [35].

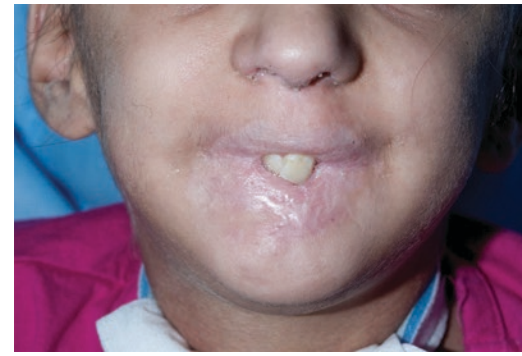

Fig. 28.2 Subjects with *WDR1* deficiency experience recurrent episodes of severe, scarring stomatitis that can result in extreme reductions in oral aperture, as pictured. Photograph courtesy of Dr. Steven Holland, M.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

28.7.4 Clinical Manifestations

28.7.4.1 Recurrent Inflammatory Episodes

Recurrent episodes of severe inflammation, marked by fever, leukocytosis and thrombocytopenia occurred in two affected sisters of the Pakistani family within the first weeks of life [35]. Episodes lasted 3–7 days and occurred every 6–12 weeks. Importantly, neither episodic fever and inflammation nor thrombocytopenia were described in the other 5 patients [34].

28.7.4.2 Immunodeficiency

Immunodeficiency with recurrent and severe infections was observed in all patients with WDR1 deficiency. Among the 7 affected patients, a wide range of serious infections were described, including pneumoniae from *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Hemophilus influenzae* and *Pneumocystis jirovecii*; urinary tract infections from *Escherichia coli* and *Enterococcus* and necrotizing cellulitis from *Streptococcus pneumoniae* [34, 35]. They were also noted to have severe courses of viral infections, with one patient succumbing to disseminated varicella infection at 8 years of age [34]. Patients were also observed to develop exaggerated or prolonged inflammatory responses in the setting of infections [34, 35].

28.7.4.3 Severe Stomatitis with Scarring and Stenosis

All patients with WDR1 deficiency experienced severe, recurrent episodes of stomatitis that led to scarring. In some cases, the scarring was so severe that it resulted in oral stenosis (Fig. 28.2).

28.7.5 Laboratory Testing

The inflammatory episodes observed in the Pakistani sisters with WDR1 deficiency were marked by profound elevation of the acute phase response as evidence by increased levels of the ESR and CRP, as well as elevation of IL-18 without a commensurate elevation of IL-18 binding protein.

Neutrophil abnormalities to varying degrees were observed in all subjects with WDR1 deficiency [34, 35]. Neutrophils from patients with WDR1 deficiency were noted to contain more filamentous actin than neutrophils from healthy subjects. Moreover, neutrophils from patients deficient in WDR1 also show impaired chemotaxis, impaired spreading and impaired cell polarization, while displaying normal staphylococcal killing and an increased neutrophil oxidative burst [35]. Although thrombocytopenia was observed in the 2 affected Pakistani sisters, the quantity and characteristics of platelets and megakaryocytes were not mentioned in the reports of the other 5 patients [34, 35].

28.7.6 Diagnosis

There are no diagnostic criteria for WDR1 deficiency. The diagnosis is made molecularly, by detecting homozygous loss of function mutations in *WDR1*.

28.7.7 Treatment

There are no effective, targeted treatments for WDR1 deficiency. The Pakistani sisters experienced partial responses to corticosteroids and colchicine, although inflammatory episodes persisted. One of them also experienced partial improvement with anakinra [35]. Additionally, two patients received autologous hematopoetic stem cell transplantation. The transplantation was noted to be "successful" in one patient [34], while the second patient was noted to be in remission off medications at 3 years post-transplant [35].

28.7.8 Outcome/Prognosis

WDR1 deficiency is a severe syndrome with life-threatening complications. As a result of their immunodeficiency, patients suffer from recurrent life-threatening infections. The severity of their infections is often exacerbated by exaggerated inflammatory responses that produce sepsis-like syndromes, and 3 out of 7 patients died from complications related to infection and/or inflammation [34, 35].

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29 Other Rare Monogenic Autoinflammatory Diseases

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Abstract

Over the past decade, major advances have been m`ade in understanding the molecular and cellular bases leading to autoinflammatory diseases, and a number of very rare entities have been described. Next-generation sequencing technologies led to the rapid identification of a number of additional genes responsible for syndromes observed in only a very small number of families or in sporadic cases. The identification of all these new genes and associated molecular pathways underlines that activation of interleukin (IL)-1β signaling is far from being the only pathogenic process involved in autoinflammatory disorders. Genetic defects found in patients with rare monogenic autoinflammatory diseases might also facilitate the study of common autoinflammatory diseases with a genetic component. Since these disorders affect multiple organs with potentially severe complications, management of patients is complex and warrants a multidisciplinary approach. Finally, it is necessary to translate discoveries of the pathophysiology of these conditions into more effective therapies, since the choice of therapeutic options often remains empirical.

Keywords

NLRP12: NOD-like receptor family pyrin domain containing 12 · NLRP1: NOD-like receptor family pyrin domain containing 1 PAAND: Pyrin-associated autoinflammation with neutrophilic dermatosis · TNFRSF11A: Tumor necrosis factor receptor superfamily member 11a · NLRC4: NOD-like receptor family CARD domain containing 4 NEMO-△CT: NEMO deleted C-terminus · Otulin HA20: A20 haploinsufficiency

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Abbreviations

ALPS	Autoimmune lymphoproliferative syndrome
ASC	Apoptosis-associated speck-like protein containing a CARD
CAPS	Cryopyrin-associated periodic syndrome
CARD	C-terminal caspase activation and recruitment domain
CNS	Central nervous system
CRP	C-reactive protein
CYLD	Cylindromatosis
DAMP	Danger-associated molecular pattern
DMARDs	Disease-modifying anti-rheumatic drugs
DUB	Deubiquitinating enzymes
EDA-ID	Ectodermal dysplasia with anhydrosis with immunodeficiency
ESR	Erythrocyte sedimentation rate
FCAS	Familial cold autoinflammatory syndrome
FIIND	Function-to-find domain
FKLC	Familial keratosis lichenoides chronica
FMF	Familial Mediterranean fever
GSDMD	Gasdermin D
GVHD	Graft versus host disease
HA20	Haploinsufficiency of A20
HLH	Hemophagocytic lymphohistiocytosis
IBD	Inflammatory bowel disease
IFN	Interferon
IKK	IκB kinase
IL	Interleukin
IL-1Ra	IL-1 receptor antagonist
KGF	Keratinocyte growth factor
LRR	Leucine-rich repeat
LUBAC	Linear ubiquitin assembly chain complex
MAS	Macrophage activation syndrome
MSPC	Multiple self-healing palmoplantar carcinoma
NAIAD	*NLRP1*-associated autoinflammation with arthritis and dyskeratosis
NBS	Nucleotide binding site
NEMO	NF-κB essential modulator
NF-κB	Nuclear factor kappa B
NLR	NOD-like receptor
NLRC4	NOD-like receptor family CARD domain containing 4
NLRP1	NOD-like receptor family pyrin domain containing 1
NLRP12	NOD-like receptor family pyrin domain containing 12
NLRP12AD	*NLRP12*-associated disorder
NOD	Nucleotide-binding oligomerization domain
ORAS	Otulin-related autoinflammatory syndrome
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PAMP	Pathogen-associated molecular pattern
PAPA	Pyogenic arthritis, pyoderma gangrenosum, acne
PBMC	Peripheral blood mononuclear cell
PSTPIP	Proline-serine-threonine phosphatase interacting protein
PYD	Pyrin domain
RIG	Retinoic acid-inducible gene
RIP	Receptor interacting protein 1
RLR	RIG-I-like receptor
RNP	Ribonuclear protein
SCC	Squamous cell carcinoma
TANK	TRAF associated NFκB activator
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNFRSF11A	Tumor necrosis factor receptor superfamily member 11a
TRAF	Tumor necrosis factor receptor-associated factors
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome

Key Points

- **Genetic testing plays a key role in the confirmation of these rare clinical diagnoses**
- **The number and diversity of signaling pathways underlying autoinflammatory conditions are steadily expanding**
- **Next generation sequencing technologies accelerate the discovery of new disease-causing genes**

29.1 Nucleotide-Binding Oligomerization Domain (NOD)-Like Receptor Family Pyrin Domain Containing 12 (*NLRP12*)-Associated Disorder (NLRP12AD)

- **Nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 12-associated disorder (NLRP12AD) presents with clinical similarities with the cryopyrin-associated periodic syndromes (CAPS) and is due to mutations in a gene of the NOD-like receptor family**
- **The NLRP12-mediated inflammasome regulates inflammatory processes, including nuclear factor kappa B (NF-κB) and interleukin (IL)-1ß signaling**

29.1.1 Introduction

The first description of nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 12 (*NLRP12*)-associated disorder (NLRP12AD) was reported in 2008 [1], followed by several studies which expanded the phenotype. NLRP12AD presents a number of clinical and biological similarities with the cryopyrin-associated periodic syndromes (CAPS, see Chap. 19). Since *NLRP3* molecular defects explain only a subset of CAPS, another disease-causing gene was searched for by a candidate gene approach. *NLRP12* was selected as a good candidate due to its similarities with *NLRP3*, including its expression pattern, high homology of the corresponding protein sequences, and involvement in inflammatory and innate immune pathways. This led to the description of the first families carrying *NLRP12* germline pathogenic variants.

29.1.2 Epidemiology

NLRP12AD has been described in only a dozen families worldwide, underlining that this disorder is a very rare condition. Patients are from various origins (Guadeloupean of African descent, Italian, Armenian and Chinese) [1–5].

29.1.3 Etiology/Genetics

NLRP12AD is an autosomal dominant disorder. Several different molecular defects have been implicated in NLRP12AD: missense pathogenic variants (p.Asp294Glu, c.882C>G; p.Arg352Cys, c.1054C>T), nonsense mutations (p.Arg284*, c.850C>T; p.Trp408*, c.1223G>A), as well as a splice site variant leading to the activation of a cryptic donor splice site and resulting in a frameshift (c.2072+3insT; p.Val635Thrfs*12). All the variants identified to date with a pathogenic effect validated by familial segregation analyses and functional studies are located in exon 3 or in its flanking intronic sequences, showing that this domain is crucial for proper function of NLRP12. Additional variants whose pathogenic effects remain to be clarified (see http://fmf.igh.cnrs.fr/infevers, Chap. 12) have also been reported [4, 6, 7]. Notably, some *NLRP12* variants have also been identified in common variable immunodeficiency [8], as well as in patients with primary immunodeficiency, which shares clinical features with NRP12AD [7].

29.1.4 Pathogenesis

NLRP12 is expressed primarily in cells from the myelomonocytic lineage [9]. NLRP12 is part of the NOD-like receptor family (NLR), which includes several dozen intracellular proteins playing a central role in the regulation of inflammatory processes and innate immune responses. The timely activation and resolution of the innate immune response is essential for host defence and tissue homeostasis. NLR proteins act as intracellular sensors of various microbial structures called pathogen-associated molecular patterns (PAMP), and of endogenous molecules released by stressed cells called danger-associated molecular patterns (DAMP) (see Chap. 4). Upon stimulation, NLRs induce the formation of intracellular macromolecular protein complexes, called inflammasomes (see Chap. 5), resulting in the activation of pro-inflammatory signalling pathways and in the secretion of interleukin-1β (IL-1β), which is a major pro-inflammatory cytokine (see Chap. 6).

NLRP12 has a tripartite architecture very similar to that of NLRP3; an N-terminal pyrin domain (PYD) is able to recruit downstream effector molecules; a central nucleotide binding site (NBS) exhibits ATPase activity and regulates NLRP12 oligomerization; C-terminal leucine-rich repeats (LRR) might be implicated in NLRP12 autoregulation, protein-protein interactions, and/or sensing of activating agents. NLRP12 has been shown to play an important role in the regulation of IL-1β activation through its participation in the formation of an NLRP12-mediated inflammasome [9]. In addition to NLRP12, this complex includes ASC-apoptosis-associated speck-like protein containing a C-terminal caspase activation and recruitment domain (CARD) and caspase 1. Upon activation of the inflammasome, pro-caspase-1 is activated to caspase 1, which in turn cleaves pro-IL-1β and pro-IL-18 to produce the biologically active IL-1β, and IL-18, respectively. As an example, the NLRP12 inflammasome has been shown to play a key role in the response to *Yersinia pestis* infection [10]. It has also been reported that NLRP12 acts as a negative regulator of inflammation by suppressing both canonical and non-canonical nuclear factor kappa B (NF-κB) pathways [11].

While inflammasome complexes are essential for pathogen clearance under physiological conditions, their aberrant activation can be detrimental. Indeed, *NLRP12* disease-causing mutations alter IL-1β signalling. In some reports *NLRP12* pathogenic variants have been shown to induce spontaneous inflammasome activation, and exacerbated secretion of IL-1β [3]. In another report, monocytes from patients display steady-state normal IL-1β secretion but the kinetics of PAMP-induced IL-1β were significantly accelerated and associated with a high production of reactive oxygen species [2]. Frameshift and nonsense mutations have also been shown to alter NF-κB signaling [1], whereas missense mutations did not seem to alter this pathway [2, 3].

29.1.5 Clinical Manifestations

The disease usually starts during the first years of life and the frequency of inflammatory episodes is difficult to predict. Attacks can last 12–24 h in some patients and 1–2 weeks in others. Inter- and intra-familial clinical heterogeneity has been reported. One of the main hallmarks of the disease is the particular sensitivity to cold exposure. Indeed, in nearly all cases reported to date, episodes are triggered by generalized exposure to cold and avoiding cold exposure results in well-being. Attacks usually include fever, arthralgia, myalgia, an urticaria-like skin rash, and systemic inflammation. Other manifestations reported in a limited number of patients include headache, sensorineural hearing loss, abdominal pain, vomiting, buccal aphthous ulcers, and adenopathy. Optic neuritis was observed in one individual.

29.1.6 Laboratory Findings

As in many other autoinflammatory disorders, patients present with systemic inflammation associated with elevated levels of C-reactive protein (CRP) during inflammatory episodes, with normalization of acute phase reactants during symptom-free intervals. Neutrophilia is observed. No autoantibodies have been identified.

29.1.7 Diagnosis

The clinical presentation of NLRP12AD is very similar to familial cold autoinflammatory syndrome (FCAS) (see Chap. 19). Indeed, FCAS is characterized by episodes lasting about 12 h comprising fever, systemic inflammation, severe fatigue, an urticaria-like skin rash triggered by generalized exposure to cold, and arthralgia. Due to these clinical similarities with FCAS, NLRP12AD is also called familial cold autoinflammatory syndrome 2 (FCAS2). These two disorders can be distinguished by genetic testing of the *NLRP3* and *NLRP12* genes.

29.1.8 Treatment and Outcome

Avoiding cold exposure and living in warm climates has proven to be beneficial in 4 patients [1, 2]. In addition, decrease in the frequency and severity of manifestations with age has been reported in 3 separate families [1, 5]. The treatment of NLRP12AD is mainly empirical. Daily colchicine treatment, given to 4 patients, showed mild efficacy with no impact on the frequency of attacks, although it reduced the height of fever in 2 patients. Nonsteroidal anti-inflammatory drugs and oral corticosteroids alleviate symptoms, at least partially [1, 2]. Two patients have been treated with anakinra, a recombinant form of the IL-1 receptor antagonist (IL-1Ra) which blocks the activity of both IL-1α and IL-1ß (see Chap. 41). Initially, patients displayed a marked clinical improvement associated with near-normalization of IL-1β secretion. However, progressive clinical relapse occurred over time associated with the increase of other pro-inflammatory cytokines leading to the reactivation of IL-1β hypersecretion; anakinra treatment was discontinued after 14 months [12].

29.2 Monogenic NOD-Like Receptor Family Pyrin Domain Containing 1 *(NLRP1)*-Associated Diseases

- ***NLRP1* germline pathogenic variants are responsible for a wide spectrum of disorders characterized by dyskeratosis which may or not be associated with autoinflammatory and autoimmune features**
- **These syndromes result mainly from the exacerbated activation of the NLRP1 inflammasome in keratinocytes**

29.2.1 Introduction

The first Mendelian disorder due to molecular defects in the *NLRP1* gene was reported in 2013 and corresponds to a particular form of corneal intraepithelial dyskeratosis [13], which was subsequently called multiple self-healing palmoplantar carcinoma (MSPC) [14]. Subsequently, additional disorders with germline *NLRP1* pathogenic variants have been identified in other skin disorders with overlapping features: familial keratosis lichenoides chronica (FKLC) [14], and *NLRP1*-associated autoinflammation with arthritis and dyskeratosis (NAIAD) [15].

Notably, polymorphisms in *NLRP1* have been associated with autoimmune disorders such as vitiligo, rheumatoid arthritis, Addison disease, and systemic lupus erythematous.

29.2.2 Epidemiology

To date, less than 10 independent families carrying *NLRP1* germline pathogenic variants have been reported worldwide. Patients originate from various countries (Tunisia, Algeria, France and Holland) [13–15].

29.2.3 Etiology/Genetics

MSPC is an autosomal dominant disorder. The first reported *NLRP1* germline mutation (p. Met77Thr; c.230T>C), which occurred *de novo* in the first reported case, was identified by exome sequencing in a three-generation family [13]. Two additional missense pathogenic variants have been identified in familial forms of MSPC: p.Ala54Thr, and p.Ala66Val [14].

A single form of FKLC due to *NLRP1* variants has been reported to date in a consanguineous family [14]. A deletion of *NLRP1* exon 5 (p. Phe787_Arg843del) was identified in the homozygous state in two brothers with a severe phenotype and in the heterozygous state in their parents with a milder FKLC presentation, consistent with an autosomal dominant transmission associated with a dose effect.

In NAIAD, molecular screening of *NLRP1* revealed a homozygous mutation (c.2176C>T;

p.Arg726Trp) in 2 cousins born of related parents and a *de novo* heterozygous mutation (c.3641C>G, p.Pro1214Arg) in a girl of Dutch origin [15].

29.2.4 Pathogenesis

NLRP1 has a large expression pattern, with high expression in the skin (especially in keratinocytes), as well as in the corneal epithelium [13, 14]. Multiple alternatively-spliced transcripts encoding distinct isoforms have been described, but their biological function remain to be investigated.

As a member of the Ced-4 family of apoptosis proteins, NLRP1 is a key protein of caspase-mediated apoptosis. It is also a member of the NLR protein family, which plays a major role in inflammatory and innate immune signaling pathways. In this regard, NLRP1 is the most prominently expressed sensor in human skin triggering inflammasome activation.

NLRP1 comprises the classical tripartite structure described previously for NLRP12, consisting of an N-terminal PYD, the central NBS, followed by LRR. Nevertheless, among all PYD-containing NLR, NLRP1 is the only one to possess an additional CARD domain preceded by a "function-to-find" domain (FIIND). The PYD of NLRP1 functions as an auto-inhibitory domain, unlike the PYD of other known NLR, while the C-terminal fragment containing the CARD is responsible for activating the inflammasome [14]. Wild-type NLRP1 is kept as an inactive monomer by the combined action of the PYD and LRR domains.

Functionally, all MSPC and FKLC pathogenic variants lead to spontaneous inflammasome assembly and IL-1β secretion. The three missense variants reported in MSPC are located within the PYD domain of the protein. The deletion of the fifth exon of *NLRP1* identified in FKLC results in an internal in-frame deletion, which removes the first of the six LRR and part of the preceding linker region. When either domain is mutated by MSPC or FKLC variants, the autoinhibitory mechanism is lost and there is an increased propensity for NLRP1 to oligomerize [14]. The variants identified in NAIAD, p.Arg726Trp and p.Pro1214Arg, are located between the NBS and LRR for the first mutation, and within the FIIND domain for the second. Their functional consequences have not been investigated to date.

The recurrent focal lesions observed in MSCP and FKLC originate from the exacerbated activation of the NLRP1 inflammasome in keratinocytes [14]. The subsequent release of cytokines from the IL-1 family likely activates a paracrine signaling network, resulting in the secondary secretion of other pro-inflammatory cytokines and growth factors (such as tumor necrosis factor (TNF)-α and keratinocyte growth factor—KGF) by neighboring keratinocytes and fibroblasts. This aberrant "wound-healing"-like response leads to epidermal hyperplasia and keratoacanthoma. The continuous unresolved inflammation facilitates malignant transformation towards squamous cell carcinomas (SCC). NLRP1 thereby plays a key role in skin inflammatory syndromes and skin cancer predisposition.

29.2.5 Clinical Manifestations

MSPC can start during infancy or adulthood. The clinical picture includes dyskeratosis in conjunctival and corneal epithelia, palmoplantar hyperkeratosis, and laryngeal dyskeratosis (Fig. 29.1) [13, 14]. The ocular involvement, which includes dyskeratotic lobules, neovascularisation and complete corneal opacification, leads to severe visual impairment with visual acuity limited to hand motion in some patients. Patients develop numerous ulcerative, hyperkeratotic nodular growths on plantar and palmar skin. Clinically, these lesions resemble keratoacanthomas. They usually regress spontaneously, but patients have increased susceptibility to malignant SCC [14]. A subset of patients displays additional features: hyperkeratosis pilaris, irregular and thickened nails, finger joint hypermobility, dysmorphic features (long philtrum, short neck, bulging chest), and raspy voice.

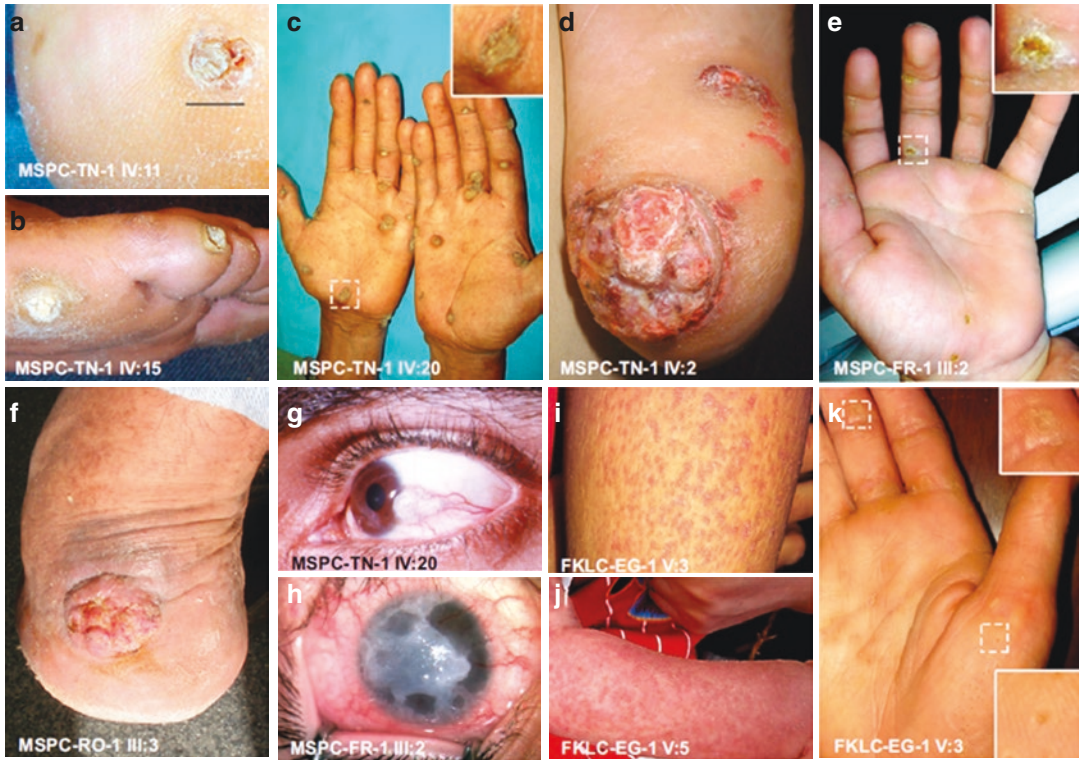

Fig. 29.1 (**a**–**k**) *NLRP1* associated autoinflammation with arthritis and dyskeratosis. (**a**) Indurated ulcer with overlying crust and surrounding hyperkeratosis on the heel. (**b**) Firm, scaly nodule on the outer part of the right foot and subungual lesion on the little toe. (**c**) Multiple discrete, self-healing, warty keratoacanthomas on both palms and fingers (one lesion detailed in inset). (**d**) "Ulcerated squamo-proliferative" plaque on the heel with a further smaller lesion on the sole. (**e**) Multiple discrete warty papules and nodules on the left palm and fingers (one lesion detailed in inset), consistent with keratoacanthoma. (**f**) Squamo-proliferative keratoacanthoma on the heel with surface ulceration. (**g**) Left eye showing conjunctival and corneal scarring with hyperemia. (**h**) Obliterative corneal scarring and surrounding conjunctival inflammation. (**i**) Multiple discrete and semi-confluent lichenoid papules on the upper arm. (**j**) Multiple discrete and semi-confluent lichenoid papules on the forearm. (**k**) Hyperkeratotic papules on the thenar eminence and palmar surface of fingers (shown in more detail in insets). Reference [14], with permission from Cell

A single consanguineous family with FKLC has been described to date [14]. FKLC shares multiple clinical features with MSPC, such as plantar keratosis and follicular hyperkeratosis. In addition, the disorder is characterized by severe generalized lichenoid papular lesions on the limbs and trunk. In this family, the phenotype was milder in parents who were heterozygous carriers for the *NLRP1* pathogenic variant, as compared to their children who carried the mutation in the homozygous state.

NAIAD combines dyskeratotic manifestations as well as systemic autoinflammatory and autoimmune features [15]. The disease starts within the first months of life. Patients display follicular palmoplantar hyperkeratosis and dyskeratotic lesions of the larynx, which can be accompanied by subglottic edema and failure to thrive. Ocular signs were reported in 2 patients: one with corneal dyskeratosis and neovascularisation associated with photophobia and one with uveitis with no sign of dyskeratosis. As for the autoinflammatory part of the phenotype, the 3 patients from 2 independent families presented with recurrent episodes of unprovoked fever lasting several days associated with systemic inflammation and polyarthritis. Patients display various signs revealing an impairment of the

immune system: chronic infections (*Giardia intestinalis*, candidiasis), autoimmune hemolytic anemia, and thyroiditis. All patients also had a vitamin A deficiency. Amelogenesis imperfecta, leading to loss of several teeth, and hepatosplenomegaly have also been reported in NAIAD.

29.2.6 Laboratory Findings

The histology of cornea, vocal cords, and skin shows dyskeratotic keratinocytes, hyperkeratosis, and epidermal hyperplasia [13, 14]. The culture of patients' keratinocytes reveals constitutive NLRP1 inflammasome activation and spontaneous secretion of very high levels of IL-1β.

In NAIAD, several additional signs of autoinflammation or autoimmunity are observed [15]. In the 3 reported patients there is systemic inflammation associated with elevated levels of CRP, caspase-1, and interleukin 18. High levels of IL-1β have also been detected in the serum of one patient. Two of the 3 patients had antinuclear antibodies. Two patients displayed high levels of IgG and one patient elevated levels of IgA. The evaluation of circulating immune cells shows neutrophilia, T-cell lymphopenia, a high level of transitional B cells, and a low number of circulating CD27+ B-cell subsets. Massive hepatosplenomegaly was observed. The histopathology of the spleen revealed extra-medullar hematopoiesis, siderosis, and sparse white pulp with very few B cells.

29.2.7 Imaging

Skeletal radiographs performed in two patients with NAIAD have revealed abnormal features in the distal femoral epiphysis and metaphyses [15].

29.2.8 Diagnosis

The clinical spectrum of *NLRP1*-associated disorders, comprising MSPC, FKLC, and NAIAD is large. All *NLRP1*-associated disorders are characterized by ocular, palmoplantar and laryngeal dyskeratosis. In the classical form of hereditary benign intraepithelial dyskeratosis, ocular lesions rarely impair vision acuity and patients do not have general involvement with pruritic hyperkeratotic scars, palmoplantar hyperkeratosis, chronic rhinitis, vocal cord involvement, and nail thickening. In FKLC, patients also display multiple lichenoid papules on the limbs and trunk. NAIAD combines signs of MSPC with autoinflammatory and autoimmune features. Clinical diagnosis of *NLRP1*-associated disorders can be confirmed by genetic testing.

29.2.9 Treatment and Outcome

Ocular topical corticosteroids are not effective in preventing the poor visual outcome in MSPC. Several deep anterior lamellar keratoplasties performed in a patient with MSPC resulted in a brief visual improvement followed by recurrence of the disease. Corneal implants performed in the same patient improved visual acuity [13].

One patient with NAIAD showed a beneficial effect of etanercept on polyarthritis [15]. Anakinra used in another patient, resulted in total resolution of arthritis and inflammatory signs and allowed the discontinuation of corticosteroids [15]. Acitretin used in two patients to treat dermatological signs, markedly reduced skin lesions. Vitamin A supplementation does not seem to improve disease manifestations.

29.3 Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND)

- **PAAND is a rare autosomal dominant disorder involving the *MEFV* gene, also responsible for familial Mediterranean fever (FMF)**
- **The mutations responsible for PAAND act by a particular mechanism disrupting pyrin binding with the regulatory 14.3.3 proteins**

29.3.1 Introduction

Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) is a very rare autoinflammatory disorder described in 2016 [16]. Its name comes from the involvement of the pyrin protein in the pathogenic process (see below) and from the two main clinical features of the disease: systemic inflammation and dermatosis.

29.3.2 Epidemiology

This syndrome was first reported in a three-generation family from Belgium comprising 22 individuals with 12 affected members [16]. A similar phenotype was also observed in 3 unrelated families, one originating from France, one from the United-Kingdom, and one from Spain [16, 17].

29.3.3 Etiology/Genetics

PAAND is an autosomal dominant disorder. The *MEFV* (MEditerranean FeVer) gene was implicated in PAAND by means of a genome-wide linkage study performed in the Belgian family mentioned previously, combined with exome sequencing of the candidate region located on chromosome 16 [16]. This led to the identification of a heterozygous pathogenic variant in exon 2, p.Ser242Arg (c.276C>G), affecting a residue highly conserved throughout evolution. The same genotype was identified in two other families with PAAND [16], while the third familial form was due to another pathogenic variant, p. Glu244Lys (c.730G>A) [17]. Notably, *MEFV* was previously identified as the gene responsible for familial Mediterranean fever (FMF; OMIM: 249100) [18, 19], the prototypic and probably most common of the monogenic autoinflammatory diseases (see Chap. 16). In FMF, most mutations are missense changes located in the last exon of the gene (exon 10), which encodes a B30.2/SPRY domain. Notably, the 2 molecular variants responsible for PAAND are located in a protein region distinct from that of typical FMF mutations (see below).

29.3.4 Pathogenesis

MEFV is expressed predominantly in granulocytes, monocytes, dendritic cells, and in fibroblasts [20, 21]. It encodes a protein of 781 amino acids called pyrin (also known as Marenostrin), which plays a key role in NF-κB signaling and IL-1ß secretion. Pyrin N-terminal part consists of a PYD, known to mediate homotypic interactions and found in proteins involved in the regulation of inflammation and apoptosis. Pyrin interacts through PYD-PYD interactions with the adaptor protein ASC, allowing the formation of a pyrin-mediated inflammasome complex thereby driving the maturation and secretion of IL-1ß, as well as cell death [22]. Ser242, which is mutated in PAAND, is a phosphorylated residue located in a 14–3-3 binding motif. The disruption of this phosphorylation by the mutation identified in patients results in the loss of 14–3-3 binding, pyrin release from the 14–3-3 inhibitory protein, and subsequent inflammasome activation [16, 23]. Consistently, patients' monocytes display an increase in IL-1ß secretion. The p.Glu244Lys mutation, located two amino acids away from the first mutant similarly alters pyrin phosphorylation and 14.3.3 binding.

Notably, bacterial effectors that activate the pyrin inflammasome, such as *Clostridium difficile* toxin B (TcdB), lead to a similar loss of Ser242 phosphorylation and 14–3-3 binding. The effect of the p.Ser242 and p.Glu244 mutations thus recapitulates the effect of pathogen sensing by disrupting the guard-like mechanism mediated by 14–3-3 inhibitory proteins. In contrast, the *MEFV* mutations responsible for FMF have no effect on 14–3-3 binding and alters pyrin activity by a different mechanism.

29.3.5 Clinical Manifestations

The main clinical hallmark of PAAND is neutrophilic dermatosis, which can be revealed by different signs: severe acne, sterile skin abscesses, pyoderma gangrenosum, hidradenitis suppurativa, or neutrophilic small-vessel vasculitis. The clinical picture also includes childhood-onset

recurrent episodes of fever lasting several weeks, neutrophilic panniculitis, arthralgia, polyarthritis, and myalgia/myositis. One patient was reported to present with dilated cardiomyopathy at the age of 13 years, followed by chronic cardiac decompensation, necessitating cardiac transplantation at the age of 20 years [16]. Clinically, PAAND can be distinguished from FMF by the presence of severe recurrent neutrophilic dermatosis, the absence of serositis, and the long duration of fever attacks lasting several weeks rather than days.

29.3.6 Laboratory Findings

Histologic examination of skin biopsies reveals a dense, predominantly neutrophilic, vascular, perivascular, and interstitial infiltrate.

Systemic inflammation is associated with increased levels of acute phase reactants such as CRP, and raised peripheral polymorphonuclear count. Measurement of cytokines in sera reveals elevated levels of several inflammatory mediators: IL-1ß, IL-1Ra, IL-6, TNF-α, total IL-18, and IL-18 binding protein.

29.3.7 Diagnosis

The diagnosis is based on the presence of the neutrophilic dermatosis associated with systemic inflammation. Notably, these cutaneous manifestations could be suggestive of the rare pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome due to mutations in the gene coding for the proline-serine-threonine phosphatase interacting protein (PSTPIP)1 protein, a pyrin-binding partner [24] (see Chap. 22). PAAND diagnosis can be confirmed by genetic testing of *MEFV*.

29.3.8 Treatment and Outcome

Successful therapy with anakinra has been reported in one patient after failure of corticosteroids and methotrexate. Clinical signs resolved rapidly accompanied by normalization of CRP levels [16]. However, failure of anakinra and corticosteroids was reported in another patient. This second patient had an initial good response to infliximab followed by clinical relapse, leading to switch treatment to adalimumab [17].

29.4 *TNFRSF11A*-Associated Autoinflammatory Disorder: Tumor Necrosis Factor Receptor-Associated Periodic Syndrome 11 (TRAPS11)

- **Tumor necrosis factor receptor-associated periodic syndrome 11 (TRAPS11) shares several clinical and biological features with TRAPS**
- **The TNF receptor superfamily member 11a (TNFRSF11A) transmembrane receptor involved in this disease regulates both autoinflammatory processes and bone metabolism**

29.4.1 Introduction

The *TNFRSF11A*-associated autoinflammatory disorder is a very rare entity, which was described in 2014. This disorder is part of the subgroup of recurrent fever syndromes. It has been proposed to call this disorder tumor necrosis factor receptor-associated periodic syndrome 11 (TRAPS11) due to its clinical similarity with the "original" TRAPS [25].

29.4.2 Epidemiology

Two unrelated families from Caucasian origin have been reported to date [26]. The first one consists of a single case. The second family comprises 2 affected members (mother and daughter).

29.4.3 Etiology/Genetics

TRAPS11 is an autosomal dominant disorder due to mutations in the *TNFRSF11A* gene, also known as *RANK*. *TNFRSF11A* belongs to the same gene family as *TNFRSF1A*, the gene implicated in TRAPS. The first reported patient with TRAPS11 presented with a complex phenotype

and a large *de novo* heterozygous complex chromosomal rearrangement encompassing a duplication of the *TNFRSF11A* gene [26]. In the 2 affected members from the second family, a frameshift mutation (p.Met416Cysfs*110; c.1245del) has been identified in the heterozygous state. Notably, other *TNFRSF11A* mutations have been implicated in bone disorders: autosomal recessive osteopetrosis and autosomal dominant osteolytic disorders (familial expansile osteolysis, and Paget disease of bone).

29.4.4 Pathogenesis

The *TNFRSF11A* gene is expressed in various cell types. The protein encoded by this gene is a receptor of the TNF receptor (TNFR) superfamily; all TNFR are transmembrane proteins with cysteine-rich domains that form homotrimers. TNFR receptor superfamily member 11a (TNFRSF11A) is a protein with various functions. It interacts with TNF receptor-associated factors (TRAF), which are recruited to transduce the signal, and thereby induces the activation of the NF-κB and MAPK8/JNK signaling pathways. This receptor and its ligand are important regulators of the interaction between T cells and dendritic cells. It is an essential mediator for osteoclast and lymph node development. TNFRSF11A is also known to regulate fever in rodents: injection of the receptor ligand (TNFSF11) into the ventricle of the brain triggers fever, and *Tnfrsf11a*-knockout mice are resistant to fever induced by proinflammatory agents [27].

The p.Met416Cysfs*110 variant identified in patients with TRAPS11 does not alter the protein expression or localization in patients' leukocytes. The p.Met416Cysfs*110 truncated protein lacks conserved self-association domains and several binding motifs for TRAF. Consistently, the p.Met416Cysfs*110 pathogenic variant alters the function of TNFRSF11A on NF-κB signaling, at baseline in decreasing its activation and in modulating the response to the TNFSF11 receptor ligand. Regarding the chromosomal rearrangement comprising the *TNFRSF11A* duplication, it supports a gain of function due to a dosage effect, although it is difficult to evaluate *TNRSF11A* contribution as compared to that of neighboring genes.

29.4.5 Clinical Manifestations

The patient carrying the complex chromosomal rearrangement including the duplication of the *TNFRSF11A* gene presented with recurrent fever starting in the neonatal period and multiple congenital anomalies including intellectual disability, tetralogy of Fallot, cleft palate, and facial dysmorphism. As for the autoinflammatory component of the phenotype, the patient experienced recurrent episodes of high fever (40 °C), lasting 8 days to 5 weeks, associated with enlarged lymph nodes, macular rash, abdominal pain and constipation. The index case of the second family is a woman whose disease started at the age of 18 years. She experienced more than one episode of inflammation per month lasting 10–21 days, associated with high fever (39–41 °C), abdominal pain, headaches, severe asthenia, hacking cough, thoracic pain, and systemic inflammation. A number of additional manifestations were observed in this patient: erythema nodosum, anterior uveitis, arthralgia/arthritis, mesenteric adenitis, stress fracture of the ankle, and amelogenesis imperfecta. The mother of this patient carrying the same molecular defect experienced recurrent pharyngitis and severe abdominal pain since infancy, associated with arthralgia and myalgia.

There is some overlap between *TNRSF11A*-associated autoinflammatory syndromes and *TNRSF11A*-associated bone disorders. Indeed, the stress fracture and dental problems observed in TRAPS11 are also found in patients with osteolytic disorders. Along with this observation, several patients with autosomal-recessive osteopetrosis and carrying *TNFRSF11A* molecular defects were found to be unable to develop fever during episodes of pneumonia [27].

29.4.6 Laboratory Findings

CRP and serum amyloid A levels measured in one patient were found to be very high during attacks and moderately elevated between episodes. Mild hypergammaglobulinemia was also observed, although markers of autoimmunity were normal.

The p.Met416Cysfs*110 variant is associated with increased secretion of several inflammatory cytokines: TNF-α, IL-18, IL-1Ra, and interferon-γ.

29.4.7 Diagnosis

Long-lasting recurrent inflammatory attacks associated with systemic inflammation, fever, and abdominal pain should be suggestive of TRAPS11. Notably, this presentation exhibits clinical similarities with TRAPS. The diagnosis can be confirmed by genetic testing of *TNFRSF11A*. In addition, due to the involvement of TNFRSF11A in inflammatory processes and bone physiology, autoinflammatory manifestations as well as bone metabolism alterations should be investigated in patients with *TNFRSF11A* pathogenic variants.

29.4.8 Treatment and Outcome

Treatment with corticosteroids and colchicine of one patient with TRAPS11 resulted only in partial resolution of symptoms. Further investigations are needed to evaluate the consequences of TNFRSF11A inhibition by soluble receptors and recombinant antibodies, since these drugs lack specificity.

29.5 NOD-Like Receptor Family CARD Domain Containing 4 *(NLRC4)*-Associated Autoinflammatory Diseases

- **The understanding of NLRC4 biology is uniquely comprehensive amongst inflammasome nucleators**
- **The spectrum of NLRC4-related disorders includes phenotypes similar to CAPS as well as life-threatening infantile macrophage activation syndrome (MAS) and enterocolitis**
- **NLRC4-MAS may implicate a role for epithelial inflammasomes linking MAS and IL-18**

29.5.1 Introduction

Investigation into the NLRC4 inflammasome occurred well before its first association with monogenic disease, largely in association with its role in experimental *Salmonella* infections [28–31]. In fact, a great deal of biochemical and structural information is available for the NLRC4 inflammasome that is not yet available for the NLRP3 and pyrin inflammasomes [32–35]. The human manifestations of NLRC4 dysregulation began to take shape in 2014, when three groups reported monogenic diseases associated with gain-of-function mutations in *NLRC4* resulting in increased/spontaneous inflammasome activation [36–38]. Like many autoinflammatory diseases, those associated with NLRC4 (NLRC4ADs) can have a significant degree of phenotypic heterogeneity. However, one particularly severe phenotype, infantile macrophage activation syndrome (MAS) with enterocolitis, seems unique to NLRC4 amongst monogenic autoinflammatory diseases.

29.5.2 Epidemiology

Causative NLRC4 mutations have been reported in 2 extended kindreds and spontaneously in another 6 patients/families. Thus, NLRC4ADs appear to be extremely rare, possibly because relatively few mutations result in gain-of-function, and because severe mutations may cause perinatal lethality. Patients are from various origins, including American, Eastern European [34, 36, 47, 48], Japanese [35, 37], Dutch [49] and Italian [39].

29.5.3 Etiology/Genetics

All published disease-associated mutations in *NLRC4* have been missense mutations associated with an amino acid substitution (p.Ser171Phe; p.Thr177Ala; p.Thr337Ser; p.Val341Ala; p.His443Pro; p.Ser445Pro). Two pathogenic mutations were detected by next-generation sequencing and verified to be high-frequency somatic mutations (occurring in 50–65% of both blood

and skin cells tested) [40]. All variants published to date map in close proximity to the nucleotide-binding pocket in the proposed NLRC4 crystal structure [41], and are thought to disrupt molecular interactions necessary for autoinhibition. Most mutations have been validated either by functional assay or familial segregation analyses. Additional variants whose pathogenic effects remain to be clarified have also been reported and can be found in the http://fmf.igh.cnrs.fr/infevers (see Chap. 12). Notably, a genome-wide association study linking IL-18 levels to patients with cardiovascular disease identified an exonic single-nucleotide polymorphism in *NLRC4* [42].

29.5.4 Pathogenesis

Extensive work in murine systems has provided a uniquely complete paradigm for NLRC4 inflammasome activation and function [41]. It is, of course, difficult to quantify the extent to which this murine model is applicable to humans, but murine and human NLRC4 are highly homologous. Normally, NLRC4 exists in an autoinhibited conformation stabilized by nucleotide binding [33]. NLRC4 is not a sensor itself. Instead, NLRC4 relies on activation by a homologous NLR protein called NAIP (or NAIP1 through NAIP5 in mice). NAIP proteins bind to certain bacterial proteins, namely flagellin monomers or needle/rod proteins of the bacterial type III secretion system, that they detect in the cytosol of macrophages, monocytes, and potentially neutrophils [31, 35, 43, 44]. Upon ligand binding, NAIPs change conformation and thereby induce a conformational change in NLRC4 that allows active NLRC4 to recruit and activate other NLRC4 molecules. NAIP-induced NLRC4 oligomerization provides a scaffold for ASC fibril formation and eventually caspase-1 recruitment and autoproteolysis.

Like other inflammasomes (see Chap. 5), activation of the NLRC4 inflammasome results in proteolytic activation of IL-1β, IL-18, and gasdermin-D (GSDMD) and resultant cytokine signaling and pyroptotic cell death. NLRC4 has some unique features, as well. NLRC4 can interact with caspase 1 directly, without ASC, but ASC appears to be required for cytokine production [45]. NLRC4 activation may also preferentially drive eicosanoid production and release, causing rapid vascular leak and death [30, 46, 47]. The inflammatory response of *Salmonella*-infected cells requires NLRC4, which can then recruit and activate NLRP3 as well [48, 49]. NLRC4 inflammasome components are also expressed and active in murine intestinal epithelial cells, where they are similarly activated but can result in both cytokine production and both pyroptosis and epithelial expulsion [30, 50]. In the absence of caspase 1, the NLRC4 inflammasome can also activate caspase 8.

The association of NLRC4AD with IL-18 and MAS appears unique amongst inflammasomopathies and may be associated with its expression in intestinal epithelia [50].

29.5.5 Clinical Manifestations

The initial reports described a dominant/*de novo* syndrome of infantile onset systemic, hyperferritinemic inflammation reminiscent of MAS accompanied by a variable, but potentially severe enterocolitis [36, 38] (Fig. 29.2). MAS is a state of extreme hyperinflammation characterized by hectic fever, hyperferritinemia, coagulopathy (manifest as a low platelet count and dropping fibrinogen and/or erythrocyte sedimentation rate [ESR]), hepatobiliary dysfunction, hepatosplenomegaly, and often hemophagocytosis, typically present at disease onset, that can last for many weeks (see Chap. 33). MAS is most commonly associated with the complex autoinflammatory disorders systemic juvenile idiopathic arthritis and adult-onset Still disease (see Chap. 32). Natural killer dysfunction, a hallmark of a similar syndrome called familial hemophagocytic lymphohistiocytosis (HLH), has not been detected except during the acute phase of NLRC4-MAS [36, 38]. Severe NLRC4-MAS has been associated with pan-enterocolitis that extends from the stomach through and including the colon [38, 51]. Other patients have had milder, largely small intestinal disease histologically similar to celiac

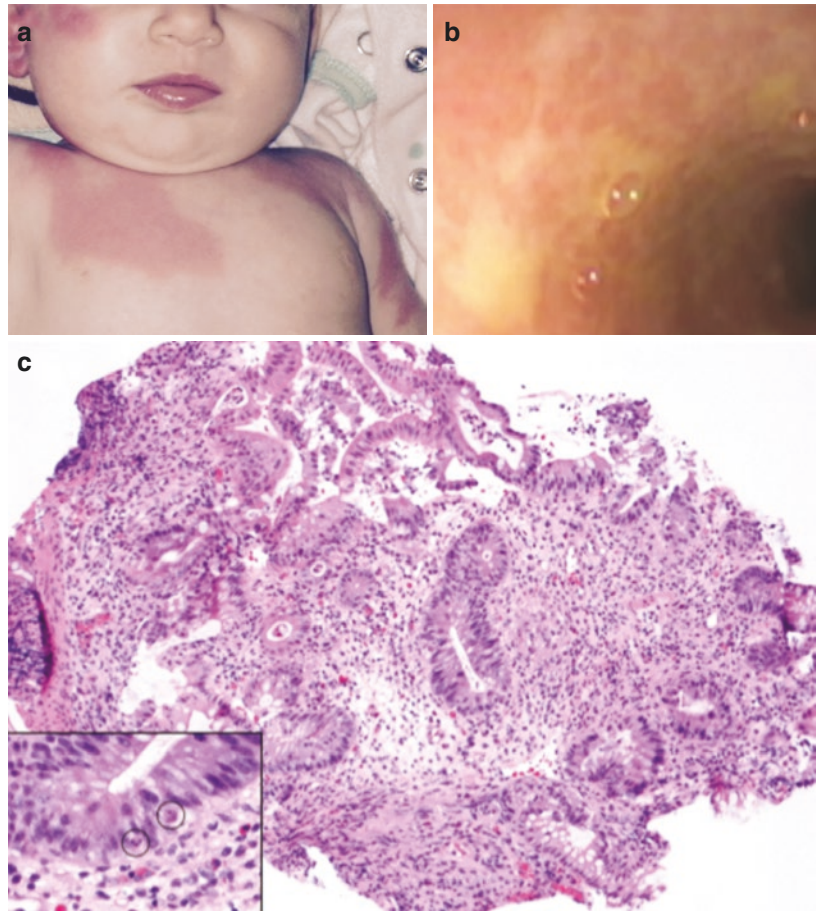

Fig. 29.2 NLRC4AD: Rash and enterocolitis in nucleotide-binding oligomerization domain (NOD)-like receptor family caspase activation and recruitment domain (CARD) domain containing 4 (NLRC4)-related macrophage activation syndrome (MAS) and enterocolitis: (**a**) erythrodermal skin lesions. (**b**) Ulcerative duodenal plaques. (**c**) Inflamed sigmoid colon with apoptotic crypt epithelial cells (inset circles); 20×, H&E. Reproduced with permission, J Allergy Clin Immunol [51]

disease [36, 38]. Curiously, NLRC4-associated enterocolitis resolved in patients who survived beyond infancy. Some patients experienced prolonged periods of seemingly complete disease quiescence off therapy, only to have severe episodes of MAS triggered by infection or stress later in life [38]. Notably, these patients appeared to have extremely elevated levels of IL-18 even during disease quiescence, a finding observed in other patients with MAS but not in other inflammasome-associated autoinflammatory diseases. Subsequent reports have largely confirmed the potential for perinatal lethality of the NLRC4-MAS phenotype, and its association with potentially pathogenic IL-18 [51, 52].

However, other groups have discovered *NLRC4* mutations associated with more typical and less lethal autoinflammatory symptoms including cold-induced urticaria, erythematous nodules, arthritis, inflammatory bowel disease, or sterile meningitis [37, 40, 53]. What distinguishes patients developing MAS from those with less severe phenotypes is unknown, although there may be consistent genotype/phenotype correlations, and disease manifestations exhibit less variability within families [41]. Notably, the histologic appearance of the skin rash in NLRC4AD may be distinct from that of *NLRP3*- and *MEFV*-related diseases; a biopsy of an erythematous/nodular rash showed a lymphohistiocytic infiltrate, whereas the rashes of other inflammasomopathies are characterized by mature neutrophils [53].

29.5.6 Laboratory Findings

Testing will be largely dictated by and dependent on clinical manifestations. During the acute phase of MAS, patients may have all the hallmarks of MAS including elevated CRP, highly elevated ferritin and soluble CD25, hypertriglyceridemia, elevated lactate dehydrogenase, anemia, leukopenia, low platelet count, elevated liver transaminases with or without direct hyperbilirubinemia, and potentially hemophagocytosis on bone marrow or tissue biopsy. The coagulopathy of MAS may progress such that fibrinogen may be elevated at presentation as part of the acute response, but drop precipitously as coagulopathy worsens. Hypoalbuminemia may be pronounced in patients with both systemic third-spacing and enterocolitis. During the convalescent phase, there may be no signs of inflammation or the ferritin may be minimally elevated. By contrast, NLRC4AD patients with more typical autoinflammatory features do not have substantial ferritin elevations and acute phase reactants track with disease activity.

29.5.7 Diagnosis

A high index of suspicion is required, and given the breadth of manifestations reported since 2014, it is likely the spectrum of phenotypes will broaden. Mutations in NLRC4 will likely be gain-of-function missense mutations and thereby detectable by standard Sanger or whole-exome sequencing strategies. However, the presence of pathogenic somatic mutations in *NLRC4* supports deeper sequencing when suspicion is high and standard techniques have not detected an *NLRC4* mutation. Because NLRC4AD has been associated with a variety of manifestations, we recommend screening for *NLRC4* mutations in patients with early-onset autoinflammatory symptoms like skin rash and unexplained fevers, very early-onset inflammatory bowel disease (see Chap. 21), and/or uncategorized MAS/HLH.

29.5.8 Treatment and Outcome

Outcomes in NLRC4AD span the spectrum from minimally symptomatic chronic urticaria to perinatal lethality. Some patients appear to require no more than intermittent non-steroidal anti-inflammatory drugs [37]. In patients with more severe urticaria or other symptoms consistent with CAPS (periodic fevers, aseptic meningitis), IL-1 inhibition with anakinra may be extremely effective [40]. However, another kindred with more variable manifestations showed a similarly variable response to IL-1 inhibition, suggesting that some genotypes, phenotypes, and/or genetic backgrounds may be less responsive to IL-1 inhibition [53]. Anakinra was effective in nearly eliminating episodes in an older child with milder NLRC4-MAS [36]. More severe NLRC4-MAS appears to require more than IL-1 inhibition, and several patients have died despite high-dose corticosteroids, IL-1 inhibition, cyclosporine, and/or etoposide [38, 51, 52]. Case reports exist of dramatic success to adding investigational IL-18 or interferon (IFN)-γ blockade, but despite the strong rationale for their use the utility of these strategies awaits formal evaluation [51]. Though numbers are very small, patients with controlled MAS appear to have normal life spans, reproductive capacity, and no increased risk of amyloidosis or malignancy.

29.6 NF-κB Essential Modulator (NEMO)-ΔCT Gain-of-Function

- **NF-κB essential modulator (NEMO) hypomorphic mutation is associated with a disease spectrum ranging from primary immunodeficiency to sterile uveitis, panniculitis/dermatitis, and enterocolitis with variable expression of impaired development of ectodermally derived tissues.**
- **Symptoms arise due to failure of NEMO to recruit negative regulators of NF-κB activation**

29.6.1 Introduction

The NF-κB family of transcription factors are important activators of innate and adaptive immunity and are essential in the development and normal function of the immune system, in addition to the embryonic ectodermally-derived structures such as the nervous system, hair follicles, sweat glands and teeth. The TNFR superfamily, toll-like receptor (TLR), retinoic acid-inducible gene (RIG)-I-like receptor (RLR) and NLR pattern recognition receptors and antigen receptors all activate the canonical IκB kinase (IKK) complex leading to NF-κB activation. Signal transduction is mediated by a series of phosphorylation events and other post-translational modifications predominated by activation of E3 ubiquitin ligases that catalyze the addition of distinct forms of polyubiquitin chains to enable proteasomal degradation or protein:protein interactions. The signal transduction pathways that lead to NF-κB activation are highly regulated, since impaired signaling leads to primary immunodeficiency and enhanced signaling leads to autoinflammatory disease. Several deubiquitinating enzymes (DUBs), including A20, cylindromatosis (CYLD), cezanne, and OTULIN act as negative regulators of NF-κB signaling and are essential for the return to homeostasis following immune activation. Mouse and human studies indicated that not only impaired negative regulation of NF-κB but also impaired activation of NF-κB can result in inflammatory disease phenotypes.

The NF-κB essential modulator (NEMO) is a scaffolding protein that recruits the catalytically active components of the IKK complex in addition to multiple other signaling effectors and negative regulators of the NF-κB pathway. The IKK complex phosphorylates the inhibitor of NF-κB, IκB, which targets IκB for K48-linked polyubiquitination and proteosomal degradation, thus enabling stimulation-induced nuclear translocation of NF-κB dimers. Amorphic NEMO mutations are lethal to males, but hypomorphic mutations can result in ectodermal dysplasia and immunodeficiency [54]. This disease spectrum was defined by familial susceptibility to mycobacterial infection, recurrent infection with pyogenic bacteria, and abnormal immunoglobulin production in the setting of variable T and B cell defects [55]. The ectodermal dysplasia results from an inability of the ectodysplasin A receptor (a TNF receptor family member) to induce NF-κB activation following ligation. Severe mutations can present with a Behçet disease phenotype in males and carrier females. The clinical and immunological phenotypes attributed to NEMO hypomorphic mutation have expanded in recent years to include its function in recruiting negative regulators, as mutant forms with this impaired function have been shown to lead to increased NF-κB activation and inflammatory disease phenotypes resembling Behçet disease.

29.6.2 Epidemiology

Estimates of NEMO hypomorphism have been placed at 1/100,000 newborns [56]. Autoimmune and autoinflammatory disease phenotypes have appeared in between 25 and 33% of individuals who have been described [55, 57].

29.6.3 Etiology/Genetics

Individuals with hypomorphic mutations in *IKBKG,* develop primary immunodeficiency with a wide phenotypic spectrum due to reduced NEMO protein function. Individuals with mutations in two distinct NEMO domains have been diagnosed with autoinflammatory phenotypes. Although this is an X-linked disorder, in addition to males who express the NEMO-ΔCT form due to a premature stop codon, in rare cases female carriers have been diagnosed with an inflammatory disease resembling Behçet disease [personal communication EPH, and [58]].

29.6.4 Pathogenesis

As stated in the introduction, NF-κB activation depends on NEMO expression. Absence of NEMO leads to impaired formation of the IKK

signaling complex and embryonic lethality in NEMO knockout mice due to massive hepatocyte apoptosis that does not occur in the absence of TNF signaling. In murine models, inflammatory skin and intestinal disease due to an absence of NEMO in keratinocytes or intestinal epithelial cells is well characterized, due to a similar TNF driven apoptotic cell death [59, 60]. Mutations associated with autoinflammatory disease in humans primarily affect two domains of the protein, the first coiled coil-leucine zipper and C terminus which affect the TRAF associated NFκB activator (TANK) interaction domain and A20 interaction domains, respectively. Mutant forms which express partial or complete zinc finger truncation exhibit gain of function in patient derived monocytes, T cells and cell lines that also fail to stabilize A20 at the TNFR following stimulation [61]. Mutant forms of NEMO with missense mutations that permit interaction with A20 and lead to impaired NF-κB activation have been associated with Behçet disease phenotypes, indicating that autoinflammatory disease in humans can result from impaired NF-κB activation in addition to impaired negative regulation of NF-κB (Fig. 29.3 and Table 29.1).

29.6.5 Clinical Manifestations

Hypomorphic NEMO mutation leads to ectodermal dysplasia with anhidrosis and immunodeficiency (EDA-ID). EDA occurs to some degree in a majority of patients manifesting as defective eccrine sweat gland and hair follicle development with facies characterized by frontal bossing and periorbital hyperpigmentation and wrinkling. Primary teeth are cone-shaped and enamel defects lead to tooth decay with delayed eruption of secondary teeth which can appear normal. Primary immunodeficiency is combined due to defective T cell proliferation, B cell antibody production and NK cell killing leading to infection with pyogenic bacteria, pneumocystis jirovecii, atypical mycobacteria and DNA herpesviruses. A variety of skin rashes have been described ranging from those resembling chronic graft versus host disease (GVHD), to features similar to seborrhoeic keratitis [62]. One report described atypical colitis and a sustained inflammatory response triggered by infection [63], while several others described early onset inflammatory disease starting in the first months of life, in individuals with the common mutation of E390Xfs4 [62, 64, 65]. Human females with incontinentia pigmenti, the disease which results from X-linked dominant mutations in NEMO, exhibit an inflammatory skin disease soon after birth, in addition to retinal abnormalities and central nervous system infarcts, seizures and developmental delay. Female mice heterozygous for null IKKg mutation have a similar self-limiting inflammatory disease but males with null mutations die in utero [66].

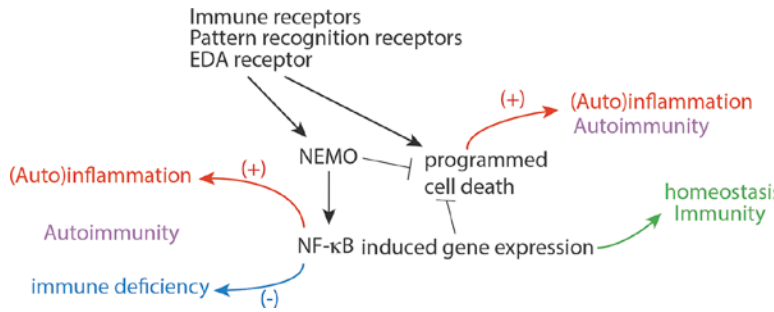

Fig. 29.3 Autoinflammatory disease pathogenesis and nuclear factor kappa B (NF-κB) essential modulator (NEMO) function. NEMO scaffolding function permits recruitment of the canonical IKK complex to immune and developmental receptors. Independent of NF-kB activation, NEMO impairs receptor-induced cell death pathways and leads to NF-kB activation induced gene expression that also prevents cell death. Both enhanced and impaired NF-kB activation can lead to autoinflammatory disease

Table 29.1 Disease associated with NF-κB essential modulator (NEMO) mutations. Cell type and context specific signaling leads to a broad spectrum of immune mediated disease

Genetics	NEMO hypomorphic (reduced activation)	NEMO hypomorphic (impaired regulation)	NEMO amorphic (no activation)
Diagnosis	EDA-ID	NEMO-ΔCT	Incontinentia Pigmenti
Sex affected	male	male/female	female
Cell type	epithelium (gut and skin) immune cell	immune cell	keratinocyte neuron
Signaling	↓ NF-κB	↑ NF-κB	↓ NF-κB
Cellular process	impaired effector cells programmed cell death	activated effector cells	impaired skin/neuronal homeostasis programmed cell death
Immune-mediated disease	primary immunodeficiency autoimmunity autoinflammation (mild)	autoinflammation autoimmunity dermatitis/IBD	neonatal dermatitis CNS infarct

NF-κB: nuclear factor kappa B; NEMO: NF-κB essential modulator; EDA-ID: ectodermal dysplasia with immune deficiency; IBD: inflammatory bowel disease; CNS: central nervous system

29.6.6 Laboratory Findings

As may be expected, elevated acute phase reactants such as CRP and ESR can be detected during episodes of active inflammatory disease. Otherwise, there are no specific clinical laboratory findings. On a research basis, gain of function NF-κB activation can be demonstrated, in contrast to classic NEMO hypomorphic mutations [61]. Due to the presence of a non-transcribed pseudogene, ψNEMO, if long read sequencing of genomic DNA is not possible, sequencing cDNA may be necessary to identify rare variants [67].

29.6.7 Diagnosis

The diagnosis is suggested by the presence of autoinflammatory disease features with or without ectodermal dysplasia and immunodeficiency. The diagnosis can be confirmed by genetic sequencing of *IKBKG* that indicates a rare NEMO variant and demonstration of functional NF-κB regulation defect. For classical NEMO hypomorphic disease, impaired TLR-induced cytokine production from peripheral blood monocytes cells (PBMC) or impaired IκBα degradation or nuclear NF-κB translocation assay can be carried out in several specialized centers.

29.6.8 Treatment and Outcome

TNF inhibition has been used effectively. Hematopoietic stem cell transplantation is also an option, and outcomes inversely correlate with the degree of ectodermal dysplasia present [68].

29.7 Haploinsufficiency of A20 (HA-20)

- **The spectrum of disease due to A20 deficiency is highly variable and includes autoantibody production, lymphproliferation, recurrent infection, uveitis, vitiligo and lupus nephritis**

- **The most common clinical features of A20 deficiency are oral, genital and gastrointestinal ulcers leading to a diagnosis of atypical Behçet disease in most patients identified to date**

29.7.1 Introduction

A20 is an E3-ligase that also possesses deubiquitinase activity [69]. The first description of autoinflammatory disease due to haploinsufficiency of A20 was made in 2016 with the description of 6 unrelated families with dominantly inherited disease [70], followed by description of Behçet-like disease in 3 generations of a Japanese family [71]. Cells from patients exhibit persistent polyubiquitination of proteins such as TRAF6, receptor interacting protein 1 (RIP1) and NEMO, that are essential components of the NF-kB activation pathway. Although enzymatic activities of A20 have been well characterized, the precise molecular mechanism by which A20 interferes with NF-kB activation remains an area of intense research.

29.7.2 Epidemiology

Eight individual families with this extremely rare disease have been reported in the literature. Ancestry of affected individuals includes Italian, Turkish, European-American, Dutch and Japanese. The sixth mutation was found in 1 of 768 individuals diagnosed with Behçet disease who underwent targeted sequencing.

29.7.3 Etiology/Genetics

Mutations in the *TNFAIP3* gene (which encodes for the A20 protein) which have been identified to date lead to generation of a premature stop codon. Mutations generally occur in the C-terminal portion of the OTU domain and include p.Trp85GlyfsX11, p.Leu227*, p.Phe224Serfs*4, p.Arg271*, p.Tyr306*, p.Pro268Leufs*19. Other mutations are located in the C-terminal to the first p.Gln415fs, or the third zinc finger, p.Thr604Argfs*93. The disease is highly penetrant and dominantly inherited.

29.7.4 Pathogenesis

Insufficiency of A20, due to reduced protein levels, leads to increased NF-kB activation in response to TNF and TLR stimulation. Disease in humans is due to haploinsufficiency, as reduced levels of full length A20 were detected in patient samples. In vitro experiments in which mutant forms of the protein were overexpressed did not lead to suppression of NF-kB activation. Cell types that have been shown to be affected by A20 deficiency in humans included non-immune cells such as skin fibroblasts, and PBMC, monocyte derived macrophages and T cells, the latter of which can be induced to express excess pro-inflammatory cytokines such as IL-9 and IL-17. Additionally, spontaneous NLRP3 inflammasome activity has been observed in cells from HA20 patients which express increased pro-IL-1β. As demonstrated by TNFR and IL-1R signaling blockade, activity of these pathways is important to maintain active disease.

29.7.5 Clinical Manifestations

HA-20 resembles Behçet disease, although some features in certain individuals led to an initial diagnosis of atypical systemic lupus erythematosus with central nervous system vasculitis, anterior uveitis, colonic ulceration [71] and an autoimmune lymphoproliferative syndrome (ALPS) phenotype [72]. Features of the disease include early onset systemic inflammation, arthritis, oral and genital ulcers and uveitis. Patients with A20 mutation develop autoantibodies, however, typical autoimmune disease is not seen [73].

29.7.6 Laboratory Findings

Acute phase reactants tend to be elevated with disease flares but normal between flares [73]. Fluctuating levels of low-titre autoantibodies,

including antinuclear antibodies, anti-dsDNA, anti-Sm/ribonuclear protein (RNP), anti-cardiolipin and lupus anticoagulant were found in the largest reported cohort. Two patients from one family had IgG subclass deficiency and lymphopenia.

29.7.7 Diagnosis

The diagnosis should be considered in patients in whom Behçet disease is suspected, especially with an autosomal dominant inheritance pattern and early age of onset. The presence of anterior uveitis may make a diagnosis of HA20 more likely than Behçet disease. Confirmation of the diagnosis requires the presence of a mutation in *TNFAIP3*.

29.7.8 Treatment and Outcome

As very few patients have been described to date, no true evidence-based treatment recommendations can be made. Some patients have had a good response to colchicine monotherapy, but most have required a combination of corticosteroids with various immunosuppressive agents including methotrexate, thalidomide, tofacitininb, anti-TNF, anti-IL-6 and anti-IL-1 agents. Rituximab has been used occasionally. Several patients have undergone autologous or allogeneic stem cell transplantation. In one patient, treatment with prednisolone and cyclosporine were found to be effective for ALPS-like symptoms, but not hepatitis, which responded to mycophenolate mofetil [73].

29.8 Otulin-Related Autoinflammatory Syndrome (ORAS)/ Otulopenia

- **OTULIN deficiency reveals a role for linear ubiquitination in development and regulation of immune pathways**
- **Early experience suggests that TNF blockade has been somewhat more effective in treating inflammatory disease than DMARDs, IL-1 blockade or prednisone**

29.8.1 Introduction

OTULIN is a deubiquitinase that regulates linear polyubiquitin modifications which are produced by the linear ubiquitin assembly chain complex (LUBAC). LUBAC is active in TNF and TLR signaling. The first descriptions of disease due to *OTULIN* deficiency, otulin-related autoinflammatory syndrome (ORAS), or otulipenia were made in 2016 with the description of 3 affected individuals with homozygous mutation in *OTULIN* due to parental consanguinity and an additional 2 unrelated patients [74, 75].

29.8.2 Epidemiology

Only 3 families (from Pakistan and Turkey) with this disorder have been described to date.

29.8.3 Etiology/Genetics

The *OTULIN* mutations described to date include chr5: 14690368T>C c.815T>C p.L272P, chr5: 14690284A>G c.731A>G p.Y244C, and chr5: 14687678delC c.517delC p.G174Dfs*2.

29.8.4 Pathogenesis

OTULIN mutations that severely limit function lead in mice to abnormal vasculature development and embryonic lethality by day 13 [76]. OTULIN inhibits NF-κB activity and deubiquitinates linear polyubiquitin chains (also referred to as M1 polyubiquitin). Enzymatic function of mutant protein tested in patient samples revealed a defect in the ablility of mutant forms of OTULIN to deubiquitinate NEMO, RIP1 and TNFR in skin fibroblasts and PBMCs in response to stimulation by TNF and IL-1β. Since both overexpression and deficiency of OTULIN lead to TNF-induced apoptosis, TNF signaling likely leads to cell death in disease, as there appears to be a requirement for restricted levels of functioning OTULIN. In the seminal description of the disease, 4 mouse models were studied that indi-

cated myeloid lineage cells to be pathogenetically important, whereas lymphocyte depletion of OTULIN did not lead to inflammatory disease phenotypes.

29.8.5 Clinical Manifestations

Patients have exhibited neonatal fever and rash with systemic inflammation that in some individuals was fatal in early childhood. Other symptoms included joint swelling, diarrhea and lipodystrophy. The skin rash is described as erythematous, pustular and scarring with nodules. A Cushingoid facial appearance was included in the symptoms, but this was possibly secondary to prolonged high dose corticosteroid dependence. Developmental delay is possible [74, 75].

29.8.6 Laboratory Findings

Acute phase reactants such as CRP and ESR are significantly increased accompanied by leukocytosis that reflects neutrophilia and elevated CD4 and CD8+ T cells, whereas absolute B cell and NK cell numbers are reduced. T and B cells proliferate normally in response to stimulation and elevated IgA was seen in two patients. On a research basis, lipopolysaccharide induced pro-inflammatory cytokine production from whole blood and isolated monocytes in patients, even in the absence of elevated serum pro-inflammatory cytokines, which appear to be variably elevated in patients [75].

29.8.7 Diagnosis

The disease should be suspected in patients with features of early-onset systemic inflammatory disease affecting skin, bone and hematopoiesis. A diagnosis can be made by genetic testing for mutations in *OTULIN* accompanied by functional tests indicating increased NF-κB activation. Skin biopsy has shown septal panniculitis with vasculitis of small and medium sized blood vessels, but this is not considered specific [75].

29.8.8 Treatment and Outcome

Treatment with anakinra has been reported to mitigate symptoms, but has not permitted discontinuation of daily corticosteroids. However, the disease was very rapidly responsive to infliximab. Etanercept may be less effective than infliximab, as it was not corticosteroid sparing in one patient.

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Part V

Complex Autoinflammatory Diseases

30

Periodic Fever, Aphthous Stomatitis, Pharyngitis and Cervical Adenitis (PFAPA) Syndrome

Kathryn M. Edwards and Michael Hofer

Abstract

Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome is a recurrent fever syndrome that usually starts in early childhood. Episodes occur every 4–6 weeks and spontaneously resolve in 3–6 days. During episodes, children have elevated acute phase reactants that return to normal between episodes. Children with PFAPA grow and develop normally, are not more susceptible to infections, and exhibit no long-term sequelae. Since its initial description in 1987, numerous cases of PFAPA have been reported throughout the world and understanding of the syndrome has greatly increased. The epidemiology, theories on causation, clinical manifestations, and therapeutic options for PFAPA will the discussed in this chapter.

Keywords

Periodic fever · Aphthous stomatitis
Pharyngitis · Cervical adenitis (PFAPA)
Periodic fever · Autoinflammation

Abbreviations

CAPS	Cryopyrin-associated periodic syndrome
CARD	Caspase activation and recruiting domains
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
FMF	Familial Mediterranean fever
HIDS	Hyperimmunoglobulinemia D syndrome and periodic fever
Ig	Immunoglobulin
IL	Interleukin
IL-1RA	Interleukin-1 receptor antagonist
INF	Interferon
IP10	Interferon γ-induced protein
LPS	Lipopolysaccharide
MKD	Mevalonate kinase deficiency
NLRP	Nucleotide-binding oligomerization domain, leucine rich repeat with pyrin domain
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic syndrome

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Key Points

- **Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome is the most common cause of periodic fever in young children**
- **Febrile episodes occur every 4–6 weeks and spontaneously resolve in 3–6 days**
- **Episodes are often accompanied by pharyngitis, aphthous stomatitis and cervical adenitis or a combination of these symptoms**
- **During episodes, children have elevated acute phase reactants that return to normal between episodes**
- **Children with PFAPA grow and develop normally, are not more susceptible to infections, and exhibit no long-term sequelae**
- **Short courses of oral corticosteroids abort the episodes**

30.1 Introduction

In 1987, 12 children from two pediatric medical centers in the United States were described with a syndrome of recurrent fever, aphthous stomatitis, pharyngitis, and cervical adenitis [1]. The episodes recurred every 4–6 weeks and resolved spontaneously in 3–6 days. Elevated acute phase reactants were detected during the episodes and resolved when the fever abated. These children grew and developed normally, were not more susceptible to infections, and exhibited no long-term sequelae. Short courses of oral corticosteroids aborted the episodes, but non-steroidal anti-inflammatory drugs did not. In 1989 the term periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) was coined to describe the syndrome and a list of diagnostic criteria were proposed [2] (Table 30.1). Since that time numerous cases of PFAPA have been reported throughout the world and our understanding of the syndrome has greatly expanded [3–12]. The epidemiology, theories on causation, clinical manifestations and therapeutic options will be the focus of this chapter.

Table 30.1 Diagnostic criteria for the periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome [2]

1. Regularly recurring fevers (>38.3 °C), usually with an early age of onset
2. Constitutional symptoms in the absence of an upper respiratory infection with at least one symptom being aphthous stomatitis, cervical adenitis or pharyngitis
3. Exclusion of cyclic neutropenia and other intermittent fever syndromes on the basis of history or laboratory studies
4. Asymptomatic intervals between episodes
5. Normal growth and development

30.2 Epidemiology

PFAPA generally begins within the first 5 years of life with a slight male predominance (55–71%) reported in many cohorts [1–12]. There appears to be no predilection for a particular ethnic or racial group, since case series have appeared from throughout the world. The only population-based study assessing the prevalence of PFAPA was reported in 2013 from one region of Norway [10]. A total of 46 children (32 males) were diagnosed with PFAPA from 2004 to 2010. Since the investigators were able to comprehensively capture all patients with PFAPA within that region, they projected the yearly incidence of PFAPA as 2.3 cases per 10,000 children less than 5 years of age. In this Norwegian cohort the median age of onset was 11 months (quartiles: 5.0, 14.8), slightly younger than generally reported in other case series. In the 37 patients followed until resolution, the median age at the time of resolution was 52.1 months (quartiles: 40.3, 71.4) [10]. Cases of PFAPA in adults have also been reported [13, 14].

30.3 Etiology

- **PFAPA is an autoinflammatory syndrome with a familial predominance**
- **PFAPA is thought to have a genetic origin that is poly- rather than monogenic**

The term “autoinflammatory” was proposed by McDermott et al. to describe a group of disorders “characterized by attacks of seemingly unprovoked

inflammation without significant levels of autoantibodies and autoreactive T cells" [15]. It is currently considered that PFAPA falls into the classification of an autoinflammatory syndrome that corresponds to overactivation of the innate immune system. Typically, patients with PFAPA present with recurrent fever flares associated with similar symptoms during each attack. A single genetic defect explains the exaggerated inflammatory response in monogenic autoinflammatory diseases, but for other autoinflammatory diseases, including PFAPA, an oligo- or polygenic origin is suspected.

30.3.1 Hereditability

After the first description, PFAPA was thought to be a sporadic disease with no genetic predisposition since it had been reported throughout the world with no increased predilection in any ethnic or racial groups. However, over time, familial clusters including siblings or parents and children were reported. In the study by Cochard et al., 57% of the patients had a positive family history for recurring fever or PFAPA (mostly parents or siblings) compared to none in a control group of healthy children [16]. Recent series show that between 10 and 78% of patients with PFAPA have family members with recurrent fever, suggesting that familial clusters are more common than originally thought [17–20]. Two recent reports have conducted comprehensive familial assessments and hypothesized an autosomal dominant inheritance pattern of PFAPA [21, 22]. Manthiram et al. evaluated 80 patients with PFAPA and found that 23% had at least one family member with symptoms suggestive of PFAPA [21], while Di Gioia et al., studied 14 families with more than 2 patients with PFAPA in each family [22].

30.3.1.1 Genomic Analysis of Patients with PFAPA

The association of PFAPA with familial Mediterranean fever (FMF) genes has been suggested. In an Israeli study of 124 patients with a clinical diagnosis of PFAPA without features of FMF, patients who were heterozygous for common *MEFV* variants (M694V, V726A, and E148Q) had shorter episodes of PFAPA, with less regularity, and fewer other associated findings, suggesting that variants in the *MEFV* gene influenced the clinical presentations of PFAPA [23]. However, these findings were not replicated in another cohort of 64 patients from Turkey, rendering the role of *MEFV* in PFAPA unclear [24]. A recent assessment of 359 patients in Turkey with PFAPA indicated that those with concomitant symptoms of FMF were less likely to fully respond to tonsillectomy [25]. Screening for mutations in *MEFV*, *TNFRSF1A*, *MVK*, *NLRP3*, *AIM2*, and *NOD2/CARD15* in PFAPA cohorts in populations from Japan, Israel, Turkey, Italy, Slovenia, and Switzerland have not consistently shown a higher prevalence of variants in these genes when compared with controls [5, 11, 22–24, 26–28]. However, these studies varied in how extensively the genes were sequenced for polymorphisms and in the allele frequency among healthy people of these different ethnic backgrounds. Kolly et al. reported that 25% of the 57 studied patients with PFAPA carried variants in the 4 genes commonly associated with monogenic autoinflammatory diseases [26]. In 12 patients, 3 different variants (Q703K, V198M and R488), considered to be of unknown significance (see Chap. 19), were found in the *NLRP3* gene coding for the nucleotide-binding oligomerization domain, leucine rich repeat-like receptors with pyrin domain (NLRP) 3 inflammasome, corresponding to a significantly higher proportion than in the general population [26].

Several additional genomic analyses have been reported in patients with PFAPA. Di Gioia et al., conducted an exhaustive genetic screening to identify responsible mutations in patients with PFAPA [22]. Linkage analysis suggested a susceptibility locus on chromosome 8, but direct molecular sequencing did not support this statistical finding. Exome sequencing did not reveal any mutation in all patients tested. The authors concluded that PFAPA is likely a complex disease of oligo- or polygenic inheritance or a group of Mendelian diseases with a common phenotype [22].

Recently, Cheung et al. reported that nearly 14% of patients in their cohort of 82 unrelated patients with PFAPA carried a frameshift mutation in *CARD8* while it was only seen in 3.2%

of healthy controls [29]. They also found that HEK298T cells transfected with the caspase activation and recruiting domains (CARD)-frameshift mutant and components of the inflammasome lacked the NLRP3 and CARD8 interaction. In addition, patients with *CARD8* mutations more often had aphthous ulcers and symptoms between flares. In contrast, another common polymorphism in the *CARD8* gene (C10X) was not found to be more common in patients with PFAPA when compared to healthy controls. These recent studies reveal that although PFAPA clusters in families and is likely to be inherited, it is unlikely to be a monogenic disease in most patients and that inflammasome/ interleukin (IL)-1 β pathway genes and genes involved in other hereditary periodic fever syndromes may modulate the clinical presentation.

30.4 Pathogenesis

- **During febrile episodes, patients with PFAPA present with increased production of pro-inflammatory cytokines, in particular interleukin (IL)-1ß**
- **Other specific gene expressions are enhanced during episodes (complement, IL1-related, interferon (IFN)-induced)**
- **Tonsils show some specific features in terms of germinal centers and distribution of inflammatory cells, and the tonsillar microbiome differs from healthy children**

30.4.1 Cytokines

A discussion of the pathogenesis of PFAPA was recently published [30]. As outlined in that publication, an infectious origin was initially suggested, but the pattern of regularly recurrent febrile episodes with similar symptoms, as well as the prompt response to a single dose of prednisone, were strong arguments against the infectious hypothesis. Because of the clinical similarities with other autoinflammatory diseases and the laboratory findings typical for innate immune activation, several investigators evaluated the activation of IL-1β and inflammasome-related pathways in patients with PFAPA [26, 31]. In 2006 Stojanov et al., determined serum and intracellular cytokine levels in 6 patients with classic PFAPA during a symptom-free period and 6–12 and 18–24 h after fever onset and compared them with age-matched, healthy controls [32]. During febrile episodes, significant increases in serum IL-6 and interferon (IFN)-γ concentrations were noted when compared to both symptom-free periods in patients with PFAPA and to healthy controls. In addition, levels of IL-1β, tumor necrosis factor (TNF)-α and IL-12p70 were significantly increased during episodes. During asymptomatic periods in patients with PFAPA, serum concentrations of IL-1β, IL-6, TNF-α and IL-12p70 remained significantly increased compared to controls. The anti-inflammatory cytokine IL-4 in the serum was at all times lower in patients with PFAPA compared to controls, but no differences in levels of intracellular IL-4 and IL-10 or serum IL-10 were noted. The observed increase of pro-inflammatory mediators, even between febrile episodes, suggests a dysregulation of the immune response in PFAPA syndrome, with continuous pro-inflammatory cytokine activation and reduced anti-inflammatory responses.

In 2013, Kolly et al., demonstrated in 15 patients with PFAPA who were studied during and between febrile episodes that inflammatory episodes were characterized by increased serum levels of IL-6, IFN-γ-induced protein 10 (IP10) and caspase-1, as compared to asymptomatic periods and to healthy controls [26]. They also found an enhanced secretion of active IL-1ß and IL-1 receptor antagonist (IL-1RA) in lipopolysaccharide (LPS)-stimulated peripheral blood lympho-monocytes during episodes.

30.4.2 Gene Expression

In 2011 Stojanov et al., employed a systems biology approach to analyze blood samples from patients with PFAPA whose genetic testing

Table 30.2 Gene expression and cytokine/chemokine production during febrile episodes of periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome [31]

Genes upregulated	
Complement	*C1QB, C2, SERPING1*
Interleukin (IL)-1–related	*IL-1β, IL-1RN, CASP1, IL-18RAP*
Interferon (IFN)-induced	*AIM2, IP-10/CXCL10*
Genes downregulated	
T cell-associated transcripts	*CD3, CD8B*
Increased serum levels cytokines/chemokines	
Chemokines for activated T lymphocytes	*IP-10/CXCL10, MIG/CXCL9, G-CSF, IL-18, IL-6*

excluded hereditary periodic fever syndromes, from healthy children, and from children with confirmed hereditary periodic fever syndromes [31]. Gene expression profiles were different during the febrile and the asymptomatic periods in patients with PFAPA (Table 30.2). These data suggested that an environmentally triggered activation of complement and IL-1β/−18 occurred during febrile episodes of PFAPA, with induction of Th1-chemokines and subsequent retention of activated T cells in the peripheral tissues. Based on the positive effect of anakinra in 5 patients, the authors concluded that IL-1 inhibition might be beneficial for the treatment of episodes of PFAPA [31].

30.4.3 Tonsil Pathophysiology

The curative role of tonsillectomy in many patients with PFAPA has focused attention on comparing the extirpated tonsils in these patients with tonsils removed from children with hypertrophic tonsils and obstructive sleep apnea. Comparisons of cytokine transcript expression in tonsils removed from patients with PFAPA and those with obstructive sleep apnea/hypertrophic tonsils have shown that tonsils removed from patients with PFAPA express less IL-4 [33]. In another study Dytrych et al., performed a comprehensive assessment of lymphocyte subsets by flow cytometry in both the blood and removed tonsils of 10 patients with PFAPA and compared them with samples obtained from subjects with obstructive sleep apnea [34]. Tonsils removed from patients with PFAPA had a lower percentage of B lymphocytes, but a higher percentage of CD8+ T lymphocytes and naïve CD4+ and CD8+ T lymphocytes than tonsils from patients with obstructive sleep apnea/hypertrophic tonsils. The tonsils removed from patients with PFAPA also had fewer CD4+ T lymphocytes with high expression of the inhibitory molecule programmed cell death protein 1 (PD-1). T cell chemokine levels were also elevated in tonsils from patients with PFAPA, but immunoglobulin and T-cell receptors did not show clonal or oligoclonal expansion.

Manthiram et al. evaluated tonsillar tissue from 16 patients with PFAPA and 16 controls with obstructive sleep apnea/hypertrophic tonsils [35]. Tonsils from patients with PFAPA had significantly smaller germinal centers and wider surface of the squamous epithelia (Fig. 30.1). The percentages of B and T lymphocytes, macrophages, and dendritic cells were comparable in germinal centers, crypts, and squamous epithelia in tonsils removed from patients with PFAPA and obstructive sleep apnea/hypertrophic tonsils. However, tonsils removed from patients with PFAPA after a longer interval from the time of the last febrile episode had a significantly larger germinal center size [35]. These results suggested that naïve, polyclonal T lymphocytes accumulate in the tonsils from the peripheral blood as part of the pathogenesis of PFAPA. However, major questions remain, among them the trigger for the influx of T cells into tonsils, the impact of these T cells on resident B lymphocytes and the triggers of the regular cyclic episodes of tonsillar inflammation and fever. The efficacy of tonsillectomy among patients who have only fever and do not strictly meet the original criteria for PFAPA and the high frequency of reduced penetrance phenotypes suggest that a broader array of phenotypes may fall under the umbrella of PFAPA [9, 36]. In addition, longitudinal studies in tonsillar tissue over time are limited by the complete removal of tonsils during asymptomatic periods and comparison with tonsils from patients with obstructive

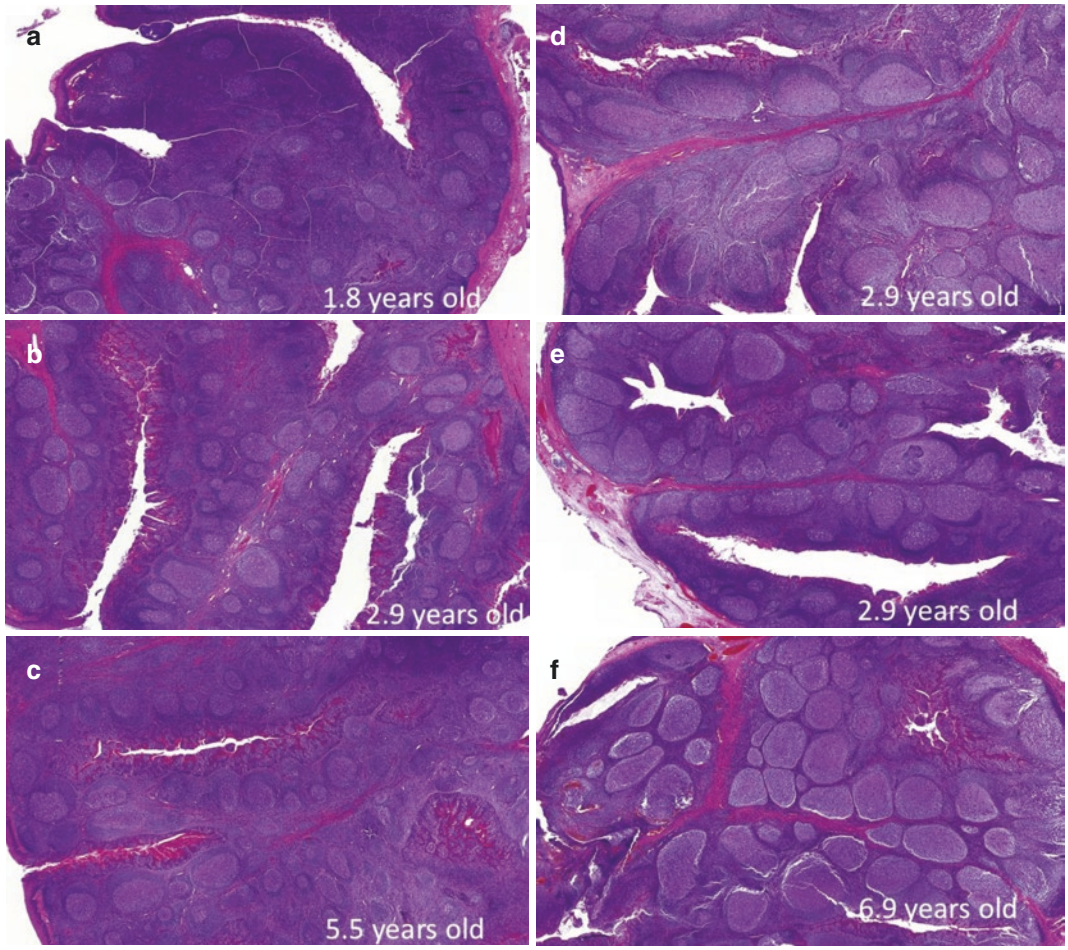

Fig. 30.1 Representative hematoxylin and eosin-stained tonsil sections at 2× magnification; **a**, **b** and **c** are from patients with periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) and **d**, **e**, and **f** are from patients with obstructive sleep apnea. The tonsils of patients with PFAPA had smaller germinal centers and wider squamous epithelia than did patients with obstructive sleep apnea (OSA). The germinal center size was also dependent on the time from the last febrile episode to tonsillectomy in patients with PFAPA. Reproduced with permission from [35]

sleep apnea and/or hypertrophic tonsils, which are inflammatory diseases as well. Whether repeat biopsies from unremoved tonsils could be performed over time to assess the natural progression of the PFAPA cycle remains speculative.

30.4.3.1 Tonsil Microbiome

The role of the tonsillar microbiome has also been evaluated in patients with PFAPA. One study assessed the viral load of Epstein-Barr virus, cytomegalovirus, human herpervirus-6 and adenovirus in the tonsils of patients with PFAPA and controls by quantitative polymerase chain reaction (PCR) [34]. They detected at least one of these viruses in 7 of 10 tonsils from patients with PFAPA but also in 7 of 9 controls. Tejesvi et al., performed bacterial, viral, mycobacterial, and fungal cultures as well as PCR for herpes viruses and visualized biofilms in tonsils of 31 patients with PFAPA and 24 patients with obstructive sleep apnea [37]. Tonsils from patients with PFAPA were more likely to contain *Candida albicans* and develop biofilms and less likely to

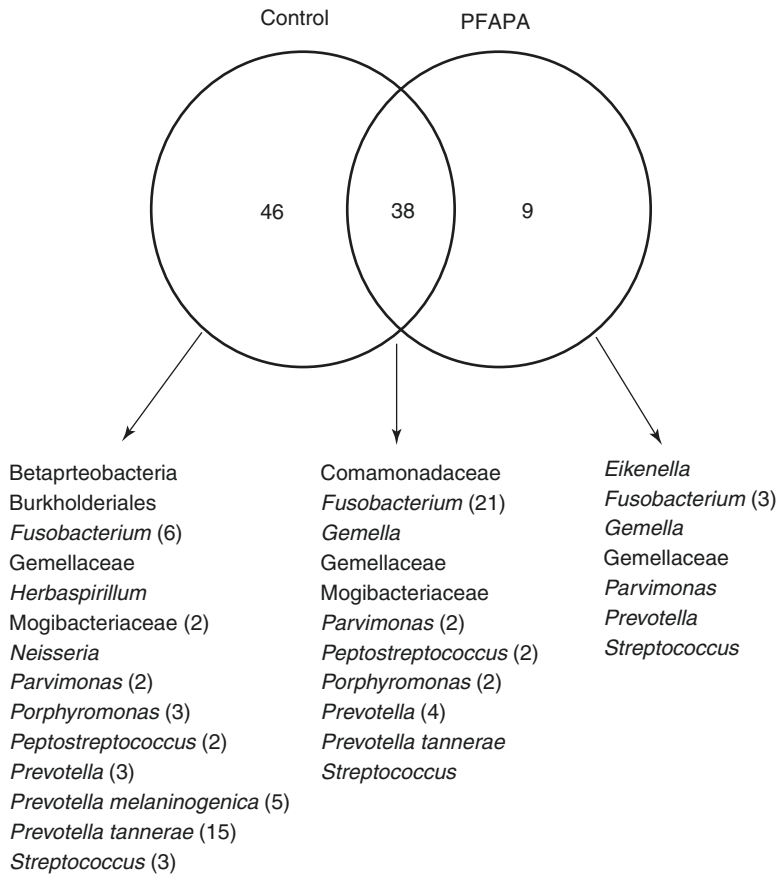

Fig. 30.2 Venn Diagram showing the core microbiome of the control and periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) samples at a 97% confidence level. The numbers in the brackets represent the numbers of operational taxonomic units in each genera. Reproduced with permission from [37]

contain *Staphylococcus aureus* and varicella-zoster-virus. Using next-generation sequencing of 16S ribosomal RNA they also thoroughly profiled the bacterial microbiota from tonsils removed from the same 30 patients with PFAPA and 24 controls (Fig. 30.2). No specific organisms were present in all tonsils removed from patients with PFAPA and absent in all patients with obstructive sleep apnea, and no differences were documented in the microbial populations by principal component analysis. However, the proportions of samples that tested positive for- and the relative abundance of particular phyla, genera, and species differed significantly between the cases with PFAPA and controls with sleep apnea. At the phylum level, tonsils from patients with PFAPA were more likely to contain cyanobacteria and *Synergistetes* than controls. At the genera level, the mean relative abundance of streptococci was lower and that of *Prevotella* higher in cases with PFAPA than in controls with sleep apnea. No differences were found between cases and controls in the frequency of nasopharyngeal pathogens like *Haemophilus* and *Mycoplasma*; moreover, with extensive sequencing, differences in the presence of *Staphylococcus aureus* that were detected by culture were not found. The lack of identification of a common microbe in the tonsils of patients with PFAPA suggests that PFAPA is not an infectious disease although thorough assessments of the virome and fungi have not yet been conducted. Nevertheless, it remains unclear whether differences in the tonsil microbiome play a causal role in stimulating the disease, or whether the differences are a result of repeated episodes of inflammation.

30.5 Clinical Manifestations

- **Regularly recurring periodic fever is the hallmark of PFAPA syndrome**
- **During febrile episodes many patients will have cervical lymphadenitis, pharyngitis and aphthous stomatitis, or a combination of these findings**
- **Patients should have normal growth and development with no symptoms or persistent laboratory abnormalities between febrile episodes**
- **Many consider a response to corticosteroids as a hallmark of the syndrome. Fever resolves within a few hours following the administration of corticosteroids**

Many of the case series of patients with PFAPA, including the original description, have comprehensively outlined the major clinical manifestations, with the similarities and differences summarized in Table 30.3. The original report in 1987 described 8/12 (67%) children having cervical adenitis, 9/12 (75%) having pharyngitis, 10/12 (83%) having aphthous stomatitis during the febrile episode and all having periodic fever [1]. In the series of 105 patients from Connecticut reported in 2001, cervical adenitis was noted in 62%, pharyngitis in 85%, and aphthous stomatitis in 38% [7]. In 2014, Hofer et al. [9] established an international web-based cohort through the Pediatric Rheumatology European Society and identified a total of 301 patients diagnosed with PFAPA by experienced pediatric rheumatologists from 15 medical centers. The clinical symptoms of that large cohort were: pharyngitis (90%), cervical adenitis (78%), oral aphthous ulcers (57%), and all three of these clinical features (44%). In that series, 10% had disease onset after 5 years, mostly between 5 and 6 years, challenging the criterion "before age of 5 years". Eight percent reported symptoms (aphthosis and malaise) outside of the febrile episodes.

Table 30.3 Frequency of findings reported in various case series of patients with periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) (also [38])

	Marshall et al. 1987 [1]	Thomas et al. 1999 [6]	Feder and Salazar 2010 [7]	Hofer et al. 2014 [9]
Onset <5 years (%)	100	100	83	90
Fever onset	Abrupt	NR	NR	NR
Episode frequency	4–6 weeks	NR	More than 6 episodes, every 2–8 weeks	4 weeks
Episode periodicity	Regular	Regular	Regular	Regular
Episode duration (days)	~5	~5	≤10	~4
Symptom reported in cohort (%)				
Constitutional symptoms (≥1)	100	100	100	82
Aphthous stomatitis	83	70	38	57
Pharyngitis	75	72	85	90
Cervical adenitis	67	88	62	78
Asymptomatic intervals	100	100	100	100
Exclusion of URI	100	85	NR	NR
Exclusion of CN	100	ICT	NR	NR
Benign course	100	100	100	100
Response to corticosteroids	100	100	100	95
Normal growth and development	100	100	100	NR
Elevated acute phase reactants during episodes	100	ICT	ICT	100

NR not reported; *URI* upper respiratory infection; *CN* cyclic neutropenia; *ICT* incomplete testing

30.5.1 Specific Features of Pediatric PFAPA

30.5.1.1 Periodic Fever

The most prominent characteristic of PFAPA is its periodic nature, which is required for diagnosis. The fever generally begins abruptly, although many parents describe a prodromal illness that begins the day before the onset of fever and is characterized by malaise, irritability, and poor appetite. Temperatures range from 38.9 to 41.1 °C, generally persist for 3–6 days, with most lasting 4–4.5 days and then abruptly returning to normal. Episodes that last longer than 7 days are very rare and should prompt consideration of other febrile syndromes [1–12].

Febrile episodes generally recur between every 2–8 weeks with most case series reporting episodes at approximately 4 week intervals. Parents can generally predict when the episodes will occur and plan trips and celebrations around these recurrences [39]. Highly irregular episode timing should prompt evaluation for alternate diagnoses, but, with time, episodes may become more irregular, especially after corticosteroid use. Between febrile episodes, children with PFAPA are asymptomatic and have normal growth and development. There are no other family members ill during fever episodes in patients with PFAPA, reinforcing that the episodes are not secondary to infectious agents. The episodes are stereotypic, again a hallmark of PFAPA, helping the physician to distinguish PFAPA from recurrent infections. Original reports highlighted the time that lapsed between the initial presentation of the syndrome and the ultimate diagnosis. This was particularly noteworthy in the series by Perko et al. where their 81 patients had a mean duration of 1.9 years (range 0.4–6.5 years) from symptom onset to the diagnosis of PFAPA [11].

30.5.1.2 Cervical Adenitis, Pharyngitis and Aphthous Ulcers

Cervical adenitis is reported in 60–100% of patients with PFAPA, mostly bilateral, with some tenderness to palpation; sometimes parents report cervical swelling. However, none of the nodes suppurate and all resolve rapidly when the fever abates [1–12].

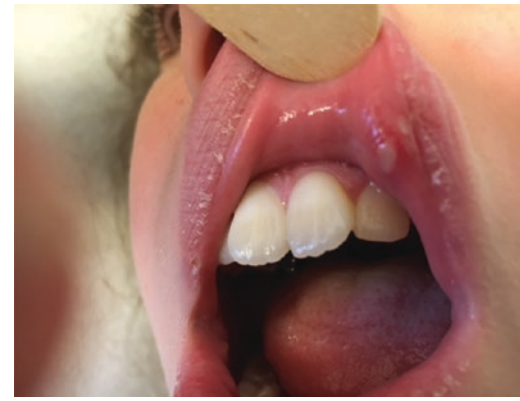

Fig. 30.3 Aphthous stomatitis in a patient with periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome

Pharyngitis/tonsillitis is reported in 65–100% of patients with PFAPA, mostly bilateral and often with accompanying exudates. Ulcers can also be seen on the palatine tonsils during febrile episodes [1–12].

Aphthous ulcers are usually noted on the inner lips or buccal mucosa and are reported in 40–80% of patients (Fig. 30.3). The ulcers are not numerous, with generally no more than 4–5 noted at any one time. These ulcers can be easily missed on physical exam and when pointed out to parents, they are often surprised by their presence. Ulcers are smaller and less painful than those in Behçet disease, likely contributing to their less frequent recognition. Some patients report aphthous ulcers between febrile episodes, and also after cessation of the fever [1–12].

30.5.1.3 Additional Symptoms

Other symptoms were reported in 76% of patients in the largest PFAPA cohort from Europe [9]. Of the 301 patients, 44% had gastrointestinal symptoms, 29% had arthralgia and myalgia, 12% had skin rashes, and 3% had neurologic symptoms, but these were not further characterized. In other series of patients with PFAPA, abdominal pain was reported in 40–65%, arthralgia in 11–42%, vomiting in 18–41% and headache in 18–65% [1–12]. Rarely, upper respiratory

symptoms, such as cough or coryza were noted. Arthritis, abdominal pain, and a prominent rash should lead one to consider other monogenic periodic fever syndromes. In fact, Gattorno and colleagues devised a diagnostic score that discriminated between patients who were genetically positive for hereditary periodic fever syndromes from those who were negative [40] (see below, Sect. 30.7.2).

30.5.1.4 Fever Alone

Lantto and colleagues reported on a series of 108 children who underwent tonsillectomy for regularly recurring fevers [36]. Half of the subjects had only recurrent fever without other manifestations of PFAPA. All of these children had complete resolution of fever after tonsillectomy. The authors suggested that PFAPA syndrome "should be suspected and tonsillectomy considered even in children with a late onset of symptoms (>5 years of age) or when fever is the only symptom during PFAPA episodes". However, the risk in considering fever alone as the sole diagnostic criterion could lead to the over-diagnosis of PFAPA and to miss other important diagnoses. However, since the etiology of PFAPA syndrome is not elucidated, one should consider patients with isolated recurrent fever as part of the PFAPA spectrum.

30.5.2 Specific Features of Adults with PFAPA

Padeh and colleagues described 15 adults with PFAPA syndrome [13]. Their febrile episodes occurred at 4.6 ± 1.3-week intervals and began at the age of 20.9 ± 7.5 years. Between episodes, patients were healthy, without any accompanying diseases. Episodes were aborted in all patients by a single 60 mg oral dose of prednisone. In another report, Rigante et al. reported on a series of 85 children and 30 adults diagnosed with PFAPA at three Italian centers and compared their clinical characteristics [14]. The mean age at diagnosis in the adults was 33 years. The frequency of febrile episodes was significantly higher in pediatric cases, while the duration of fever was significantly longer among adults. Clockwork periodicity of fever and recurrent pharyngitis were more frequently observed in childhood, but no differences were identified between children and adults in rates of aphthosis and cervical adenitis. Pharyngitis was significantly more common in children (98%) than in adults (77%). Joint symptoms, myalgia, headache, fatigue, ocular signs, and rashes were more common in adults. Corticosteroids were effective in resolving the fever in 99% of children and 88% of adults. Tonsillectomy was performed in 3 adult patients, 2 when they were children and 1 in adulthood. Tonsillectomy was effective in only 1 adult who underwent the tonsillectomy as a child, but the disease relapsed when he was 25 years of age. These data suggest that there is substantial clinical overlap in the symptoms experienced by adults and children with PFAPA.

Additionally, Cantarini and colleagues evaluated 359 adults with recurrent fever in their clinic and found 17 that fulfilled the criteria of PFAPA [41]. All were Caucasian, 10 (59%) were males, their recurrent febrile episodes begun at a mean age of 25.9 ± 8.3 years with a mean of 8.3 ± 5.2 episodes per year and a mean duration of 5.5 ± 1.8 days. Forty-one percent had cervical adenitis, pharyngitis, and aphthous stomatitis, while 59% had a combination of two of these symptoms. Corticosteroids were given to 82% of the patients and 79% of those treated had a prompt response. Tonsillectomy was performed in 53% of the patients but only 2 had resolution of their symptoms.

30.6 Laboratory Testing

There is no specific diagnostic laboratory test for PFAPA syndrome. The diagnosis depends on a constellation of clinical symptoms and physical findings, accompanied by elevated inflammatory markers during an episode that normalize when the fever abates. Normal inflammatory markers in between the episodes are useful to distinguish PFAPA from the monogenic autoinflammatory diseases. In a comprehensive review of the laboratory findings collected in 94 patients with PFAPA at two medical centers in the United States, 29 of a total of 284 (10%) throat cultures

obtained yielded Group A *Streptococcus*, one of 111 urine samples revealed a urinary tract pathogen, and none of 105 blood cultures demonstrated bacterial pathogens [6]. The mean leukocyte count in this series of patients during episodes of PFAPA was 13,000 μ/L with 62% polymorphonuclear leukocytes and the mean platelet count was 296,000 μ/L with a maximum platelet count of 524,000 μ/L. An elevated mean erythrocyte sedimentation rate (ESR) of 41 mm/h was also recorded. In a large European cohort, C-reactive protein (CRP) levels were measured during febrile episodes in 190 patients and were determined to be >30 mg/L in 155 (82%) patients, >50 mg/L in 131 (69%) and >100 mg/L in 77 (41%) [9].

In an attempt to evaluate other acute phase reactants in patients with PFAPA, serum procalcitonin concentrations were determined in 6 patients with PFAPA during febrile episodes and 32 controls with various other diseases [42]. No elevations in procalcitonin levels were seen during any of the febrile episodes in the patients with PFAPA, suggesting that episodes are not infectious in nature and that procalcitonin levels could be used to distinguish PFAPA from infectious illnesses. Due to the limited number of patients with PFAPA tested to date, additional studies of procalcitonin in PFAPA are warranted.

Serologic studies were obtained in the series of 94 patients with PFAPA [6]. Antinuclear antibody was negative in 29/30 (97%) patients who were tested; rheumatoid factors were negative in all 12 patients tested, and no elevations in antistreptolysin O were seen in the 14 patients tested [6]. These values were all consistent with what is seen in healthy children. Some minor alterations were noted in immunoglobulin (Ig) concentrations in this series [6]. In an Israeli cohort of patients with PFAPA the serum IgD level was elevated (>100 U/mL) in 12/18 (67%) patients tested (140.2 ± 62.4 U/mL), but did not reach those seen in patients with hyperimmunoglobulinemia D syndrome/mevalonate kinase deficiency (HIDS/MKD) [3]. However, these elevations were not documented in other reports [7]. IgE levels were elevated in 8 of 16 (50%) patients with PFAPA tested in one report, with levels ranging from 31 to 999 IU/L [6]. Other series have not documented elevations of IgE. Decreased serum Ig levels in a child with recurrent fever should lead one to suspect an immunodeficiency and should trigger further investigations, such as checking for vaccine antibody responses and lymphocyte immunophenotyping. Dysregulated immunity syndromes should be included in the differential diagnoses of patients with recurrent fevers.

Many imaging studies have been performed in children with PFAPA including chest and sinus radiographs, gastrointestinal series, computed tomography scans of the brain and abdomen, gallium and bone scans, all of which were reported as negative.

30.7 Diagnosis

- **Diagnosis is based on criteria proposed by Thomas in 1999, but the criteria used in the literature vary widely**
- **New classification criteria based on a consensus among a panel of experts and statistical analysis of a large cohort of patients are under development**
- **The hallmark of diagnosis is periodic febrile episodes with laboratory signs of inflammation, asymptomatic periods with negative inflammatory markers and the exclusion of other immunologic diseases**
- **The phenotype of PFAPA may be observed in patients with genetic variants of unknown significance in other monogenic periodic fever syndromes**

30.7.1 Diagnostic and Classification Criteria

The original diagnostic criteria include: regularly recurring fevers (>38.3 °C), usually with an early age of onset; constitutional symptoms in the absence of upper respiratory infections with at least one symptom being aphthous stomatitis, cervical adenitis or pharyngitis; exclusion of cyclic neutropenia and other recurrent fever syndromes on the basis of history or laboratory assessment; asymptomatic intervals between episodes; and

normal growth and development [2] (Table 30.1). Since then, others have used variations on the criteria, while some have required that the diagnosis be made by rheumatologists or other specialists in managing recurrent fever syndromes [9]. Since these first criteria were never validated, a consensus is needed on the classification criteria to allow studies on treatment and outcome based on comparable groups of patients.

Several attempts have been made to develop consensus criteria for PFAPA syndrome. Federici et al., developed a clinical set of criteria for the 4 "classic" monogenic periodic fever syndromes, including FMF, HIDS/MKD, TNF receptor-associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndrome (CAPS) and also for PFAPA syndrome based on a statistical analysis of patients with these diagnoses [43]. For patients with monogenic periodic fever syndromes the gold standard was confirmatory genetic analysis, but for PFAPA, there is no gold-standard. Univariate and multivariate analyses identified clinical criteria that were associated with the diagnosis of each monogenic periodic fever syndrome. Four classification scores were then established based on receiver operating curves and used to evaluate additional patient cohorts (see Chaps. 11 and 14). The criteria appear to be both sensitive and specific for these monogenic periodic fever syndromes [43]. In another study, Vanoni et al. queried an international group of adult and pediatric rheumatologists for a consensus process aiming to develop classification criteria for the 4 "classic" monogenic periodic fever syndromes and for PFAPA syndrome [44]. They used an initial open-ended questionnaire to identify the variables they thought most relevant to a diagnosis of PFAPA. In a second survey, respondents were asked to select from the list of variables chosen to be the most important in the first survey. The 5 top criteria included regular periodicity, aphthous stomatitis, response to corticosteroids, cervical adenitis, and well-being between episodes. The performance of these items is currently being tested through analysis of real patient data and the clinical relevance confirmed by a panel of experts during a consensus conference. The results of these deliberations have not yet been published.

In another independent survey querying pediatric rheumatologists and infectious diseases subspecialists in North America, practice patterns utilized in patients with PFAPA were assessed [45]. Responses were obtained from 277 participants (123 of 424 (29%) members of the Childhood Arthritis and Rheumatology Research Alliance and 154 of 980 (16%) members of the Pediatric Infectious Disease Society) with most respondents agreeing that patients must have the following features of the diagnostic criteria to classify as PFAPA: stereotypical febrile episodes (95%), asymptomatic intervals between episodes (93%), and normal growth and development (81%). However, 71% of the respondents did not require an age of onset <5 years, 33% did not require regular intervals between episodes, and 79% did not require the concomitant signs of aphthous stomatitis, adenitis, or pharyngitis during episodes. Over half (58%) considered the resolution of an episode after use of corticosteroids to be diagnostic of PFAPA. Subspecialists in pediatric rheumatology and infectious diseases showed limited adherence to the earlier published criteria for diagnosing PFAPA suggesting heterogeneity in the characteristics of patients. Further standardization of the diagnostic criteria is needed (see Chap. 11).

30.7.2 Differential Diagnosis and Diagnostic Clues

The diagnosis of PFAPA is made on clinical grounds and is a diagnosis of exclusion. Other causes must be considered including recurrent infections, (very) early onset inflammatory bowel disease, fever associated with malignancy, and cyclic neutropenia. Monogenic fever syndromes must also be considered and excluded.

The most important aspect of making the diagnosis of PFAPA is taking a careful history and documenting the dates of febrile episodes and examining the patient during an episode (see Chap. 11). As mentioned in the earlier sub-section on diagnostic criteria, standardized criteria are in the process of evolution and no universally accepted diagnostic criteria have been developed. Important clues for diagnosis include:

- Multiple documented stereotypic febrile episodes occurring at regular intervals associated with the elevation of inflammatory markers, like ESR or CRP. Intervals between episodes and the clinical manifestations during febrile episodes should be similar within each patient. Although it is not uncommon for patients to intermittently skip episodes, irregularity in the intervals and variation in the characteristics of febrile events themselves are uncommon and should prompt one to question the diagnosis of PFAPA.
- During febrile episodes many patients will have cervical lymphadenitis, pharyngitis, aphthous ulcers, or a combination of these findings. It is critical to examine patients during the episodes to observe for the presence of these characteristic findings. Atypical symptoms during episodes such as severe abdominal pain, chest pain, arthritis, rash, or significant vomiting or diarrhea should prompt evaluation for other conditions. Genetic testing for monogenic fever syndromes should be considered in patients with atypical PFAPA presentations. Gattorno et al., attempted to determine whether there were clinical differences between children presenting with the clinical criteria of PFAPA and those who had positive and negative genetic testing for monogenic fever syndromes [4]. Genetically positive patients had higher frequencies of abdominal pain and diarrhea ($P < 0.001$), vomiting ($P = 0.006$), rash and arthralgia ($P = 0.01$). Genetically negative patients had a higher frequency of exudative pharyngitis ($P = 0.01$) and genetically undetermined patients showed the same pattern of symptom frequency as patients with negative genetic testing.

In a European study of 228 patients with a history of periodic fever, genetic screening was performed to detect monogenic fever syndromes and a diagnostic score, termed the Gaslini score, was developed comparing clinical features of mutation-positive and negative patients. Young age at onset ($P = 0.003$), positive family history of periodic fever ($P = 0.039$), thoracic pain ($P = 0.05$), abdominal pain ($P < 0.001$), diarrhea ($P = 0.028$), and lack of oral aphthosis ($P = 0.007$) were significantly associated with a higher Gaslini score and identification of gene mutations for monogenic periodic fever syndromes [40].

There are several findings that are nearly always seen in patients with PFAPA and include normal growth and development with no symptoms or persistent laboratory abnormalities between febrile episodes. Most physicians who take care of patients with PFAPA consider a prompt response to corticosteroids as a hallmark of the syndrome. With the administration of corticosteroids fever resolves within a few hours. With the exception of HIDS/MKD, febrile episodes in the other monogenic periodic fever syndromes generally do not abate with a single dose of corticosteroids. The diagnosis of PFAPA should be reconsidered if episodes are not responsive to corticosteroids and if more than one dose of 2 mg/kg of prednisone is needed (Fig. 30.4). It is also important to exclude other periodic fever syndromes, recurrent infectious illnesses, cyclic neutropenia, immunodeficiency and autoimmune diseases. If patients are noted to be neutropenic either prior to or during a febrile episode the potential for cyclic neutropenia should be considered. Repeated white cell counts and differentials should be obtained every several days for the duration of the interval between febrile episodes. Finally, laboratory evidence of persistently elevated acute-phase reactants, including elevated ESR or CRP, during asymptomatic periods suggests another autoinflammatory syndrome or chronic illness with intermittent flares rather than a true periodic disorder and would need further evaluation.

30.7.2.1 Monogenic Autoinflammatory Fever Syndromes

Recurrent fevers that are not truly periodic (i.e., do not have a consistent interval between episodes) suggest one of the monogenic autoinflammatory fever syndromes, including HIDS/MKD, FMF, TRAPS, or CAPS. Screening panels for mutations in genes associated with the known monogenic periodic fever syndromes or autoinflammatory diseases are available through several reference laboratories. In case of a

well-recognized pathogenic mutation in these genes, the diagnosis of PFAPA can be excluded. However, the presence of a variant of unknown significance has been shown in patients with PFAPA. For example, patients with the R92Q variant in the *TNFR1A* gene have been compared to genetic negative patients with PFAPA and patients with TRAPS with structural mutations; patients with the R92Q variant had a phenotype closer to PFAPA than to TRAPS, and have been reported to have attenuated disease that spontaneously remits [46]. A PFAPA-like phenotype can also be seen in some patients with FMF.

30.8 Treatment

- **A single dose of corticosteroids at the onset of fever is usually able to abort the episode, but the interval between episodes may be shortened**
- **Prophylactic colchicine administration may reduce the frequency of episodes**
- **Tonsillectomy has a good efficacy in inducing remission, and may be particularly useful in patients with frequent episodes and decreased quality of life**
- **The long-term outcome of PFAPA syndrome is favorable with a spontaneous remission after a few years and the absence of complications**

Several treatments have been used in patients with PFAPA. In the earliest description of PFAPA published in 1987, all patients had received antibiotics early in their course, usually with the presumptive diagnosis of streptococcal pharyngitis, with little benefit [1]. Two of the original 12 children with PFAPA were treated with therapeutic doses of non-steroidal anti-inflammatory drugs also with little benefit. Other therapeutic options were described in that paper and in many subsequent studies reported since that time [1, 6–12]. The primary treatment options are corticosteroids for treatment of individual episodes, cimetidine or colchicine as prophylactic therapy, and tonsillectomy (±adenoidectomy) for potentially curative therapy. Each of these options will be reviewed (Table 30.4) and a proposed treatment algorithm (Fig. 30.4) will be presented at the end of this section.

30.8.1 Corticosteroids

Three children in the original report of PFAPA in 1987 received short courses of prednisone that resulted in rapid disappearance of the fever, particularly if treatment was given at the onset of the episode [1]. In another publication from the same center in 1999, a more comprehensive description of the efficacy of various therapeutic agents was presented [6]. Acetaminophen, antibiotics, and non-steroidal anti-inflammatory drugs were reported to have only modest therapeutic benefit. Prednisone was reported to be very effective in promptly reducing fever. Most patients responded to a single dose of 1–2 mg/kg of prednisone, but some patients required an additional dose. Corticosteroid therapy was not associated with the prevention of further episodes, but patients continued to respond to corticosteroids during subsequent episodes. However, several families reported that fever cycles became more closely spaced after treatment with corticosteroids [6, 7]. Other forms of corticosteroids may be used like betamethasone or dexamethasone, but we prefer prednisone/prednisolone due to shorter half-life and less CNS penetration.

In 2006 Tasher et al., reported on 54 patients with PFAPA who responded promptly to corticosteroid therapy using a lower dose [8]. They routinely prescribed 0.6 mg/kg/day with a range of 0.15–1.5 mg/kg/day. Fifty-one of their 54 (94%) patients required a single dose of corticosteroid and the other three patients required a second dose within 24 h. Resolution of fever after corticosteroid administration was seen in a mean of 10 h. Although, it did not prevent subsequent episodes of fever, patients responded successfully to corticosteroid therapy during subsequent episodes. In nearly half the cases (48%), corticosteroid treatment did not change the course of the disease. In 31% episodes became less frequent. However, in 19% of the patients, families described an increased frequency of subsequent episodes. Most of the parents (65%) did not report any adverse effects of corticosteroid treat-

Table 30.4 Therapeutic options for the treatment of periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome

Therapy	Dose	Responses	References
Antibiotics		No response	[1]
Corticosteroids	0.6–2 mg/kg of prednisone/ prednisolone (alternative 0.1–0.2 mg/kg of betamethasone)	Complete response in 63–97% within a few hours	[6–9, 12, 48, 49]
Cimetidine	20–40 mg/kg/day in divided doses every 12 h (maximum dose 1200 mg/day)	26–46% effective	[6, 7, 12, 50]
Colchicine	0.5–1.2 mg/day in children 4–6 years old; 1–1.8 mg/day in children >6 years of age	40–80% effective	[3, 49, 51–53]
Surgical	Tonsillectomy or adeno-tonsillectomy	63–100% effective, but literature with low level of evidence	[36, 55–60]
Interleukin (IL)-1 inhibition	Anakinra 1 mg/kg	Effective in 6/6 cases treated	[31]

ment, but in those that reported adverse effects, the most common (33%) was restlessness.

Feder et al., reported on 105 cases of PFAPA from Connecticut [7]. Overall, 72 of 105 (69%) patients were treated with prednisone and all but two reported prompt resolution of the fever, within 2–24 h. The prednisone dose was generally 1 mg/kg with the administration of the same dose of prednisone after 12 h if fever was not aborted. However, nearly half of the patients that received corticosteroids reported that subsequent febrile episodes recurred more frequently.

In 2011 Wurster et al., reported that corticosteroids, usually prednisone, was "very effective" in 37 of 44 (84%) patients [12]. In the large Eurofever study, Hofer et al., reported that corticosteroids was used in 147 of the 301 (49%) patients with PFAPA [9]. After a single dose of corticosteroids, usually 1 mg/kg given at the onset of fever, complete resolution of symptoms occurred in 63% of the patients, 32% had partial resolution (not defined in the paper), and only 5% were non-responders. In a subsequent publication from the Eurofever group, Vanoni et al., noted that the corticosteroid response could be used to distinguish attacks of PFAPA from other hereditary periodic fever syndromes, since only HIDS/MKD was associated with a prompt therapeutic response [47].

Few randomized clinical trials have been conducted with corticosteroid therapy in patients with PFAPA. In one, the effectiveness of 2 different doses of corticosteroids were compared in patients with PFAPA [48]. A total of 41 patients with PFAPA were randomized to receive a dose of prednisolone at either 0.5 mg/kg/day or 2 mg/kg/day. The lower prednisolone dose was as effective as the higher dose in terms of rates of response and recurrence. Finally, in the therapeutic review of autoinflammatory syndromes by the Eurofever consortium, 81 of the 92 (88%) patients with PFAPA enrolled in the registry had received corticosteroids at the onset of episodes with fever totally abating in 90% of patients while 7% had a partial response [49]. Thus, the body of data strongly supports the role of corticosteroids as an effective therapy in patients with PFAPA. However, the shortened interval between episodes after corticosteroid use can be problematic in a subset of the PFAPA population. The treatment is usually well tolerated except for some reports of restlessness. Many parents are concerned about the potential risks of corticosteroids and are reluctant to consider this therapy.

30.8.2 Cimetidine

Cimetidine, a histamine (H)2 blocker shown to have immunomodulatory properties, was proposed as a prophylactic therapy for PFAPA by Feder in 1992 and given at a dose of 150 mg twice daily (or 20–40 mg/kg/day) for 6–12 months [50]. In the 1999 review by Thomas et al., parents reported cimetidine to be 43% effective in preventing episodes of PFAPA [6]. However, in the

subsequent follow-up study by the same group in 2011, cimetidine was reported to be less effective in preventing episodes, with cessation of the episodes in only 26% of the patients [12]. In the study of 105 patients from Connecticut, cimetidine resulted in the prevention of febrile episodes in 27% of patients [7], similar to the rate reported by Thomas et al. None of the 92 patients with PFAPA reported from the Eurofever registry and none of the 42 patients in the Norwegian study were treated with cimetidine [10, 49]. Further, no randomized clinical trials have evaluated its therapeutic impact. Its use is rarely reported from sites other than those in the United States, with successful resolution of the episodes in only about 25% of subjects. Since these studies are not placebo-controlled, it is difficult to know how these rates of resolution would compare to placebo alone.

30.8.3 Colchicine

Although colchicine binds to tubulin changing the cytoskeleton of inflammatory cells, its mechanism of action as an anti-inflammatory medication is not completely understood (see Chap. 40). Colchicine was initially used in patients with PFAPA because of its success in preventing attacks of FMF and the clinical similarities of PFAPA and FMF. In 2008 Tasher et al., reported on the treatment of 9 patients with PFAPA with closely spaced intervals between episodes of <14 days with a daily dose of 0.5–1 mg of colchicine [51]. In 89% significantly increased intervals between episodes were noted. Aviel Butbul et al., conducted a randomized, open-label study of 18 Israeli patients with PFAPA, comparing colchicine (8 patients) and corticosteroid therapy [52]. Eight of the 18 (44%) patients in the study carried *MEFV* mutations. Patients treated with colchicine had significantly fewer episodes than those treated with corticosteroids.

Dusser et al., in France performed a retrospective, multicenter study of 20 children with PFAPA receiving colchicine prophylaxis [53]. Nine (45%) children responded with total resolution or a two-fold reduction of episodes, while others did not respond. Among the 9 responders, 5 (56%) had mutations in the *MEFV* gene. The authors suggested that colchicine appeared effective in patients with less complete PFAPA and with *MEFV* heterozygosity. In contrast, Padeh et al., reported that in 10 patients with PFAPA, 6 of whom were heterozygotes for *MEFV* gene mutations, colchicine was only partially effective and was discontinued [3]. In the Eurofever registry study, febrile episodes completely resolved in 3 patients with PFAPA treated with colchicine, while 2 had only partial responses. Colchicine was well tolerated with only minor gastrointestinal side effects reported that were responsive to dose modifications [49].

30.8.4 Other Drugs

The leukotriene inhibitor montelukast, a drug with anti-inflammatory properties used in asthma has been empirically found to reduce the frequency of febrile episodes flares in a few patients, but reports in the literature are scarce and the response to therapy is not well characterized [45]. Because of the role of IL-1ß in the pathogenesis of PFAPA, IL-1 blocking agents may represent a good therapeutic option. Stojanov et al., treated 5 children with a single dose of anakinra (IL-1RA) and observed a rapid improvement of both clinical and laboratory parameters [31]. A trial with canakinumab (anti-IL-1ß antibody) is under way (clinicaltrials.gov NCT02775994) to evaluate the effect of a single dose of canakinumab on the frequency of febrile episodes.

30.8.5 Tonsillectomy

Shortly after the description of PFAPA, case reports emerged showing that tonsillectomy was associated with cessation of febrile episodes [7, 54]. In the report by Thomas et al., 11 patients with PFAPA underwent tonsillectomy with 7 (64%) having complete resolution of episodes, 2 (18%) a partial response and 2 (18%) had no response [6]. In the report by Wurster et al., 12 patients underwent tonsillectomy or adeno-tonsillectomy with 9 (75%) reporting a reduction in

the severity and frequency of episodes and 6 (50%) describing a total cessation of episodes [12]. Feder et al. in their report on 105 patients with PFAPA reported that 11 underwent a tonsillectomy and 9 (82%) had prevention of all subsequent episodes [7].

The most comprehensive randomized clinical trials for the therapy of PFAPA evaluated the impact of tonsillectomy. The first trial conducted by Renko et al., enrolled 26 children diagnosed with PFAPA into a prospective, randomized, controlled trial [55]; however, some of the patients had only fever during episodes and did not met classic criteria for diagnosis of PFAPA. Six months after randomization all 14 children in the tonsillectomy group and 6 of 12 children in the control group were free of symptoms. Six months later tonsillectomy was performed on 5 of 6 of the patients in the control group who still had symptoms. Following tonsillectomy all symptoms resolved, but the high rate of spontaneous remission in that study questioned the need for a surgical intervention. In a subsequent trial, Garavello et al., reported on the impact of adeno-tonsillectomy in 39 patients with PFAPA who were randomized to either undergo adeno-tonsillectomy or symptomatic care [56]. Patients were followed for 18 months. The percent of subjects experiencing complete resolution was 63% in the adeno-tonsillectomy group and 5% in the control group, a statistically significant reduction. In 2012, Licamelli et al., reported a prospective case series of 102 patients with well-defined PFAPA who underwent adeno-tonsillectomy in a large academic medical center [57]; 99 (97%) had complete resolution of their symptoms immediately after surgery.

A Cochrane review was published in 2014 and summarized the evidence from the 2 randomized trials [58]. The authors concluded surgery conferred significant beneficial effects compared to no surgery "on immediate and complete symptom resolution and a substantial reduction in the frequency and severity of any further symptoms experienced." However, they encouraged further research and cautioned that "children with PFAPA syndrome recover spontaneously and medication can be administered to try and reduce the severity of individual episodes."

In 2016, Lantto et al., reviewed the medical records of 3852 children from Finland who underwent tonsillectomy at their medical center between 1990 and 2007 and identified 108 children with regularly recurring fever [36]. These children were invited to an outpatient visit for evaluation. The authors reported that tonsillectomy was an effective treatment not only for patients with PFAPA who met strict criteria for diagnosis, but also for children with regularly recurring episodes of high fever who failed to meet the classic definition of PFAPA. More recently a group of Turkish investigators reported on 23 patients with PFAPA who underwent tonsillectomy ± adenoidectomy between January 2009 and November 2014 [59]. Twenty-one (91%) of the patients had complete resolution immediately after surgery and one patient had one attack 24 h after surgery, but no further attacks since. They concluded that "tonsillectomy is a good option for the treatment of PFAPA syndrome".

Eight of 92 patients with PFAPA from the Eurofever registry underwent tonsillectomy (± adenoidectomy) with complete resolution in 4 (50%), and partial response in 3 (37.5%) and 1 (12.5%) failure [49]. Recently, Førsvoll and Oymar reviewed all publications of tonsillectomy in PFAPA, for a total of 28 case series including 555 patients. They concluded that tonsillectomy might have a curative effect on patients with PFAPA, but the evidence is of moderate quality [60]. There is still no long-term (more than 5 years) data on recurrence of febrile episodes following tonsillectomy. Based on the previously cited literature, there is good evidence for the effectiveness of tonsillectomy in PFAPA syndrome; nevertheless, spontaneous remission of episodes might occur during follow-up that challenges the need for a surgical intervention with potential serious complications; especially for a benign disease that is easy to control in most cases with a single dose of corticosteroid at the onset of fever. Clinical or laboratory markers are needed to help to select the patients who would benefit the most from tonsillectomy. It is not clear from the literature if tonsillectomy alone should be performed, or adeno-tonsillectomy. Surgical therapy can be recommended in children

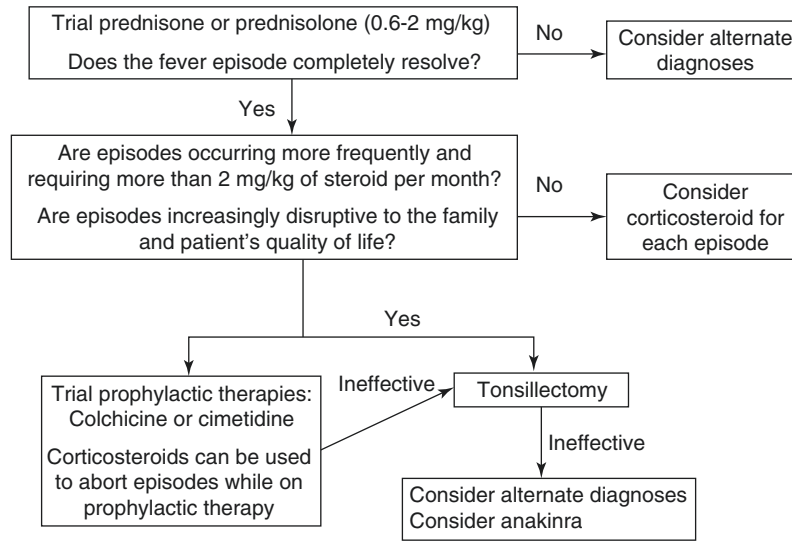

Fig. 30.4 Algorithm for the treatment of periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome

with PFAPA when episodes occur very frequently, the disease has a significant impact on the quality of life and/or may have a negative impact on school performance.

A number of algorithms have been proposed for the treatment of PFAPA and international collaborative groups are working on a common approach. One potential approach is presented in Fig. 30.4. As stated earlier, cimetidine is used much more commonly in the United States and colchicine in Europe and Israel.

30.9 Outcome/Prognosis

In 1999, a 10-year follow-up of the children diagnosed with PFAPA from the initial cohort and additional patients collected over a 10-year period was attempted [6]. Parental contact and review of the medical records of 83 of 94 available children revealed that 41% no longer had febrile episodes, while the remainder still had episodes, but less frequently. The affected children had no long-term sequelae. Corticosteroids were highly effective in controlling symptoms, but episodes recurred after therapy. Tonsillectomy and cimetidine treatment were associated with total remission in some patients. Israeli investigators described similar findings in 28 patients with classic PFAPA syndrome [3]. Affected children grew normally, had no associated diseases and no long-term sequelae. Episodes were aborted by a single dose of corticosteroids at the beginning of the episode in all patients who were treated; in 9 (32%) patients, episodes completely resolved after 6–10 years. In 3 patients, complete resolution occurred after tonsillectomy.

In 2006, other Israeli investigators described a group of 54 patients with PFAPA that they had followed since 1996 [8]. Patients had a higher rate of abdominal pain (65%) and a lower rate of aphthous stomatitis (39%), but all other clinical features were characteristics of PFAPA. Episodes were aborted with low doses of corticosteroids and tonsillectomy was associated with cessation of episodes in all 6 patients who underwent the procedure. In 2010, 105 patients who met the diagnostic criteria for PFAPA were described from one academic medical center in Connecticut [7]. Individual episodes of fever usually resolved with a single oral dose of corticosteroids, but the following episode occurred after a shorter interval of time. In 20% of these patients, episodes resolved after approximately a duration of 3 years, but in 63% there were persistent episodes. Cimetidine therapy was associated with resolution in 27% of the patients and tonsillectomy aborted subsequent attacks in all 11 patients who underwent this procedure.

In 2011, the same group that reported the long-term follow-up study in 1999 again

attempted to contact the original cohort of patients [12]. Fifty-nine patients were contacted with an interval ranging from 12 to 21 years since the original diagnosis of PFAPA. Fifty (85%) had complete symptom resolution, with a mean duration of symptoms of 6.3 years (95% CI, 5.4–7.3). None developed sequelae. Nine (15%) patients continued to have persistent symptoms for a mean duration of 18.1 years (95% CI, 17.4–18.8). There were no differences in the initial presentation between patients with PFAPA whose symptoms did or did not resolve. Patients with persistent symptoms had shorter episodes than at the onset with the mean duration of fever >38.3 °C decreasing from 3.6 days to 1.8 days at follow-up ($P = 0.01$). In addition, the mean symptom-free interval between episodes increased from 29 to 159 days ($P < 0.005$). Of the 44 patients treated with corticosteroids, 37 (84%) had prompt symptom resolution. Twelve patients underwent tonsillectomy or adeno-tonsillectomy, 9 had a marked reduction in symptoms; of these 6 (67%) had complete resolution.

30.10 Future Challenges

PFAPA is an autoinflammatory syndrome characterized by common and nonspecific manifestations, and is probably due to polygenic gene variants inducing systemic inflammatory hyperresponsiveness, affecting the oropharyngeal area. Validated classification criteria are essential to help better understand the disease as well as the response to treatment and the outcome. Even if progress has been achieved over the last decades, the exact etiology of the syndrome needs to be clarified as well as the role of a genetic origin. Biomarkers would be of a great help for the diagnosis and to separate different forms of the syndrome by specific pathophysiology. It might also help in the choice of the treatment and the selection of patients who would the most benefit from tonsillectomy. Finally, there is a need for randomized trials to measure the efficacy of the different proposed treatments, related especially to the spontaneous resolution in most patients.

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Chronic Non-Bacterial Osteomyelitis

31

Christian M. Hedrich and Hermann J. Girschick

Abstract

Chronic non-bacterial osteomyelitis (CNO) is an autoinflammatory bone disorder originally reported in the early 1970s. It typically presents in late childhood to early adolescence with bone pain and swelling. The most commonly affected areas include the metaphyses of long bones, the clavicle and the vertebral bodies. There are no diagnostic criteria making CNO a diagnosis of exclusion. While a variety of treatments have been reported to be effective, to date there are no clinical trials upon which a firm evidence-based decision of which treatment to use can be made. Recent work in mice and humans has led to new understanding of both genetic associations and the immunologic mechanisms that appear to be involved in the pathogenesis of CNO.

Keywords

Chronic non-bacterial osteomyelitis · Chronic recurrent multifocal osteomyelitis · Bisphosphonates · Microbiome

Abbreviations

ASC	Apoptosis-associated speck-like protein containg a CARD
CARRA	Childhood Arthritis & Rheumatology Research Alliance
Casp1	Caspase-1
CCL	Chemokine (C-C motif) ligand
CMO	Chronic multifocal osteomyelitis
CNO	Chronic nonbacterial osteomyelitis
CRMO	Chronic recurrent multifocal osteomyelitis
CRP	C-reactive protein
DAMP	Danger-associated molecular pattern
DC	Dendritic cell
DIRA	Deficiency of the interleukin-1 receptor antagonist
DMARD	Disease modifying anti-rheumatic drug
ERK	Extracellular signal-regulated kinase

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ESR	Erythrocyte sedimentation rate
FBLIM	Filamin-binding LIM protein
FBP	Formin binding protein
GM-CSF	Granulocyte and monocyte colony stimulating factor
IL	Interleukin
IL-RN	Interleukin-1 receptor antagonist
LPIN2	Lipin-2, a phosphatidate phosphatase
MAP kinase	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein 1 (also CCL2)
MIG	Monokine induced by gamma interferon (also CXCL9)
MIP	Macrophage inflammatory protein
MRI	Magnetic resonance imaging
MTX	Methotrexate
NF-κB	Nuclear factor-κB
NK cells	Natural killer cells
NLRP	NACHT, LRR and PYD domains-containing protein
NSAIDs	Nonsteroidal anti-inflammtory drugs
PAMP	Pathogen-associated molecular pattern
PAPA	Pyoderma gangrenosum and acne syndrome
PBMC	Peripheral blood mononuclear cells
PRRs	Pattern recognition receptors
Pstpip	Proline-serine-threonine phosphatase-interacting protein
RANK	Receptor activator of nuclear factor-κB
RANKL	Receptor activator of nuclear factor-κB ligand
RANTES	Regulated on activation, normal T cell expressed and secreted protein (also CCL5)
SAPHO	Synovitis acne pustulosis hyperostosis osteitis syndrome
STAT	Signal transducer and activator of transcription
sJIA	Systemic juvenile idiopathic arthritis
TIRM	Turbo inversion recovery measurement
TNF	Tumor necrosis factor
VAS	Visual analogue scale

Key Points

- **CNO is an autoinflammatory bone disorder**
- **The pathogenesis is undefined but theories include both genetic and environmental contributions**
- **The course is often prolonged and while most patients do well, some have severe complications including pathologic fractures, growth deformities and pain amplification syndromes**
- **Although evidence-based data are limited, treatments including NSAIDS, bisphosphonates and anti-TNF agents generally help achieve a good outcome for most patients**

31.1 Introduction

Chronic non-bacterial osteomyelitis (CNO) is an autoinflammatory bone disorder. It covers a wide clinical spectrum with single site involvement and/or a short course at one end and chronically active and/or recurring multifocal disease at the other end of the spectrum (chronic recurrent multifocal osteomyelitis: CRMO) [1–7]. Both CNO and CRMO most commonly occur in children and adolescents, and can sometimes cause severe sequelae, including bone fractures and/or hyperostoses, growth abnormalities, neurological symptoms if vertebral bodies are involved, psychosocial problems, pain amplification and others [7–10]. Although there has been significant progress over the past years, the exact molecular pathophysiology of CNO remains unknown. However, CNO appears to be a genetically complex disorder with potentially variable molecular pathomechanisms resulting in similar clinical phenotypes. Treatment is empiric, but quite effective in most patients [6].

In this chapter, the clinical presentation of CNO/CRMO, important differential diagnoses, current pathophysiological hypotheses, and treatment options will be discussed.

Table 31.1 Some alternative and historic names for CNO/CRMO

Chronic nonbacterial osteomyelitis (CNO)	Chronic multifocal symmetrical osteomyelitis
Chronic recurrent multifocal osteomyelitis (CRMO)	Sternoclavicular hyperostosis
Nonbacterial osteomyelitis (NBO)	Sternoclavicular pustulotic osteitis
Synovitis, acne, pustulosis, hyperostosis, arthritis syndrome (SAPHO)	Diffuse sclerosing osteomyelitis
Chronic sclerosing osteitis	Multifocal recurrent periostitis
Pustulotic arthrosteitis	Bone lesions of acne fulminans
Chronic multifocal cleidometaphyseal osteomyelitis	Clavicular hyperostosis and acne arthritis
Chronic symmetrical osteomyelitis	Garre's osteomyelitis

Modified and extended after [16]

31.2 Definition and Nomenclature

Chronic non-bacterial osteomyelitis was first proposed as a disease entity by Giedion et al. in 1972 who described the clinical presentation of four children with aseptic subacute osteomyelitis [11]. Several years later, Probst et al. and Bjorksten et al. reported on the recurrent nature of this entity and were the first to use the term *chronic recurrent multifocal osteomyelitis* (CRMO) [12, 13]. Based on the observation that not all cases of non-infectious osteomyelitis involved multiple bones or were recurrent, more recently the term CNO has been suggested as an "umbrella term" for all forms of non-infectious osteomyelitis, with CRMO being the most severe presentation. To date, many terms have been used to describe CNO and related entities, thus complicating data interpretation and reviewing the literature (Table 31.1) [9]. In adolescents and particularly in adult patients, CNO can be associated with joint and skin manifestations, in which case it is usually referred to as SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome [14]. All individual symptoms of SAPHO can also occur in children with CNO/CRMO. Whether CRMO and SAPHO are the same disorder with slightly different presentations in different age groups or whether they are strongly related entities currently remains uncertain [6]. Based on the key features of systemic inflammation in the absence of high-titer autoantibodies, and lacking involvement of autoreactive lymphocyte populations (at least during early stages), CNO is currently considered as an autoinflammatory condition. CNO type lesions are prominent in two well-defined monogenic autoinflammatory conditions, Majeed syndrome and DIRA (deficiency of the interleukin-1 receptor antagonist) [5, 15].

31.3 Epidemiology

CNO is considered a rare disorder. Data on incidence and prevalence are sparse. Cumulatively, several hundred cases have been reported in the literature from many different countries [7, 8, 17–26]. Recent data suggest that the incidence of CNO and bacterial osteomyelitis may be almost equal in individuals of Caucasian ancestry [7, 20]. However, secondary to sometimes rather mild and unspecific clinical symptoms, mild cases of CNO may elude diagnosis and therefore not counted in studies. Though initial reports were from Scandinavia and central Europe, all ethnicities from/in all geographic regions can be affected. CNO mostly affects children and adolescents with a peak onset between 7 and 12 years. However, it can affect all age groups, with the exception of very rare occurrence in young children under 3 years, in whom a wide differential diagnosis including infectious osteomyelitis and monogenic autoinflammatory disorders should be carefully considered [7, 20, 26, 27].

31.4 Etiology and Pathogenesis

CNO is considered an autoinflammatory condition [5, 15]. It meets the definition of an autoinflammatory condition based on the following criteria:

- CNO is an idiopathic inflammatory condition
- Typically, high-titer antibodies cannot be detected
- Autoreactive lymphocyte populations do not play a role (at least at disease-onset)

The initial suspicion that CNO may be an infectious condition was excluded by several studies confirming that cultures and/or nucleic acid amplification of bacterial pathogens remain negative in bone biopsies from CNO patients. Furthermore, antibiotic treatment fails to induce long-term remission [10, 19, 24, 27–34]. Few reports on the detection of *Propionibacterium acnes*, *Mycoplasma spp.*, or *Staphylococcus spp.* in bone biopsies most likely were due to contamination with skin commensals [10, 24, 25, 29, 32, 33, 35–37]. However, the ability of certain bacteria to alter immune responses and the detection of *Propionibacterium spp.* in acne lesions raises the question of whether pathogens may contribute to bone inflammation in an indirect manner (see below: The potential involvement of the microbiome).

31.4.1 Mechanisms of Cytokine Dysregulation in CNO/CRMO

Recently, it became apparent that disrupted cytokine and chemokine expression patterns from innate immune cells contribute to the pathophysiology of CNO/CRMO. Monocytes from CRMO patients express increased amounts of pro-inflammatory cytokines (IL-1ß, IL-6, TNF-α) and chemokines (IL-8, IP-10, MCP-1, MIP-1a, MIP-1b) while failing to express the immune regulatory proteins IL-10, IL-19, and the IL-1 receptor antagonist as compared to cells from healthy controls (Table 31.2) [38–41]. Impaired expression of the immune-regulatory cytokines IL-10 and IL-19 is (at least partially) caused by reduced activation of the mitogen activated protein kinases (MAPK) extracellular signal–regulated kinases (ERK) 1 and 2 [38, 40]. Notably, the expression of other MAPK-induced pro-inflammatory cytokines including TNF-α and IL-6 is increased in monocytes from CNO/CRMO patients [41]. This may be explained by the fact, that the alternative p38 MAPK pathway is not altered in CNO/CRMO monocytes [40].

MAP kinases are involved in gene regulation at various levels. Impaired activation of ERK1 and 2 results in altered phosphorylation and shuttling of the transcription factor signaling protein 1 (SP-1) to the cell nucleus. In turn, reduced nuclear levels of activated Sp-1 result in reduced recruitment of Sp-1 to regulatory elements within the *IL10* and the *IL19* promoter and impaired gene transcription [40, 41]. MAP kinases ERK1 and 2 are furthermore involved in the induction of epigenetic remodeling though the phosphorylation of histone proteins. Thus, reduced ERK activation results in altered histone H3S10 phosphorylation at the *IL10* promotor [40, 41]. Histone 3 S10 phosphorylation is an activating epigenetic modification, mediating "opening" of regulatory regions to transcription factor:DNA interactions [46, 47]. Reduced H3S10 phosphorylation therefore results in even more significantly reduced Sp-1 recruitment to *IL10* regulatory elements. Taken together, altered ERK1 and 2 activation in monocytes from CNO patients centrally contributes to impaired IL-10 and IL-19 expression and a disruption of balanced pro- and anti-inflammatory cytokine expression (Fig. 31.1a) (Table 31.2) [38, 40, 41].

Increased NLRP3 inflammasome activation in IL-10-deficient animals has been linked to inflammatory bone loss [48]. Scianaro et al. [49] suggested increased NLRP3 inflammasome activation contributing to the inflammatory phenotype in CRMO, showing increased mRNA expression of inflammasome components (Apoptosis-associated speck-like protein containg a CARD [ASC], NLRP3, caspase-1), increased IL-1β mRNA expression and protein release from PBMCs from CRMO patients with active disease as compared to patients in remission or controls. Hofmann et al. linked reduced expression of immune regulatory cytokines IL-10 and IL-19 with increased IL-1β mRNA expression and protein release in monocytes from CRMO patients [38]. Notably, increased inflammasome activation and IL-1β secretion is reversible by co-culture with recombinant IL-10 or IL-19 [38], further suggesting a regulatory function of IL-10 and IL-19 on inflammasome activation.

Taken together, recent observations resulted in the hypothesis that imbalanced expression of pro- (IL-1, IL-6, TNFα, IL-20) and anti- inflammatory

Table 31.2 Dysregulated cytokines, chemokines and secreted proteins in CNO/CRMO

Protein	Serum levels	Supernatants	Biological function of dysregulated protein	Biological sources
IL-10	Not detectable	↓	Immune regulatory cytokine, including control of cytokine release by monocytes and macrophages	Many cell types, including monocytes, macrophages, T cells, B cells, epithelial cells, keratinocytes, etc.
IL-19	Not detectable	↓	Immune regulatory cytokine, control of cytokine release by monocytes, monocyte apoptosis	Monocytes, and potentially others?
IL-1 receptor antagonist		↓	Immune regulatory protein, control of IL-1 effects	Monocytes, macrophages
IL-1β	Not detectable	↑	Pro-inflammatory cytokine, induction of cytokine and chemokine release from various cell types, osteoclast activation, endothelial adhesion-molecule expression, etc.	Monocytes, macrophages, neutrophils, dendritic cells, mast cells, B cells, fibroblasts, keratinocytes
IL-6	↑	↑	Pro-inflammatory cytokine, activation and proliferation of various immune cells, effects on cytokine release from monocytes, osteoclast activation	Monocytes, macrophages, lymphocytes, dendritic cells, fibroblasts
IL-12	↑		Th1 proliferation, maturation, T cell cytotoxicity, B cell activation	Macrophages, neutrophils, activated B cells, dendritic cells
TNF-α		↑	Pro-inflammatory cytokine, monocyte activation, cytokine and prostaglandin release, osteoclast activation	Monocytes, macrophages, neutrophils, eosinophilic granulocytes, dendritic cells, mast cells, T, cells, B cells, NK cells, fibroblasts, keratinocytes, glial cells, osteoblasts, smooth muscle cells
IL-20		↑	Pro-inflammatory cytokine, regulation of keratinocyte growth, role in tissue damage, osteoclast activation	Monocytes, keratinocytes
GM-CSF		↓	Granulocyte and monocyte maturation, hematopoietic effects, prostaglandin release, dendritic cell maturation, etc.	Macrophages, T cells, endothelia, fibroblasts
IL-8/ CXCL8		↑	Pro-inflammatory chemokine, neutrophil trafficking	Monocytes, macrophages, other cells including epithelial cells, airway smooth muscle cells, and endothelial cells
IP-10/ CXCL10		↑	Pro-inflammatory chemokine, Th1 response; T cell and NK cell trafficking	Monocytes, endothelial cells, fibroblasts, and others
MCP-1/ CCL2	↑	↑	Pro-inflammatory chemokine, inflammatory monocyte trafficking	Primarily: monocytes, macrophages, dendritic cells
MIG/ CXCL9		↑	Pro-inflammatory chemokine, Th1 response; T cell and NK cell trafficking	Epithelia, endothelia, hematopoietic cells
MIP-1a/ CCL3		↑	Pro-inflammatory chemokine, macrophage and NK cell migration, T cell:DC interaction	Monocytes, macrophages, T cells

(continued)

Table 31.2 (continued)

Protein	Serum levels	Supernatants	Biological function of dysregulated protein	Biological sources
MIP-1b/ CCL4	↑	↑	Pro-inflammatory chemokine, macrophage and NK cell migration, T cell: dendritic cell interaction	Monocytes, macrophages, T cells
Soluble IL-2 receptor (sIL-2R)	↑		Cytokine receptor, increased sIL-2R levels may be observed in association with several immunological abnormalities	The truncated sIL-2R is generated by proteolytic cleavage of membrane-bound IL-2Rα on the surface of T cells
Eotaxin-1/ CCL11	↓		Pro-inflammatory chemokine, eosinophil and basophil migration	Monocytes, macrophages, T cells, epithelia, fibroblasts, eosinophilic granulocytes
RANTES/ CCL5	↑		Pro-inflammatory chemokine, macrophage and NK cell migration, T cell: dendritic cell interaction	T cells, epithelia, fibroblast, thrombocytes

Protein levels as compared to healthy controls [15, 39]. Supernatants were from cultured monocytes under resting and/or stimulating conditions (lipopolysaccharide) [15, 42–45]

cytokines (IL-10 and IL-19) (Table 31.1) may result in increased osteoclast differentiation and activation through enhanced interaction between receptor activator of nuclear factor-κB (RANK) and its soluble ligand RANKL on osteoclast precursor cells (Fig. 31.1a, Box 31.1) [6, 50–52].

31.4.2 Genetic Associations in CNO/CRMO

Studies in monogenic/familial disorders, involving "CNO" as a key clinical feature provided insights that also contributed to a better understanding of sporadic CNO. There are at least three genetically determined human diseases in which CNO type bony lesions are prominent: Majeed syndrome (*LPIN2* mutations) [53], deficiency of interleukin-1 receptor antagonist (DIRA, mutation in *IL1RN*) [54], and pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPA, mutations in *PSTPIP1*) [55, 56]. In these syndromes, increased activation of inflammasomes and/or increased IL-1 signaling centrally contribute to inflammation. These disorders are discussed in Chaps. 22 and 27 [50]. While some authors consider cherubism within this group of disorders, there is debate about whether or not to include it within the autoinflammatory bone diseases.

Genetic predisposition also appears to be involved in the pathophysiology of sporadic CNO. Occasional familial clusters (approximately in 1–2%) of CNO/CRMO and high incidences of other inflammatory conditions, including psoriasis and inflammatory bowel disease in CNO patients and first degree family members strongly suggest a genetic component in the pathophysiology of CNO/CRMO [1, 7, 19, 20, 57–61]. Several studies investigated genetic associations in CNO/CRMO cohorts. Since there are overlapping features between CNO and some monogenic diseases, the *IL1RN* gene (that carries mutations in patients with DIRA) was screened for CNO-associated mutations. However, no CNO-associated mutations were found [62]. Golla et al. performed an association study in CNO patients and identified a potential susceptibility locus on chromosome 18q21.3–22 [61]. However, subsequent investigations failed to confirm these findings.

Expression of the immune-regulatory cytokine IL-10 is to some extent predetermined by genetic variants within the *IL10* proximal promoter. Three promoter haplotypes rs1800896 (−1082A>G), rs1800871 (−819T>C), and rs1800872 (−592A>C) form three haplotype blocks (GCC, ACC, and ATA) that influence transcription factor recruitment to regulatory elements, including Sp-1 [63, 64]. Hofmann et al. demonstrated an enrichment of *IL10* promoter haplotypes that encode for "high" IL-10 expression (GCC) in a German cohort of CNO/CRMO patients using target-gene analyses [64, 65]. This observation was somewhat surprising considering the aforementioned failure to produce IL-10 in CRMO

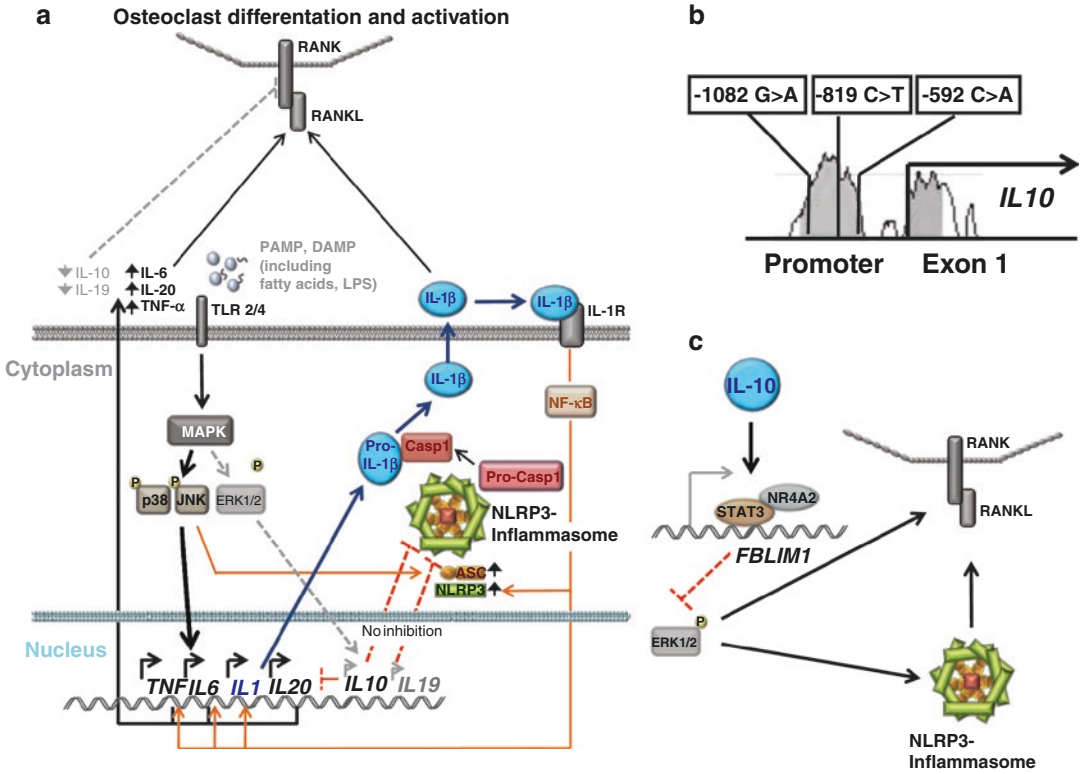

Fig. 31.1 Molecular pathophysiology of CNO/CRMO. (**a**) Sensing of danger signals occurs by pattern recognition receptors (PRRs), such as the membrane associated Toll like receptors (TLRs) and the predominantly cytoplasmic localized NOD-like receptors (NLRs). In response to the recognition of danger signals by monocytes/macrophages, multiprotein complexes, so-called inflammasomes, are activated. The NLRP3 inflammasome comprises NLRP3, ASC and procaspase-1. Inflammasomes mediate caspase-1 activation, which results in cleavage of pro-IL-1β into its active from IL-1β. In monocytes from European CRMO patients, MAP kinases Erk1 and 2 signaling is impaired, resulting in reduced expression of the immune regulatory cytokines IL-10 and IL-19. Conversely, JNK and p38 MAPK are unaffected, resulting in the expression of pro-inflammatory cytokines (TNFα, IL-6, IL-1β, IL-20). Reduced expression of IL-10 and IL-19 results in increased inflammasome activation and IL-1β release. As suggested by the literature, pro-inflammatory cytokines TNFα, IL-6, IL-20, IL-1β increase the interaction between RANK receptors on osteoclast precursor cells and their soluble ligand RANKL, which induces osteoclast differentiation and activation. (MAPK: mitogen-activated protein kinase; CRMO: chronic recurrent multifocal osteomyelitis; Erk1: extracellular signal-regulated kinase-1; TLR: Toll-like receptor; IL: interleukin; JNK: Jun kinase; TNF: tumor necrosis factor; NF-κB: nuclear factor-κB; Casp1: caspase-1; PAMP: pathogen-associated molecular pattern; DAMP: danger-associated molecular pattern; RANK: receptor activator of nuclear factor-κB; RANKL: RANK ligand.); ASC Apoptosis-associated speck-like protein containg a CARD (modified after [15]). (**b**) IL10 promoter polymorphisms rs1800896 (−1082A>G), rs1800871 (−819T>C), and rs1800872 (−592A>C) form three haplotype blocks (GCC, ACC, and ATA) that influence transcription factor recruitment to regulatory elements. (**c**) FBLIM1 is an anti-inflammatory molecule controlling bone remodeling through the regulation of RANKL activation through ERK1/2 phosphorylation. The *FBLIM1* gene is regulated by the transcription factor STAT3. Since IL-10 induces STAT3, aforementioned *IL10* promoter haplotypes may be involved in the pathophysiology of CNO (STAT Signal transducer and activator of transcription; FBLIM Filamin-binding LIM protein)

monocytes. However, observations may suggest that individuals with CRMO-associated molecular disturbances and *IL10* promoter haplotype blocks encoding for "low" IL-10 expression (ATA or ACC) may develop more severe disease and therefore not be diagnosed with CNO/CRMO (Fig. 31.1b) [41, 66].

Recently, Cox et al. identified a CRMO susceptibility gene using whole-exome sequencing [67, 68]. In two unrelated CNO patients from South Asia a homozygous and a compound heterozygous mutation in the filamin-binding domain of the *FBLIM1* gene were detected [67, 68]. Of note, the patient with the homozygous mutation in

the *FBLIM1* gene was from a consanguineous family and additionally presented with psoriasis. To our current understanding, FBLIM1 acts as an anti-inflammatory molecule controlling bone remodeling through the regulation of RANKL activation through ERK1/2 phosphorylation [67, 68]. *Trans*-activation of the *FBLIM1* gene is regulated by the transcription factor STAT3 (signal transducer and activator of transcription) [67, 68]. Since IL-10 induces STAT3 activation, aforementioned *IL10* promoter haplotypes may be involved in the pathophysiology of CNO. Indeed, Cox et al. demonstrated that both individuals carried such *IL10* promoter haplotypes that code for "low" IL-10 expression. Since IL-10 induces the activation of STAT3," low" or absent IL-10 expression may very well result in reduced STAT3 activation and down-stream effects on FBLIM1 expression in the reported individuals (Fig. 31.1c) [68]. Notably, Hofmann et al. did not identify individuals carrying ACC and ATA promoter haplotypes in a large European CNO/CRMO cohort. Together with the fact that reported *FBLIM1* mutations also rarely occur in healthy individuals, these observations suggest that the combination of *FBLIM1* variants together with *IL10* promoter haplotypes encoding for "low" gene expression may result in CNO/CRMO [41, 66].

Box 31.1 Pathophysiological Concepts in CNO/CRMO

- **Monocytes from CRMO patients fail to produce IL-10**
- **Altered MAP kinase activation centrally contributes to reduced IL-10 expression**
- **Failure to produce IL-10 contributes to inflammasome activation and a severe imbalance between pro- and anti-inflammatory cytokines**
- **Imbalanced cytokine expression may result in increased osteoclast differentiation and activation, and subsequent bone inflammation**
- **Mutations in *FBLIM1* may result in osteoclast differentiation and activation, and subsequent bone inflammation**

31.4.3 Murine Models of CNO/CRMO

Three well-defined murine models are available to study non-bacterial osteomyelitis. Mice deficient of proline-serine-threonine phosphatase-interacting protein 2 (*Pstpip2*) develop bone inflammation, elevated pro-inflammatory cytokines in the blood, extramedullary hematopoiesis and skin inflammation, resembling very severe CRMO.

(1) *Lupo* mice carry a chemically induced homozygous mutation in the *Pstpip2* gene (c.Y180C; p.I282N) (Fig. 31.2) [69, 70]. (2) Chronic multifocal osteomyelitis (*cmo*) mice spontaneously acquired homozygous mutations in *Pstpip2* (c.T293C, p.L98P). (3) Targeted knockout of exons 3 and 4 of *Pstpip2* resulted in paw swelling, synovitis, hyperostosis and osteitis, resembling SAPHO syndrome. Osteomyelitis was observed in paws, joints and skin, and characterized by increased levels of neutrophil-attracting chemokines and IL-1β [71].

Though centrally involved in the pathophysiology of murine CNO, the exact molecular contribution of *Pstpip2* mutations to sterile bone inflammation remains unclear [71]. Pstpip2 belongs to the F-BAR (Fes/CIP4 homology-Bin/Amphiphysin/Rvs) domain containing protein superfamily, linking membrane remodeling with actin dynamics associated to endocytic pathways and filopodium formation [72]. Pstpip2 interacts with formin binding protein 17 (FBP17) through its F-BAR domain. Recruitment of FBP17 and Pstpip2 to the plasma membrane enables activation of actin polymerization at podosomes. In the absence of Pstpip2, actin polymerization is increased by through the FBP17-WASP (Wiskott-Aldrich syndrome protein) complex [73]. Pstpip2 deficient macrophages exhibit abnormal podosome formation, leading to a more invasive phenotype.

The pro-inflammatory cytokine IL-1β plays a central role in the pathophysiology of osteomyelitis in *cmo* mice [74, 75]. IL-1 receptor inhibitor (IL-1RI)- or IL-1β-deficient animals (but not IL-1α-defective animals) were completely protected from the development of osteomyelitis [74, 75]. *Cmo* mice deficient of the inflammasome components NLRP3, ASC or

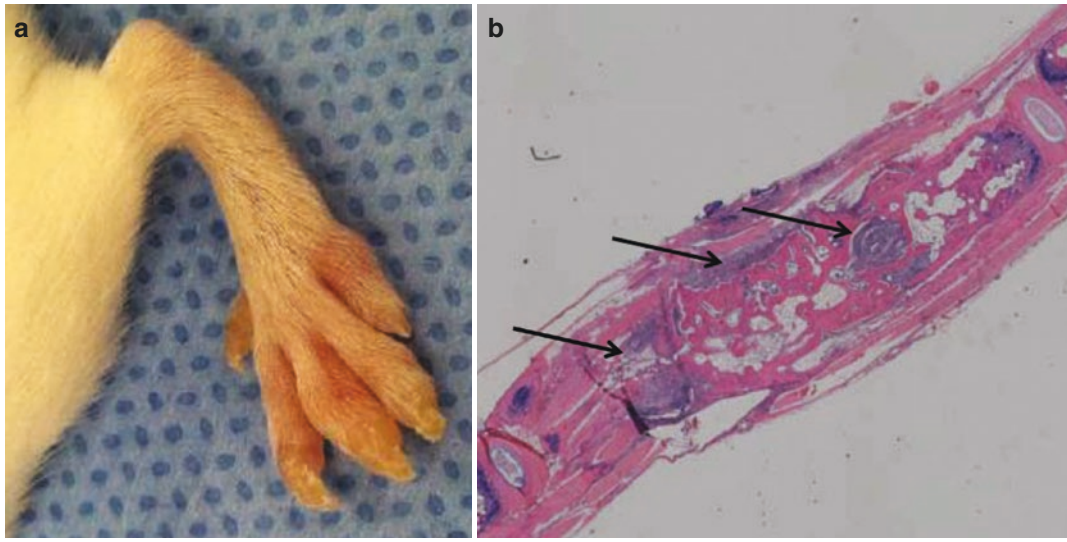

Fig. 31.2 Clinical characteristics of cmo mice. (**a**) Paws of cmo mice exhibit erythema and swelling in all 5 digits. (**b**) Histology of vertebra from the tail of a cmo mouse showing mixed inflammatory Infiltrates (arrows) and destruction of the bone architecture. Images were provided with friendly permission by Prof. Polly Ferguson, University of Iowa, Iowa City, USA

caspase-1, however, developed CNO. These observations suggest the involvement of alternative kinases or proteases other than caspase-1 in the activation of IL-1β, such as neutrophil serine proteases or caspase-8 [76]. Indeed, Cassel et al. demonstrated that IL-1β production was reduced by treatment of *cmo* bone marrow with a serine protease inhibitor (diisopropylfluorophosphate) but not with the pan-caspase-1 inhibitor z-YVAD-fmk [74], suggesting the involvement of neutrophils in the pathogenesis of *cmo*. These findings were confirmed by Lukens et al. [75], who also demonstrated that pharmacological depletion of neutrophils with the monoclonal antibody anti-Ly6G protected *cmo* mice from the development of osteomyelitis [77]. Notably, *cmo* mice deficient of caspase-1 or − 8 still develop CNO, while animals deficient of both caspases are protected from disease [77], suggesting redundant roles of caspases *in vivo*.

Though *Pstpip2*-deficient animals resemble severe CRMO with systemic features of autoinflammation, it is worth mentioning that mutations in the human equivalent *PSTPIP2* have not been reported in CNO/CRMO patients. Furthermore, mutations in both *cmo* and *lupo* mice are located in a region of the gene that is not present in humans. Taken together, these data suggest that *Pstpip2*-deficient animals are a valuable model for autoinflammatory bone disorders, resulting in a CRMO-like phenotype. However, the clinical picture results from pathological activation of inflammasomes that is distinct from pathomechanisms in human CRMO. This indicates that variable pathomechanisms may result in the same clinical picture of CNO/CRMO (also in individual human CNO/CRMO patients).

31.4.4 The Potential Involvement of the Microbiome

Recently, it has become apparent that host interactions with skin and gut microbiota affect immune homeostasis [78]. Alterations to the microbiome can result in inflammation and the expression of autoimmune/−inflammatory disease. Lukens et al. [77] suggest that dietary manipulation of the microbiome in *cmo* mice can prevent osteomyelitis. However, the exact molecular mechanisms involved remain unclear. The fact that patients with CRMO exhibit associations with severe acne,

inflammatory bowel disease [7], and other inflammatory conditions (see above) underscores the potential of these observations for disease prevention and treatment [78], and may at least partially explain why antibiotic treatment was considered somewhat effective in CNO/CRMO at least while treatment was being administered [28, 29].

Taken together, currently available data on the pathophysiology suggest that CNO is a complex genetic disorder with variable related molecular pathomechanisms resulting in a clinical picture that fits the descriptive diagnosis CNO/CRMO. Further studies targeting the molecular pathophysiology of CNO/CRMO will likely provide additional (and individually variable) pathomechanisms that may be applied as disease biomarkers for the diagnosis, treatment, and outcome assessment in CNO/CRMO. Furthermore, molecular patterns may be targeted in future therapeutic approaches.

31.5 Clinical Manifestations

As mentioned above, the clinical presentation and severity of CNO may vary significantly between affected individuals [17]. CNO and its most severe form, CRMO, frequently involve the metaphyses of long bones, the pelvic bones, the vertebral column, or the shoulder girdle/clavicle [3, 7, 8] (Fig. 31.3) [7–9]. Clinical signs of osteitis include pain, (rarely) redness, local heat and/or swelling (Fig. 31.4). The presentation may be insidious and many patients have a long delay in diagnosis. Some CNO/CRMO patients develop inflammatory organ involvement, including psoriasis and palmoplantar pustulosis (~8%) (Fig. 31.5a), inflammatory bowel disease (~10%), and severe acne (~10%). Some CNO/CRMO patients develop peripheral arthritis or sacroiliitis (Fig. 31.5b) [7–9], and some patients may progress from childhood CNO to spondyloarthropa-

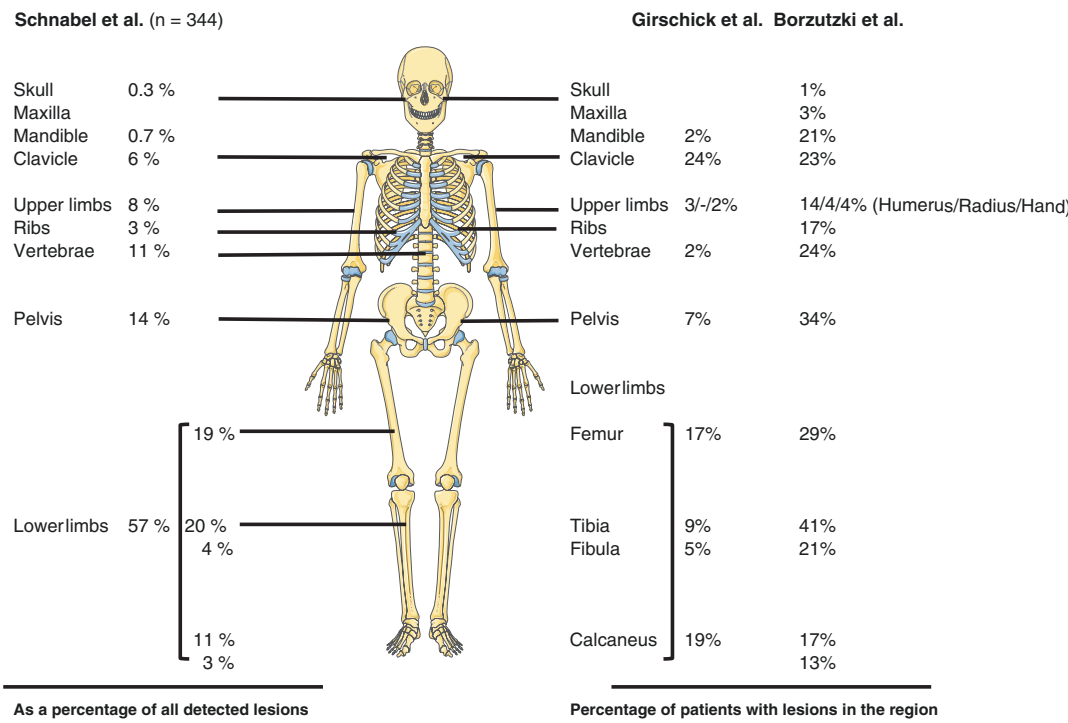

Fig. 31.3 Patterns of bone inflammation in CNO/CRMO. Relative frequencies of regional bone involvement in CNO/CRMO are shown. The study of Schnabel et al. ([7], left) provides relative frequencies based on the number of total bone lesions in all included patients. The studies of Girschick et al. and Borzutzky et al. provide numbers based on the presence of regional bone involvement in a subset of individuals [8, 9]

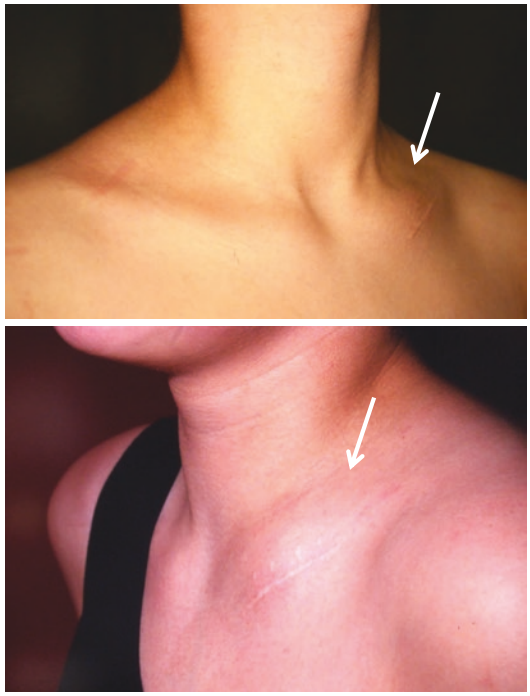

Fig. 31.4 Clavicular swelling in a 14-year-old female patient with CNO. Typical clinical picture in a 14-year-old girl with monofocal CNO of the left clavicle. The patient exhibited painful clavicular swelling in the absence of additional symptoms or laboratory anomalies. The scar resulted from bone biopsies

thies later in life [7, 25, 79]. When compared to younger children, extraosseous symptoms are more common in adolescents and adults and frequently occur in the context of the aforementioned SAPHO syndrome [14]. In addition to these relatively "common" associations, generalized pustulosis [80], Sweet syndrome [53, 81, 82], pyoderma gangrenosum [27, 83, 84], celiac disease [19, 27, 37], Takayasu arteritis [21, 85, 86], granulomatosis with polyangiitis (GPA) [27, 34], sclerosing cholangitis [27, 36], parenchymal lung disease [87, 88], and some others have occasionally been reported in CNO/CRMO patients.

31.5.1 Clinical Manifestations of CNO/CRMO

- Bone inflammation is the key feature of CNO/CRMO
- Other organ systems can be involved, including:
  - The skin: psoriasis, palmoplantar pustulosis, severe acne
  - The gut: inflammatory bowed disease (Crohn's disease, ulcerative colitis)
  - Arthritis (also distal from bone inflammation)
- SAPHO syndrome of the adolescent and adult age group combines symptoms of synovitis, acne, pustulosis, and hyperostosis

31.5.2 Differential Diagnosis and Diagnostic Approach

Since diagnostic criteria are lacking, CNO/CRMO remains a diagnosis of exclusion [1, 3–6]. Clinical symptoms include bone pain, local swelling, redness (rarely) and heat, sometimes low-grade fever, and pathological fractures (usually of vertebral bodies). Arthritis was reported to be present in some patients [7–9].

Routine laboratory inflammatory parameters (white blood cell count, C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) are usually within normal limits or only mildly elevated [7, 39]. HLA B27 testing delivered inconsistent results. Some authors reported high incidences of HLA B27 positivity in CRMO cohorts, while others did not see higher frequencies when compared to regional healthy controls, even in patients who develop spondylarthropathies [3, 7, 10, 25].

The differential diagnosis include primary or secondary bone tumors, hematologic malignancies, Langerhans cell histiocytosis, infections, osteonecrosis, metabolic bone disorders, and rare genetic disorders [1, 3–6, 10, 32, 50]. A clinical score has been developed to aid in the diagnosis of CNO and assist in deciding whether to do a bone biopsy (see below) [89].

Independent of results in available scoring systems, a large cohort of patients diagnosed with CNO included two children carrying mutations in the tissue non-specific alkaline phosphatase gene (*TNSALP*). In those individuals, the diagnosis hypophosphatasia was made based on

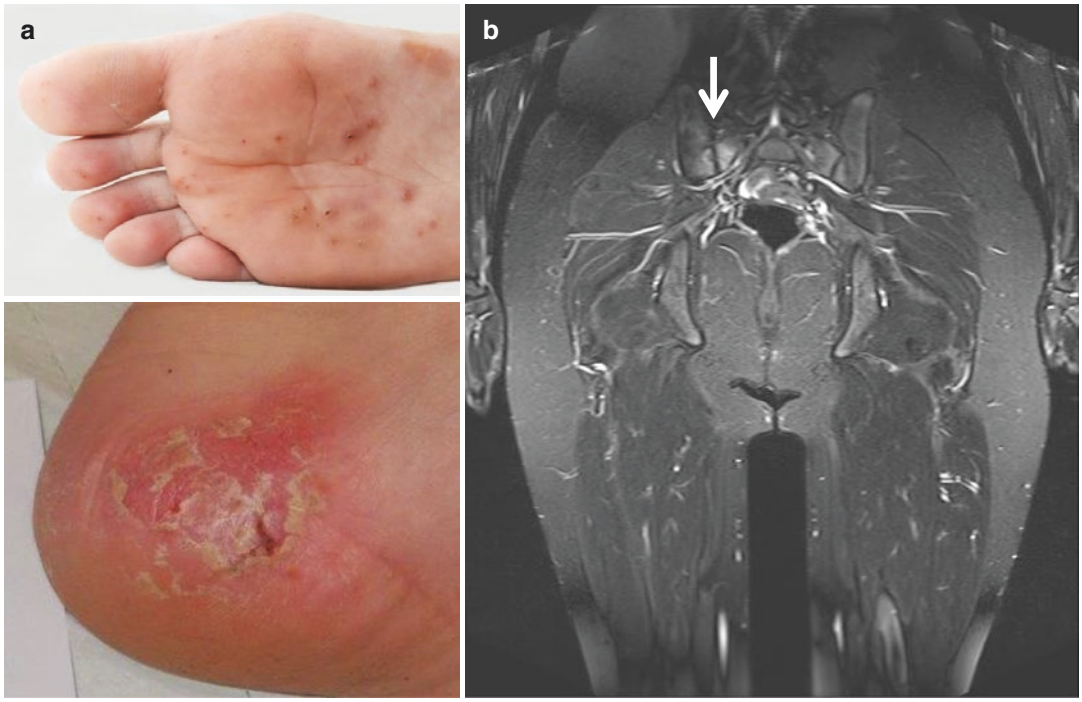

Fig. 31.5 Extraosseous symptoms in patients with CNO/CRMO. (**a**) Palmoplantar pustulosis in an 11-year-old patient with severe CRMO (upper panel), and in a 13-year-old female patient with monofocal CNO (lower panel). (**b**) Whole body MRI scans (coronary TIRM sequences), showing juxta articular signal enhancement in the os sacrum and the right pelvic bone as signs of sacroiliitis in an HLA-B27 negative 14-year-old patient with severe CRMO (MRI images with friendly permission of Gabriele Hahn, Pediatric Radiology, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany). *TIRM* turbo inversion recovery measurement

genetic testing [90, 91]. Furthermore, a number of CNO patients carried intronic or exonic polymorphisms in *TNSALP* (c.330C>T, c.787T>C, c.862+20G>T, c.862+51G>A, c.862+58C>T, c.863-7T>C, c.863-12C>G, c.876A>G, c.1565T>C), which may alter gene expression or function and result in CNO and/or a CNO-like picture (91). Taken together, hypophosphatasia has to be considered in the differential diagnosis of CNO in patients with serum alkaline phosphatase levels below the low normal range for age.

31.5.3 Imaging

Imaging techniques are centrally important tools in the diagnosis of CNO/CRMO, and the exclusion of other diagnoses [7]. Chronic inflammatory bone lesions may be detected in plain radiographs as radiolucent, osteolytic, or sclerotic lesions [13, 16, 31, 92]. In early stages, radiographs may remain normal. Magnetic resonance imaging (MRI) techniques are particularly sensitive in early stages of disease, since they can detect bone edema before bone erosions and sclerosis develop. Furthermore, MRI allows for the assessment of surrounding tissues. At the time of diagnosis, strongly T2 weighted sequences (Turbo Inversion Recovery Measurement, TIRM) and/or gadolinium-enhanced T1 sequences with fat saturation are usually used to identify inflammatory bone lesions and/or periosseous involvement (Fig. 31.6a) [16, 93–96]. At least at the time of diagnosis, whole body imaging should be performed to identify clinically silent lesions, particularly in the vertebral column (Fig. 31.6b, c). Whole body imaging should be performed using MRI techniques (TIRM) [95]. Bone scintigraphy should only be considered when MRI is not available; technical limitations in the growing skeleton (signal from growth plates, etc.), and high radiation [94] associated with scintigraphy

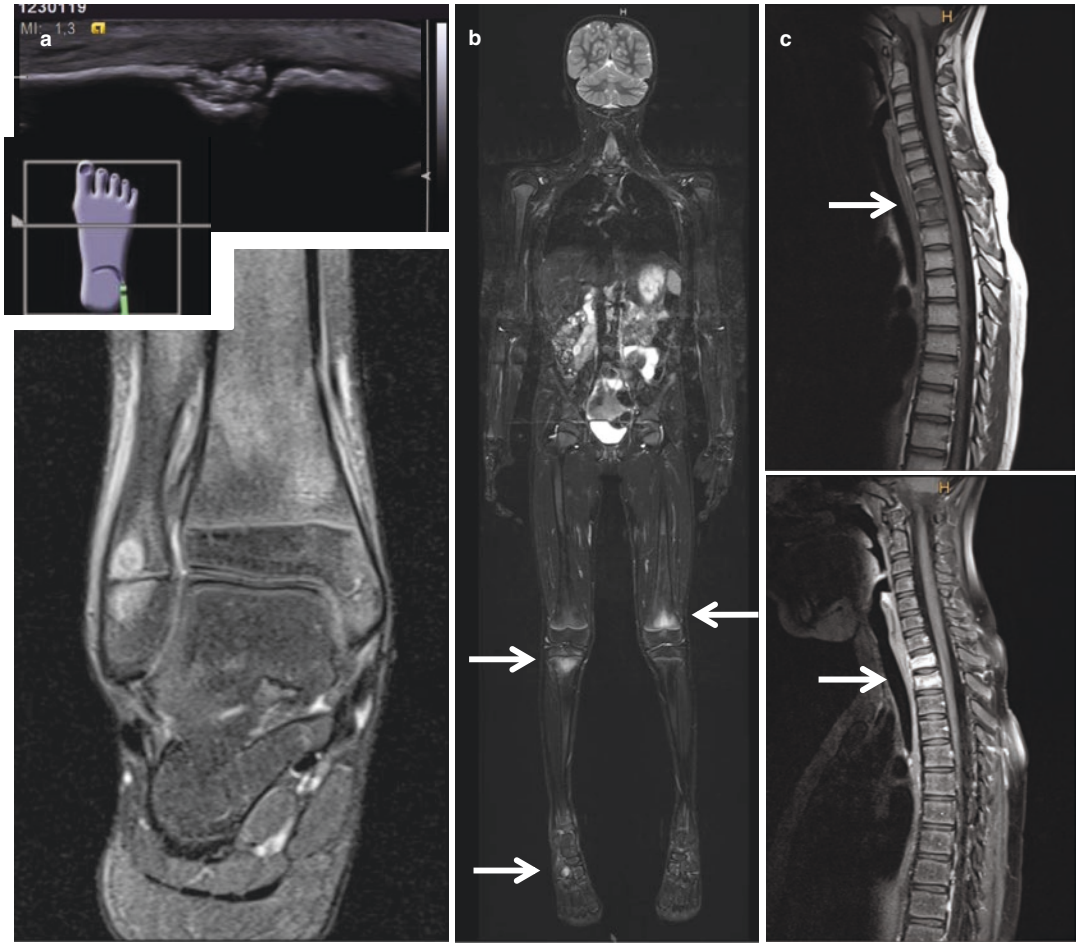

Fig. 31.6 Imaging findings in patients suggestive of CRMO. (**a**) Ultrasound in a 6-year-old patient with swelling and warmth over the lateral right ankle showing cortical incongruity. MRI scans (contrast enhanced coronal T1 sequences with fat saturation) unveiled enhancement in the metaphysis and epiphysis of the distal right fibula. (**b**) Coronary TIRM sequences in the same patients showed hyper intense areas in the distal femoral metaphysis of the left leg, the proximal tibia metaphysis and the tarsus on the right side. (**c**) MRI scans of the vertebral column in a 16-yeal-old patient with CRMO. In the upper panel, native sagittal T1 sequences indicate flattening of the first and second thoracic vertebrae with reduced signal intensity. In the lower panel, contrast enhanced sagittal T1 sequences with fat saturation show flattening of the first and second thoracic vertebrae with contrast uptake (MRI images with friendly permission of Gabriele Hahn, Pediatric Radiology, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany)

also make MRI the imaging modality of choice. Another important implication of imaging techniques is the assessment of disease activity during follow-up, and the identification and monitoring of disease-associated sequelae, such as fractures, inflammatory involvement and tissue damage to surrounding anatomical structures (95). For most questions, MRI should be considered the gold standard. Plain radiographs may be used for follow-up of fractures or sclerotic bone lesions.

31.5.4 Bone Biopsy

If the diagnosis is not clear, a *bone biopsy* should be performed to exclude malignancy, infection or other systemic disorders (e.g. Langerhans cell histiocytosis) [32]. The histologic findings vary significantly depending on the age of the lesions (Fig. 31.7). In early stages, neutrophilic granulocytes and monocytes/macrophages are the predominant cell types. Later during disease, dense

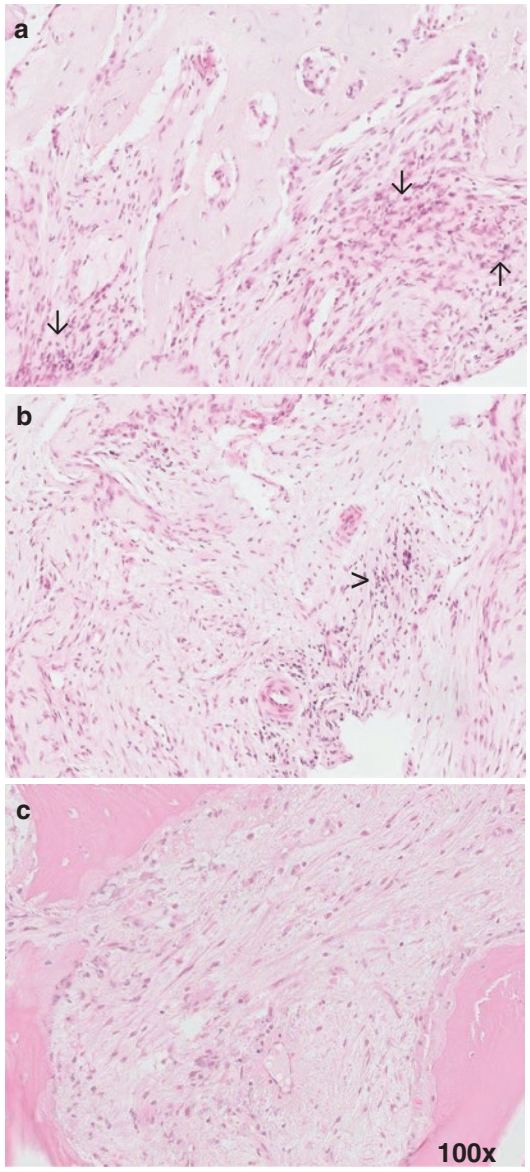

Fig. 31.7 Histologic features of CNO/CRMO in different stages of disease. (**a**) Early phase CRMO: dense infiltrate of inflammatory cells, predominantly neutrophilic granulocytes (↓) and monocytes (↑); increased osteoblast activity and bone remodeling. (**b**) Chronic phase CRMO: moderately dense infiltrate of inflammatory cells, predominantly plasma cells and mononuclear cells (>). Bone remodeling, and marrow fibrosis, and an increased number of blood vessels with endothelial cell proliferation can be seen. (**c**) Late stage CRMO: chronic fibrotic stage with only few inflammatory mononuclear cells (Magnification ×100)

infiltrates of lymphocytes and plasma cells can be seen, indicating a (secondary) activation of the adaptive immune system. After months or years of inflammation bone fibrosis in the absence of immune cells can be the predominant feature [5, 22, 30, 32, 33]. Since cellular infiltrates are not specific for CNO/CRMO and can also occur during infections, microbiological workup requires to be performed, and histologic exams mainly allow the exclusion of differential diagnoses.

31.5.5 Biomarkers

Easily accessible *biomarkers* can aid in the diagnosis of some inflammatory disorders (e.g. S100 proteins in systemic juvenile idiopathic arthritis) [97]. Preliminary data indicate that serum biomarkers may also be used to diagnose CNO/CRMO and measure disease activity (Table 31.2). The currently available, but still preliminary, set of serum biomarkers allows differentiating between newly diagnosed and treatment-naïve patients with CRMO, Crohn disease, and healthy controls. Proposed biomarkers include monocyte-derived chemokines MCP-1 and MIP-1b, pro-inflammatory cytokines IL-6 and IL-12, the mast cell derived chemokine eotaxin, RANTES, the soluble IL-2 receptor, and the IL-1 receptor antagonist. In addition to serum biomarkers, cytokine and chemokine expression from isolated immune cells may be used to support the diagnosis CNO/CRMO [39].

31.6 Treatment

Key Points

- **NSAIDs inhibit cyclooxygenase enzymes and reduce inflammasome activation. Thereby, NSAIDs may target the generation and activation of osteoclasts as well as the cytokine imbalance**
- **Classical DMARDs (methotrexate; sulfasalazine) alter cytokine expression and**

immune-cell function, and may therefore be beneficial in some patients

- **Corticosteroids suppress prostaglandin production through the inhibition of phospholipase A1, and suppress NFκB-mediated pro-inflammatory cytokine expression**
- **TNF inhibitors (at least partially) restore the imbalance between pro- and anti-inflammatory cytokine in CNO/CRMO**
- **Bisphosphonates inhibit osteoclast activity; pamidronate exerts incompletely understood effects on inflammatory cytokine expression**

The treatment of CNO/CRMO is empiric and largely based on personal experience, expert opinion, case reports and small case series. Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally used as first-line therapy (Table 31.3). They provide (at least some) symptomatic relief in patients with bone pain [9, 19, 21, 23, 24, 39, 98, 99]. Several retrospective and one prospective observational study indicate that NSAIDS are effective in a large subset of patients within the first 1–2 years of treatment. However, flare rates of above 50% after 2 years underscore the chronic character of CNO/CRMO [7, 17]. Anti-inflammatory effects of NSAIDs are mainly achieved through cyclooxygenase inhibition. Recently, NSAIDs were demonstrated to exert variable suppressive effects on inflammasomes [100]. Efficacy of NSAIDs in CNO/CRMO may therefore be explained by the involvement of pro-inflammatory monocytes in the pathophysiology of CNO/CRMO that likely contribute to the generation and activation of osteoclasts. Prostaglandins are involved in osteoclast activation, and the inflammasome activation is a hallmark of pro-inflammatory monocytes in CNO/CRMO [6].

Data from Hofmann et al. indicate that NSAIDs alone may not be sufficient in CNO/CRMO patients presenting with arthritis or spinal involvement, since 2/7 patients with spinal involvement treated with naproxen developed fractures [99]. For these patients and those who fail to respond to NSAID treatment, additional therapeutic agents have been discussed.

Table 31.3 Important differential diagnoses to CNO/CRMO

Malignancies
Leukemia
Lymphoma
Primary bone tumors (osteosarcoma, Ewing's sarcoma, etc.)
Metastases
Systemic disease
Langerhans cell histiocytosis
Collagen tissue disease with bone involvement and/or osteonecrosis
Benign tumors
Osteoid osteoma
Osteoblastoma
Fibrous dysplasia
Enchondromatosis
Hemangiomatosis
Infections
Bacterial osteomyelitis
Typical or atypical mycobacteriosis (immunodeficiency needs to be considered)
Fungal infections (immunodeficiency needs to be considered)
Metabolic bone disease
Hypophosphatasia
Hypertrophic osteoarthropathy
(Rare) genetic disorders
Autoinflammatory disoders with CNO (DIRA, PAPA, Majeed syndrome)
Cherubism
Other differential diagnoses
Osteonecrosis
Bone cysts
Bone bruise
Arthritis with bone edema (sometimes in JIA)
Hypermobility and bone edema
Scurvy

However, treatment decisions must be taken with caution, since (1) findings on MRI may be over-interpreted in clinically asymptomatic patients, (2) lesions may resolve without even becoming clinically apparent or causing sequelae, and (3) (at least some) therapeutic agents may cause side-effects and generate significant cost. On the other hand, vertebral involvement holds the risk of fractures and associated sequelae, and

long-lasting uncontrolled disease activity contributes to unfavorable long-term outcomes with polyarthritis/spondylarthropathy, pain amplification, and others [3, 6, 10].

Corticosteroids, classical disease modifying anti-rheumatic drugs (DMARDs), including sulfasalazine and methotrexate (MTX), biologic treatments (mostly anti-TNF agents), and bisphosphonates have all been reported to be effective in the treatment of patients with CNO [1, 3–6, 10, 39, 66]. Recently, the North American Childhood Arthritis & Rheumatology Research Alliance (CARRA) proposed three alternative consensus treatment plans for CNO patients refractory to NSAID treatment and/or with vertebral involvement (Fig. 31.8) [101].

Similar to NSAIDs, corticosteroids suppress prostaglandin production, which is achieved through the inhibition of phospholipase A1. Furthermore, corticosteroids dampen the expression of NFκB-mediated pro-inflammatory cytokine expression, including IL-1, IL-6, and TNF-α [6]. Most physicians prescribe short oral bursts of 2 mg/kg/day prednisone equivalent over 5–10 days. Sometimes, low-dose prednisone equivalent (0.1–0.2 mg/kg/day) is used as bridging therapy until DMARDs reach full efficacy. Corticosteroids quickly and effectively control inflammatory activity in most CNO/CRMO patients but rarely induce long-term remission. A large proportion of patients flare after discontinuation. Due to corticosteroid-associated side effects, their long-term use should be limited. Therefore, corticosteroids may be used to achieve rapid control of inflammatory activity, e.g. until other treatment regimens are established (e.g. DMARDs, biologicals, etc.) (Table 31.4) [6, 9, 21, 23].

Tumor necrosis factor α centrally contributes to bone inflammation in CNO/CRMO. Thus, blockade of TNF-α appears a promising therapeutic intervention. Indeed, several small case

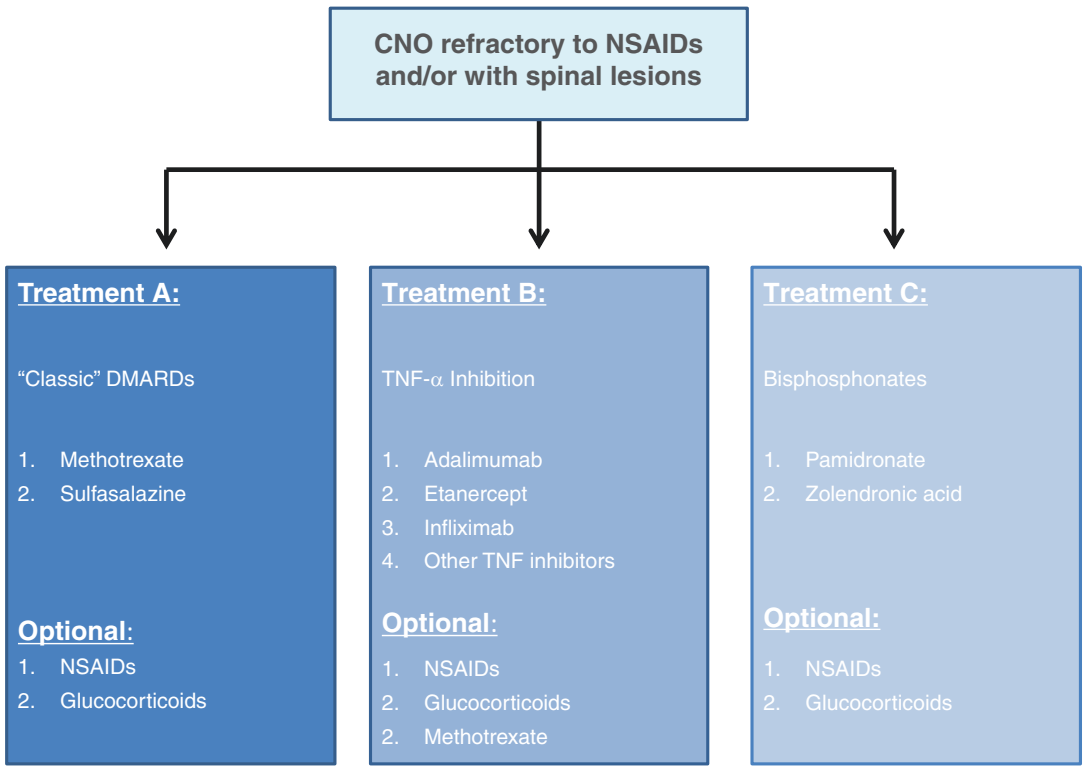

Fig. 31.8 CARRA Consensus treatment plans for CNO refractory to NSAID treatment and/or with vertebral lesions [101]

Table 31.4 Commonly used treatment options in CNO/CRMO

NSAIDs	Corticosteroids	Biologic agents	Bisphosphonates	DMARDs
Celecoxib	Prednisone equivalent	Adalimumab	Pamidronate	Methotrexate
Diclofenac		Etanercept	Zolendronic acid	Sulfasalazine
Ibuprofen		Infliximab		
Meloxicam				
Naproxen				
Piroxicam				

Treatment options are provided in **alphabetical** order and do not suggest a prevalence in usage. Standard doses as per juvenile idiopathic arthritis recommendations may be used. Dosing for glucocorticoids and bisphosphonates is given in the text. Regional approval for the usage in children by regulatory agencies may vary. No medication has been approved specifically to treat for CNO. Thus, listed medications are suggested by the authors based on personal experience and literature review. *DMARDs* disease modifying anti-rheumatic drugs

series report induction of clinical and radiological remission in CNO/CRMO in response to TNF blockade. Furthermore, patients with additional CNO-associated extra osseous manifestations (e.g. arthritis, psoriasis, IBD) may particularly benefit from anti-TNF agents. However, in view of the off-label character and associated costs, biologic treatment should only be considered for severe CNO/CRMO refractory to other treatment (Table 31.4) [6, 8, 10, 17, 19, 23, 102].

The bisphosphonates pamidronate and occasionally zolendronic acid were used in CNO/CRMO. Both bisphosphonates inhibit osteoclast activity, and pamidronate exerts incompletely understood effects on inflammatory cytokine expression (6). Pamidronate was reported to induce rapid and long-lasting remission in most CNO/CRMO patients [6, 10, 18, 103–105]. According to published reports, two alternative treatment regimens can be used: 1 mg/kg/dose (max. 60 mg/dose) every month, or 1 mg/kg/dose (max. 60 mg/dose) on 3 consecutive days every 3 months for 9–12 months. Zhao et al. reported rapid response to treatment with zolendronic acid. Since patients were also treated with infliximab, an assessment of the exact contribution of each therapeutic agent is difficult [106]. Provided potential side-effects, including growth retardation, and the fact that bisphosphonates remain in the system for many years, they should only be considered in cases refractory to other treatment options or in individuals with primary vertebral involvement and structural damage (Table 31.3) [6, 10].

Both favorable and poor outcomes of treatment with DMARDs (MTX; sulfasalazine) have been reported in case series and single case reports (Table 31.4) [19] [3, 6, 8, 10, 25].

Provided the previously described overactivation of inflammasomes and increased IL-1 release from PBMCs and monocytes from CNO/CRMO patients, surprisingly few cases of anti-IL-1 treatment have been reported in CNO/CRMO. In the few cases reported to date, the recombinant IL-1 receptor antagonist anakinra was used and showed mixed response with variable outcomes. The potential explanation for the poor response may include low tissue concentrations, pathophysiological heterogeneity in CNO/CRMO, among others.

Finally, anecdotal reports suggest effectiveness of colchicine, hyperbaric oxygen, calcitonin, interferon-α, and interferon-γ. However, the case numbers are too small to assess effectiveness or recommend treatment of CNO/CRMO with these agents [10].

31.6.1 Treatment Monitoring

In the absence of established and widely accepted tools to measure disease activity and/or treatment response, treatment decisions can be difficult and largely depend on the experience of the individual health care provider. Various parameters have been reported in the literature, including the PedCNO score (consisting of pain visual analogue scale [VAS] scores of the patient, disease activity VAS scores by

Table 31.5 Criteria for treatment failure after 3 months (as suggested by CARRA) [19, 23]

Treatment failure is defined as failure to improve on ≥4/6 criteria
Patient pain as measured by VAS
Total number of clinically active lesions
Number of radiological lesions (on WB-MRI or bone scintigraphy)
Size and degree of bone marrow edema of CNO lesion on imaging and/or presence of soft tissue swelling/inflammation related to CNO
Physician VAS
Abnormal ESR and/or CRP after exclusion of other potential causes

VAS visual analogue scale; *WB-MRI* whole body magnetic resonance imaging; *ESR* erythrocyte sedimentation rate; *CRP* C-reactive protein

physician, number of lesions on MRI, and ESR) [99], tissue inflammation on MRI [106], childhood health assessment questionnaire (CHAQ), and others. For the monitoring of treatment responses, the aforementioned CARRA consensus treatment plans include suggestions for the definition of treatment failure that are similar to the PedCNO score and involve patient pain scores, number of clinically active lesions, number of radiologically active lesions, severity of radiographically determined lesions, physician VAS, and abnormal ESR and/or CRP (Table 31.5). In accordance with previously published reports, remission was defined as resolution of pain and bone marrow edema on MRI, and normalization of ESR and CRP [8, 19, 26]. Though criteria may prove beneficial in determining disease activity, with regards to treatment decisions, they have not been validated and are at this point merely suggestions while treating CNO patients [101].

It appears generally accepted that response to treatment should be assessed no sooner than 3 months after its initiation. CNO patients with involvement of the vertebral column are usually exempt from this "rule". In the authors' institutions, patients with structural damage to vertebral bodies on MRI usually receive pamidronate as first-line treatment. In individuals with inflammatory involvement of vertebral bodies without structural damage, the strategy is less clear. Based on the extent and symptoms, patients may receive NSAIDs plus a short course of oral corticosteroids to induce timely remission or TNF inhibitors as first-line options. Generally, patients with vertebral involvement require close clinical and radiographic monitoring. Treatment responses should be assessed 4–6 weeks after initiation, and treatment should be escalated at such early time points, especially in patients with vertebral involvement, since compression fractures are a considerable threat [23, 101]. The required duration of treatment also remains unclear. Provided recent data suggesting CNO/CRMO to be a chronic disease with flares even years after initial treatment responses, patients are often treated for 6–12 months past the induction of clinical and radiological remission. However, this "routine" is empiric and not based on trials targeting treatment durations and outcomes [1, 2, 4–6, 10, 23, 27, 66].

31.7 Outcomes

In most cases, disease activity in CNO/CRMO is "waxing and waning" with periods of clinical remission and disease activity. Courses are highly variable with sometimes spontaneous resolution after several weeks or months. However, more frequently prolonged courses over several years are noted. Older studies suggested that CNO/CRMO may be mostly benign and resolve without sequelae [9, 24]. More recent studies, however, suggest that CNO/CRMO is a chronic disease with a high risk of relapses, resulting in disease-related sequelae in a subset of patients, particularly with prolonged and insufficiently controlled inflammatory activity [23, 27]. Particularly in cases with spinal involvement, pathologic fractures can occur (42–49% of patients with vertebral involvement) [17, 19, 23] (Fig. 31.9a, b). Other sequelae include bone sclerosis and hyperostosis, leg length discrepancies, muscle atrophy (also secondary to nerve affection; Fig. 31.9c), polyarthritis and spondyloarthropathies (Fig. 31.5b), psychosocial problems, and pain amplification syndromes [9, 19, 23, 27, 34, 92].

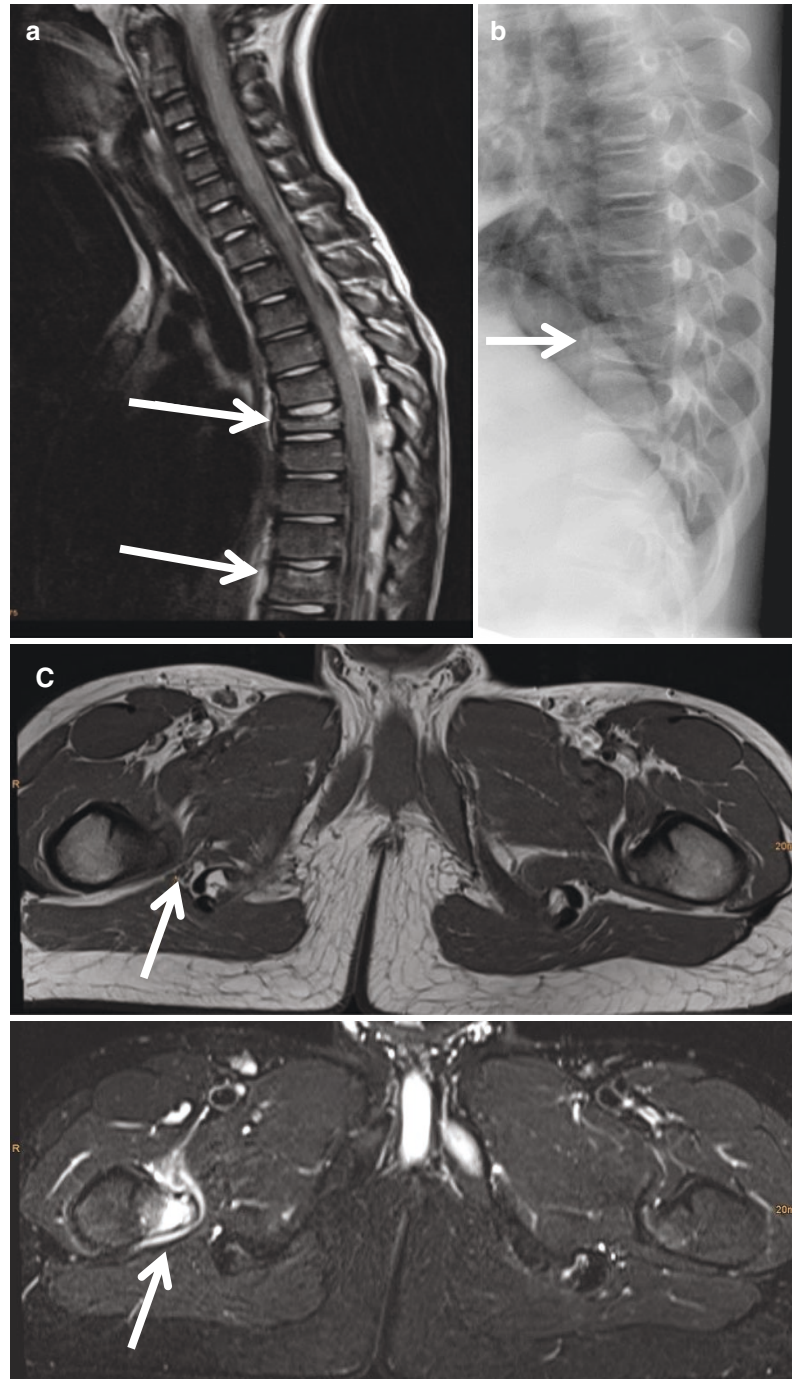

Fig. 31.9 Complications in patients with CRMO. (**a**) Sagittal T2 sequences, showing flattened thoracic vertebral bodies 6 and 10 as a result of bone inflammation in a 14-year-old boy. (**b**) Plain radiograph of the thoracic spine, showing flattened vertebral body because of bone inflammation in CRMO. (**c**) Upper panel: Native transverse T1 sequences indicating the location of the ischial nerve in close proximity to the ischial tuberosity (arrow). Lower panel: transvere TIRM sequences unveiled CRMO-associated bone inflammation of the lesser trochanter (arrow) resulted in paraosseous inflammation, affecting the ischial nerve in a 15-year-old boy who initially presented with weakness of the right leg and muscle atrophy. (MRI images and plane radiographs with friendly permission of Gabriele Hahn, Pediatric Radiology, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany). *TIRM* turbo inversion recovery measurement

31.8 Conclusions

CNO with its most severe form CRMO is an autoinflammatory bone disorder. Associations with other autoimmune/inflammatory disorders, particularly of the skin and gut have been reported. Recent data suggest a complex pathophysiology with several genetic factors, and distinct molecular disturbances (that may vary between patients) resulting in pronounced

cytokine dysregulation, and bone inflammation. Cytokine dysregulation and osteoclast activation can be targeted in therapeutic approaches. Therapeutic options include NSAIDs, short-term corticosteroids, TNF inhibitors, bisphosphonates, and DMARDs. Recent cohort studies indicate that prolonged disease activity may result in less favorable outcomes. Thus, early and sufficient treatment, particularly in individuals with spinal involvement, should be considered to prevent prolonged courses and severe complications.

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32

Systemic Juvenile Idiopathic Arthritis and Adult Onset Still Disease

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Abstract

Systemic juvenile idiopathic arthritis (sJIA) and adult onset Still disease (AOSD) are characterized by the triad of fever, rash and arthritis, together with high-grade systemic inflammation and other manifestations such as serositis, lymphadenopathy and hepatosplenomegaly. Patients exhibit considerable heterogeneity in disease severity and course, with some experiencing episodes of life-threatening inflammation termed macrophage activation syndrome. Pathophysiologic investigation into sJIA/AOSD implicates dysregulated immune control, with potential roles for both innate and adaptive immune cells, and for cytokines including interleukin (IL)-1β, IL-6, IL-18 and interferon (IFN)-γ. Therapeutic intervention with antagonists of IL-1 and IL-6 provide important clinical benefit for many patients.

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Keywords

Systemic juvenile idiopathic arthritis · Adult-onset Still disease · Fever · Arthritis · Interleukin · Inflammasomes · Macrophage activation syndrome · Anakinra · Rilonacept · Canakinumab · Tocilizumab

Abbreviations

ACR	American College of Rheumatology
AOSD	Adult-onset Still disease
CRP	C-reactive protein
DAMP	Damage-associated molecular pattern
DIC	Disseminated intravascular coagulation
DMARD	Disease-modifying anti-rheumatic drug
ESR	Erythrocyte sedimentation rate
FAMIN	Fatty acid metabolism-immunity nexus
GWAS	Genome-wide association study
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocyosis
HSCT	Hematopoietic stem cell transplantation
IL	Interleukin
IL-(X)R	Interleukin (X) receptor
IL1ra	IL-1 receptor antagonist
ILAR	International League of Associations for Rheumatology
INF	Interferon
IVIG	Intravenous immunoglobulin
JAK	Janus kinase
JIA	Juvenile idiopathic arthritis

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MAS	Macrophage activation syndrome
MHC	Major histocompatibility complex
MKD	Mevalonate kinase deficiency
NK	Natural killer
NSAIDs	Non-steroid anti-inflammatory drugs
sIL-(X)R	Soluble interleukin (X) receptor
sJIA	Systemic juvenile idiopathic arthritis
STAT	Signal transducer and activator of transcription

Key Points

- **Systemic juvenile idiopathic arthritis (sJIA) and adult onset Still disease (AOSD) represent a continuous clinical spectrum**
- **Autoinflammatory features of sJIA/AOSD include fever and rash, as well as brisk response to cytokine antagonism in many patients; however, the disease is rarely familial, exhibits genetic association with the human leukocyte antigen region, and resolves in many patients**
- **Prominent clinical features of sJIA/AOSD include marked systemic inflammation, in some patients leading to the "cytokine storm" of macrophage activation syndrome, as well as chronic peripheral arthritis that can be destructive and refractory to therapy**
- **Antagonists of interleukin (IL)-1 and IL-6 provide substantial therapeutic benefit in most sJIA/AOSD patients**

32.1 Introduction

- Systemic juvenile idiopathic arthritis (sJIA) and adult onset Still disease (AOSD) differ in classification criteria but appear clinically and biologically similar and are commonly regarded as the same disease
- Disease onset peaks in early childhood, and in young adulthood for AOSD, with an approximately equal gender balance in most series

Systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still disease (AOSD) are uncommon, highly inflammatory diseases characterized by intermittent fevers, rashes, and arthritis. The presence of fever, lymphadenopathy, organomegaly and pericarditis (but without rash) in some children with arthritis was reported by George Frederic Still in 1897, leading to the eponym "Still disease" to describe at first the whole spectrum of arthritis in childhood and now more specifically its febrile variant [1]. An adult-onset phenotype with similar clinical features was described in 1971 by Eric G. L. Bywaters, who considered the syndrome identical with pediatric Still disease [2].

32.1.1 Classification of sJIA and AOSD

Classification criteria for sJIA and AOSD are distinct. The International League of Associations for Rheumatology (ILAR) classifies sJIA as one of 6 major forms of chronic arthritis in children. Definitional requirements include age of onset before the 16th birthday and the presence of inflammatory arthritis for at least 6 weeks [3]. Additional features that contribute to the diagnosis include an evanescent rash, lymphadenopathy, organomegaly and serositis (Table 32.1, left). AOSD is commonly classified according to the Yamaguchi criteria, in patients whose symptoms begin at age 16 and above [4, 5]. According to these criteria, patients may be diagnosed with AOSD in the presence of a combination of symptoms and/or laboratory features, including fever, arthralgia, and/or rash (Table 32.1, right). Unlike sJIA, overt joint inflammation is not required, while sore throat is included and serositis is not.

Both ILAR and Yamaguchi classification criteria were developed to standardize research, and neither is intended for diagnostic use. Given the imperative to initiate treatment of children presenting with acute systemic disease, sJIA is frequently diagnosed clinically before arthritis has been present for 6 weeks, and sometimes in the absence of any arthritis at all [6, 7]. For this reason, the Yamaguchi criteria (which do not require arthritis) are more sensitive that the ILAR criteria for sJIA [8]. The term sJIA is sometimes extended to

Table 32.1 Classification criteria of systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still disease (AOSD) adapted from Petty [3] and Yamaguchi [4]

Systemic juvenile idiopathic arthritis	Adult onset Still disease
Arthritis in one or more joints with or preceded by fever of at least 2 weeks' duration that is documented to be daily ("quotidian") for at least 3 days, and accompanied by one or more of the following: 1. Evanescent (nonfixed) erythematous rash 2. Generalized lymph node enlargement 3. Hepatomegaly and/or splenomegaly 4. Serositis. **Notes:** 1. To be considered a form of JIA, arthritis must begin before the 16th birthday, persist for at least 6 weeks, and have no other known cause 2. Exclusions: a. Psoriasis or a history of psoriasis in the patient or a first-degree relative b. Arthritis in an HLA-B27 positive male beginning after the 6th birthday c. Ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, reactive arthritis, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative d. The presence of IgM RF on at least 2 occasions at least 3 months apart	**Major criteria** 1. Fever of 39 °C or higher, lasting 1 week or longer 2. Arthralgia lasting 2 weeks or longer 3. Typical rash[a] 4. Leukocytosis (10,000/mm^3) including 80% or more granulocytes **Minor criteria** 1. Sore throat 2. Lymphadenopathy and/or splenomegaly[b] 3. Liver dysfunction[c] 4. Negative RF and negative ANA[d] **Exclusions** I. Infections (especially, sepsis and infectious mononucleosis) II. Malignancies (especially, malignant lymphoma) III. Rheumatic diseases (especially, polyarteritis nodosa and rheumatoid vasculitis with extraarticular features)

RF rheumatoid factor; *ANA* antinuclear antibody

Classification as AOSD requires 5 or more criteria including 2 or more major criteria. All criteria are applicable only in the absence of other clinical explanations. Any disease listed under "Exclusions" should be excluded.All criteria are applicable only in absence of other clinical explanations

[a]Macular or maculopapular nonpruritic salmon-pink eruption usually appearing during fever

[b]Lymphadenopathy is defined as a recent development of significant lymph node swelling, and splenomegaly is confirmed on palpation or by an ultrasound

[c]Liver dysfunction is defined as an abnormally elevated level of transaminases and/or lactate dehydrogenase, which is attributed to liver damage associated with this disease but not with drug allergy/toxicity or other causes. For the differentiation, it is recommended to see if liver function returns to normal upon discontinuation of hepatotoxic drugs or not, before applying this criterion

[d]RF in serum must be negative by routine test for the detection of IgM RF, and serum ANA must be negative by routine immunofluorescence test

patients presenting before the age of 18 [9]. Despite differences in nomenclature, sJIA and AOSD are commonly regarded as essentially the same condition and will be discussed as such here [10].

32.2 Epidemiology

The epidemiology of sJIA has been defined in a number of observational series [6, 11–13]. These studies show a broad peak in early childhood, greatest between the ages of 1 and 6, tailing off thereafter (Fig. 32.1). In this respect sJIA mimics JIA as a whole, which exhibits an incidence peak in early childhood [14]. Unlike other types of JIA, sJIA has an appreciable incidence in the first year of life, although it is rare before 9 months of age. Demographically, sJIA exhibits a rough balance between male and female, potentially with a slight female predominance. Consistent seasonal variation in disease onset has not been observed [15, 16].

Worldwide, the proportion of the sJIA relative to the other JIA subtypes varies strikingly. In Europe and North America, sJIA represents approximately 5–15% of all JIA, with an

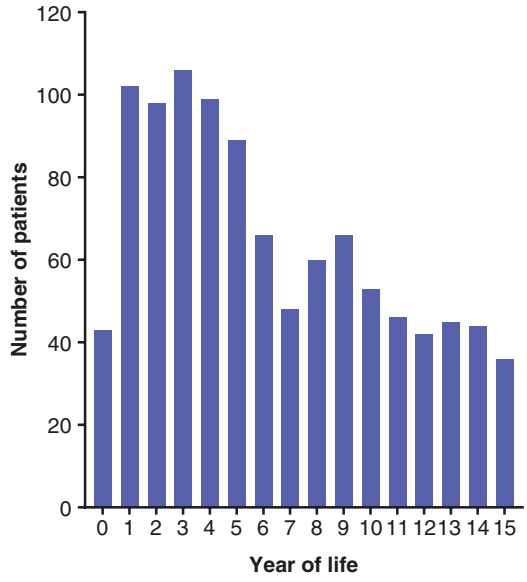

Fig. 32.1 Age of onset of systemic juvenile idiopathic arthritis, reflecting 1043 patients from Ogilvie [11], Behrens [6], Russo [12] and Klotsche [13]

incidence estimated at 0.3–0.8 per 100,000 children under the age of 16 years [17–21]. By contrast, in India and Japan, sJIA has been reported to account for 25% and almost 50% of all JIA, respectively [22, 23]. The basis for these regional differences is unknown, and may in part overestimate the proportion of sJIA patients as a result of referral bias that favors inclusion of patients with more severe or dramatic presentation.

The epidemiology of AOSD is less well defined. Its prevalence is estimated in the range of 0.1 to 1 per 100,000 [24, 25]. Most series demonstrate a relatively young age of onset, usually averaging around 20–40 years, although there may be a second "peak" between the ages of 30 and 50 years [2, 24, 25]. As with sJIA, the gender distribution of AOSD is split roughly evenly between males and females, although series in Japan often skew strongly to women [25].

32.3 Etiology and Pathogenesis

- **Genetic data suggest that sJIA/AOSD is in most cases a polygenic disease**
- **Interleukin (IL)-1β and IL-6 appear key to the pathogenesis of sJIA/AOSD, with additional roles for IL-18, interferon (IFN)-γ, and S100 proteins**
- **Aberrant function of natural killer cells, monocytes and neutrophils may contribute to dysregulated inflammation**
- **Systemic manifestations may differ in pathogenesis from arthritis, with implications for treatment**

A mechanistic understanding of sJIA/AOSD must account for several key features. Patients experience marked systemic inflammation, in some cases taking the form of an overt "cytokine storm" in macrophage activation syndrome (MAS) (see Chap. 33). Inflammatory arthritis is a frequent although not invariable clinical feature, and may be more resistant to usual therapies than other forms of JIA. Antagonism of the cytokines IL-1 or IL-6 can produce rapid improvement in many patients. Disease onset is typically abrupt. Patients may enter an afebrile (but sometimes persistently inflammatory) arthritic phase. In some patients the disease eventually resolves completely, such that more than half ultimately enter extended drug-free remission (see Prognosis & Outcome below). At present, the basis for this complex of features remains poorly defined. Clinical heterogeneity suggests underlying biological heterogeneity, and it is likely that sJIA/AOSD can reflect dysfunction in a range of pathways. To date, most pathophysiologic investigation has focused on sJIA; to the extent available, data from AOSD are comparable. We will summarize the available data, followed by a synthesis.

32.3.1 Genetics

Genetic investigation into sJIA has encompassed both population-level and target-gene studies. At a population level, the most prominent genetic association resides within the human leukocyte antigen (HLA) locus on chromosome 6. A comparison of 770 sJIA patients with almost 7000 controls identified carriage of major histocompatibility complex (MHC) class II allele *HLA-DRB1*11* as associated with an odds ratio of 2.3 [26]. This allele is also associated with oligoarticular and

polyarticular JIA, although other HLA associations that confer risk or protection for these JIA subtypes were not observed [10, 27]. *HLA-DRB1*11* is rare in Japanese populations, where AOSD is associated instead with *HLA-DRB1*15* [28]. MHC II linkage typically implies CD4 T cell-driven pathology, although mechanisms exist for triggering antigen presenting cells via MHCII in the absence of a T cell/antigen complex [26, 29]. To date, a detailed search for T cell clonal expansion in sJIA/AOSD has not been reported.

A genome-wide association study (GWAS) in the same sJIA cohort identified one non-HLA region attaining genome-wide significance, *LOC284661* (odds ratio 2.4) [30]. This region on the short arm of chromosome 1 marked by the genetic variant rs72632736 includes several putative transcription factor binding sites and a long non-coding RNA but no coding DNA; the nearest gene is *AJAP1*, encoding adherens junction-associated protein 1. How this locus mediates sJIA risk is unknown. Twenty-three additional loci achieved a suggestive level of significance. Comparison with loci implicated by GWAS in rheumatoid factor negative oligoarticular/polyarticular JIA or adult rheumatoid arthritis (mostly rheumatoid factor positive) identified no overlap, confirming that sJIA is distinct genetically from these disease families [10, 30].

Such association studies test the impact of genetic polymorphisms that are common, typically with a frequency of at least 1–5%, most of which have small functional impact. Rare variants of greater functional consequence will not be detected by GWAS. To identify these, approaches required include classic genetic linkage analysis, candidate gene studies, and whole genome/whole exome sequencing.

Linkage studies employ classic Mendelian genetics, but require kindreds in which the phenotype appears in either dominant or recessive form. While familial sJIA is exceeding rare, several kindreds were identified in consanguineous Saudi Arabian families, and positional techniques implicated a homozygous coding variant in *LACC1* [31]. Additional cases of childhood-onset arthritis associated with homozygous interruption of *LACC1* were identified in a consanguineous Lebanese family [32]. LACC1 has been identified as a regulator of fatty acid metabolism, now termed FAMIN (fatty acid metabolism-immunity nexus) [33]. FAMIN deficiency impairs the energy reserves of macrophages and potentially neutrophils, including impaired generation of reactive oxygen species. This may help to explain the association of the *LACC1* region not only with sJIA but also with Crohn disease and leprosy [34, 35]. Paradoxically, while macrophages deficient in FAMIN exhibit reduced IL-1β production, FAMIN-deficient mice demonstrate hyperinflammatory responses to immune stimulation, potentially due to aberrant function of energetically-deprived macrophages [33]. Given the role of lipid metabolism in mevalonate kinase deficiency (MKD), metabolic dysregulation could emerge as a common theme in inflammatory disease (see Chap. 17).

Candidate gene studies have been employed to interrogate specific pathophysiologic pathways in sJIA. The most successful of these have taken their cue from the similarity of MAS to primary hemophagocytic lymphohistiocyosis (HLH), a family of diseases mediated by defects in immune autoregulation through cell-cell killing. Patients with sJIA can exhibit heterozygous defects in HLH-related pathways, including *Munc13-4*, *PRF1*, *LYST*, and *STXBP2* [36–38]. Most but not all patients with such mutations have developed clinical MAS (see Chap. 33). Other candidate gene studies have identified possible associations with genes encoding IL-6, TNF, macrophage migration inhibitory factor, SLC26A2, IL-1, and pyrin, among others [28, 39–44]. Of these, only *IL1RN* encoding the IL-1 receptor antagonist (IL-1ra) could be confirmed in the sJIA GWAS cohort, implicating variants associated with higher expression of this gene as protective for sJIA [45].

32.3.2 Cytokines and Chemokines

A role for cytokines in sJIA has been firmly established by the clinical efficacy of cytokine blockade, beginning with IL-1 [46, 47]. Elevation of a range of cytokines and chemokines in peripheral blood has been well documented, including IL-6 and IL-18 [48–50]. Less is known about sJIA

synovial fluid, but IL-6 and the chemokines CCL3, CXCL8, CXCL9, and CXCL10 appear enriched compared with matched plasma [49, 51].

32.3.2.1 IL-1

IL-1 is a potent pro-inflammatory mediator that encompasses two distinct cytokines, IL-1α and IL-1β (see Chap. 6). IL-1β, but not IL-1α, requires processing by intracellular protein complexes termed inflammasomes to assume its active form (see Chap. 5). The receptor for both cytokines, IL1R1, is widely distributed; a decoy without the capacity to signal, IL1R2, is expressed in monocytes, neutrophils and B cells [52]. The pro-inflammatory effect of IL-1 is constrained endogenously by IL-1ra that competes with cytokine for receptor binding, and by soluble IL1R2. The efficacy of IL-1β antagonism in clinical trials, as well as corroborative data in a related mouse model, implicates IL-1β as the form of IL-1 most relevant to sJIA [53, 54]. Potent in low concentrations, IL-1β is often difficult to measure directly in sJIA blood, although sJIA serum/plasma can induce an IL-1 signature in exposed healthy donor leukocytes [47]. Overproduction of IL-1β by activated sJIA mononuclear cells has been reported by some but not all investigators [47, 55]. IL-1 triggers pro-inflammatory responses and elaboration of other pro-inflammatory mediators, including IL-6. These effects are mediated through innate and stromal lineages, such as neutrophils, endothelial cells, and osteoclasts, as well as T lymphocytes, in which IL-1 favors Th17 differentiation in both CD4 and γδT cells [54, 56]. IL-1 directly mediates fever through stimulation of the hypothalamus, and is the most powerful human endogenous pyrogen [57]. While the operative sources, targets, and effects of IL-1 in sJIA remain to be established, its importance is clear from the genetic linkage of sJIA risk with *IL1RN* and from the efficacy of IL-1 blockade for fever in most patients and arthritis and other manifestations in many [7, 45, 47, 53, 58].

32.3.2.2 IL-6

IL-6 is produced by many lineages (see Chap. 6). Its receptor, IL-6R, is expressed by hepatocytes, neutrophils, monocytes/macrophages, and some lymphocytes. IL-6 bound to IL-6R engages membrane-bound gp130 to signal via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway ("classic signaling"). IL-6R may be also secreted, through metalloproteinase cleavage from membranes and by production of an alternatively-spliced form, binding IL-6 with an affinity similar to membrane-bound receptor. The complex of soluble (s) IL-6R with IL-6 remains capable of engaging with gp130 to initiate signaling ("trans-signaling"). Gp130 is more widely expressed than membrane-bound IL-6R, expanding the range of cells that can respond to IL-6 and accounting for much of its pro-inflammatory activity. Trans-signaling is in turn antagonized by soluble gp130, which competes with membrane-bound gp130 for binding to the IL-6/sIL-6R complex. As a result, the extent to which IL-6 stimulates IL-6R-expressing cells and gp130-expressing cells depends on the levels of sIL-6R (required for trans- but not classic signaling) and of sgp130 (blocks trans- but not classic signaling) [59].

IL-6 was implicated in sJIA by de Benedetti and colleagues, who noted a marked increase in levels of circulating IL-6 in patients with sJIA that correlated better with the number of affected joints than with fever [48]. In particular, the concentration of IL-6/sIL-6R complexes was greatly elevated, closely paralleling the level of circulating acute phase reactants [60]. A blocking antibody against IL-6R, interrupting both classic signaling and trans-signaling, improves both fever and arthritis in sJIA, confirming a pathogenic role for this cytokine in sJIA [61, 62]. IL-6 has also been implicated in growth failure and in impaired cell-cell killing by NK cells [63, 64]. How IL-6 mediates sJIA is incompletely understood. Despite the association of impaired NK function with MAS, IL-6R antagonism does not appear to reduce the incidence of this complication in sJIA [62, 65].

32.3.2.3 IL-18

IL-18 is an IL-1-family cytokine that, like IL-1β, is produced as an inactive precursor and requires proteolytic cleavage to become bioactive (see Chap. 6). It is widely expressed, both by hematopoietic

and non-hematopoietic lineages, and (like IL-1α but not IL-1β) may be expressed in the absence of cell activation. Most reports indicate that, like IL-1β, IL-18 is predominantly activated via inflammasomes. However, it may also be released in proform by cellular injury, whereupon it can be activated by extracellular proteases including neutrophil proteinase 3. It signals via a paired receptor consisting of two chains, IL-18Rα and IL-18Rβ, the latter induced through exposure to cytokines including IL-12 and IL-15. IL-18 receptor is expressed on lymphocytes, including Th1 and cytotoxic cells, as well as macrophages, neutrophils and chrondrocytes [66]. Productive engagement of the receptor induces cell activation and release of IFNγ. Under homeostatic conditions, IL-18 likely serves to maintain barrier integrity, particularly in the gut. During inflammation, IL-18 potentiates the effects of cytokines such as IL-2, IL-12, and IL-15 in driving cytokine production and cytotoxicity. The effects of IL-18 are inhibited by a soluble antagonist, IL-18 binding protein (IL-18BP), that prevents binding to its receptor. Most circulating IL-18 is inactive due to such binding, but elevated levels of free IL-18 are observed in active sJIA/AOSD and in MAS [67–70].

IL-18 is present at extremely high concentrations in many patients with sJIA, distinguishing this disease from other forms of arthritis [71, 72]. Circulating levels are particularly elevated during MAS, and both sJIA and AOSD patients expressing a high level of IL-18 are particularly predisposed to this complication, whereas IL-6-predominant patients tend to have worse arthritis [50]. Unlike IL-1β, IL-18 does not directly cause fever. It can, however, induce the pro-apoptotic protein FasL on hepatocytes, potentially contributing to transaminitis associated with MAS [67]. Interestingly, in treated sJIA, IL-18 declines weeks to months after normalization of other cytokines and inflammatory markers, and indeed may remain very elevated in some individuals despite years of clinically inactive disease [73].

Cellular responses to IL-18 are aberrant in some sJIA patients due to impaired phosphorylation of the IL-18R β chain and thereby disrupted downstream signaling [72, 74]. This phenomenon does not simply reflect receptor desensitization due to tonic exposure to high levels of IL-18 [74]. Interestingly, intact IL-18 signaling has been observed in several patients with MAS, suggesting that loss of susceptibility to IL-18 is a homeostatic mechanism that, when lost, may contribute to the pathogenesis of this syndrome through activation of cells such as NK cells to produce IFNγ [72, 75].

32.3.2.4 Interferon (IFN)-γ

IFN-γ is produced by multiple hematopoietic and some non-hematopoietic lineages, and has roles in both innate and adaptive immunity (see Chap. 6). Identified initially through its ability to interfere with viral replication, IFN-γ enhances expression of antigen-presenting molecules in addition to other activating effects on macrophages, NK cells, and other cell types. Levels of IFN-γ and of a more easily-measurable proxy, CXCL9, are not typically elevated in sJIA, but rise strikingly during MAS [72, 76]. Production of IFN-γ can be induced by IL-18, potentially explaining the concordant elevation of these cytokines in MAS [77]. The potential role of IFN-γ in MAS is discussed in Chap. 33.

32.3.2.5 Other Soluble Mediators

The S100 proteins are a large family of calcium-binding proteins, typically active as dimers (homo- or heterodimeric) or even as higher-order oligomers (see Chap. 9). Two members of this family, S100A12 and the heterodimer S100A8/A9 (also known as MRP8/14), are damage-associated molecular patterns (DAMPs) produced by neutrophils and other cells and engage inflammatory mechanisms through binding to toll-like receptor (TLR) 4. Measurement of S100 proteins can help distinguish active sJIA from infection-related fever, and may represent a positive feedback loop that can perpetuate and amplify inflammation in sJIA [78–80].

32.3.3 Cellular Mediators

32.3.3.1 Natural Killer Cells

Active sJIA may be accompanied by impaired killing function in NK cells, and sometimes but

not invariably with low circulating NK cell counts [37, 72, 74, 81, 82]. Lower perforin content is sometimes observed as well, and in some patients can be attributed to heterozygous mutations in the gene encoding this protein [37, 72, 75, 83]. NK cells in sJIA exhibit transcriptional evidence of activation by IL-6 and IL-1β, but usually not by IL-18, despite high circulating levels of this cytokine (see above) [72]. IL-6 can impair NK function, suggesting that defective NK function is at least in part secondary to the inflammatory milieu [64]. Irrespective of the pathway to dysfunction, the reduced ability of NK cells to constrain activated immune cells (as well as to kill cells infected with viruses) could contribute to dysregulated inflammation in sJIA. NK cells also elaborate IFN-γ and S100A9, though their quantitative importance as primary sources of these and other mediators is unknown [72].

32.3.3.2 Lymphocytes

A role for CD4 (helper) T cells in sJIA is suggested by the genetic association with *HLA-DRB1*11*, although this association could also be mediated through non-T cell mechanisms [26]. Arthritis in mice lacking IL-1ra is mediated by CD4 T cells as well as γδT cells, the latter driven by IL-1 to produce IL-17 as an essential pro-inflammatory mediator [54, 84, 85]. Supported by these observations, it has been proposed that sJIA may be mediated by similar pathways, and indeed enhanced expression of IL-17 by circulating γδT cells has been observed in some patients with sJIA [29, 86, 87]. CD8 (cytotoxic) T cells have been observed to exhibit lower levels of perforin in sJIA, suggesting impaired cell-cell killing function. These levels normalize with autologous hematopoietic bone marrow transplantation, suggesting that this defect is induced rather than constitutive [83].

32.3.3.3 Monocytes

Monocytes participate in immune responses both within the circulation and after egress into the tissues, where they can differentiate into macrophages with either pro-inflammatory (often termed M1) or immunomodulatory (M2) function. An elevated circulating monocyte count is common in active sJIA. The subset distribution of monocytes is not markedly altered, but they may display an increase in both M1 and M2 markers during flares, as well as lower responsiveness to interferon signaling [88, 89]. Interestingly, although production of IL-1β protein by sJIA monocytes exceeds that from healthy donor cells, release of this cytokine *in vitro* is reduced [55, 88]. A highly-multiplexed analysis of circulating leukocytes from patients with sJIA in clinical remission off treatment identified monocytes as a key focus of functional aberrancy, with hyper-responsiveness to bacterial products and to ligands for TLR4 and TLR8. Monocytes exhibited enhanced intracellular accumulation but not secretion of IL-1β, as well as aberrant *in vitro* differentiation into macrophages, phenotypes potentially mediated through increased expression of the inflammasome regulator activin A and decreased expression of the transcription factor AHR (aryl hydrocarbon receptor), a modulator of monocyte development [90]. These findings suggest that intrinsic defects in the monocyte lineage could contribute to exaggerated inflammatory responses in sJIA, although it is also possible that the effects observed manifest past or ongoing subclinical disease activity.

32.3.3.4 Neutrophils

Active sJIA is associated with characteristic changes in circulating peripheral blood, most strikingly neutrophilia [55, 91]. Neutrophils are also prominent in the synovial fluid of sJIA patients, as in other types of arthritis. Neutrophils are a potential source for S100 proteins as well as of cytokines including IL-1β [78, 79, 91–94]. Murine studies implicate neutrophils as essential contributors to the initiation and perpetuation of joint inflammation [94, 95]. The degree to which neutrophils mediate the pathophysiology of sJIA is unknown.

32.3.4 Pathogenesis of sJIA/AOSD

Biological findings in AOSD, where available, are reminiscent of sJIA. For example, levels of IL-6, IL-18 and S100 proteins are elevated in

AOSD [68, 96–99]. NK cell function is impaired in active AOSD [100, 101]. As discussed below, AOSD responds to blockade of IL-1 and IL-6 in a manner similar to sJIA.

The available evidence does not yet fully define the pathogenesis of sJIA/AOSD. A central feature is a loss of control of inflammatory mechanisms, presumably triggered by an environmental trigger in the context of a suitable genetic predisposition. Aberrancy in monocytes and other innate lineages may be particularly critical [33, 90]. Genetic or acquired defects in cell-cell killing by NK and CD8 T cells could contribute to impaired immune auto-regulation through failure to eliminate activated cells, particularly in patients who go on to develop overt MAS. IL-1β and IL-6 are both dominant mediators of the clinical phenotype, with probable roles for IL-18 and S100 proteins as amplifying factors. How the initial systemic phase leads to chronic articular inflammation remains incompletely understood. Murine data implicate IL-1-driven development of arthritogenic T lymphocytes, both CD4 T cells and γδT cells, acting via mediators including IL-17 [54]. Such development could potentially explain superior responsiveness to IL-1 blockade in early disease, because the resulting T cell-driven arthritic phase would be expected to exhibit less responsiveness to IL-1 antagonism (the "biphasic hypothesis") [29, 86]. An alternate possibility, not mutually exclusive with the biphasic hypothesis, is that differences in response to therapy reflect biologic subcategories within sJIA/AOSD [55]. How immune control is restored to enable drug-free remission in some patients, but not others, remains to be determined.

32.3.4.1 Is sJIA/AOSD an Autoinflammatory Disease?

Consistent with their inclusion in this volume, sJIA and AOSD are often referred to as autoinflammatory diseases. This disease category encompasses inflammatory diseases mediated in the absence of an autoantigen, typically through inborn or sometimes acquired defects in immunoregulatory pathways. It is well recognized that many autoimmune diseases also leverage autoinflammatory-type mechanisms, with autoimmunity and autoinflammation representing a spectrum rather than a dichotomy [102]. Where sJIA/AOSD resides on this spectrum remains to be determined. Autoinflammatory features include its presentation with fever and rash; appearance early in life in many patients; roughly similar frequency among males and females; the absence of known autoantibodies; and brisk responsiveness to IL-1 blockade in many patients. Unlike some autoinflammatory diseases, sJIA/AOSD is very rarely familial; it exhibits genetic association with a class II HLA allele; it is rare in the first 6 months of life; and it resolves completely in many patients. Whether autoinflammatory mechanisms will fully account for the pathogenesis and clinical presentation of sJIA/AOSD will emerge more clearly as understanding improves [29].

32.4 Clinical Manifestations

- **sJIA/AOSD begins with a systemic phase characterized by fever and rash that often evolves into an arthritic phase characterized by destructive joint inflammation**
- **Extra-articular manifestations of sJIA/AOSD include lymphadenopathy, serositis, and elevation of inflammatory markers; pulmonary complications are increasingly recognized**
- **Transition to MAS may be triggered by intercurrent infections or medication changes, and is marked by persistent fevers, organomegaly, hyperferritinemia, and blood markers of disseminated intravascular coagulation**

The clinical manifestations of sJIA and AOSD are divided conventionally into systemic and arthritic features. In this context, "systemic" refers to fever, rash, lymphadenopathy, serositis and other non-articular inflammatory manifestations as well as the clinical and laboratory manifestations associated with systemic inflammation and MAS. This dichotomy is useful because

Table 32.2 Clinical manifestations in 7 series of systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still disease (AOSD)

	sJIA	sJIA	sJIA	AOSD	AOSD	AOSD	AOSD
	Mozziconacci Prieur 1983/4	Schneider 1992	Russo 2013	Pouchot 1991	Gerfaud 2	Ichida 2014	Ruscitti 2016
n	**100**	**38**	**132**	**62**	**57**	**71**	**100**
Age of onset (years)	4.5	6.3y	5	24	36	32	45
Female	51%	55%	59%	45%	53%	65%	66%
Fever	100%	100%	100%	100%	99%	99%	100%
Rash	90%	75%	71%	54%	77%	79%	78%
Arthritis	80%	92%	100%	94%	95%	93%	86%
LAD	50%	41%	48%	75%	60%	61%	57%
HSM	29%	41%	≥31%	≥55%	≥30%	≥48%	79%
Serositis	>20%	41%	15%	≥53%	≥19%	≥8%	≥15%
Sore throat	nr	nr	nr	92%	82%	57%	64%
MAS	(8% died)	(3% died)	15%	(2 died with DIC)	14%	≥4%	13%

Age of onset is reported variably as mean or median. *LAD* lymphadenopathy; *HSM* hepatomegaly and/or splenomegaly; *MAS* macrophage activation syndrome; *nr* not reported; *DIC* disseminated intravascular coagulation. References: Mozziconazzi/Prieur [209, 210], Schneider [211], Russo [12], Pouchot [104], Gerfaud-Valentin [212], Ichida [213], Ruscitti [214]

patients typically exhibit periods in which either systemic or arthritic symptoms dominate the clinical picture, potentially with different therapeutic implications. Essentially all patients exhibit systemic symptoms at the start of the disease course, a point at which arthritis is usually but not invariably present. Many patients then transition to a phase in which arthritis predominates, although systemic symptoms may remain to a greater or lesser degree, sometimes escalating intermittently to become the focus of clinical attention. The clinical manifestations of sJIA and AOSD as reported in selected series are presented in Table 32.2.

32.4.1 Fever

Fever is the hallmark of sJIA/AOSD, present in essentially all patients at disease onset and distinguishing this condition from other forms of chronic arthritis in adults and children. Typically, fevers begin abruptly and exhibit a high spiking character, peaking over 102°F/39°C but returning to normal several times a day. Classically, body temperature may even fall below normal, accentuating the hectic spiking character of the temperature swings (Fig. 32.2).

32.4.2 Rash

The large majority of patients with sJIA and AOSD develop an erythematous, migratory rash ("Still rash") that appears intermittently, often together with fever (Table 32.2). Rash can precede appearance of fever, and can persist after resolution of the fever and other systemic symptoms. The rash is typically pale pink in color ("salmon pink") but can be more deeply erythematous as well, and can appear urticarial. It is often concentrated on the trunk and proximal limbs. The rash is typically asymptomatic but may be pruritic in some patients; Koebnerization (exacerbation of the rash in skin that is scratched) is common and can impart a linear appearance (Fig. 32.3). It may be more difficult to detect in darkly pigmented skin. Associated ecchymosis does not occur, and the rash does not scar. Skin biopsies describe perivascular infiltrates of mononuclear cells or neutrophils with increased expression of S100 proteins [103–106]. While not all patients exhibit rash, particular caution is required in patients without rash to ensure that conditions such as infection and malignancy are not mistakenly diagnosed as sJIA/AOSD (see Diagnosis). Brown or violaceous papules and

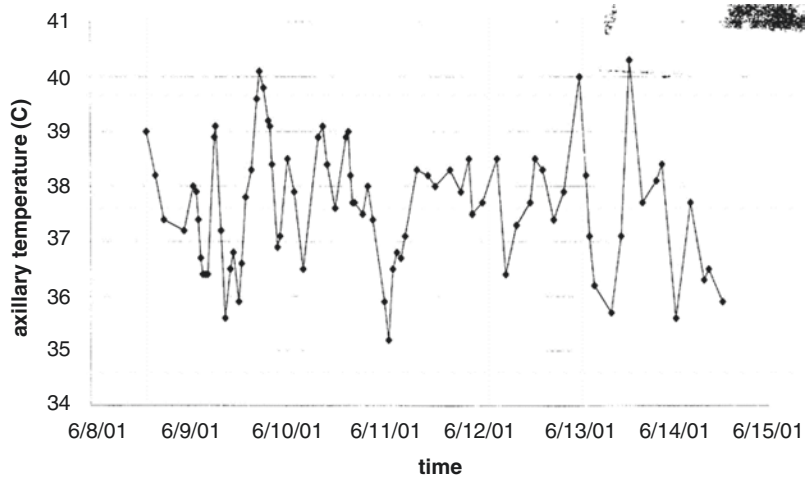

Fig. 32.2 Fever curve in systemic juvenile idiopathic arthritis (sJIA). Serial temperature measurements in 11-month-old boy with sJIA over the period of 1 week, demonstrating characteristic pattern of spiking temperatures interspersed with periods of mild hypothermia

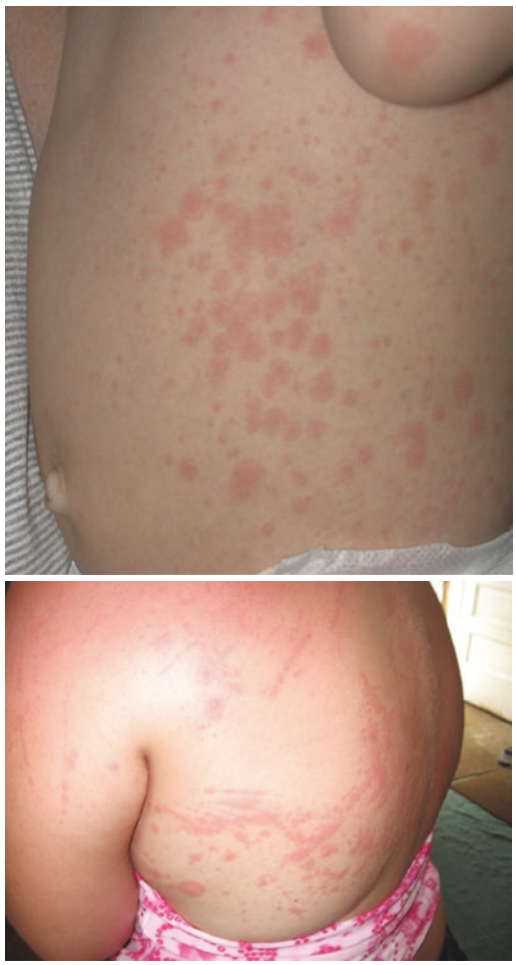

Fig. 32.3 Still Rash. Images of two patients with systemic juvenile idiopathic arthritis and characteristic erythematous macules and papules on trunk and proximal extremities; Koebnerization is illustrated in second image

plaques that are more persistent than the typical Still rash may also occur [106, 107].

32.4.3 Lymphadenopathy and Hepatosplenomegaly

Enlargement of lymphoid tissue is common in patients with sJIA/AOSD who are systemically active (Table 32.2). Lymphadenopathy with follicular hyperplasia may be generalized and is typically mild to moderate in severity and painless. A range of histopathologic findings is observed [108]. Tender lymphadenopathy should arouse suspicion of infectious lymphadenitis or Kikuchi syndrome [109]. Hepatomegaly may be accompanied by elevation in transaminases, though such elevation may also occur without corresponding physical findings. Liver pathology is non-specific, with periportal inflammation and Kupffer cell hyperplasia [108]. Splenomegaly is rarely associated with clinical hypersplenism. Extensive lymphadenopathy and organomegaly should raise concerns for malignancy and for MAS.

32.4.4 Serositis

Serositis refers to inflammation of the pericardium, pleura, or peritoneum, and may be painful or not. Serositis in observed in 15–40% of patients with sJIA/AOSD (Table 32.2). The most common manifestation is pericarditis, which may

escape notice unless echocardiography or other imaging is performed. Occasionally the pericardial effusion is prominent enough to cause hemodynamic compromise and require drainage. Small pleural effusions are relatively common, but effusions large enough to require drainage should raise the possibility of other conditions such as infections or malignancy. Abdominal serositis is rarely clinically prominent; accordingly, marked peritonitis should raise concern for alternate diagnoses, such as appendicitis.

32.4.5 Sore Throat

Sore throat is a clinical hallmark of AOSD, observed in 57–92% of patients and incorporated as a minor criterion into the Yamaguchi criteria (Table 32.2). The pathophysiological correlate for this symptom is not well defined. Data regarding sore throat in sJIA are not regularly reported; in one small series, sore throat affected 6 of 24 patients (24%) [110]. Whether the apparent paucity of this symptom in sJIA reflects a true difference between sJIA and AOSD, or rather the young age of most sJIA patients and therefore an inability to report accurately, is unknown.

32.4.6 Macrophage Activation Syndrome (MAS)

MAS is one of the most important systemic manifestations of sJIA/AOSD, and carries most of the mortality associated with this condition (See Chap. 33). The term is employed to describe the hyperinflammatory extreme of systemic inflammation, wherein the disease transforms into a "cytokine storm" leading to immune-mediated organ dysfunction. MAS is most frequently observed close to disease onset or during active systemic disease. However, MAS can appear abruptly even in patients whose disease is under apparently good control, either "out of the blue" or triggered by infection in approximately one third of episodes, in particular with herpesviruses (most frequently Epstein-Barr virus, cytomegalovirus, and human herpesvirus 6) [111]. Overt MAS affects 7–17% of patients with sJIA and AOSD (Table 32.2) but milder MAS-like clinical, laboratory, and histological features are observed in 30–50% of patients with sJIA/AOSD [112].

Clinical manifestations of MAS include persistent high fevers, rather than the spiking fevers typical of systemically-active disease; hemodynamic instability ranging from mild hypotension to shock; hepatosplenomegaly; and dysfunction of brain (seizures, coma), heart (congestive heart failure), lungs (acute respiratory distress syndrome) and liver. Disseminated intravascular coagulation (DIC) is evident on peripheral blood indices, in particular thrombocytopenia, either absolute (i.e. below the lower limit of normal) or relative compared to the degree of thrombocytosis expected for the extent and chronicity of systemic inflammation [113]. The hallmark of MAS is hyperferritinemia, often 10 to 100-fold above upper limits of normal, although this marker may be blunted in patients receiving IL-6 blockade [65]. Other laboratory markers of MAS include leukopenia and anemia; abrupt elevation in hepatic transaminases; a decline in erythrocyte sedimentation rate (ESR) resulting from DIC-driven consumption of fibrinogen; and elevation in triglycerides and soluble IL-2 receptor (CD25). Bone marrow may show hemophagocytosis, although this finding is neither necessary nor sufficient for the diagnosis of MAS [112].

Although there are no validated diagnostic criteria, the recent development of classification criteria for sJIA-associated MAS, is a major step forward in helping to advance the study of this condition [113]. According to these criteria, a febrile patient with known or suspected sJIA is classified as having MAS if ferritin > 684 ng/mL is associated with any two of the following: platelet count $\leq 181 \times 10^9$/L, AST >48 U/L, triglyceride >156 mg/dL, and fibrinogen ≤360 mg/dL. Closely following the trajectory of laboratory features of MAS is crucial and may be more important than absolute threshold laboratory values in establishing an early diagnosis [114]. Compared with sJIA-associated MAS, patients with primary HLH are generally younger at disease onset and affected

with more severe organomegaly and cytopenias [115]. The current guidelines for the diagnosis of primary HLH are insufficiently sensitive for the diagnosis of MAS in sJIA, and their application in this context could result in unnecessary delay in diagnosis and treatment [116].

The clinical presentation, diagnosis, pathogenesis, and treatment of MAS are reviewed in detail in Chap. 33.

32.4.7 Arthritis and Other Musculoskeletal Manifestations

Inflammation in the joints is a major clinical manifestation of sJIA and AOSD, and plays a dominant role in long-term prognosis and functional outcome. Arthritis can first manifest later in the disease course, months or rarely even years after disease onset. In some patients early treatment appears to prevent the development of overt synovitis. As a result, it is not uncommon to entertain the diagnosis of sJIA even without unequivocal evidence of joint inflammation [6, 7].

Several features characterize the arthritis associated with sJIA and AOSD. Multiple joints may be affected, with a predilection for hips, wrists, ankles, and cervical spine (Table 32.3). Arthritis may be unusually destructive, with relatively rapid progression to joint space loss, erosions and ankylosis (Fig. 32.4). Histological data are scant, but limited reports show a chronic mixed inflammatory infiltrate with proliferation of the synovial lining and infiltration of the deeper synovial tissues with mononuclear and plasma cells [104]. Synovial fluid analysis characteristically exhibits a polymorphonuclear leukocytosis, sometimes with extremely high cell counts that mimic septic arthritis. The pathophysiology of arthritis in sJIA/AOSD is not well understood, though animal models of arthritis driven by deficiency of IL-1ra implicate T lymphocytes (including γδ T cells) as primary drivers of disease [54, 84, 85]. Arthritis may improve transiently during pregnancy and relapse after parturition, although flares during pregnancy have also been observed [104, 117].

Tenosynovitis involving the tendons of the wrists, fingers and ankles, as well as synovial cysts around the shoulder, wrist and knee may accompany arthritis, especially when it follows a polyarticular course [118]. Myalgia is common with active systemic symptoms and frank myositis has been noted on muscle MRI [119].

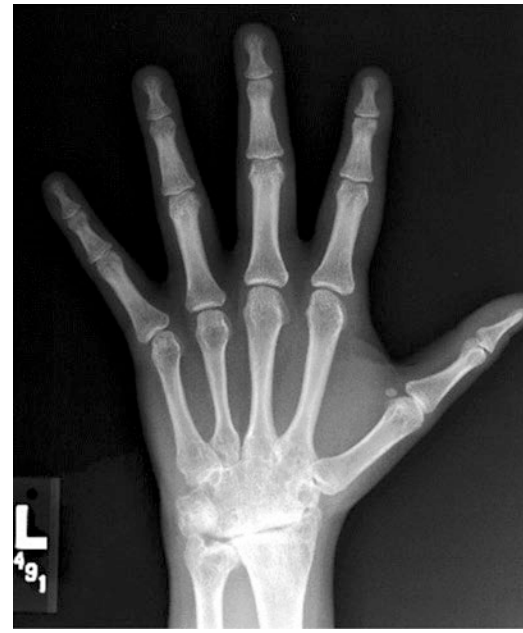

Fig. 32.4 Destructive arthritis in systemic juvenile idiopathic arthritis (sJIA). Radiograph from a 21-year-old patient with chronic sJIA, onset age 4 years, illustrating nearly complete carpal fusion as well as cystic changes in distal radius and ulna, with sparing of finger joints

Table 32.3 Joint involvement in systemic juvenile idiopathic arthritis (JIA) and adult-onset Still disease (AOSD)

Knees	56–98%
Wrists	70–77%
Ankles	39–88%
Cervical spine	24–70%
Hips	32–57%
Elbows	30–60%
TMJ	>41%
Hands	35–56%

TMJ temporomandibular joint
References: Mozziconazzi/Prieur [209, 210], Behrens [6], Pay [110], Russo [215], Pouchot [104], Masson [216]

32.4.8 Pleuropulmonary Manifestations

Patients with sJIA/AOSD may develop a range of changes affecting the lungs. Pleuritis, often manifested as asymptomatic pleural effusion, is the most common. Acute respiratory distress syndrome can accompany MAS. Patients may also develop pulmonary hypertension, interstitial lung disease, and pulmonary alveolar proteinosis [120]. The pathogenesis of these latter manifestations is unknown. Patients may present with clubbing and nailfold erythema. Many but not all have been exposed to antagonists of IL-1 and/or IL-6, potentially reflecting the severity of their disease [120]. Reports of severe pulmonary manifestations appear to be increasing. Whether this increase reflects ascertainment bias, changing management (increased biologic use, reduced steroid exposure), or other cause is unknown.

32.5 Laboratory Testing

32.5.1 Hematopoietic and Metabolic Parameters

Patients with active sJIA commonly exhibit elevation of the leukocyte count, usually with a neutrophil predominance, although immature forms (bands) are not usually present. A striking leukemoid reaction with leukocyte counts exceeding 60–70 × 10^3 cells/μL can be seen in the absence of infection. Anemia may be present, typically with red blood cell parameters consistent with anemia of chronic disease, potentially reflecting elevated hepcidin levels [121]. Thrombocytosis parallels the inflammatory state, reflecting the role of IL-6 as a promoter of thrombopoietin production [122]. Worsening in these parameters can herald MAS, including leukopenia, anemia, and relative thrombocytopenia [113]. Mild transaminitis is frequent, though hepatic function is intact; marked elevation and impaired function (such as hyperbilirubinemia) suggest MAS or drug toxicity. Renal function is not directly affected by sJIA/AOSD but may become impaired in MAS. In patients with poorly-controlled chronic disease, renal AA amyloidosis may occur (see below); assessment for urine microalbumin can help screen for this complication in patients with longstanding active disease.

32.5.2 Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP)

Patients with sJIA/AOSD exhibit elevation in inflammatory markers, including ESR and CRP, often to extreme levels. Elevation is most marked in phases of systemic activity, but may persist during chronic afebrile disease out of proportion to the extent of arthritis. ESR falls in MAS through DIC-mediated consumption of fibrinogen, despite elevated or rising CRP. Elevation of ESR and most especially CRP are driven by IL-6 stimulation of hepatocytes, and may be markedly suppressed by IL-6 antagonists even in incompletely-controlled disease [65].

32.5.3 Ferritin

A hallmark laboratory abnormality of sJIA and AOSD is elevated ferritin. Ferritin is a multimeric iron chelator produced by hepatocytes, macrophages (including hepatic Kuppfer cells), and other cells, including the intestinal mucosa, protecting these cells from the toxic consequences of intracellular accumulation of ferrous (Fe^{2+}) iron [123–126]. In individuals without inflammatory disease, the level of ferritin reflects body iron stores. Levels are low in iron-deficiency anemia and elevated in conditions such as hemochromatosis. Expression of ferritin is induced by cytokines including IL-1, and therefore increases with inflammation [127–129]. Patients with sJIA and AOSD often exhibit ferritin elevation out of proportion to other inflammatory markers. Abrupt increases are a reliable marker of transition to MAS [113, 130, 131]. Like other laboratory features of MAS, hyperferritinemia may be blunted by IL-6 blockade, rendering MAS more difficult to recognize [65].

The mechanistic basis and pathophysiologic relevance of hyperferritinemia in sJIA/AOSD are incompletely understood. Ferritin is released by macrophages engaged in hemophagocytosis of erythrocytes, potentially as a mechanism to clear iron; ferritin may also be released through hepatocellular injury [123]. Secreted ferritin is commonly glycosylated [132]. Patients with sJIA/AOSD commonly demonstrate an elevated fraction of non-glycosylated ferritin (>20%), even in remission [131, 133, 134]. The processes that mediate this change are unknown, but could include accelerated production that outstrips the glycosylation machinery or release of intracellular aglycosylated ferritin through cell injury.

32.5.4 D-Dimer and Other Markers of DIC

Although overt symptomatic DIC is restricted to frank MAS, markers of subclinical DIC are common and indeed almost ubiquitous in active sJIA/AOSD. The most useful of these is the D-dimer, reflecting crosslinked fibrinogen. In its native state, fibrinogen is a linear molecule consisting of a central E domain and two peripheral D domains connected by arms of coiled polypeptide chains termed α, β and γ. Crosslinking by thrombin into fibrin directly connects the D domains, that can then be detected in blood upon fibrinolysis by plasmin. Mild elevation of D-dimer is common in sJIA/AOSD and may be diagnostically useful, although tumors and infections can also elevate D-dimer [135]. Marked increases in D-dimer accompany overt DIC in MAS.

32.5.5 S100 Proteins

The S100 proteins S100A12 and S100A8/A9 are both elevated in sJIA (see Pathogenesis). The degree of elevation in sJIA has been found to be greater than in some other febrile sJIA mimics, including systemic infections, leukemia and lymphoma [78, 79]. S100A8/A9 levels are elevated in AOSD as well [99]. At present, commercial testing for these mediators is available in Europe but not yet in the United States or Canada.

32.5.6 Cytokines and Other Mediators

Striking elevation of cytokines accompanies active sJIA. Elevated IL-18 is particularly suggestive of this form of arthritis [49]. Data from Japanese sJIA and AOSD patients found higher levels of IL-6 relative to IL-18 in patients with marked arthritis, while IL-18-dominant patients appeared prone to MAS [50, 73, 98]. High levels of IL-18 correlated with reduced response to corticosteroids [136]. IL-1β is usually difficult to measure in circulation, although administration of canakinumab may render an immobilized pool detectable [137]. Procalcitonin is a sensitive inflammatory marker that is elevated in sJIA/AOSD but does not discriminate this syndrome from other inflammatory disorders [138]. Levels of IL-18 and IL-6 can overlap with those observed in sepsis, at least in adults [139]. Whereas IFN-γ is difficult to measure directly, the chemokines CXCL9 and CXCL10 represent stable IFN-γ-induced proteins that are measurable in blood and may help presage the development of MAS [76].

32.6 Imaging

Imaging is not specific for sJIA and AOSD. Plain radiographs can assist in the assessment of joint injury from arthritis (Fig. 32.4). Gadolinium-enhanced magnetic resonance imaging can distinguish chronic injury, stress fractures and avascular necrosis from active synovitis. Echocardiography can identify pericardial effusions associated with pericardial serositis, and chest imaging (plain radiographs and high-resolution computed tomography) together with formal pulmonary function testing assist with the diagnosis of pulmonary complications. Iimaging modalities such as dual-energy X-ray absorptiometry (DEXA) scanning are useful to quantify osteopenia mediated by sJIA/AOSD or by its treatment.

32.7 Diagnosis

The classification criteria for sJIA and AOSD (Table 32.1) guide diagnosis, but need not be fulfilled before a diagnosis (sometimes provisional) is made and treatment initiated. The differential diagnosis of sJIA/AOSD resembles that of fever of unknown origin, and varies with the age of the patient (Table 32.4). The diagnostic mimics of greatest consequence are systemic infections, such as tuberculosis, endocarditis and osteomyelitis, as well as malignancy. In children, the malignancies that may mimic sJIA are leukemia (principally acute lymphoblastic leukemia), lymphoma, and neuroblastoma. In adults, malignancies presenting with fever include leukemia, lymphoma, and renal cell cancer. Other malignancies can present with fever as well, such that patients should be screened for malignancies as appropriate for age, gender and risk factors.

Malignancy is a particular concern in patients with MAS, since leukemia and lymphoma are common non-rheumatologic causes of MAS in both adults and children [140–142]. Intravascular or hepatosplenic lymphomas can be associated with MAS and can be particularly difficult to diagnose because of the absence of lymphadenopathy, requiring biopsy of associated tissues including skin or liver. MAS induced by infection with viruses, including Epstein-Barr virus, cytomegalovirus, and influenza, can mimic MAS from sJIA/AOSD.

In both adults and children, other inflammatory disorders can present with fever, rash, arthritis, and systemic inflammation. These include inflammatory bowel disease, vasculitis (Kawasaki disease, polyarteritis nodosa, Takayasu arteritis, giant cell arteritis, and others), and Sweet syndrome (febrile neutrophilic dermatosis). In adults, Schnitzler syndrome presents as intermittent fever, urticarial rash, and monoclonal gammopathy (usually IgM), sometimes with joint pain and swelling; some of these patients evolve to overt hematoproliferative malignancy (see Chap. 37) [143, 144].

Table 32.4 Differential diagnosis of systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still disease (AOSD)

• Infection
– Bacterial—conventional and bartonella, mycoplasma, tuberculosis, fungal, Lyme disease
– Endocarditis
– Osteomyelitis or other abscess
– Viral—including EBV, CMV, HIV
• Malignancy
– Leukemia
– Lymphoma
– Solid tumor (including neuroblastoma[a] and Wilms' tumor[a])
• Autoinflammatory disease
– Cryopyrin associated periodic syndromes
– Familial Mediterranean fever (prolonged febrile myalgia syndrome)
– Mevalonate kinase deficiency/ hyperimmunoglobulin D syndrome
– NLRC4-MAS
– Tumor necrosis factor associated periodic syndrome (TRAPS)
• Other acute/chronic inflammatory disease
– Acute rheumatic fever
– Castleman disease
– Giant cell arteritis[b]
– Inflammatory bowel disease
– Kawasaki disease[a]
– Kikuchi-Fujimoto disease
– Langerhans cell histiocytosis
– Reactive arthritis
– Sarcoidosis
– Schnitzler syndrome[b]
– Serum sickness
– Sweet syndrome
– Systemic lupus erythematosus
– Takayasu arteritis
• Congenital immunodeficiency
– Autoimmune lymphoproliferative syndrome (ALPS)

EBV Epstein-Barr virus; *CMV* cytomegalovirus; *HIV* human immunodeficiency virus; *NLRC4* NLR family CARD domain-containing protein 4; *MAS* macrophage activation syndrome

[a]Partial list of potential diagnostic mimics of sJIA/AOSD. Causes restricted to children are marked

[b]Causes restricted to adults are marked

Since treatment with corticosteroids can complicate evaluation for leukemia and lymphoma, a thorough evaluation is imperative before initiating corticosteroid therapy, potentially including bone marrow biopsy and abdominal ultrasound. Particular caution is advised in the evaluation of patients presenting in an atypical manner (absent or atypical rash, low-grade or intermittent fevers, cachexia, arthralgia rather than arthritis, and mild or atypical laboratory findings). In such cases, a therapeutic trial with a short-acting IL-1 antagonist (anakinra) is unlikely to obscure subsequent evaluation for malignancy, should the patient fail to respond (see treatment below).

32.8 Treatment

- **Traditionally, therapy of new-onset sJIA/AOSD has included NSAIDs and corticosteroids; however, limited efficacy and/or toxicity have spurred a search for better alternatives**
- **High-quality randomized controlled trials confirm the efficacy of IL-1 and IL-6 blockade in sJIA; theoretical considerations support the use of these agents as first-line treatment, but supportive clinical data remain limited to observational series**
- **Therapeutic alternatives for patients who fail to respond to blockade of IL-1 and IL-6 are poorly established, with evidence for efficacy limited to case series and clinical experience**

The treatment of sJIA and AOSD has undergone rapid evolution. Historically, patients were given non-steroid anti-inflammatory drugs (NSAIDs) and corticosteroids, often at high doses and for prolonged periods, in the hope of rendering the disease tolerable until resolution occurred in patients destined for a monophasic course. Arthritis was addressed, often sub-optimally, with disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate and tumor necrosis factor (TNF) inhibitors. However, the advent of IL-1 and IL-6 antagonists has revolutionized care, and many patients now receive these drugs as first-line treatment. The treatment of MAS is reviewed separately (see Chap. 33).

32.8.1 NSAIDs

NSAIDs represent the traditional first-line treatment for sJIA and AOSD. Controlled studies documenting efficacy are not available. Clinical experience indicates that some patients respond well, albeit usually incompletely. In one prospective series of 21 patients with new-onset sJIA treated with indomethacin, complete remission after at least 1 week of treatment was obtained in 1 patient (4%), illustrating the modest efficacy of this option, at least in the short term [58].

32.8.2 Corticosteroids

In patients responding incompletely to NSAIDs or with severe systemic disease such as symptomatic pericarditis, corticosteroids are usually initiated, typically at a dose of 0.5–2 mg/kg/day. While effective for both systemic and arthritic symptoms, therapy is typically prolonged. For example, in a recent Canadian inception cohort, more than 60% of patients with sJIA remained under corticosteroid therapy for at least 6–9 months after diagnosis [145]. In patients with especially severe disease, or with MAS, intravenous pulse therapy can be helpful (e.g. IV methylprednisolone 30 mg/kg/dose, up to 1000 mg, often on 3 successive days). Alternate-day corticosteroid therapy has been employed to control refractory systemic symptoms, potentially with reduced toxicity, but is not always effective [146].

The goal should always be to wean corticosteroids to the minimum dose required. Consensus criteria have been developed for initiating, increasing and weaning corticosteroids in a clinical trial setting for sJIA [147]. Intraarticular injection of corticosteroid can assist with individual joints, but benefit is often short-lived, in contrast to patients with oligoarthritis or polyarthritis JIA.

32.8.3 IL-1 Inhibitors

Antagonists of IL-1 were the first biologic response modifiers shown to have dramatic and specific effect on sJIA [46, 47]. Three agents are clinically available: the recombinant IL-1ra anakinra, which inhibits both IL-1α and IL-1β (2–5 mg/kg SC); a humanized antibody specific for IL-1β, canakinumab (4 mg/kg monthly SC); and a novel protein, rilonacept, based structurally on the IL-1 receptor that serves to "trap" IL-1β, IL-1α, and also IL-1ra (4.4 mg/kg loading dose then 2.2 mg/kg weekly SC) (see Chap. 42 for dosing and adverse events). Randomized controlled trials have been conducted with all three agents. Anakinra was more effective than placebo in a study of 24 patients with sJIA arthritis, approximately one third of whom also had active systemic features [148]. Canakinumab was evaluated in two randomized controlled trials enrolling over 170 children with sJIA, all with ongoing systemic symptoms, finding remission of fever in more than 95% and pediatric American College of Rheumatology (ACR) 90 improvement in almost 50% [53]. These studies led to regulatory approval of canakinumab for the treatment of sJIA in many countries. Rilonacept was studied in over 70 patients, approximately 20% of whom had fever. Almost two-thirds achieved a modified pediatric ACR70 response [149]. A meta-analysis has suggested that rilonacept may be the least potent of these agents at the dose regimen studied [150]. Anakinra and canakinumab have been effective in AOSD [151–154]. Combination therapy of anakinra with the T cell costimulatory inhibitor abatacept has been reported [155]. High-dose IL-1 blockade may contribute to treatment of MAS [156].

Some data suggest that use of IL-1 blockade at the onset of disease could be particularly effective. A retrospective series of patients treated with anakinra as a component of first-line therapy for sJIA found chronic arthritis at 6 months in only 11% of patients, while 8 of 10 anakinra monotherapy patients entered immediate complete remission without corticosteroids [7]. These results were mirrored in a prospective series, wherein 14/20 patients treated with anakinra required no additional therapy, and by other series that found complete response to correlate with initiation of anakinra soon after disease onset [58, 91, 157]. Both observational and pharmacokinetic data suggest that younger children may require higher per-kg dosing of anakinra to obtain equivalent efficacy [7, 158]. The clinical response to IL-1 blockade early in the course of disease compares favorably with that observed later in the disease course, although response is observed in some established patients as well [55, 91, 159, 160]. These data have raised the possibility that early therapy might alter the course of sJIA ("window of opportunity"), forestalling development of chronic arthritis, a hypothesis that is currently under prospective clinical study [86, 161].

32.8.4 IL-6 Inhibitors

Tocilizumab is a humanized monoclonal antibody that blocks IL-6R, both in circulation and on the cell surface. A randomized controlled trial in Japan established the efficacy of this agent for sJIA. Responders to open label tocilizumab 8 mg/kg IV every 2 weeks were randomized to ongoing tocilizumab treatment or placebo. Eighty percent of patients on tocilizumab maintained a response compared to 20% of patients on placebo. In the open label extension phase, 90% of patients achieved a JIA pediatric ACR70 response [61]. A second study, conducted in 112 children in Europe and the Americas, utilized 12 mg/kg every 2 weeks for children <30 kg and 8 mg/kg every 2 weeks for children ≥30 kg IV, documenting almost 60% pediatric ACR90 responses even in a population of patients with severe, chronic,

refractory sJIA [62]. Like IL-1 blockade, IL-6 inhibition was highly effective at control of fever, with resolution in 35/41 (85%) patients with such symptoms [62]. Accordingly, tocilizumab has been approved for sJIA in multiple countries. Longer-term data for safety are reassuring, with partial growth recovery, although progression of radiographic joint injury has been reported in some patients [162–164]. Compelling but uncontrolled data support the efficacy of tocilizumab for articular and systemic manifestations in AOSD as well [165–167]. Dosing and adverse events are discussed in Chap. 43.

32.8.5 Methotrexate and Other Non-Biologic DMARDs

In sJIA, methotrexate has not proved to be helpful in treating systemic symptoms. Observational data suggest efficacy for arthritis, but a small controlled study found at best modest improvement compared with placebo [168, 169]. A clinical series in AOSD suggested that methotrexate could help limit corticosteroid exposure [170]. Leflunomide has not been studied in sJIA/AOSD, but has anecdotally proven useful. Uncontrolled studies suggest that cyclosporine A has limited benefit in sJIA, but it has assumed a role in treating sJIA-associated MAS, where it was employed in over 60% of patients in a large international series [111, 171, 172]. Use of tacrolimus, another calcineurin inhibitor, has been reported in case series [173].

32.8.6 TNF Inhibitors

Inhibition of TNF appears generally less effective in sJIA/AOSD than in other inflammatory arthritides. Observational data document pediatric ACR50 responses to etanercept in approximately 30% of sJIA patients compared with approximately 60% of patients with other JIA subtypes [174–177]. Comparable observations of partial response have been made in small series of AOSD [178–181]. TNF inhibitors are reserved for sJIA/AOSD patients with refractory arthritis in the absence of systemic symptoms and who have failed to respond to IL-1 and IL-6 blockade. A monoclonal antibody such as infliximab or adalimumab is preferred to etanercept, and is employed at the high end of the dose range.

32.8.7 Thalidomide and Lenalidomide

Successful use of thalidomide has been reported in both sJIA and AOSD [182–184]. Concurrent treatment with multiple other agents, in uncontrolled studies, renders the efficacy of thalidomide difficult to evaluate. Neuropathy is perhaps somewhat less common with the thalidomide analog lenalidomide. Teratogenicity and cost render these agents uncommon therapeutic choices.

32.8.8 Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) has been employed in sJIA for decades, with anecdotal reports of success, including for refractory systemic symptoms [185]. IVIG was tested in one randomized controlled trial; however, insufficient patient numbers and high dropout precluded a statistical conclusion [186]. IVIG is sometimes used as adjunctive treatment for sJIA-associated MAS, particularly when there is a reluctance to use systemic corticosteroids while the diagnosis is being confirmed. Otherwise IVIG is reserved for circumstances in which other agents have failed, are unavailable, or are contraindicated.

32.8.9 Other Therapeutic Agents

Significant clinical and laboratory improvement has been reported in sJIA patients treated with a combination of intravenous pulse methylprednisolone, relatively low-dose intravenous cyclophosphamide and methotrexate [187, 188]. An uncontrolled single-center study of B cell depletion with rituximab in 46 sJIA patients, in the setting of other background therapies, suggested substantial response [189]. These approaches

have not been widely adopted given the advent of more established biologic agents with relatively favorable safety profiles.

32.8.10 Hematopoietic Stem Cell Transplantation

A small number of patients with refractory sJIA have been treated with hematopoietic stem cell transplantation (HSCT) [190–192]. Accompanying immunological characterization has identified repair of restricted T regulatory cell receptor diversity as a potentially contributor to the therapeutic effect [193]. At present, HSCT is reserved by most clinicians for extreme refractory disease in view of the number of other options available and the associated morbidity and mortality.

32.8.11 Synthesis of Current Therapy of sJIA/AOSD

The available data are compatible with a wide range of clinical practice [9, 194]. For new-onset disease, the authors currently favor initiation of anakinra, a short-acting IL-1 antagonist, although the other IL-1 inhibitors, canakinumab and rilonacept, and the IL-6 inhibitor, tocilizumab may also be effective. There have been no direct comparative effectiveness trials of biologic agents in sJIA. Although the heterogeneity of published studies makes comparative analysis difficult, a meta-analysis suggested that patients with active sJIA may be less likely to respond to rilonacept than to canakinumab or tocilizumab [150]. Most patients respond at least partially to anakinra, with rapid resolution of fevers, helping to confirm the diagnosis [7, 58]. Absence of a striking response should trigger further consideration of alternate diagnoses, including malignancy and infection, as well as active MAS. Initiation of anakinra without concomitant corticosteroids is optimal to permit an unobstructed assessment of its efficacy, but we add corticosteroids to the initial regimen if active or incipient MAS is suspected, or if there are prominent systemic disease manifestations such as symptomatic pericarditis, because IL-1 blockade is not sufficient by itself to treat these complications [7, 195]. Canakinumab, rilonacept and tocilizumab may all be effective in sJIA that is refractory to treatment with anakinra, though where arthritis is prominent tocilizumab may be preferable. Studies are in progress that will help test whether first-line biologic treatment is indeed superior to non-biologic initial treatment [161].

Therapy of established sJIA/AOSD varies with disease manifestations. Systemic symptoms respond to blockade of IL-1 or IL-6, as well as to corticosteroids. Inflammatory arthritis responds to blockade of the same cytokines, and to corticosteroids, but potentially also to methotrexate and TNF blockade. Recurrent MAS-like manifestations are typically addressed with corticosteroids in the short-term, together with (high-dose) IL-1 blockade, a calcineurin inhibitor, and/or IVIG. The role for other anti-rheumatic agents including the T cell costimulatory blocker abatacept, the JAK inhibitor tofacitinib, and the IL-17 antagonist secukinumab remain undefined, as are the roles of antagonists of IL-18, IFN-γ and IL-33 in therapeutic development. Options for highly refractory disease include combination therapy, rituximab, cyclophosphamide, and HSCT.

32.9 Outcome/Prognosis

32.9.1 Monophasic, Polyphasic and Persistent Courses

Historical data provide insight into the natural history of sJIA and, to a lesser extent, of AOSD. Patients may follow one of three courses, typically in escalating order of severity. Patients with *monophasic* disease run their course over a period of 12–24 months, followed by permanent drug-free remission. Those with *polyphasic* disease manifest several discrete monophasic-like episodes separated by periods of drug-free remission. Patients with *persistent* disease demonstrate continually active disease, except as suppressed

by therapy, and are unable to attain drug-free remission [196]. In patients with persistent disease, systemic symptoms typically give way to a phase characterized by chronic arthritis. Each of the three courses may be punctuated by periodic flares, potentially including MAS. The distribution of patients across these three subtypes is incompletely defined, but in sJIA appears to be roughly 30–40% monophasic, 10–20% polyphasic, and 50–60% persistent; AOSD may manifest a somewhat lower rate of persistent arthritis (Table 32.5).

Implicit in these statistics is that drug-free remission is a relatively common outcome. Long-term follow-up data suggest that at least 30–50% of patients will follow this course [197–199]. How these patients recover their immune "equilibrium" is one of the most interesting and important questions in sJIA/AOSD research.

32.9.2 Mortality

Although some patients with sJIA/AOSD recover completely, some patients succumb to disease. In a U.S. study of almost 1000 children with sJIA, the observed death rate was 0.6% with a standardized mortality ratio of 1.8 [200]. MAS is a leading cause of death, with mortality as high as 10–20% (see Chap. 33). Mortality may result from pulmonary disease, including interstitial lung disease and pulmonary hypertension [120]. Patients may succumb to infection-related death related to immunosuppressive therapy. Amyloidosis, a complication as frequent as 15% in early European series, is now quite rare [201–203]. Long-term morbidity and mortality arising from accelerated cardiovascular disease is expected from experience with other arthritides, but has to date not been studied in sJIA/AOSD.

32.9.3 Growth Failure and Osteoporosis

Active sJIA is markedly detrimental to skeletal growth and bone health. Studies of mice implicate IL-6 as a major contributor to growth restriction, and human data confirm catch-up growth in sJIA patients receiving IL-6 blockade [63, 164]. Corticosteroids also directly impair growth and bone mineralization. In series dating from the pre-biologic era, patients with sJIA commonly exhibited loss of stature as well as osteoporosis [201, 202, 204–206]. Early institution of effective therapy minimizing corticosteroid exposure should help to mitigate these effects. Treatment with recombinant human growth hormone, in selected patients, may improve growth and perhaps even adult height; however, patients may not reach their full genetic growth potential [207, 208].

32.9.4 Articular Outcomes

Historically, sJIA had the worst articular outcome of any form of JIA, with high-grade disability and/or joint replacement rates in excess of 30% [7]. Frequent complications included fusion of wrists and ankles, instability at the high cervical spine, and hip destruction requiring joint replacement. However, the advent of effective biologic therapies has begun to alter this prognosis. Current series document a frequency of chronic arthritis below 20%, in many patients without substantial corticosteroid exposure [7, 58, 157].

32.10 Summary

SJIA and AOSD encompass a spectrum of diseases characterized by the abrupt-onset of systemic inflammation accompanied by fever, rash and arthritis, and in some by life-threatening MAS. Substantial phenotypic breadth is observed, with respect to clinical presentation as well as response to therapy, suggesting underlying pathophysiologic heterogeneity that remains poorly understood. The advent of cytokine blockade directed against IL-1 and IL-6 has transformed the care of affected patients and thereby the long-term prognosis, although some individuals remain resistant to all available options.

Table 32.5 Clinical course of systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still diseases (AOSD)

	sJIA	sJIA	sJIA	sJIA	sJIA	sJIA	sJIA	sJIA	AOSD	AOSD	AOSD	AOSD
Disease course	Schaller (1972)	Svantesson (1983)	Mozziconazzi/Prieur (1983/4)	Lomater (2000)	Fantini (2003)	Singh-Grewal (2006)	Bloom (2009)	Russo (2013)	Pouchot (1991)	Gerfaud-Valentin (2014)	Ruscitti (2016)	Asano (2017)
N	**32**	**33**	**100**	**80**	**88**	**45**	**31**	**132**	**62**	**57**	**100**	**87**
Monophasic				11%		42%		23%	34%	30%	29%	22%
Intermittent				34%		7%		19%	24%	44%	22%	64%
Persistent	**59%**	**>49%**	**50%**	**55%**	**>58%**	**51%**	**83%**	**58%**	**36%**	**26%**	**33%**[a]	**14%**

Data from selected series. References: Schaller [217], Svantesson [202], Mozziconazzi/Prieur [209, 210], Lomater [218], Fantini [219], Singh-Grewal [196], Bloom [220], Russo [12], Pouchot [104], Gerfaud-Valentin [212], Ruscitti [214], Asano [28]

[a]Missing patients reflect 16% "AOSD-related death"

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Macrophage Activation Syndrome in Rheumatic Diseases

33

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Abstract

Macrophage activation syndrome is a hemophagocytic syndrome presenting as a complication of a rheumatic disease. Excessive activation and expansion of T lymphocytes and macrophagic histiocytes in MAS leads to a cytokine storm and hyperinflammation associated with extreme hyperferritinemia, cytopenias, liver dysfunction and coagulopathy resembling disseminated intravascular coagulation. It is a life-threatening condition and may progress to multiple organ failure. High dose glucocorticoids and cyclosporine A are most commonly used to treat MAS. Anakinra and intravenous immunoglobulin may be effective in some patients. Etoposide should be considered in more severe cases. Treatments under investigation include strategies aimed at neutralization of IFN-γ and IL-18.

Keywords

Macrophage activation syndrome · Systemic juvenile idiopathic arthritis · Still disease
Hemophagocytic macrophages
Hemophagocytic lymphohistiocytosis

Abbreviations

AOSD	Adult onset Still disease
ATG	Anti-thymocyte globulin
BM	Bone marrow
CMV	Cytomegalovirus
CRP	C-reactive protein
DIC	Disseminated intravascular coagulation
EBV	Epstein Barr virus
ESR	Erythrocyte sedimentation rate
HLH	Hemophagocytic lymphohistiocytosis
IRF5	Interferon regulatory factor 5
MAS	Macrophage activation syndrome
NK cells	Natural killer cells
SJIA	Systemic juvenile idiopathic arthritis
TLR	Toll-like receptor

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Key Points

- ***Macrophage activation syndrome is a hemophagocytic syndrome*** **presenting as a complication of a rheumatic disease**
- **Due to the abundance of histiocytes in the inflammatory lesions and clinical similarities with hemophagocytic lymphohistiocytosis (HLH), MAS is classified as a histiocytic disorder, and the term secondary HLH is often used to describe MAS**
- **Excessive activation and expansion of T lymphocytes and macrophagic histiocytes in MAS lead to a cytokine storm and hyperinflammation that may be life-threatening**
- **Clinically, hyperinflammation in MAS leads to extreme hyperferritinemia, cytopenias, liver dysfunction and coagulopathy resembling disseminated intravascular coagulation**
- **Due to its life-threatening nature, MAS should be recognized promptly to ensure timely administration of immunosuppressive therapy**

33.1 Introduction

Macrophage activation syndrome (MAS) is a potentially fatal complication of rheumatic diseases that is caused by excessive activation and expansion of T lymphocytes (predominantly $CD8^+$) and of macrophages exhibiting hemophagocytic activity [1–6]. These events lead to overproduction of cytokines and a hyperinflammatory state associated with cytopenias, liver dysfunction and coagulopathy resembling disseminated intravascular coagulation (DIC). Extreme hyperferritinemia is another striking laboratory feature of MAS. Bone marrow (BM) biopsy may help establish the diagnosis in difficult cases since the *presence of hemophagocytic macrophages in BM is the pathognomonic feature of MAS* (Fig. 33.1). Although BM is the most common site for diagnostic biopsy, inflammatory infiltrates composed predominantly of T lymphocytes and macrophagic histiocytes can be found in almost any organ and may cause severe organ dysfunction. MAS is a life-threatening condition and may progress to multiple organ failure. The reported mortality rates reach 20–30% [7, 8].

33.2 Epidemiology

- In rheumatology, MAS most commonly presents as a complication of systemic juvenile idiopathic arthritis
- The reported mortality rates can reach 20–30%.

Although MAS can occur in many inflammatory disorders, it is seen most frequently in systemic juvenile idiopathic arthritis (SJIA) and in its adult equivalent, adult-onset Still disease (AOSD) [7, 9, 10]. The reasons for this association remain unclear. SJIA is classified as a subtype of juvenile idiopathic arthritis, although it is increasingly recognized as a distinct disease. Recent data from a genome wide association study suggest that SJIA has a genetic architecture that is markedly different from other forms of JIA [11]. Whereas other subtypes of JIA have features of classic autoimmune diseases, SJIA may better be described as an autoinflammatory syndrome [12, 13] with only some features of classic autoimmunity [12]. Indeed, the pathophysiology of SJIA (and AOSD) seems to be driven by continuous activation of innate immune pathways leading to dysregulated production of pro-inflammatory cytokines, mainly IL-1β [14, 15] and IL-6 [16–18] (see Chap. 32).

Estimates for the prevalence of JIA range from 16 to 400 cases per 100,000 children, with SJIA accounting for 4–17% of all JIA cases [19]. Based on several reports originating from large pediatric rheumatology centers, approximately 7–17% of patients with SJIA develop full blown MAS [8, 20], while mild "subclinical" MAS may be seen in as many as one third of SJIA patients with active systemic disease [21, 22]. The Division of Rheumatology at Cincinnati Children's Hospital, a large tertiary center in the United States, currently follows approximately 50 patients with SJIA, and this number has been relatively stable over the last 10 years. At this center, 2–3 patients with SJIA are diagnosed with

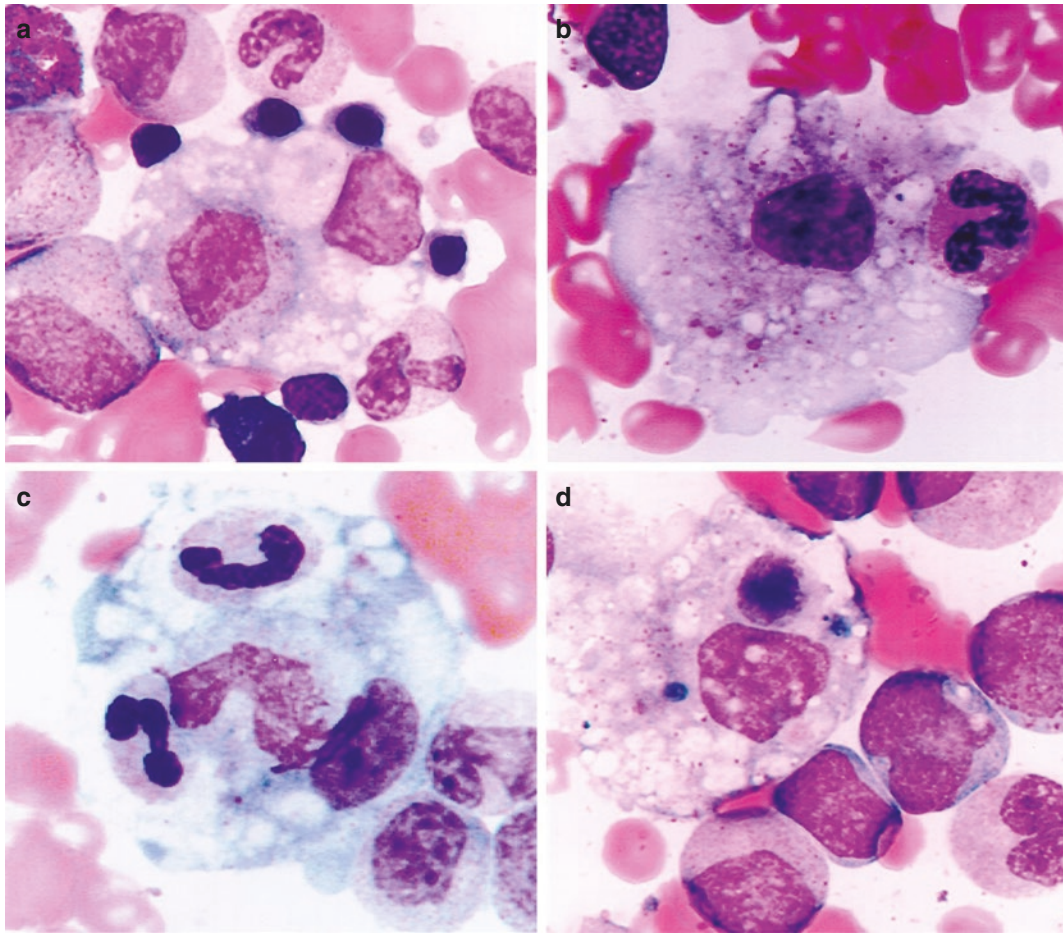

Fig. 33.1 Bone marrow hemophagocytic macrophages in MAS. Bone marrow aspirate specimen revealing activated macrophages (H&E stain), original magnification ×1000). (**a**) Myelocyte within activated macrophage. In addition, there are multiple adherent red blood cell and myeloid precursors. (**b**) Activated macrophage engulfing a neutrophilic band form. (**c**) Neutrophilic band forms and metamyelocyte within an activated macrophage. Nuclei of band forms appear condensed. (**d**) Activated macrophage with hemosiderin deposits and a degenerating phagocytosed nucleated cell. (from Prahalad et al. J Rheumatol 2001;28:2122)

MAS every year, suggesting a crude incidence of MAS in SJIA to be in the range between 4 and 6 cases per 100 patient/years (Grom, unpublished observations).

Besides SJIA, systemic lupus erythematosus (SLE) and Kawasaki disease are two other rheumatologic conditions in which MAS appears to occur somewhat more frequently than in other rheumatic diseases [23, 24]. In most patients who develop MAS, the MAS occurs at some time during the course of their primary rheumatic disease, but MAS occurring at the initial presentation of a rheumatic illness is not uncommon [25]. In a large retrospective, multicenter survey, 23% of the episodes were reported as occurring at SJIA onset with diagnoses of MAS and SJIA being established simultaneously [26].

33.3 Nomenclature

33.3.1 MAS and HLH

The abundance of tissue macrophages, or *histiocytes*, exhibiting hemophagocytic activity in inflammatory lesions in MAS suggests that MAS

belongs to a group of histiocytic disorders collectively known as *hemophagocytic lymphohistiocytosis or HLH* [27, 28]. HLH is a more general term that describes a spectrum of disease processes characterized by accumulations of histologically benign well-differentiated mononuclear cells with a macrophage phenotype [29, 30]. The current classification of histiocytic disorders distinguishes *primary,* or *familial HLH*, and *secondary*, or *reactive HLH*. Clinically, however, they may be difficult to distinguish from each other. *Primary HLH* (p*HLH*) is a constellation of rare autosomal recessive immune disorders linked to genetic defects in various genes all affecting the cytolytic pathway. The clinical symptoms of pHLH usually become evident within the first months of life. Secondary HLH tends to occur in older children or adults. It may be associated with an identifiable infectious episode, most often Epstein-Barr virus (EBV) or cytomegalovirus (CMV) infection. The group of secondary hemophagocytic disorders also includes *malignancy associated HLH*. The distinction between primary and secondary HLH is becoming increasingly blurred as new genetic causes are identified, some of which are associated with less severe and somewhat more distinct clinical presentations [31]. Some of these may present later in life due to heterozygous or compound heterozygous mutations in cytolytic pathway genes that confer a partial dominant negative effect on the cytolytic function [32]. The exact relationship between HLH and MAS is an area of extensive investigations, and some rheumatologists believe that MAS should be categorized as *secondary HLH occurring in a setting of a rheumatic disease (*or MAS-HLH*).* However, the pathophysiologic cause of MAS is far from settled, and others support the concept that while the clinical endpoints are common between MAS and HLH, the initiating factors are different and thus the nomenclature should remain separate. While there are multiple processes underway to try to standardize the nomenclature for these various syndromes, at this point, best practice would be to use the term primary HLH for cases with known molecular diagnoses or a clear genetic component. For so-called secondary cases, there is not yet a prescribed specific terminology, but inclusion of known secondary causes can add clarity to the specific syndromes being described (e.g., SJIA-MAS, EBV-HLH).

33.4 Etiology

33.4.1 Genetic Defects in Primary HLH

In primary HLH, the uncontrolled proliferation of T cells and macrophages has been linked to various genetic defects leading to decreased NK cell and cytotoxic T cell function (Table 33.1). In about 30% of pHLH patients the cytolytic defect is due to mutations in the gene encoding perforin [33]. Perforin is a protein which cytolytic cells utilize to induce apoptosis of target cells such as tumor cells or cells infected by viruses. In about 10% of patients with primary HLH, the disease is caused by mutations in another gene, such as *MUNC13-4* [34]. The protein encoded by this gene is involved in the release of perforin into the immune synapse with a target cell. Although the cytolytic cells of patients

Table 33.1 Genes associated with primary hemophagocytic lymphohistiocytosis

Genes associated with cytotoxic vesicle function
PRF1
MUNC 13-4
STX11
STXBP2
Other vesicular transport/biogenesis genes
RAB27A
LYST
AP3B1
BLOC1S6
X-linked proliferative disorder genes and other EBV-HLH associated genes
SH2D1A
BIRC4
CD27
ITK
MAGT1
Inflammasome genes
NLRC4
Metabolic
SLC7A7

EBV Epstein Barr virus; *HLH* hemophagocytic lymphohistiocytosis

with *MUNC13-4* mutations produce sufficient amounts of perforin, their ability to kill target cells is greatly diminished. More recently, mutations in two other genes encoding proteins that facilitate granule fusion in intracellular trafficking events leading to the release of perforin have been linked to the development of primary HLH: *syntaxin 11,* a member of the SNARE protein family [35], and *syntaxin binding protein 2* (*STXBP2*, known as *MUNC18-2*) [36]. Other genes implicated in the development of pHLH are listed in Table 33.1.

Even moderate defects in the cytolytic pathway may prolong the survival of target cells and increase immune synapse time, ultimately leading to overproduction of proinflammatory cytokines including IFN-γ [37]. Cytolytic cells can also directly induce apoptosis of overly activated immune cells [38]. These observations led to the hypothesis that in HLH, failure to induce apoptosis of target cells by cytolytic cells leads to persistent expansion of activated T lymphocytes and macrophages and escalated production of cytokines, thus creating a 'cytokine storm'.

33.4.2 Cytolytic Dysfunction in MAS

Similar to pHLH, depressed cytolytic function is observed in SJIA patients with MAS [39]. Others have shown that loss of cytolytic capacity is correlated to the activity of the underlying SJIA [40], suggesting that background inflammation is a contributing factor. In particular, IL-6, a pivotal proinflammatory cytokine in SJIA, has been shown to induce defective expression of perforin and decreased NK cell cytotoxic activity [41]. Genetic explanations for loss of cytolytic function in SJIA have also been sought. Heterozygous hypomorphic mutations in HLH-associated genes are detected in at least one-third of MAS patients [42, 43]. Functional studies of some of these mutations show that these variants might partially reduce cytolytic activity [44], and these genetic functional losses might be further amplified by the SJIA inflammatory milieu. Although patients with such variants appear at a higher risk for MAS recurrence, their pathogenic significance still needs to be clarified [42]. Indeed, in some reports, depressed cytolytic function is not identified in SJIA [45], suggesting that there may not be complete overlap between defective CD8 or NK killing and the presence of SJIA/MAS and therefore providing room for other mechanisms to result in disease.

33.5 Pathogenesis

- **Similar to HLH, in the inflammatory lesions of MAS there is marked predominance of activated histiocytes exhibiting hemophagocytic activity and CD8+ T cells**
- **The activated immune cells produce massive amounts of inflammatory cytokines creating a "cytokine storm"**
- **In animal models of HLH/MAS, IFN-γ is emerging as the pivotal cytokine**
- **Expansion of macrophages appears to be IFN-γ-driven**
- **CD8+ T lymphocytes are the major source of IFN-γ**
- **Strikingly high levels of IL-18 are observed in MAS and HLH, but its pathophysiologic significance is still unclear**

Since it is a monogenic disorder, pHLH is perhaps the easiest to dissect in terms of fundamental pathophysiology. Use of gene-targeted mice to delete the known genetic causes of pHLH in humans has yielded much understanding of the cellular and humoral causes of disease. Initial work in perforin deficient mice suggested that loss of cytolytic function in $CD8^+$ T-cells was the root cause of pHLH [46]. $CD8^+$ T-cells that are impotent in killing their targets are unable to clear antigen presenting cells, leading to overstimulation of the $CD8^+$ T-cells via the T-cell receptor. This in turn causes these cells to over-produce IFN-γ, which leads to systemic toxicity. In these mouse models, deletion of $CD8^+$ T-cells causes a reduction in IFN-γ, and improved subsequent survival. Neutralization of IFN-γ directly via monoclonal antibodies also leads to increased survival. Similar results have been obtained in mice deficient in other HLH-associated genes including *Munc13-4* and *Rab27a* [47]. Indeed, such data have led to trials of IFN-γ blockade in pHLH

patients. In further accordance with these ideas, animal data testing the function of the common chemotherapeutics used to treat pHLH suggest that their main mechanism of action is promoting death of activated T-cells, including $CD8^+$ cells. Studies of bone marrow transplants in mice demonstrate that restoration of even 10–15% of $CD8^+$ T-cells with normal cytolytic function via bone marrow transplant is sufficient to protect from disease, providing the pathophysiologic rationale for transplantation in patients as well.

More recently, a role for non T-cell receptor-triggered signals in pHLH has also been demonstrated. In the pHLH animal models utilizing perforin- and *Munc-13-4* deficient mice, MyD88 signaling is also required to develop the HLH phenotype [48, 49]. It has further been shown that, at least in perforin deficient mice, the MyD88 signaling is triggered through the ST2 receptor, whose ligand is IL-33 [49]. Neutralization of ST2 in these animals prevents pathogenic IFN-γ secretion from perforin deficient CD8+ T-cells and improves survival. Thus, a two-step process is required for pHLH development, the inability to clear an antigenic response that leads to an exaggerated T-cell receptor stimulation of $CD8^+$ T-cells, and signaling through ST2 via MyD88 that permits pathogenic amounts of IFN-γ to be produced by these cells. This makes ST2 blockade an additional attractive target for therapy in pHLH.

While the central role of IFN-γ in the murine model of perforin deficient pHLH has been well proven, the significance of this cytokine is less clear in some other animal models. Thus, when immunocompetent BALB/c mice are infected with the β-herpesvirus murine CMV, IFN-γ deficient animals developed severe hyperinflammation reminiscent of a secondary HLH [50]. This observation suggests that in some forms of HLH, IFN-γ might actually play an immunoregulatory role.

Given the success of the models of pHLH in illustrating the mechanisms by which pHLH occurs, it has been tempting to extend these ideas to related syndromes such as MAS. Consistent with parallels to pHLH, in MAS, there is an abundance of IFNγ- producing CD8+ T cells in inflammatory lesions in MAS patients [51] as well as the fact that cyclosporin A, a therapeutic agent that acts predominantly on T cells, is very effective in treatment of the majority of MAS patients [4]. Furthermore, cytotoxic function appears to be impaired in certain subsets of SJIA patients, perhaps specifically in those at risk for MAS [40]. Others have demonstrated an enrichment of heterozygous mutations in known pHLH genes in MAS populations, suggesting that hypomorphic lesions in cytolytic pathways may result in disease [42]. However, in this same study, genetic defects in cytotoxicity were not universally found suggesting that cytolytic dysfunction may not be the only means by which MAS occurs. Alternatively, perhaps the vesicular transport defects that affects perforin function only mildly in these patients may also affect other cellular functions yet to be explored (such as cytokine secretion, for instance), and these are the true cause of both MAS and SJIA pathophysiology. Much more work is needed in the genetics and cellular biology of MAS patients to address these questions.

Animal models of MAS are imperfect, and reflect a lack of a clear genetic cause. Nonetheless, these models have been instructive into both the overlaps and differences between MAS and pHLH. Repeated Toll-like receptor 9 (TLR9) activation in mice leads to a syndrome with many of the features of mild MAS [52]. This model is made more fulminant with the concurrent blockade of IL-10, which leads to massive hemophagocytosis and death. SJIA patients with activating polymorphisms of the TLR9 signaling molecule IRF5 have a four-fold increased risk of MAS [53], consistent with the idea that chronically activated TLR9 creates an immune milieu that would also predispose to MAS. Additional parallels to excessive TLR signaling and MAS come from the fact that gene expression signatures, reflecting continuous activation of TLR–IL-1R induced signaling pathways, have also been reported in SJIA [54]. Interestingly, in the repeated TLR9 activation MAS model, multiple groups have shown that blockade of IFN-γ also reverses disease, suggesting a common link with pHLH [52, 53]. However, in the situation of TLR9 stimulated mice with simultaneous IL-10

blockade, IFN-γ neutralization did not reverse all disease manifestations, opening up the possibility of the contribution of other cytokines, as well in the presence of an insufficient regulatory IL-10 response [55].

IL-6, a cytokine targeted therapeutically in SJIA has also been implicated in murine models of MAS. Mice that are made to overproduce IL-6 develop a MAS like syndrome when stimulated with the TLR4 agonist LPS [56]. Thus, in two animal models of TLR simulated MAS, it appears a "second-hit" such as impaired IL-10 action, or elevated IL-6 action, is required for full blown disease. This may reflect the SJIA scenario where the IL-6 axis is overactive, and a second signal in the form of infectious particles carrying TLR ligands may cause the patient to develop MAS physiology.

Translational studies in SJIA patients suggest that elevated serum IL-18 is a risk factor for the development of SJIA/MAS [57], leading to the idea that this cytokine is also important for disease development. A common link to the "IFNγ-centric" models is that IL-18 is perhaps most noted for its ability to stimulate IFN-γ production by T-cells and natural killer cells. The role of IL-18 has been examined in perforin-deficient mice infected with murine CMV. Uncontrolled viral replication of these mice is associated with many features of HLH and MAS including pancytopenia, hepatic dysfunction, hemophagocytosis, and death [58]. Administration of synthetic IL-18BP ameliorated liver damage in these mice; however, production of proinflammatory cytokines was considerable and no change in overall survival was observed. Direct evidence for a role for IL-18 in MAS in humans comes from a report of a patient with an activating mutation of the NLRC4 gene, which was described to cause a syndrome of MAS accompanied by gastrointestinal inflammation [59] (see Chap. 29). In contrast to the lack of efficacy seen in the murine perforin deficient model of pHLH, treatment of the NLRC4-MAS patient with a neutralizing IL-18 binding protein led to cessation of disease and a decrease in IFN-γ biomarker activity, providing the first in human evidence of this pathway at least in some forms of MAS.

33.5.1 "Cytokine Storm" in MAS

A 'cytokine storm' is the final pathophysiologic pathway in both MAS and HLH, and blocking various cytokines or their signaling pathways can be an attractive therapeutic strategy. With growing numbers of available biologics targeting cytokines and small molecules inhibiting cytokine signaling such as JAK/STAT inhibitors, the interest in the relative significance of various cytokines in MAS is increasing.

33.5.1.1 IFN-γ

The role of IFN-γ in both SJIA and MAS has been the focus of several recent studies. So far, there is little evidence to suggest that IFN-γ plays a major role in the pathogenesis of SJIA itself. Three independent gene expression studies have failed to find a prominent IFN-γ-induced signature in the peripheral blood monocytes of children with active SJIA in the absence of clinical features of MAS [54, 60, 61]. Consistent with this observation, serum IFN-γ levels usually remain within the normal range in patients with SJIA regardless of disease activity [62]. Furthermore, the expression of IFN-γ-induced chemokines (CXCL9 and CXCL10) in synovial tissue from SJIA patients is markedly lower that in the tissues from patients with oligoarticular or polyarticular JIA [62]. Interestingly, monocytes from SJIA patients incubated with exogenous IFN-γ often have exaggerated responses to this cytokine [62], suggesting that the absence of the IFN-γ signature in SJIA is not caused by abnormal responsiveness to IFN-γ.

In contrast to SJIA, a recent study of longitudinal cytokine changes in serum of SJIA/MAS patients has demonstrated that IFN-γ itself and IFN-γ-induced chemokines increased markedly with the emergence of clinical features of MAS and returned to normal ranges after its resolution [63]. Furthermore, the levels of IFN-γ and IFN-γ-induced chemokines strongly correlated with many laboratory features of MAS in patients with clinical features of MAS, but not in patients with a conventional SJIA flare without MAS [63]. No similar correlations were observed with TNF and IL-6. Based on these observations, CXCL9 was

proposed as sensitive biomarker of MAS activity. Combined with the data obtained in several animal models, these new observations in humans strongly implicate IFN-γ as an important player in MAS pathogenesis [63, 64].

33.5.1.2 IL-1 and IL-6

Since episodes of MAS are often triggered by SJIA flares, targeting IL -1β [14, 15] and IL-6 [16–18], the two pivotal cytokines in SJIA pathophysiology, was expected to have therapeutic benefits in MAS as well. The observed MAS rates in the phase III clinical trials of tocilizumab (anti-IL6R antibody) and canakinumab (anti-IL1β antibody) have shown, however, that therapeutic strategies aimed at the inhibition of either IL-1β or IL-6 do not provide full protection against MAS even if the underlying SJIA is well controlled [65–67]. These observations have led to the conclusion that neither IL-1β nor IL-6 alone are the key players contributing to development of MAS. However, there have been numerous case reports describing successful treatment of MAS with anakinra, a recombinant IL-1 receptor antagonist. Since anakinra blocks activity of both IL-1β and IL1-α, a potential role for IL1-α in MAS has been suggested [68–70]. However, MAS has also been observed in patients treated with rilonacept [71], which also neutralizes IL1-α. The consensus at this stage, is that there might be pathophysiologic heterogeneity among MAS patients that would explain why some SJIA/MAS patients respond to IL-1 blockade while others develop MAS during continuous treatment with IL-1 blocking biologics. Additionally, the treatment of MAS may require higher doses than those used to treat SJIA.

33.5.1.3 IL-18

Strikingly high serum levels of IL-18 have been observed in patients with SJIA [57, 72, 73], in sharp contrast to only moderately elevated levels of IL-18 seen in other rheumatic diseases [74]. Patients with high levels of IL-18 more often have systemic manifestations rather than arthritis as the predominant feature of SJIA [57] and also seem to be more likely to develop MAS. The emergence of MAS features in these patients corresponds with a further increase of IL-18 levels. Levels of IL-18 possibly reflect the extent of macrophage activation as macrophages seem to be the main source of IL-18 in patients with MAS [72]. Intriguingly, it has been suggested that the IL-18 receptor is hypofunctional in SJIA [75], which would seem to be at odds with IL-18 playing a pathogenic role. However, the spectrum of IL-18 receptor dysfunction is broad. It is possible that it is precisely those patients that have the most functional IL-18 receptor activity that are the ones at risk for MAS.

33.6 Clinical and Laboratory Manifestations

- **Massive systemic inflammatory response in MAS leads to cytopenias, liver dysfunction, coagulopathy consistent with DIC, and extreme hyperferritinemia**
- **In a patient with a rheumatologic condition, a fall in the erythrocyte sedimentation rate (ESR) and platelet count in combination with persistently high C-reactive protein (CRP), hyperferritinemia, and increasing levels of serum D-dimers should raise a suspicion of MAS**
- **Since the presence of hemophagocytic macrophages in the bone marrow is a pathognomonic feature of MAS, bone marrow biopsy may assist with the diagnosis**
- **The absence of hemophagocytiosis does not rule out MAS**

The clinical findings in overt MAS often evolve rapidly and may progress to multiorgan failure. High persistent fever (unlike the intermittent fever of SJIA), hepatosplenomegaly, generalized lymphadenopathy and changes in mental status are common. Coagulopathy resembling DIC [1–6] can be associated with hemorrhagic skin rashes ranging from mild petechiae to extensive ecchymotic lesions. At later stages, patients may develop epistaxis, and hematemesis secondary to upper gastrointestinal bleeding. Rectal bleeding can occur as well. Erythroderma, generalized macules and papules, and morbilliform

eruptions are other types of rash that may be seen in MAS. Mental status changes, seizures and coma are the most common manifestations of CNS disease [26]. Cerebrospinal fluid examination in these patients usually reveals pleiocytosis with mildly elevated protein [2, 4]. Deterioration in renal function has been noted in several series, and in one report was associated with particularly high mortality [8]. Pulmonary infiltrates have been observed in some patients, and hemophagocytic macrophages can be found in bronchoalveolar lavage fluid [4].

The early features that should raise the suspicion for clinical MAS are typically found in laboratory evaluation. A sudden fall in at least two of three blood cell lines (leukocytes, erythrocytes or platelets) is one of the early findings. Perhaps the most striking laboratory change in MAS is increasing serum ferritin. Although diagnostic/classification criteria set levels of 500 and 684 ng/mL as cutoffs for HLH and SJIA-MAS respectively, levels are often greater than 10,000 ng/mL and can reach into the millions. The reasons for this elevated ferritin are not clear and are likely multifactorial. Ferritin has been shown to be transcriptionally regulated by NFkB [76], a factor activated by inflammatory cytokines. It is additionally translationally regulated by IL-1β by elements in the 5′ region of its mRNA [77]. Thus, the hypercytokinemia of MAS and HLH could certainly provide direct signals for increasing ferritin levels. Teleologically, it makes sense that increased ferritin might be desirable to deal with the need to sequester free iron that may be generated by the erythrophagocytosis as well as the body's perceived need to sequester iron from pathogens that it presumes to be present and driving systemic inflammation. While extremely high ferritin is often a good serologic marker of HLH and MAS, high levels are not pathognomonic and can be seen in a wide variety of conditions. Thus, ferritin needs to be interpreted in the context of the other features of the disease to support a diagnosis.

Decreasing erythrocyte sedimentation rate (ESR) despite persistently high C-reactive protein (CRP) is another laboratory feature suggestive of MAS. Falling ESR usually parallels decreasing serum levels of fibrinogen secondary to fibrinogen consumption and liver dysfunction. Most patients with MAS develop marked hepatomegaly with some developing mild jaundice. Liver function tests typically reveal high serum transaminases activity but only mildly elevated levels of serum bilirubin. Serum ammonia levels are typically normal or only mildly elevated. Other laboratory markers of MAS include hypertriglyceridemia and hypoalbuminemia.

33.7 General Diagnostic Approach

The early diagnosis of MAS is often difficult. There is no single clinical or laboratory feature that is specific for MAS, including hemophagocytosis, and many clinical features of MAS overlap with those seen in the underlying rheumatic diseases. The MAS clinical presentation also overlaps with sepsis-like syndromes associated with infection. This is further complicated by the fact that MAS may also be triggered by a flare of the underlying rheumatic disease or infection. As a general rule, in a patient with active underlying rheumatologic disease, persistent fevers, decreasing ESR, fibrinogen and platelet count in combination with increasing in serum ferritin levels should raise a suspicion for MAS [78]. Increasing liver enzymes, aspartate aminotransferase in particular, is another characteristic laboratory change. Bone marrow biopsy may help establish the diagnosis. Indeed, the presence of increased hemophagocytosis in bone marrow is the pathognomonic feature of MAS (Fig. 33.1). However, the demonstration of hemophagocytosis may be difficult due to sampling error, particularly at the early stages of the syndrome. In such cases, additional staining of the bone marrow with anti-CD163 antibodies may be helpful. Features consistent with MAS include massive expansion of highly activated histiocytes (Fig. 33.2); the absence of overt hemophagocytosis does not rule out the diagnosis of MAS in these patients.

It has been recently recognized that as many as one third of SJIA patients with active systemic JIA may have "**occult**" or "**subclinical MAS"** [21, 22]. These patients typically have moderate

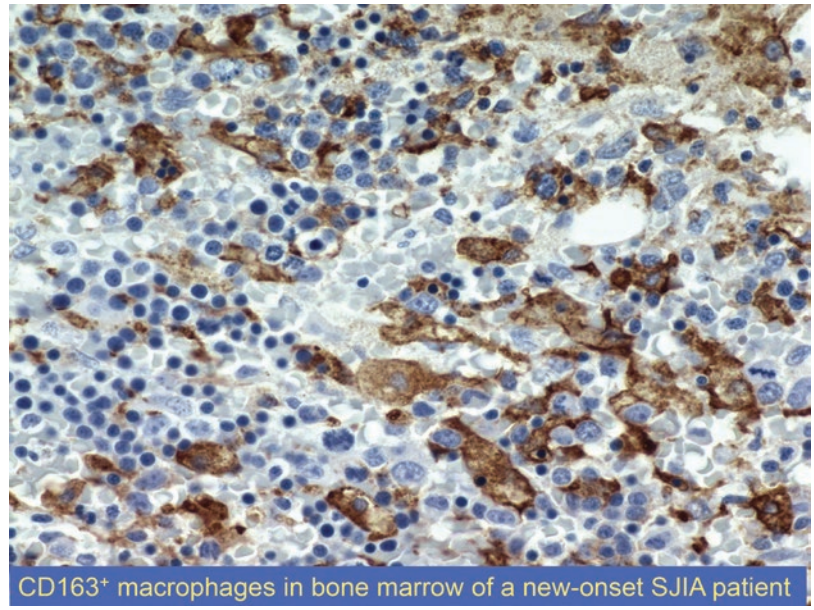

Fig. 33.2 Bone marrow biopsy from a patient with SJIA and subclinical MAS. Immunostaining with monoclonal antibodies specific for CD 163. Brown staining identifies CD163+ cells many of which have foamy cytoplasm reflecting highly activated status. (from Hinze et al. Arthritis Res Ther 2010;12:R123)

hyperferritinemia, highly increased CRP, moderately decreased hemoglobin, and relatively low platelet counts (e.g., low end of the normal range despite highly increased inflammatory markers suggestive of mild relative thrombocytopenia). These patients may also have mild hepatosplenomegaly and mildly elevated liver enzymes. Serum fibrinogen tends to remain in the normal range despite highly increased CRP. Increased serum soluble IL2Rα chains presumably shed from overly activated T cells, might be another important laboratory marker distinguishing this group of patients [21]. Bone marrow examination in patients with "subclinical MAS" typically reveals extensive expansion of highly activated foamy macrophages with only occasional cells exhibiting overt hemophagocytic activity (Fig. 33.2). Additional immunochemistry staining with anti-CD163 antibodies usually helps visualize such expansion. Early recognition of this pattern is important because even mild treatment modifications such as a moderate increase in the dose of corticosteroids are likely to prevent the development of full blown MAS in these patients.

In a large international collection of patients with SJIA associated MAS, about 23 percent of the reported episodes occurred at SJIA onset [26]. While in a patient known to have SJIA the clinical suspicion of MAS in the presence of the typical clinical manifestations may be relatively obvious, when MAS occurs at SJIA onset it is important to differentiate it from primary HLH. With the goal to identify laboratory features that would differentiate MAS from HLH, Lehmberg et al. compared the clinical presentation between the two groups [79]. Neutrophil counts and CRP were significantly higher in patients with MAS/SJIA. In contrast, highly increased levels of sIL2Rα were seen more frequently in HLH. More recently with the same goal in mind, Minoia et al. developed the **MAS/HLH (MH) Score** comprised of six demographic, clinical, and laboratory variables: age at onset, neutrophil count, fibrinogen, splenomegaly, platelet count, and hemoglobin (Table 33.2). The **MH Score** appears to discriminate between

Table 33.2 The macrophage activation syndrome—hemophagocytic lymphohistiocytosis score (MH score) [80]

	The best fit
Age at onset, years	0 (>1.6); 37 (≤1.6)
Neutrophil count, × 10^9/L	0 (>1.4); 37 (≤1.4)
Fibrinogen, mg/dL	0 (>131); 15 (≤131)
Splenomegaly	0 (no); 12 (yes)
Platelet count, × 10^9/L	0 (>78); 11 (≤78)
Hemoglobin, g/dL	0 (>8.3); 11 (8.3)

The cut-off value for the MH score ≥60 provides a sensitivity of 91% and a specificity of 93% in discriminating primary hemophagocytic lymphohistiocytosis from macrophage activation syndrome

pHLH and MAS with a high degree of sensitivity and specificity and could be easily calculated in any clinical setting [80]. Although the MH Score still needs to be validated prospectively, it is highly likely that these criteria will help identify patients who need early introduction of aggressive treatment as well as further genetic evaluation for pHLH.

MAS is being increasingly recognized in other conditions such as systemic lupus erythematosus (SLE) [23], and Kawasaki Disease [24]. Since these underlying conditions may also contribute laboratory abnormalities, diagnosing MAS becomes more difficult. For instance in SLE, cytopenias, elevated transaminases, and fever may be due underlying disease activity, rather than the onset of MAS. For this reason it is likely that the MAS diagnostic guidelines developed for SJIA-MAS [see below] may not perform well when applied to patients with rheumatic diseases other than SJIA. However, although some disease classifiers change with different underlying conditions, other seem to be more universal. For instance, serum ferritin and lactate dehydrogenase and the histopathologic demonstration of hemophagocytosis may be more generally applicable to the diagnosis of MAS, even if these are not necessarily specific nor sensitive individually. Nonetheless, the presence of these universal markers of MAS can aid in diagnosis despite the triggering disease itself confounding other classic diagnostic tests.

Striking clinical similarities between MAS and HLH has led some to advocate the use of the HLH-2004 diagnostic guidelines developed by the HLH Study Group of the International Histiocyte Society [81]. However, some of the HLH clinical and laboratory criteria, such as splenomegaly and hyperferritinemia, are common in active rheumatic diseases themselves and, therefore, do not distinguish MAS from underlying disease activity. Other HLH criteria, such as cytopenias, and hypofibrinogenemia, become evident only in the later stages of MAS. This is particularly evident in SJIA patients who often have highly increased white blood cell and platelet counts as well as serum levels of fibrinogen as a part of the inflammatory response. Therefore, when these patients develop MAS, they reach the degree of cytopenias and hypofibrinogenemia seen in HLH only later in the clinical course. As a result, the HLH-2004 diagnostic guidelines when applied to SJIA patients with suspected MAS are highly specific but not sufficiently sensitive to diagnose the condition in its early stages when further deterioration could be prevented with relatively mild treatment.

In 2014, a set of classification criteria for MAS complicating systemic JIA was developed through a combination of expert consensus and analysis of patient data (Table 33.3). In cross-validation analyses, the criteria revealed a sensitivity of 0.72–0.76 and a specificity 0.97–0.99 [82]. These criteria still need to be prospectively validated in an independent cohort of patients and certainly adjusted for MAS presenting as a complication of rheumatic diseases other than SJIA.

Another potential limitation of the 2014 MAS classification criteria is related to the recent evidence suggesting that background treatment with biologics might modify clinical presentation of MAS. Indeed, the 2014 MAS classification criteria were developed using clinical data that had been generated prior to the introduction of IL-1 and IL-6 inhibitors now widely used for the treatment of SJIA. While IL-1 and IL-6 inhibitors effectively control the disease in the majority of SJIA patients, they do not provide full protection against MAS. Schulert et al. have recently performed a systematic literature review with the goal to assess performance of the 2014 MAS classification criteria for SJIA patients who

Table 33.3 The classification criteria for macrophage activation syndrome in systemic juvenile idiopathic arthritis [82]

A febrile patient with known or suspected systemic juvenile idiopathic arthritis is classified as having macrophage activation syndrome if the following criteria are met:
Ferritin >684 ng/mL
And
Any 2 of the following:
Platelet count $\leq 181 \times 10^9$/L
Aspartate aminotransferase >48 U/L
Triglycerides >156 mg/dL
Fibrinogen ≤360 mg/dL

developed MAS while treated with biologic medications [83]. Patients who developed MAS while treated with the monoclonal anti-IL1β antibody canakinumab trended towards lower ferritin at MAS onset than the historical cohort, but there were no differences in other cardinal clinical or laboratory features. In comparison, patients who developed MAS while treated with the anti IL-6 receptor tocilizumab were less likely to be febrile, and had notably lower ferritin (1152 vs. 5353 ng/mL, $p < 0.001$). Other features of MAS were more pronounced in patients treated with tocilizumab, including lower platelet counts, lower fibrinogen and higher aspartate aminotransferase levels. As a result, the MAS classification criteria were less likely to classify tocilizumab treated patients as having MAS compared to the historical cohort or canakinumab treated patients (56.7% vs. 78.5% and 84%, $p < 0.01$).

33.8 Treatment

- **High dose glucocorticoids and cyclosporine A are most commonly used to treat MAS**
- **Anakinra and intravenous immunoglobulin (IVIG) may be effective in some patients**
- **Etoposide should be considered in more severe cases**
- **Treatments under investigation include strategies aimed at neutralization of IFN-γ and IL-18 activity**

33.8.1 Most Common Treatments

MAS is a life-threatening condition and, therefore, requires prompt recognition and initiation of immediate therapeutic intervention to prevent a potentially fatal outcome. There have been no clinical trials in MAS, however, and standardized treatment guidelines have not been developed. In a multi-national multicenter retrospective study of 362 patients with MAS, nearly all patients received high dose glucocorticoids as the initial treatment. The initial glucocorticoid regimen may include intravenous methylprednisolone pulse therapy (e.g. 30 mg/kg for three consecutive days) followed by 2–3 mg/kg/day in 4 divided doses. If response to glucocorticoids is not satisfactory, cyclosporine A (2–7 mg/kg/day) is usually added to the treatment regimen based on several reports describing the rapid resolution of MAS features in response to this medication [3, 4, 6, 84]. With trough levels in the 150–200 ng/mL range, improvement in the laboratory features of MAS typically occur within 24–48 h. Cyclosporine is preferentially used orally, and careful monitoring for toxicity is required, especially if it is administered intravenously. In additional to the known side effects, in MAS patients the use of cyclosporine has been associated with pulmonary hypertension and the posterior reversible encephalopathy syndrome.

Since a 'cytokine storm' is the final pathophysiologic pathway in both MAS and HLH, blocking various cytokines could be an attractive therapeutic strategy. The utility of anti-cytokine biologics in MAS treatment, however, remains unclear. Although TNFα inhibiting agents have been reported to be effective in occasional MAS patients, other reports describe patients in whom MAS developed while they were on TNFα-inhibiting agents. Since, at least in SJIA, MAS episodes are often triggered by the disease flare, biologics that neutralize IL-1, a cytokine that plays a pivotal role in SJIA pathogenesis, have been tried by many authors with conflicting results. Recent case series have suggested that anakinra might be effective in many patients with SJIA-associated MAS, particularly when used in higher doses [70]. It should however be pointed out that in established SJIA, continuous treatment with standard doses of anti-IL-1 and IL-6 biologic therapies does not prevent completely the occurrence of MAS even if the underlying disease responds well to the treatment [85].

Intravenous immunoglobulin treatment has been a successful treatment in virus-associated reactive HLH [86]. Rituximab, a treatment that depletes B lymphocytes, the main type of cells harboring EBV virus, has been successfully used in EBV-induced lymphoproliferative disease [87, 88] and could be considered in EBV-driven MAS.

If MAS remains active, despite the use of glucocorticoids, cyclosporine A, and anakinra, the HLH-2004 treatment protocol developed by the HLH Study Group of the International Histiocyte Society [81] may be considered. This protocol, in addition to glucocorticoids and cyclosporine A, includes etoposide (or VP16), a podophyllotoxin derivative that inhibits DNA synthesis by forming a complex with topoisomerase II and DNA. Etoposide is metabolized by the liver and then both the unchanged drug and its metabolites are excreted through the kidneys. Since patients who require the use of etoposide are very likely to have hepatic and renal involvement, caution should be exercised to properly adjust the dosage and thus limit the potential side effects such as severe bone marrow suppression. Although successful use of etoposide in MAS has been reported, potential toxicity of the drug is a major concern, particularly in patients with hepatic impairment. Reports describing deaths with the use of etoposide caused by severe bone marrow suppression and overwhelming infections have been published. The use of lower doses of etoposide (50–100 mg/m^2 range rather than 150 mg/m^2 as suggested by the HLH-2004 protocol) has been advocated by some groups [89].

Recently, it also has been suggested that in unresponsive patients anti-thymocyte globulin (ATG) may be an alternative to etoposide [90, 91]. ATG depletes both CD4+ and CD8+ T cells through complement-dependent cell lysis. Mild depletion of monocytes is noted in some patients as well. Although in the reported cases this treatment was well-tolerated, infusion reactions are frequently reported with the use of ATG and adequate laboratory and supportive medical resources must be readily available if this treatment is used.

33.8.2 Experimental Treatments

Findings in animal models and translational studies in HLH patients support IFN-γ blockade as novel therapy for pHLH: a Phase II-III trial of an anti-IFNγ antibody is currently underway, and a pilot trial in MAS patients unresponsive to standard treatment is planned.

Neutralization of IL-18 may be another therapeutic strategy in MAS. In a patient with an inflammasomopathy caused by gain-of function mutations in *NLRC4*, administration of the recombinant IL-18BP resulted in rapid and sustained improvement including the resolution of all MAS-like features [92]. It remains unclear whether a similar therapeutic intervention might be effective in MAS as well, but the ongoing Phase 1 clinical trial of the recombinant IL-18BP in AOSD may provide some answers.

Based on their essential roles in transmitting cytokine-induced signals, the JAK/STATs have become a target for pharmacologic manipulation in inflammatory diseases. Ruxolitinib, a potent inhibitor of JAK1 and JAK2, has been recently shown to ameliorate the disease influencing patterns of JAK/STAT–dependent gene expression in animal models of pHLH [93], but at this stage, it still remains to be determined whether this treatment may be applied to patients with MAS as well.

33.9 Prognosis

MAS is a life-threatening condition with significant morbidity and mortality. A large scale retrospective study of children with SJIA/MAS estimated the mortality rate was at 8% [26]. In contrast, in a retrospective study of 41 adults, mortality was estimated at 42.5% [94]. While treatment regimens continue to vary widely, and the potential availability of new therapeutic options expands, undoubtedly the prognosis remains guarded in this population. More rapid diagnosis and aggressive therapy have aided in improving outcomes. Since a proportion of patients develop recurrent episodes, close monitoring is required to ensure disease does not relapse. While serum ferritin and sIL2Rα are used as biomarkers for disease activity at the present, future work to better define markers more proximal to the underlying pathophysiology may aid in tracking disease activity and prognosis.

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Gouty Inflammation

34

Naomi Schlesinger and Johnson C. Kay

Abstract

Inflammation and hyperuricemia drive the gout cascade. Understanding of the role of inflammation, including involvement of the innate immune system, helps to improve the diagnosis and therapeutic course of the disease. Treatment of acute and chronic gouty inflammation is of utmost importance in preventing long-term disability. In this chapter, we discuss the clinical presentation of gout and its diagnosis, immunopathogenesis, and treatment. The anti-inflammatory therapies used to combat gouty inflammation are highlighted.

Keywords

Gout · Inflammation · Hyperuricemia Monosodium urate crystal · NLRP3 (cryopyrin) inflammasome · Interleukin-1 Anti-inflammatory drugs · Prophylaxis

Abbreviations

ACP	American College of Physicians
ACR	American College of Rheumatology
ACTH	Adrenocorticotropic hormone
DAMP	Damage-associated molecular patterns
DECT	Dual-energy computed tomography
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
IL	Interleukin
MSU	Monosodium urate
MTP	Metatarsophalangeal
NLRP3	NACHT, LRR and PYD domains-containing protein 3
NSAID	Non-steroidal anti-inflammatory drugs
SU	Serum urate
TA	Triamcinolone acetonide
TLR	Toll-like receptor
TNF	Tumor necrosis factor
URL	Urate lowering therapy
US	Ultrasound

Key Points

- **Inflammation and hyperuricemia drive the gout cascade**
- **Monosodium urate crystal deposition in tissues triggers a powerful inflammatory cascade, activating the NLRP3 inflammasome**

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- **Gout is associated with increases in the pro-inflammatory cytokines interleukin-1, interleukin-6, interleukin-8, and tumor necrosis factor-α**
- **Acute gout is periodic, recurrent, short lived attacks of autoinflammation**
- **Acute and chronic anti-inflammatory treatment, in addition to urate lowering therapy, are important in the optimal management of gout**

34.1 Introduction

Gout is the most common inflammatory arthritis in humans. An estimated 8.3 million adults in the United States, a prevalence of 3.9%, suffer from gout [1]. Gout is a systemic metabolic disease. Humans do not express the enzyme urate oxidase, or uricase, which converts urate to the more soluble compound allantoin. This may lead to elevated serum urate (SU) levels, known as hyperuricemia. Gout has several clinical stages including: asymptomatic hyperuricemia, acute gout, intercritical period between acute flares, and chronic tophaceous gout. Acute flares are inflammatory episodes triggered by monosodium urate (MSU) crystal deposition promoted by long-term hyperuricemia.

Urate production is increased in males after puberty and in females post menopause. The predominant cause of hyperuricemia in most patients is undersecretion of urate by the kidneys. Lower clearance of urate is seen in all gout patients compared with normal controls. It is therefore not surprising that up to 73% of all gout patients have mild to severe renal insufficiency [2]. The SU level is elevated when it exceeds 6.8 mg/dL, the limit of solubility of MSU in serum at 37 °C (98.6 °F). The SU level is the single most important risk factor for development of gout, and a sustained elevation of SU is essential for the development of inflammatory disease. An elevated SU level, however, is not diagnostic of gout by itself. In fact, most patients with hyperuricemia never develop gout.

In addition to renal impairment, gout patients frequently have other comorbidities including cardiovascular disease, diabetes, hypertension, hyperlipidemia, and obesity. Hyperuricemia is associated with endothelial dysfunction, which may contribute to risk of heart disease [3]. Gout is also associated with systemic inflammation, frequently with increases of the erythrocyte sedimentation rate and C-reactive protein [4]. Prior studies have linked chronic systemic inflammatory conditions like rheumatoid arthritis and systemic lupus erythematosus to increased risk of heart disease not explained by traditional cardiac risk factors as may be the case in patients with gout [5, 6].

Inflammation and hyperuricemia drive the gout cascade. Most publications discuss the urate burden, omitting discussion of the importance of inflammation and its treatment in gout. New advances in molecular biology reveal that at the base of the inflammatory cascade,-stimulated by MSU crystals, are many complex cellular mechanisms mainly involving the inflammasome. The inflammasome, an intracellular protein complex (see Chap. 5), is considered the hallmark of autoinflammatory syndromes and plays a key role in the powerful inflammatory cascade in acute and chronic gouty inflammation. Treatment of acute and chronic gouty inflammation is of utmost importance in combating the disease. In this chapter, we will discuss the clinical presentation of gout and its diagnosis, immunopathogenesis, and treatment, highlighting the anti-inflammatory therapies used to combat gouty inflammation.

34.2 Pathogenesis

Key Points

- **Monosodium urate (MSU) crystals act as damage-associated molecular patterns (DAMPs), signaling macrophages to secrete pro-inflammatory cytokines**
- **MSU crystals engage caspase-1, activating the NLRP3 inflammasome**
- **Interleukin (IL-1)-1 plays a crucial component in the inflammatory cascade**
- **IL-1β is the leading cytokine of a macrophage-released mix that also includes tumor necrosis factor (TNF)-α, IL-6, IL-8, and other inflammatory mediators**

Damage-associated molecular patterns (DAMPs) are released upon cellular injury to activate pattern recognition receptors on innate immune cells and amplify organ damage (see Chap. 4). MSU crystals (NaC5H3N4O3.H20) act as damage-associated molecular patterns (DAMPs) mainly to neutrophils and macrophages [7]. Urate DAMP signaling activates macrophages to secrete pro-inflammatory cytokines including interleukin (IL)-1β [8] (see Chap. 6). When MSU crystals were injected into the peritoneal cavity of rats, peritoneal neutrophil accumulation was induced at the site of the MSU crystal deposition, measured 6 h after MSU crystals were injected. In contrast, neutrophil infiltration did not occur in mice lacking the IL-1 receptor. Thus, experimental data from MSU crystal-induced peritonitis and MSU crystals injected intra-articularly provided evidence for the role of IL-1 in gouty inflammation [9]. Two lines of study have been employed - a murine IL-1R1 knockout model, and the effects of IL-1 blockade (by antibodies against IL-1β, IL-1Rα or by murine IL-1 Trap). These inhibitors of IL-1 were as effective as genetic deletion of IL-1R1 in reducing inflammation and hyperalgesia in these murine models [10, 11].

IL-1 and signaling through the IL-1 receptor were found to be crucial components of the inflammatory cascade triggered by MSU crystals. MSU crystals engage the NLRP3 inflammasome, resulting in the production of active IL-1β [12] (see Chap. 5). The NLRP3 inflammasome is a protein complex expressed abundantly in neutrophils and macrophages that regulates caspase-1 and activates IL-1β.

Cleavage of pro-IL-1β releases the mature p17 form of IL-1β resulting in the active IL-1β; active IL-1β is then secreted from the cell to act as an intercellular signal [13]. These two signals provide the minimum requirements for inflammasome activation; however, activation only by these signals is associated with production of IL-1β, which peaks by 24 h [13]. It is unclear why gout flares occur only intermittently despite continuous presence of MSU crystals and why flares often follow rich meals and alcohol overconsumption. New research suggests that this is due to free fatty acids that induce release of large amounts of IL- 1β following engagement of TLR2 [14].

Thus, in addition to the intracellular NLRP3 receptor, the extracellular toll-like receptor (TLR)2 and TLR4 may also play a role in the innate immune response to MSU deposition (see Chap. 4). Macrophages isolated from TLR2−/− and TLR4−/− mice show impaired uptake of MSU crystals and reduced pro-inflammatory cytokine production, suggesting these TLRs to be essential for MSU crystal-induced inflammation.

IL-1β is the leading cytokine of a macrophage-released mix that also includes TNF-α, IL-6, IL-8 and other inflammatory mediators. IL-1β stimulates synoviocytes and endothelial cells to produce chemokines such as IL-8 that attracts and activates neutrophils. These cytokines are activated during gout flares and contribute to acute gout by production of reactive oxygen species, lysosomal enzymes, prostaglandins, and leukotrienes.

34.3 Clinical Presentation

Key Points

- **Acute gout is characterized by exquisite pain from the rapid onset of joint synovitis, typically monoarticular and most commonly initially involving the first metatarsophalangeal joint**
- **Acute gout is associated with increases of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α**
- **The gold standard for definitive diagnosis of gout is joint aspiration for monosodium urate crystal analysis**
- **Hyperuricemia is the most important risk factor for gout, but gender, trauma, alcohol, diet, diuretics, and seasonal variations also play a role**
- **Ultrasonography and duel-energy computed tomography may help with the diagnosis of gout**

34.3.1 Acute Gout

The usual course of an untreated patient with gout is one of recurrent inflammatory flares of intense synovitis that last several hours to weeks [15]. Acute gout flares are periodic, recurrent, short-lived episodes of autoinflammation. Acute gout is characterized by a rapid onset and buildup of pain. The flare typically begins approximately 2 h before the patient usually wakes up, in the late night or early morning. During an acute flare the patient endures exquisite pain associated with signs of synovitis including warmth, erythema (Fig. 34.1), edema, and decreased range of motion of the affected joint(s). The initial episode is usually monoarticular, at least in men.

The lower extremity joints are most commonly involved, with the first metatarsophalangeal (MTP) joint being the initially involved joint, in approximately half of gout patients. Acute synovitis of the first MTP joint is referred to as podagra. The predilection for lower extremity joints is multifactorial with the susceptibility of lower extremity joints to osteoarthritis and local anatomical considerations such as temperature, pH, biomechanical stress, and trauma that contribute to ideal conditions for MSU crystal formation and deposition.

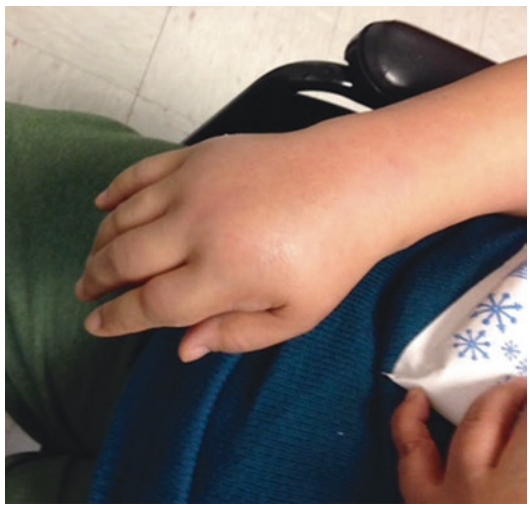

Fig. 34.1 Acute gout: gouty cellulitis, a red, swollen hand and wrist

In post-menopausal women, flares are frequently oligoarticular with the fingers commonly involved, usually where there are changes secondary to osteoarthritis like Heberden nodes. Systemic symptoms of fatigue, fever, and chills may accompany acute gout due to increased production of pro-inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor-α (TNF-α).

Local trauma, alcohol overconsumption, high purine diet intake, and diuretic use are implicated as factors that can precipitate an acute flare of gout. In the hospital setting [2], acute gout flares often occur postoperatively or with severe acute medical illnesses. Changes in the body's total urate pool can precipitate a flare due to homeostatic mechanisms which mobilize the deposited MSU crystals. This is also commonly seen in patients newly initiated on urate-lowering therapy (ULT) and can be mitigated by slow titration of the dose upward and the addition of concomitant drug prophylaxis.

Seasonal factors may influence the risk of gout flares, with increased frequency reported in the spring [16]. SU levels are highest in the summer. Cortisol levels, however, are low in the spring while high absolute neutrophil count and high levels of plasminogen activator inhibitor-1 (PAI-1) are common during the spring. The seasonal variation provides clues as to why gout flares may be more frequent in the spring. Further study is needed to clarify whether changes in hormones, patients' diet, acute-phase reactants, activity of the immune system, and lipid metabolism have a role in affecting seasonal variation.

34.3.2 Chronic Tophaceous Gout

Chronic tophaceous gout [17] usually develops after greater than 10 years of acute gout flares, although rarely patients can present with tophi as the initial manifestation of their disease. A tophus means "chalk stone" in Latin and appears as firm swellings. Tophi may appear at any site, but most commonly appear on the digits of the hands and feet as well as in the olecranon bursa (Fig. 34.2). Tophi of the helix or antihelix of the ear are classic, but currently are seen infrequently. Tophi may be associated with a destructive deforming arthritis.

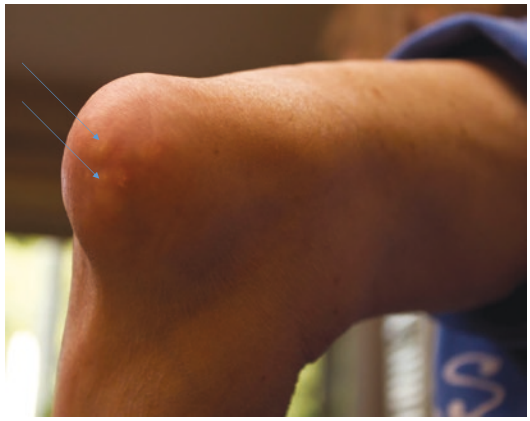

Fig. 34.2 Chronic tophaceous gout: enlarged olecranon bursa (elbow) with tophaceous deposits (whitish subcutaneous deposits, see arrows)

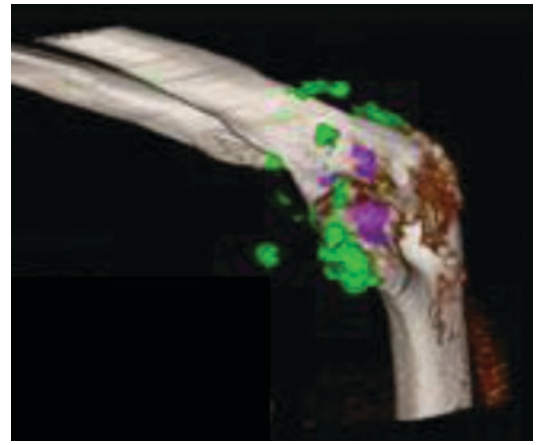

Fig. 34.3 A 65 year old white male with long standing chronic kidney disease and hyperuricemia showed up in the emergency room with a swollen olecranon bursa. He refused to undergo a joint or bursa aspiration. The dual energy CT (DECT) detected tophaceous deposits of monosodium-urate crystals (green, arrow). A diagnosis of acute gout was suspected and the patient was successfully treated. Photo courtesy of Dr. Gabriel Breuer, Head, Rheumatology Unit. Shaare Zedek Medical Center, Jerusalem, Israel

34.4 Diagnosis

A typical clinical history of an acute flare is that of a severely painful joint, classically the first MTP joint, of sudden onset- reaching its pain peak within 2–4 h that may wake the patient from sleep [15]. Even when the clinical appearance strongly suggests gout, the diagnosis must be confirmed by needle aspiration for crystal analysis. A definitive diagnosis of gout requires aspiration of the acutely inflamed joint or suspected tophus [18]. MSU crystals can be observed in more than 95% of patients experiencing flares of acute gout [18].

The joint aspiration procedure, however, is invasive and not always possible or conclusive. Although not intended for use in diagnosis, the 2015 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) gout classification criteria can be instructive for highlighting key features of the condition [19]. The criteria also highlight the gold standard presence of MSU crystals in synovial fluid or tophus aspiration as sufficient for classification of gout.

SU level is the most important risk factor for developing gout. Hyperuricemia is associated with an increased gout risk, although SU levels may be normal during an acute flare in up to 49% of patients [20]. Patients may also have renal disease worsening under-excretion of SU or taking medications that can elevate SU.

Advanced radiology can help with the diagnosis of gout. On ultrasonography (US), the "double contour sign" is highly specific for gout [21]. The double-contour sign is observed on US as a hyperechoic band over anechoic cartilage, and is believed to be indicative of MSU crystals overlying articular cartilage. Dual-energy computed tomography (DECT) is an advanced imaging modality that enables visualization of MSU crystal deposits by analysis of the chemical composition of the scanned materials [22]. DECT provides good diagnostic accuracy for detection of MSU deposits in patients with gout (Fig. 34.3). However, sensitivity is lower in patients with recent-onset disease.

34.5 Treatment

Key Points

- **Colchicine, nonsteroidal anti-inflammatory drugs and corticosteroids are commonly used to terminate acute flares of gout.**

Anti-inflammatory prophylaxis with colchicine and NSAIDs are used to prevent further acute flares

- **Interleukin-1 inhibitors should be considered in patients without infection, who have frequent gout flares and contraindications to use of colchicine, NSAIDs, or corticosteroids**
- **Urate lowering therapy to target a serum urate level <6 mg/dL could prevent monosodium urate crystal deposition and thus avert inflammation and joint destruction**
- **Non-pharmacologic treatments including lifestyle modifications, topical ice, and cherry juice should also be considered**

The treatment of gout includes: treating the acute flare, lowering the urate pool to prevent MSU crystal deposition, and providing anti-inflammatory treatment as prophylaxis to prevent future gout flares. In this chapter highlighting the autoinflammatory aspects of gouty inflammation, acute and chronic anti-inflammatory treatment of gout will be emphasized, without elaborating on ULT, which aides in the long-term dissolution of MSU crystals.

Several guidelines and recommendations for the management of gout have been published including the 2012 ACR guidelines [23] and the 2016 updated EULAR recommendations [24]. The 2016 EULAR recommendations support starting treatment of a flare as early as possible with oral colchicine, nonsteroidal anti-inflammatory drugs (NSAIDs), or corticosteroids, but no specific anti-inflammatory drug is stated as preferable. (ULT should be considered with a definite diagnosis of gout from the first presentation, reflecting the opinion that delayed treatment may allow further MSU crystal deposition and thus promote inflammation and joint destruction. Flare prophylaxis is also recommended during the first 6 months of ULT.

Monitoring serum urate levels regularly is important for treatment success. The 2016 EULAR recommendations [24] support treating to a target serum urate level of <6 mg/dL in gout patients and a level of <5 mg/dL in those with severe gout and tophi to facilitate faster MSU crystal dissolution. Allopurinol is recommended for first-line ULT in patients with normal kidney function, starting at a low dose and increasing every 2–4 weeks until the target serum urate level is reached. If the serum urate target cannot be reached, allopurinol should be switched to febuxostat.

The 2016 American College of Physicians (ACP) gout clinical guidelines [25] recommend treating acute gout with corticosteroids, NSAIDs, or colchicine, using low doses of colchicine or NSAIDs as prophylaxis for greater than 8 weeks. Compared with the 2016 EULAR guidelines, the ACP guidelines recommend against initiating long-term ULT after a first flare or in patients with 2 or fewer flares per year, but to discuss benefits, harms, costs, and individual preferences before initiating ULT in patients with recurrent gout flares. The ACP guidelines suggest that evidence is insufficient for monitoring SU levels in gout patients in a treat-to-target SU strategy.

The ACP recommendations seem to view gout from a primary care perspective with gout as an intermittent disease for which the main goal is to eradicate the acute flares, suggesting implementation of the "treat-to-avoid-symptoms" approach. The ACR/EULAR guidelines, on the other hand, recommend treatment as early as possible with ULT, which reflects rheumatologists' view of gout as a chronic progressive disease, requiring long term ULT. Many of the current recommendations and guidelines for the management of gout lack robust evidence and rely on poor evidence, indicating gaps in our understanding of this common disease. More research is needed to move towards an evidence-based approach and improve patient care.

34.5.1 Non-Pharmacologic Treatments of Inflammation in Gout

Non-pharmacologic treatments include topical ice application in acute gout as well as chronic lifestyle modifications including dietary interventions such as cherry consumption, hydration, low purine diet, and decreasing alcohol intake.

34.5.1.1 Use of Topical Ice in Acute Gout

Cooling can have a marked effect on joints. Icing of the knee for more than 10 min reduces the intra-articular temperature by 2–3 °C for several hours [26]. In animal models of gout, cooling joints reduced intra-articular temperatures, hyperemia, cellular infiltration, and crystal-induced inflammation [27]. We previously studied the effect of topical ice application on acutely inflamed joints in gout patients [27]. The patients described symptomatic improvement with topical ice treatment as compared with previous flares. The response to topical ice was dramatic with significant reduction in pain compared with the control group. Patients who used topical ice (for half-an-hour, four times per day, for 1 week) in addition to pharmacological treatment (oral prednisolone and colchicine) rated their pain 3.33 points lower on a 0 to 10-point pain scale (33% absolute improvement). Complete resolution at 1 week was seen only in those treated with topical ice in addition to pharmacological treatment. Topical ice application exerts an anesthetic effect in acutely inflamed joints [27].

34.5.1.2 Use of Cherries

Eating one-half pound of cherries or drinking an equivalent amount of cherry juice prevented gout flares. Black, sweet yellow, and red sour cherries were all effective [28]. In a pilot, prospective randomized controlled trial [29], gout patients treated with tart cherry juice concentrate had a significant decrease in the number of acute flares within 4 months of initiating ingestion of a cherry juice concentrate ($p < 0.05$), an effect not seen in the control group ingesting pomegranate juice concentrate. A significant 55% of patients ingesting cherry juice concentrate were flare-free and stopped their regular intake of NSAIDs within 60 days of initiating the cherry juice concentrate. None of the patients in the pomegranate group stopped any of their medications.

In a retrospective study, regular intake of cherry juice concentrate led to a significant reduction in flares over a minimum period of 4 months. In those not on ULT, there was no reduction in the SU level over the same period, suggesting the reduction in flares was not as a result of a change in SU [29]. The mechanism by which cherries and cherry juice concentrate prevent gout flares is unclear: antioxidant and anti-inflammatory effects are suggested. Preliminary studies indicate that in-vitro cherry juice concentrate can reduce the release of IL-1 by monocytes activated by MSU crystals [29].

34.5.2 Pharmacologic Treatments of Inflammation in Gout

Drug options for acute gout include colchicine, NSAIDs, corticosteroids (intra-articular or systemic: oral, intravenous, intra-muscular), and IL-1 inhibitors (approved by the European Medicines Agency but not approved by the Food and Drug Administration-FDA) [30]. Drug options for gout prophylaxis include colchicine, NSAIDs and IL-1 inhibitors in trials.

There is an unmet need regarding anti-inflammatory drugs in acute gout. Early treatment of an acute gout flare with low dose oral colchicine (FDA approved dose of 1.2 mg followed by 0.6 mg 1 h later) led to ≥50% reduction in pain in only 37.8% of patients at 24 h [31]. In a Cochrane review of 23 trials with 2200 participants [32], 47% of participants taking NSAIDs reported at least a 50% reduction in pain after 24 hours as compared to those taking placebo (risk ratio (RR) 2.75, 95% confidence interval (CI) 1.13 to 6.72). The relative efficacy of colchicine versus NSAIDs is not known. IL-1 inhibitors were effective in combating gouty inflammation both acutely and chronically [33, 34], but were not approved by the FDA. Due to failure of available drugs to decrease pain and inflammation quickly in acute gout, combination therapy is often used, but needs further study.

Anti-inflammatory drug prophylaxis is recommended for all gout patients when ULT is initiated. Oral colchicine is a first-line gout prophylaxis treatment, but requires dose adjustment in chronic kidney disease for drug interactions. Low-dose NSAIDs may also be considered a first-line drug for gout prophylaxis, unless there is a medical contraindication which may be

common due to comorbidities and drug interactions. Use of anti-inflammatory medications as prophylaxis in gout requires further study of which drugs are most effective and the duration needed.

34.5.2.1 Oral Colchicine

Colchicine binds to tubulin dimers, thus preventing microtubule assembly and thereby disrupts the NLRP3 inflammasome-driven caspase-1 activation and IL-1β processing [35]. These effects may be mediated at least in part by a decrease in the expression of adhesion protein expression on endothelial cells and inhibition of IL-1 induced L-selectin [36]. In addition, colchicine affects microtubule-based inflammatory cell chemotaxis, generation of leukotrienes and cytokines, as well as phagocytosis (see Chap. 40).

Colchicine is considered a first-line treatment for acute gout when initiated within 36 hours or less from the onset of the flare. The FDA-approved colchicine dose for the treatment of acute gout flares is 1.2 mg followed by 0.6 mg an hour later for a total dose of 1.8 mg [31].

One hundred and eighty-four patients with acute flares of less than 12-h duration were enrolled in a study that compared low-dose colchicine (1.8 mg total over an hour), high-dose colchicine (4.8 mg total over 6 h), and placebo. The primary endpoint was a 50% reduction or greater in pain within 24 h of enrollment. Low-dose colchicine resulted in a similar efficacy with fewer side effects as compared to high-dose colchicine [31]. The most common side effects reported were diarrhea, nausea, vomiting, and nasopharyngeal pain. These side effects were considered mild and resolved with dose reduction. More severe side effects were observed with overdoses of colchicine, including bone marrow suppression with agranulocytosis.

34.5.2.2 Nonsteroidal Anti–Inflammatory Drugs (NSAIDs)

NSAIDs may be considered as a first-line treatment for acute gout flares in the absence of comorbidities. In clinical trials, different NSAIDs all have similar efficacy in treating acute gout [37]. Selective cyclooxygenase (COX)-2 inhibitors and non-selective NSAIDs also have similar efficacy with the advantage that the COX-2 inhibitors are associated with fewer gastrointestinal adverse events [37]. The NSAIDs currently approved by the FDA for the treatment of acute gout include naproxen, indomethacin, and sulindac. A recent randomized, clinical trial compared low-dose colchicine versus naproxen in patients with acute gout. Initial findings suggest that both naproxen and low-dose colchicine are effective treatments for acute gout, however, they also suggest that naproxen worked more quickly and was associated with fewer adverse-effects and less use of rescue analgesia than low-dose colchicine [38].

34.5.2.3 Corticosteroids

Corticosteroids are recommended as a first-line treatment option for acute gout, especially when use of NSAIDs or colchicine is not effective, poorly tolerated, or contraindicated. Corticosteroids may be a safer treatment option for patients with acute gout who have underlying renal impairment, a common comorbidity in patients with gout.

In two randomized, placebo-controlled trials of a 5-day course of oral prednisolone (one evaluating a dose of 30 mg/d and the other a dose of 35 mg/d), the efficacy of prednisolone 30 mg/d was equivalent to that of standard regimens of indomethacin and naproxen versus prednisolone 35 mg/d [39, 40].

Monoarticular gout flares are often managed with intra-articular corticosteroids. Oligoarticular flares may also be managed with intra-muscular corticosteroids. Evidence, however, for the use of intra-articular or intra-muscular corticosteroids in acute gout is limited.

The ACR guidelines for the management of acute gout recommended that prednisone or prednisolone be started at a dose ≥0.5 mg/kg/d for 5–10 days followed by discontinuation or alternately for 2–5 days at the full dose followed by 7 to 10 day taper [41]. The maximum dose was not specified.

34.5.2.4 Adrenocorticotropic Hormone (ACTH, Corticotropin)

ACTH stimulates the adrenal cortex to produce corticosteroids and interferes with the acute inflammatory response. It plays a role in the inhibition of inflammation in a corticosterone independent manner in adrenalectomized rats [42]. Efficacy in acute gout is achieved via rapid peripheral suppression of leukocyte activation by the activation of the melanocortin type 3 receptor (MC3R). MC3R is present on cells of the brain, placenta, gastrointestinal tract, and macrophages (but not in the adrenal gland) [43].

A retrospective study of 181 patients who received a synthetic ACTH intramuscularly injection for their acute gout flare found that 78% had a significant response to treatment and all responded to a repeated single dose of ACTH [44]. The ACR guidelines for the management of gout recommended that ACTH can be used for the treatment of acute flares with a single subcutaneous injection (25–40 IU) which may be repeated every 24–72 h, as clinically indicated. ACTH is not approved by FDA for the treatment of acute gout and is very expensive in the United States, which limits its use.

34.5.2.5 Interleukin–1 (IL–1) Inhibitors

Anakinra is a recombinant human interleukin-1 receptor antagonist (IL-1Ra) (see Chap. 41). Due to its short plasma terminal half-life of 4–6 h, anakinra requires daily subcutaneous injections. Only pilot studies have been published regarding use of anakinra in patients with gout [45, 46]. A recent study of 40 patients with contraindications to standard-of-care anti-inflammatory medications showed a good response to anakinra injections [47]. Median pain on a 100 mm visual analogue scale (VAS) was rapidly reduced from 70–80 to 20–35 in the anakinra-treated group, as well as a reduction in C-reactive protein levels from 55.8–238.8 to 5–29 mg/L. Currently, a randomized controlled trial of anakinra in acute gout is in progress.

Canakinumab is a recombinant, human anti-human-IL-1β monoclonal antibody with a long half-life of approximately 28 days (see Chap. 41). In a multicenter study [48], patients with acute gout who were refractory or had contraindications to NSAIDs and/or colchicine were randomized to receive a single SC injection of 150 mg canakinumab or a single intramuscular dose of triamcinolone acetonide (TA) 40 mg. Canakinumab provided superior pain relief with a more rapid onset of action than TA. A greater reduction in pain intensity from baseline was seen in canakinumab versus TA-treated patients from 6 h onward, and was significantly greater at 24, 48, and 72 h after treatment (differences of −11.5 mm ($p = 0.04$), −18.2 mm ($p = 0.002$), and −19.2 ($p < 0.001$), respectively). In addition, the time to a 50% reduction in pain was significantly shorter with canakinumab than TA (median of 1 vs. 2 days; $p < 0.001$).

In another randomized double-blind active-controlled multicenter trial assessing prophylaxis for gout flares during initiation of ULT, a single dose of canakinumab effectively reduced the risk of flares in patients starting treatment with allopurinol [33]. All canakinumab doses investigated were superior to colchicine 0.5 mg once daily for flare prevention over the 16-week study period. At week 16, the mean number of gout flares per patient was reduced by 62–72% for canakinumab doses ≥50 mg compared with colchicine ($p \leq 0.0083$). In addition, there was a 64–72% reduction in the risk of experiencing ≥1 gout flare for canakinumab doses ≥50 mg compared with colchicine ($p \leq 0.05$). The percentage of patients experiencing ≥1 gout flare was significantly lower for all canakinumab doses (15–27%) compared with colchicine (44%, $p < 0.05$).

Canakinumab is the only IL-1 inhibitor approved in the European Union [31] for treatment of patients with frequent gout flares (≥3 flares in the previous 12 months) in whom NSAIDs and colchicine are contraindicated, not tolerated, or do not provide an adequate response, and in whom repeated courses of corticosteroids are not appropriate. Canakinumab provides effective pain relief during acute flares as well as

prolonged suppression of gout flares in a patient population with limited treatment options.

Rilonacept is a dimeric fusion protein consisting of the ligand-binding domains of the extracellular portions of the human IL-1 receptor component (IL-1RI) and IL-1 receptor accessory protein (IL-1RAcP) linked in-line to the Fc portion of human IgG1 (see Chap. 41). The FDA Arthritis Advisory Committee recommended rejection of rilonacept in its application for the short-term treatment of gout flares, expressing concerns regarding long-term safety and efficacy.

The 2012 ACR guidelines for the management of gout [23] addressed the use of one injection of canakinumab 150 mg SC or anakinra 100 mg SC daily for 3 consecutive days as an option for severe flares of acute gouty arthritis refractory to standard treatment. Given the lack of randomized studies for anakinra and unclear risk to benefit ratio for canakinumab, the ACR task force panel assessed the role of IL-1 inhibitor therapy in acute gout as uncertain and stated that anakinra doses and length of treatment should be further studied in randomized trials. The 2016 EULAR recommendations for the management of gout, however, do recommend considering IL-1 blockers for treatment in patients without current infection, who have frequent flares and contraindications to colchicine, NSAIDs, and corticosteroids.

34.6 Conclusion

In summary, inflammation and hyperuricemia drive the gout cascade. Better understanding of the immunopathogenesis of this inflammatory disease will lead to improved diagnosis and therapies. Treatment of acute and chronic inflammation is of utmost importance in combating gout and preventing long-term disability.

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Behçet Disease

35

Ahmet Gül

Abstract

Behçet disease, a multifactorial systemic inflammatory disorder of unknown etiology, is characterized by recurrent oral and genital aphthous ulcers, and uveitis as cardinal features, and also other manifestations involving the skin and mucosal tissues, eyes, joints, blood vessels, lungs, central nervous and gastrointestinal systems. Some of its recurrent manifestations overlap with the clinical findings of autoinflammatory disorders. However bilateral posterior or panuveitis with typical features, variable vessel vasculitis with a preference for the venous side, and parenchymal neurologic involvement as subacute brainstem syndrome constitute its distinctive clinical findings. Behçet disease is strongly associated with human leukocyte antigen (HLA)-B*51, and an epistatic interaction between HLA-B*51 and one of the endoplasmic reticulum-associated aminopeptidase 1 (ERAP1) haplotypes implicate the critical role of peptide-HLA complex in the disease pathogenesis. Additional non-HLA genetic variants associated with Behçet disease contribute to the changes in sensitivity to microbial and other environmental triggers resulting in a hyperinflammatory response involving mainly innate immunity, Th1 and Th17 type adaptive response and endothelial activation. Treatment of Behçet disease is empiric, and should be tailored according to the severity of manifestations. Clinical findings and their recurrences can be managed by anti-inflammatory and immunosuppressive drugs, including corticosteroids, colchicine, apremilast, azathioprine, cyclosporine as well as monoclonal anti-tumor necrosis factor agents and interferon α, and when necessary with other targeted biologic treatments.

Keywords

Behçet disease · Aphthous ulcer · Vasculitis
Uveitis · Human leukocyte antigen (HLA)-B*51 · Endoplasmic reticulum aminopeptidase (ERAP)-1

Abbreviations

5-ASA	5-Aminosalicylate
ASC	Apoptosis-associated speck-like protein containing CARD
CAPS	Cryopyrin-associated periodic syndrome
CARD	Caspase activation and recruitment domains
ERAP	Endoplasmic reticulum aminopeptidase

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FMF	Familial Mediterranean fever
HA20	Haploinsufficiency of A20
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
hnRNP	Heterogeneous nuclear ribonucleoprotein
IFN	Interferon
IL	Interleukin
JAK	Janus kinase
MHC	Major histocompatibility complex
MKD	Mevalonate kinase deficiency
NF-M	Neurofilament-medium
NK	Natural killer
NLRP3	Nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3
PAPA	Pyogenic arthritis, pyoderma gangrenosum and acne
STAT	Signal transducer and activator of transcripton
TNF	Tumor necrosis factor
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome

Key Points

- **Behçet disease is a multifactorial systemic inflammatory disorder with recurrent manifestations involving mucocutaneous, ocular, articular, cardiovascular, neurologic and gastrointestinal tissues**
- **Some of its recurrent manifestations overlap with the clinical findings of autoinflammatory disorders**
- **Several environmental agents, such as streptococcal and viral antigens, are possible triggers of disease manifestations in genetically susceptible individuals**
- **Behçet disease is strongly associated with human leukocyte antigen (HLA)-B*51, and an epistatic interaction between HLA-B*51 and one of the endoplasmic reticulum-associated aminopeptidase 1 (*ERAP1*) haplotypes implicate the critical role of peptide-HLA complex in the disease pathogenesis**
- **Bilateral posterior or panuveitis with typical features, vasculitis affecting all types and sizes of vessels with a preference for the venous side, and parenchymal neurologic involvement presenting as subacute brainstem syndrome constitute distinctive clinical findings of Behçet disease**
- **Treatment of Behçet disease is empiric, and should be tailored according to the severity of manifestations**

35.1 Introduction

Behçet disease was originally described in 1937 as a distinct entity characterized by the three-symptom-complex; recurrent oral aphthous ulcers, genital ulcers, and uveitis. It is a multisystem inflammatory disease of unknown etiology with recurrent manifestations involving the skin and mucosal tissues, eyes, joints, blood vessels, lungs, central nervous and gastrointestinal systems [1].

Behçet disease is considered as a systemic vasculitis, and the revised International Chapel Hill Consensus Conference defines it as a "variable vessel vasculitis" because of its unique pattern affecting all types and sizes of blood vessels [2]. Because of the predominance of neutrophils in the inflammatory infiltrates of active lesions, it is also classified among the neutrophilic dermatoses [3, 4].

Recurrent hyperinflammatory manifestations of Behçet disease, which are mediated primarily by the cells and molecules of the innate immune system and significant contribution of genetic factors to its pathogenesis fit well with the updated definition of autoinflammatory diseases [5]; and some of these manifestations overlap with the clinical findings of hereditary autoinflammatory disorders. However, because of its complex genetic background and strong association with the major histocompatibility complex (MHC) Class I antigen, human leukocyte antigen (HLA)-B*51, it has been placed in the middle of the inflammatory disease continuum, spanning from the monogenic autoinflammatory to the monogenic autoimmune disorders (see Chap. 38) [6].

35.2 Epidemiology

Behçet disease is prevalent in the Eastern Mediterranean countries and to some extent along the Silk Road with decreasing rates eastward up to Korea and Japan. The highest prevalence rates are reported from Turkey and Jordan (Table 35.1) [7–9]. The geographic distribution correlates with the frequency of the HLA-B*51 allele in the healthy population [10]. Behçet disease can be seen much less frequently in other parts of the world [9]; the disease course may be less severe and women are overrepresented in non-endemic regions [11].

In endemic areas, both sexes are affected equally. However, more severe manifestations of Behçet disease including uveitis, large vessel vasculitis and parenchymal neurologic involvement occur more frequently in males [12].

Age at onset of Behçet disease is usually in the third decade, and it very rarely starts before puberty and in elderly people. Younger patients (<25 years) usually have a tendency for a more severe disease course.

Table 35.1 Epidemiological features of Behçet disease

Prevalence	Pooled overall estimate 10.3/100,000 [9]
	Highest rates in Turkey (420/100,000) and Jordan (660/100,000); lowest rates in northern Europe, North America and sub-Saharan Africa
Male:female ratio	Approximately 1:1 in endemic regions
	Female dominance in non-endemic areas
Age at onset	Most common in the third decade
	Rare before puberty and among the elderly
Morbidity and mortality	More severe course in young males
	Increased morbidity and mortality in patients with vascular and parenchymal neurologic involvement
	Increased morbidity associated with ocular involvement

35.3 Etiology

- **Behçet disease is a multifactorial inflammatory disorder**
- **HLA-B*51 is the strongest genetic susceptibility factor for Behçet disease, and an epistatic interaction with one of the endoplasmic reticulum-associated aminopeptidase 1 (ERAP1) haplotypes determines its pathogenic role**
- **Several non-HLA genetic variants contribute to disease susceptibility by affecting the sensitivity of host to different environmental triggers and polarization of immune response**
- **Several environmental agents, such as streptococcal and viral antigens, are possible triggers of clinical manifestations in genetically susceptible individuals**

Behçet disease is considered as a multifactorial inflammatory disorder, and several environmental agents, such as streptococcal and viral antigens, are claimed as triggers of manifestations in genetically susceptible individuals [1].

35.3.1 Major Histocompatibility Complex (MHC) Genetic Associations

Familial aggregation with high sibling recurrence risk ratio (11.4–52.5) supports the contribution of genetics, and a positive family history can be observed in 3–20% of the patients [13]. Association of HLA-B*51 with Behçet disease is the strongest genetic finding identified so far [14]. HLA-B*51 association has been validated in both endemic and non-endemic populations, especially in patients with the complete form of the disease [15, 16]. Although the mechanism of action of this strong association has yet to be clarified, recent genetic findings revealing the epistatic interaction between HLA-B*51 and endoplasmic reticulum-associated aminopeptidase (ERAP)1 polymorphisms suggest that the peptides which should be loaded onto the antigen-binding groove

of the B*51 allele for proper folding, play a critical role in the pathogenesis [17]. HLA-B*51 carriers who are homozygous for the disease associated-haplotype 10 of the *ERAP1* gene have more than a ten-fold risk for Behçet disease compared with those carrying neither risk factor [18].

Weaker associations with HLA-A*26, HLA-B*15, HLA-B*27 and HLA-B*57 as susceptibility, and with HLA-B*49 and HLA-A*03 as protective alleles and location of the amino acid variants affecting the disease risk further support the critical role of peptide binding to MHC Class I in the pathophysiology of Behçet disease [19].

35.3.2 Non–MHC Genetic Associations

Genome-wide association and immunochip studies revealed several non-HLA gene variants as risk factors for Behçet disease including variants encoding for interleukin (IL)-23R, IL-10, CCR1, STAT4, IL-1α-IL-1β, IRF8, CEBPB-PTPN1, ADO-EGR2, RIPK2, LACC1, and FUT2 [17, 20, 21] (Table 35.2). Targeted sequencing of innate immune response genes also documented that Toll-like receptor (TLR) 4 variants and the familial Mediterranean fever (FMF) associated *MEFV* gene p.Met694Val variant increase the risk for Behçet disease [22].

Table 35.2 Genes associated with an increased risk for Behçet disease

Human leukocyte antigen (HLA) alleles
HLA-B*51
Other HLA alleles with weaker effects
Susceptibility alleles:
HLA-B*15, HLA-B*27, HLA-B*57
HLA-A*26
Protective alleles:
HLA-B*49
HLA-A*03
Non-HLA Genes
Immune response
IL-10, IL-23R, IL-1α/IL-1β, CCR1, STAT4, IRF8, CEBPB-PTPN1, RIPK2, ADO-EGR2
Sensing and processing of danger signals
ERAP1, MEFV, TLR4, NOD2, LACC1, FUT2

Genetic studies of Behçet disease identified several polymorphisms shared with spondyloarthropathies, especially Crohn disease, and implicate the role of recognition of pathogen or danger associated molecular patterns at the mucosal surfaces and regulation and polarization of innate and adaptive immune responses in the pathogenesis [1, 21].

35.3.3 Epigenetics and Environmental Factors

A genome-wide association study identified 383 differentially methylated CpG sites in monocytes and 125 sites in CD4 T cells of patients with Behçet disease compared with healthy controls, especially in the genes that regulate cytoskeletal dynamics [23]. A response to treatment was associated with reversal of the direction of aberrant DNA methylation patterns [23].

Hulusi Behçet suggested viruses as well as focal infections in the oral cavity as possible triggers of the disease manifestations. Hypersensitivity to streptococcal antigens, including some uncommon serotypes of *Streptococcus sanguinis* and herpes simplex virus have since been associated with the pathogenesis [24–31], and a cross-reactive immune response between microbial and self heat shock protein antigens has long been suggested as a potential pathogenic mechanism [26, 32].

35.4 Pathogenesis

- **HLA-B*51 and non-HLA genetic factors play a critical role in the hyperinflammatory response which is mediated mainly by the innate immune system. This response is considered to be induced by non-specific trauma or different microbial agents**
- **Hypersensitivity to streptococcal antigens has been claimed as a pathogenic mechanism for Behçet disease**
- **Vasculitis associated with a mixed cellular perivascular infiltrate and a thrombotic tendency has been suggested as the underlying pathology of disease manifestations**

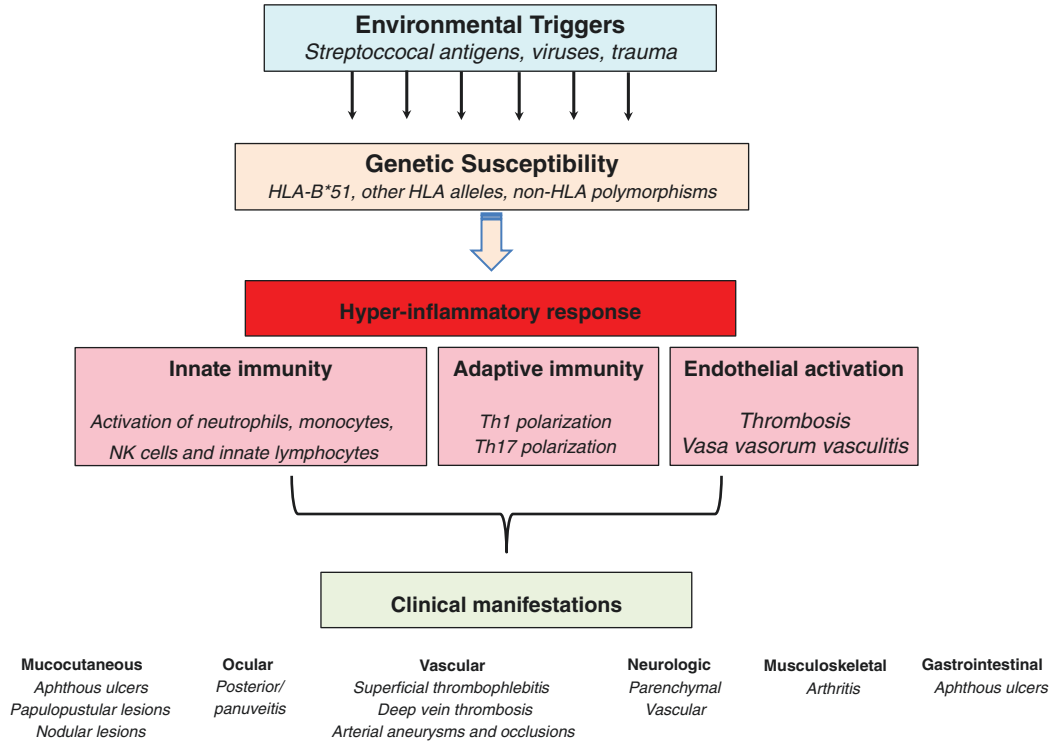

Fig. 35.1 A summary of the pathogenesis of Behçet disease

- **The skin pathergy reaction to hypodermic needle trauma exemplifies the non-specific hyperinflammatory responses observed in the patients**

The pathogenesis of Behçet disease is yet unknown, but its manifestations are associated with a hyperinflammatory response, mainly involving the innate immune system, and considered to be induced by non-specific trauma or different microbial agents (Fig. 35.1). Hyperactivity of neutrophils and monocytes manifesting with increased expression of proinflammatory cytokines including IL-1, IL-6, IL-8 and tumor necrosis factor (TNF) as well as increased generation of superoxides have been documented [33, 34]. In addition to the findings of activated innate immune response, oligoclonal expansions of T cells with Th1 and Th17 type polarization in association with the disease activity have also been reported [1].

35.4.1 Human Leukocyte Antigen (HLA)–B*51 Interaction with Endoplasmic Reticulum–Associated Aminopeptidase 1 (ERAP1) Haplotypes

Despite the strong association of Behçet disease with HLA-B*51, the precise pathogenic mechanism associated with this Class I MHC allele has yet to be clarified. An epistatic interaction between HLA-B*51 and one of the ERAP1 haplotypes (Hap10) was documented [17, 18], and homozygosity for ERAP1 Hap10 was later shown to be associated with a significantly altered peptidome, which was enriched for lower affinity peptides for HLA-B*51 [35]. This peptidome changes may affect the disease susceptibility either by causing an unfolded protein response due to weak peptide-heavy chain interactions or changing the recognition of peptide-HLA complex by T cells and natural killer (NK) cells.

Epistatic interaction of certain ERAP1 haplotypes with HLA-B*27 and HLA-C*06 was previously recognized in ankylosing spondylitis and psoriasis, respectively; identification of a similar interaction in Behçet disease led to the proposal of a new pathogenic mechanism named as "MHC-I-opathy" (see Chap. 38), which suggests a critical role of certain HLA Class I alleles and ERAP1 haplotype pairs in shaping the disease phenotype and possibly activating a shared inflammatory pathway involving innate immunity and Th17 polarization [36].

Several non-HLA polymorphisms are also thought to be contributing disease susceptibility by either affecting the strength and polarization of the inflammatory response as well as by changes in the sensing and processing of different environmental triggers and endogenous danger signals. These are suggested to be responsible for the hyperinflammatory response with neutrophil-rich innate immune reaction and adaptive immunity associated with the activation of Janus kinase (JAK)/signal transducer and activator of transcripton (STAT) pathway and resulting in recurrent characteristic disease manifestations (Table 35.2) [37–39].

35.4.2 Oral Hygiene and Microbiome

Oral hygiene is impaired in Behçet disease, and is associated with the disease severity [40]. Changes in the commensal microbes of oral cavity were reported by different investigators, especially for the increased colonization of rare strains of *Streptoccocus sanguis* among the microbiota, and a recent study showed a significant difference for *Streptoccocus sanguis* colonization in aphthous ulcer sites of patients with Behçet disease compared to healthy controls [41]. Hypersensitivity to streptococcal antigens has been claimed as a pathogenic mechanism for Behçet disease [31], and induction of different disease manifestations was reported by streptococcal antigens [24, 31, 42]. A cross-reactivity between streptococcal and mycobacterial heat shock protein 65-kDa and self-antigens was suggested to explain the immunoreactivity observed in patients with Behçet disease [26, 43, 44]. Heat shock protein 65-kDa epitopes recognized by lymphocytes of patients with Behçet disease were shown to induce uveitis in Lewis rats [45], and oral tolerization studies using the heat shock protein peptide fragment 336–351 linked to the cholera toxin B subunit was found to be helpful in the prevention of recurrent uveitis attacks in patients with Behçet disease [46].

35.4.3 The Role of the Adaptive Immune System

The adaptive immune response against several self-antigens including α-enolase, retinal proteins such as interphotoreceptor retinoid binding protein and retinal S antigen, tropomyosin, PINK1, SWAP70, heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1, and more recently against neurofilament-medium (NF-M) was previously reported, but these observations were not considered as evidence of an autoimmune pathogenic mechanism in Behçet disease [32, 44, 47–50]. NF-M has a structural homology to bacterial heat shock protein 65-kDa [32], thus immunoreactivity of serum samples of patients with Behçet disease with NF-M filaments in brain, retina and scrotal skin tissues of mice could be a potential example of an autoimmune response induced by molecular mimicry [32]. However, the immune reactivity against NF-M was detected in patients with and without neurologic involvement [32], which suggested a secondary response related to the damage of affected tissues or vascular barriers exposing organ specific antigens to the immune system rather than a primary pathogenic mechanism.

35.4.4 Vasculitis and Venous Thrombosis

Vasculitis associated with a mixed cellular perivascular infiltrate and a thrombotic tendency has been suggested as the underlying pathology of

Behçet disease manifestations. Pathogenic mechanisms leading to the characteristic predilection for venous involvement are not known, and endothelial activation has been considered as the main cause of the prothrombotic state. Although inflammation-associated changes in the coagulation system, such as increased fibrinogen carbonylation and impaired fibrinogen function as well as enhanced reactive oxygen species production could be detected in patients with Behçet disease, these findings could not discriminate patients with and without thrombosis [51]. The only factor found to so far to be associated with a predilection for thrombosis in patients with Behçet disease was an imbalance between serum microparticles expressing the tissue factor pathway inhibitor and tissue factors of the coagulation cascade [52].

35.4.5 Pathergy and Autoinflammatory Mechanisms

The skin pathergy response to hypodermic needle trauma is a characteristic pathologic finding of Behçet disease, and it exemplifies the nonspecific hyperinflammatory responses observed in the patients. A similar pathergy response can be induced in patients with Behçet disease by intradermal injection of uric acid crystals to the forearm, seen as a larger erythematous reaction at 48 h compared to healthy controls [53]. Uric acid crystals trigger an inflammatory response induced by the nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3 (NLRP3)-inflammasome. Similarly, in the nodular skin lesions of Behçet disease, increased expression of NLRP3 and apoptosis-associated speck-like protein containing caspase activation and recruitment domains (ASC) has been documented, compared to lesions of "classic" erythema nodosum [54]. These observation supports the involvement of autoinflammatory components in the pathogenesis of the hyperinflammatory response seen in Behçet disease.

35.5 Clinical Manifestations

- **Aphthous ulcers, papular-pustular and erythema nodosum-like lesions are common manifestations and can be seen also in several other conditions**
- **Skin pathergy reaction supports the diagnosis depending on the clinical context, since it is rarely seen in other conditions**
- **Posterior or panuveitis with typical features, inflammatory major vessel involvement with thrombotic tendency, and parenchymal neurologic involvement are distinctive features of the disease**
- **Young male patients develop serious manifestations of the disease more frequently and have a more severe course**

Behçet disease is characterized by recurrent inflammatory manifestations (Figs. 35.2, 35.3 and 35.4). Some of the manifestations, such as oral aphthous, acne-like lesions, pseudofolliculitis, or erythema nodosum-like lesions are common and can be seen in several other conditions (Table 35.3), while some, like genital ulcers or superficial thrombophlebitis are less common and the differential diagnosis includes only a few other disorders.

Hulusi Behçet considered the condition to be a separate entity due to the development of distinctive features of ocular inflammation in his patients. The presence of typical ocular, vascular and parenchymal neurologic manifestations plays a critical role in the differential diagnosis. The recurrent manifestations of Behçet disease overlap with the clinical picture of hereditary autoinflammatory disorders (Table 35.4) or multifactorial conditions such as Crohn disease, and may cause a diagnostic challenge especially in the pediatric age group [55, 56].

There is no data to reliably differentiate clinical manifestations of Behçet disease between children and adults. The first manifestation in children usually starts between the ages of 7–12 years, and they usually develop fewer typical findings during childhood [57]. Familial aggregation is seen more frequently in pediatric

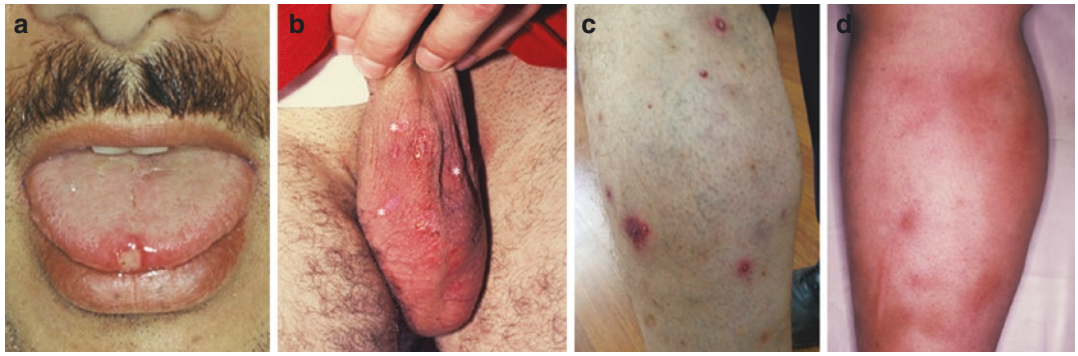

Fig. 35.2 Mucocutaneous manifestations of Behçet disease (**a**) Oral aphthous ulcer on the tongue (**b**) Genital ulcers on the scrotum—asterisks indicate scars of previous ulcers (**c**) Pustular and (**d**) Nodular skin lesions on the calf

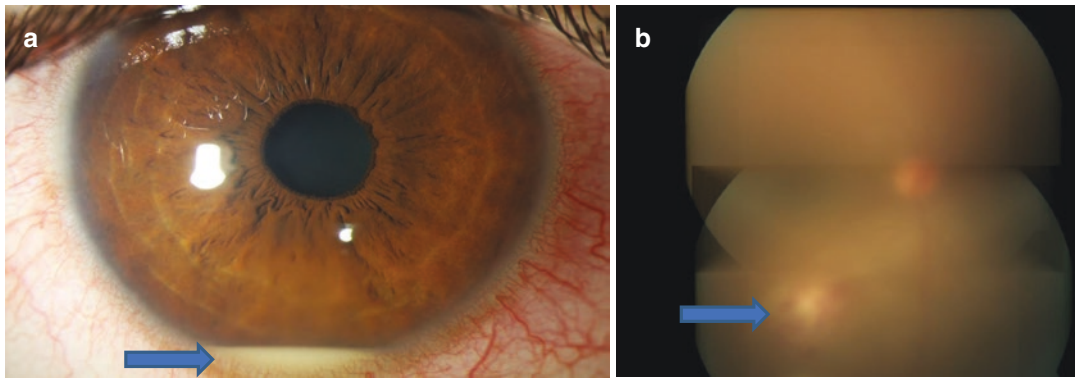

Fig. 35.3 Ocular findings of 28 year-old male presenting with finger count vision in the right eye (Courtesy of Professor İlknur Tugal-Tutkun). (**a**) Slit lamp photograph shows ciliary injection and hypopyon (arrow) in the right eye (**b**) Composite color fundus photograph of the same eye shows 3+ vitreous haze and a retinal infiltrate with surrounding hemorrhages (arrow) inferotemporal to the optic disc

cases [58]. Males fulfilling the classification criteria before the age of 25 years have a greater frequency of neurological, ocular and vascular involvement [57].

35.5.1 Mucocutaneous Manifestations

Recurrent oral aphthous ulcers are the most common manifestation of the disease (Fig. 35.2a). They are indistinguishable from aphthous stomatitis or canker sores, and appear as minor (<10 mm), major (≥10 mm) or herpetiform, oval or round shaped, superficial, non-scarring ulcers with a white or greyish pseudomembrane necrotic base, surrounded by erythema [59]. Although it usually precedes other manifestations, especially in children, often by many years, up to 15–20% of the patients may not develop aphthous ulcers at the disease onset. Smoking may suppress their appearance, and local trauma and oral hygiene problems may increase the attack frequency.

Genital aphthous ulcers are less common and have a tendency to leave a scar (Fig. 35.2b, asterisks indicate ulcer scars). These ulcers usually occur on the scrotum of males and labia majora of females, however they can be seen in perianal and perineal areas, and groins as well [59].

Papular-pustular skin findings are also common manifestations, and they can be seen as acne or folliculitis-like lesions. Lesions are similar to

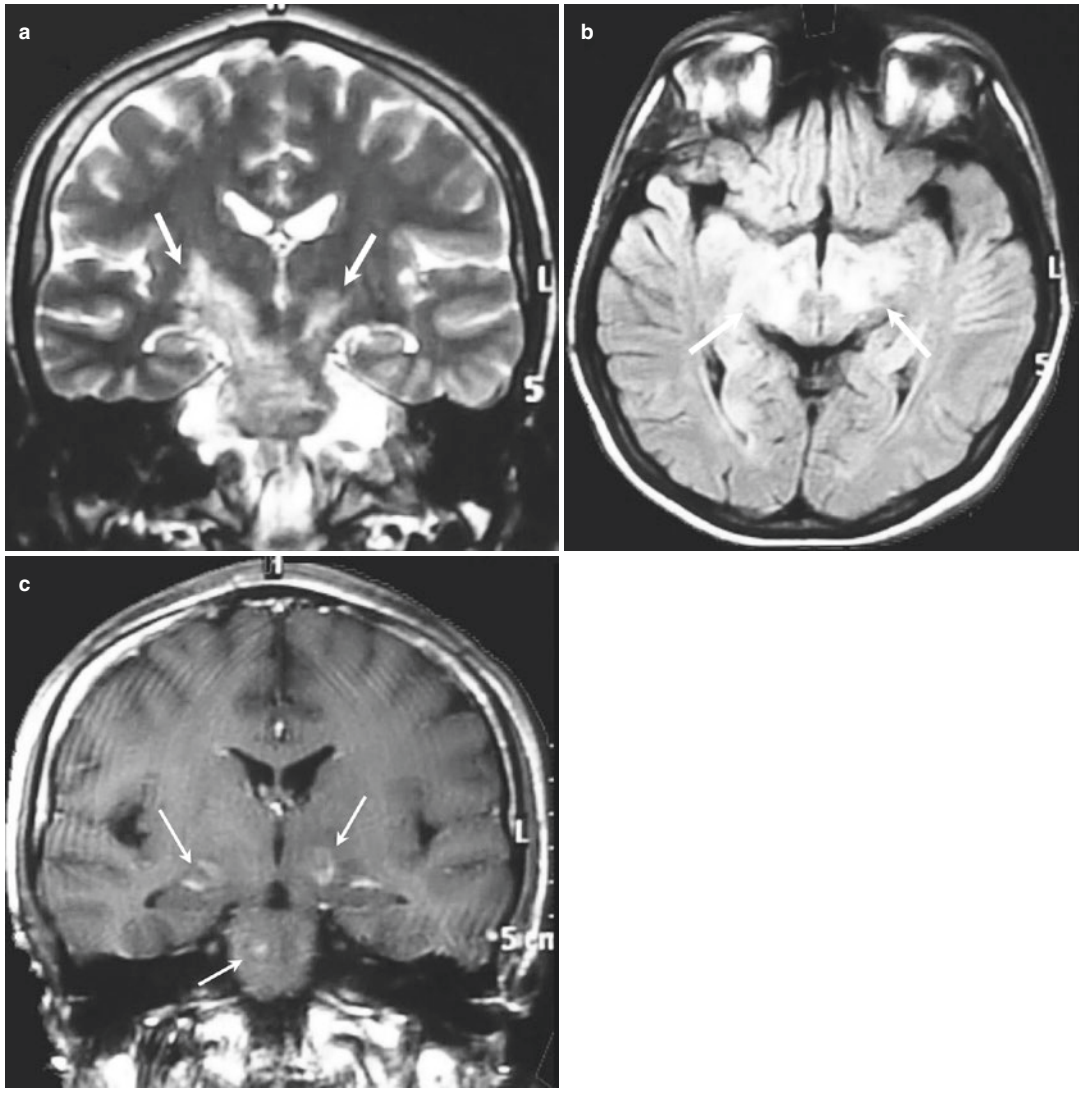

Fig. 35.4 Parenchymal neurologic involvement (arrows indicating the locations of the lesions) (Courtesy of Professor Murat Kürtüncü). (**a**) Coronal T2 weighted image of the patient. The bilateral lesion extends from the thalamus to mesencephalon (**b**) Axial FLAIR weighted image of the patient at the level of the mesencephalon. Both cerebral peduncles and substantia nigra are affected. Red nuclei are spared relatively (**c**) Axial contrast enhanced T1 weighted images. There is patchy contrast enhancement of the lesion

ordinary acne, but when they occur on the buttocks and lower limbs they may be more suggestive of Behçet disease (Fig. 35.2c).

Erythema nodosum-like lesions are seen more frequently in females (Fig. 35.2d). These lesions usually develop in the pretibial area as painful erythematous nodules, but they can also be seen on the face, neck, arms and buttocks. They resolve spontaneously within 3 weeks and frequently leave a residual pigmentation [59]. Superficial thrombophlebitis may also present with painful erythematous nodular lesions, as linear or nodular swellings on the inflamed subcutaneous veins.

Skin pathergy reaction is a hyperreactive erythematous papular-pustular lesion developing at

Table 35.3 Overall frequencies of clinical manifestations of Behçet disease

Manifestations	Frequency (%)
Common/overlapping[a]	
Oral aphthous ulcers	97–100
Papular-pustular lesions	70–90
Erythema nodosum-like lesions	40–60
Arthritis	40–50
Less common/overlapping	
Skin pathergy reaction	30–80
Genital ulcers	0–90
Superficial thrombophlebitis	15–30
Gastrointestinal involvement	2–30
Distinctive features	
Uveitis	30–50
Deep vein thrombosis	10–15
Arterial aneurysms	5
Neurologic involvement	5–10

[a]Overlapping with other inflammatory/autoinflammatory diseases

Table 35.4 Manifestations of Behçet disease overlapping with monogenic autoinflammatory disorders

Manifestations	Monogenic autoinflammatory disorders
Oral aphthous ulcers	MKD, HA20, CAPS
Genital ulcers	MKD, HA20
Papular-pustular lesions	PAPA, HA20
Pathergy reaction	PAPA
Uveitis	HA20, CAPS, TRAPS, Blau syndrome (granulomatous)
Arthritis	FMF, TRAPS, PAPA, Blau syndrome, CAPS, MKD
Meningoencephalitis	FMF, CAPS, interferonopathies
Orchiepididymitis	FMF

MKD mevalonate kinase deficiency; *HA20* haplotype insufficiency for A20; *CAPS* cryopyrin-associated periodic syndrome; *PAPA* pyogenic arthritis, pyoderma gangrenosum and acne syndrome; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *FMF* familial Mediterranean fever

the site of a needle prick. Similar hyperinflammatory lesions can also be induced at other sites by local injury such as oral or genital aphthous ulcers following needle prick or other forms of trauma. Therefore, it is important to avoid unnecessary invasive procedures involving veins and arteries, which may result in thrombophlebitis or aneurysm formation, respectively.

35.5.2 Ocular Manifestations

Typical presentation of ocular involvement in Behçet disease is bilateral non-granulomatous posterior or panuveitis. Diffuse vitritis due to posterior segment involvement may extent to the anterior chamber, and some patients with severe inflammation may also develop hypopyon (Fig. 35.3a, b). Spontaneously resolving superficial infiltrates are typical findings of the uveitis of Behçet disease, and some patients may also develop recurrent branch retinal vein occlusions, retinal hemorrhages (Fig. 35.3b), and peripheral retinal occlusive periphlebitis. Episodes of posterior or panuveitis affect the visual acuity, and recurrences may result in total loss of vision.

35.5.3 Vascular Manifestations

Behçet disease has a unique place among systemic vasculitides by affecting all types and sizes of blood vessels with the predominance of the venous side [2]. Vessel wall inflammation is associated with a tendency for thrombosis and a very low risk of pulmonary thromboembolism due to the thrombus adherent to the underlying endothelium.

Superficial thrombophlebitis, seen as a painful nodular skin lesion, is the most frequent type of vascular finding, and occurs more frequently in male patients. Superficial thrombophlebitis has prognostic importance, since it may be the earliest sign of vascular involvement in Behçet disease.

Deep vein thrombosis is seen most frequently in lower extremities [60]. However, all veins, including inferior and superior cava veins, hepatic veins, and cerebral sinuses can be affected. Recurrent episodes may result in postthrombotic syndrome and chronic venous ulcers, and patients with Budd-Chiari syndrome may have a worse prognosis.

Arterial involvement occurs most frequently as aneurysms rather than occlusions, and aneurysms of Behçet disease usually develop as irregularly shaped saccular pseudoaneurysms with a mural thrombus. Pulmonary arterial

aneurysms are the most common form of arterial involvement, which often present with massive hemoptysis. Venous thrombosis is frequently seen in these patients as an accompanying finding. Some patients develop pulmonary artery thrombosis without aneurysm formation. The abdominal aorta and other large or medium-sized arteries are affected less frequently, and extra-pulmonary arterial involvement develops at later ages compared to the age of onset of venous and pulmonary artery involvement [60].

35.5.4 Neurologic Manifestations

Typical neurologic involvement of Behçet disease manifest as subacute brainstem syndrome. Unifocal or multifocal inflammatory parenchymal lesions (Fig. 35.4) result in pyramidal signs, hemiparesis, ataxia, sphincter disturbances and behavioral changes [61, 62]. Psychiatric disorders without neurologic involvement are not expected in Behçet disease, but depression and suicidal thoughts can be seen in patients with major organ involvement during the active phase of the disease [63].

A smaller group of patients (20%) with neurologic involvement develop intracranial hypertension and papilledema due to cerebral venous thrombosis; this vascular patient group has a better prognosis than patients with parenchymal disease.

35.5.5 Other Manifestations

35.5.5.1 Musculoskeletal Manifestations

Acute non-erosive mono- or oligoarthritis can be seen, usually in the lower extremities and less frequently in upper extremities. Chronic arthritis is rare. Recent genetic findings of Behçet disease support shared inflammatory pathways with spondyloarthropathies [21, 36]. However, axial involvement is rare, and classification of Behçet disease among the spondyloarthropathies has been questioned by many.

35.5.5.2 Gastrointestinal Manifestations

Aphthous ulcers can occur anywhere in the gastrointestinal mucosa, and the ileocecal region is affected more commonly with solitary big oval or round shaped ulcers. Abdominal pain and diarrhea are the main findings, and vasculitic ulcers also have a tendency for bleeding and perforation. Gastrointestinal involvement has been reported more frequently in Eastern Asian countries. Differential diagnosis with Crohn disease is critical because of overlapping intestinal and extraintestinal manifestations [64, 65].

35.5.5.3 Others

Parenchymal lung disease with organizing pneumonia, cardiac involvement, orchiepididymitis are other rare manifestations observed in patients with Behçet disease.

35.6 Diagnosis

- **There are no pathognomonic clinical and laboratory findings of Behçet disease, and a constellation of symptoms is necessary to confirm the diagnosis**
- **Skin pathergy test supports the diagnosis strongly when positive, and non-invasive imaging methods are helpful for screening and monitoring organ involvement**

35.6.1 Laboratory Investigations

Laboratory and imaging findings may help confirm the diagnosis in patients with the right constellation of clinical manifestations [66]. However, there are no pathognomonic clinical and laboratory findings of Behçet disease.

Acute phase reactants are usually not elevated in patients with mucocutaneous involvement, and they may provide limited help in patients with major organ involvement. Cerebrospinal fluid findings of pleocytosis including neutrophils and lymphocytes, elevated protein and IL-6 levels (with normal glucose values) are considered very helpful in the differential diagnosis of parenchymal neurologic disease [61, 62].

HLA-B*51 is seen more frequently in patients with the complete form of the disease, especially in patients from endemic areas. However, HLA-B*51 testing has no diagnostic or prognostic value in both endemic and non-endemic areas.

35.6.2 Pathology

There is no pathological finding diagnostic for Behçet disease. The papular-pustular lesions of Behçet disease could not be distinguished from acne vulgaris by the presence of vasculitic or other histologic findings [67]. On the other hand, leukocytoclastic vasculitis in the interlobular septa, lobular infiltrates, and lack of the histopathologic hallmark of erythema nodosum, the so-called Miescher radial granulomas, which are well-defined radially arranged nodular aggregates of small histiocytes, may be helpful distinguishing lesions of Behçet disease from classic erythema nodosum [68].

Perivascular mixed cellular infiltrates can be seen frequently as a sign of vascular pathology, and vasculitis of the vasa vasorum has been documented in Behçet disease patients with large vessel involvement [69].

35.6.3 Imaging

Non-invasive imaging methods such as contrast-enhanced magnetic resonance imaging (MRI), and MR- or computed tomographic-angiography are frequently used for the diagnosis and follow-up of neurologic and vascular involvements. Similarly, fundus fluorescein angiographic investigation may provide supportive findings such as diffuse fern-like capillary leakage, optic disc hyper-fluorescence and retinal capillary non-perfusion in patients with ocular involvement.

35.6.4 Pathergy Test

Skin pathergy reaction has been considered helpful in the diagnosis of Behçet disease, but a trend for lower positivity has been recognized during recent years. Positive skin pathergy reaction can be seen in up to 60–80% of the patients in endemic regions and much less frequently in non-endemic areas. It supports the diagnosis of Behçet disease depending on the clinical context, since pathergy is rarely seen in other neutrophilic dermatoses such as Sweet syndrome and pyoderma gangrenosum [70].

Following a trauma to the skin of the forearm by oblique insertion of 20G hypodermic needle (Fig. 35.5a), patients with Behçet disease may develop an erythematous papule or more rarely a pustule similar to the spontaneously appearing skin lesions (Fig. 35.5b). The varying amount of trauma induced due to thickness and bluntness of the needle may affect the extent of the inflammatory reaction, and persistence of the erythematous induration at 48 h is required for a positive test [71].

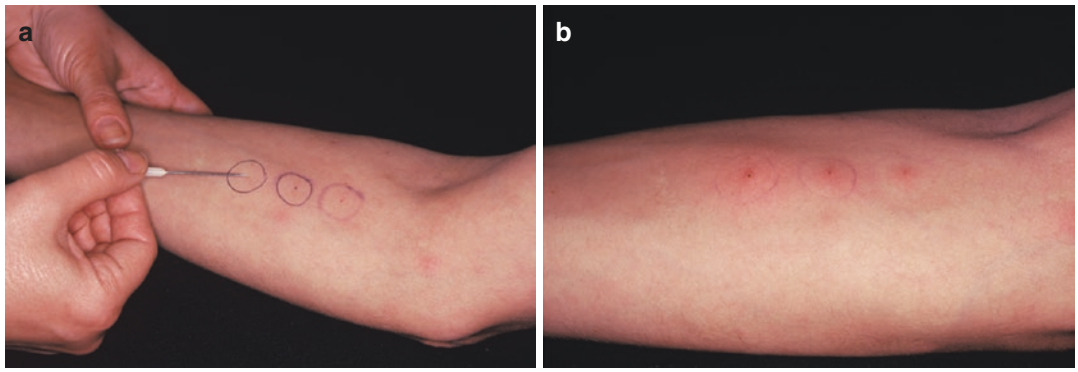

Fig. 35.5 Skin pathergy reaction tested by hypodermic needle trauma (**a**) Oblique insertion of No.1 (20G) hypodermic needle into the skin of forearm. (**b**) Positive skin pathergy reaction at the needle prick site manifest as an erythematous papules at 48 h

35.6.5 Diagnostic Criteria and Differential Diagnosis

Different sets of criteria have been developed for the diagnosis and classification of adult and pediatric patients with Behçet disease based on a constellation of certain manifestations (Table 35.5) [72–74].

These criteria have limitations, such as missing the diagnosis (*false negative*) in some of the 15–20% of patients who do not have oral aphthous ulcers at disease onset, since the *International Study Group Criteria* requires the presence of oral aphthous ulcers in all patients [72]. On the other hand, classification of the patients with oral and genital aphthosis as having Behçet disease according to the *International Criteria for Behçet Disease* (*false positive*) constitutes another major problem [73, 75]. Development of both oral and genital aphthous ulcers, also known as complex aphthosis or bipolar aphthosis, can be seen in several other condi-

Table 35.5 Three commonly used diagnostic and/or classification criteria for Behçet disease

A. International Study Group (ISG) diagnostic criteria [72]

For the diagnosis of Behçet disease the patient must have recurrent oral ulceration plus at least two of the other criteria in the absence of any other explanation for the clinical findings (95% sensitivity and 98% specificity)

1. Recurrent oral ulceration

Minor aphthous, major aphthous, or herpetiform ulcers observed by the physician or reliably described by the patient, which recurred at least three times over a 12-month period

Plus two of the following criteria:

2. Recurrent genital ulceration

Aphthous ulceration or scarring observed by the physician or reliably described by the patient

3. Eye lesions

Anterior or posterior uveitis or cells in the vitreous body on slit-lamp examination; or retinal vasculitis detected by an ophthalmologist

4. Skin lesions

Erythema nodosum, pseudofolliculitis, papular-pustular lesions or acneiform nodules not related to corticosteroid treatment or adolescence

5. Positive pathergy test

Test interpreted as positive by the physician at 24–48 hours

B. International criteria for Behçet disease (ICBD) diagnosis and classification [73]

Point score system: a score of ≥4 indicates diagnosis of Behçet disease (94.8% sensitivity and 90.5% specificity)

Sign/symptom	Points
Ocular lesions	2
Genital aphthosis	2
Oral aphthosis	2
Skin lesions	1
Neurological manifestations	1
Vascular manifestations	1
Positive pathergy test[a]	1[a]

C. Consensus classification of pediatric Behçet disease [74]

Three of six items are required for classification (91.7% sensitivity and 42.9% specificity)

Sign/symptom	Description	Value
Recurrent oral aphthosis	At least three episodes/year	1
Genital ulceration or aphthosis	Typically with scar	1
Skin involvement	Necrotic folliculitis, acneiform lesions, erythema nodosum	1
Ocular involvement	Anterior uveitis, posterior uveitis, retinal vasculitis	1
Neurological signs	With the exception of isolated headaches	1
Vascular signs	Venous thrombosis, arterial thrombosis, arterial aneurysm	1

[a]Pathergy test is optional, and when pathergy test result is available, one additional point is given for a positive result

tions, including inflammatory bowel disease, gluten sensitive enteropathy, cyclic neutropenia, human immunodeficiency virus (HIV) infections, herpes infections, immunodeficiency syndromes, deficiencies of iron, vitamin-B12 and folate, Lipschütz ulcers (also known as ulcus vulvae acutum or reactive non-sexually related acute genital ulcers), and idiopathic complex aphthosis [75]. Complex aphthosis can also be a manifestation of autoinflammatory mevalonate kinase deficiency (MKD, see Chap. 17) and haploinsufficiency of A20 (HA20-see Chap. 29) (Table 35.4) [55, 56].

Since some of the manifestations are quite common and can be seen in other conditions, identification of distinctive characteristics of ocular, vascular and parenchymal neurologic lesions is critical in the diagnosis (Table 35.3).

Differential diagnosis of Behçet disease with Crohn disease is always confusing due to the overlapping manifestations including oral and genital ulcers, erythema nodosum and peripheral arthritis. Therefore, characterization of intestinal and ocular lesions is critical for proper classification. For intestinal lesions, round-shaped, focal lesions as solitary or multiple ulcers suggest Behçet disease; on the other hand longitudinal and segmental/diffuse lesions favor the diagnosis of Crohn disease [76]. For the evaluation of ocular lesions, bilateral uveitis affecting the posterior segment can also be seen in Crohn disease [77], however presence of typical findings such as spontaneously resolving superficial retinal infiltrates, diffuse vitritis and peripheral retinal occlusive periphlebitis support the diagnosis of Behçet disease [78].

Similarly, the recently described rare monogenic disorder HA20 can easily be misdiagnosed as Behçet disease (see Chap. 29) [79]. Disease onset during early childhood, autosomal dominant inheritance, recurrent episodes of fever, oral ulcers healing with scars, isolated anterior uveitis, or choroiditis with necrotizing inflammation as well as presence of autoantibodies or autoimmune features favors the diagnosis of HA20 [56]. MKD should also be considered in juvenile patients with bipolar aphthosis and febrile episodes [55, 74].

35.7 Treatment

- **Treatment of Behçet disease is empiric, and it should be tailored according to the severity of manifestations**
- **Treatment should aim to limit the tissue damage in acute attacks and to prevent recurrences during follow-up**

Behçet disease runs a relapsing and remitting course and its treatment should aim to limit the tissue/organ damage associated with enhanced inflammatory response and prevent recurrences. Treatment of Behçet disease is empiric, and should be tailored according to the type and severity of organ involvement as well as patients' preferences (Fig. 35.6) [80].

Colchicine at doses of 1–2 mg/day are frequently used for the treatment of mucocutaneous manifestations and arthritis. Colchicine has been shown to be effective for erythema nodosum-like lesions and genital ulcers, especially in females [81]. Thalidomide at doses of 100 mg/day is an effective option for refractory oral and genital ulcers and follicular lesions, however the teratogenicity risk, and its association with polyneuropathy and nodular skin lesions limits its use [82]. Dapsone (100 mg/d) can also be used for mucocutaneous manifestations in selected cases based on a the results of a small placebo-controlled trial [83]. Some studies also report favorable clinical observations with lenalidomide and pentoxyphylline as well, despite lack of controlled studies. Low dose corticosteroids and azathioprine are frequently tried in severe cases. Recent randomized controlled trial data supports the use of apremilast, a phosphodiesterase 4 (PDE4) inhibitor for refractory oral ulcers with a good safety profile [84, 85].

For patients with arthritis refractory to colchicine, low-dose oral corticosteroids, intraarticular corticosteroid injections, and immunosuppressive drugs, or when necessary monoclonal anti-TNF antibody agents can be considered.

Treatment of posterior/panuveitis flares requires high dose systemic corticosteroids and immunosuppressive treatment; and azathioprine (2.5 mg/kg), cyclosporine (3–5 mg/kg) or their

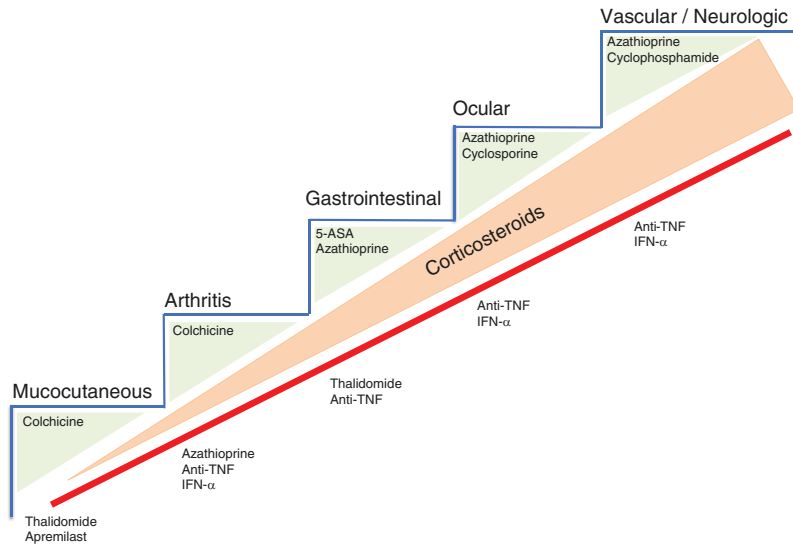

Fig. 35.6 Treatment of Behçet disease should be tailored according type and severity of organ involvement, using more potent drugs in patients with more severe disease (below the red line). *ASA* aminosalicylates; *TNF* tumor necrosis factor; *INF* interferon

combination should be started initially in any patient with posterior segment involvement. For refractory patients or patients with severe sight-threatening uveitis, interferon-α or monoclonal anti-TNF antibodies could be considered [80].

For the management of deep vein thrombosis, systemic high dose corticosteroids and immunosuppressive treatment are required. No additional favorable effect of anticoagulant treatment has been documented [86], and there is no consensus about the use of anticoagulants on top of immunosuppressive medications [80]. On the other hand, anticoagulant treatment should be considered for refractory cases with a potential for the development of post-thrombotic syndrome, if there is no risk of bleeding from arterial aneurysms.

Arterial aneurysms are managed with high dose corticosteroids and cyclophosphamide, and for refractory cases monoclonal anti-TNF antibodies are recommended. For selected cases, surgery, endovascular stents or embolization can be tried despite high complication rates [80].

For the management of parenchymal neurologic lesions, high dose corticosteroids and an immunosuppressive agent such as azathioprine are recommended [80]. Cyclosporine should be avoided because of increased risk of neurologic involvement associated with its usage [80]. Monoclonal anti-TNF antibodies and interferon-α can be alternative treatments for refractory cases. For the treatment of cerebral venous thrombosis, high-dose corticosteroids should be used with later tapering, and anticoagulants may be added for a short duration [80].

Acute exacerbations of gastrointestinal lesions are treated with corticosteroids and disease-modifying agents such as 5-aminosalicylate derivatives or azathioprine, depending on the severity of lesions, and monoclonal anti-TNF antibodies can be tried patients with more severe manifestations [80]. Urgent surgical intervention should be requested for patients with perforation, major bleeding, and obstruction [80].

For patients with refractory disease, usage of biologic agents targeting IL-1, IL-6 receptor, or p40 subunit of IL-12/IL-23 have also been reported with favorable results [87].

35.8 Outcome and Prognosis

Behçet disease has a worse prognosis in young and male patients (Table 35.1). Amelioration of disease manifestations can be observed when patients become older [80]. Vascular and neurologic manifestations are associated with increased morbidity and mortality, which may reach as high as 9.8% in 20 years [12, 66, 80]. Ocular manifestations are associated with increased morbidity,

and effective use of immunosuppressive treatments has resulted in better outcomes with about 20% or less of patients with a final visual acuity of less than 20/200 Snellen scale within 7 years of disease course compared to much higher frequencies before 1990 [88]. Non-pharmacological approaches such as improvements in oral hygiene, employment status and lifestyle are also important components of managing Behçet disease possibly by reducing the contribution of triggering microbial insults [89–91].

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Idiopathic Recurrent Pericarditis

36

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Abstract

Idiopathic recurrent pericarditis (IRP) is a subset of pericarditis characterized by frequent true recurrences (free interval between two flares longer than 4 weeks). The term "idiopathic" refers to a non-specific etiology and is used even if an autoinflammatory pathway is suspected. The diagnosis is based on the association of typical symptoms and signs (mainly pericardial chest pain plus pericardial rub or electrocardiographic alterations or pericardial effusion). The monitoring of inflammatory markers and the use of imaging techniques help to guide management. Standard treatment includes the combination of non-steroidal anti-inflammatory drugs (NSAIDs) plus colchicine. In cases that do not respond to this combination, therapy with interleukin-1 inhibition has been remarkably effective.

Keywords

Pericarditis · Recurrent pericarditis
Autoimmune diseases · Autoinflammatory diseases · Colchicine · Immunotherapy
Anakinra · Echocardiography
Cardiac magnetic resonance

Abbreviations

AHA	Anti-heart antibodies
AIDA	Anti-intercalated-disk antibodies
AIRTRIP	The Anakinra-Treatment of Recurrent Idiopathic Pericarditis
ANA	Anti-nuclear antibodies
AP-1	Activator protein-1
APC	Antigen-presenting cell
ASA	Acetylsalicylic acid
CEACAM1	Carcinoembryonic antigen cell adhesion molecule 1
CMR	Cardiac magnetic resonance
CMV	Cytomegalovirus
CRP	C-reactive protein
CT	Computerized tomographic
DAMP	Damage-associated molecular patterns
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ESC	European Society of Cardiology

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ESR	Erythrocyte sedimentation rate
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigens
HSV-1	Herpes simplex virus
IL	Interleukin
INF	Interferon γ
IRP	Idiopathic recurrent pericarditis
IVIg	Intravenous immunoglobulins
MHC	Major histocompatibility complex
MICA	MHC class I chain related protein A
NF-kB	Nuclear factor-kappa B
NSAIDs	Nonsteroidal anti-inflammatory drugs
PAMP	Pathogen-associated molecular patterns
PPS	Postpericardiotomy syndrome
STIR	Short-tau inverted recovery
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic fever syndrome

Key Points

- **Idiopathic recurrent pericarditis may often have an autoinflammatory pathogenesis**
- **Idiopathic recurrent pericarditis is a diagnosis of exclusion (approach guided by pre-test probability that a specific condition is present)**
- **Inflammatory markers and imaging can support the diagnosis and management**
- **Interleukin-1 antagonists are very effective in difficult-to-treat patients**

36.1 Introduction

Pericarditis is a clinical disorder in which similar clinical pictures may be sustained by different mechanisms. The spectrum of possible causes is broad and the mechanisms not completely understood; in about 70% of pediatric patients and more than 80% of adult patients a specific etiology cannot be detected and pericarditis is therefore considered idiopathic [1, 2], even if the etiopathogenesis is presumed to be viral or immune-mediated. Typically the disease has a good prognosis with a full recovery within several weeks. However in about 20–40% of cases relapses occur. Recurrences are one of the most challenging management issues and a common reason of concern for both the physician and the patient. In recent years idiopathic recurrent pericarditis (IRP) has been considered to be autoinflammatory in its behavior. The dramatic beneficial effect of the interleukin (IL)-1 receptor antagonist anakinra has solidified this notion and led to the consideration of a new pathogenetic scheme [3, 4].

36.2 Definition/Classification

The recent European Society of Cardiology (ESC) guidelines have defined acute pericarditis as an inflammatory pericardial syndrome with specific manifestations, including chest pain (85–90% of cases), usually sharp, improved by sitting up and leaning forward, the presence of a pericardial friction rub (≤1/3 of cases), electrocardiogram (ECG) changes (up to 60% of cases)—with new widespread ST elevation or PR depression in the acute phase, and pericardial effusion (up to 60% of cases) assessed by echocardiography [2]. Supportive findings include elevation of inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and the white blood cell count or other imaging evidence of pericardial inflammation [2].

Recurrent pericarditis is defined as a relapse of disease after a documented first acute episode followed by a symptom-free interval of at least 4 weeks or longer, corresponding to the completion and usual duration of anti-inflammatory therapy in most non-complicated cases [2]. It is suggested to differentiate recurrent pericarditis from *incessant* pericarditis, in which symptoms persist for more than 4–6 weeks but less than 3 months, and from *chronic* pericarditis, in which the symptoms last longer than 3 months [2].

36.3 Epidemiology

There are limited data documenting the precise incidence of idiopathic recurrent pericarditis. Acute pericarditis is common. An Italian registry reported an incidence as 27.7 cases per 100,000 population per year [2] but a recent study from Denmark documented a higher incidence (almost 168 cases per 100,000 population per year) [5]. The Italian study addressed only data from emergency rooms and hospital admissions, and the diagnosis of acute pericarditis was confirmed by the investigators, while the Danish study included also outpatients visits and probably also pericardial effusions from other causes; this may explain the apparent discrepancy between the two studies. The frequency of recurrences varied in different studies between 20 and 30% after the first episode and between 20 and 50% after the first relapse [2]. Higher frequencies are generally related to an inadequate treatment of the previous attack. A positive family history has been described in 10% of patients with recurrent idiopathic pericarditis [6].

36.4 Etiology

The causes of pericarditis are numerous and heterogeneous. Multiple triggers can initiate or precipitate the inflammatory reaction. Causes can be divided into two major categories: infectious (any type of microorganisms) and non-infectious (autoimmune, neoplastic, metabolic, traumatic or iatrogenic, drug-related and miscellaneous) [2]. In approximately 70% of pediatric patients and more than 80% of adults a specific etiology cannot be detected and pericarditis is therefore considered idiopathic.

36.5 Pathogenesis

- **Many non-specific stimuli (including viruses) may trigger attacks of acute pericarditis**
- **An autoinflammatory pathway with inflammasome activation and IL-1 release has a pivotal role in many patients**

The pathogenesis of recurrent pericarditis is currently unknown. Infectious (mainly viral), autoimmune or autoinflammatory mechanisms have been proposed.

An infectious hypothesis is supported by molecular analysis on pericardial fluid and epicardial biopsies that identified a virus in almost 20% of cases [1]. However, standard laboratory techniques are not diagnostic in the majority of cases. Recurrent attacks may result from an inability to clear a presumed viral infection. This could explain the increased risk of relapse in patients treated with corticosteroids. Anti-viral therapy is generally not considered.

Some laboratory findings seem to confirm an autoimmune pathway: antinuclear antibodies (ANA) [7] are found in about 40% of patients with recurrences as well as anti-heart and anti-intercalated-disk autoantibodies, in 50 and 25% of patients with IRP, respectively [8]. Other findings suggestive of an autoimmune pathway include the detection of proinflammatory cytokines, such as IL-6, IL-8 and interferon (INF)-γ in pericardial fluid, and the association of IRP with human leukocyte antigen (HLA)-A*02, HLA-Cw*07, and HLA-DQB1*0202, and in a lower frequency HLA-DQB1*0302 [9]. In addition, recurrent pericarditis is not an infrequent manifestation of systemic autoimmune disease, usually during a flare. It can occur in the context of systemic lupus erythematosus (20–50% of patients), vasculitides, rheumatoid arthritis and Sjogren syndrome [1, 9]. Post cardiac injury syndrome may be considered a model of autoimmune pericarditis. This syndrome is triggered by damage to pericardial tissue or the presence of blood in the pericardial cavity, related to open heart surgery and other minor procedures or during myocardial ischemia. The exposure of pericardial antigens may result in an autoimmune response activating T- and B lymphocytes. However, the role of autoantibodies in the process is not clear, as they may only be a consequence of the antigenic exposure rather than a cause of the inflammatory process [10]. While ANA may be detected, these are generally at low titres (1/40 to 1/80), are not disease-specific and

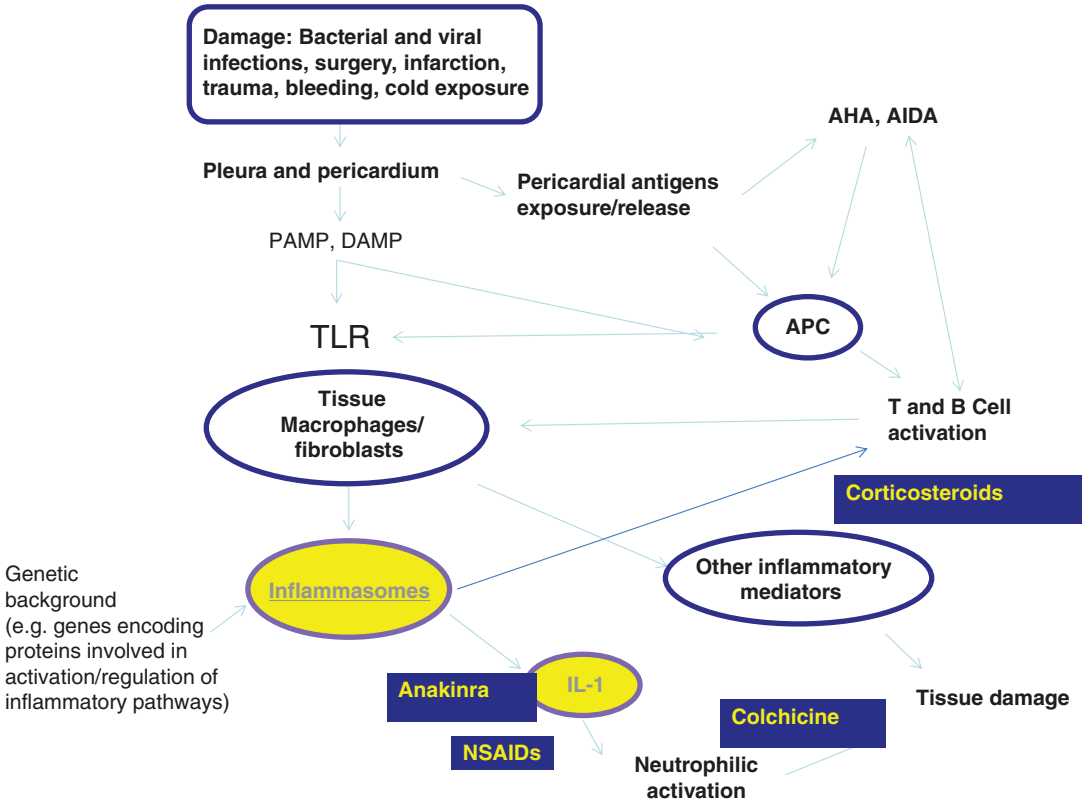

Fig. 36.1 The interplay among etiological agents, genetic factors and the immune system as determining the course of pericarditis and relapses. Drugs interfere with the release of inflammatory cytokines, acting in different steps of inflammatory cascade. *PAMP* pathogen-associated molecular patterns; *DAMP* damage-associated molecular patterns; *TLR* toll-like receptor; *AHA* anti-heart antibodies; *AIDA* anti-intercalated-disk antibodies; *IL-1* interleukin-1; *NSAIDs* nonsteroidal anti-inflammatory drugs; *APC* antigen-presenting cell

have limited clinical significance [11], as they are equally distributed in patients with IRP with or without rheumatologic disorders.

The third hypothesis proposes the involvement of the innate immune response. It derives from the recent demonstration of the spectacular efficacy of the IL-1 receptor antagonist anakinra in the treatment of recurrent pericarditis [3, 4, 12]. Recurrent episodes of pericarditis can be observed in several monogenic autoinflammatory diseases such as familial Mediterranean fever, mevalonate kinase deficiency and tumor necrosis factor (TNF) receptor-associated periodic fever syndrome (TRAPS) [13]; we observed that 6% of IRP patients carried a mutation in the *TNFRSF1A* gene (often the non-specific R92Q mutation) [14]. The dramatic effect of IL-1 inhibition suggests an important role for the inflammasome in the pathogenesis of the disease [15].

In summary, it appears that while the initial eliciting causes of pericarditis may differ the mechanisms that sustain at least the recurrence of the disease are autoinflammatory in nature, with a leading role for IL-1 (Fig. 36.1).

36.6 Clinical Manifestations

The main symptom of recurrent pericarditis is anterior chest pain, worsened by lying supine and improved by leaning forward and described by the patient as similar to previous attacks. Also, at least one objective finding of pericarditis (pericardial rub, ECG changes, and pericardial effusions) may be present [2]. These symptoms are often attenuated during recurrences, mainly during treatment. In the subset of patients with more aggressive manifestations, fever, involvement of other serosal

Table 36.1 Comparison of clinical features, etiologies and outcomes in pediatric patients (Finetti, Raatikka, Imazio) and adults (CORP2) with recurrent pericarditis according to largest published studies

Feature	Raatikka [18]	Finetti [3]	Imazio [17]	CORP 2 [19]
Number of patients studied	15	15	110	240
Fever	12 (80%)	8 (53%)	84 (76%)	73 (30%)
Chest pain	15 (100%)	13 (87%)	103 (93.6%)	239 (100%)
Pericardial rub	n/a	5 (33%)	31 (28%)	82 (34%)
ECG	10/13 (77%)	13 (87%)	49 (44%)	25% [24]
Pericardial effusion	15 (100%)	13 (87%)	86 (78%)	138 (57%)
Elevated CRP	14 (93%)	13 (87%)	102 (93%)	174 (72%)
Tamponade	1 (7%)	n/a	15 (14%)	2 (1%)
No specific etiology	8 (53%)	13 (87%)	98 (89%)	198 (82%)
PPS	7 (47%)	n/a	10 (9%)	21 (9%)
ANA positivity	1(N = 14) (7%)	n/a	18 (16%)	43% [7]
ASA/NSAIDs	4 (27%)	13 (87%)	89 (81%)	240 (100%)
Colchicine	4 (27%)	14 (93%)	68 (62%)	120 (50%)
Corticosteroids	11 (73%)	15 (100%)	70 (65%)	16 (7%)
Anakinra	0	13 (87%)	12 (17.1%)	0
Constriction (transient)	0 (0.0%)	n/a	3 (3%)	4 (7%)
Pleuropulmonary involvement	10 (67%)	n/a	54%	36% [16]
Liver involvement	n/a	n/a	9 (8%)	8% [16]

ECG electrocardiogram; *CRP* C-reactive protein; *PPS* postpericardiotomy syndrome; *ANA* anti-nuclear antibodies; *ASA* acetylsalicylic acid; *NSAIDs* nonsteroidal anti-inflammatory drugs, *n/a* not applicable

membranes (pleuropulmonary involvement in 36% of adults and 55% of children, peritoneal involvement in 5%) and elevation of liver enzymes (8% in adult) can be present [16].

In the pediatric age group the clinical presentation is often more acute and inflammatory, with more frequent pleuropulmonary and systemic involvement. However, ANA are present less frequently in children. Overall, the autoinflammatory pattern is often more evident in children with high fever, strikingly elevated CRP and pleuropulmonary involvement [3, 17] (Table 36.1).

36.7 Laboratory Testing

No specific laboratory marker is diagnostic for pericarditis. The ESC has proposed a general diagnostic approach to acute pericarditis, defining the first level investigations [2]. These include complete blood count, markers of inflammation, renal and liver function tests, thyroid function, markers of myocardial involvement (i.e. troponin, serum level of creatine kinase), ECG, echocardiography, chest radiograph and limited additional tests in related to the suspected etiology and clinical presentation [2]. The specific clinical manifestations in an individual patient should guide the diagnostic approach. Additional testing should be related to the suspected origin and clinical presentation (low-risk vs high risk; hospitalization vs non-hospitalization), based on the pre-test probability that a specific condition is present, according to the ESC guidelines [2]. In cases of recurrence three specific causes must be excluded: tuberculosis, malignancy and systemic autoimmune disease. Viral serological tests are considered futile, since viral identification has no impact on therapy and prognosis. A possible exceptions are those for human immunodeficiency virus (HIV) and hepatitis C virus (HCV). If a viral etiology is strongly suspected, generally in the first attack, a genome search with PCR is now preferred for most viruses to serology, including parvovirus, herpes simplex virus-1 and -2 (HSV 1 and 2), cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus and enteroviruses (echo and coxsackie viruses) [2].

In case of a positive family history for pericarditis or autoinflammatory (periodic fever) syndromes genetic tests for monogenic syndromes are indicated [13, 14].

CRP and ESR and other parameters of inflammation, even if not specific, are important to help

define the intensity of the inflammatory process [20]. A small study reported the use of the carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1) and the major histocompatibility complex (MHC) class I chain related protein A (MICA) as biomarkers, but further investigations are needed to clarify their possible application [21].

36.8 Imaging

Transthoracic echocardiography is the preferred imaging modality for the diagnosis of pericarditis [2]. It is a simple, non-invasive and low-cost imaging technique that can be easily performed at the bedside and in urgent/emergency settings. Furthermore it is safe and can be repeated many times without health risks. It supports the diagnosis by identifying a pericardial effusion and can show complications such as tamponade, constriction, ventricular dysfunction, etc. (Fig. 36.2). However, echocardiography does not provide precise information regarding the inflammatory process. In fact, reports of increased thickness or hyperechogenicity of the pericardium are not specific and are often limited by artefacts.

Cardiac magnetic resonance (CMR) (Fig. 36.3) is a second-level imaging technique and is helpful to study pericardial and myocar-

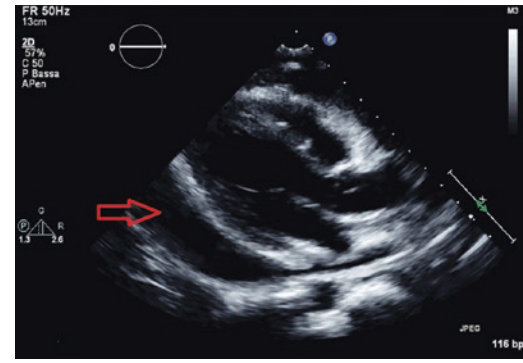

Fig. 36.2 Echocardiographic findings in acute pericarditis. The red arrow indicates a large circumferential pericardial effusion

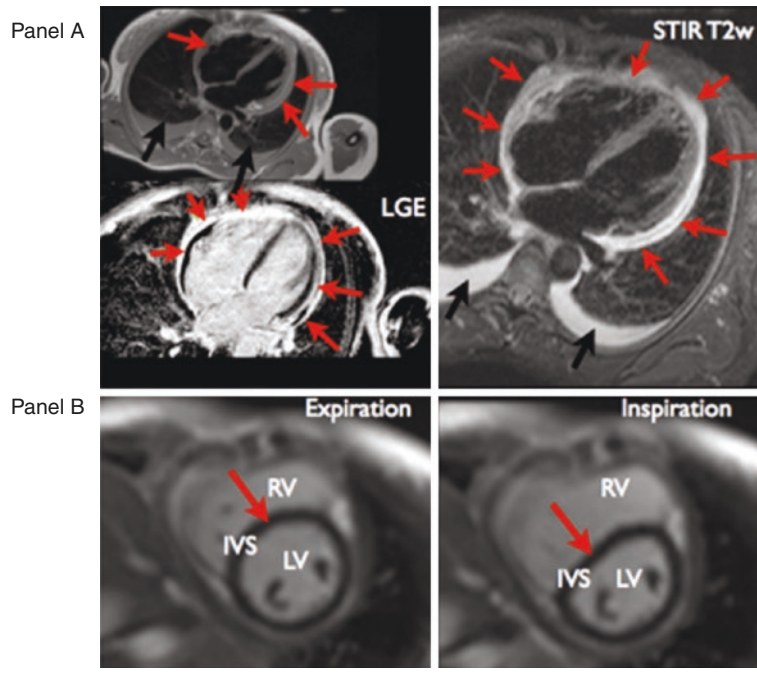

Fig. 36.3 Pericardial thickening with pericardial inflammation on CMR study (see red arrows in panel (**A**), pericardial edema on STIR T2w image and pericardial late gadolinium enhancement, concomitant pleural effusion is marked with black arrows). On panel (**B**) septal bounce is seen (see red arrows) due to exaggerated interventricular interdependence by real-time CMR imaging, expression of transient pericardial constriction. *CMR* cardiac magnetic resonance; *STIR* short-tau inverted recovery; *LGE* late gadolinium enhancement; *RV* right ventricle; *IVS* interventricular septum; *LV* left ventricle

dial tissues [2, 22]. On T1-weighted imaging the normal pericardium appears like a thin hypointense ("dark") curvilinear structure surrounded by hyper-intense ("bright") mediastinal and epicardial fat. CMR can assess pericardial thickness (normal value is <4 mm) and pericardial edema. On T2-weighted short-tau inverted recovery (STIR) fast spin-echo sequences, pericardial edema appears bright. The tissue edema is not well defined if there is a concomitant effusion, which appears as bright as the edema. Following intravenous administration of paramagnetic gadolinium chelates, enhancement may show extension of inflammation into the surrounding epicardial fat, which suggests severe inflammation. CMR is useful also in showing myocardial inflammatory involvement, fibrosis and evolving constrictive pericarditis. The technique has some disadvantages, first of all its limited availability and its costs and also the need of breath-holding and regular heart rhythms to get a better picture quality. Contraindications include pacemakers, claustrophobia and renal insufficiency.

Computerized tomographic (CT) scanning is considered a complementary imaging modality (Fig. 36.4). It is useful in order to assess anatomic features and presence of calcifications [2,

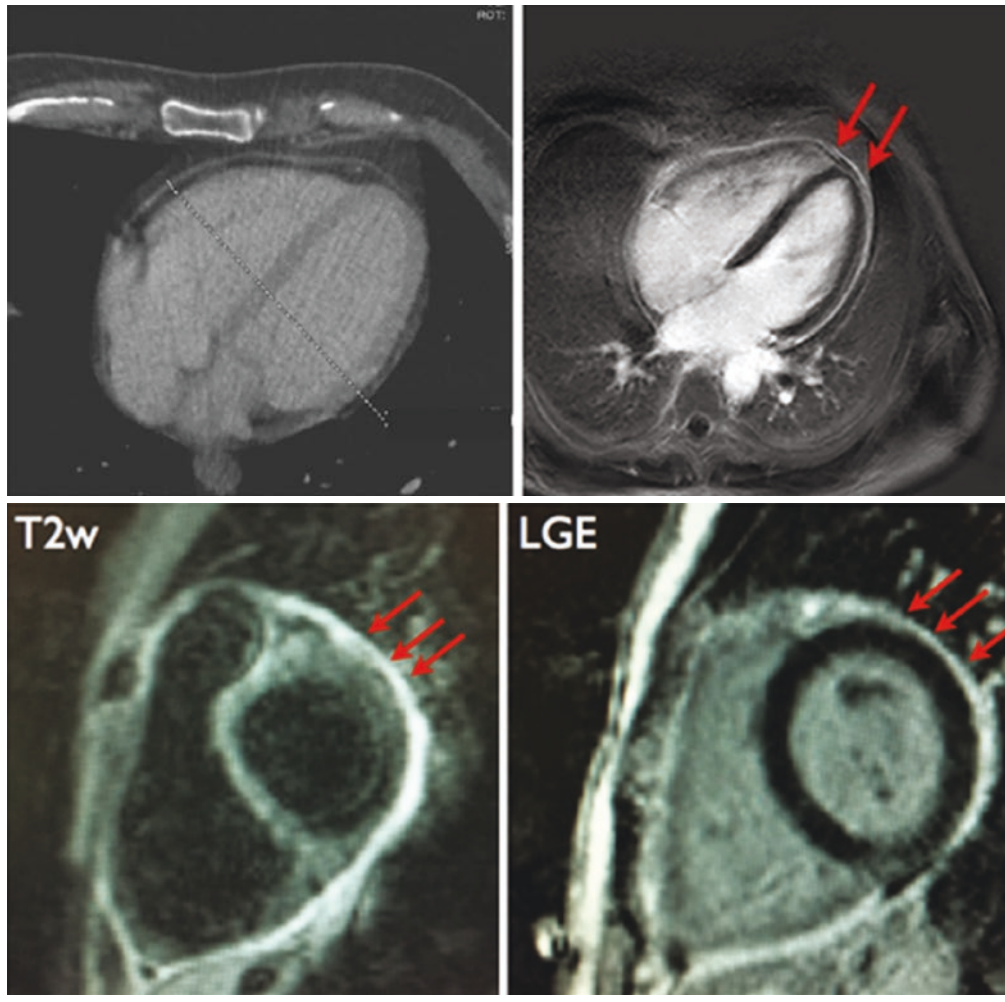

Fig. 36.4 Pericardial inflammation is detected by mild contrast-enhancement of the pericardium by CT (TAC). Pericardial edema is particularly evident by cardiac magnetic resonance on STIR T2w image and as pericardial late gadolinium enhancement (see red arrows)

23]. On CT, the normal pericardium is visible as a thin curvilinear structure surrounded by the hypodense mediastinal and epicardial fat, and has a thickness ranging from 0.7 to 2.0 mm. CT can depict focal effusions, precisely quantify the amount of fluid and, depending on attenuation values of fluid (HU), it can provide information about the nature of the effusion. Low attenuation values (e.g. 0–20 HU) suggest a transudate, intermediate values (e.g. 20–60 HU) are indicative of exudative effusions, while high attenuation values (>60 HU) suggest haemorrhage. Intravenous administration of iodinated contrast material may allow the detection of pericardial inflammation because of the enhancement of the inflamed pericardium after contrast injection. CT is the first choice in the pre-operative work-up in case of pericardiectomy, in order to appraise the extension of calcification of the pericardium. It is also useful to exclude specific causes, mainly neoplastic disease, if extended to the remainder of the chest and abdomen [23]. Its limits are mainly related to radiation exposure and renal insufficiency, if iodine contrast is used.

36.9 Treatment

- **The therapy of pericarditis is based on the association between high-dose non-steroidal anti-inflammatory drugs, low-dose colchicine and eventually also corticosteroids at low doses**
- **Immunotherapy should be considered in case of treatment-resistant cases. These include azathioprine, intravenous immunoglobulins and interleukin-1 antagonists**
- **IL-1 antagonists (anakinra) are very effective in difficult-to-treat patients**

In the past the treatment of pericarditis was largely empirical, because its etiology and mechanisms were usually unknown. Better understanding of the inflammatory basis of pericarditis has led to more rational use of treatment and a novel therapeutic approach.

The cornerstone of therapy for pericarditis remains anti-inflammatory therapy with either aspirin or non-steroidal-anti-inflammatory drugs (NSAID) plus colchicine [2]. NSAIDs must be taken at high anti-inflammatory doses (Tables 36.2 and 36.3).

Table 36.2 Aspirin and non-steroidal anti-inflammatory drugs in the treatment of pericarditis: recommended regimens in adults (modified from guidelines). The wide dosages ranges are based on weight, age, severity of the attack and subjective tolerability

Drug	Treatment dose	Length of treatment	Tapering
Aspirin	500–1000 mg every 6–8 h (1.5–4 g/d)	FIRST attack: 2–4 weeks RECURRENCES: several weeks to months The optimal length of treatment is debatable, and CRP should probably be used as a marker of disease activity to guide management and treatment length. Gradual tapering (every 1–2 weeks and only if the patient is asymptomatic and CRP is normal), is recommended	Decrease the total daily dose by 250–500 mg every 2–4 weeks
Ibuprofen	600–800 mg every 8 h (1600–3200 mg daily)		Decrease the total daily dose by 200–400 mg every 2–4 weeks
Indomethacin	25–50 mg every 8 h (150 mg daily)		Decrease the total daily dose by 25 mg every 2–4 weeks
Naproxen	250–500 mg every 12 h; maximal daily dose 1500 mg for limited time period (<6 months). Dosage expressed as naproxen base; 200 mg naproxen base is equivalent to 220 mg naproxen sodium		Decrease the total daily dose by 125–250 mg every 1–2 weeks

Start at the lower end of dosing range and titrate upward. According to local availability of the different agents we recommend intravenous use of NSAIDs in hospitalized symptomatic patients
Geriatric dosing: refer to adult dosing. Use lowest recommended dose and frequency
Renal impairment dosing: CrCl <30 mL/min: NSAIDs use is not recommended (for aspirin: use is not recommended if CrCl <10 mL/min)
Hepatic impairment dosing: use with caution; dose adjustment may be required [2]
CRP C-reactive protein; *NSAIDs* nonsteroidal anti-inflammatory drugs

Table 36.3 Aspirin and non-steroidal anti-inflammatory drugs: recommended doses in children with pericarditis (modified from guidelines)

Drug	Treatment dose	Length of treatment and tapering
Ibuprofen	30–50 mg/kg/24 h divided every 8 h; maximum: 2.4 g/day	FIRST attack: 2–4 weeks. RECURRENCES: several weeks to months The optimal length of treatment is debatable, and CRP should probably be as a marker of disease activity to guide management and treatment length. A gradual tapering (every 2–4 weeks and only if the patient is asymptomatic and CRP is normal), should be considered
Indomethacin	Children ≥2 years: oral: 1–2 mg/kg/day in 2–4 divided doses; maximum dose: 4 mg/kg/day; not to exceed 150–200 mg/day)	
Naproxen	Children >2 years: oral suspension is recommended: 10 mg/kg/day in 2 divided doses (up to 20 mg/kg/day has been tolerated)	

Start at the lower end of dosing range and titrate upward. *CRP* C-reactive protein

Colchicine is recommended without a loading dose and using weight-adjusted doses (i.e. 0.5 mg once daily if body weight is<70 kg or 0.5 mg twice daily if it is ≥70 kg, for ≥6 months) in adults, in order to improve remission rates and prevent recurrences [2, 24]. In children with IRP commonly used doses are 0.5 mg/day in children younger than 5 years and 1–1.5 mg/day in older children [2]. Higher doses, sometimes used in familial Mediterranean fever, have generally not been considered (see Chap. 41 for further details on the mechanism and use of colchicine).

In cases of incomplete response corticosteroids may be added particularly in adults to aspirin/NSAIDs and colchicine as a triple therapy, but at low to moderate doses (i.e. prednisone 0.2–0.5 mg/kg/day in adults) [2].

Corticosteroids block transcription factors such as nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1) which are involved in the transcription of several inflammatory mediators.

Although corticosteroids provide rapid relief of symptoms, they increase the risk of the pericarditis turning into chronic disease or increased number of recurrences [2, 16, 17, 19, 22, 24], and have many side effects. They should be used only in selected patients with specific indications (i.e. systemic inflammatory diseases, post-pericardiotomy syndromes, impending cardiac tamponade, pregnancy), NSAID contraindications (true allergy, high risk of bleeding, renal insufficiency), and intolerance or resistance to standard therapy [2]. If corticosteroids are used, they should be tapered very slowly. Therapy and subsequent tapering should be guided by CRP values as an indicator of the inflammatory process and the potential for relapse. Long-term corticosteroid use is particularly worrisome in pediatric patients due to their multiple side-effects, including growth impairment.

36.9.1 Immunotherapy and IL–1 Inhibition

Immunotherapy is an alternative approach to the treatment of resistant IRP [2]. Three medications have been proposed: azathioprine, intravenous immunoglobulin (IVIg) and anakinra.

Data of the efficacy of azathioprine as therapy for IRP are few and based only on case reports or case series of adults and one retrospective cohort study [25].

The use of IVIg was reported in two small case series and one retrospective analysis describing only 14 cases [2]. IVIg has a rapid onset of action and can be used as a corticosteroid-sparing agent. However, the high cost and lack of good evidence do not really support its routine administration.

Anakinra is a recombinant IL-1 receptor antagonist that inhibits the action of IL-1 (see Chap. 42). The IL-1 intracellular signaling pathway is involved in the activation of T cells, stimulation of metalloproteinases, prostaglandin release by macrophages and in chemotaxis of monocytes, lymphocytes and polymorphonuclear leukocytes. Anakinra was initially developed for

the treatment of rheumatoid arthritis but has found its niche in the treatment of many autoinflammatory diseases. Its efficacy in IRP was first recognized in the pediatric population [3, 17], and this proof of concept was of paramount importance. Data derived from case reports, cohort studies, one retrospective analysis and a meta-analysis, confirmed these findings. A recent double-blind randomized controlled trial (AIRTRIP-The Anakinra-Treatment of Recurrent Idiopathic Pericarditis) formally demonstrated the spectacular effects of anakinra in 21 patients with corticosteroid-dependent and colchicine-resistant recurrent pericarditis with elevated CRP [4]. Anakinra proved highly effective, achieving quick symptoms relief in a few days and allowed steroid discontinuation in all patients within 6 weeks. It is administered as a once daily subcutaneous injection at 1–2 mg/kg/d (maximal 100 mg) for several months [4]. After clinical stabilization recurrences can occur if tapering is too rapid. Tapering regimes are not well established yet. A possible scheme might be to withdraw a dose every month after a full control of the disease has been reached: e.g. 1st step 100 mg/d, every day for 6 months; 2nd step in the 7th month, 100 mg/d, 6 times per week; 3rd step 100 mg/d, 5 times per week for 1 month, and so on until the 7th step, 100 mg once per week, in the 14th month. A critical point for recurrence might be at the dose of 2 doses weekly.

The drug is generally well tolerated. The most common adverse events are skin reactions at the site of injection, neutropenia and mild elevation of transaminases.

In children anakinra might now be considered prior to corticosteroids, to avoid their side-effects in the growing child. There are only few case reports on the use of other anti-IL1 antagonists in IRP.

36.10 Outcome/Prognosis

Severe complications are uncommon in IRP [2]. Cardiac tamponade is rare and generally occurs at the beginning of the disease. Constrictive pericarditis has not been reported in IRP, despite numerous recurrences [16, 26], and the overall risk is lower than that recorded after a first episode of acute pericarditis (less than 1%) [27]. Thus, it is important to reassure patients about their prognosis, explaining the nature of the disease and its likely course. Drug treatment should take into account this favourable outcome to avoid more toxic agents. However, the quality of life can be severely impaired in patients with repeated recurrences and corticosteroid dependence [2]. IRP may last for years with recurrent flares if not properly treated. IL-1-inhibitors have proved to be rapidly acting and highly efficient also in refractory cases and their role in the management of IRP is becoming more prominent. In our experience most patients continue treatment for 1–3 years, at low doses. At present, approximately 40% have discontinued treatment. In case of recurrence during anakinra tapering, NSAIDs may be useful to control mild recurrences [4].

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Schnitzler Syndrome

37

Heleen D. de Koning and Karoline Krause

Abstract

Schnitzler syndrome (SchS) is a late-onset autoinflammatory disease characterized by the association of a chronic urticarial rash and monoclonal gammopathy with signs and symptoms of systemic inflammation. Clinical efficacy of IL-1ß blocking drugs revealed the key role of IL-1ß in the pathophysiology of SchS. This was corroborated by *in vitro* and genetic studies. Anti-IL-1ß treatment abrogates the systemic inflammation, but leaves the monoclonal gammopathy unaffected. The role of the monoclonal gammopathy (cause or consequence) is the major question that remains to be resolved.

Keywords

Schnitzler syndrome · Autoinflammatory Urticaria · Monoclonal gammopathy Interleukin-1

Abbreviations

CAPS	Cryopyrin-associated periodic syndrome
CNO	Chronic non-bacterial osteomyelitis
CRP	C-reactive protein
CSU	Chronic spontaneous urticaria
ESR	Erythrocyte sedimentation rate
Ig	Immunoglobulin
IL-1	Interleukin-1
MGUS	Monoclonal gammopathy of unknown significance
MRP	Myeloid-related protein
NUD	Neutrophilic urticarial dermatosis
PBMCs	Peripheral blood mononuclear cells
SchS	Schnitzler syndrome
TLR	Toll-like receptor

Key Points

- **The main features of Schnitzler syndrome include a chronic urticarial rash, monoclonal gammopathy and signs and symptoms of systemic inflammation**
- **Schnitzler syndrome is an IL-1ß driven autoinflammatory disease**
- **IL-1ß blocking therapy abrogates the systemic inflammation and all symptoms, but does not influence the monoclonal gammopathy**

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37.1 Introduction

In 1972, the French dermatologist Liliane Schnitzler first described an enigmatic inflammatory disorder in a 63-year-old male patient presenting with a 4-year history of chronic urticarial rash, monoclonal gammopathy of the IgMκ class and persistent systemic symptoms including recurrent fever attacks, bone pain with radiologic signs of osteosclerosis, lymphadenopathy and elevated erythrocyte sedimentation rate (ESR) [1]. Investigations for underlying infectious or neoplastic disorders were all negative and the patient was treated with prednisone and chlorambucil to which he responded with moderate improvement of his general symptoms but persistence of urticarial exanthema and elevated ESR. After 20 years of follow-up, the patient developed lymphoplasmocytic lymphoma and died.

Following additional case reports of French origin, Schnitzler syndrome (SchS) was considered a unique entity characterized by recurrent urticarial exanthema and monoclonal gammopathy of unknown significance (MGUS) combined with signs of systemic inflammation such as fever episodes and musculoskeletal symptoms [2]. Based on its interleukin-1 (IL-1)-related phenotype closely mimicking adult-onset cryopyrin-associated periodic syndrome (CAPS), dysregulated inflammasome function and its immediate responsiveness to IL-1 blockade, SchS is classified as an acquired late-onset autoinflammatory disease [3, 4].

The paraprotein, as the main criterion of the disease, typically belongs to the immunoglobulin M (IgM) class or less often to the IgG class [5]. Currently, it remains unknown whether the paraprotein functions as initiator of IL-1ß-mediated inflammation, or results from excessive inflammation.

37.2 Epidemiology

Since its initial description, more than 320 individual cases of SchS, most of Caucasian ancestry, have been reported in the literature. Most reports are from central European countries with the highest numbers of patients coming from France and Germany. However, numerous cases from other parts of the world including American and Australasian countries were described as well. Despite increased disease awareness during the last several years, SchS is still believed to be underdiagnosed and often associated with a diagnostic delay of years or even decades. A recent large French-Italian study described a mean diagnostic delay of 31 months among SchS patients treated at expert centers [6]. Disease onset ranges from 13 to 76 years with a median age of 51 years. SchS affects both males and females with a ratio of 1.5:1 [7].

37.3 Etiology and Pathogenesis

- IL-1β hyperactivation causes the systemic inflammation in SchS
- Somatic mosaicism of mutations in the *NLRP3* gene was found in two variant SchS patients
- The role of the paraprotein is still unknown

Previously, SchS was considered to have an autoimmune or hematological origin. More recently, clinical and laboratory findings pointed towards a crucial role of the innate, rather than the acquired, immune system. For example, in 70% of skin biopsies, immune depositions were absent, and in the remaining samples, findings were heterogeneous and non-specific [7]. Earlier studies in MGUS and multiple myeloma suggested a role for chronic antigenic stimulation via microbial agents or autoantibodies in the development of monoclonal gammopathy [8, 9]. Hence, it is unlikely that the paraprotein in SchS initiates the inflammation in the skin in an antibody-specific manner. It is not known whether it can activate the innate immune system. In most cases, it is unknown whether the monoclonal gammopathy developed prior to or after the clinical symptoms appeared. Further, the concentration of the paraprotein does not correlate with disease activity [10]. However, it cannot be excluded that the paraprotein might be involved in the formation of the second hit required for inflammasome signaling.

The first clue to the autoinflammatory nature of the disease came from the remarkable efficacy of treatment with anakinra, an IL-1 receptor antagonist [11, 12]. This therapy was tried in light of the efficacy in CAPS and the clinical similarities of SchS and CAPS. Anakinra blocks both IL-1α and ß, but IL-1ß appears to be the key mediator of inflammation, as treatment with the IL-1ß antibody canakinumab proved to be highly effective [13, 14]. This was supported by *ex vivo* data. First, hypersecretion of IL-1β, IL-6 and TNF-α by peripheral blood mononuclear cells (PBMCs) of a patient was found. This hypersecretion was blocked by the *in-vitro* addition of the caspase-1 inhibitor YVAD [4]. In another patient, monocyte IL1ß gene expression was inhibited by anakinra treatment [15].

In a study of eight patients sequentially treated with anakinra, no therapy and canakinumab, an mRNA microarray of PBMCs showed distinct transcriptomes for controls, symptomatic patients and anti-IL-1-treated patients. *In vitro*, LPS stimulation induced higher IL-6 and IL-1ß production in PBMCs from symptomatic SchS patients compared to healthy controls. Interestingly, patient cells were relatively hyporesponsive to poly:IC and Pam3Cys; TLR3 and TLR2 ligands, respectively, so there seems to be distinct sensitivity of PBMCs of SchS patients to stimulation of different pattern recognition receptors. Hence, these findings require further investigation [10]. Serum IL-6, IL-1 receptor antagonist and IL-6 levels in lysates of freshly isolated PBMCs, as well as serum myeloid-related protein (MRP8)/14 and S100A12 levels correlated with disease activity [10, 14]. Elevated levels of IL-6 were reported in 10 other patients [7]. As yet, it is not known if all of the effects of IL-1ß in SchS are mediated via IL-6, but the high efficacy of IL-6 blockade in 3 (of 3) patients suggests a pivotal role of IL-6 [16]. IL-1ß was found in the skin of a SchS patient [17]. Intriguingly, this cytokine was present in dermal mast cells in both lesional and non-lesional skin of eight patients, and not in the skin of healthy controls or in patients with chronic non-specific urticaria (CNU) [18]. This was confirmed by a recent study, which additionally showed the presence of IL-6 in mast cells and the presence of IL-1ß and IL-18 in neutrophils [19]. Based on these data, immunohistochemical staining of a cytokine panel could help distinguish SchS from CSU skin lesions. Replication studies are needed, as IL-1ß-positive neutrophils were found in the latter study, whereas only mast cells stained positive for IL-1ß in the former.

The autoinflammatory phenotype and central role of IL-1ß rendered a causative mutation in an inflammasome-related gene likely. *NLRP3* was the most analyzed gene in SchS due to its causative role in CAPS. The Q703K polymorphism was found in three patients [14, 20], the V198M variant, of which the pathophysiological relevance is unknown, was present in two cases [21, 22], and no mutations were found in 16 cases. However, a CAPS-associated F523L mutation and a pathogenic K435E variant of the *NLRP3* gene were identified in two variant IgG-type SchS patients. Intriguingly, in these cases the mutant cells displayed somatic mosaicism and only occurred in the myeloid lineage. PBMCs of both patients spontaneously produced large amounts of IL-1ß *in vitro* [23]. As mosaicism can be missed by older detection techniques such as Sanger sequencing, next generation sequencing might reveal additional cases of mosaicism of mutations in inflammasome-related genes in SchS patients in the future.

37.4 Clinical Manifestations

- A chronic urticarial rash affects all patients
- Other symptoms include intermittent fever, arthralgias and bone pain
- Quality of life is markedly impaired

With the onset of the first clinical symptoms in middle-aged individuals, SchS shows a chronic disease course and persists for life. In most patients, urticarial rash, the main clinical symptom which is present in all cases, is also the first symptom. The rash is primarily located on the trunk and extremities, sparing the head, hands and feet (Fig. 37.1). Individual lesions are urticarial or erythematous macules or maculopapules, that last between several hours and a

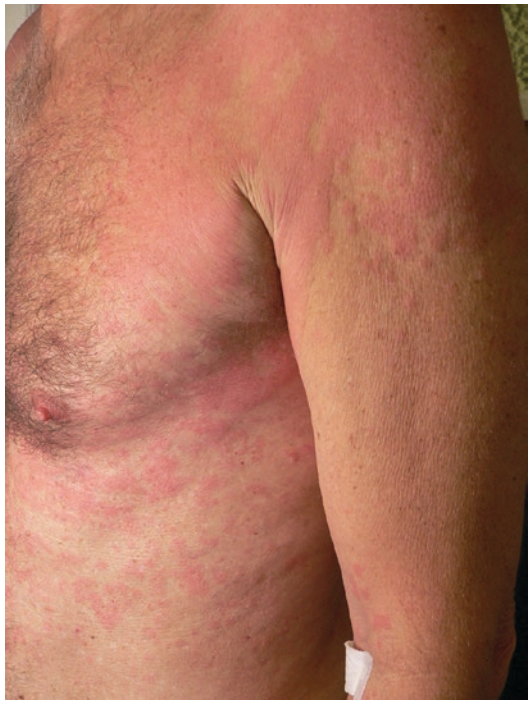

Fig. 37.1 Urticarial rash in a 62-year-old male patient with SchS

maximum of 24 h, in contrast to rather fleeting histamine-mediated classical urticaria. Similar to CAPS patients, the urticarial exanthem is non-pruritic or only mildly pruritic and/or burning and shows a diurnal pattern with maximum intensity in the evening hours [24]. The majority of patients report daily rashes. Nevertheless, some individuals experience skin symptoms only occasionally and have no skin lesions in between.

Systemic symptoms in SchS are variable and depend on individual disease activity. In most patients, systemic symptoms develop months or even years after the initial urticarial rash. However, reports of non-specific musculoskeletal pain and malaise preceding the skin symptoms exist as well. Intermittent, non-periodic, fever episodes are reported in 70% of cases (Table 37.1). Many patients suffer from daily attacks of fever up to 40 °C or below-normal temperatures with chills in the evening accompanied by urticarial rash. Others experience only a few fever episodes lasting for a couple of days per year and others completely lack episodes of fever. Another typical feature of SchS

Table 37.1 Clinical features[a]

	Percentage	Number of cases
Clinical features		
Chronic urticaria	100%	323
Pruritus	24%	69
Intermittent fever	70%	227
Arthralgia, rarely overt arthritis	68%	220
Bone pain	53%	172
Weight loss	15%	48
Lymphadenopathy	25%	80
Hepatomegaly	9%	29
Splenomegaly	6%	19
Neuropathy	7%	21
Laboratory investigations		
Elevated ESR/CRP	97%	194/199
Leukocytosis	78%	134/172
Anemia	65%	66/102
Paraprotein	**100%**	**323**
IgMκ	80%	259
IgMλ	8%	27
IgGκ	7%	21
IgGλ	2%	6
Other (including biclonal)	3%	10
Bence Jones proteins	25%	16/65

[a]Updated and adapted from De Koning [7]

is chronic bone pain, which commonly affects the tibia and/or pelvic bones and worsens over time. Also, two thirds of patients report arthralgia of the large joints and diffuse myalgia, whereas genuine arthritis is a rare finding. Additional general symptoms owing to the chronic inflammation include fatigue and weight loss. Also, reactive lymphadenopathy and hepatosplenomegaly are described. Differences in the clinical phenotype of SchS and CAPS (e.g. bone pain, reactive lymphadenopathy and hepatosplenomegaly, all of which are rarely found in CAPS) may result from the monoclonal gammopathy. Neurologic manifestations occur in a minority of patients and include polyneuropathy and positional vertigo. The neuropathy usually affects the sensorimotor function of the distal lower extremities [25], progresses during the course of the disease and is not responsive to IL-1 blockade. Distal acquired demyelinating sensory neuropathy is common in IgM MGUS and in part is linked to neural antigens such as myelin-associated glycoprotein [26], which was occasionally reported in SchS as well [25]. Interestingly and comparable to CAPS patients, several patients experience higher disease activity during the cold winter months. Cold temperatures (besides stress) are a major trigger of skin and systemic symptoms in a subset of patients.

Besides the physical symptoms of the disease, most patients also experience considerable quality of life impairment with impact on social and family life as well as work performance [13, 14].

37.5 Blood and Urine Markers

In most patients with SchS, inflammation markers such as C-reactive protein (CRP) and ESR are constantly elevated and correlate well with disease activity. Further signs of systemic inflammation are leukocytosis (78%) with neutrophilia and/or monocytosis and mild to moderate normocytic or microcytic anemia in 65% of patients (Table 37.1). In addition, other serum biomarkers of autoinflammatory diseases, including serum amyloid A, S100 proteins S100 A12 and IL-1 receptor antagonist (IL-1Ra) levels are markedly increased in SchS [13, 14].

Monoclonal gammopathy reveals a spike in the gamma band of the serum electrophoresis and needs to be confirmed by immune fixation. Most often, the paraprotein belongs to the IgM class and exhibits a kappa light chain. IgM with light chain lambda or IgG kappa/lambda are commonly found. However, single cases with biclonal gammopathy combining IgM and IgG or even IgA gammopathy have been described [14]. Increased serum concentrations of free light chains and an abnormal kappa/lambda ratio are found as well. In 25% of patients (usually those with very high paraprotein concentrations), immunoglobulin light chains can be detected as Bence Jones proteins in the urine. Over time, most patients followed for several years or decades, show a gradual increase in paraprotein levels.

Initial laboratory tests in SchS should include inflammation markers, differential blood count, serum electrophoresis and immune fixation. Also, free light chain concentrations, Bence Jones proteins, serum calcium, alkaline phosphatase and ß2 microglobulin levels are recommended as prognostic markers of lymphoproliferative disease development [5]. Depending on the individual clinical presentation, further laboratory tests are performed to exclude hematologic, infectious, autoimmune, immunodeficiency or other autoinflammatory diseases. After the clinical diagnosis of SchS is established, laboratory measures should be routinely monitored to prevent long-term complications. CRP levels, blood count and kidney function should be assessed every 3 months. Quantitative paraprotein levels should be followed up according to the diagnostic guidelines for MGUS (<10 g/L every 12 months; <30 g/L every 6 months; >30 g/L every 3 months) [5].

37.6 Bone Marrow Examination

A bone marrow biopsy is performed in symptomatic SchS patients with a newly diagnosed monoclonal gammopathy to exclude hematologic malignancy. In most cases plasma cells are less than 10% of the cellular aspirate without signs of

other B-cell lymphoproliferative disorders. In some reports, moderate plasmacytosis was found, and few low-grade B-cell malignancies were mentioned [7]. The establishment of the absence or presence of a low-grade malignancy can be difficult in some of the cases of monoclonal gammopathy.

37.7 Skin Histopathology

Histopathologic findings in lesional skin of SchS are characterized by a neutrophil-rich perivascular and interstitial dermal infiltrate (Fig. 37.2). Compared to lesional skin of chronic spontaneous urticaria, there is no significant dermal edema. The histologic features are consistent with neutrophil urticarial dermatosis (NUD) which may also be present in other autoinflammatory disorders such as CAPS or adult-onset Still's disease and autoimmune diseases such as systemic lupus erythematosus [27]. Signs of vasculitis, e.g. erythrocyte extravasation, fibrinoid deposits or necrosis of the vessel wall, are usually absent. In some publications, the microscopic findings are classified as urticarial vasculitis [25], which may be motivated by signs of mild leukocytoclasis and rather broad interpretation of histopathologic criteria for urticarial vasculitis.

37.8 Imaging

The clinical presentation of chronic bone pain originates from osteosclerotic changes and increased bone formation. A retrospective study of 22 patients with SchS revealed osteosclerosis in 14 (64%) of these patients involving particularly the distal femur, proximal tibia and innominate bones. Osteosclerotic changes of the proximal tibia and distal femur are denominated as "hot knee sign" and these changes are known to occur in Erdheim-Chester disease, a rare non-Langerhans cell histiocytosis. Conventional radiography is less sensitive in detecting osteosclerotic changes as compared to bone scan, MRI and PET scan techniques. Some authors suggest bone scan as most appropriate method to screen for osseous abnormalities in patients with suspected SchS [28]. Indeed, a review reported the presence of osteosclerosis in 39% of conventional X-rays whereas increased uptake was present in 85% of reported bone scans [7].

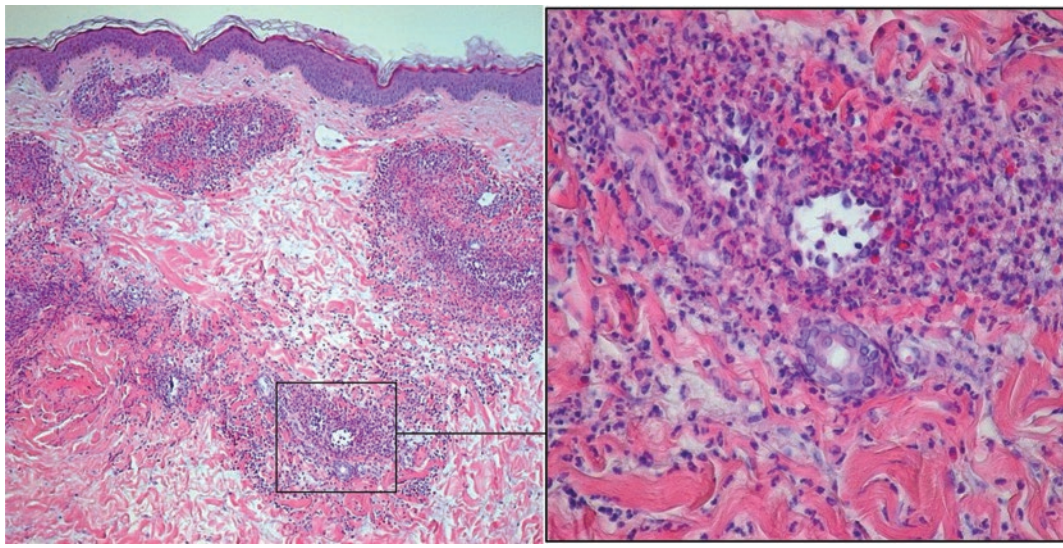

Fig. 37.2 Routine histology of SchS lesional skin: dermal infiltrate of mainly neutrophils, without edema. Original magnification 50×

In cases with clinical signs of significant lymphadenopathy or hepatosplenomegaly, exploratory sonography is recommended.

37.9 Diagnosis

A diagnosis of SchS relies on the combination of clinical criteria. These were first defined by Lipsker et al. in 2001 [29] and re-defined following reports on numerous novel cases and an expert consensus meeting in 2012 [5]. The diagnostic criteria, referred to as the Strasbourg criteria, include two obligatory criteria: chronic urticarial rash and monoclonal gammopathy (IgM or IgG) (Table 37.2). Minor criteria of the disease include recurrent unexplained fever, abnormal bone remodeling, a neutrophilic dermal infiltrate on skin biopsy and leukocytosis and/or CRP elevation. For a definite diagnosis of SchS, both obligatory criteria and at least two minor criteria must be fulfilled in cases with IgM paraprotein. In less common cases with IgG paraprotein, three minor criteria must be present. A probable diagnosis of SchS can be made, if both obligatory criteria and either one minor criterion (IgM) or two minor criteria (IgG) are found. A recent validation study assessed the sensitivity and specificity of the diagnostic criteria for SchS. For this study, 42 patients with established clinical diagnosis of SchS and 38 controls (12 patients with adult-onset Still disease, 7 with CAPS, 9 patients with Waldenström's disease and 10 with chronic spontaneous urticaria) treated at 14 centers in France and Italy between 2009 and 2014 were included. For the Strasbourg criteria, a sensitivity of 81% and 93% and a specificity of 100% and 97% were found, respectively, for a definite and probable diagnosis. In this study, the older Lipsker diagnostic criteria attained a higher sensitivity of 100%. However, selection bias is likely as many of the included patients were previously diagnosed with SchS based on the Lipsker criteria and not the Strasbourg criteria, as the latter only became available in 2013 [6].

Table 37.2 Diagnostic Strasbourg criteria for Schnitzler syndrome[a]

Obligate criteria
Chronic urticarial rash +
Monoclonal IgM or IgG
Minor criteria
Recurrent fever[b]
Objective findings of abnormal bone remodeling with or without bone pain[c]
A neutrophilic dermal infiltrate on skin biopsy[d]
Leukocytosis and/or elevated CRP[e]
Definite diagnosis if
Two obligate criteria AND at least two minor criteria if IgM, and three minor criteria if IgG
Probable diagnosis if
Two obligate criteria AND at least one minor criterion if IgM, and two minor criteria if IgG

[a]Adapted from Simon et al. [5]
[b]A valid criterion if objectively measured. Must be >38 °C, and otherwise unexplained. Occurs usually—but not obligatory—together with the skin rash
[c]As assessed by bone scintigraphy, MRI or elevation of bone alkaline phosphatase
[d]Corresponds usually to the entity described as 'neutrophilic urticarial dermatosis' (Medicine 2009;88:23–31); absence of fibrinoid necrosis and significant dermal edema
[e]Neutrophils >10,000/mm^3 and/or CRP >30 mg/L

37.10 Differential Diagnosis

The differential diagnosis of SchS includes several skin, bone, systemic, hematologic and infectious diseases, as individual characteristics can mimic many other diseases. Hence, diagnoses that should be excluded are shown in Table 37.3.

37.11 Treatment

- IL-1β blocking treatment is almost invariably highly effective
- Anti-IL-6 treatment may be an effective alternative
- Paraprotein levels seem unaffected by IL-1 blockade

Numerous different treatment options for SchS have been published. Still, approved therapies are completely lacking in SchS, and patients

Table 37.3 Differential diagnosis

Skin
Chronic spontaneous urticaria
Delayed pressure urticaria
Urticarial vasculitis
Other urticarial autoinflammatory diseases (adult-onset Still's disease [AOSD], Cryopyrin-associated periodic syndrome [CAPS])
Fever of unknown origin
Infection
Malignancy
Autoimmune disease (e.g. systemic lupus erythematosus, cryoglobulinemia)
Immunodeficiency
Bone inflammation
Chronic non-bacterial osteomyelitis (CNO)
Erdheim-Chester disease
POEMS syndrome
Systemic mastocytosis
Lymphoma
Hematology
MGUS
Waldenström's disease
Multiple myeloma
Other lymphoproliferative diseases
Cryoglobulinemia

rely on off-label treatment and/or participation in clinical studies. Historically, patients were treated with anti-inflammatory, immunosuppressive and anti-neoplastic drugs including systemic corticosteroids, non-steroidal anti-inflammatory drugs, alkylating agents, colchicine, dapsone, cyclosporin, azathioprine and many other agents. Most of these drugs showed only limited or no efficacy. Also, treatment strategies targeting the urticarial rash such as antihistamines or UV therapy were not effective. Since IL-1 blocking drugs became available and demonstrated rapid and high efficacy in initial case reports [11, 12], many patients with SchS were shown to benefit from this treatment. The highest evidence of efficacy, with 97 treated patients described in single cases or case series, arises from the use of the IL-1 receptor antagonist anakinra. More than 90% of SchS patients exhibited a good or complete clinical response to anakinra, 100 mg per day given by SC injection (Table 37.4). The monoclonal IL-1ß specific antibody canakinumab was assessed in two clinical trials (one of them placebo-controlled) with 8 and 20 patients respectively and variable doses of 150 to 300 mg given every 4 weeks to on demand treatment [13, 14]. Both studies showed high clinical and laboratory efficacy within a few days with a lasting effect after discontinuation for up to 8 months. Only few clinical data in SchS are available from treatment with rilonacept, an IL-1 soluble receptor transfusion protein. A small open-label study with 8 patients who received weekly rilonacept injections of 160 mg, reported good responses in the majority of patients [30]. Only three patients were described who did not adequately respond to anti-IL-1 treatment, although they were clinically diagnosed as typical SchS patients. In these patients, IL-6 blockade (tocilizumab 8 mg/kg, given intravenously every 4 weeks), as used for systemic juvenile idiopathic arthritis, was very effective [16]. This might imply that these patients form a specific subset in which IL-6 and not IL-1β is the key cytokine in the pathophysiology. It would be interesting to test anti-IL-6 treatment in anti-IL-1-responding SchS patients, as thus far it has only been given to 3 anti-IL-1 unresponsive patients.

Irrespective of the type of therapy, almost all patients with SchS were shown to require continuous treatment. Exceptions were a few patients that experienced remissions of several months after canakinumab withdrawal. Although cytokine-targeted drugs are highly effective, they only reduce symptoms and do not cure the disease.

37.12 Outcome/Prognosis

Patients with SchS usually have a normal life expectancy. The disease itself, however, follows a chronic course, as only one case of a partial clinical remission has been reported (prof. dr. Lipsker,

Table 37.4 Treatment[a]

	Efficacy %		Efficacy # cases		Reported	
	High	Partial	High	Partial	None	# Cases
Anti-IL-1Ra (anakinra)	95%	2%	92	2	3[b]	97
Anti-IL-1β antibodies (canakinumab)	81%	19%	25	6	0	31
Anti-IL-6 antibodies (tocilizumab)	75%	25%	3	1	0	4
Fusion protein IL-1R (rilonacept)	50%	38%	4	3	1	8
Anti-CD20 rituximab	25%	16%	5	3	12	20
IFNα	20%	35%	4	7	9	20
Corticosteroids	18%	46%	34	86	66	186
Thalidomide	19%	25%	3	4	9	16
Colchicine	14%	6%	7	3	41	51
Pefloxacine	13%	63%	2	10	4	16
Cyclosporine	10%	14%	3	4	22	29
PUV-A[c]	8%	62%	1	8	4	13
Alkylating agents	7%	20%	4	12	44	60
Cox-inhibitors	6%	33%	6	31	57	94
Hydroxychloroquine	7%	7%	1	1	13	15
Dapsone	5%	5%	2	2	35	39
Histone deacetylase inhibitor (ITF2357	0%	75%	0	3	1	4
Doxepine	0%	50%	0	3	3	6
Bisphosphonates[d]	0%	33%	0	3	6	9
I.v. Immunoglobulins	0%	25%	0	2	6	8
Psoralene	0%	25%	0	1	3	4
UVB phototherapy	0%	25%	0	1	3	4
Antihistamines[c]	0%	10%	0	15	132	147
Plasmapheresis	0%	7%	0	1	13	14
e.c. Immunoadsorption	33%	0%	1	0	2	3
Bortezomib	0%	100%	0	1	0	1
Dihydroergotamine	0%	100%	0	1	0	1
Azathioprine	0%	0%	0	0	26	26
Anti-TNF	0%	0%	0	0	10[b]	10
Chloroquine	0%	0%	0	0	6	6
Sulfasalazine	0%	0%	0	0	3	3
Fludarabine	0%	0%	0	0	1	1
UVA phototherapy	0%	0%	0	0	1	1
Sulphones	0%	0%	0	0	1	1
Leflunomide	0%	0%	0	0	1	1

[a]Updated and adapted from De Koning [7]
[b]Exacerbation in one case
[c]Only against urticaria partially effective
[d]Only against bone pain partially effective

Strasbourg, personal communication), and no curative therapies have been identified to date. After a median follow-up of 8 years, lymphoproliferative malignancies developed in 12% of patients. Two-thirds of these cases were diagnosed with Waldenström macroglobulinemia.

Amyloidosis was reported in 6 patients [7], a relatively small risk for such a long-standing proinflammatory state. Interestingly, only the AA subtype occurred, not the AL subtype, despite the presence of a monoclonal gammopathy.

37.13 Conclusion

SchS is a chronic late-onset autoinflammatory disease. IL-1ß hyperactivation is the pivotal pathophysiological mechanism and IL-1 blockade is a highly effective treatment. The role of the paraprotein is still unclear, but evidence is mounting that it is a consequence rather than cause of the proinflammatory state. Somatic mosaicism was found for *NLRP3* mutations in 2 patients, and might be found in more patients in genes of the IL-1 pathway as detection techniques become more sensitive.

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Part VI

The Relationship Between Autoinflammation and Other Inflammatory and Common Diseases

38

Autoinflammation and Autoimmunity

Dennis McGonagle and Abdulla Watad

Abstract

The elucidation of the genetic basis for hereditary recurrent fever syndromes validated the role of innate immune dysregulation in diseases formerly viewed as autoimmune. Recognizing the non-autoimmune nature of tumor necrosis factor receptor-associated periodic syndrome (TRAPS), one such syndrome, and the lack of evidence for autoantibodies or B- or T-cell involvement in the context of the emergent genetics, led to the proposal of the term autoinflammation in 1999. While formally coining a new term for this type of inflammation against self, the definition was essentially stating what inflammation was not, rather than what it was. Based on the lack of an association with humoral or cellular mediated immunity and the propensity for recurrent seemingly unprovoked attacks of inflammation, fevers, elevation of inflammatory markers, without high-titer autoantibodies or antigen-specific T lymphocytes, the new designation of autoinflammatory disorders also included some conditions that would have previously been considered autoimmune, e.g. Behçet disease (BD). BD is a prime example of the two-tiered classification of inflammation against self since BD has a strong population level human leukocyte antigen (HLA)-B51 association. Given the classically defined role of major histocompatibility complex (MHC)-I molecules in peptide presentation to T cells, this incriminates adaptive immunity in BD immunopathology, which was supported by clinical therapeutics, where immunosuppressant agents like azathioprine had a proven role in disease management. The purpose of this chapter is to summarize the overlap and differences between autoinflammatory and autoimmune disorders.

Keywords

Autoimmunity · Autoinflammation
Periodic fever · Immunological diseases continuum · TNF-alpha · IL-1

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Abbreviations

AAV	ANCA-associated vasculitis
ACPA	Anti-citrullinated protein antibodies
ALPS	Autoimmune lymphoproliferative syndrome
AOSD	Adult-onset Still disease
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
ATD	Autoimmune thyroid disorder
BD	Behçet disease
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase activation and recruitment domains
CMML	Chronic myelomonocytic leukaemia
DITRA	Deficiency of IL-36 receptor antagonist
DM	Dermatomyositis
DMARD	Disease modifying anti-rheumatic drugs
ERAP-1	Endoplasmic reticulum aminopeptidase 1
FMF	Familial Mediterranean fever
GCA	Giant cell arteritis
GWAS	Genome-wide association studies
HIDS	Hyperimmunoglobulinemia D syndrome
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IFN	Interferon
IL	Interleukin
IPEX	Immune dysregulation polyendocrinopathy enteropathy x-linked syndrome
MAS	Macrophage activation syndrome
MDS	Myelodysplastic syndrome (MDS)
MHC	Major histocompatibility complex
MKD	Mevalonate kinase deficiency
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa B
NLR	Nucleotide-binding oligomerization domain-like receptors
NOD	Nucleotide-binding oligomerization domain
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PAPA	Pyogenic arthritis pyoderma gangrenosum and acne syndrome
PBS	Primary biliary cirrhosis
PM	Polymyositis
PsA	Psoriatic arthritis
RA	Rheumatoid arthritis
RAEB	Refractory anemia with excess blasts
RCMD	Refractory cytopenia with multilineage dysplasia
SJIA	Systemic juvenile idiopathic arthritis
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SNS	Self/non-self
SpA	Spondyloarthropathies
SSc	Systemic sclerosis
STAT	Signal transducer and activator of transcription
T1DM	Type 1 diabetes mellitus
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome

Key Points

- **Mutations in genes implicated in innate immunity spawned the autoinflammation concept and has allowed "inflammation against self" to be viewed in a new light**
- **There is occasional ambiguity concerning the diagnosis of autoimmune diseases and autoinflammatory disorders due to genetic, clinical and therapeutic aspects that are shared by both diseases**
- **The immunological diseases continuum is a useful tool for better understanding of the link between autoimmunity and autoinflammatory disorders and overlaps between the two**

38.1 Introduction

Given the close functional integration and interdependence of innate and adaptive immune system function at multiple levels it was evident that

autoinflammation, as defined in the relatively rare monogenic disorders, likely had a much more general relevance to medicine and immunology. Autoinflammation was defined as a disorder of innate immunity (in clear contradistinction to autoimmunity), where self-directed inflammation, induced by local factors at sites that lead to activation of innate immune cells, results in target tissue damage (Box 38.1). For example, disturbed homeostasis of canonical cytokine cascades (as in the recurrent fever syndromes), aberrant bacterial sensing (as in Crohn disease), and tissue microdamage predispose to site-specific inflammation that is independent of adaptive immune responses [1]. This resulted in the emergence of the immunological disease continuum classification of inflammation against self (Fig. 38.1). Consequently, for the classical autoimmune diseases this implied tolerance failure in the primary and secondary lymphoid organs with normal tissue being later subject to an immunological attack. Some diseases considered as classical autoimmune were then recognized to have a primary autoinflammatory initiation with

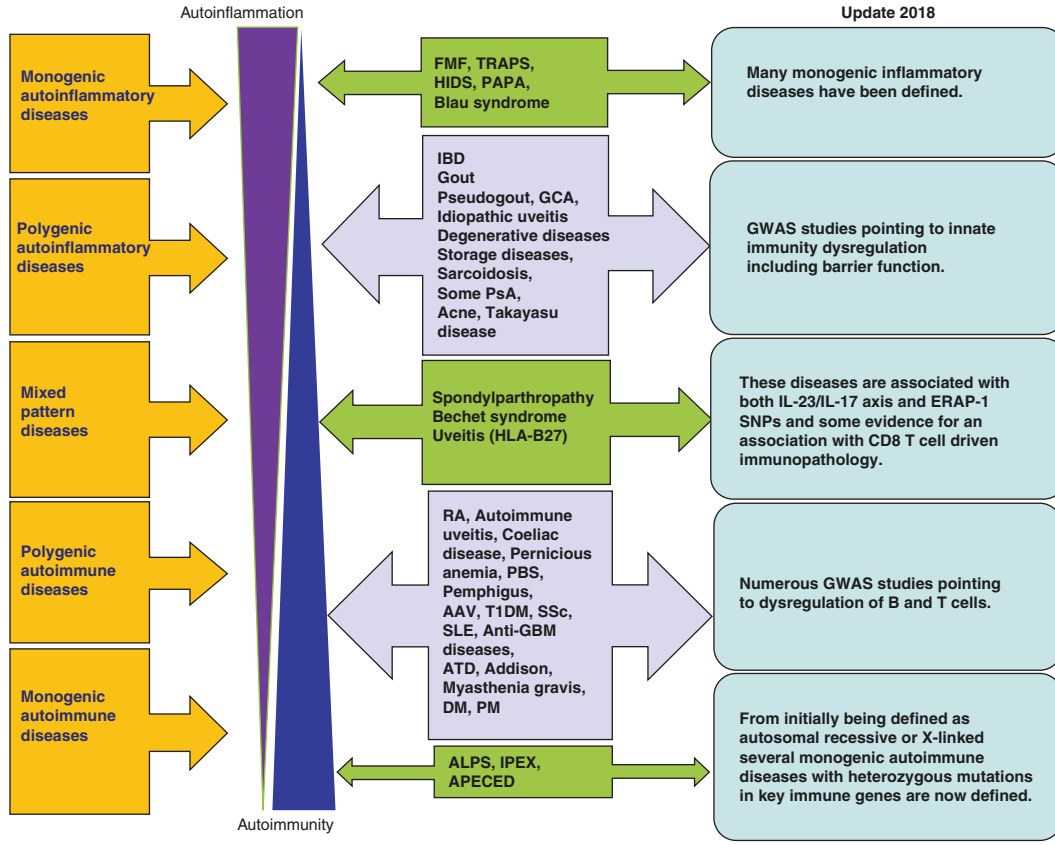

Fig. 38.1 The immunological disease continuum with pure autoimmunity and pure autoinflammation or innate immunopathology at opposites. *FMF* familial Mediterranean fever; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *HIDS* hyperimmunoglobulinemia D syndrome; *PAPA* pyogenic arthritis pyoderma gangrenosum and acne syndrome; *IBD* inflammatory bowel disease; *GCA* giant cell arteritis; *PsA* psoriatic arthritis; *PBS* primary biliary cirrhosis; *AAV* ANCA-associated vasculitis; *T1DM* type 1 diabetes mellitus; *SSc* systemic sclerosis; *SLE* systemic lupus erythematosus; *ATD* autoimmune thyroid disorder; *DM* dermatomyositis; *PM* polymyositis; *ALPS* autoimmune lymphoproliferative syndrome; *IPEX* immune dysregulation polyendocrinopathy enteropathy x-linked syndrome; *APECED* autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; *GWAS* genome-wide association studies; *ERAP-1* endoplasmic reticulum aminopeptidase 1; *SNP* single nucleotide polymorphism

secondary T-cell mediated target tissue autoimmune reactions that resulted in the emergence of intermediate diseases between autoimmunity and autoinflammation (Fig. 38.1).

The innate immune basis for autoinflammation was reaffirmed and refined in later viewpoints that autoinflammation simply represented non-infectious diseases involving innate immunity [2]. The underlying cardinal innate immune mechanisms underpinning these disorders were later proposed to include interleukin (IL)-1β activation disorders, nuclear factor kappa B (NF-κB) activation syndromes, protein misfolding disorders, complement regulatory diseases, disturbances in cytokine signaling, and macrophage activation syndromes (MAS) [3] (see Chap. 10), but it is clearly much broader than this. Thus, after the late nineteenth century work on humoral and cellular immunity and late twentieth century research on rare monogenic inflammatory disorders, a unified theory of non-infectious inflammation against self emerged, whereby innate immune mediated immunopathology and autoimmunity were closely integrated, just as the physiological immune response often encompasses and integrates innate and adaptive immunity.

38.2 The Immunological Disease Continuum

From this platform of an immunological disease continuum of inflammation against self, with pure autoinflammation (innate immunity) and autoimmunity at opposite boundaries, some important subgroups have emerged. Kastner et al. [4] proposed the term "horror autoinflammaticus" that highlighted the innate immune system dysregulation in these conditions. Then, the recognition of gain- or loss-of-function in the immune system along the continuum helped to conceptualize the complexity of autoimmunity and autoinflammation in relationship to immunodeficiency states [4] (Figs. 38.2 and 38.3). It also emerged that two key cytokines linked to innate immunity, namely type 1 interferon (IFN) and IL-1β are polarized too, with the former being more strongly

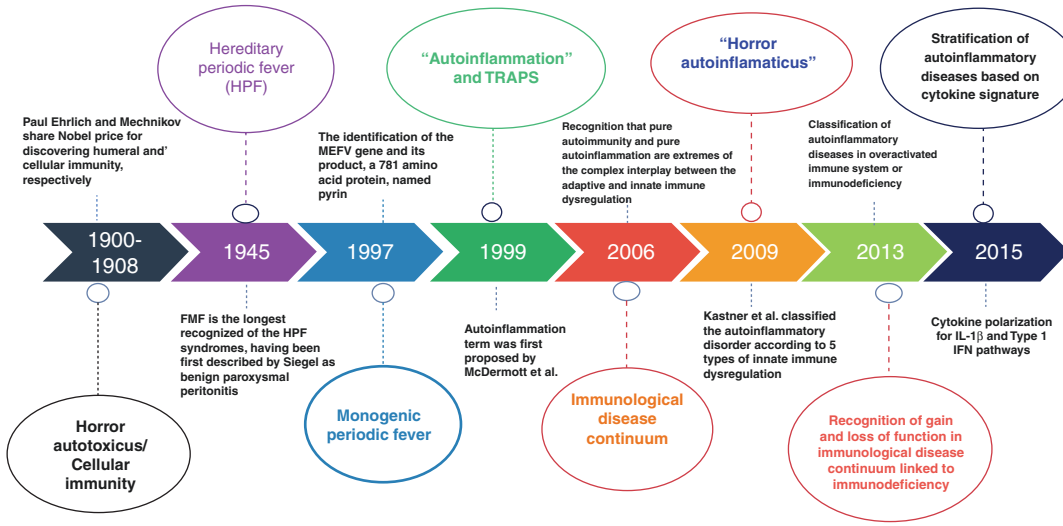

Fig. 38.2 This figure illustrates some important milestones in the evolution of the classification of autoinflammatory and autoimmune disease inter-relationships during the last decades. *FMF* familial Mediterranean fever; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *IL* interleukin; *IFN* interferon; *HPF* hereditary periodic fever; *MEFV* Mediterranean fever

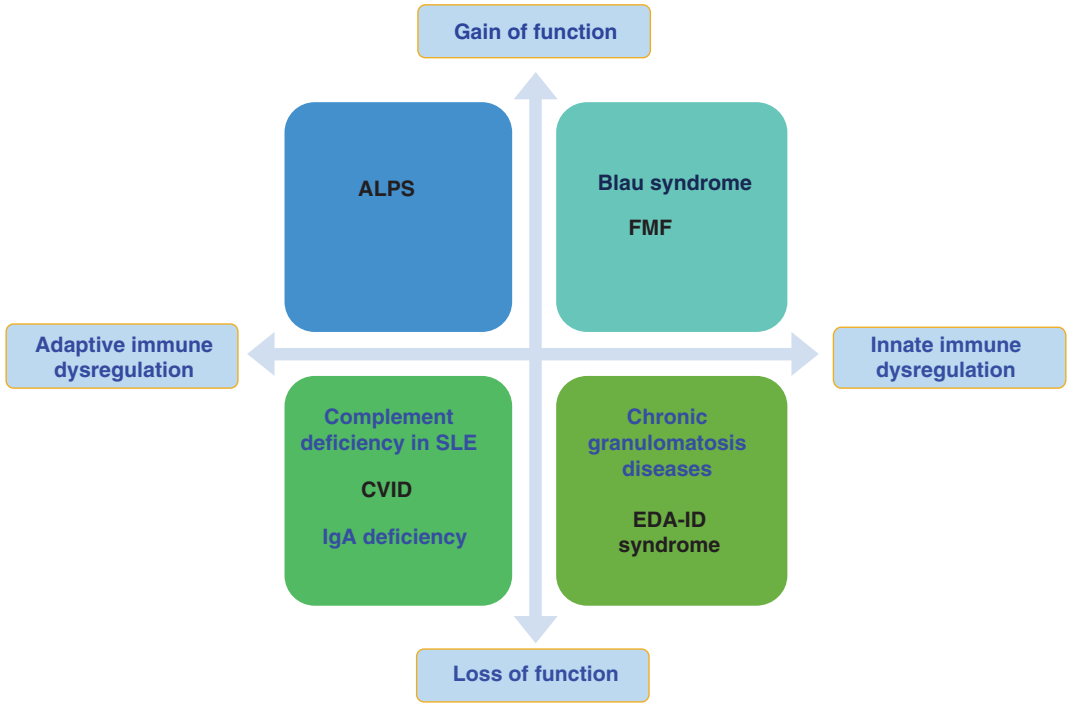

Fig. 38.3 Immunological disease continuum viewed in terms of gain- and loss-of-function mutations in innate and adaptive immunity. Based on references [2, 6]. *ALPS* autoimmune lymphoproliferative syndrome; *FMF* familial Mediterranean fever; *SLE* systemic lupus erythematosus; *CVID* common variable immunodeficiency; *EDA-ID* anhidrotic ectodermal dysplasia with immunodeficiency; *IFN* interferon; *CTLA4* cytotoxic T-lymphocyte–associated antigen 4

associated with autoimmune disease, namely monogenic forms of systemic lupus erythematosus (SLE), and the latter linked to pure innate immune driven pathology. The recognition of Type I IFN dysregulation driving autoimmunity whilst nucleotide-binding oligomerization domain-like receptors (NLR) perturbation driving classical autoinflammatory diseases that do not exhibit autoantibody formation led to the realization that these pivotal cytokines polarize immune disease classification (Figs. 38.2 and 38.4) [5]. From the original recognition of autoinflammatory diseases being linked to NLR cytoplasmic resident innate immune receptors (NLRP3 in particular) [6], other inflammasomes including NLRC4 have been implicated in innate immune mediated pathology [7] (see Chap. 5). Remarkably, NLR family members are consistently linked to both monogenic and polygenic autoinflammatory disease whereas toll-like receptors (TLR) are not, possibly indicating functional redundancy in the latter receptors.

38.2.1 Inflammation against Self: Self/Non–Self Discrimination *Versus* Danger Signals

The historical understanding of non-infectious inflammation was based on self/non-self (SNS) discrimination and immune tolerance failure which dominated immunological thinking until the mid 1990s. The importance of SNS discrimination in the organ transplant rejection setting powerfully validated the concept in the clinical arena. However, this model is largely based on

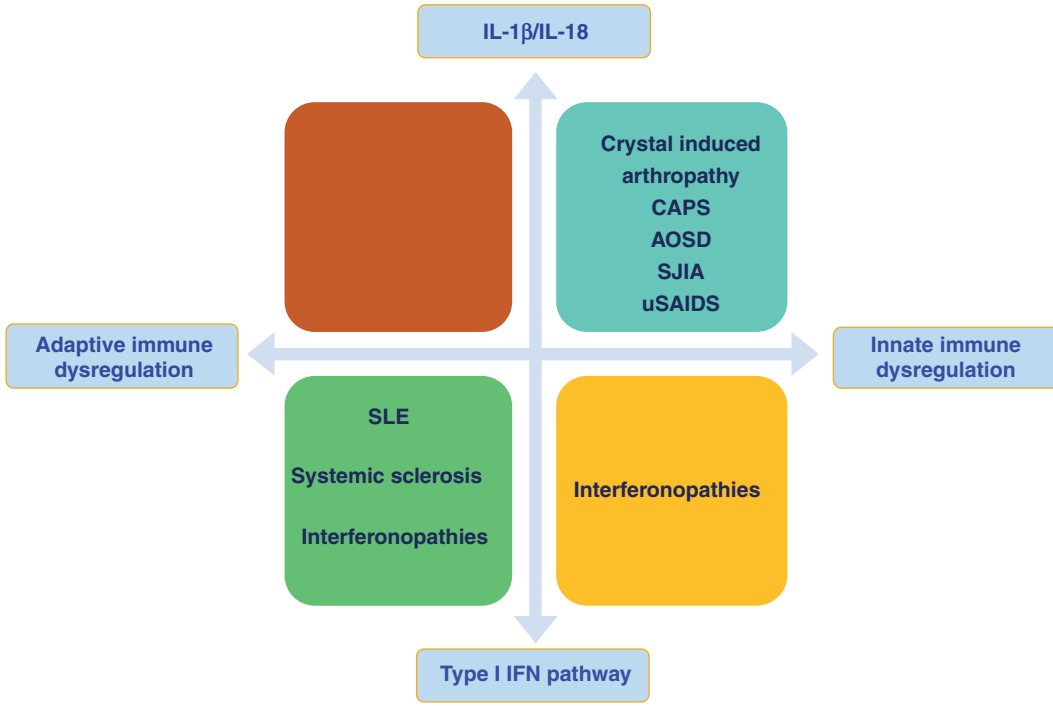

Fig. 38.4 Immunological disease continuum viewed in terms of cytokines pathways mutations in innate and adaptive immunity. Based on references [2, 5]. *IL* interleukin; *CAPS* cryopyrin-associated periodic syndromes; *AOSD* adult-onset Still disease; *SJIA* systemic juvenile idiopathic arthritis; *uSAIDS* undifferentiated systemic autoinflammatory disorder

the autoimmunity concept of dysregulated T- and B-cell function. In the 1990s, Matzinger advocated the danger theory of inflammation against self, whereby the immune system was not so much concerned with SNS discrimination but with responding to danger signals [8]. This theory was proposed as an alternative to the classical SNS model and was enthusiastically embraced by some, but not all immunologists. The discovery of pattern recognition receptors, most notably TLR, provided a biochemistry basis for how local danger signals could activate immunity [9]. Even more relevant was the recognition that damaged self-tissues, including degraded proteins and nucleic acids in the wrong cellular compartments, cytoplasmic and extra-cellular and rather than nuclear, were capable of triggering immune activation [10]. Collectively, these observations provided strong support for the role of danger signals in disease initiation.

In 2002, Matzinger developed the danger signal hypothesis postulating that the target tissues actually controlled the immune response by sending alarm signals [11]. This is especially noteworthy given that the originally described autoinflammatory disorders have a tissue tropism that cannot be explained by autoimmune mechanisms. Broadly, these disorders have a strong, but not exclusive, predilection for the "moving parts" of the body including skin, joints, peritoneal cavity, pleural cavity and pericardium but not for example the non-mechanically stressed endocrine organs. Other diseases that were subsequently shown to have an autoinflammatory genesis, including pyogenic arthritis pyoderma gangrenosum acne (PAPA) syndrome, showed a tissue tropism that was linked to injury in the target tissues. Also, according to the Matzinger viewpoint cytokines were also danger signals and around the turn of the millennium two such cytokines were

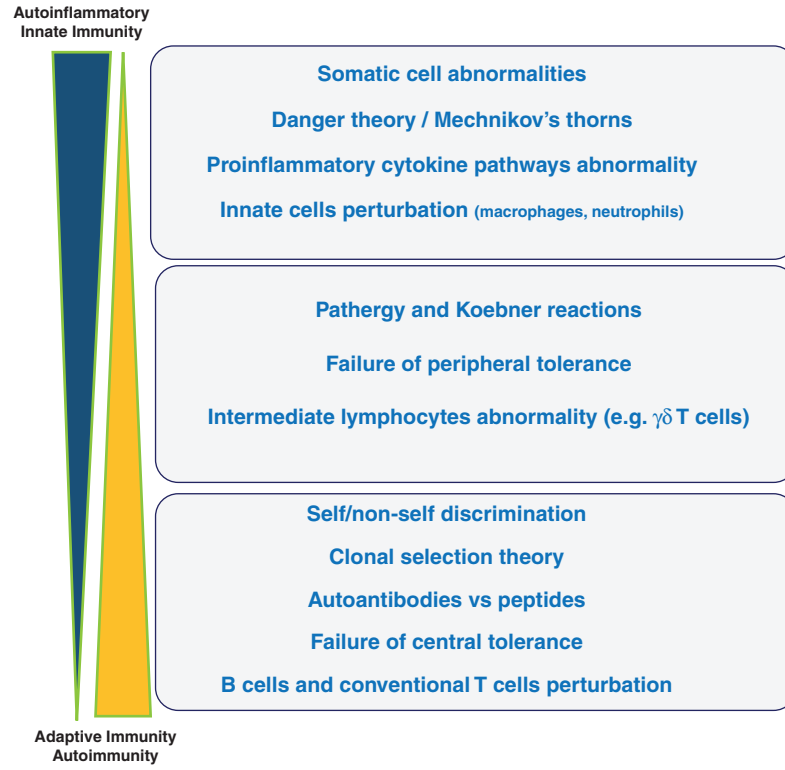

Fig. 38.5 The theoretical concepts that underpin autoinflammation and autoimmunity are best viewed along the immunological disease continuum model

pre-eminent in the autoinflammation field: IL-1β and tumor necrosis factor (TNF) [11].

The implications of these clinical and theoretical considerations were the reconciliation of seemingly contradictory immunological terminology along the immunological disease continuum of innate and adaptive mediated inflammation. Hence, SNS discrimination underlines peripheral and central tolerance failure and B- and T-cell dysfunction and autoimmunity, while tissue specific danger signals are involved in innate immunopathology or autoinflammation (Fig. 38.5). The intrinsic dysregulation of TNF receptor (TNFR) and NLRP3 inflammasomes in the monogenic autoinflammatory diseases with increased levels of danger cytokine signals nicely fits into the non-autoimmune model for inflammation that essentially captured hyperinflammatory responses within the innate immune system. Thus, hitherto confusing immunological lexicon could be more comfortably accommodated along the immunological disease continuum [2, 12].

38.2.2 Autoinflammation: Proof of Concept of the Pivotal IL-1β Connection *Via* Therapy

The "classically described" monogenic autoinflammatory disorders are clinically and genetically heterogeneous [2, 12] but despite this there has been a remarkable therapeutic convergence. The original definition of the cryopyrin-associated periodic syndromes (CAPS) group of disorders led to the recognition that *NLRP3* mutations resulted in dysregulated and constitutive overproduction of IL-1β [13]. Subsequently, the demonstration of a rapid onset and potent action of anakinra, an IL-1 receptor antagonist therapy, a strategy that was suboptimal in the management of autoimmune disorders such as rheumatoid arthritis (RA), confirmed the role of the IL-Iβ pathway in the autoinflammatory setting [14]. Given the genetics of TRAPS and its link to the TNF pathway, it was somewhat surprising that IL-1 antagonism was effective for

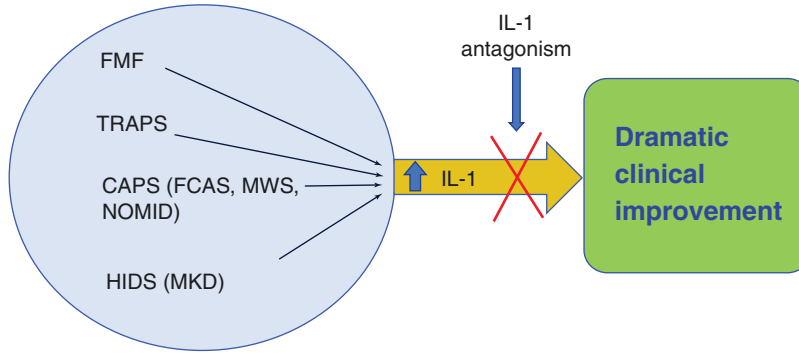

Fig. 38.6 The original defined monogenic autoinflammatory disorders all show clinical responses to interleukin (IL-1) antagonists. The classical autoimmune diseases do not generally show such remarkable responses. *FMF* familial mediterranean fever; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *CAPS* cryopyrin-associated periodic syndromes; *FCAS* familial cold autoinflammatory syndrome; *MWS* Muckle-Wells syndrome; *NOMID* neonatal-onset multisystem inflammatory disease; *HIDS* hyperimmunoglobulinemia D syndrome; *MKD* mevalonate kinase deficiency

TRAPS [15]. Patients with TRAPS had prompt response to anakinra and disease relapse after treatment withdrawal, with therapy re-introduction leading to a dramatic response. Likewise, IL-1 blocking strategies have proven to be beneficial in hyperimmunoglobulinemia D syndrome/mevalonate kinase deficiency (HIDS/MKD) and familial Mediterranean fever (FMF) [16, 17]. Indeed, IL-1 blocking therapy has proven to be highly effective in patients with non-monogenic hyperinflammatory disorders as well, including crystal arthropathy, systemic juvenile idiopathic arthritis (SJIA) and adult-onset Still disease (AOSD) [18]. In fact, the concept of the intertwined nature of autoinflammation and IL-1 has contributed to the "real world" clinical practice of using IL-1 blocking strategies in adult and pediatric cases with inflammatory phenotypes that exhibit autoinflammatory features (Fig. 38.6) [19]. Indeed, just as corticosteroids might be used as a therapeutic trial to help diagnose and simultaneously treat polymyalgia rheumatica, so is the case with poorly defined suspected autoinflammatory disorders, where rapid responses to IL-1 antagonism supports the diagnosis of an autoinflammatory disease [19].

38.2.3 Autoinflammation Underpinning Autoimmunity

The immunological disease continuum placed SLE towards the boundary of autoimmunity but even in 2006 it was recognized that soluble innate immunity, or the complement system, was associated with various SLE features with C2, 3 and 4 deficiencies leading to an SLE phenotype [20] (Fig. 38.7). Hence, it was posited that at the population level, SLE sat closer to the adaptive boundary or self-directed inflammation, but innate immunity might also be involved. However, beyond complement, Mendelian disorders collectively termed "the interferonopathies", due to dysregulated metabolism of self-nucleic acids, with associated elevated levels of type-I IFN also resulted in autoantibody associated SLE [21]. This realization emerged from a series of elegant papers in Aicardi-Goutières syndrome by Crow and colleagues [22] (see Chap. 24). Indeed, several monogenic diseases leading to an SLE phenotype were reported recently such as DNase II deficiency, leading to SLE consequent to impaired nucleic acid metabolism [23]. For SLE in particular, there is a strong immunological basis for aberrant handling of self-nucleic acids consequent to dysregulation of DNA and RNA metabolizing

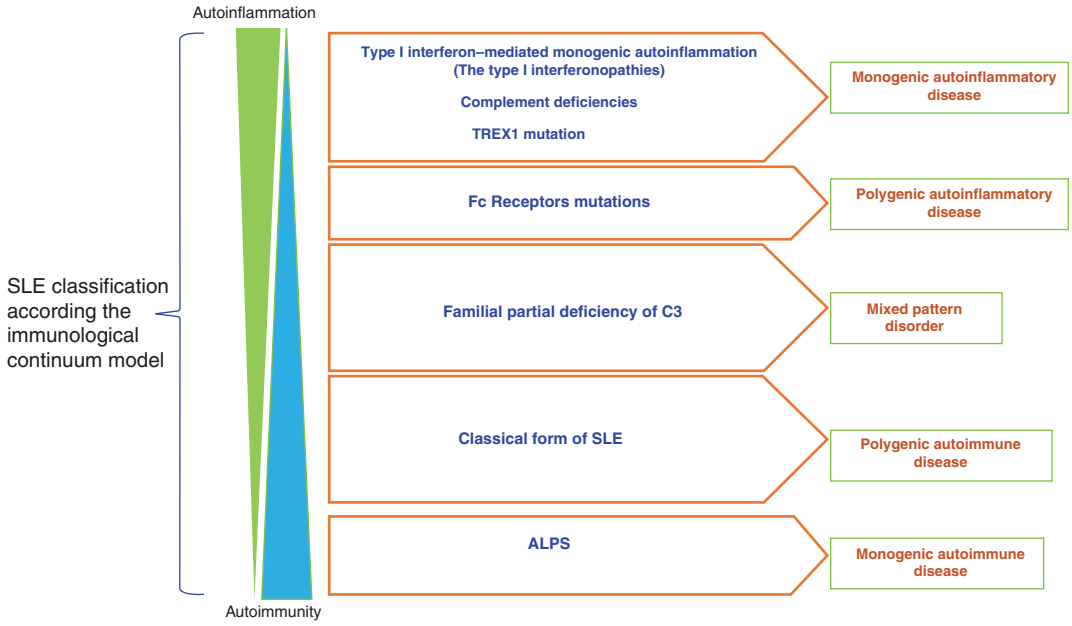

Fig. 38.7 SLE is a one of the best examples in which the immunological continuum model can be applied showing the diversity of clinical manifestation according the involvement of different genetic and immune system components. The figure is based on references [2, 6]. *SLE* systemic lupus erythematosus; *ALPS* autoimmune lymphoproliferative syndrome; *TREX1* three prime repair exonuclease 1

enzymes that leads to nucleoprotein-nucleic acid interactions with TLR7 and TLR9 related pathways and autoimmunity development [24]. Thus the IFN and IL-1β axis have defined a major cytokine split within the immunological disease continuum (Fig. 38.4), with pure autoinflammatory disease at one end and innate dysregulation of IFN leading to classical autoantibody mediated disease or an SLE-like pattern of disease at the other [6]. This has major implications for the proper stratification of SLE (Fig. 38.7).

38.2.4 Intermediate Diseases Between Autoinflammation and Autoimmunity: Major Histocompatability Complex (MHC)-I Associated Disorders

The group of clinically overlapping disorders, collectively termed the spondyloarthropathies (SpA), are generally autoantibody negative, and include psoriasis, psoriatic arthritis, BD, ankylosing spondylitis, Crohn disease, ulcerative colitis and uveitis and other related disorders. Historically these have been difficult to conceptualize in relationship to autoinflammation and autoimmunity. There are several monogenic autoinflammatory syndromes that seem to be closely related to these conditions, including deficiency of IL-36 receptor antagonist (DITRA), Majeed syndrome and PAPA syndrome [25].

Focusing on BD as an example, the lack of autoantibodies, periodicity of attacks and neutrophil related inflammation originally lead to calls for BD to be designated as autoinflammatory (see Sect. 38.2) Genetically, BD has been linked to heterozygous mutations in the *MEFV* gene [26], rather than homozygous or compound heterozygous mutations that usually characterize FMF. The *MEFV* gene encodes for pyrin and is predominantly expressed by innate immune cells [3]. The BD phenotype also exhibits a site-specific injury known as pathergy phenomenon

(see Chap. 35). Moreover, sometimes and especially in early phases of the disease course there is ambiguity concerning the diagnosis of BD and FMF due to genetic, clinical and therapeutic aspects that are shared by both diseases, thus strengthening the association of BD to autoinflammation [27].

Within this group of disorders, psoriasis also exhibits a link between site specific physical injury and disease development- a phenomenon termed the Koebner response. The primary pathogenic process begins in the tissues where an abnormal response to stress or injury at the entheses, adjacent bone or disturbance of the bowel barrier function triggers activation of the innate immune system. Hence, the SpA concept is strongly linked to clinical features including site specific injury leading to local innate immune activation. Furthermore, several monogenic autoinflammatory disorders including DITRA, DIRA and CARD14-associated psoriatic disease clinically resemble the SpA group of disorders [28].

However, at the population level, the genetic architecture of the SpA group diseases is very distinct from autoimmunity, with the SpA related-disorders converging on the IL-23/17 cytokine axis and cell mediated immunity dysregulation. The SpA related disorders also converge on common MHC-I associations of HLA-Cw06 (psoriasis), HLA-B51 (BD) and HLA-B27 (AS and uveitis) [29]. Furthermore, epistatic endoplasmic reticulum aminopeptidase 1 (ERAP)-1 polymorphisms are associated with these disorders, which incriminates peptide loading for presentation to CD8 T-cells. Consequently, these disorders have been designated as intermediate disorders between innate and adaptive immunity or MHC-I-opathies [29–31]. It is thought that the MHC-I association of BD, namely the HLA-B51 link, through local factors can trigger secondary adaptive immune CD8 T-cell responses with prominent neutrophilic inflammation that culminate in exacerbation and recurrence of disease manifestations [29]. Accordingly, BD and allied disorders that were broadly classified under the seronegative SpA concept were designated as being primary autoinflammatory or initiated by site specific innate immune dysregulation with subsequent adaptive CD8 T-cell driven immunopathology. While this concept appears increasingly robust in the case of HLA-Cw06 associated psoriasis and HLA-B27 associated uveitis, it certainly needs much more research in the case of ankylosing spondylitis [29], where a role of CD8 T-cells or response to T-cell blocking therapies needs to be defined.

The IL-17 cytokine axis is remarkable in that deficiency of IL-17A, IL-17F and related pathways is strongly associated with a propensity for immunodeficiency and specifically fungal infections [32]. The targets for these fungal infections include the nail, scalp and genital regions, which is especially noteworthy since the involvement of these sites in subjects with psoriasis leads towards an increased propensity for PsA. Indeed, patients with PsA treated with IL-17A blockers had a higher prevalence of candida infection in comparison to those treated with placebo [32]. Moreover, single nucleotide polymorphisms (SNPs) in the IL-23 pathway such as IL23A and IL23R confer susceptibility to PsA, implying a central role of the IL-23/IL-17 axis in PsA disease pathogenesis [33, 34]. Thus, the original use of "pure autoinflammation" and "pure autoimmunity" with the placement of the MHC-I associated disorders as intermediate diseases with site specific autoinflammation and adaptive CD8 T-cells responses has been strengthened in recent years with the emergence of SNPs in ERAP-1, which trims peptides for MHC-I presentation. Thus, IL-1β, type I IFN and IL23/17 genetic associations define distinct immunological diseases. The fundamental differences between these disorders and classical autoimmunity are summarized in Table 38.1.

38.2.5 Autoimmunity–Autoinflammatory Overlap

The phenotype of both SJIA in children and AOSD are viewed as part of the autoinflammatory spectrum (see Chap. 32). This is supported

Table 38.1 The main differences between autoimmune and monogenic autoinflammatory disorders

Variable	Autoimmune disease	Monogenic autoinflammatory diseases	MHC-I-opathy
Epidemiology	Common	Rare	Rare
Gender	Female predominance	None	Disease dependent
Age of onset	Disease dependent	Generally young	Generally young
Primary site of disease	Lymphoid organ	Tissue target	Tissue target
Immunopathogenesis	Predominantly adaptive system dysregulation	Predominantly innate system dysregulation	Innate system dysregulation with secondary MHC-I/ERAP-1 related IL-23/17 cytokine axis dysregulation
Main cellular involvement	B and T cells	Neutrophils, macrophages	Myeloid cells Innate lymphocytes CD8 T cells
Genetic predisposition	MHC II associations	Cytokine and bacterial sensing pathways	MHC-I, ERAP-1/2, IL-23/IL-17 axis
Therapy	DMARDs, B-cell depletion	Anti-cytokines (e.g. anti-IL-1)	Anti-cytokines (IL-23/17 pathway) but not B-cell depletion
Natural history	Progressive	Recurrent episodes	Waxing and waning

MHC major histocompatibility complex; *DMARD* disease modifying anti-rheumatic drug; *IL* interleukin; *MHC* major histocompatibility complex; *ERAP-1* endoplasmic reticulum aminopeptidase 1

by evidence for an IL-1 transcriptional signature and responses to anti IL-1 therapy [35, 36]. Poorly defined, or what was historically termed "atypical AOSD", is now designated as autoinflammatory or undefined systemic autoinflammatory disease [19]. While SJIA has a rare monogenic variant reported in Arab populations, the typical cases of AOSD have an MHC-II association that points towards an overlap between autoinflammation and autoimmunity [37, 38]. The reported IL-1 signature in the context of the clinical phenotypes and response to IL-1 blockade is perhaps the strongest confirmatory evidence that AOSD and SJIA are part of the autoinflammatory phenotypes [35, 36]. At any rate, the recognition of the immunological disease continuum provides a platform for future studies to more accurately classify such disorders.

Rare instances have been described of the co-occurrence of complex phenotypes where patients have classical RA with MHC-II associated anti-citrullinated protein antibodies (ACPA) but simultaneously exhibit an associated autoinflammatory phenotype with sudden onset of severe attacks that are self-limiting with joint erythema. These attacks have a variable response to colchicine and in some cases anakinra but poor response to traditional treatment strategies using disease modifying anti-rheumatic drugs (DMARDs) and anti-TNF biologics [39]. We envisage that this is due to the complex interaction of both innate and adaptive immune mechanisms that are both independently capable of driving inflammatory arthritis. The recognition that autoinflammatory aspects that occur in common autoimmune diseases explain a variable response to therapy needs assessment in longitudinal studies [40].

As an example of the complexity between autoimmunity and autoinflammation, SNPs in the *TNFAIP3* gene, encoding the NF-kB regulatory protein A20, are associated with an array of classical autoimmune disorders but also disorders that are closer to the autoinflammation end of the spectrum [41]. In murine models, conditional knockout of *TNFAIP3* in keratinocytes, enterocytes, B cells or dendritic cells can create completely different inflammatory phenotypes including colitis, rash, RA-like arthropathy, SLE and Sjogren syndrome [42]. In humans, heterozygous loss-of-function mutations in the

TNFAIP3 gene are associated with a BD-like phenotype characterised by mucosal ulceration, uveitis and arthritis [43, 44]. The emerging lesson would appear to be that genes with widespread expression in both innate and adaptive immune cells may lead to very complex or overlapping phenotypes.

38.3 Other Immunological Aspects of Autoinflammatory Disorders

38.3.1 Autoinflammation and Cancer

Sporadic adult autoinflammatory disorders can be linked to an underlying lymphoproliferative disease. This is best defined for Schnitzler syndrome where cases typically present in adulthood with urticaria, fever and bone pain [45] (see Chap. 37). Although no genetic abnormalities have been defined in the NLRP3 inflammasome or other molecules, Schnitzler syndrome responds well to therapy with IL-1 inhibition [46, 47]. The vast majority of cases have a monoclonal IgM gammopathy and a significant number of patients go on to develop a lymphoproliferative disorder [48]. Beyond the well-recognised association between Schnitzler syndrome and autoinflammation there is a more poorly defined link with malignancy.

Beyond Schnitzler syndrome, which is a classical disease to illustrate the link between autoinflammation and cancer, a national French survey found an association between myelodysplastic syndrome (MDS) and hyper-inflammation [49]. Amongst 123 cases defined with MDS, the main clinical manifestations consisted of non-infectious fever with constitutional symptoms, skin involvement and arthritis. While many of these cases had bona fide autoimmune diseases, over 10% had innate immunopathology including Sweet syndrome and pyoderma gangrenosum [49]. Within the MDS group, chronic myelomonocytic leukemia (CMML), refractory anemia with excess blasts (RAEB), refractory cytopenia with multilineage dysplasia (RCMD), and refractory cytopenia with unilineage dysplasia were the most commonly defined entities that were linked to systemic inflammation. From a therapeutic perspective, most of these cases showed corticosteroid responsiveness. However, many patients required DMARD therapy and eventually went on to biological therapy. To emphasise, some of these cases had autoimmune diseases but for various biological therapies the responses rates were under 50%. While the association between classical autoimmune diseases and lymphoproliferative disease is well established with diffuse large B-cell lymphoma in RA, marginal zone lymphoma in Sjogren syndrome and T-cell lymphoma in celiac disease the link between autoinflammation and hematological malignancy is less well understood [50] (see Chap. 39). Given that the MDS group of disorders are essentially linked to the progenitor cells that are linked to myeloid lineage development, it is no surprise that a parallel situation of innate immune system related hematological disorders overlaps with malignancy [51].

The opposite is also true, autoinflammation tendencies might even protect against cancer (see also Chap. 39). The constitutive gain of function in the *MEFV* gene in FMF has recently been associated with a lower rate of cancers raising the possibility that innate immunity or aberrant immune activation propensities may be protective against cancer [52]. However, the link between malignancy and autoinflammation and cytokine pathways seems to be very complicated, indeed, in a study aimed to evaluate the effect of canakinumab on the atherosclerosis process, it was found that blocking IL-1β reduced the incidence and mortality of lung cancer [53]. This shows that the innate immune system plays an important role in malignancies and tumorigenesis and could be equally important to the adaptive immune system.

38.3.2 Autoimmunity, Autoinflammation and Immunodeficiency

A historical misconception was that gain-of-function in the immune system resulted in autoimmunity and loss-of-function resulted in

immunodeficiency. Recognition of the immunological disease continuum with innate and adaptive immune responses defining distinct disease boundaries led to the realization that complex phenotypes with either loss- or gain of immune function could occur within the disease spectrum, as pointed out by Grateau et al. [5] (Fig. 38.3) (see Chap. 28). A wide range of mechanisms is emerging to explain these features. For autoimmune disorders, high titre autoantibodies that neutralize IL-17 family members account for immunodeficiency in the monogenic autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) disease that is associated with multi-organ failure in the endocrine system. In the setting of signal transducer and activator of transcription (STAT)-1 gain-of-function the phenotype is associated with several autoimmune diseases, the counter-regulatory effects of the STAT-1 pathway suppresses IL-17 driven response and makes subjects susceptible to secondary infection. Furthermore, loss-of-function of immunity related to reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is associated with infections but also a Crohn-like disease phenotype that can be treated successfully with anti-TNF therapy [54]. The complex functional integration and a myriad of redundant and counter-regulatory pathways in the immune system underscores this complex immunological triumvirate (Fig. 38.3).

38.4 Clinical Approach to Distinguish Autoinflammation and Autoimmunity

38.4.1 Clinical Investigations

The approach to distinguish between autoinflammation and autoimmunity begins with a careful medical and family history, general physical examination, laboratory tests including complete blood count, general biochemistry, serum complement and immunoglobulin levels, inflammatory biomarkers and relevant autoantibodies. The detailed evaluation for autoinflammatory diseases is described in Chap. 11.

By the time autoinflammation is considered as a diagnosis most patients will already have had an extensive work up including a detailed infectious disease assessment with blood cultures and viral and bacteriological serologic studies. In our experience, underlying chronic disease, including bronchiectasis, may be difficult to recognize, especially if patients do not have significant purulent sputum expectoration. The second major differential diagnosis is underlying malignancy and lymphoproliferative diseases in particular, since myeloid or lymphoid dysregulation can lead to inflammation before malignancy manifests itself. In our experience, some cases of unexplained inflammatory conditions with therapy-resistant disease do have bone marrow findings indicative of probable, but not definite, underlying lymphoproliferative disease at post-mortem examination, although earlier bone marrow examinations had remained inconclusive.

By the time adult subjects with suspected autoinflammatory disease are formally referred for assessment it is likely they will already have had CT imaging of the neck, chest, abdomen and pelvis to search for lymphoproliferative disease, infectious source or large vessel vasculitis. Both upper and lower gastrointestinal tract endoscopy may also have been performed especially if weight loss is prominent. Echocardiography including transesophageal examination is often ordered to exclude bacterial endocarditis. The role of fludeoxyglucose positron emission tomography (FDG PET) has been recently clarified in subjects with inflammation of unknown origin and is informative in subjects over 50 years of age where it is useful in the evaluation of giant cell arteritis which may present with a prominent inflammatory phenotype with fever and constitutional symptoms [55].

Occasionally patients may present with prominent fevers and serositis and constitutional symptoms that appear autoinflammatory in nature. On detailed immunoblot assessment for autoantigens, one autoantibody may be found, not fitting the clinical pattern, only to evolve a classical connective tissue disease pattern at a later stage including autoimmune myositis. Therefore, a high degree of clinical vigilance is needed in the assessment of poorly defined autoinflammatory disease.

38.4.2 Molecular Investigations

The foremost test for the assessment of the autoinflammatory disorders is an initial screen of genes known to be associated with monogenic autoinflammatory disorders (see Chap. 12). In adult autoinflammatory disease clinics, cases tend to be sporadic rather than familial. The complexity of the genetic analysis is increasing, for example the recognition that heterozygous mutations in the *MEFV* gene may be associated with FMF or other inflammatory phenotypes [56]. Whilst homozygous mutations are needed for autoimmune or autoinflammatory phenotypes in animal models, e.g. for CTLA4 or TNFAIP3, heterozygous mutations in the latter are associated with the emergence of a BD-like phenotype [57]. Uncommon variants of NOD2 or TNFR1 are not infrequently found in patients in autoinflammatory disease clinics and their full meaning awaits to be elucidated.

An informatics approach has been used to evaluate whether it is possible to decipher between autoimmune and autoinflammatory disorders by choosing entities from both ends of the spectrum and performing a meta-analysis of publicly available gene expression datasets generated from peripheral blood mononuclear cells [58]. The most striking feature of this dichotomous autoimmune and autoinflammatory disease analysis was that common pathway dysregulation occurred in both settings including TLR, P13k-AKT and NF-κB signalling [58]. The common denominator between autoimmunity and autoinflammation was the integration of signals in both innate and adaptive cell signalling, including immune cell polarization, migration, growth, survival and differentiation.

38.4.3 Clinical Recognition of Sporadic Hyper–Inflammatory States

The monogenic autoinflammatory disorders have provided a strong platform in pediatric medicine for genetic testing and the number of genes is expanding all the time. However, rheumatology, infectious disease and hematology and other specialties consistently see sporadic hyper-inflammatory ill-defined states. The immunological disease continuum provides a platform for a systematic assessment of such cases and whether they are autoinflammatory or autoimmune. The recognition of the sporadic autoinflammatory states that are termed undefined systemic autoinflammatory disease represent an example of this [19]. These cases may present with fever, prominent cutaneous and musculoskeletal manifestations and are resistant to conventional DMARDs that are used for RA and other diseases. Such patients typically undergo repeated hospital admissions under different specialties and repeated investigations that fail to turn up a specific diagnosis. They typically show a good response to high dose corticosteroids, but are unable to wean and develop corticosteroid-related toxicity. The slow onset of action of DMARDs results in several months of inefficacious treatment trials in these cases. However, based on the remarkable efficacy of IL-1 antagonism in the well-defined monogenic autoinflammatory disorders, as shown in Fig. 38.3, IL-1β blocking strategies also often work in many, but not all of these cases [19].

38.5 Severe Complications of Autoinflammatory Disorders *Versus* Autoimmune Disorders

The severity spectrum of autoinflammatory disorders spans from very mild disease to extreme forms leading to high rates of mortality. Patients can present with poorly defined autoinflammatory-driven shock and circulatory collapse resembling sepsis. This actually may be part of the autoinflammatory disease spectrum under the guise of MAS (see Chap. 33). Indeed, MAS and severe sepsis share many clinical, laboratory, and pathologic features, including ferritin elevations, various cytopenias, hepatic dysfunction with transaminitis in particular, coagulopathy with a disseminated intravascular coagulation and tissue hemo-

phagocytosis [59]. In MAS, these features are associated with hyperferritinemia and it is likely that IL-1 is the apical cytokine that may drive a cytokine storm since its blockade was associated with a dramatic improvement in survival [60]. Further studies are needed to formally examine whether early therapy with IL-1 blockade will be effective in this extreme form of autoinflammation. Recently, IL-18, an IL-1 family member that is also regulated by inflammasomes, has emerged as potentially one of the most pivotal cytokines in these poorly designated life-threatening autoinflammatory disorders [61]. In contrast, severe life-threatening autoimmune diseases have a tendency to exhibit a predominant attack of a single organ system and lack the severe constitutional features with some exceptions including catastrophic antiphospholipid syndrome, severe pulmonary involvement in anti-GBM syndrome or severe autoimmune nephritis.

38.6 Therapeutic Implications of Autoinflammation and Autoimmunity

Not only are the autoimmunity and autoinflammation concepts underscored by common anatomical and genetic factors but their successful therapeutic manipulation further dichotomizes immunopathogenesis and appropriate disease classification (Fig. 38.8). The emerging therapeutic responses represent an overarching concept but there are

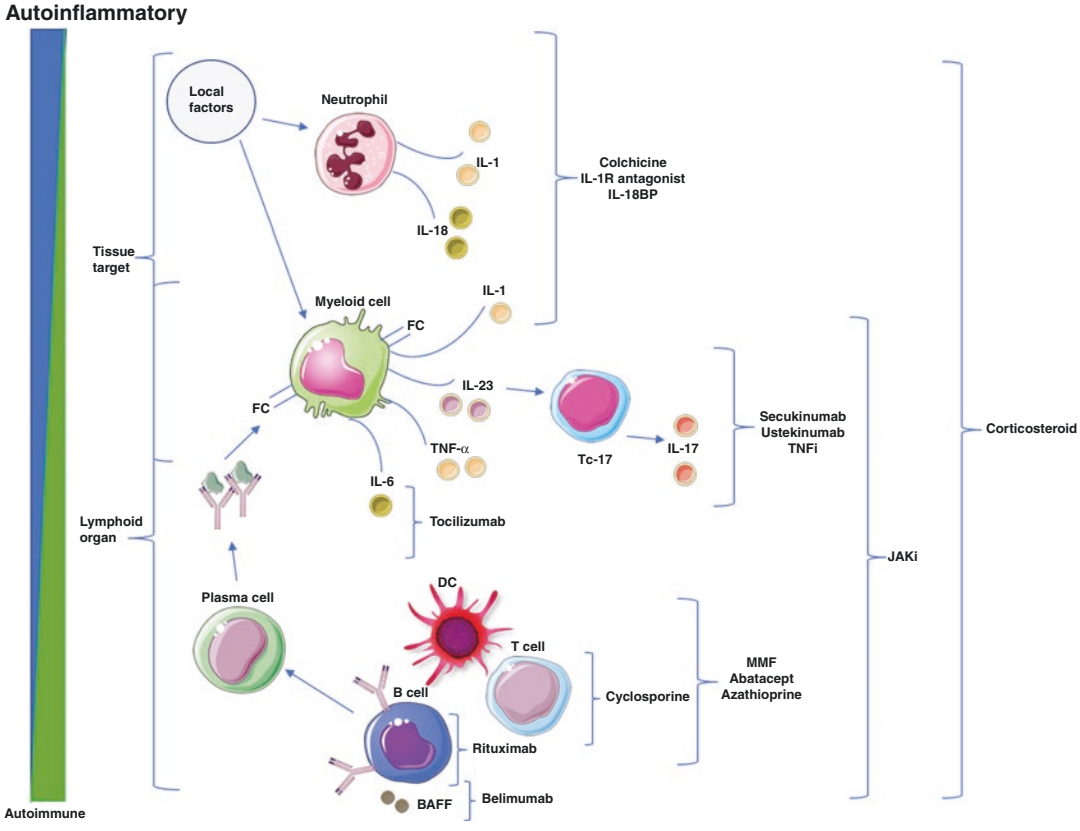

Fig. 38.8 Autoimmunity and autoinflammation classification is reflected in differential therapeutic targeting. Features of this figure are reproduced from https://smart.servier.com (Servier medical art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License), and were changed in terms of shape and size. *MMF* mycophenolate mofetil; *BAFF* B-cell activating factor; *IL* interleukin; *IL-1R* IL-1 receptor; *IL-18BP* IL-18 binding protein; *TNF* tumour necrosis factor; *TNFI* TNF inhibitor; *JAKI* Janus kinase inhibitor

some therapeutic exceptions in these groups. For example, molecules that selectively target B cells (primarily anti-CD20 therapy with rituximab) are effective for many of the classical autoantibody associated diseases. At the other extreme, the predominantly autoinflammatory diseases often show responses to anti-IL-1 strategies (Fig. 38.6). The non-autoantibody mediated SpA related diseases generally show good responses to cytokine blockers and historically the anti-TNFs. The "intermediate diseases" or "MHC-I-opathy" disorders show good responses to therapies that antagonize the IL-23/17 axis likely reflecting how local tissue resident factors ultimately orchestrate the so-called type 17 innate and adaptive lymphocytes to drive IL-17 related pathology. Small molecules that antagonize conventional T cells work over a wide range of inflammatory disorders, but generally less well than cytokine blockers. Corticosteroids work across the entire spectrum of inflammatory diseases likely reflecting their myriad of effects on the immune system. Finally, the heterogeneity and complexity of the immune system needs to be noted, reflecting the fact that some cytokine blockers, especially IL-6 antagonism, is effective for several adaptive immune disorders as well as innate/autoinflammatory disorders like for TRAPS and others (see Chap. 42).

38.7 Conclusion

The ongoing identification of new genetic variants in both autoinflammatory and autoimmune diseases highlights the complexity of these disorders including complex overlapping phenotypes. A careful scrutiny of self-directed inflammation indicates that innate immune driven or autoinflammatory disease is conceptually, clinically and therapeutically very different from the autoimmune disorders. The emergent knowledge has facilitated clinical translational strategies for recognising and treating sporadic inflammatory disorders. The application of the continuum model for classification of immunological disease seems to be a practical and useful tool to approach the diagnosis and treatment of such diseases.

Box 38.1 Definitions of Autoimmunity and Autoinflammation

Generic definition of autoimmunity

Self-directed inflammation, whereby aberrant dendritic cell, B- and T cell, responses in primary and secondary lymphoid organs lead to breaking of tolerance, with development of immune reactivity towards native antigens. The adaptive immune response plays the predominant role in the eventual clinical expression of disease. Organ-specific autoantibodies may predate clinical disease expression by years and manifest before target organ damage is discernible.

Proposal for a definition of autoinflammation

Self-directed inflammation, whereby local factors at sites predisposed to disease lead to activation of innate immune cells, including macrophages and neutrophils, with resultant target tissue damage. For example, disturbed homeostasis of canonical cytokine cascades (as in the recurrent fever syndromes), aberrant bacterial sensing (as in Crohn disease), and tissue microdamage predispose one to site-specific inflammation that is independent of adaptive immune responses.

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39

Interleukin-1 Mediated Autoinflammation from Heart Disease to Cancer

Charles A. Dinarello

Abstract

Interleukin-(IL)-1α and IL-1β are highly active proinflammatory cytokines which lower pain thresholds and damage tissues. Monotherapy blocking IL-1 in hereditary autoinflammatory syndromes results in a rapid and sustained reduction in disease severity. But blocking IL-1 activity is also effective in treating common conditions such as gout and post-myocardial infarction heart failure. Targeting IL-1 in a broad spectrum of new indications is ongoing. There are several trials of IL-1 inhibition in cancer. Initially believed to be contraindicated, targeting IL-1 in cancer has expanded greatly. Anti-IL-1α has been used to treat patients with metastatic lung and colorectal cancers, advanced pancreatic cancer, human epidermal growth factor receptor 2 (HER2) negative breast cancer and with smoldering myeloma. A placebo controlled randomized trial of canakinumab in over 10,000 subjects revealed a marked decrease in the incidence and survival of patients with lung cancer. In each of these cancers, a role for IL-1 mediated autoinflammation is a fundamental mechanism of action.

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Keywords

Inflammation · Cytokines · Osteoarthritis Cancer · Heart · Atherosclerosis · Biologics Anakinra · Canakinumab

Abbreviations

3-MCA	3-methylcholanthrene
AOSD	Adult-onset Still disease
BMI	Body mass index
CANTOS	Canakinumab Anti-inflammatory Thrombosis Outcomes Study
CAPS	Cryopyrin-associated periodic syndrome
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
FMF	Familial Mediterranean fever
HER2	Human epidermal growth factor receptor 2
IL	Interleukin
IL-1R1	IL-1 receptor 1
IL-1Ra	IL-1 receptor antagonist
LBM	Lean body mass
MABp1	Monoclonal antibody p1
MCD-1	Macrophage-derived chemoattractant-1

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MDSC	Myeloid-derived suppressor cells
NIHSS	National Institutes of Health stroke scale
NK	Natural killer
NLRP3	Nucleotide-binding domain and leucine-rich repeat pyrin containing 3
NOMID	Neonatal-onset multisystem inflammatory disease
PD-1	Programmed cell death protein 1
STEMI	ST-elevated myocardial infarction
TAM	Tumor-associated macrophages
TNF	Tumor necrosis factor
TRAPS	TNF receptor-associated periodic syndrome
WT	Wild type

Key Points

- **In addition to interleukin (IL)-1-acting as an inflammatory mediator in hereditary autoinflammatory disorders, IL-1-mediated (auto)inflammation also underlies several common diseases. These include gout, osteoarthritis, heart failure, epilepsy and atherosclerosis**
- **Cancer is an (auto)inflammatory disease. Death of tumor cells release the IL-1α precursor which is active and stimulates IL-1β. IL-1β drives synthesis and processing of additional IL-1β**
- **IL-1β is a new target for treating cancer**

39.1 Introduction

The term interleukin (IL)-1 is often used without distinguishing between the two gene products, IL-1α and IL-1β. This is because both cytokines bind to the same signaling receptor, the IL-1 receptor 1 (IL-1R1), and hence there is no significant difference between the biological activities of these cytokines. The IL-1 receptor antagonist (IL-1Ra) is also an endogenous member of the IL-1 family; IL-1Ra binds to the IL-1R1 and thereby blocks the activity of both IL-1α and IL-1β [1].

The central role of IL-1 in the pathogenesis of many disorders of autoinflammation is well established. Monocytes from patients with some autoinflammatory diseases release more IL-1β, but not tumor necrosis factor (TNF)-α, compared to healthy persons [2–4]. These autoinflammatory diseases can be regarded as 'natural experiments', which reveal the clinical and pathologic consequences of dysregulated IL-1-mediated inflammation in humans. Lessons from autoinflammatory diseases extend and apply beyond this group of rare conditions: deregulated activation of the myeloid compartment and IL-1 also mediate several common diseases, which can also be classified as autoinflammatory disorders (i.e. gout (see Chap. 34), pericarditis (see Chap. 36), or at least include autoinflammation as part of disease pathogenesis (e.g. heart failure, diabetes, myocarditis) [5].

Anakinra, a recombinant form of IL-1Ra, is used to treat a broad variety of diseases, ranging from common conditions such as rheumatoid arthritis, gout, and recurrent idiopathic pericarditis, to rare hereditary diseases ([1], see Chap. 41). Because of the safety and rapid onset of action, IL-1 inhibition with anakinra can be used as a diagnostic as well as a treatment tool for patients with undefined signs or symptoms of autoinflammation [6]. Another drug which blocks IL-1 signaling is canakinumab, a monoclonal antibody against IL-1β (see Chap. 41). A recently reported randomized, double-blind trial of canakinumab performed over 4 years in 10,061 patients with previous myocardial infarction, called the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial, offers new insights in the role of IL-1β in heart disease, as well as in osteoarthritis and cancer [7]. Other recent trials with IL-1 inhibition have also revealed a role for IL-1 in cancer.

This chapter will focus on the role IL-1 mediated inflammation and autoinflammation in several common disorders and especially in diverse forms of cancer.

39.2 The Role of IL-1 Mediated Inflammation in Several Common Disorders

39.2.1 Osteoarthritis

There is clinical and experimental evidence that IL-1 is involved in the pathogenesis of osteoarthritis. Previous studies have evaluated the efficacy of direct instillation of anakinra into affected knee joints of patients with osteoarthritis. However, these injections yielded limited clinical benefit, which did not extend beyond 1 month from administration (possibly due to short-term persistence of anakinra in the joint space) [8, 9]. Anakinra has demonstrated some efficacy against joint pain and swelling in erosive osteoarthritis of the hand [10]. IL-1 inhibition with antibodies to the IL-1 receptor has also been evaluated, again with only modest improvement [11]. Recent data from the CANTOS trial support a role for IL-1β in osteoarthritis. Although this was not the intent of the study, the incidence of osteoarthritis reported as an adverse event was significantly lower in the canakinumab group than in the placebo group [7]. Patients receiving 150 mg of canakinumab every 3 months reported a lower incidence of osteoarthritis (1.67 per 100 person-years for placebo versus 1.12 for canakinumab, $p < 0.001$). The evidence from the CANTOS database is strong because of (1) the large number of patients enrolled worldwide (n = 10,061) and long-term follow-up, (2) the randomized, placebo controlled nature of the trial and (3) the specificity of IL-1β neutralization. The demographics of the entire CANTOS population included subjects with a relatively advanced age (mean 61.1 years), high body mass index (BMI) (mean 29.7–29.9) and type 2 diabetes in approximately 40% of the participants [7], each of which is characteristic of the population of patients with osteoarthritis. Not unexpectedly, there was also a significant reduction in gouty arthritis [7]. Although treatment with canakinumab was effective in reducing the incidence of osteoarthritis, systemic treatment with anakinra or canakinumab is an unlikely therapy for this disease.

39.2.2 IL–1 and Heart Disease

39.2.2.1 Acute Myocardial Infarction

The first studies of anakinra in acute ischemic heart disease involved patients who had suffered a ST-elevated myocardial infarction (STEMI) [12, 13]. Seventy-two hours after an acute myocardial infarction, despite optimal standard of care, inflammation develops due to myocardial ischemia. The C-reactive protein (CRP) reaches a peak level, which correlates with the size of the area of infarction. Infiltration of neutrophils and monocytes into the area surrounding the ischemic tissue contributes to further damage, which is significantly reduced in animal models by treatment with anakinra [14]. In the human studies in STEMI, anakinra was administered subcutaneously at a dose of 100 mg/d for 2 weeks, following stent placement and in addition to optimal standard of care. In a pilot study of 10 patients with STEMI, treatment with anakinra resulted in a significant reduction in CRP levels [12], and a reduction of the progressive inflammatory response and myocardial damage. Twelve weeks following myocardial infarction, the heart function of patients was evaluated as residual left ventricular ejection fraction. Compared to the placebo-treated group, patients treated with anakinra seemed to exhibit an improved functional status, but this did not reach statistical significance [12]. A second trial was performed in 30 patients [13]. Again, anakinra significantly reduced CRP levels 72 h after myocardial infarction; after 10–14 weeks, this reduction in CRP correlated with a reduction in left ventricular end-systolic volume [13]. Patients treated with anakinra exhibited an overall reduction in the development of heart failure (New York Heart Association grade III and IV) compared to placebo-treated patients [13].

Subsequent studies confirmed that anakinra treatment effectively dampens inflammation

associated with myocardial infarction. Anakinra treatment was started after standard of care for the acute event in 182 patients with non-STEMI myocardial infarction and continued for 14 days [15]. For 7 days following the acute event, a significant reduction in CRP levels was observed in patients receiving anakinra compared to placebo (expressed as log-transformed area under the curve); levels rose again 16 days after cessation of anakinra [15].

In the previously mentioned CANTOS trial [7], 10,061 patients with previous myocardial infarction and a high-sensitivity CRP level of 2 mg or more per liter were included. The trial compared three doses of canakinumab (50 mg, 150 mg, and 300 mg, administered subcutaneously every 3 months) with placebo. The primary efficacy end point was nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death. Antiinflammatory therapy targeting the IL-1β innate immunity pathway with canakinumab at a dose of 150 mg every 3 months led to a significantly lower rate of recurrent cardiovascular events than placebo, independent of lipid-level lowering (risk of primary end point 15% lower than that in placebo group) [7].

39.2.2.2 Heart Failure

Several years ago, *ex vivo* studies with human atrial heart strips revealed that IL-1β suppresses cardiac contractility, even at picomolar concentrations [16]. In recent years, various studies examined the effects of anakinra on heart failure with poor exercise tolerance and signs of systemic inflammation. For example, mice treated with a single dose of recombinant human IL-1β have a 76% reduction in response to isoproterenol and a 32% reduction in left ventricular function. In a clinical trial, seven patients with heart failure and elevated markers of systemic inflammation despite standard of care treatment received 100 mg of anakinra daily for 14 days. Compared to baseline, treatment with anakinra was associated with a statistically significant improvement in oxygen consumption, a marker of exercise capability [17]. This study first established a role for anakinra treatment in patients with refractory heart failure.

Besides impaired left ventricular contractility, heart failure with preserved ejection fraction (or diastolic heart failure) can also occur and be associated with reduced exercise tolerance. In a double-blind, randomized, placebo controlled study patients with this condition treated with anakinra at a dose of 100 mg/d for 14 days exhibited a significant increase in oxygen consumption of 1.2 mL/kg/min and a concomitant 74% reduction in CRP levels [18].

Patients with acute, decompensated heart failure often exhibit signs of systemic inflammation. Thirty patients with acute decompensated heart failure with an ejection fraction less than 40%, and elevated CRP were randomized to receive either anakinra or placebo [19]. Upon entering the trial, patients received either 100 mg anakinra or placebo twice daily for 3 days followed by 11 days of once daily dosing. Three days into the trial, CRP levels decreased by 61% from baseline in the anakinra group compared to a 6% reduction in the placebo treated group [19]. Although the study was not powered to determine a clinical benefit, it showed that IL-1 inhibition with IL-1 receptor blockade reduced the systemic inflammation associated with acute heart failure. In all these trials of heart failure, patients received anakinra for only 14 days. Although clinical and objective data indicate a functional improvement as well as reduced inflammation already after short-term treatment, it is possible that a prolonged course of anakinra would result in a more marked benefit. For example, patients hospitalized with an episode of acute decompensated heart failure are at high risk for repeated hospitalizations due to recurrent episodes. Therefore, a trial of anakinra (100 mg/d) was conducted comparing two different treatment durations (2 vs. 12 weeks) in patients discharged from the hospital following an episode of acute decompensated heart failure. In this study, patients treated with 12 weeks of anakinra had reduced hospital readmission rates and improved aerobic capacity,

oxygen consumption, and quality of life compared to patients receiving either placebo or 2 weeks of anakinra [20]. Of note, patients receiving anakinra for rheumatoid arthritis exhibited improved cardiac contractility, even within 3 h of a single administration [21].

39.2.2.3 Myocarditis and Pericarditis

Clinical observations indicate a central role of IL-1 in the pathogenesis of cardiac inflammation. For example, myocardial involvement is part of the clinical spectrum characteristically mediated by IL-1. IL-1 blockade is highly effective in these conditions, as exemplified by several published cases of myocarditis associated with systemic juvenile idiopathic arthritis and adult-onset Still disease promptly controlled by anakinra [22, 23] (see Chap. 32). However, there is emerging evidence that anakinra can be effective in the treatment of fulminant myocarditis irrespective of the initiating trigger or underlying condition [22, 24, 25]. In patients with myocarditis-associated acute heart failure, beneficial effects of anakinra on myocardial contractile function are particularly striking, and generally consistent with the observed benefit in patients with heart failure. It remains to be determined whether anakinra may increase myocardial function in non-acute myocarditis as it does during the acute condition. Blocking TNF-α in myocarditis is contraindicated. There are case reports of successful treatment with anakinra for myocarditis in patients non-responsive to anti-IL-6 [26].

Pericarditis can be a manifestation of autoinflammatory conditions. For details on idiopathic recurrent pericarditis see Chap. 36.

39.2.3 IL–1 and the Central Nervous System

Neurologic complications observed in patients with cryopyrin-associated periodic syndrome (CAPS) reveal the effects of IL-1-mediated inflammation in the brain (see Chap. 19). Common clinical manifestations include headache (including migraine), sensorineural hearing loss, papilledema due to elevated intracranial pressure, and mental impairment [27]. IL-1 blockade with anakinra, and to a lesser degree with canakinumab, reverses neurologic inflammation and related symptoms, including mental and hearing impairment [28–32]. The first evidence that anakinra administered peripherally crossed the blood brain barrier and reduced severity of a disease primarily localized to the central nervous system came from neonatal-onset multisystem inflammatory disease (NOMID) [3] (see Chap. 41). Specifically, 12 children with NOMID were treated with 1–2 mg/kg/d of subcutaneous anakinra. The median cerebrospinal fluid (CSF) level of IL-1Ra was 211 pg/mL before treatment, but rose to 1136 pg/mL after 3 months of treatment [3]. These effects were associated with a remarkable decrease in the severity of various manifestations of NOMID, including elevated intracranial pressure, leptomeningitis, and sensorineural hearing loss, as well as reduced cerebrospinal fluid (CSF) levels of IL-6.

39.2.3.1 Cerebrovascular Accidents and Trauma

In a placebo-controlled study intravenous anakinra was administered to 13 patients with subarachnoid hemorrhage due to aneurysmal rupture [33]. Within 72 h of the acute event, patients received a bolus infusion of 500 mg of anakinra followed by a steady infusion of 10 mg/kg/h for 24 h. At 24 h, CSF levels of IL-6 were reduced in the anakinra compared to the placebo group (clinical outcomes were not studied as part of this trial) [33].

A related study investigated the dose regimen necessary to obtain a CSF concentration of anakinra of 100 ng/mL. This concentration was deemed neuro-protective based on studies of rats subjected to brain ischemia [34]. As for human reference, this target concentration is 100-fold greater than that in the CSF of children receiving subcutaneous anakinra 100 mg/d for 3 months

[3]. In this study, patients with subarachnoid bleeding received incremental doses of intravenous anakinra [35]. Specifically, patients received a bolus dose of anakinra (100–500 mg) followed by a 4-h infusion of anakinra from 1 to 10 mg/kg/h. Levels of anakinra were monitored in the plasma and CSF (collected through a cerebral ventricular drain). A target CSF level of 100 ng/mL was achieved with the highest regimen (a bolus of 500 mg followed by 4 h of anakinra at 10 mg/kg/h) [35, 36]. The authors concluded that anakinra passively enters the brain in patients with a subarachnoid hemorrhage: therefore, a high-dose regimen of anakinra may reduce inflammation, infiltration of neutrophils, and edema at the site of the lesion. In a subsequent randomized study, patients with subarachnoid hemorrhage received anakinra 100 mg twice daily subcutaneously within 3 days of a stroke and for the following 21 days. Anakinra treatment significantly reduced levels of the inflammatory markers IL-6, CRP, and fibrinogen. Although these studies were not powered to determine clinical effects, scores of the Glasgow Outcome Scale at 6 months were possibly better, albeit not significantly, among patients receiving anakinra. Whether dampening of IL-1-mediated inflammation will result in improved neurological outcomes remains to be determined in adequately powered, randomized, placebo-controlled studies.

In a different study, intravenous anakinra was administered to patients admitted to the hospital within 6 h of an acute thrombotic stroke [37]. This trial included 34 patients and was randomized and placebo controlled. Anakinra was administered at a high dose of 2 mg/kg/h for 72 h, analogous to inception trials of anakinra in septic shock. Compared to placebo-treated controls, patients treated with anakinra had lower IL-6, CRP, and neutrophil levels [37]. Although the study was not powered for detecting significant improvements in neurological outcomes, the subgroup of patients with cortical infarcts receiving anakinra performed better compared to the placebo group, as expressed in several standardized scoring scales (National Institute of Health stroke scale (NIHSS), Barthel index, modified Rankin scale).

Additional evidence that anakinra crosses the blood brain barrier and exerts anti-inflammatory effects in the brain comes from studies of traumatic brain injury, a major cause of death and disability worldwide, particularly in young persons. In a randomized, open-label trial, 20 patients who had suffered diffuse traumatic brain injury within the previous 24 h received either anakinra, 100 mg/d for 5 days, or placebo. A central microdialysis catheter was placed in each patient as part of standard of care. Prior to administration of anakinra, the mean level of IL-1Ra in the CSF was 78 pg/mL but rose to 138 pg/mL 12 h after the first dose [38]. In general, inflammatory cytokines in the CSF were lower in patients treated with anakinra; of these, macrophage-derived chemoattractant-1 (MDC-1) was remarkably lower compared to patients treated with the placebo [38]. The study was not powered to evaluate clinical improvement, although the marked decrease in CSF levels of cytokines and MDC-1 argue in favor of beneficial anti-inflammatory effects.

39.2.3.2 Epilepsy

Although IL-1α is found in brain astrocytes and microglia, available data point at IL-1β as the main cytokine contributing to epileptic seizures [39]. Several studies have focused on febrile seizures since these are among the most common type of seizure activity. Using an animal model for febrile seizures, an agonist role in the hippocampus for IL-1β and an antagonist role for endogenous IL-1Ra have been reported [40]. Other studies examined circulating cytokines in patients with recurrent seizures, and revealed elevated levels of IL-6 and IL-1Ra in the post-acute period [41]. In one study, high levels of IL-1β were also observed during acute episodes of recurrent temporal lobe epilepsy [42]. Some studies have reported polymorphisms in IL-1α, IL-1β and IL-1Ra in subjects who develop epilepsy as adults [43–47].

39.3 The Role of IL–1 Mediated Inflammation in Cancer

Whether IL-1 is "good" or "bad" for cancer is a challenging issue similar to the role of IL-1 in infection. Clinically, we know that reducing IL-1, particularly IL-1β using canakinumab, increases the risk of serious infections [1, 7]. Blocking IL-1α and IL-1β with anakinra also increases the risk of infection; however, because anakinra has a very short half-life, the danger of anakinra in infection is less than that of the long-lasting canakinumab (see Chap. 41). Ample evidence in mice supports a role for IL-1 in protecting the host from various bacterial and fungal infections. On the other hand, treating mice with antibodies to IL-1β can increase survival. In that case, the reduction in IL-1-mediated systemic inflammation contributes to the survival of the mouse.

Chronic inflammation is at the root of most solid tumors. In mice deficient in IL-1β and subjected to local carcinogenesis, a reduction in inflammation resulted in slower development of tumors that developed only in some of the mice while they developed in all wild-type mice [48]. Yet, IL-1-mediated local inflammation in carcinogenesis is a double edge sword. For instance, once a cancer develops, for example melanoma in the skin or a local adenoma in the colon, IL-1β as well as IL-1α are needed to increase the maturation of dendritic cells in the recognition of neoantigens. Here, IL-1 serves as an adjuvant for anti-tumor immunity. When successful, the tumor neoantigens are recognized by IL-1-primed dendritic cells, which instruct the T-cell receptor to mount a T-cell response to generate tumor specific CD8 cytotoxic T-cells. IL-1 also serves as an adjunct for expansion of CD8 cells, which eliminate the nascent tumor cells. We assume that this scheme takes place often and many nascent tumors are eliminated by immune destruction. If, however, IL-1 plays a major role in assisting the host in the recognition and processing of tumor neoantigens, patients receiving IL-1 blocking therapies should have a higher incidence of cancer. At present, there are no long-term studies of patients receiving IL-1 blocking therapies that reveal an increase in cancer incidence. Quite the contrary. In a cohort of cigarette smokers at high-risk to develop cancer receiving canakinumab for 4 years as part of a worldwide study to reduce recurrent myocardial infarction (the CANTOS trial), the incidence of cancer was significantly reduced [49]. In patients with early stage multiple myeloma called smoldering myeloma, anakinra significantly increased overall survival and prevented progression to active myeloma in some patients for over 10 years [50].

39.3.1 The Properties of IL–1 that Directly Relate to Cancer Progression

39.3.1.1 Carcinogenesis

A role for IL-1 in carcinogenesis is primarily one of local inflammation. In models with known carcinogens in mice, there are studies demonstrating a role for IL-1 in the processes of malignant transformation. For example, in the model of intradermal 3-methylcholanthrene (3-MCA)-induced carcinogenesis, histologic analyses revealed fibrotic structures forming a capsule surrounding droplets of the carcinogen in olive oil, resembling foreign body-like granulomas. These appeared 10 days after injection of 3-MCA and persisted until the development of local tumors [48]. However, in mice deficient in IL-1β there were significantly fewer tumors and the onset of the development of tumors was delayed in those that did develop a tumor when compared to wild-type mice [48]. Fewer leukocytes infiltrated the site of the carcinogen injection in mice deficient in IL-1β. At Day 70, there was a dominance of macrophages in wild type (WT) and mice deficient in IL-1α, which were nearly absent in mice deficient in IL-1β These studies support a role for local IL-1β in tumor microenvironment. Unexpectedly, tumor incidence was similar in WT and mice deficient in IL-1α. In mice deficient in IL-1Ra (so increased IL-1 signaling), tumor development was more rapid than those in WT

mice. In these IL-1 Ra deficient mice, an intense neutrophilic response was observed. When mice deficient in IL-1Ra-were treated with recombinant IL-1Ra, the neutrophilic response was significantly reduced. In contrast, blocking endogenous TNF-α had no effect. This observation supports the concept that differences in carcinogenesis are due to the balance of IL-1 and IL-1Ra at the site of local inflammation.

39.3.1.2 IL–1 and the Tumor Microenvironment

In the tumor microenvironment, one considers the role of IL-1 from the infiltrating myeloid cells as well as IL-1 production from the tumor cells. In addition, the production of IL-1Ra from the infiltrating myeloid cells is of considerable relevance. It is possible that tumors also produce IL-1Ra. In health, the IL-1α precursor is found in nearly all epithelial cells such as the entire gastrointestinal tract, the epithelial cells of the lung, the ductal epithelium of the breast, prostatic cells, the hepatocyte and lining of the bladder and the renal epithelium. Therefore, tumors originating from these cells contain the IL-1α precursor. Melanoma cells, B –cells of lymphoma, Hodgkin lymphoma cells and the plasma cells of multiple myeloma contain the IL-1β precursor [51]. In general, the level of IL-1α or IL-1β often correlates with poor outcomes [52]. The IL-1β in the tumor microenvironment may be from the tumor cell itself but is more likely to be from infiltrating myeloid cells, such as monocytes, neutrophils, dendritic and natural killer (NK) cells. T-cells are often present in the tumor and can be a source of either IL-1α or IL-1β. Regardless of whether IL-1 is of tumor cell origin or from infiltrating bone marrow-derived cells, IL-1 plays a major role in inducing angiogenesis [53–55]. As the tumor increases in size due to neovascularization, IL-1 induction of matrix metalloproteinases facilitates the entry of tumor cells into the circulation which results in local or distant metastasis. IL-1 itself is a growth factor for malignant cells but IL-1 also induces growth factor production from resident stromal cells. For example, in multiple myeloma, IL-1β from the malignant plasma cells in the bone marrow induces IL-6 from the stromal cells; IL-6 is a growth factor for plasma cells [56].

39.3.1.3 IL–1 in the Systemic Response to Cancer

Although levels of circulating IL-1β or IL-1α are low and often below the level of quantification in cancer [57], the systemic response to cancer reveals that the inflammation of cancer is due, in part, to IL-1. In two studies treating patients with metastatic colorectal cancer with a neutralizing antibody to IL-1α, the levels of IL-6 fell consistently as did the platelet counts [58, 59]. Not unexpectedly, the inflammation of cancer increases the levels of circulating of IL-1Ra [51]. In several studies, the levels of IL-1Ra reflect IL-1 driven inflammation, as is the case with non-cancer inflammation such a patients with coronary artery inflammation [60]. However, in some cancers, the expected elevated levels of IL-1Ra are low [51].

39.3.2 IL-1 Inhibition in Treating Cancer

Recent trials have supported a role for IL-1 in cancer. As discussed below, patients with cancer are being treated with anakinra as well as a monoclonal antibody that targets IL-1α. Anti-IL-1α has been used to treat end-stage metastatic cancers [58, 61]. In a placebo-controlled randomized trial in over 300 subjects with advanced metastatic colorectal cancer, the primary and secondary endpoints were met [59]. Anakinra has been administered to patients with smoldering myeloma [50, 62], advanced pancreatic cancer [63, 64], colorectal cancer [65] and HER2 negative breast cancer [66]. In the CANTOS trial, the overall survival of patients with lung cancer was 77% compared to placebo treated patients [49].

39.3.2.1 Anakinra for Pre–Multiple Myeloma

The first study to use an IL-1 blocking therapy to treat cancer was in patients with a diagnosis of pre-myeloma stage. Anakinra treatment was assessed in patients with smoldering or indolent myeloma, and first reported by Lust and coworkers in 2009 [62]. The treatment was the standard dose of 100 mg daily of anakinra plus a weekly low dose of 20 mg of oral dexamethasone. The aim for using anakinra in these patients was to reduce progression to overt myeloma, a consistently fatal disease even with advanced chemotherapeutics and bone marrow transplantation. The mechanism of action of IL-1 blockade was based on data that IL-1β released from bone marrow plasma cell induces IL-6 production from marrow stromal cells; furthermore, IL-6 is a growth factor for the malignant plasma cell [56]. Since the amount of IL-1β released from malignant plasma cells is low, anakinra would reduce IL-6 with greater efficacy compared to neutralizing IL-6. In addition, experimental data revealed that although anakinra was highly effective in reducing IL-1β induced IL-6 *in vitro*, adding dexamethasone resulted in the death of the plasma cells [56, 62, 67].

A follow-up assessment of the treated cohort of patients has recently been reported [50]. A reduction in serum CRP level of ≥40% from baseline was used as a biomarker of response to anakinra. Of the 47 patients, 22 patients did not achieve this 40% decrease in CRP levels; the median progression free survival in this subgroup was 11 months. However, in 25 patients with a decrease in CRP levels of ≥40%, the median progression free survival was 104 months ($p < 0.001$). Moreover, the median overall survival of the responders was not reached whereas the overall survival of those patients without a 40% reduction in CRP was 7.9 years ($p < 0.001$).

The clinical assessment of patients with smoldering myeloma includes a therapeutic intervention or a "wait and see" approach. Since the progression to active myeloma in many patients occurs within 6 months to 1 year, blocking IL-1 with anakinra might be a strategy for preventing progression of the disease. Each of the 47 patients in the study met established criteria for smoldering myeloma or indolent myeloma [50]. Patients who did progress had statistically significant evidence of more active disease at time of enrollment. The standard of care for patients with active multiple myeloma is often autologous bone marrow transplantation, but this is not standard therapy in older patients. The overall survival data of the 47 patients include those who underwent transplantation. Many of the 25 patients with a CRP decrease of ≥40% who did not undergo bone marrow transplantation remained without progression to active disease. As such, a universally fatal disease was held at bay by daily administration of anakinra with weekly low-dose dexamethasone. It is possible that anakinra therapy in those without a 40% decrease in CRP levels (considered non-responders) was started at a time when progression had already occurred. In contrast, when anakinra was started at an earlier stage of the disease, the progression free survival as well as the overall survival was significantly greater.

39.3.2.2 Anakinra for Solid Tumors

Despite a considerable number of reports in various animal models, there has been limited use of anakinra to treat solid tumors in humans. Models of breast cancer are of particular interest based on data that the levels of IL-1β in biopsies and excised tumors from patients with hormone receptor negative breast cancer correlate with a poor prognosis [68]. A polymorphism in the IL-1β gene (rs1143627) has been proposed to be related to increased risk of breast cancer but a meta-analysis of several studies revealed that this polymorphism was not a risk factor [69].

39.3.3 How Much of Anakinra Therapy in Cancer Is Blockade of IL–1α Activity?

Since IL-1α and IL-1β can play a role in augmenting immune recognition, a prevailing, but inaccurate, idea is that blocking IL-1β or IL-1α would contribute to the immunosuppression of cancer and be contraindicated. However, reversing the immunosuppression of cancer has been validated with blocking cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and programmed cell death protein-1 (PD-1). Therefore, there is now less reluctance to neutralize an immunostimulatory cytokine such as IL-1α. In what may be a landmark study, 52 patients with refractory, end-stage cancer (18 different tumor types) received a course of MABp1, a neutralizing antibody to IL-1α [61]. Within the treated population, a significant number of patients responded with an increase in lean body mass (LBM), decreased constitutional symptoms and extended life compared to non-responders.

The study, albeit small, is the first to specifically neutralize IL-1α, a highly inflammatory member of the IL-1 family. It is a unique contribution for many reasons, particularly in end stage cancer patients. Since blocking the IL-1 receptor with anakinra or neutralizing IL-1β with canakinumab or rilonacept [1] is without serious adverse effects, it was not unexpected that there were no serious adverse effects with use of anti-IL-1α. Importantly, the study evokes an examination of likely mechanisms, which could account for the objective and constitutional endpoints. First, the data demonstrate that treatment reduces systemic inflammation, since a decrease in circulating IL-6 levels remains one of the most consistent observations of blocking IL-1 [1]. The source of the inflammatory trigger is likely the tumor itself, as all cancer cells of epithelial cell origin contain IL-1α in its precursor form. Inflammation also is due to invading stromal cells into the tumor microenvironment. As tumors outgrow their vascular supply, they become necrotic, the IL-1α precursor is readily released and triggers local production of chemokines, which facilitate an influx of neutrophils and monocytes [1]. Unlike the precursor of IL-1β, the IL-1α precursor is fully active [70]. Neutralization of local IL-1α likely reduces the infiltration of tumor-associated macrophages and myeloid derived suppressor cells, which contribute to the immunosuppression of cancer mediated by inflammation [71].

At some point, this local inflammation must become systemic in order to account for one of the clinical endpoints of the study, the association of increased survival (19.3 months) with increased LBM in patients with colorectal cancer compared to 6.6 months in those who failed to increase LBM. The association of non-cancer chronic inflammation with loss of LBM is well established and IL-1 can directly induce muscle protein breakdown [72]. The study also reports a reduction in fatigue, which is consistent with the use of anakinra in patients with inflammatory diseases unrelated to cancer [1]. In the early 1990s either IL-1β or IL-1α was administered to patients with suppressed bone-marrow due to chemotherapy in order to stimulate hematopoiesis. Although effective, picomolar concentrations of IL-1α were toxic causing development of fever, severe fatigue, loss of appetite, myalgias and frank hypotension [73]. Since picomolar concentrations of IL-1α induced these symptoms, demonstrating elevated circulating IL-1α should not be a criterion for the rationale of blocking IL-1α in cancer. IL-1α is also present on platelets which may account for the systemic effects such as elevated serum levels of IL-6. In fact, the longstanding reports of platelet involvement in metastasis, which would include platelet-endothelial cell interaction [74], may now be, in part, understood by neutralization of IL-1α. This study is the first clinical evidence that endogenous IL-1α-induced IL-6 contributes to the thrombocytosis of cancer [73].

Additional possible mechanisms of action with neutralization of IL-1α include decreased angiogenesis [53] and decreased immunosuppression [75]. IL-1α neutralization also includes direct anti-tumor properties by inhibition of tumor growth. With IL-1α presence in non-cancerous as well as cancerous cells and given the broad inflammatory properties of IL-1α, no one

mechanism accounts for the study's observations [61]. There are several remaining questions. Is refractory end-stage cancer with progressive loss of LBM sufficient to initiate treatment with IL-1α neutralization? Given the near total lack of adverse effects, the long-term safety of IL-1 blockade [1] and the efficacy of monoclonal antibody p1 (MABp1), there are few reasons to withhold treatment in this population. Would neutralizing IL-1α exhibit a greater efficacy if used earlier as an adjunct during chemotherapy? For example, would neutralizing IL-1α potentiate the anti-tumor effect of tyrosine kinase inhibitors? Since we know that IL-1 induces myeloid suppressor cells [71], would neutralizing IL-1α be beneficial if used in combination with anti-CTLA4 or anti-PD-1?

39.3.4 CANTOS Trial Reveals a Role for IL–1β in the Progression of Cancer

In the CANTOS trial, 10,061 patients were randomized to receive 50, 150, 300 mg of canakinumab or placebo every 3 months. Patients with a known cancer were excluded from enrollment [7]. During the 4-year duration, these subjects were evaluated for a diagnosis of cancer by oncologists blinded to the whether the patients were treated with canakinumab or placebo. There were 196 deaths from cancer in the overall study. The first observation was remarkable: in patients receiving the 300-mg dose of canakinumab, there was a 51% reduction in the incidence rate of death from any cancer when compared to the placebo group (0.31 per 100 person-years versus 0.64 per 100 person-years, $p = 0.0009$) [49]. The baseline CRP levels in those patients who were determined to have lung cancer were 6.0 mg/L compared to 4.2 mg/L in patients with no cancer ($p < 0.0001$). Similarly, the baseline IL-6 in the cancer group was 3.2 pg/mL compared to 2.6 pg/mL in those without a diagnosis of cancer ($p < 0.0001$). The incidence of lung cancer diagnosis was reduced by 67% in patients receiving the 300-mg dose of canakinumab ($P = 0.00008$). In this group of patients with lung cancer, treatment with the 300 mg dose of canakinumab reduced fatal lung cancer by 77% (0.0002).

39.3.5 Can Autoinflammation Explain Cancer Progression?

The reduction in the incidence and survival of cancer demonstrated in the CANTOS trial reveals the role of IL-1 driven inflammation in the progression of cancer. As shown in Fig. 39.1, there are several known inflammatory properties of IL-1, which mediate cancer progression. A fundamental concept in autoinflammation is that regardless of the initiating event, as inflammation increases, the production of IL-1 also increases and contributes to more inflammation. As seen in Fig. 39.1, cancer progression from its early stage of malignant cell transformation during carcinogenesis in lung cancer, vascularization and local spread to invasion into the circulation and lymphatic system suggest autoinflammatory mechanisms independent of T or B cell function. The suppression of acquired immune responses in cancer is specific for the neoantigens of the tumor but the IL-1-driven immunosuppression is nonspecific and therefore due to autoinflammation. The data from the subjects with lung cancer in the CANTOS trial provide evidence that any of the mechanisms shown in Fig. 39.1 may contribute to the impressive reduction in incidence and increased survival [49]. The CANTOS trial [7] as well as anti-IL-1α treatment of advanced metastatic cancer [61] support the concept that IL-1 blockade is a safe, non-toxic, durable checkpoint inhibitor, reversing the immunosuppression of cancer-induced inflammation. One can speculate whether reversing the immunosuppression of cancer by the toxic checkpoint inhibitors may be better served when used in combination with IL-1 blockade. Would treating very early cancer with IL-1 blockade monotherapy prevent cancer progression?

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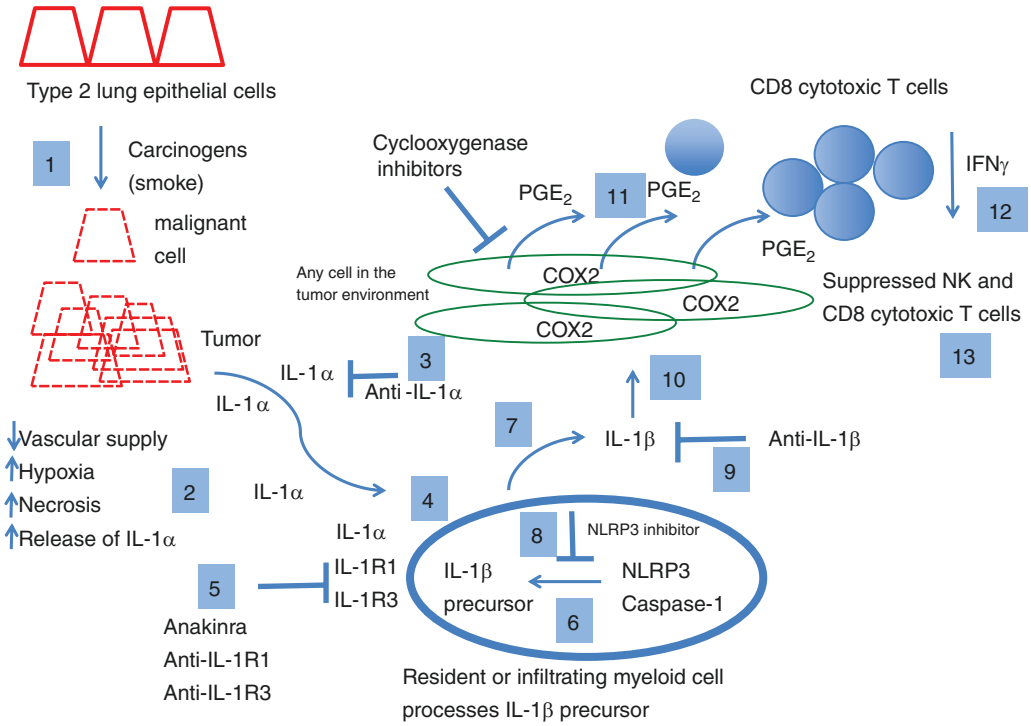

Fig. 39.1 The role of interleukin (IL)-1 in the pathogenesis of lung cancer. (1) Carcinogens, such as smoke in the lungs, induce mutations and transform epithelial cells into a malignant phenotype. IL-1 may play a role at this stage, as mice deficient in IL-1β do not develop tumors to the carcinogen 3-methylcholanthrene [48]. (2) As the tumor increases in size, neoangiogensis is not sufficient, hypoxia leads to frank necrosis and the IL-1α precursor is released. Most tumors of mesenchymal origin contain constitutive IL-1α precursor, which is released with tumor necrosis. The IL-1α precursor is fully active [70]. (3) At this point, neutralizing antibodies targeting IL-1α prevent activities such as binding to IL-1 receptors on nearby cells. Neutralizing IL-1α has been reported in three trials of human cancer, including a randomized. Placebo controlled trial in 300 patients with advanced metastatic colorectal cancer [58, 59, 61]. (4) IL-1α binds to the IL-1R1 on resident macrophages in the tumor microenvironment; these can be tumor-associated macrophages (TAM) or infiltrating myeloid-derived suppressor cells (MDSC), either of granulocytic or monocytic lineage. (5) Anakinra, which blocks IL-1R1, prevents the downstream signals from the IL-1R complex. Similarly, antibodies that block IL-1R1 or IL-1R3 also prevent the downstream signals from the IL-1R complex. (6) One of the downstream signals from the IL-1R is transcription and synthesis and release of IL-1 itself from the resident or infiltrating myeloid cells, a marker of autoinflammation. Mechanistically, the inactive IL-1β precursor accumulates in the cytosol and awaits activation of nucleotide-binding domain and leucine-rich repeat pyrin containing 3 (NLRP3) and cleavage by caspase-1. Activation of the NLRP3 inflammasome likely takes place by increasing concentrations of adenosine triphosphate (ATP) in the tumor microenvironment due to necrotic cells. In a model of breast cancer in mice, blocking NLRP3 reduces IL-1 and there is a reduction in the tumor [66]. (7) Active IL-1β is released from the cell. (8) At this stage, inhibitors of NLRP3 reduce the processing and release of active IL-1β. (9) Antibodies that neutralize IL-1β prevent its activities. (10) IL-1β binds to IL-1R on any cell in the tumor microenvironment and induces cyclooxygenase-2 (COX2). Prostaglandin E2 (PGE2) increases in the microenvironment. (11) Inhibition of COX2 reduces PGE2 levels. (12) PGE2 suppresses interferon (IFN)-γ from T-cells, particularly in CD8 cytotoxic lymphocytes (CTL). (13) Immunosuppression is accomplished by reduced anti-tumor properties of natural killer (NK) cells and CD8 cytotoxic T lymphocytes (CTL)

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Part VII

Pharmaceutical Agents for Treatment of the Autoinflammatory Diseases

Colchicine

40

Eldad Ben-Chetrit

Abstract

Colchicine is an alkaloid which was originally extracted from bulbs of a plant called Colchicum autumnale (meadow saffron). Its active pharmacological component was isolated in 1820 and in 1833 the active ingredient was purified and named colchicine. It consists of three hexameric rings termed A, B, and C. It was first recommended for the treatment of gout by Alexander of Tralles in the sixth century AD. Later it has been employed for suggested and approved indications including primary biliary cirrhosis (PBC), alcohol induced hepatitis, psoriasis, Behçet disease, Sweet syndrome, scleroderma, sarcoidosis and amyloidosis. Perhaps the most effective results have been obtained in the prophylaxis of familial Mediterranean fever (FMF). Colchicine is absorbed in the jejunum and ileum and is trapped in the body tissues. It is metabolized in the liver and the intestine by cytochrome P (CYP) 450 3A4 and P-glycoprotein (PGY) 1. Colchicine is excreted mainly by the biliary system, intestines and the kidneys. It has a narrow therapeutic range, but with normal liver and kidney functions is relatively safe and can be used during pregnancy, nursing and in infants. The main mechanism of action of colchicine is probably through interaction with microtubules affecting leukocyte chemotaxis, thereby suppressing inflammation. The blood level of colchicine may be affected by concomitant drug administration and therefore, caution should be exercised when such medications are added.

Keywords

Colchicine · Familial Mediterranean fever (FMF) · Gout · Inflammasome

Abbreviations

ABCB1	ATP-binding cassette subfamily B member 1
AGREE	Acute Gout Flare Receiving Colchicine Evaluation
ARDS	Adult respiratory distress syndrome
COPPS	COlchicine for the Prevention of the Post-pericardiotomy syndrome
CRP	C-reactive protein
DIC	Disseminated intravascular coagulation
ER	Extended release
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
FMF	Familial Mediterranean fever
GEF	Guanine nucleotide exchange factor

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HCC	Hepatic cell carcinoma
HPLC-MS	High performance liquid chromatography with mass spectrometry
IL	Interleukin
ILGF1	Insulin-like growth factor 1
IV	Intravenous
MDR1	Multiple drug resistant 1
MSU	Monosodium urate
NLRP3	NOD receptor family pyrin 3
NOD	Nucleotide-binding oligomerization domain
PAAND	Pyrin associated autoinflammatory disease with neutrophilic dermatosis
PBC	Primary biliary cirrhosis
PGY1	P-glycoprotein 1
PKN	Protein kinases
PRR	Pattern recognition receptor
RhoA	Rat sarcoma homolog gene family, member A
SAA	Serum amyloid A
TGF	Transforming growth factor
TNF	Tumor necrosis factor

Key Points

- **Colchicine is an alkaloid which is used for familial Mediterranean fever (FMF), gout, Behçet disease and recently for unusual additional conditions such as cardiovascular diseases**
- **Colchicine is effective in suppression of inflammation by different mechanisms. However, inhibition of neutrophil chemotaxis seems to be its main anti-inflammatory effect**
- **Colchicine is a relatively safe medication for pregnant or nursing patients with FMF provided that their liver and kidney functions are intact**
- **Colchicine is metabolized by cytochrome 3A4 which can be inhibited by many drugs leading to colchicine intoxication when given concomitantly**
- **Colchicine intoxication is a life-threatening condition. Therefore, one should be extremely cautious in using the drug in patients with renal and/or liver disturbances**

40.1 Introduction and Historical Notes

Colchicine is an alkaloid which has been used for centuries for treating gout [1]. The source of its name is Colchis, a kingdom on the Black Sea in Western Georgia. Its history is related to Medea who was the daughter of Aeetes, ruler of Colchis. Aeetes kept the "golden fleece" under heavy guard. His daughter, Medea used to "harvest grasses and to extract harmful juices squeezed from twisted roots" [2]. Among the most potent products squeezed from these bulb-corms was the juice of *Colchicum autumnale*, the yellow crocus of Colchis. On his quest to fetch the "golden fleece" from Colchis, Jason met Medea and fell in love with her. The princess used her potions (most of which consisted of concentrated colchicine) to help Jason poison the warriors and dragons that stood guard over the fleece.

The use of the bulb-like corms of Colchicum for gout traces back to 550 A.D., as the "hermodactyl (Iris)" recommended by the Byzantine physician, Alexander of Tralles (today—Aydin in Turkey) [3]. While the Greeks and Romans knew about the use of colchicine for gout, the drug wasn't available in pure form until the late nineteenth century. The active pharmacological component of the plant, colchicum, was isolated in 1820 and in 1833, P.L. Geiger purified the active ingredient, which he named colchicine [4].

In the nineteenth century, Alfred Garrod, Dyce Duckworth, and many others, reached a consensus that colchicine was relatively specific for the treatment of gout [5]. Pernice, an Italian pathologist, found that when therapeutic doses of colchicine were given to experimental animals, lesions were produced in the nuclei of gastric and intestinal cells as these cells were arrested in metaphase [6]. In 1967, Ed Taylor and Gary Borisy used tritiated colchicine to identify the target of colchicine in dividing and non-dividing cells [7]. The protein they identified was the dimeric building block of microtubules, subsequently given the name "tubulin" by Mohri [8]. We now know that the traffic of intracellular materials is carried over tracks formed by microtubules [9].

In the last 50 years, colchicine has been employed for an increasing number of diseases, in addition to gout, including familial Mediterranean fever (FMF), idiopathic recurrent pericarditis, Behçet disease, Sweet syndrome, systemic sclerosis, amyloidosis and hepatic cirrhosis [1] (Table 40.1). In acute gout, colchicine is effective in alleviating the acute attack and as a prophylactic medication. In Behçet disease, colchicine is effective mainly in the treatment of mucosal ulcers, especially in female genitalia. In systemic sclerosis, colchicine may decrease the stiffness of the skin whereas in amyloidosis it may result in regression of amyloid deposition loci where serum amyloid A (SAA) fibers are deposited [10, 11]. Since 1972, colchicine has gained popularity as being the main effective remedy for FMF [12]. In this disease, colchicine prevents the occurrence of the acute inflammatory episodes and the development of amyloidosis. However, colchicine is not effective in controlling acute attacks when administered once they occur.

Table 40.1 Suggested and approved indications for colchicine use

Crystal-induced arthropathy
Gout
Pseudogout
Liver diseases
Primary biliary cirrhosis
Alcohol induced hepatitis
Prevention of hepatocellular carcinoma in cirrhosis
Skin diseases
Psoriasis
Scleroderma
Dermatitis herpetiformis
Bullous dermatosis
Heart diseases
Acute pericarditis
Chronic relapsing pericarditis
Prevention of coronary artery disease
Prevention of post-operative atrial fibrillation
Miscellaneous
Familial Mediterranean fever (FMF)
Behçet disease
Amyloidosis
Sweet syndrome
Sarcoidosis

40.2 Chemistry and Pharmacology

- **Colchicine is an alkaloid which is absorbed in the jejunum and ileum and is trapped in the body tissues**
- **Colchicine is metabolized in the liver and the intestine by cytochrome P (CYP) 450 3A4 and P-glycoprotein (PGY) 1**
- **Colchicine is excreted mainly by the biliary system, intestine and the kidneys**
- **The half-life of colchicine is between 7 and 9 h following oral ingestion**
- **Caution should be exercised in patients with liver or kidney disease or damage**

Colchicine is a tricyclic, lipid—soluble alkaloid with the chemical formula; N-(5, 6, 7, 9, tetrahydro-l, 2,3,10, tetramethoxy-9 oxobenzo[a] hep-tain-7-yl) acetamide n (Fig. 40.1). Pharmacokinetic studies of colchicine are limited, and their results somewhat contradictory because of the different methods used for its measurement. Previously, most studies employed thin-layer chromatography, whereas recently a more sensitive radioimmunoassay and high performance liquid chromatography with mass spectrometry (HPLC- MS) has been used [13].

40.2.1 Absorption and Elimination

Colchicine can be administered by mouth as 0.5, 0.6 and in some localities 1 mg tablets. Until recently an intravenous (IV) formula was also available. However, in 2014, the United States Food and Drug Administration (FDA) withdrew approval following several cases of fatal toxicity [14]. Formerly, it was thought that colchicine is almost completely absorbed after oral administration. However, recent studies have shown that its bioavailability ranged from 24 to 88% [15]. The exact site of absorption is unknown, but the drug seems to reach the jejunum and ileum because dysfunction of these bowel regions is common in cases of chronic colchicine overdose [16].

The plasma membrane-localized multidrug transporter P-glycoprotein 1 (PGY 1) [also known

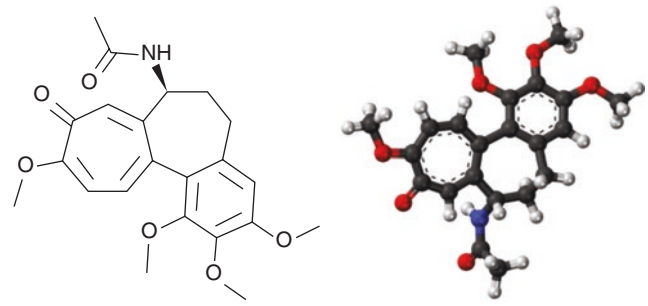


Fig. 40.1 Chemical structure of colchicine CHsO~H CH2C) k~ /2O ′~OCH3

as ATP-binding cassette subfamily B member 1 (ABCB1), multiple drug resistant 1 (MDR1), and CD243] transports multiple classes of substrates across cell membranes [17, 18]. Altered intestinal PGY1 expression can change the absorption of drugs transported by this peptide, including colchicine. Therefore, PGY-1 is critical in the regulation of its bioavailability and plasma concentrations, which can lead to sub-optimal therapeutic effects or, alternatively, to drug toxicity [19, 20]. Also, PGY 1 clearly participates in the removal of drug metabolites through bile, the intestinal lumen, and urine [17–20].

Colchicine is predominantly eliminated by biliary excretion and through the stool, with gastrointestinal tract lining cell turnover playing a variable role in this process [17, 18, 21]. Normally, a lesser, but significant role in colchicine metabolism (~20%) is played by enteric and hepatic cytochrome P450 3A4 (CYP450 3A4), which catalyzes the demethylation of colchicine to 2 and 3 demethtylcolchicine, which are inactive metabolites [21]. Renal elimination has been estimated to be responsible for 10–20% of drug disposition in normal subjects. However, CYP3A4 and renal disposition of colchicine can be significantly impacted by certain drug–drug interactions that affect PGY-1 (ABCB1), as well as hepatobiliary dysfunction and aging [21, 22].

The bioavailability of oral colchicine tablets is comparable in the young and elderly, but colchicine pharmacokinetics differ markedly, with volume of distribution at steady state (*V*ss) and total body clearance significantly reduced in the elderly, and the plasma *C*max significantly higher at equivalent colchicine doses [22].

Over the last decade, several single nucleotide polymorphisms of PGY 1 (ABCB1) were identified with the potential to influence expression and quantitative transporter function [23]. For instance, 5–10% of patients diagnosed with FMF do not respond to treatment with colchicine. Compared with non-responders (who usually have more severe disease)**,** treatment responders had a twofold greater concentration of colchicine in mononuclear cells, which was attributed to a potential genetic effect independent of *MEFV* mutations [23, 24]. Specifically, the distribution of ABCB1 3435 C and T-genotypes was significantly different between colchicine-responders and non-responders, with the 3435C allele significantly increasing the risk of resistance to colchicine, whereas in patients with the 3435T genotype a decreased colchicine dose was needed to obtain an adequate response [23].

40.2.2 Pharmacokinetic Studies of Colchicine

Wallace and Ertel [25] were pioneers in studying the pharmacokinetics of colchicine. They found that after IV administration of 1 mg of the drug to healthy volunteers, the peak plasma concentration averaged 0.32 + 1.17 μg/100 mL. After IV administration of 2.0 mg of colchicine, a rapid drop of plasma levels occurred during the first 10 min, followed by a logarithmic decline [26]. The rapidity of colchicine disappearance from the plasma and its persistent excretion days after drug ingestion suggest that it is trapped in body tissues for a prolonged period. Indeed, the

apparent volume of distribution calculated for orally administered colchicine was 4.25 ± 2.90 L/kg, indicating intensive tissue binding of the drug [27]. An example for such binding is the detection of colchicine in leukocytes 10 days after a single IV dose [28]. Furthermore, it was shown that in plasma 40% of the colchicine is bound to albumin [29]. After oral administration of colchicine, the Tmax (the time needed to reach peak plasma levels) is 1–3 h [14]. However, maximal anti-inflammatory effects develop over 24–48 h based upon intra-leukocytes accumulation of the drug [30].

Initial studies reported terminal elimination half-life time ($T_{1/2}$) from 19 min (after IV administration) to 16 h (after oral ingestion) [31]. In an additional study, the mean $T_{1/2}$ was 9.3 h, similar to findings in patients with FMF without renal or liver disease ($T_{1/2}$ of about 9 ± 4 h) [27, 32]. In a study in which we evaluated the pharmacokinetics of colchicine in patients with FMF with and without renal or liver disease, we found that colchicine clearance was significantly impaired in those with kidney or liver failure [27]. The $T_{1/2}$ of colchicine in patients with severe renal failure was two- to threefold longer and in a patient with both renal failure and cirrhosis tenfold longer than in patients with normal renal and liver function. Leighton et al. [33] reported similar results in patients with liver cirrhosis. These findings suggest that patients with either liver or renal disease should be closely monitored even when treated with conventional doses of colchicine.

40.3 Biological Effects

- **Colchicine binds non-polymerized tubulin forming a tubulin-colchicine complex**
- **Heterodimers of α- and β-tubulin form microtubules that can elongate and contract as filaments**
- **Colchicine, can bind the tubulin molecule and inhibit its polymerization into microtubules in vitro**
- **Since microtubules are involved in cell division, in signal transduction, regulation of gene expression and cell migration, colchicine can inhibits these functions and especially neutrophil chemotaxis**

Older studies claimed that most of the effects of colchicine at the cellular level are attributed to its interaction with tubulin, the main building block of microtubules [34]. Colchicine binds in an equimolar and poorly reversible manner to soluble non-polymerized tubulin with high activation energy, forming a tubulin-colchicine complex [21, 30, 34]. Heterodimers of α- and β-tubulin form dynamic polymers termed microtubules that can elongate and contract as filaments, to change the structure and function of the cytoskeleton, exemplified by the interphase microtubule network and the mitotic spindle. Microtubules are involved not only in cell division, but also in signal transduction, regulation of gene expression, migration, and secretion [35].

Microtubules are widely distributed in organelles present in nerve cells, ciliated cells, leukocytes, and sperm tails. It was shown that tropolone methyl ester, which is a precise analog of the ring C of colchicine, can bind the tubulin molecule and inhibit its polymerization into microtubules in vitro. Furthermore, mescaline, which is an analog of the methoxyphenyl moiety of ring A of colchicine, also may inhibit microtubular assembly [34]. These data suggest that the action of colchicine is dependent on its two rings which bind microtubules, inhibiting the movement of intracellular granules, thereby disturbing the secretion of various components to the cell exterior.

Colchicine has an inhibitory effect on several leukocyte functions such as adhesiveness, ameboid motility, mobilization and degranulation of lysosomes [1]. However, the most potent effect of colchicine is on leukocyte chemotaxis [36]. Of all the effects of colchicine, only the inhibition of chemotaxis was shown to occur at concentrations as low as 1×10^{-8} mol/L. A higher dose is necessary to inhibit other effects. It was suggested that the primary anti-inflammatory effect of colchicine is derived from its potent inhibitory effect on leukocytes chemotaxis [37]. However, additional

studies have shown that colchicine decreases the expression of adhesion molecules on neutrophil membranes, leading to significant inhibition in migration and interaction with endothelial cells [38]. Other investigators have shown that colchicine may modulate cytokine production by polymorphonuclear cells.

Several studies have shown a relatively high concentration of colchicine in leukocytes explaining its potential effect on these cells [24, 30]. However, the cause for the special "affinity" of colchicine to leukocytes was unclear. We and other investigators, have shown that granulocytes have low activity of the PGY1 efflux pump and therefore when colchicine enters these cells it accumulates in their cytoplasm [39, 40]. Conversely, lymphocytes and mononuclear cells have a higher activity level of PGY1. Therefore, some of the colchicine entering these cells is effluxed, explaining why the level of colchicine in granulocytes exceeds that of lymphocytes and monocytes.

Table 40.2 Biologic effects of colchicine

Abrogates the microtubule self-assembly by forming complex with tubulin
Reduces the generation of tumor necrosis factor (TNF)-α by macrophages
Abrogates E-selectin-mediated adhesiveness of endothelium to neutrophils
Alters distribution of adhesion molecules on endothelial cells
May suppress phospholipase A2 activation (at high concentrations)
May inhibit lysosomal enzyme release, and phagocytosis
Raises *MEFV* gene expression in a cell line of peritoneal fibroblasts
May modulate the expression of numerous genes in endothelial cells
Suppresses the activation of caspase-1
May inhibit superoxide production by neutrophils
Leads to release of guanine nucleotide exchange factor (GEF)-H1 factor thereby activating rat sarcoma homolog gene family, member A (RhoA)
Reduces neutrophils elasticity and relaxation inhibiting chemotaxis
Inhibits liver fibrosis by inducing stellate cell apoptosis
Inhibits anti-transforming growth factor (TGF)-β1 activity

40.4 Anti–Inflammatory Mechanisms of Colchicine

- **The anti-inflammatory mechanisms of colchicine may involve activation of the rat sarcoma homolog gene family, member A (Rho A) protein, direct interaction with the *MEFV* gene or pyrin, the protein product of the gene**
- **Colchicine interacts with tubulin thereby affecting chemotaxis and modulating adhesion molecules on the membrane of leukocytes**
- **Changes in neutrophils elasticity and relaxation caused by colchicine are the most effective steps in inhibiting neutrophil chemotaxis**
- **The anti-fibrotic action of colchicine may explain its role in preventing amyloidosis**

Several mechanisms have been ascribed to the therapeutic action of colchicine (Table 40.2). Bessis and Gorius discovered that colchicine disrupts microtubules in a dose-dependent fashion [41]. Colchicine does not enhance microtubule dissolution but abrogates the process of microtubule self-assembly by forming tubulin-colchicine complexes [42, 43]. Colchicine reduces the generation of tumor necrosis factor (TNF)-α by macrophages and its receptors on endothelial cells [44, 45]. Colchicine also has been shown to interfere with the interaction of neutrophils and the vascular endothelium by abrogating their binding to adhesion molecules. Colchicine abrogates the E-selectin-mediated adhesiveness of the cytokine-stimulated vascular endothelium to neutrophils. It also alters the distribution of the adhesion molecules on the surface of endothelial cells and neutrophils, significantly reducing their interaction [46]. In addition, at high concentrations colchicine suppresses phospholipase A_2 activation, lysosomal enzyme release and phagocytosis [47]. Conversely, colchicine does not exert its anti-inflammatory effect through inhibition of cyclooxygenases [48].

In 1997, the gene likely to cause FMF was isolated and cloned [49, 50]. Following the isolation of the *MEFV* gene and the finding that it is fully expressed in neutrophils, a question was raised regarding the possibility of a direct effect of col-

chicine on the gene or its protein. In a study where we tested this hypothesis, we showed that colchicine did not up-regulate the expression of *MEFV* gene in neutrophils [51]. However, colchicine increased the *MEFV* gene expression in a primary cell line of peritoneal fibroblasts. The exact significance of this finding is unknown. Nevertheless, it should be borne in mind that peritoneal cells comprise an important target in the acute attack of FMF (peritonitis), suggesting a potential local effect of colchicine.

A few studies suggested that colchicine modulation of pyrin expression and interaction with pyrin in the cytosol contributed to its efficacy in FMF [52, 53]. At relatively high concentrations, colchicine also modulates the expression of numerous genes in cultured endothelial cells, with a significant delay in the onset of action [54]. This may explain the observation that initiation of colchicine treatment during acute attacks of FMF does not effectively terminate them.

Colchicine has recently been shown to suppress the activation of caspase-1, the enzymatic component of the nucleotide-binding oligomerization domain (NOD) receptor family pyrin 3 (NLRP3) inflammasome. Caspase-1 suppression blocks conversion of pro-interleukin (IL)-1β to active IL-1β, leading to secondary reduction in cytokines such as TNF-α and IL-6. The effect of colchicine on this process may be upstream of the inflammasome, rather than a direct effect [55]. To date, inflammasome inhibition has been assessed at colchicine concentrations 10 to 100-fold higher than that achieved in the serum. Whether colchicine inhibits caspase-1 at physiologic concentrations, or whether colchicine accumulation in leukocytes is sufficient to block the inflammasome, remains to be determined.

Another mechanism by which colchicine may suppress inflammation is by inhibition of superoxide production by neutrophils. Chia et al. demonstrated that colchicine inhibits monosodium urate (MSU)-induced superoxide production by murine peritoneal macrophages in vivo at doses 100 times lower than that required to inhibit neutrophil infiltration [56]. This suggests that superoxide anion production is more sensitive to suppression by colchicine than microtubule formation involved in cell migration.

40.5 How Does Colchicine Prevent Attacks of FMF?

- **Rat sarcoma homolog gene family, member A (Rho A) protein is a peptide which controls the action of GTPases thereby affects tubulin dynamics**
- **Pyrin is a specific immune sensor (pattern recognition receptor—PRR) for bacterial modifications of Rho and GTPases**
- **Activation of RhoA inhibits pyrin activity while inactivation of RhoA causes over activation of pyrin resulting in increased production of interleukin (IL)-1, thereby enhancing inflammation**
- **Colchicine may activate RhoA by guanine nucleotide exchange factor (GEF)-H1, thereby suppressing pyrin activity and inflammation**
- **Colchicine also disrupts microtubules structure reducing neutrophils membrane elasticity and relaxation, thereby preventing their extravasation from the blood vessels to the inflammatory site**

To understand this process, we first need to explain the cellular role of pyrin. Pyrin is the protein encoded by the *MEFV* gene, which is mutated in FMF (see Chap. 16). Rat sarcoma homolog gene family, member A (Rho A) protein is an intra-cellular peptide which controls the action of GTPases. GTPases are important enzymes in regulation of actin and tubulin dynamics. Actin-tubulin interaction has a major role in the motility and chemotaxis of neutrophils [37, 42–44].

Bacterial toxins such as that of Clostridium may modify the effect of Rho on GTPases, thereby inhibiting actin activity and leukocytes chemotaxis. Xu et al. found that pyrin is a specific immune sensor (pattern recognition receptor—PRR) for bacterial modifications of Rho and GTPases [57]. Pyrin does not directly recognize Rho modification, but probably senses events downstream of Rho modification. Xu et al. showed that activation of RhoA inhibits pyrin activity while inactivation of RhoA causes over activation of pyrin resulting in increased production of IL-1, thereby enhancing inflammation. Clostridium toxin inactivates RhoA thereby leading to less production or recruitment of

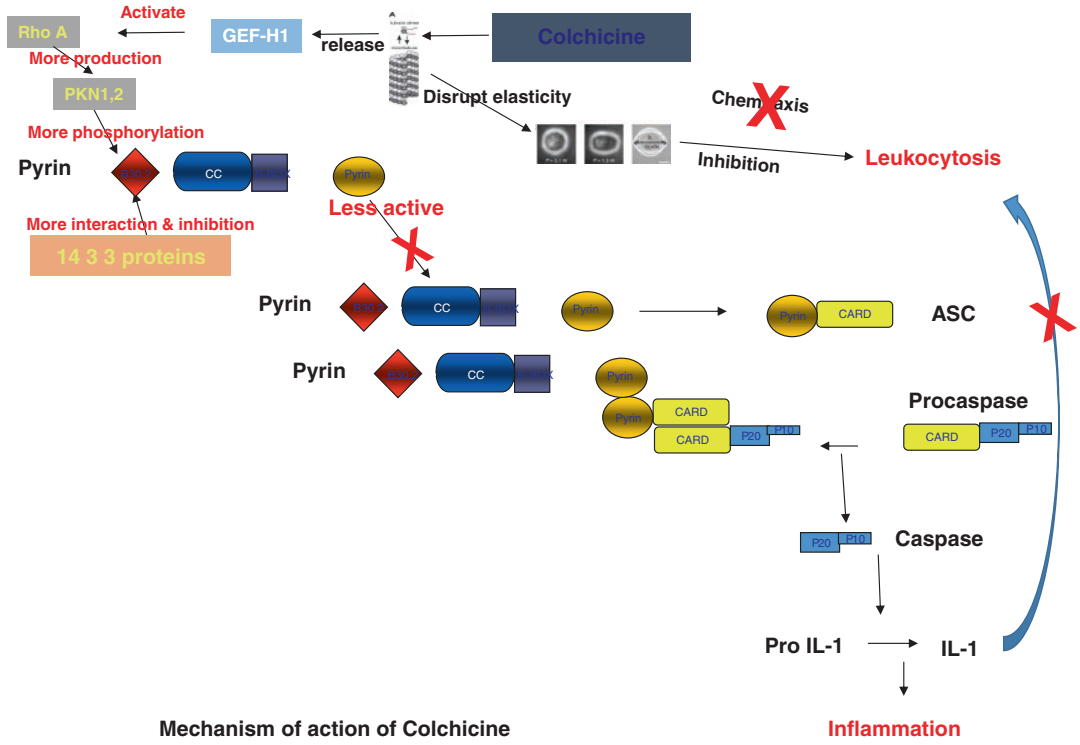


Fig. 40.2 Schematic illustration of the inflammatory cascade of the pyrin inflammasome and the putative role of colchicine in inhibiting this process (see text)

protein kinases (PKNs) and thereby less phosphorylation of pyrin [57]. 14 3 3** peptides (**the name reflect the migration pattern on 2D DEAE gel of these acidic proteins family) are group of proteins which interact with phosphorylated pyrin [58]. Decreased phosphorylation of pyrin leads to less interaction and reduced binding by 14 3 3 peptides. This increases the amount of free pyrin which can recruit apoptosis-associated speck-like protein containing a CARD (ASC) and procaspase −1 to construct the pyrin inflammasome, leading to increased secretion of IL-1β and an enhanced inflammatory process (Fig. 40.2).

Colchicine may also suppress inflammation in FMF by the following mechanism. It binds to tubulin and depolymerizes microtubules, resulting in the release of the RhoA activator, guanine nucleotide exchange factor (GEF)-H1, which is inactive when bound to microtubules [59, 60]. Thus, colchicine indirectly activates RhoA. Activation of this protein results in enhancement of pyrin phosphorylation, thereby higher binding by the 14 3 3 peptides and hence, less free pyrin is available for constructing the inflammasome, required for secreting inflammatory cytokines, including IL-1.

In a recent study, we looked more closely to the mechanistic features of the effect of colchicine on the neutrophil membrane [61]. We found that colchicine disrupts the microtubules structure and reduces neutrophil elasticity and relaxation, thereby preventing their extravasation from the blood vessels to the site of inflammation. This may be the final and most effective step in inhibiting chemotaxis.

40.6 The Anti-Fibrotic Action of Colchicine

Colchicine has anti-fibrotic effects. In a rat model of hypertensive chronic kidney disease, colchicine inhibited renal fibrosis via inhibition of RhoA signaling and infiltration of inflammatory cells [62]. In another rat model, colchicine inhibited liver

fibrosis by inhibiting the activation of hepatic stellate cells and inducing stellate cell apoptosis [63]. In an encapsulating peritoneal sclerosis model, colchicine inhibited anti-transforming growth factor (TGF)-β1 activity [64].

40.7 The Safety Profile and Toxicity of Colchicine

- **Colchicine is a relatively safe medication but has a narrow therapeutic window**
- **The metabolism of colchicine is affected by the functions of cytochrome P (CYP) 3A4, P-glycoprotein (PGY) 1, the liver and the kidneys and by additional medications taken concomitantly**

Colchicine is a relatively safe medication but has a narrow therapeutic window [65, 66]. Colchicine is well tolerated when taken in age dependent doses that are less than 2 mg/day in children (up to 12 year old) and 3 mg/day in adults with normal liver and kidney function, when not taking concomitant drugs that may affect its pharmacokinetics. Nevertheless, therapeutic oral doses of colchicine (1–2 mg/day), may cause cramping, abdominal pain, hyperperistalsis, diarrhea and vomiting. These effects are usually mild and transient. When abdominal cramps persist, lowering colchicine dose may be effective. When diarrhea persists dividing the dose of colchicine (twice daily) may be helpful. In resistant cases, a short course of anti-diarrhea medications such as loperamide may be beneficial [67]. Decreasing use of lactose containing products or using lactase prior to their consumption may also reduce symptoms. Leukopenia is a very rare adverse event in therapeutic dose and neuromuscular complications may occur when renal functions are impaired or with use of concomitant drugs such as clarithromycin.

40.7.1 Colchicine Overdose

Colchicine overdose may lead to a cholera-like syndrome associated with dehydration, shock, multiple organ failure, alopecia, disseminated intravascular coagulation (DIC), seizures, coma and death [68].

Colchicine doses of 0.5–0.8 mg/kg are highly toxic, and doses of more than 0.8 mg/kg are typically lethal [69]. Cumulative doses of colchicine causing toxicity when administered intravenously were 18 mg given over 11 days, 10 mg in 5 days and even 8 mg given within 3 days. The lowest reported oral dose causing lethal colchicine toxicity was 7 mg given over 3 days to a 39-year old male [70].

The course of colchicine intoxication can be divided into three stages, with overlap between the stages. In the first stage, gastrointestinal symptoms dominate. There may be excessive fluid loss through diarrhea, leading to volume depletion and dehydration. This stage develops within 24–72 h following ingestion of the drug. The second stage is dominated by multi-organ failure which may include: bone marrow failure, renal insufficiency, adult respiratory distress syndrome (ARDS), arrhythmias, disseminated intravascular coagulation (DIC), neuromuscular disturbances and alopecia. This stage develops over 3–7 days. Patients surviving this stage may enter the third stage which is characterized by bone marrow recovery and rebound leukocytosis, resolution of organ failure and regrowth of hair.

Clinical management of colchicine intoxication is basically supportive. In a single case, treatment with F(ab) fragments of anti-colchicine antibodies was successful [71]. Unfortunately, these antibodies, raised in goats, are not currently available.

One of the problems of managing colchicine overdose is that it is not dialyzable using regular dialysis membranes. Recently, new high flux polysulfone membranes have been introduced to improve dialysis [72]. Many medications and substrates which were non-dialyzable with older membranes are now dialyzable. Colchicine is one of the medications of which steady state levels were reduced when given to patients with FMF on high flux dialysis [73]. However, our study showed that the rate of excretion of colchicine by these membranes is far less than the rate needed for effective treatment of colchicine intoxication.

40.8 Colchicine Drug-Drug Interaction

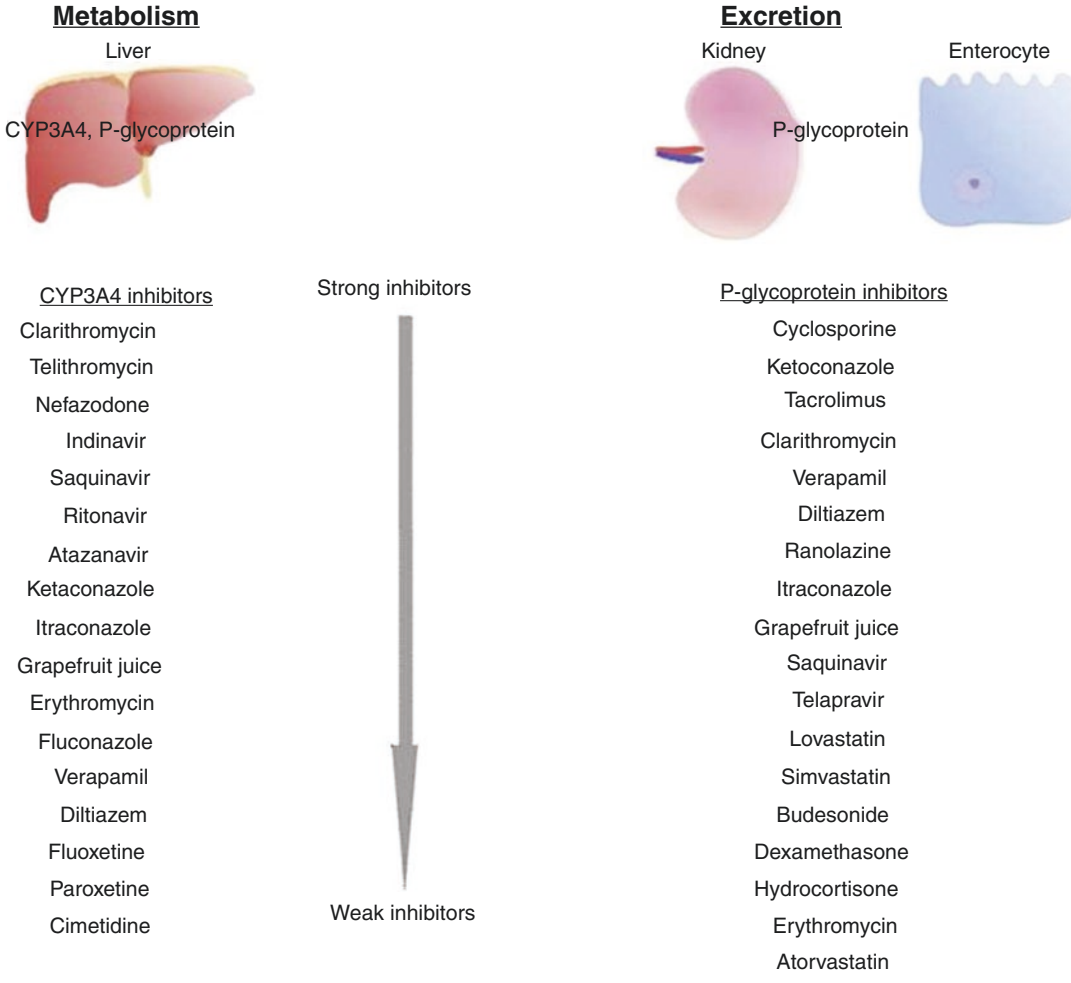
- **Cytochrome P (CYP)3A4 is the most abundant of the human P450 enzymes and metabolizes multiple structural classes of drugs including colchicine**
- **Macrolides, anti-fungal and anti human immunodeficiency virus (HIV) drugs may have specific inhibitory effect on CYP3A4, thereby increasing colchicine blood levels**
- **Taking these drugs concomitantly with colchicine requires a reduction of the dose of colchicine to prevent toxicity**
- **On the other hand, colchicine can alter absorption of other compounds or medications from the intestines**

Drug–drug interactions have increasingly become apparent as a cause of colchicine toxicity in patients treated with "standard" daily prophylactic regimens. ABCB1 (PGY1) can undergo conformational changes with expression modulation thereby promoting clinically significant colchicine drug– drug interactions [16, 18, 20, 74]. Cyclosporine is a prime example of a drug that can inhibit or modulate the ABCB1 transporter [75]. Potentiation of colchicine neuromyopathy can occur within weeks of commencement of cyclosporine therapy. This complication is often associated with cyclosporine nephropathy and a decline in the glomerular filtration rate [75, 76]. Notably, cyclosporine was found to delay colchicine-induced diarrhea in an animal model system, likely due to modulation of intestinal ABCB1. Hence, it is suspected that cyclosporine could mask the gastrointestinal side effects of colchicine in humans that might otherwise be a clue to the development of systemic colchicine toxicity.

CYP3A4 is the most abundant of the human P450 enzymes and metabolizes multiple structural classes of drugs (e.g. cyclosporine, quinidine, testosterone, nifedipine, etc) [77, 78]. CYP3A4 is a focal point of many drug– drug interactions and dietary and herbal interactions. CYP3A4 may be stimulated by its substrates ("normotropic cooperativity") or its effectors ("heterotropic cooperativity"), which renders prediction of drug–drug interactions difficult [79, 80]. These substances may be divided into three groups. The first group contains drugs, such as cimetidine, which have an inhibitory effect on the entire cytochrome system. Indeed, in animal studies, it was shown that concomitant administration of cimetidine and colchicine resulted in a significant rise in serum colchicine concentration. The second group contains substances that have a specific inhibitory effect on the isoform CYP3A4 which metabolizes colchicine. These include erythromycin, ketoconazole and other medications. The third group includes drugs that are also metabolized by CYP3A4, such as cyclosporine and nifedipine, and may compete with colchicine for binding to the enzyme. The interaction in these cases is dictated by the affinity of each medication for the enzyme. Thus, co-administration of medications and substances metabolized by the same cytochrome system may lead, in principle, to an increase of one or more of the drugs, exposing the patient to a higher risk of toxicity.

Certain drugs increase the potential for colchicine toxicity via dual modulation of ABCB1and CYP3A4 (Table 40.3). These include the macrolide antibiotics erythromycin and clarithromycin, and the statins, e.g. lovastatin, simvastatin and atorvastatin [81]. Clarithromycin is a particularly striking case in point and has been associated with at least 2 fatalities with concomitant colchicine use [82]. However, azithromycin (a weak CYP3A4 inhibitor) had minimal effects on colchicine concentration and terminal elimination half-life, and decreased total apparent oral clearance by only 30%. Azithromycin should be recommended as a safer alternative to clarithromycin in patients taking colchicine [81]. Mutual potentiation by colchicine and statins of myopathy (sometimes including rhabdomyolysis) is a notable concern [83, 84]. Significantly, case studies have reported acute myopathy after concurrent use of colchicine with a statin that was either not metabolized or minimally metabolized by the CYP3A4 isoenzyme [78, 85]. In this context, fluvastatin can disrupt the integrity of the cytoskeleton and is linked with vacuolization and other

Table 40.3 A list of drugs which may inhibit the cytochrome P (CYP)3A4 and P-glycoprotein affecting colchicine metabolism (reproduced from Slobodnick et al. [69] with permission)



pathology in muscle, which is pertinent given the capacity of colchicine to promote vacuolization in muscle by disruption of the microtubule network.

A new set of evidence-based guidelines provides an algorithm for reducing colchicine doses to prevent toxicity in patients who are concomitantly taking other drugs [23]. The researchers conducted a series of studies designed to show the effects of a single-dose of colchicine given with known inhibitors of CYP3A4 or PGY1. Among the drugs tested were: cyclosporine, ketoconazole, ritonavir, clarithromycin, azithromycin, verapamil extended release (ER), and diltiazem ER. It was shown that the mean maximum concentration of colchicine was 100% higher when colchicine was co-administered with ketoconazole compared with colchicine alone. The mean maximum concentration of colchicine was 185% higher and the mean total colchicine exposure was 290% higher when colchicine was combined with ritonavir as compared with colchicine alone [86].

Colchicine treatment can alter absorption of other compounds or medications from the intestines. It may induce malabsorption of vitamin B12 by reducing the number of B12 intrinsic factor receptors as shown in the intestinal mucosa of guinea pigs [87]. Colchicine-induced lactose intolerance occurs in a significantly higher per-

centage of patients with FMF treated with oral colchicine compared with non-treated patients [88]. Reduction in iron absorption was also observed among patients with FMF taking colchicine.

40.9 Long-Term Effects of Colchicine Treatment

- **Colchicine rarely causes oligo/azoospermia**
- **Colchicine does not affect sperm motility**
- **It is safe to take colchicine during pregnancy and nursing provided the liver and kidney functions are normal**
- **Colchicine is relatively safe in treating children and toddlers and does not affect their growth**

40.9.1 Colchicine and Male Fertility

Colchicine is a drug which may affect the function of microtubules in various cells. In high concentrations, it may inhibit mitosis within the process of cell division. Therefore, concern was raised as to the effect of colchicine on sperm proliferation and motility in patients taking colchicine.

A case report by Merlin described a patient with gout who developed azoospermia following treatment with colchicine [89]. Re-challenge again induced azoospermia. Because patients with FMF receiving colchicine are often in their child-bearing years, the concern about fertility is pertinent. Indeed, rabbits treated with a relatively high dose of colchicine showed various degenerative changes of the testes, including loss of differentiation from spermatogonia to spermatozoa [90]. However, Cohen et al. performed cytogenetic evaluation in patients with FMF receiving long-term colchicine. Mitotic rates, percentage of tetraploidy, and chromosomal breakage rates were determined in lymphocytes [91]. No significant differences were found between the patients and controls.

In a study by Levy et al., 6 patients receiving long term colchicine therapy were evaluated. No effect on fertility was noted and levels of spermatocytes, testosterone stimulating hormone, luteinizing hormone and prolactin were all within normal limits [92]. Another study showed that four out of 16 males with FMF receiving colchicine suffered from infertility [93]. One had azoospermia and the others had a normal spermatogram, but the sperms could not penetrate the ova normally.

Since sperm motility and hence ovum penetration depends upon microtubule function, we hypothesized that colchicine may affect the movement of sperm. Accordingly, we studied the effect of colchicine on sperm motility in an in-vitro system employing the "swim up" technique for sperm selection [94]. Sperm motility was inhibited significantly only after an incubation period of at least 18 h, with a minimal colchicine concentration of 10 μg/mL. Because plasma colchicine concentration under therapeutic dose is about 3–9 ηg/mL, the amount of colchicine needed for affecting sperm motility in vitro is 3000-fold higher. Thus, it seems unlikely that standard colchicine treatment would inhibit sperm motility unless the drug has a very high and special affinity to the testes.

The frequency of oligo or azoospermia with colchicine depends on the underlying disease. Bremner and Paulson failed to show any effect on spermatogenesis in six healthy volunteers who received commonly used doses of colchicine for 4–6 months [95]. Conversely, in a study of 62 Turkish men treated with colchicine for Behçet disease, oligospermia was evident in 23 (37%) patients and azoospermia in two patients [96]. If corroborated, these findings suggest that infertility and disturbed spermatogenesis result not only from colchicine use but also may depend on other factors such as genetic background or underlying disease. The vasculitis associated with Behçet disease may further contribute to this complication by adding local ischemia to the potential toxicity of colchicine.

Based upon the above observations, it is tempting to ascribe the development of azoospermia in patients with FMF to colchicine. However, in three cases of azoospermia we performed a testicular biopsy which demonstrated amyloido-

sis of the testes [97]. Thus, amyloidosis of the testes should also be considered in patients with FMF presenting with azoospermia (see Chaps. 15 and 16).

Another concern related to male fertility is the question on the outcome of pregnancies induced by male patients with FMF. In a study by Zemer et al. of 1000 patients with FMF, 24 females conceived while their male partners were treated with colchicine [98]. There was no mention concerning fertility or delivery problems. Due to limited data on this issue, some physicians in the past used to advise to discontinue colchicine 3 months before attempting to conceive. In a prospective study, we followed the outcome of pregnancies and deliveries of 60 female partners of males with FMF, 53 were on colchicine when their partners conceived [99]. As a control group, we followed the outcome of pregnancies and deliveries in 230 healthy women married to healthy men. Our findings revealed no difference regarding the rate of early or late abortions, or of congenital malformations. Therefore, it seems that there is no need for males with FMF to discontinue colchicine prior to planning conception.

40.9.2 Colchicine and Female Fertility

The potential effects of colchicine on microtubules, cell division and growth raise a serious concern as to the female reproduction system.

40.9.2.1 Menstruation

FMF attacks may be triggered in some patients by their menstrual period [100]. The association of FMF attacks and menstruation raises the possibility of hormonal influences (see Chap. 16). Indeed, it was shown that estrogen significantly decreases intercellular adhesion molecules [101]. Furthermore, it was demonstrated that estrogens inhibit tubulin assembly by interacting directly with tubulin 6S sites analogous to colchicine sites [102]. Moreover, estrogen is metabolized by the 3A4 liver CYP-450 complex and competes in binding it with colchicine. Thus, it is tempting to speculate that estrogens mimic the effect of colchicine on tubules and adhesion molecules, thereby enhancing the effect of colchicine. During menstruation, there is a sharp decrease in estrogens, thus their accumulative suppressive effect on inflammation is suddenly diminished. In addition, the reduction in estrogen, allows for a more effective metabolism of colchicine by the 3A4 cytochrome (less inhibitory competition by estrogen) so that the effective level of colchicine may be further reduced. This situation may lead to menstruation associated FMF attack. To control these attacks, it is recommended to increase the dose of colchicine (by 0.5 mg) for 2 days prior to the onset of the menstrual period and for two more days after its onset (see Chap. 16).

40.9.2.2 Pregnancy

Theoretically, colchicine may affect female fertility by affecting the ovaries via its potential effect on cell division. However, this has not been shown. Serious concern was raised regarding a teratogenic effect of colchicine. Therefore, in the 1970s physicians advised their patients to discontinue colchicine 3 months before planning conception and during pregnancy. Sporadic reports claimed that colchicine was safe during pregnancy. Furthermore, in a study which followed 36 pregnant women with FMF treated with colchicine, the outcome of the newborns was the same as in an untreated control group [103]. In another study, all 13 women with FMF, who had 16 pregnancies and were on colchicine, gave birth to normal children [104]. However, Rabinovitch et al. reported that 4 newborns out of 2000 (1:500) deliveries of FMF patients were born with trisomy 21, twice the expected rate of a comparable normal population [105]. It was not clear whether colchicine therapy itself plays a role with this increment outcome. Recently, Diav-Citrin et al. examined the safety of fetuses by following mothers exposed to colchicine during pregnancy. In a prospective observational comparative cohort study between 1994 and 2006 they found that colchicine did not appear to be a major human teratogen, and, probably, has no cytogenetic effect [106].

We followed the outcome of pregnancy in a group of patients with FMF who took colchicine during pregnancy and compared them with a group of patients with FMF who were not treated with colchicine and with a group of healthy pregnant individuals [107]. We showed that colchicine was not associated with a higher rate of miscarriage or stillbirth. There was no reduction of the duration of pregnancy or the birth weight of the babies. Based upon these results, we do not recommend amniocentesis for patients with FMF solely because of treatment with colchicine during pregnancy.

40.9.3 Colchicine and Nursing

Leaflets of pharmaceutical companies and textbooks of pharmacology warn women not to nurse their babies while treated with colchicine. Milunsky and Milunsky found that colchicine was present in the breast milk of patients taking the drug [108]. We also measured the levels of colchicine in sera and milk of 4 women with FMF at various time points after drug ingestion [109]. Colchicine was detected in all samples of sera and milk, with similar concentrations. However, the estimated daily amount of colchicine ingestion by the nursing babies was less than one tenth the therapeutic dose (per kilogram) given to adult patients with FMF. This rough estimation was concordant with our favorable clinical experience in following more than 50 children of mothers who continued to breastfeed while taking colchicine. Therefore, we suggest that breast feeding is safe while taking colchicine.

40.9.4 The Effect of Colchicine on Child Growth and Development

Since growth depends upon cell division, the potential effect of colchicine on child growth and development may raise concerns. The diagnosis of FMF can be made as early as several months of age. Initially, we were reluctant to start treatment with colchicine before the age of 4 years. During this period, children continued to suffer from recurrent attacks of FMF and were also at risk of developing amyloidosis. Furthermore, they exhibited growth delay when compared with healthy children of the same age. Following the start of colchicine treatment and control of FMF attacks, their appetites improved and a marked growth spurt was evident. We followed seven children since the age of 5–6 years and measured their height and weight every 6 months for a period of 10 years [110]. Their growth while treated with colchicine was within the normal expected percentile range. Similar results were observed in larger and more recent studies [111]. Savgan-Gurol et al. evaluated the growth process and insulin like growth factor-1 (IGF-1) levels in children with FMF [112]. They found that IGF-1 levels of children with FMF did not differ from their healthy peers. However, there was a positive correlation between the rate of growth and the cumulative colchicine dose. Therefore, they suggested that colchicine enhances the growth of children with FMF by suppressing disease activity and inflammation.

40.10 Treatment Indications

- **Colchicine is the first line therapy for the treatment of acute gouty arthritis and FMF**
- **Due to the anti-inflammatory and anti-fibrotic effects of colchicine on multiple pathways, the therapeutic use of colchicine has extended beyond these diseases**
- **Recent studies have showed that colchicine may have a beneficial effect in preventing secondary cardiovascular events, and cardiac dysrhythmias**

40.10.1 Familial Mediterranean Fever (FMF) (Chap. 16)

40.10.1.1 How to Use Colchicine in Daily Practice

The literature suggests that the minimal daily dose for preventing the development of amyloidosis in adult FMF patients is 1 mg/day, even if

attacks can be suppressed with a lower dose [113]. Nevertheless, several series from Japan, reported that their adult patients with FMF were controlled by low-dose colchicine (0.5 mg daily). It is hypothesized that the reason for that is their carriage of mutations (in exon 2 and 3) which are known to cause a milder disease [114].

The dose of colchicine should not exceed the maximal tolerated dose and should not be more than 3 mg per day in adults without comorbidity and 2 mg per day in prepubertal children [115].

It is of paramount importance to avoid toxicity due to concomitant administration of CYP 3A4 or PGY1 inhibitors (Table 40.3). Drug-drug interactions need to be considered and the dose of colchicine should be appropriately adjusted. It is necessary to closely monitor the kidney and liver function of these patients (see Sect. 40.7 for details). We recommend taking the entire colchicine dose at once in order to improve compliance. If the patient develops diarrhea due to ingestion of a relatively high single dose of colchicine the dose should be divided to twice per day without affecting its effectiveness [116].

40.10.2 **Gout** (Chap. 34)

Although colchicine has been used to treat gout for centuries, relatively few controlled trials have assessed its efficacy. The most recent major trial, Acute Gout Flare Receiving Colchicine Evaluation (AGREE), randomized 184 patients with acute gout to receive a lower-dose colchicine regimen (1.2 mg dose followed by one 0.6 mg dose 1 h later), a traditional higher dose regimen (1.2 mg dose followed by 0.6 mg every hour for a maximum of 4.8 mg) or placebo within 12 h of the onset of attack [117]. The lower- and higher-dose regimens demonstrated similar efficacy (37.8% vs. 32.7% achieving ≥50% improvement within 24 h), but adverse events in the lower-dose regimen were similar to placebo. Accordingly, the lower-dose regimen was approved by the FDA. American College of Rheumatology guidelines recommend the lower-dose colchicine regimen as a first-line therapy option for acute gouty attacks [118].

In addition to its role in acute gout, colchicine is used prophylactically to reduce gout flare frequency, particularly when patients are initiating urate-lowering therapy. An analysis of three randomized controlled trials found that colchicine use for up to 6 months during initial urate lowering provided greater prophylaxis of flares than its use for only 8 weeks [119].

40.10.3 Pseudogout

There is only limited evidence for the use of colchicine in prophylaxis of pseudogout, although this is recommended practice [120]. The rationale is the shared NLRP3-inflammasome and IL-1 driven inflammatory pathogenesis of urate and calcium pyrophosphate crystal deposition. Recent European League Against Rheumatism (EULAR) guidelines recommend giving colchicine for acute attacks at a dose of 0.5 mg three times daily with or without a 1 mg loading dose and for prophylaxis 0.5 mg per day. These recommendations are based largely on expert opinion and a single uncontrolled trial [120, 121].

40.10.4 **Behçet Disease** (Chap. 35)

Colchicine is recommended in the management of Behçet disease, particularly for the mucocutaneous manifestations (oral and genital ulcers) and joint symptoms, based on controlled trials performed to date [122, 123].

40.10.5 Hepatic Disorders

Over the years colchicine has been studied for disorders of hepatic fibrosis. Primary biliary cirrhosis is a rational potential indication, given the intense concentration of colchicine in bile and the evolving understanding of the capacity of colchicine to modulate bile composition [124]. However, a meta-analysis combining the results of 15 randomized controlled trials encompassing 1714 subjects with alcoholic or non-alcoholic liver cirrhosis, demonstrated no significant

effects of colchicine on mortality, liver related mortality, complications and other outcomes [125]. In a recent double blind, randomized controlled trial in 74 subjects with chronic liver cirrhosis who could not be treated with interferon-α, Muntoni et al. demonstrated that colchicine at a dose of 1 mg per day significantly increased survival (94.6% vs. 78.4% p = 0.001), and decreased serum procollagen III (a biomarker of liver fibrosis) over a follow up period of 4.4 years [126].This suggests that there may be a beneficial effect of colchicine for selected subjects with liver cirrhosis. Additional research has assessed whether colchicine can delay the development of hepatic cell carcinoma (HCC) in patients diagnosed with hepatitis virus-related liver cirrhosis [127]. While the effect of colchicine on the progression of cirrhosis was disappointing, colchicine suppressed the development of HCC. Nine percent of patients treated with colchicine developed HCC versus 29% of untreated patients (P −0.0001), and the time to development of HCC was longer in the colchicine-treated group.

40.10.6 Neutrophilic Dermatoses

Colchicine is beneficial in neutrophilic skin conditions. Sweet syndrome typically occurs in women aged 30–50 years and is characterized by fever, neutrophilia, arthralgia, tender erythematous skin lesions, and neutrophilic infiltrates in the upper dermis. Maillard et al. gave colchicine to 20 patients with Sweet syndrome (0.5 mg three times daily for 10–21 days). They reported that 18 patients experienced resolution of fever, skin lesions, arthralgia and neutrophilia within 14 days [128].

Interestingly, in pyrin associated autoinflammatory disease with neutrophilic dermatosis (PAAND) colchicine is not effective and anti-IL 1 agents are required for disease control [129].

40.10.7 Colchicine and Cardiovascular Disease

The accumulating understanding of the role of inflammation in cardiovascular diseases has been accompanied by recognition of the potential anti-inflammatory benefit of colchicine in these settings.

40.10.7.1 **Pericarditis** (Chap. 36)

Practice guidelines from the European Society of Cardiology advocate that colchicine appears to be effective when added to a nonsteroidal anti-inflammatory drug regimen or as monotherapy to treat an initial attack or to prevent recurrence of pericarditis [130]. These recommendations have subsequently been supported by randomized trials [131, 132]. Colchicine was as effective at reducing multiple recurrences of pericarditis as it was for first recurrences. [133, 134] Similar benefits were observed for treatment of an initial attack of acute pericarditis [135, 136].

40.10.7.2 Coronary Artery Disease (Chap. 39)

C-reactive protein (CRP) is a biomarker of inflammation and infection. Elevated high-sensitivity (hs) CRP is both a predictor and a pathogenic factor in vascular events such as coronary artery disease [137]. A recent pilot study evaluated whether low-dose colchicine as an anti-inflammatory treatment could lower hs-CRP levels in patients with stable coronary artery disease whose CRP remained elevated despite aspirin and high-dose atorvastatin therapy [138]. Low-dose oral colchicine (0.5 mg twice daily) was associated with decreased hs-CRP levels by 60%. However, in a separate, randomized study of 80 patients with acute coronary syndrome or stroke, Raju et al. found no significant association between colchicine use (1 mg daily) and CRP reduction, suggesting either that the dose studied was insufficient in the acute setting, or that colchicine may be more effective when administered as a prophylactic rather than as a treatment agent [139]. More recently, Nidorf et al. evaluated the effect of colchicine (0.5 mg daily) on secondary cardiovascular events in 532 patients with stable coronary artery disease already on aspirin and/or clopidogrel and statin therapy [140]. Patients were followed for a median of 3 years. The primary outcome,-the composite incidence of acute syndromes, out-of-hospital cardiac arrest, and non-cardio-embolic ischemic stroke-occurred in 5.3% of patients

treated with colchicine versus 16% of patients receiving placebo. These results warrant further study of the potential benefit of colchicine in the prevention of acute coronary syndrome.

There is growing evidence that inflammation plays a role in the re-stenosis process. Therefore, one study randomized 196 patients with diabetes mellitus and coronary artery disease who underwent percutaneous coronary intervention with bare-metal stent placement to receive either colchicine 0.5 mg or placebo twice daily for 6 months [141]. Subsequent angiography indicated that the rate of angiographic in-stent restenosis was 16% in the colchicine group versus 33% in the placebo group (p = 0.007).

40.10.7.3 Secondary Atrial Fibrillation

Post-operative atrial fibrillation is a common occurrence after cardiac surgery and is presumed to be driven by surgery-related inflammation. The COlchicine for the Prevention of the Post-pericardiotomy Syndrome (COPPS) atrial fibrillation substudy demonstrated that post-operative administration of colchicine was associated with a 45% reduction in the incidence of post-operative atrial fibrillation [142]. However, a second multicenter, randomized, double-blind, placebo-controlled trial (COPPS-2) found no reduction in post-operative atrial fibrillation among patients receiving colchicine [143].

Colchicine may also reduce the risk of recurrence of atrial fibrillation after ablation therapy. In a randomized, double-blind, placebo-controlled study by Deftereos et al., 3 months of colchicine monotherapy was associated with a 16% reduction in the incidence of atrial fibrillation after pulmonary vein isolation with radiofrequency ablation [144]. The colchicine group also experienced a significant decrease in IL-6 and CRP levels.

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41 Interleukin (IL)-1 Blocking Compounds and Their Use in Autoinflammatory Diseases

Tilmann Kallinich and Fabrizio de Benedetti

Abstract

Autoinflammatory diseases are driven by an excessive production of proinflammatory cytokines. Hypersecretion of interleukin (IL)-1β plays a pivotal role in the pathogenesis of many of these disorders and explains a large part of the clinical manifestations of these multisystem diseases. Several drugs which block the IL-1 pathway have been developed. In this chapter, we summarize the properties of the three compounds, namely anakinra, rilonacept and canakinumab, which are currently approved for treatment of a variety of autoinflammatory diseases. We focus on their mode of action, summarize the data of their use in autoinflammatory diseases derived from case reports, case series, clinical trials, and registries, outline their pharmacokinetic and pharmacodynamics properties and discuss safety aspects of these medications.

Keywords

Interleukin (IL-1) · IL-1 receptor antagonist Anakinra · Rilonacept · Canakinumab

Abbreviations

ACR	American College of Rheumatology
ADA	Anti-drug antibodies
AISLE	Autoinflammatory syndrome associated with lymphedema
AOSD	Adult-onset Still disease
AP1S3	Adaptor related protein complex 1 sigma 3
APLAID	Autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation
AUC	Area under the curve
CAPS	Cryopyrin-associated periodic syndrome
CARD	Caspase recruitment domain
CNS	Central nervous system
CSF	Cerebrospinal fluid
DADA2	Deficiency of adenosine deaminase 2
DIRA	Deficiency of the IL-1 receptor antagonist
DITRA	Deficiency of the IL-36 receptor antagonist
DMARD	Disease modifying antirheumatic drugs
EMA	European Medicines Agency

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ESRD	End-stage renal disease
FDA	Food and Drug Administration
FMF	Familial Mediterranean fever
HOIL	Heme-oxidized iron regulatory protein 2 ubiquitin ligase-1
IC50	50% inhibitory concentration
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
IL-1R-AcP	IL-1 receptor accessory protein
LPS	Lipopolysaccharide
MAS	Macrophage activation syndrome
MKD	Mevalonate kinase deficiency
MTX	Methotrexate
NLR	NOD-like receptor
NOMID	Neonatal onset multisystem inflammatory disease
PAAND	Pyrin-associated autoinflammation with neutrophilic dermatosis
PAPA	Pyogenic arthritis, pyoderma gangrenosum and acne
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
PFIT	Periodic fever, immunodeficiency, and thrombocytopenia
RA	Rheumatoid arthritis
SAPHO	Synovitis, acne, pustulosis, hyperostosis, osteitis
SIFD	Sideroblastic anemia with immunodeficiency, fevers, and developmental delay
sJIA	Systemic juvenile idiopathic arthritis
SOBI	Swedish Orphan Biovitrum
TNFRSF	TNF receptor superfamily member
TRAP	Target-related affinity profiling
TRAPS	Tumor necrosis factor receptor-associated periodic *s*yndrome

Key Points

- **Interleukin (IL)-1 plays a pivotal role in various autoinflammatory diseases**
- **Anakinra, rilonacept and canakinumab are specific inhibitors of IL-1 signalling**
- **These IL-1 blocking drugs have been widely used to treat autoinflammatory conditions**
- **IL-1 blocking drugs differ in their mechanisms of action, pharmacokinetic and pharmacodynamic properties but offer fairly similar safety profiles**

41.1 Rationale for Interleukin (IL)-1 Inhibition in Autoinflammatory Diseases

Interleukin (IL)-1α and IL-1β bind to the IL-1 receptor type 1, which is expressed by nearly every human cell and trigger a cascade of inflammatory processes (see Chap. 6). Exposing individuals to exogenously produced IL-1 leads to high levels of inflammation which demonstrates the strong impact of the molecule on the initiation and maintenance of inflammatory processes [1]. The discovery of the genes involved in some monogenic autoinflammatory diseases and their direct impact on inflammasome function and IL-1β secretion has led to major efforts to specifically block this pathway with the aim of controlling various human diseases [1].

Indeed, a natural occurring IL-1 inhibitor exists; an IL-1 inhibitory molecule was first described in urine from patients with monocytic leukemia more than 30 years ago [2]. This molecule was later cloned and denoted as IL-1 receptor antagonist (IL-1Ra) because of its IL-1 antagonizing function [3]. The authors cited in their introduction, that "an IL-1 inhibitor could also be useful as a therapeutic agent for treatment of inflammatory diseases" [3]. Three molecules have been developed as specifically targeting IL-1, anakinra, rilonacept and canakinumab. These will be described in detail in this chapter. Two novel IL-1 inhibitors are being developed. One, targeting the IL-1 receptor, is being developed with the affibody technique [4]. The second one is a heterodimeric fusion protein that inhibits IL-1β/IL-1F2-induced signaling.

41.2 Anakinra

- **The interleukin-1 receptor antagonist (IL-1Ra) efficiently blocks IL-1α and IL-1β signal *in vitro***
- **Anakinra is effective in a variety of autoinflammatory diseases**
- **The recombinant human IL-1Ra anakinra has a short half-life time and penetrates the central nervous system**

The naturally occurring IL1-Ra, as well as the recombinant pharmacological compounds, blocks the action of IL-1α and IL-1β by competitively binding to the IL-1 receptor. This binding prevents the interaction of a second cell-membrane molecule termed IL-1 receptor accessory protein (IL-1RAcP), which is necessary for initiating cell stimulation, resulting in no cellular response. Furthermore, membrane-bound as well as soluble IL-1RII may bind IL-1 and therefore may prevent interaction with cell surface IL-1RI (Fig. 41.1) [5]. A 50% inhibition of IL-1-induced biological responses requires amounts of IL-1Ra in large excess (greater than 1000-fold) of the circulating amounts of IL-1α or IL-1β [6].

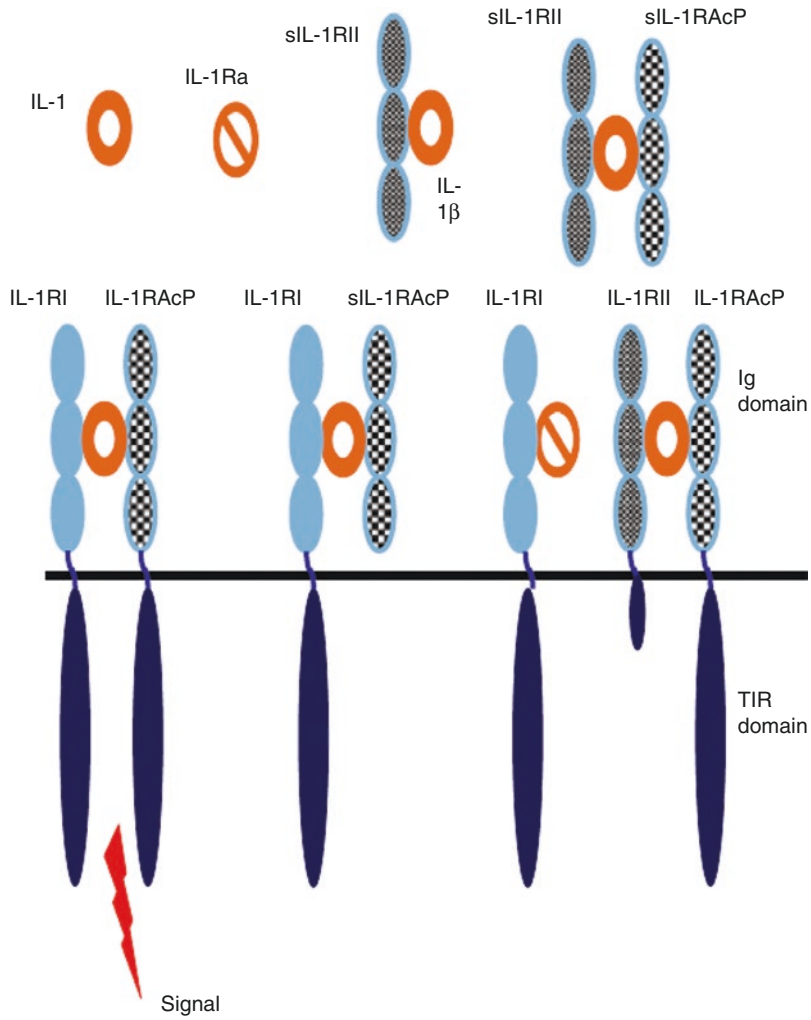

Fig. 41.1 Interaction of interleukin (IL)-1 and IL-1 receptor antagonist (IL-1Ra) with their soluble and membrane-bound ligands. The binding of IL-1 to IL1-RI and IL-1RAcP leads to signalling transduction. Binding of soluble IL-1RA as well as soluble and bound IL-1RII are capable to inhibit IL-1 medited cell stimulation (taken with permission from Arend et al., Immunol Rev, 2008, 223:20). A further mechanism of blocking IL-1 action is the interaction of membrane bound as well as soluble IL-1RAcP with IL-1 and IL-1RII. *IL* interleukin; *IL-1Ra* IL-1 receptor antigonist; *IL-1RAcP* IL-1 receptor accesory protein; *Ig* immunoglobulin; *TIR* toll IL-1 receptor

The efficacy of recombinant IL-1Ra was proven in a variety of subsequently performed *in vitro* and *in vivo* experiments:

- In synovial cells and cultured articular cartilage IL-1Ra inhibits the IL-1β-mediated production of hyaluronic acid, matrix metalloproteinase and PGE2/collagenase [6–9], Furthermore, it alters IL-1-specific effects on bone resorption [10].
- IL-1-induced T cell proliferation can be blocked by the addition of IL-1Ra [8].
- In a rodent model of streptococcal cell wall induced arthritis early administration of recombinant IL-1Ra prevented the development of arthritis [11]. The same effect was described in lipopolysaccharide (LPS)-induced arthritis in rabbits [12], as well as in collagen-induced arthritis in mice [13]. In other animal models for arthritis, recombinant IL-1Ra failed to prevent inflammation, indicating that IL-1 is not the key mediator for the development of arthritis in every experimental system [14, 15].

These observations indicated the effective use of recombinant IL-1Ra for the control of IL-1-mediated inflammatory processes *in vitro* and in *vivo*. Subsequently, a clinical research program for the administration of the commercially available recombinant IL-1Ra anakinra was initiated. Anakinra (Kineret®, Swedish Orphan Biovitrum-SOBI) is a recombinant, non-glycosylated form of the human IL-1Ra produced in *E. coli* using recombinant DNA techniques. It is structurally identical to the human IL-1Ra except for an additional preceding methionine residue at the NH_2-terminus [16]. It consists of 153 amino acid proteins and has an approximate molecular weight of 17.3 kDa.

Anakinra is currently labelled by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for subcutaneous administration to treat adults with rheumatoid arthritis (RA) and patients with cryopyrin-associated periodic syndromes (CAPS), systemic juvenile idiopathic arthritis (sJIA) from the age of 8 months and adult-onset Still disease (AOSD).

Meta-analysis studies have demonstrated a significant difference in the proportion of patients with RA achieving an American College of Rheumatology (ACR) 20 response with anakinra vs. placebo [17–19]. However, the modest absolute improvement, especially when compared to other biologics, together with the observed increase of infections, limits the utility of anakinra for the treatment of RA. But in order to describe the pharmacokinetic properties as well as the safety profile of anakinra we refer to human studies using anakinra in various inflammatory diseases, including RA, sJIA and CAPS. Table 41.1 summarizes observations of the use of anakinra in various autoinflammatory diseases and other diseases with autoinflammatory aspects (for further detail see specific disease chapters).

41.2.1 Pharmacokinetics and Pharmacodynamics of Anakinra

41.2.1.1 Serum Levels After Intravenous and Mucosal Administration

In a phase I study intravenous administration of 1 mg/kg and 10 mg/kg anakinra led to peak plasma levels of 3.1 μg/mL and 29 μg/mL, respectively. The terminal half-life was 108 min [87]. The first pharmacokinetic study in patients came from trials performed in sepsis. In these studies patients received 100 mg anakinra intravenously as loading dose followed either by 17, 67 or 133 mg/h of anakinra, for 3 days. The mean IL-1Ra plasma level during the infusion was 3.5 ± 2.9, 14.8 ± 8.12 and 25.2 ± 13.2 μg/mL, respectively. These concentrations were markedly greater than the mean endogenous IL-1Ra plasma concentrations measured during the infusion of placebo (8.6 ± 13.3 ng/mL) [88].

Pharmacokinetic steady state is reached after 4 weeks of treatment with once daily subcutaneous administration of anakinra [89]. Following

Table 41.1 Uses of anakinra

Use of anakinra in autoinflammatory diseases					
Chapter	Disease	Doses administered (children)	Doses administered (adults)	Degree of efficacy	Evidence level
16	Colchicine refractory FMF	50–300 mg/day [20]	50–300 [20], 100 mg/day [21]	Significant [20, 21]	RCS [20, 21]
17	MKD	1 mg/kg/day [22]	[a]100 mg/day [22], 100 mg/day [23]	Significant [22, 23]	OLS [22], CR [23]
18	TRAPS	1.5 mg/kg/day [24]	100 mg/day [25, 26]	Significant [24–26]	CR [25], OLS [24], CS [26]
19	CAPS	1–1.5 [27], 1–2 [28], 1.5–8 mg/kg/day [29]	100 mg/day [30, 31], 1–2 mg/kg/day [28], 1.5–8 mg/kg/day [29]	Dramatic [27, 28, 30, 31]	CR [27, 30], OLS [28, 31], CS [29]
20	Blau syndrome	3 mg/kg/day [32]		Significant [32]	CR [32]
22	PAPA	1 mg/kg/day [33]	100 [34]	Significant [33, 34]	CR [33, 34]
25	Monogenic inflammatory bone diseases	Majeed syndrome: 1.7 mg/kg/day [35] DIRA: Up to 4 mg/kg/day [36–38]		Significant [35] Dramatic [36–38]	CR [35] CR [36, 38], CS [37]
26	Monogenic psoriasis	DITRA: 4 mg/kg/day [39]	Not specified [40], 100 mg/day [41]	Significant [39, 41] Moderate [40]	CR [39–41]
28	Monogenic autoinflammatory disease with immunodeficiency	APLAID: intravenous dosage not specified, local administration [42]	APLAID	APLAID: Moderate [42]	CR [42]
29	Other rare autoinflammatory diseases	NLRC4: not specified [43]	PAAND: 100 mg/day [44]	NLCR4: moderate [45] significant [43] PAAND: significant [44]	NLRC4: CR [43, 45] PAAND: CR [44]
30	PFAPA	[a]1 mg/kg/day [46]	[a]100 [47]	Moderate	CS [46], CR [47]
31	Chronic non-bacterial osteomyelitis	Median 2 mg/kg/day [48]	SAPHO: 100 mg/day [49]	Significant [48, 49]	CS [48, 49]
32	Systemic juvenile idiopathic arthritis	1.3–4 mg/kg/day [50–53]		Significant [50, 52–55]	RCT [53], registry [54, 55], RCS [52], PCS [50], TP [51]
32	Adult-onset Still disease		100 mg/day [56, 57]	Significant	MA of CS and surveys [56], RCT [57]
34	Gout and crystal disease		100 mg/day [58, 59]	Pending [58] Significant [59]	RCT [58], RCS [59]
35	Behçet disease		100–200 mg/day [60, 61]	Significant	RCS [61], OLS [60]
36	Recurrent idiopathic pericarditis	1–2 mg/kg/day [62, 63]	100–150 mg/day [64, 65]	Significant	RCT [66], CR/CS [62–65]
37	Schnitzler syndrome		100 mg/day [67, 68]	Significant [67–69] Moderate [70]	CR [67, 68], CS [69, 70]

(continued)

Table 41.1 (continued)

Use of anakinra in autoinflammatory diseases					
Chapter	Disease	Doses administered (children)	Doses administered (adults)	Degree of efficacy	Evidence level
Use of anakinra in other diseases with autoinflammatory aspects					
NA	Hidradenitis suppurativa		100 mg/day	Significant [71]	RCT [71]
39	Heart failure after acute myocardial infarction		100 mg/day	Unclear [72]	RCT [72]
NA	Recent onset type 1 diabetes mellitus		100 mg/day	Not effective [73]	RCT [73]
NA	Ulcerative colitis in patients with chronic granulomatous disease		Not available	Unclear [74]	CS [74]
NA	Autoimmune inner ear disease		100 mg/day	Significant [75]	OLS [75]
39	Inflammation following subarachoid hemorrhage		100 mg/day	Significant [76]	OLS [76]
39	Exercise intolerance due to systolic heart failure		100 mg/day	Moderate [77]	OLS
39	Smoldering or indolent multiple myeloma		100 mg/day [78]	Moderate [78]	OLS [78]
NA	Rheumatoid arthritis		30, 75, 100 mg/kg/day [79]; 0.04, 0.1, 0.4, 1.0 or 2.0 mg/kg/day [80]; 100 mg/day [81, 82]	Moderate [79–82]	RCT [79–82]

DADA2 inconsistent results, insufficient evidence [83–85]; *NLRP12* insufficient evidence [86]; hydatidiform mole/NLRP7, X-linked inhibitor of apoptosis, otulopenia, TNFRSF11A, NLRP1, CARD14, AP1S3, AISLE, SIFD, HOIL, PFIT, interferonopathies: no reports found

FMF familial Mediterranean fever; *MKD* mevalonate kinase deficiency; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *CAPS* cryopyrin-associated periodic syndrome; *PAPA* pyogenic arthritis, pyoderma gangrenosum and acne; *DIRA* deficiency of the IL-1 receptor antagonist; *DITRA* deficiency of the IL-36 receptor antagonist; *APLAID* autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation; *NLRC4* NLR family CARD domain containing 4; *PAAND* pyrin-associated autoinflammation with neutrophilic dermatosis; *PFAPA* periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis; *SAPHO* synovitis, acne, pustulosis, hyperostosis, osteitis; *MAS* macrophage activation syndrome; *DADA2* deficiency of adenosine deaminase 2; *NLRP* NOD-like receptor family, pyrin domain; *CARD* caspase activation and recruitment domains; *SIFD* sideroblastic anemia, immunodeficiency, fevers, and developmental delay; *PFIT* periodic fever, immunodeficiency and thrombocytopenia; *RCT* randomized controlled trial; *RCS* retrospective cohort study; *PCS* prospective cohort study; *CS* case series; *CR* case report; *TP* treatment protocol; *OLS* open label study; *MA* meta-analysis; *NA* not applicable

[a]On demand at start of febrile episode

single subcutaneous dose at 1–2 mg/kg/day, peak concentrations are in the range of 1 μg/mL or less.

In a rodent disease model, absolute bioavailability after intratracheal and intranasal administration of recombinant IL-1Ra were 94.3% and 24.8%, respectively [90].

41.2.1.2 Pharmacokinetics in Children

In children, the pharmacokinetics of anakinra following subcutaneous administration is described by a one compartment model with first order absorption [91]. The body weight is the sole covariate that explains variability in pharmacokinetics. The study demonstrated that children, especially those with a body weight below 10 kg, required higher dosages to normalize C-reactive protein (calculated daily dose to reach the mean anakinra steady-state concentration of 0.4 mg/L [91]: <10 kg approximately 3 mg/kg/day, 10–50 kg approximately 2 mg/kg/day, >50 kg approximately 1 mg/kg/day). Therefore, in the young child anakinra might have to be administered in higher doses (up to 10 mg/kg/day) in order to achieve symptom control (Table 41.1). A recently performed study in children with CAPS (median age of 11.4 (4.2–42.2) years) treated with varying doses of subcutaneous anakinra showed a median peak steady-state serum concentration of 3.6 μg/mL and a median half-life time of 5.7 h (EMA Assessment Report, Anakinra).

41.2.1.3 Pharmacokinetics in Chronic Renal Failure/End-Stage Renal Disease

Analysis in rats suggested that approximately 80% of the drug is excreted by renal clearance [92]. In humans, it has also been shown that the mean plasma clearance of anakinra strongly depends on intact kidney function (137 ± 21 mL/min in healthy subjects, 20 mL/min in patients with end-stage renal disease). The dialysis process has a minimal effect on the removal of anakinra [93]. No dose adjustment is probably needed in subjects with mild to moderate renal impairment. In patients with end stage renal disease longer intervals with lower dosages are advisable, e.g. injection every second or third day.

41.2.1.4 Central Nervous System (CNS) Levels After Intravenous and Subcutaneous Administration

The involvement of the central nervous system (CNS) in autoinflammatory diseases raises the issue of CNS pharmacokinetics of anakinra. On one hand, stable IL-1 homeostasis is critical for the maintenance of the hippocampal-dependent memory [94]. Therefore, cognitive functions might be impaired through a CNS blockade of IL-1. On the other hand, a clinical study demonstrated that administration of anakinra led to improvement of cognitive function and memory in patients with CAPS. Thus, in a clinical setting, anakinra seems to have a beneficial effect with respect to cerebral functions [95].

An early animal study showed that a substantial amount (1/3 to 2/3) of serum IL-1Ra crosses the intact blood-brain barrier [96]. Another study in non-human primates revealed that drug exposure in the cerebrospinal fluid (CSF) was 0.28% compared to serum. The average CSF concentration after an intravenous dose of 3 mg/kg was 1.8 ng/L, which is 30-fold higher than endogenous CSF levels of IL-1Ra. In this study, the relative amount of drug penetration was not dose dependent [97].

In neonatal onset multisystem inflammatory disease (NOMID) subcutaneous anakinra administration led to a fivefold increase of IL-1Ra in the CSF (0.21 ng/mL before treatment vs. 1.14 ng/mL after 3 months of treatment) [28].

Further interest in the CNS concentration of anakinra came from the observation that control

of inflammation can be a therapeutic approach for patients with cerebral hemorrhage. Several studies, including animals as well as human patients with cerebral hemorrhage, demonstrated therapeutic levels of anakinra in CNS after intravenous administration [98–101].

41.2.2 Development of Anti-Drug-Antibodies

The development of anti-drug-antibodies was observed in 2.7% of patients at one time during a 24 weeks treatment period; none of these detected antibodies was neutralizing [80]. In another study, 4 of 454 (<1%) patients developed anti-drug antibodies at two or more follow-up visits during a 24-week treatment period [79]. Thus, the occurrence of anti-drug-antibodies is a rare event but long-term analyses are warranted.

41.2.3 Safety Aspects

41.2.3.1 Data from Randomized Controlled Trials in Patients with Rheumatoid Arthritis

A Cochrane analysis was performed from 5 randomized controlled trials of RA involving 2876 patients (781 placebo, 2065 anakinra) [17]. No significant difference in total adverse events was observed between the anakinra (without methotrexate) and placebo groups. However, in the anakinra/methotrexate versus anakinra/placebo subgroup, there was a statistically significant difference in the total number of adverse events (RR 1.11; 95 CI 1.03–1.20).

In one study, serious adverse events occurred in 2.1% of the anakinra group vs. 0.4% in the placebo group. But as in other studies, this difference was not statistically significant [81, 82].

Another meta-analysis studied 1414 patients with RA and significant co-morbidities [102] and concluded, that high doses of anakinra (≥100 mg/day) were associated with an increased risk of serious infection in patients with pre-existing co-morbidities (high vs. low dose anakinra OR = 9.63 (95% CI: 1.31 to 70.91), high dose anakinra vs. placebo OR 3.40 (95% CI 1.11 to 10.46)). The results were not statistically significant when patients without comorbidities were excluded [103]. The most frequently observed serious infection was pneumonia; no opportunistic infections were observed.

Although unusual or opportunistic infections (e.g. tuberculosis, histoplasmosis) have been observed in patients using other biologics, no increased incidence of such infections has been observed with anakinra [82].

41.2.3.2 Data from Long-Term Observation Studies

Two studies followed 1116 and 1346 patients with RA, respectively, for 36 months [104, 105]. No increase of cumulative exposure event rates were observed during the follow-up [105]. However, serious infections were observed more often in anakinra treated patients (2.1% vs. 0.4% [104]; 5.37 events/100 patient-years vs. 1.65/100 patient-years [105]. Most frequent was pneumonia and cellulitis. Three cases of opportunistic infections were reported [105]. As demonstrated in other studies, a high prevalence of injection site reactions was reported (72.6% vs. 32.9%). During 36 months of treating 1346 patients, 4 experienced decreased hemoglobin levels, 5 had increased liver enzymes and 7 developed thrombocytopenia [105]. In the limited long-term follow-up data there was no signal for an increased incidence of malignancies.

41.2.3.3 Data from Registries

In the British Society of Rheumatology Biologics Register, 111 patients with rather severe disease and early anakinra treatment were registered. These patients had higher rates of serious infections, especially of the skin and respiratory tract, compared to patients treated with conventional disease modifying anti-rheumatic drugs (DMARDs) (92/1000 vs. 34/1000 patient years) [106]. To note, patients treated with anakinra had more severe disease and higher exposure to corticosteroids.

41.2.3.4 Data from Patients with Autoinflammatory Diseases

Due to the rarity of CAPS and the overwhelming efficacy of IL-1 blockade, no randomized trials of anakinra have been performed. In studies with a rather short observation period no serious adverse events were reported [28, 107]. In a long-term study with 43 severely affected CAPS patients the most frequently reported serious adverse event was pneumonia, which might be explained by the role of IL-1β in the resistance to Streptococcus pneumoniae infections [108], and gastroenteritis [29].

Twenty-four children were enrolled in a randomized trial for efficacy of anakinra in sJIA (ANAJIS trial). Within this trial, 6 serious adverse events were observed, 4 of which were infectious [49].

41.2.3.5 Injection Site Skin Reactions

Injection site skin reactions are by far the most frequently described adverse event of anakinra administration, which is most likely caused by spontaneous mast cell degranulation due to components of the solution, but antihistamines have only limited beneficial effects [109]. Other possible explanations are immunoglobulin E mediated reactions [110], as well as delayed reactions to anakinra characterized by the presence of perivascular T-lymphocytes [111]. Injection site skin reactions are observed in up to 71% of patients [17] and is often associated with an immediate sensation of stinging and burning. A delayed reaction usually presents with rash, swelling and pain. Typically, the first reaction is observed within the first weeks of treatment initiation [112]. Skin reactions usually decline over time even with continuation of therapy.

To limit injection site reactions different approaches have been suggested, e.g. cooling of skin before and after the injection, applying local anesthetic cream, distraction of the skin with a comb or vibrator and warming the medication to room temperature. Various desensitization protocols in case of acute and delayed reactions have been suggested [111, 113].

In summary, the incidence of serious infectious might be increased under anakinra therapy, especially in case of pre-existing co-morbidities. Thus far, no signals for an increased incidence of malignancies have been described. Longer follow-up periods of large number of patients are warranted.

41.2.3.6 Pregnancy, Breast Feeding

There are no adequate and well-controlled studies of anakinra in pregnant women. Rat and rabbit models for fetal exposure to anakinra revealed no evidence of impaired fertility or harm. Anakinra should be used during pregnancy only if clearly needed. In an international cohort, 29 pregnancies exposed to anakinra were identified, 23 through maternal exposure, 6 through paternal exposure. There was no history of infection in either the mothers or the infants exposed to anakinra [114]. One miscarriage and a single case of ectopic neurohypophysis with growth hormone deficiency and left renal agenesis were reported. Ten babies were breast fed by mothers taking anakinra with no reported infections or developmental abnormalities. Further reports described the use of anakinra during pregnancy [115, 116]; one infant was born with renal agenesis [117].

41.2.3.7 Practical Aspects

Although no increased risk for a reactivation of latent tuberculosis was described so far, testing for latent tuberculosis should follow local guidelines. Neutrophil counts should be checked prior to initiating and monthly for 3 months while receiving anakinra and thereafter quarterly. It is recommended to stop the administration of anakinra if neutrophil counts decrease to below 1.5×10 [9]/L. [118] Although it is recommended in the prescribing information to suspend anakinra during a severe infection, it is important to note that even in the studies of high dose intravenous administration used for sepsis anakinra was not associated with drug-specific toxicity [74, 75].

41.3 Rilonacept

- **Rilonacept is a dimeric fusion protein composed of the ligand binding domains of the extracellular portions of the human IL-1R1 and the IL-1R-AcP, and is effective in blocking IL-1-signals by "trapping" the cytokine**
- **Rilonacept is a medium- to long-lasting IL-1α and IL-1β blocker**
- **Rilonacept is effective in cryopyrin-associated periodic syndrome (CAPS), gout, systemic juvenile idiopathic arthritis (sJIA) and familial Mediterranean fever (FMF)**

Rilonacept (Arcalyst®, Regeneron, Tarrytown, United States) represents another principle to block the IL-1β/α signalling pathways. Rilonacept is a dimeric fusion protein composed of the ligand binding domains of the extracellular portions of the human IL-1R1 and the IL-1R-AcP (see above) linked to the human IgG1 Fc domain [119]. Rilonacept is expressed in recombinant Chinese hamster ovary cells. The dimeric glycoprotein has a protein molecular weight of 201 kDa and contains about 25% glycosylation, yielding a total molecular weight of approximately 252 kDa [120, 121].

Rilonacept blocks IL-1β signalling by acting as a soluble decoy receptor that binds IL-1β and prevents its interaction with cell surface receptors. Rilonacept also binds IL-1α and IL-1Ra with reduced affinity. The equilibrium dissociation constants for rilonacept binding to IL-1β, IL-1α and IL-1Ra were 0.5 pM, 1.4 pM and 6.1 pM, respectively. Binding of IL-1β and IL-1Ra to rilonacept creates an inactive complex that clears slowly and accumulates *in vivo*, therefore the level of the complex represents the amount of synthesized cytokines. Rilonacept is currently licensed only in the United States and labelled by the FDA for CAPS.

41.3.1 Pharmacokinetics and Pharmacodynamics of Rilonacept

41.3.1.1 Serum Levels After Intravenous Administration

The C_{max} and the area under the curve (AUC) of rilonacept increase proportionally when applied in different subcutaneous dosages. Mean steady-state trough values after once weekly administration of 160 mg of rilonacept by subcutaneous injection ranges from 100 to 133 nM (20–27 μg/mL), thus the linear kinetics of rilonacept are well established. The terminal-phase elimination half-life is approximately 1 week and is dose-independent. In patients with CAPS a steady-state is reached after a 6-week treatment period. The magnitude of dose administration does not affect its terminal half-life and has a small effect on clearance within a broad dose range [120, 121].

41.3.1.2 Pharmacokinetics in Children

In two studies analysing pharmacokinetic data from children with sJIA, the serum half-life varied from 6.9 to 8.9 days. In line with this observation, the total clearance (CL/F) was higher in the first trial compared to the second (0.83 L/day vs. 0.55 L/day, respectively) [122, 123]. This discrepancy is most likely due to differing demographic characteristics and is unlikely to result in dose variations. The levels of rilonacept are inversely related to serum albumin, thus in severe inflammatory disease with hypoalbuminemia, rilonacept could be more available to proteolytic catabolism due to saturation of the protection mechanism [123].

41.3.1.3 Pharmacokinetics in Chronic Renal Failure/End-Stage Renal Disease

Rilonacept, like etanercept, is a large molecule that is expected to be cleared primarily by the reticuloendothelial system rather than the kidney [124]. In patients with end-stage renal disease (ESRD) on dialysis the terminal half-life is comparable to the results from patients with CAPS (7.63 vs. 8.86 days, respectively) [121]. This observation was confirmed in another small series analysing patients with ESRD on hemodialysis treated with rilonacept [125]. Based on these observations, a dose adjustment does not seem to be required in patients with ESRD.

41.3.1.4 Central Nervous System (CNS) Levels After Subcutaneous Administration

Data on penetration of rilonacept into the brain are limited. It is unlikely that the 252 kDa protein crosses the blood-brain-barrier. Observed effects on CNS symptoms are likely to be caused by decreasing IL-1β levels in the peripheral blood.

41.3.2 Development of Anti-Drug-Antibodies

In a series of 57 patients with CAPS treated with rilonacept 24% developed anti-drug antibodies during the first 24 weeks. In two of these patients, levels of anti-rilonacept-antibodies were considered high (>1:800). Two patients demonstrated a notable reduction in total rilonacept levels that may have been associated with the appearance of neutralizing anti-drug antibodies. In a randomized study in patients with sJIA, frequent occurrence of injection site skin reactions was associated with the development of anti-rilonacept antibodies. There appeared to be little or no correlation between the antibodies and low rilonacept sera levels or insufficient clinical response [126].

41.3.3 Safety Aspects

41.3.3.1 Data from Randomized Controlled Trials in Patients with Gouty Arthritis

A large randomized trial with rilonacept was performed in 241 patients with gout. Over a 16-week period patients received placebo, 80 mg or 160 mg rilonacept twice weekly, respectively [127]. Except for injection site reactions, the incidence of adverse events was similar among the treatment groups. Headache and upper respiratory tract infections were the second most observed events. In each treatment group 3 patients reported serious adverse events, which were not considered to be treatment related. There were no reports of tuberculosis or other opportunistic infections. Similar results were obtained from the PRESURGE-2 trial in patients with gouty arthritis (n = 248) [128]. The RESURGE trial was primarily conducted to evaluate the safety of rilonacept in patients with gout (n = 1315) [129]. Again, the incidence of adverse events was similar between the treatment and placebo group except for injection site reactions (6.2% vs. 0.3%). Equally, the observed rates of serious infections were comparable (0.5% with rilonacept, 0.9% with placebo). Treatment with rilonacept was associated with small increases in alanine aminotransferase, aspartate aminotransferase, triglycerides and creatine phosphokinase. 3.3% of rilonacept treated patients developed transient neutropenia (<1500 cells/μL).

41.3.3.2 Data from Long-Term Observation Studies

In the largest long-term study 101 patients with CAPS had a follow-up for 96 weeks. The most commonly reported adverse event were injection site reactions and upper respiratory tract infections. The nine reported serious adverse events were considered by the investigators to be unrelated to the study medication [130]. In a long term extension trial with patients with sJIA (n = 23) treated for 24 months, no deaths, malignancies or opportunistic infections were observed [126].

41.3.3.3 Data from Patients with Autoinflammatory Diseases

The use of rilonacept for autoinflammatory diseases is described in Table 41.2 (for detailed discussion see specific chapters). In patients with CAPS treated with rilonacept (n = 44) upper respiratory infections, headache, diarrhea and arthralgia were reported as the most common adverse events [131]. During the open-label phase of this study 20% of patients developed infections of which one was a staphylococcus aureus infection. In randomized trials with patients with sJIA (RAPPORT trial, n = 71) and familial Mediterranean fever-FMF (n = 14) no new safety signals were observed [122, 132]. In a cohort of six patients with deficiency of IL-1 receptor antagonist (DIRA) no clinical difference in the number or severity of adverse events were observed when comparing the groups receiving 2.2 mg/kg/week or 4.4 mg/kg/week, respectively [133]. Rilonacept was also well tolerated in patients with Schnitzler syndrome [134].

41.3.3.4 Pregnancy, Breast Feeding

There are no adequate well-controlled studies of rilonacept in pregnant women. One cynomolgus monkey fetus exposed to rilonacept developed multiple fusion and absence of the ribs and thoracic vertebral bodies and arches [119]. Mice exposed to rilonacept showed a threefold increase in the number of stillbirths in dams treated with rilonacept. Therefore, rilonacept should be used during pregnancy only if the benefit justifies the potential risk to the fetus. It is unknown whether rilonacept is excreted in human milk, thus caution should be exercised when rilonacept is administered to a nursing woman [119].

Table 41.2 Use of rilonacept in autoinflammatory diseases

Chapter	Disease	Doses administered to children [mg/kg/week]	Doses administered to adults [mg/week]	Degree of efficacy	Evidence level
16	Colchicine resistant FMF	2.2 [132]	160 [132]	Significant [132]	RCT [132]
19	Cryopyrin-associated periodic syndrome	2.2 [130]	160 [130, 131], 100–320[a] [135]	Dramatic [130, 131, 135]	OLS [130, 135], RCT [131]
25	Monogenic bone diseases (DIRA)	2.2–4.4 [133]		Significant [133]	OLS [133]
32	Systemic juvenile idiopathic arthritis, adult-onset Still disease	sJIA: 2.2/4.4[a] [122, 126]	AOSD: 160 [136]	Significant [122, 126, 136]	CR [136], RCT [122, 126]
34	Gout and crystal disease		160[a] [137, 138], 160 [129], 80–160 [128], 80–160[a] [127]	Significant [127–129, 137, 138]	RCT [127–129, 137, 138]
37	Schnitzler syndrome		160[a] [134]	Significant [134]	OLS [134]

Reports of rilonacept use were not found in other autoinflammatory diseases
FMF familial Mediterranean fever; *DIRA* deficiency of the IL-1 receptor antagonist; *sJIA* systemic juvenile idiopathic arthritis; *AOSD* adult-onset Still disease; *RCT* randomized controlled trial; *OLS* open label study; *CR* case reports
[a]Loading dose

41.3.3.5 Practical Aspects

During therapy, lipids, neutrophils and liver enzymes should be monitored every 2–3 months, at least at the beginning of therapy [119]. Although increased incidence of mycobacterial infection was not described in the trials, testing for latent tuberculosis might be indicated according to local guidelines. So far, no drug-specific high-dose toxicity has been described [138].

41.4 Canakinumab

- **Canakinumab is a fully human IgG monoclonal antibody to IL-1β**
- **Canakinumab is a long-lasting IL-1β blocker**
- **Canakinumab is effective in several autoinflammatory diseases**

Canakinumab (Ilaris®, Novartis, Switzerland) is a fully human monoclonal antibody of the IgG1/k isotype. It binds IL-1β with high affinity (in the low nanomolar range) preventing binding of IL-1β to the IL-1 receptors. It does not bind IL-1α, and, therefore, differently from anakinra and rilonacept, inhibits biological functions of IL-1β, but not of IL1α. The knowledge on the biological role of IL-1α is very limited; nevertheless, when choosing an IL-1 inhibitor, this difference in the mechanism of action must be taken into account.

Canakinumab is currently labelled by the United States FDA and by the EMA for subcutaneous administration to treat children (from the age of 2 years) and adults with CAPS, children (from the age of 2 years) with sJIA and adults with AOSD. Furthermore, it is labelled for the treatment of children (from the age of 2 years) and adults with colchicine-resistant FMF, mevalonate kinase deficiency (MKD) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), as well as (only in Europe) adults with gouty arthritis with frequent attacks.

Besides the indications included in the labels, canakinumab has been used in a variety of autoinflammatory diseases and other diseases with autoinflammatory aspects (summarized in Table 41.3).

Table 41.3 Uses of canakinumab

Use of canakinumab in autoinflammatory diseases					
Chapter	Disease	Doses administered (children)	Doses administered (adults)	Degree of efficacy	Evidence level
16	Colchicine resistant FMF	2 mg/kg q4w or q8w	150–300 mg q4w or q8w	Significant	OLS [139, 140], RCT [141]
17	MKD	2 mg/kg q4w or q8w	150–300 mg q4w or q8w	Significant	OLS [142], RCT [141]
18	TRAPS	2 mg/kg q4w or q8w	150–300 mg q4w or q8w	Significant	OLS [143],RCT [141]
19	CAPS	2–10 mg/kg q4w or q8w	150–300 mg q4w or q8w	Significant	OLS [144, 145], RCT [146]
32	Systemic juvenile idiopathic arthritis	4 mg/kg q4w		Significant	RCT [147, 148]
32	Adult-onset Still disease		150–300 mg q4w or q8w	Significant	CS [149, 150]
20	Blau syndrome	2 mg/kg q4w		Evident	CR [151]
22	PAPA		150 mg q8w	Significant	CR
NA	Pyoderma gangrenosum		150–300 mg q8w	Significant to moderate	CS [152]
25	Majeed syndrome	4 mg/kg q4w		Significant	CR [35, 153]
30	PFAPA		150 mg q8w	Significant	CR [154]

(continued)

Table 41.3 (continued)

Use of canakinumab in autoinflammatory diseases					
Chapter	Disease	Doses administered (children)	Doses administered (adults)	Degree of efficacy	Evidence level
34	Gout and crystal disease		150 mg (single dose)	Significant	RCT [155–157]
35	Behçet disease				RCS, CR [61, 158–160]
37	Schnitzler syndrome		150–300 mg q4w or q8w	Significant	OLS, RCT [161, 162]
Use of canakinumab in diseases with autoinflammatory aspects					
39	Atherosclerotic disease		50, 150, 300 mg every 3 months	Moderate effect	RCT [163, 164]
NA	Type 1 diabetes (recent onset)	2 mg/kg q4w	2 mg/kg qw4	Not effective	RCT [73]
NA	Type 2 diabetes		150 mg (single dose)	Not effective	RCT [165]
NA	Rheumatoid arthritis		150 mg q4w	Modest	RCT [166]

FMF familial Mediterranean fever, *MKD* mevalonate kinase deficiency; *TRAPS* tumor necrosis factor receptor-associated periodic syndrome; *CAPS* cryopyrin associated periodic syndrome; *PAPA* pyogenic arthritis, pyoderma gangrenosum and acne; *PFAPA* periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis; *q4w* every 4 weeks; *q8w* every 8 weeks; *OLS* open label study; *RCT* randomized controlled trial; *CS* case series; *CR* case report; *RCS* retrospective cohort study; *NA* not applicable

41.4.1 Pharmacokinetics and Pharmacodynamics of Canakinumab

41.4.1.1 Serum Levels After Subcutaneous Administration

A wealth of data is available regarding pharmacokinetic properties obtained from healthy volunteers and from patients with CAPS, sJIA, gout, recurrent autoinflammatory fever syndromes, asthma and psoriasis [167–170]. Following a single subcutaneous injection, canakinumab is absorbed slowly reaching maximal serum concentrations in approximately 7 days. Cmax ranges from 20 to 30 μg/mL after a single 150 mg dose and ranges from 30 to 40 μg/mL after a single dose of 300 mg. Bioavailability is approximately 70% in line with other IgG monoclonal antibodies. As with all human IgG, elimination occurs through endocytosis, with very little, if any, contribution from renal excretion or biliary secretion. The average half-life is approximately 26 days, again, similar to other human IgG monoclonal antibodies. After repeated injections, time to steady state was estimated to range between 110 and 130 days [167]. In patients with sJIA treated with 4 mg/kg every 4 weeks Cmax at steady state was approximately 40 μg/mL. As expected, in children a faster rate of clearance relative to body weight, was observed, based on an allometric relation, and similar to other monoclonal antibodies [170].

Even in diseases in which IL-1β plays a pivotal role, circulating IL-1β is detected with difficulty possibly due to rapid clearance from the circulation or to the fact that the IL-1β produced at a high rate in peripheral inflamed tissues may not reach the systemic circulation. Following administration of canakinumab, IL-1β/canakinumab complexes are formed. When bound in these complexes, the elimination rate of IL-1β slows down significantly reaching that of IgG. A model has been generated that allows the estimation of the constitutive production rate of IL-1β in patients with CAPS, approximately 30 ng/day [167]. Despite the formation of these complexes, target mediated drug disposition, a phenomenon by which clearance of a monoclonal antibody is increased by high levels of target/antibody complex, was not observed. This is probably due the low daily rate of IL-1β production in the majority of patients.

41.4.1.2 Pharmacokinetics in Chronic Renal Failure/End-Stage Renal Disease

As expected for human IgG, impairment of renal function does not affect the pharmacokinetics of canakinumab, as shown in 4 patients with CAPS with moderate to severe end-stage renal failure [167].

41.4.1.3 Dosing Regimens

In adult patients with CAPS the recommended starting dose is 150 mg every 8 weeks. In children aged 2 years and older the initial dose depends on the weight (between 7.5 and 15 kg body weight 4 mg/kg, between 15 and 40 kg 2 mg/kg). In CAPS, open label observations suggest that children, particularly those with the most severe phenotype of NOMID, require higher doses, up to 8 mg/kg every 4 weeks to achieve optimal control [144, 145]. These observations might be explained by a combination of faster clearance in children as a function of body weight (demonstrated in CAPS and particularly in sJIA) and a higher rate of IL-1β-production in patients with a more severe phenotype. In sJIA and AOSD, the suggested starting dose is 4 mg/kg up to a maximum of 300 mg every 4 weeks [147]. In patients with colchicine-resistant FMF, MKD or TRAPS the suggested starting dose is 150 mg or 2 mg/kg in children with body weight ≥ 7.5 kg and ≤ 40 kg every 4 weeks [141]. The dose can be increased up to 300 mg every 4 weeks (or 4 mg/kg in children, as above). In patients with MKD a requirement for the higher dose was more frequent, suggesting higher production rate of IL-1β in this disease [141]. In patients with gouty arthritis, the suggested dose is 150 mg administered subcutaneously as a single dose during an attack.

41.4.1.4 Central Nervous System (CNS) Levels After Subcutaneous Administration

The issue of blood brain barrier and canakinumab penetrance in the CNS is linked to CNS involvement in autoinflammatory diseases, particularly in NOMID. The available data have been obtained in a limited number of patients with NOMID. In a study of 6 patients with NOMID, all patients had to escalate doses from the standard dosing regimen, to the maximal allowed dose in 5 patients (600 mg or 8 mg/kg in children <40 kg every 4 weeks) with one patient receiving 600 mg every 6 weeks [171]. The median level of canakinumab in the CSF was 210 ng/mL (range 0–650 ng/mL) with corresponding plasma levels ranging from 53 to 189 μg/mL Since *in vitro* the IC50 of canakinumab is 7.1 ng/mL, the concentrations detected in the CSF should have a pharmacodynamic effect. Indeed, CSF counts of white blood cells were inversely related to canakinumab levels. However, none of the 6 patients reached complete clinical remission. In a subsequent study, in which patients with CAPS/NOMID were switched from anakinra to canakinumab, CSF counts of white blood cells and levels of IL-6 were higher while receiving canakinumab compared to when the same patients were receiving anakinra [172]. Altogether, albeit limited, these data suggest that effective neutralization of IL-1β with canakinumab in the CNS may be difficult to obtain.

41.4.2 Development of Anti-Drug Antibodies

Antibodies against canakinumab have been found in approximately 2% of the patients. No neutralizing antibodies were detected. No apparent correlation of antibody development to clinical response or adverse events was observed.

41.4.3 Safety Aspects

As canakinumab binds to marmoset IL-1β with similar affinity of that to human IL-1β, toxicity studies have been performed in marmosets. Plasma concentration in large excess (>70-fold higher) than those reached in humans are very well tolerated. The safety of canakinumab was analyzed in randomized controlled trials in autoinflammatory diseases, as well as in a large trial

in atherosclerotic disease. A long-term observational registry in CAPS has also provided some additional data. As in autoinflammatory disease trials, exposure to placebo was very limited, particularly in trials involving children. Thus, comparison of the frequencies of safety events with patients receiving placebo is of limited value. However, in the trial of atherosclerotic disease, more than 10,000 patients were recruited with approximately 3000 receiving placebo. In this large trial, in patients with a median age of 61 years and several comorbidities, neutropenia and thrombocytopenia, albeit rare, were more common in the canakinumab group (0.10 and 0.60/100 patient-years, respectively) than in the placebo group (0.06 and 0.43/100 patient-years, respectively). Also, more deaths were attributed to infection or sepsis in the canakinumab group than in the placebo group (0.31 vs. 0.18/100 patient-years). Overall no statistically significant difference in the rates of adverse events, serious adverse events, or serious infectious adverse events was observed [163]. In this study, among patients treated with canakinumab, the rate of serious adverse events and serious infections were 11.8/100 patient-years and 3.1/100 patient-years, respectively [163]. In the markedly smaller trial of 181 patients with FMF, MKD or TRAPS the rate of serious adverse events and of serious infections were 24.3/100 patient-years and 7.4/100 patient-years, respectively [141]. In the trials in sJIA, because of the complexity of the designs and variability of the exposure to canakinumab and placebo, rates of events were not reported. In the long-term registry of 285 patients with CAPS receiving canakinumab for up to 5 years, rates of serious adverse events and of serious infections were 15.4/100 patient-years and 5.4/100 patient-years, respectively [173]. In general, although children tended to have a higher percentage of serious adverse events and infectious events the differences were unremarkable. In the trial on atherosclerotic disease, a reduced mortality from cancer was also observed. A post-hoc analysis showed also a reduction in the incidence of new lung cancer during the trial [174].

In summary, canakinumab appears to be well tolerated in the treatment of autoinflammatory diseases. Very few patients have been discontinued from trials because of safety events. Infections of mild intensity (upper airway and urinary tract) appear to be the most frequently reported events. Serious infections have been reported: therefore, patients treated with canakinumab should be carefully monitored for these occurrences. Given the expected duration of the treatment, long-term assessment of drug safety is paramount in registries.

41.4.3.1 Pregnancy, Breast Feeding

Embryofetal toxicity studies in pregnant marmoset did not reveal undesirable effects. Scant information is available on the effect of canakinumab on pregnancy and offspring in humans. Only one international cohort reported on 8 pregnancies in 7 women receiving canakinumab. Seven pregnancies were uneventful, all reaching full term and normal weight [114]. One miscarriage was reported in a woman who had a previous miscarriage while on anakinra. Five infants were born from 3 fathers who were on long-term canakinumab, with no developmental abnormalities reported. No infections or developmental abnormalities were reported in 4 babies breast fed by mothers receiving canakinumab.

41.4.3.2 Vaccination

In a randomized controlled study in healthy subjects, canakinumab at 300 mg/kg (single dose) did not affect the safety or the induction or persistence of antibody response to unadjuvanted influenza or alum-adjuvanted conjugated meningococcal C vaccination [175]. A rather large set of data is available from a long-term prospective registry in patients with CAPS receiving canakinumab, with 68 patients receiving a total of 159 vaccine injections. In patients with CAPS, severe reactions (mainly local reactions around the injection site, but occasionally also systemic reactions) were observed only after receiving the pneumococcal vaccine [176]. Reactions to pneumococcal vaccines have been previously described in patients with CAPS [177, 178]. Indirect evidence suggest that these reactions are secondary to a specific effect of pneumococcal antigens on

inflammasome activation rather than to the co-administration of canakinumab. At present no recommendation on pneumoccoccal immunization can be made. Clinicians should weigh potential benefits with the concern regarding reactions. Tetanus/diphtheria and influenza vaccines did not appear to be associated with reactions. There is a lack of data on the safety of live attenuated vaccinations.

41.4.3.3 Practical Aspects

No increased risk for a reactivation of latent tuberculosis has been described with canakinumab. In the large trial on atherosclerotic disease, the incidence rate of tuberculosis was identical in the placebo and canakinumab groups [163]. Nevertheless, the prescribing information sheet recommends testing for latent tuberculosis according to local guidelines. Canakinumab should not be initiated in patients with neutropenia and neutrophil counts should be checked after 1 or 2 months of treatment and then periodically (i.e. every 3–4 months).

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42

Corticosteroid, Other Biologic and Small Molecule Therapies in Systemic Autoinflammatory Disorders

Helen J. Lachmann

Abstract

Not all patients with autoinflammatory disorders respond to treatment with colchicine or anti-interleukin (IL)-1 agents. Although no other biologics nor small molecule pharmaceuticals are currently licenced for use in autoinflammatory disease several agents are used. The pharmacology, mechanisms of action and safety data of these medications are summarised in this chapter. These include corticosteroids, tumor necrosis factor (TNF), IL-6 and Janus kinase (JAK) inhibitors. There are published consensus criteria which provide guidance to the management of familial Mediterranean fever (FMF), cryopyrin-associated periodic syndromes (CAPS), tumor necrosis factor receptor associated periodic syndrome (TRAPS) and mevalonate kinase deficiency (MKD).

Keywords

Corticosteroids · Monoclonal antibodies
Etanercept · Infliximab · Adalimumab
Interleukin-6 · Tocilizumab
Janus kinase inhibitors · Baricitinib
Tofacitinib

Abbreviations

CANDLE	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
CAPS	Cryopyrin-associated periodic syndromes
CAR	Chimeric antigen receptors
CNO	Chronic non-bacterial osteomyelitis
CRP	C-reactive protein
DADA2	Deficiency of adenosine deaminase 2
FDA	Food and Drug Administration
FMF	Familial Mediterranean fever
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GTI	Glucocorticoid Toxicity Index
IL	Interleukin
INF	Interferon
JAK	Janus kinase
MKD	Mevalonate kinase deficiency
NF-κB	Nuclear factor kappa B
NLRP	Nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain
NSAIDs	Nonsteroidal anti-inflammatory drugs
PAPA	Pyogenic arthritis, pyoderma gangrenosum, acne
PFAPA	Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
SAA	Serum amyloid A
SAVI	STING-associated vasculopathy with onset in infancy

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SHARE	Single Hub and Access point for Paediatric Rheumatology in Europe
SOSC	Suppressor of cytokine signaling
STAT	Signal transducer and activator of transcription
TNF	Tumor necrosis factor
TRAPS	Tumor necrosis factor receptor-associated periodic syndrome
TYK	Tyrosine kinase
UK	United Kingdom
USA	United States of America

Key Points

- **Not all autoinflammatory disorders respond to modulation of the interleukin (IL)-1 pathway**
- **There are published consensus criteria for the management of familial Mediterranean fever (FMF), cryopyrin-associated periodic syndromes (CAPS), tumor necrosis factor receptor-associated periodic syndrome (TRAPS) and mevalonate kinase deficiency (MKD)**
- **No other biologics or small molecule pharmaceuticals are currently labelled for use in autoinflammatory disease**
- **Biologics must be given parenterally and are prohibitively expensive; novel approaches currently being explored include drug repurposing and development of inflammasome inhibitors**

42.1 Introduction

A remarkable feature of the autoinflammatory diseases has been the almost complete responses seen once the appropriate treatment is started. The dramatic responses to colchicine in familial Mediterranean fever (FMF) and anti-interleukin (IL)-1 therapies in cryopyrin-associated periodic syndromes (CAPS) and tumor necrosis factor receptor associated periodic syndrome (TRAPS) have been covered elsewhere (see Chaps. 40 and 41) but these agents are not a complete panacea. The experience of other therapies which help with the management of these disorders and their pharmacology, mechanisms of action and safety data are summarised in this chapter. Treatment of systemic autoinflammatory diseases is a rapidly moving field and it is likely that our armamentarium will expand to include newer biologics targeting other cytokines, for example interferon (IFN) γ, IL-17 (secukinumab), IL-12/23 receptor (ustekinumab), novel agents designed to specifically target autoinflammatory pathways and repurposed old drugs.

42.2 Corticosteroids

- **The only indication for corticosteroids in familial Mediterranean fever (FMF) is prolonged febrile myalgia**
- **In other autoinflammatory diseases short-term corticosteroids can be effective in terminating acute inflammatory attacks**
- **Long-term use of corticosteroids should be avoided due to the high risk of serious adverse effects**

Corticosteroids are among the most widely used anti-inflammatory drugs and 1% of the population of the United States of America (USA) is estimated to be taking them at any time [1]. Their ability to down regulate expression of multiple genes involved in inflammatory pathways (including those encoding cytokines and chemokines, their receptors, adhesion molecules, inflammatory enzymes) have proved useful in autoinflammatory disorders, including many such as gout, chronic non-bacterial osteomyelitis (CNO), systemic juvenile idiopathic arthritis and Behçet disease which are discussed elsewhere in this book (see Chaps. 31, 32, 34, 35). Corticosteroids have been given as both short-term and, more problematically, long-term therapy and via oral and parenteral routes [2].

42.2.1 Mechanisms of Action

The anti-inflammatory effect of cortisol was first recognised by Hench in patients with coincidental Cushing syndrome and rheumatoid arthritis in 1949 [3]. The mechanisms of action of corticosteroids are complex, wide-ranging and remain incompletely understood. They undoubtedly affect gene expression; corticosteroids are taken up intracellularly and bind to cytoplasmic receptors. The resultant receptor-steroid complex migrates into the nucleus, binds to DNA and over a period of a few hours to a day or so alters protein synthesis. Many of these effects are mediated by transrepression with inhibition of the effect of proinflammatory transcription factors such as nuclear factor kappa B (NF-κB) or by reducing or reversal of deacetylation of acetylated histones [4]. Other effects result from upregulation of genes with anti-inflammatory functions such as mitogen-activated protein kinase phosphatase-1, IL-10, and corticosteroid-induced leucine zipper, an inhibitor of NF-κB. In addition corticosteroids can have non-genomic actions which may be apparent more rapidly. These are thought to be mediated by membrane-coupled receptors and have been reported to inhibit neutrophil degranulation and appear to contribute to some of their neuropsychiatric side effects [5].

Table 42.1 Significant adverse effects associated with long-term corticosteroid therapy

Skin Thinning and striae Purpura Acne Hirsutism Increased risk of squamous cell and basal cell carcinoma Cushingoid appearance: Moon face, buffalo hump, truncal weight gain	**Immune response** Increased risk of infection, particularly atypical or opportunistic Increased risk of herpes zoster reactivation Reduced response to vaccination
Ocular Posterior cataracts Glaucoma	**Renal** Fluid retention Hypertension Hypokalaemia
Metabolic Insulin resistance Abnormal lipid metabolism Central obesity Accelerated atherosclerosis and increased risk of cardiovascular events	**Central nervous system** Hypomanic symptoms Depressive symptoms Akathisia Insomnia Psychosis (at high doses)
Musculoskeletal Myopathy - typically proximal Osteoporosis Osteonecrosis	Growth retardation (in children)
Gastrointestinal Gastritis, peptic ulcers and bleeding Visceral perforation	**Endocrine** Diabetes mellitus Suppression of hypothalamic-pituitary-adrenal axis

42.2.2 Adverse Effects

Unfortunately, long-term use of systemic corticosteroids carries a serious risk of significant, poorly reversible adverse effects which limit their use particularly at doses of more than 10 mg/day in adults and for more than a few months [6, 7]. These range from suppression of the hypothalamic-pituitary-adrenal axis leading to adrenal atrophy, to an increased risk of cardiovascular events, metabolic syndrome with weight gain, abnormal glucose tolerance and development of diabetes, infections, gastrointestinal bleeding and perforation, myopathy, osteoporosis and cataract formation (Table 42.1). Some of the metabolic consequences are thought to be due to transactivation of a variety of genes involved in carbohydrate, lipid and protein metabolism following binding to corticosteroid response elements whereas the increased risk of infection seems to be due to transrepression, and osteoporosis to a combination of both, resulting in decreased transcription of osteocalcin and increased osteoblast apoptosis. Genetic polymorphisms in pathways involving steroid metabolism, steroid receptors, and transport proteins have been suggested to have an important role in the development of corticosteroid-induced adverse effects.

Attempts to define the incidence and burden of corticosteroid adverse effects have been limited perhaps because the adverse profile of an affordable group of drugs, which have been in

widespread use for many decades, has become too broadly accepted [8]. A Glucocorticoid Toxicity Index (GTI) has recently been developed by a group of clinicians representing multiple specialities including pediatricians and adult physicians utilising multi-criteria decision analysis [9]. This generates a weighted score from a series of domains (body mass index, glucose tolerance, blood pressure, lipids, bone density, steroid myopathy, skin, neuropsychiatric problems, infection, endocrine, gastrointestinal, musculoskeletal and ocular toxicity) which were felt not to be confounded by comorbidity or life style, to assess the burden of corticosteroid adverse effects and changes over time.

42.2.3 Use of Corticosteroids in Familial Mediterranean Fever (FMF)

The only widely accepted use of corticosteroids in FMF is for the treatment of protracted febrile myalgia (see Chap. 16). This rare complication of FMF was first described in 1994 and seems more common in patients homozygous for the severe M694V mutation. It presents as a prolonged (4–6 weeks) episode of muscle pain affecting the limbs, without rhabdomyolysis, and a marked systemic inflammatory response. Treatment is usually with non-steroidal anti-inflammatory drugs (NSAIDs) or oral corticosteroids [10] and a comparative study suggested equal efficacy [11]. A 2017 study reported a rapid response to intravenous pulse corticosteroids (methylprednisolone) at a dose of 10 mg/kg followed by oral corticosteroids tapered over 6 weeks [12].

42.2.4 Use of Corticosteroids in TNF-Receptor Associated Periodic Fever Syndrome (TRAPS)

Corticosteroids are widely used to terminate acute attacks of TRAPS (see Chap. 18) but data come from small retrospective studies only [13–15]. Treatment is generally reported to be effective in terminating attacks but episodic treatment does not reduce the frequency of attacks. Some centres have reported using intravenous methylprednisolone, but this is not widespread [14]. Long-term corticosteroids have been used in patients refractory to other treatment but are associated with severe adverse effects. Data from the Eurofevers/Eurotraps registry demonstrate that corticosteroids were completely effective in terminating acute attacks in 42% of 48 cases and partially effective in a further 52% [16]. Most patients require doses of between 0.5 and 1 mg/kg/day to control symptoms. Intermittent corticosteroids do not prevent subsequent symptomatic attacks, nor subclinical inflammation between attacks. Almost 80% of patients initially treated with corticosteroids were converted to maintenance therapy with a specific cytokine blocking therapy.

Consensus recommendations for the management of autoinflammatory diseases were developed as part of the European project Single Hub and Access point for Paediatric Rheumatology in Europe (SHARE) and published in 2015 [17]. These recognise that short-term corticosteroids, with or without NSAIDs, are effective for terminating inflammatory attacks but that their beneficial effect can decline over time so that increasing doses are required to achieve an equivalent response.

Our practice is to use short, less than 2 week, courses of prednisone at doses of 0.5–1 mg/kg/day to manage intermittent acute attacks with a rapid reduction in dosage as symptoms remit. Long-term maintenance corticosteroids are not used due to the high risk of adverse effects. Prompt conversion to biologic therapy is indicated when disease is refractory to corticosteroids; or relapses rapidly after corticosteroid withdrawal; or requires frequent doses of corticosteroids with a cumulative dose approaching 7 mg/day/annum in adults; or exhibits subclinical biochemical evidence of inflammation between attacks.

42.2.5 Use of Corticosteroids in Mevalonate Kinase Deficiency (MKD)

Corticosteroids are used in acute attacks of mevalonate kinase deficiency (MKD) in a very similar way to their use in TRAPS (see Chap. 17). In the

Eurofever registry, corticosteroids were used in 49 of 114 (43%) patients to treat fever attacks. Complete suppression of inflammatory episodes was reported for 19 (39%) (16 of whom had not received biologics), and some improvement was reported for 21 (43%) patients. Five of seven patients who received maintenance corticosteroids experienced some benefit [16]. Other older series also suggest the response to corticosteroids are often disappointing in the longer term [18].

42.2.6 Use of Corticosteroids in Idiopathic Recurrent Pericarditis

Corticosteroids are often used as symptom control and to shorten attacks of idiopathic recurrent pericarditis but there has been longstanding concern that they increase the risk of recurrent attacks [19] (see Chap. 36). The results of colchicine trials in prophylaxis of recurrent pericarditis support this concern [20]. Current advice is that corticosteroids should be used only after failure of aspirin/NSAID and colchicine [21]. Prednisolone doses of 0.25–0.5 mg/kg/day for 4 weeks followed by slow tapering have been recommended. Recurrences are common below doses of 10–15 mg/day and are difficult to manage. In patients requiring unacceptably prolonged or high doses of corticosteroids prompt introduction of alternative immunosuppressive or anti-cytokine therapy should be considered [21].

42.2.7 Use of Corticosteroids in Periodic Fever, Aphthous Stomatitis, Pharyngitis, Cervical Adenitis (PFAPA) Syndrome

A diagnostic feature of periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome is the dramatic and rapid response to a single dose of oral corticosteroids [22] (see Chap. 30). Prednisolone doses of 0.5–2 mg/kg have been used with similar efficacy [23]. A rapid resolution of fever, within 12–24 h, has been reported in 63% of cases and only 5% show no response [24]. The major downside of using corticosteroids in PFAPA is that they do not prevent further attacks and the interval between episodes may be shortened in 25–50% of cases. If corticosteroids are used very frequently there is a risk of cushingoid side effects, otherwise adverse effects are rare; restlessness is the most commonly reported [25].

42.2.8 Use of Corticosteroids in Deficiency of Adenosine Deaminase 2 (DADA2)

Treatment of deficiency of adenosine deaminase 2 (DADA2) has so far only been reported in retrospective case series (see Chap. 23). High dose corticosteroids appear highly effective at controlling disease activity in DADA2 in the short term. However, in a multicentre series, all patients with chronic disease relapsed as the dose of corticosteroids was reduced. Indeed, if used as monotherapy, corticosteroid tapering carries a risk of severe complications such as cerebrovascular accident and intussusception [26].

42.3 Anti-Tumor Necrosis Factor (TNF) Agents

- **In systemic autoinflammatory diseases etanercept and anti-TNF antibodies may have different effects**
- **Etanercept is beneficial in some cases of TRAPS whereas anti-TNF monoclonal antibodies should be avoided**
- **In Blau syndrome response to TNF blockade appears variable but anti-TNF antibodies may be a better choice of agents than etanercept**
- **Anti-TNF agents are widely used to treat DADA2**

Tumor necrosis factor (TNF)-α is a cytokine with pleiotropic effects including both proinflammatory and immune regulatory functions. Its activities are mediated via interaction with two distinct cell surface receptors, p55/TNFRI and p75/TNFRII with separate signal transduction pathways. The receptors are expressed on the majority of cells and

the binding of both the membrane-bound and soluble forms of TNF (sTNF) results in inflammatory responses. These may be cell type specific including enhanced cytotoxicity, T-cell and macrophage migration, granuloma formation, IL-10 production, B cell proliferation and immunoglobulin production, T cell HLA-DR and CD25 expression and granulocyte-macrophage colony-stimulating factor (GM-CSF) production [27].

There are currently five anti-TNF agents labelled for use in humans: etanercept (and biosimilars), infliximab (and biosimilars), adalimumab (and biosimilars), golimumab and certolizumab (Table 42.2). Although all five agents target TNF, etanercept is a receptor analogue and the others are monoclonal antibodies. Etanercept appears to induce less T cell apoptosis than infliximab but inhibits signalling by lymphotoxin-α. Lymphotoxin-α is a key cytokine in the regulation of the mucosal immune system and its inhibition may contribute to the relative lack of effect of etanercept in inflammatory bowel disease. Differences between agents certainly seems to have clinical significance in TRAPS. The adverse event profile also varies subtly particularly with respect to the risk of developing tuberculosis. The lower (although real) risk with etanercept may reflect lesser inhi-

Table 42.2 Comparison of labelled anti-tumor necrosis factor (TNF) agents

	Etanercept (ETA)	Infliximab (INF)	Adalimumab (ADA)	Golimumab (GOL)	Certolizumab (CER)
Structure	Human fusion protein of two TNFR2 receptor extracellular domains and the Fc portion of human IgG	Mouse-human chimeric monoclonal antibody	Fully human monoclonal antibody	Fully human monoclonal antibody	Humanised PEGylated Fab′ antibody fragment
Target	Trimeric soluble TNF-α, transmembrane TNF-α	Monomeric and trimeric soluble TNF-α, transmembrane TNF-α	Monomeric and trimeric soluble TNF-α, transmembrane TNF-α	Monomeric and trimeric soluble TNF-α, transmembrane TNF-α	Monomeric and trimeric soluble TNF-α, transmembrane TNF-α
Route of administration	Subcutaneous	Intravenous	Subcutaneous	Subcutaneous or intravenous	Subcutaneous
Standard maintenance dose in adults	50 mg	5 mg/kg	40 mg	50 mg	200 mg
Dose frequency	Weekly	Every 4–8 weeks	Every 2 weeks	Monthly	Every 2 weeks
Half-life (days)	3	9.5	14	12	14
Labelled indications	Rheumatoid arthritis, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis, polyarthritis juvenile idiopathic arthritis	Crohn disease, ulcerative colitis, rheumatoid arthritis, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis	Rheumatoid arthritis, polyarthritis juvenile idiopathic arthritis, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis, Crohn disease, ulcerative colitis, hydradenitis supperative, non-infectious intermediate, posterior and panuveitis	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, ulcerative colitis	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis

bition of T cell activation and CD4+ cells than infliximab or adalimumab. Malignancies have been a theoretical concern, although recent data, at least in adults with rheumatoid arthritis and children with chronic inflammatory diseases, including juvenile idiopathic arthritis are very reassuring [28, 29]. Other adverse effects include demyelinating syndromes, worsening of congestive cardiac failure, immunogenicity, infusion/injection reactions and hypersensitivity, hepatotoxicity and hematological disorders.

42.3.1 Use of Anti-TNF Agents in Familial Mediterranean Fever (FMF)

In general anti-TNF agents have proved disappointing in FMF. The exception is the association between FMF and both ankylosing spondylitis and sacroiliitis (see Chap. 16). In these patients, there is evidence that effective treatment of the arthritis with anti-TNF therapies may improve FMF disease control [30].

42.3.2 Use of Anti-TNF Agents in Tumor Necrosis Factor Receptor Associated Periodic Syndrome (TRAPS)

The experience of anti-TNF agents in TRAPS has generally been of limited benefit and its use has been almost entirely superseded by far more effective anti-IL-1 agents (see Chap. 18). TNF antibodies are contraindicated as they have been reported to worsen disease. A possible molecular mechanism for this phenomena has been proposed, wherein failure to shed infliximab-bound TNF/TNFRI from the cell surface triggers enhanced anti apoptotic c-Rel activation, and increased secretion of pro-inflammatory cytokines [31].

The soluble TNF receptor fusion protein, etanercept, was widely used in TRAPS. Multiple case reports in the early 2000s had suggested that more than 80% of patients had a response to initial treatment, of whom about 25% had a very good clinical response. A weakness of this literature was that there was very little data on using etanercept for more than a few months and loss of efficacy, potentially complicated by the development of AA amyloidosis, seemed common. An open-label study of etanercept in 15 patients reported a partial response over 14 weeks in terms of symptoms and to a lesser extent a decrease in acute phase reactants. Long-term follow-up showed a median duration of etanercept treatment of 3.3 years, which is very short for a life-long disease, with the most common reason for discontinuation being lack of efficacy. Etanercept usage was not associated with a reduction in NSAID or corticosteroid use [32].

42.3.3 Use of Anti-TNF Agents in Mevalonate Kinase Deficiency (MKD)

As with FMF and TRAPS, blockade of TNF has proved partially effective at best. In the largest published series of 114 patients, 27 received etanercept with disappointing efficacy. Only 2 (7%) had a complete response and there was no response in 11 patients (41%) [33].

42.3.4 Use of Anti-TNF Agents in Blau Syndrome

The evidence base for the treatment of Blau syndrome is extremely scanty (see Chap. 20). Although there are case reports of benefit from anti-TNF antibodies, particularly with respect to visceral granulomatous involvement, this is not clear cut in larger series [34–36]. Treatment of Blau-associated uveitis has recently been reported in 38 patients Thirty-seven (97%) were treated with systemic medication, with 68% receiving a combination of systemic corticosteroids with immunosuppressive drugs and/or biologics. Within these combinations, systemic corticosteroids were the most commonly used (68%), followed by methotrexate (47%), adalimumab (45%), infliximab (13%), mycophenolate mofetil (8%), thalidomide (5%), and canakinumab (3%). Choice of immunosuppressants varied among the participating centers, and therefore efficacy of individual agents was difficult to ascertain, but the trend over follow-up remains of cumulative worsening of visual function [37].

42.3.5 Use of Anti-TNF Agents in Deficiency of Adenosine Deaminase 2 (DADA2)

TNF blockade is the current treatment of choice in patients at risk of severe complications of DADA2 such as stroke or peripheral vascular complications. In a recently published Italian series, nine of the ten patients who received anti-TNF treatment with etanercept achieved a complete remission with a median treatment duration of 3.9 years (range 0.9–13 years); only one patient was still corticosteroid dependent [26]. In a United Kingdom (UK) series excellent responses were also seen with the anti-TNF antibodies infliximab and adalimumab [38], suggesting that the benefits of TNF blockade are not dependent on the type of agent used.

42.3.6 Use of Anti-TNF Agents in Chronic Non-Bacterial Osteomyelitis (CNO)

There are no randomized-controlled trials of treatment in chronic non-basterial osteomyelitis (CNO). TNF inhibitors have been used and there are just over 50 reports in the literature, generally in refractory disease, including the use of infliximab, adalimumab and etanercept. The majority of case reports describe benefit although the data are retrospective and follow-up generally short [39]. Consensus treatment plans were proposed in 2017 for the first 12 months of therapy for patients who have proved refractory to NSAID monotherapy and/or with active spinal lesions. One of the three options includes the use of anti-TNF agents, either alone or in combination with methotrexate [40].

42.3.7 Use of Anti-TNF Agents in Pyogenic Arthritis, Pyoderma Gangrenosum, and Acne (PAPA) Syndrome

There has been almost no systematic assessment of treatment efficacy in this exceptionally rare disease. Therapies targeting TNF-α including etanercept, adalimumab and infliximab have been used and case reports suggest some benefits in controlling skin and joint disease manifestations [41].

42.4 Anti-Interleukin (IL)-6 Agents

- **Experience of IL-6 blockade is largely in patients who have proved refractory to other agents**
- **IL-6 blockade can be effective and may be most useful in patients with AA amyloidosis**

IL-6 is a pleiotropic cytokine that is secreted by a range of cells including neutrophils, monocytes/macrophages, endothelial cells, fibroblasts and T-cells and plays a major role in systemic inflammation, immunity, reproduction, hematopoiesis, neural development and bone metabolism. It stimulates a number of cellular responses including proliferation, differentiation (particularly of T-cells, B-cells and neurons), survival, and apoptosis and the hepatocyte acute-phase reaction, including raised levels of C-reactive protein (CRP) and serum amyloid A (SAA) protein.

IL-6 binds to a specific receptor on target cells. The IL-6/IL6-R complex results in signal transduction by activating a homodimer of the ubiquitously expressed signal-transducing β-receptor gp130. Ligand binding results in Janus kinase (JAK), particularly JAK1, activation and phosphorylation of tyrosine residues in the intracellular portion of receptor, thereby recruiting signal transducer and activator of transcription (STAT) molecules, which are also phosphorylated by JAKs. Phosphorylated STAT translocates into the nucleus activating gene transcription (Fig. 42.1).

Regulation of IL-6 is complex and includes mechanisms to reduce cytokine signalling. One of the main negative feedback regulators of the IL-6 signalling axis is suppressor of cytokine signalling (SOCS) 3, which itself is a STAT3 target gene. SOCS3 binding to the cytoplasmic portion of gp130 and JAK, results in recruitment of an ubiquitin ligase complex and their degradation. In addition, soluble IL-6R and soluble gp130 are

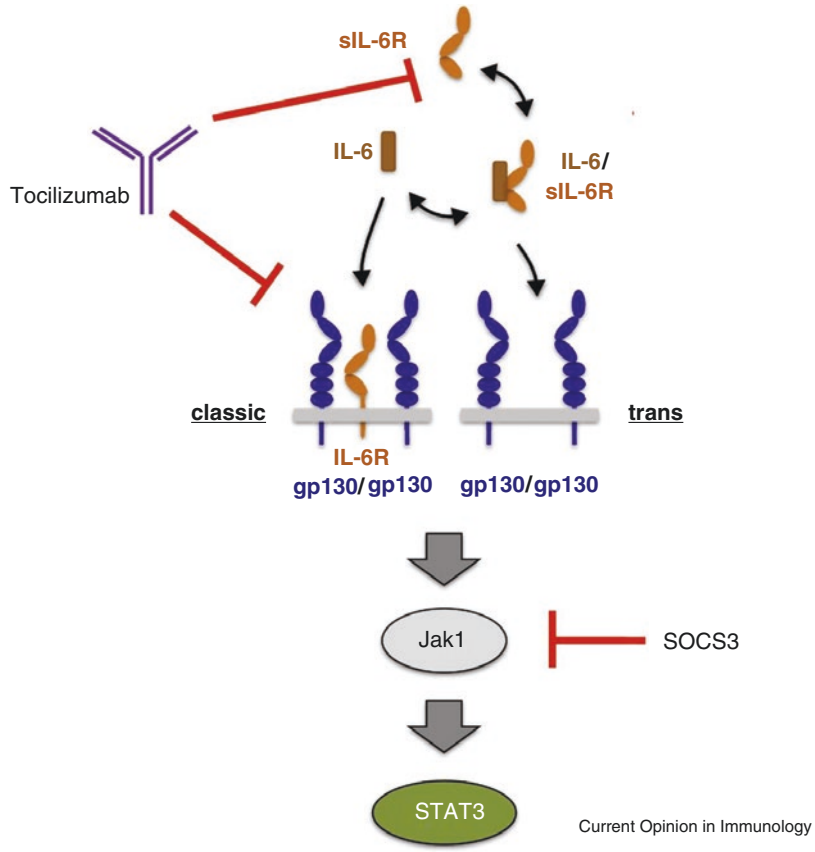

Fig. 42.1 Interleukin (IL)-6 classic and trans-signalling. IL-6 can either signal via the membrane-bound receptor (classic signalling), or in complex with the soluble IL-6 receptor (trans-signalling). Both pathways induce homodimerization of gp130 leading to the activation of intracellular signalling pathways, in particular phosphorylation of signal transducer and activator of transcription (STAT)3 via the tyrosine kinase Janus kinase (JAK)1. Negative feedback inhibition is achieved through induced expression of suppressor of cytokine signalling (SOCS3). Reproduced with permission from Garbers et al. Curr Opin Immunol 2015 [42]

present in plasma at low levels. Circulating IL-6 can bind to soluble receptor with an affinity of approximately 1 nM; this is dramatically increased by recruitment of sgp130 with the complex having a binding affinity of 10 pM. As the IL-6/sIL-6/sgp130 complex acts as an endogenous antagonist of cytokine activity it acts as an important buffer during inflammatory episodes [42].

IL-6 signal transduction can occur via membrane bound or soluble IL-6R, the former known as classic and the latter as trans-signalling. Binding of IL-6 to its membrane bound receptor appears to promote generally desirable anti-inflammatory outcomes such as healing, tissue regeneration and defence against bacterial infection. In contrast, the less desirable pro-inflammatory actions of IL-6 appear to be largely mediated via trans-signalling suggesting that its specific blockade might be beneficial. Olamkicept, a recombinant IL-6 soluble receptor antagonist, is currently in clinical trials in inflammatory bowel disease [42].

There are currently three anti-IL-6 agents labelled for use in humans: tocilizumab, sarilumab and siltuximab (Table 42.3). Tocilizumab is currently labelled by the USA Food and Drug Administration (FDA) for the treatment of: rheumatoid arthritis; systemic juvenile idiopathic arthritis; polyarthritis juvenile idiopathic arthritis; giant cell arteritis; and chimeric antigen receptors (CAR) T cell-induced (used for treatment of hematologic malignancies) severe or life-threatening cytokine release syndrome. Sarilumab is labelled at present only for the treatment of rheumatoid arthritis and siltuximab for the treatment of multicentric non-viral associated Castleman disease. The adverse event profile associated with IL-6 blocking agents includes hypersensitivity, infections including hepatitis, malignancies notably lymphoma, changes of blood counts, lipid profile, gastrointestinal perforations, deranged liver function with elevated transaminases and cardiovascular events [43, 44].

Table 42.3 Comparison of labelled anti-interleukin (IL-6) agents

	Tocilizumab	Sarilumab	Siltuximab
Structure	Recombinant humanized monoclonal antibody	Fully human IgG1 monoclonal antibody	Chimeric human-murine monoclonal antibody
Target	Both soluble and membrane bound IL-6 receptor	Both soluble and membrane-bound IL-6Rα	Both soluble and membrane bound IL-6 receptor
Route of administration	Subcutaneous	Subcutaneous	Intravenous
Standard maintenance dose in adults	162 mg	200 mg	11 mg/kg
Dose frequency for adults	Weekly	Every 2 weeks	Every 3 weeks
Half-life (days)	1.6	Initially 8–10 In steady state 21	21
Labelled indications	Systemic and polyarthritis juvenile idiopathic arthritis[a], rheumatoid arthritis, giant cell arteritis, treatment of CAR- T cell-induced cytokine release syndrome	Rheumatoid arthritis	Multicentric Castleman disease

Ig immunoglobulin; *IL* interleukin; *CAR* chimeric antigen receptors
[a]Dose for systemic juvenile idiopathic arthritis in children is 12 mg/kg intravenous administration every 2 weeks in children <30 kg and 8 mg/kg in heavier children; dose for polyarthritis juvenile idiopathic arthritis is 10 mg/kg intravenous every 4 weeks in children <30 kg and 8 mg/kg in heavier children

42.4.1 Use of Tocilizumab in Familial Mediterranean Fever (FMF)

Tocilizumab was reported to be effective in a Japanese patient with colchicine-resistant FMF complicated by recurrent fasciitis and myositis [45] and in number of cases of FMF complicated by AA amyloidosis (see Sect. 42.4.4).

42.4.2 Use of Tocilizumab in Tumor Necrosis Factor Receptor Associated Periodic Syndrome (TRAPS)

One patient with a cysteine mutation who had failed to respond to etanercept and had a partial response to anakinra (recombinant IL-1 receptor antagonist), complicated by the development of neutropenia, was treated with tocilizumab with a good clinical and biochemical response, sustained over 6 months of follow up [46].

42.4.3 Use of Tocilizumab in Mevalonate Kinase Deficiency (MKD)

MKD is often difficult to treat with disappointing responses. Although current data suggest IL-1 blockade is probably the most effective current therapy, there are several reported cases describing the use of tocilizumab in treatment refractory cases with encouraging responses [47–50] (Table 42.4).

42.4.4 Use of Tocilizumab in AA Amyloidosis

Tocilizumab has been reported to be effective in patients with AA amyloidosis complicating FMF, MKD and uncharacterised autoinflammatory diseases. Many of these patients had long-standing severe disease resistant to conventional therapies. In AA amyloidosis the major aim of treat-

Table 42.4 Summary of case report use of tocilizumab in mevalonate kinase deficiency (MKD)

	Shendi et al. [47] (n = 1)	Stoffels et al. [48] (n = 2)		Lane et al. [49] (n = 2)		Muster et al. [50]
Patient	**1**	**2**	**3**	**4**	**5**	**6**
Tocilizumab dose (mg/kg) and route of administration	7 IV	8 IV	8 IV	8 IV	8 IV	8 IV
Frequency of administration (weeks)	4	4	4	4	4	4
Age at onset of treatment (years)	13	Not described	Not described	24	13	36
Treatment prior to tocilizumab	Colchicine, prednisolone, etanercept, anakinra	Anakinra	Anakinra	Anakinra, etanercept	Etanercept	NSAID, simvastatin, anakinra
Duration of treatment (months)	20	5	5	24	13	48–60
Clinical outcome	CR	CR	CR	CR		PR
Serologic outcome	CR	Not described	PR	CR		PR
Adverse events	URI	Not described	Not described	Not described		Not described
Comments	CR at dose of 8 mg/kg but due to AE dose halved; subsequently increased inflammatory markers Stable on 7 mg/kg			MKD complicated by AA amyloidosis; remained on therapy with prednisolone 0.5 mg/day	Stabilized on monotherapy	After starting hospital admissions dropped 11/year to 3/year Given in combination with IV methylprednisolone first 3 years then as monotherapy

IV intravenous; *NSAID* non-steroidal anti-inflammatory drug; *CR* complete response; *PR* partial response; *URI* upper respiratory infection; *AE* adverse events

ment is to reduce the production of the hepatic acute phase response protein SAA, which is the amyloid fibril protein [51]. IL-6 is a major stimulant of the hepatic acute phase response so its use in AA amyloidosis is rational. Responses have been generally encouraging and suggest that effective treatment results in improvement of clinical symptoms as well as biochemical inflammation.

Ugurlu et al., reported a series of 12 Turkish patients with FMF complicated by AA amyloidosis. Four patients had coexistent ankylosing spondylitis and one had Crohn disease. Tocilizumab was given in addition to colchicine and was well tolerated. Treatment resulted in a biochemical response in all cases with decreased proteinuria without loss of renal function during follow-up. Ten patients had no clinical FMF attacks on treatment, one had episodes of erysipelas like erythema and one reported an amelioration in the attack frequency [52]. Lane et al. reported a series of 14 patients with AA amyloidosis treated with tocilizumab, of whom one had MKD, one

unclassified systemic autoinflammatory disease and one with Castleman disease. The remaining cases included 7 patients with refractory rheumatoid arthritis and 4 with systemic juvenile idiopathic arthritis. Tocilizumab effectively reduced the acute phase response in all cases with symptomatic benefit and improved quality of life measures. The series included four patents with renal transplants and two on dialysis; even in these immune compromised patients' treatment was well tolerated. Adverse events included respiratory and urinary tract infections, post-transplant Epstein Barr virus viremia, transient neutropenia and abnormal liver function tests. No patients discontinued treatment permanently as a result of adverse effects. There was evidence of amyloid regression on imaging in nine cases and no patient had worsening disease. No patients progressed to renal failure and proteinuria improved in all assessable cases [49].

42.5 Janus Kinase (JAK) Inhibitors

- **Janus kinase (JAK) inhibitors are the first agents to show benefit in the interferonopathies, although as yet there are very few published data**
- **The major adverse effect concern at present is the risk of viral reactivation, especially herpes zoster**

The JAKs are cytoplasmic protein tyrosine kinases that play a critical role in cellular response to cytokines including type I cellular immune responses and type II, antibody mediated responses. A simplified summary of the role of the JAK-STAT pathway is that binding of a cytokine induces dimerization of its cognate receptor (Fig. 42.2). This activates JAK resulting in reversible phosphorylation of the receptor dimer. STAT binds to the phosphorylated receptor and in turn is phosphorylated by JAK. The resultant dimer of

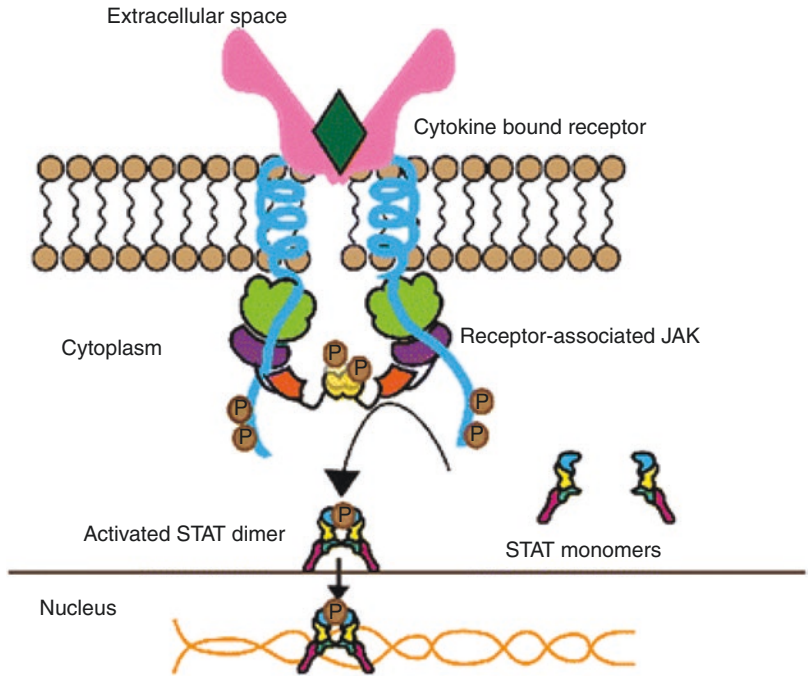

Fig. 42.2 Cytokine signalling through the Janus kinase (JAK)-signal transduction and activation of transcription (JAK/signal transducer and activator of transcription-STAT) pathway. Binding of a cytokine to the receptor leads to activation and phosphorylation of JAK and phosphorylation of the receptor. This in turn leads to phosphorylation and dimerization of STAT. Activated STAT dimer migrates to the nucleus and binds to specific DNA-binding sites regulating gene transcription. This culminates in alteration of cellular function. Reproduced with permission from Banerjee et al. Drugs 2017 [53]

phosphorylated STAT translocate to the nucleus thereby activating gene transcription [53]. Mutations in JAK-STAT pathways have been implicated in a variety of diseases including severe combined immunodeficiency, rheumatoid arthritis, systemic lupus erythematosus, Crohn disease and Behçet disease [54].

There are four JAKs: JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2). They are each associated with specific cytokine receptors and STATs (Table 42.5).

JAK inhibitors are conventional small molecule pharmaceuticals which are orally active. The currently labelled drugs are summarised in Table 42.6. Most of the safety data derive from tofacitinib studies and preliminary data suggest that although the agents have different selectivity for JAK types the side effect profile is broadly similar. All these agents carry a risk of inducing cytopenia, dyslipidemia and abnormal liver function tests. The major adverse events reported to date are infections. Bacterial infections including pneumonia, urinary tract infections and soft tissue infections occur at a rate broadly similar to that seen with use of biologics in rheumatoid arthritis. It seems likely this is the case for tuberculosis too. In contrast, use of JAK inhibitors has

Table 42.5 Janus kinases (JAKs) and signal transducers of activation (STATs) with associated cytokines

JAK	Major role in cytokine signalling	STAT
JAK1	IL-2, IL-4, IL-6, IL-7, IL-9, IL-10, IL-13, IL-15, INF-α/β, NF-γ, IL-21	STAT 1, 2, 3, 5, 6
JAK2	IL-3, IL-5, IL-6, IL-12, IL-13, IL-19, IL-23, GM-CSF, G-CSF, GH, erythropoietin, INF-γ	STAT 1, 3, 4, 5, 6
JAK3	IL-2, IL-4, IL-7, IL-9, IL-15, IL-21	STAT 3, 5, 6
TYK2	IFNα/β, IFNγ, IL-6, IL-12, IL-23	STAT 1, 2, 3, 4, 6

TYK tyrosine kinase; *IL* interleukin; *INF* interferon; *NF* nuclear factor; *GM-CSF* granulocyte-macrophage colony-stimulating factor; *G-CSF* granulocyte colony-stimulating factor; *GH* growth hormone

Table 42.6 Comparison of currently labelled Janus kinase (JAK) inhibitors

	Tofacitinib	Baricitinib	Ruxolitinib
Major Target	JAK 1, JAK3, and JAK 2 to a lesser extent	JAK 1 and 2	JAK 1 and 2
Route of administration	Oral or topical	Oral or topical	Oral
Standard maintenance dose in adults	5–10 mg	4 mg	5–20 mg (depending on platelet count)
Dose frequency	Twice per day	Once per day	Twice per day
Labelled indications	Rheumatoid arthritis as monotherapy or combined with methotrexate	Rheumatoid arthritis as monotherapy or combined with methotrexate[a]	Disease related splenomegaly in myelofibrosis
Clinical trials	Psoriasis and psoriatic arthritis, ulcerative colitis, vitiligo, alopecia areata, systemic juvenile idiopathic arthritis	Psoriasis, diabetic nephropathy, systemic lupus erythematosus, atopic dermatitis	
Major adverse events	Abdominal pain/gastritis; nasopharyngitis; anemia; dyspnea/cough; diarrhea; dyslipidemia; headache; hypertension; insomnia; leukopenia; peripheral edema; pyrexia; raised liver enzymes; rash; vomiting; weight gain; infection (including serious bacterial, fungal, viral and mycobacterial infection); lymphopenia; neutropenia	Gastroenteritis; herpes simplex; herpes zoster; BK viremia; dyslipidemia; thrombocytosis; venous thrombosis; upper respiratory tract infection; urinary tract infection; acne; neutropenia; weight gain	Anemia; thrombocytopenia; neutropenia; bleeding and weight gain

[a]Approved for rheumatoid arthritis by the United States of America Food and Drug Administration at a 2 mg/day dose

a much higher risk of viral infections, particularly reactivation of herpes zoster. The risk in rheumatoid patients seems to be potentiated when taken together with corticosteroids and/or methotrexate [45]. To date there is no convincing evidence of an increased risk of malignancy but given the small number of exposed patients and relative short length of follow up subtle alteration in risk would not be expected to have become apparent as yet [54]. Inhibitors targeting JAK2 carry a risk of resistance to growth hormone, which is potentially clinically significant in children and perhaps venous thrombotic events (at higher doses). Certainly a postulated cause of short stature in chronic renal failure is impaired phosphorylation of JAK2/STAT5b after growth hormone stimulation [55].

42.5.1 Use of Janus Kinase (JAK) Inhibitors in Interferonopathies

Evidence of a chronically elevated IFN response gene signature in chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) and STING-associated vasculopathy with onset in infancy (SAVI) patients suggested a pathologic role of increased IFN signalling. Although treatment is being used in individual patients very little data have been published. A compassionate use program in pediatric patients has been on-going at the USA National Institutes of Health since 2011. Preliminary results on 18 patients (10 with CANDLE, four with SAVI and four with presumed CANDLE-related conditions) treated with baricitinib for a mean 3.0 years were published in 2018 [56]. Half of the CANDLE patients were maintained in clinical remission and clinical features; inflammatory biomarkers and the interferon signature improved in patients with SAVI. Three patients discontinued treatment, two with genetically undefined disease for inefficacy and one with CANDLE for tubulointerstital nephritis and BK viremia. One patient developed herpes zoster. BK viremia developed in 44% of patients with low, stable viral titres on continued treatment. These patients did not develop clinical sequelae and remain under observation. In a pharmacodynamics paper the authors suggested dosing regimens dependent on weight and renal function, with the starting dose of 4 mg twice daily in patients with >40 kg weight and normal renal function. This dosing was sufficient to decrease STAT phosphorylation in four cell populations and reduce downstream markers of INF signalling, as assessed by a 25-gene panel [57].

42.6 Future Developments in Therapeutics

- **There is an unmet need for treatment in many rare systemic autoinflammatory diseases**
- **Even where effective treatments exist biologics are prohibitively expensive for many health care systems and require parenteral administration**

42.6.1 Inflammasone Inhibitors

The increasing evidence that activation of nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain 3 (NLRP3) may play a role in the pathogenesis of common acquired diseases such as type 2 diabetes, atherosclerosis, obesity, gout, neurodegenerative diseases, fibrotic pulmonary diseases, liver and kidney disease and malignancy (see Chap. 39) has fuelled interest in developing small molecules as cost effective inhibitors of the inflammasome. One such agent, MCC950, has been shown to be a potent selective NLRP3 inhibitor in mice models of CAPS. The compound not only prevented activation of IL-1β by caspase 1 but also prevented maturation of IL-18 and pyroptosis. The compound was not disastrously immunosuppressive as it did not block the major antimicrobial inflammasomes NLRC4 and NLRP1, nor IL-1β maturation mediated by serine proteases and caspase-8 [58].

42.6.2 Repurposing of Drugs for Use in Systemic Autoinflammatory Diseases

Rare diseases disproportionately affect the young, often the very young, and are associated with poor outcomes. Currently there are few available therapeutic options; in fact, more than 95% of rare diseases have no labelled treatments. Consequently, there is intense pressure to find effective, affordable treatments. Repurposing of drugs is an appealing approach which carries less commercial risk, saving time and money by focussing on agents which have already met regulatory requirements and undergone post-market monitoring. In the past identification of novel indications relied on clinical acumen and serendipity; the recognition of colchicine as prophylactic treatment in FMF is a beautiful example of this (see Chaps. 16 and 40). Advances in computing which enable data mining from the academic literature, regulatory documentation, clinical trials and electronic health records combined with research tools such as high through-put "signalome" screening allows for rapid identification of and screening of potential therapeutics. Work using these approaches in TRAPS has suggested fluoroquinolone antibiotics may downregulate disease specific signalling pathways [59]. Similarly squalene synthase inhibitors, previously developed for the treatment of hyperlipidemia, are now being explored in the treatment of MKD [60].

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