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.

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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 keratoacan-thoma. (**f**) Squamo-proliferative keratoacanthoma on the heel with surface ulceration. (**g**) Left eye showing con-junctival and corneal scarring with hyperemia. (**h**) Obliterative corneal scarring and surrounding conjuncti-val 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 pal-mar 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 muta-tion in the homozygous state.

NAIAD combines dyskeratotic manifesta-tions as well as systemic autoinflammatory and autoimmune features [15]. The disease starts within the first months of life. Patients display follicular palmoplantar hyperkeratosis and dys-keratotic 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 neovasculari-sation 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 char-

acterized 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 inf-

liximab 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 gas-dermin-D (GSDMD) and resultant cytokine signaling and pyroptotic cell death. NLRC4 has some unique features, as well. NLRC4 can inter-act 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]
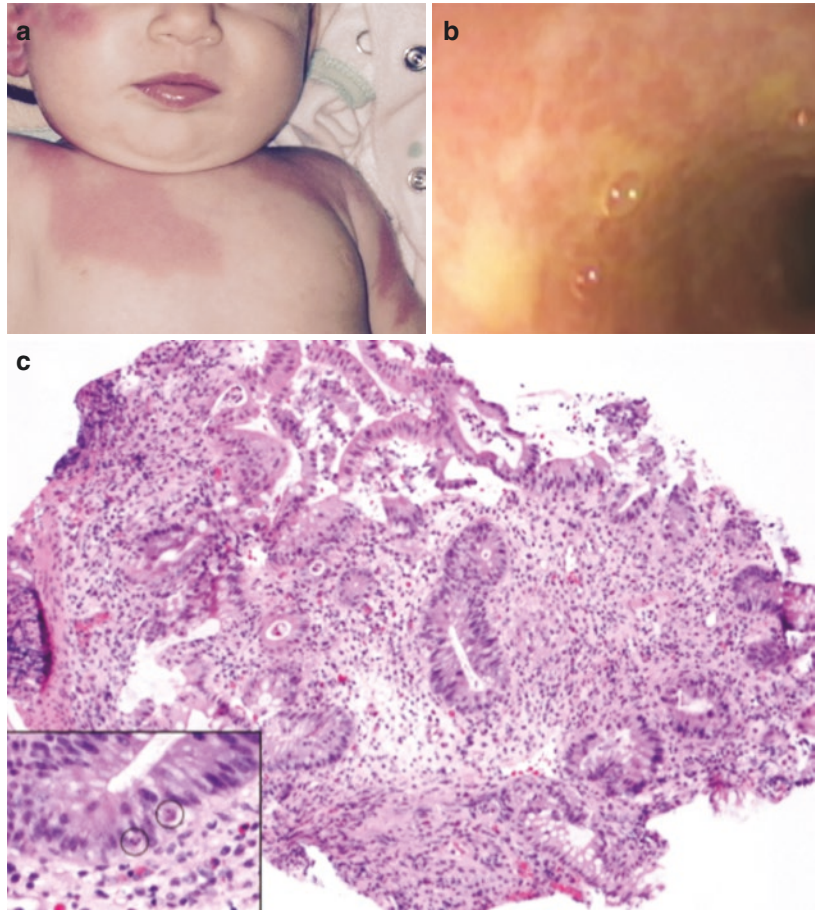
disease [36, 38]. Curiously, NLRC4-associated enterocolitis resolved in patients who survived beyond infancy. Some patients experienced prolonged periods of seemingly complete disease quiescence off therapy, only to have severe episodes of MAS triggered by infection or stress later in life [38]. Notably, 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 typi-cal 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 posttranslational 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 myco-

bacterial 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]].
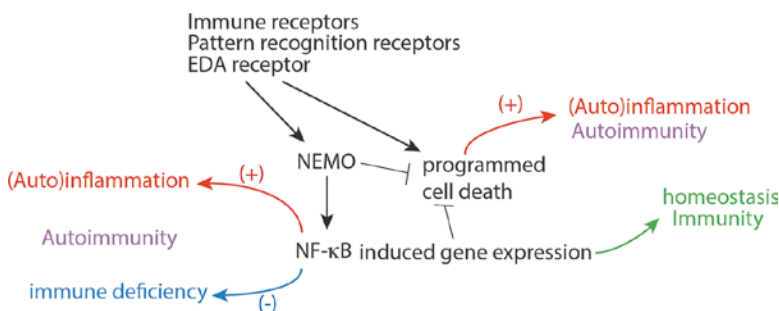
29.6.4 Pathogenesis

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

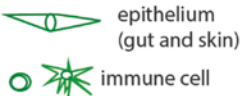Hypomorphic NEMO mutation leads to ectodermal dysplasia with anhidrosis and immunodeficiency (EDA-ID). EDA occurs to some degree in a majority of patients manifesting as defective eccrine sweat gland and hair follicle development with facies characterized by frontal bossing and periorbital hyperpigmentation 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 hypo-morphic 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 auto-antibody 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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