Rare Diseases of the Immune System
Series Editors: Lorenzo Emmi · Domenico Prisco

Mario Milco D'Elios
Cosima Tatiana Baldari
Francesco Annunziato *Editors*

Cellular Primary Immunodeficiencies

Foreword by: Donatella Lippi

Springer

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Dedicated with love to our children,

Alessandro, Bernardo, Emanuele, Sofia

Erica, Marco, Andrew

Lorenzo, and Mattia;

to all our patients with primary immunodeficiencies and their families whose continuous support has guided us over the years;

to our nurses; and to all our students!

Foreword

Primary immunodeficiencies (PIDs) are a large and growing group of over 350 different disorders, caused when some components of the immune system (mainly cells and proteins) do not work properly. This cold definition does not justify the whole complex of studies and researches behind this problem, nor of the relapses that involve the expectations of patients.

PIDs have a very short history as the role of the immune system has recently been discovered in its details. Historians of Medicine may trace back the history of Immunology to Edward Jenner's discovery that vaccination with cowpox protects against smallpox, but the observation that the same disease did not return a second time to a recovered individual had already been done in the 5th century BC by Thucydides.

It seems preposterous to collect occasional observations made by individual scholars in antiquity, long before the germ theory of disease and the theory that bacterial virulence could be attenuated by culture in vitro and used as vaccines was confirmed and disseminated among scientists and population. The true history of immunology belongs to the twentieth century.

Indeed, the formulation of Koch-Henle's postulates paved the way for modern immunology, which begins with the research of Metchnikoff, who discovered the phenomenon of phagocytosis in starfish and applied it to macrophages in humans.

The timeline of the history of immunology includes the names of Paul Ehrlich, Karl Landsteiner, Felix Haurowitz, Niels Jerne, and MacFarlane Burnett, but the path is still in progress and many challenges await those who deal with these issues.

Nowadays, scientists know that primary immunodeficiency diseases (PIDs) are a heterogeneous group of inherited disorders, with defects in one or more components of the immune system, characterized by increased incidence of infections, autoimmunity, and malignancies. PIDs were long considered rare diseases; it is not the case anymore today because their number is constantly growing; one of the major problems, however, is that the lack of awareness causes many related pathologies to be underestimated, generating a rise in morbidity and mortality rates as well.

PIDs are not contagious, as they are caused by hereditary or genetic defects, and although some disorders already reveal themselves at birth or in early childhood, they can affect anyone, regardless of age or gender; therefore, an early diagnosis of primary immunodeficiency diseases is fundamental to define their effects on medical management.

Some disorders concern a single part of the immune system while others may affect one or more of its mechanisms. Over 300 different forms of PIDs have already been identified and compared to previous studies; there has been a great deal of diagnostic and classification effort. PIDs may have different and articulated external manifestations, but they all share the same underlying problem; one or more functions of the immune system are not working as they should. The consequence of the failure of the immune system is that people suffering from PID are more exposed to infections, which can affect different organs of the body.

On the one hand, therefore, in this disorder group, medical treatment could really adhere to the idea of precision medicine, through the unveiling of the mechanisms that made it possible to isolate the various morbid entities; on the other hand, research has brought to overcoming the pathophysiological approach to address the treatment choice and, last but not least, the relationship with patients that represent the ultimate target of translational medicine. As a matter of fact, a main area where precision medicine joins immunological studies is in the exploration of immunotherapies to help providing proper treatments.

The life of patients affected by PIDs is thus a difficult life, always placed under the Sword of Damocles of the activation of an infection, which can cause a serious or severely disabling disease. Many patient associations have been founded in order to understand the problem from a scientific point of view, as non-professionals, to share experiences, and disseminate knowledge of resources or specialized centers. Luckily, with the appropriate medical care, many patients live full and independent lives.

This book collects the synergies that come from many areas of the scientific world, enhancing skills and giving space to a chorus of voices, a testimony to the effort made by medicine in this field. It is therefore not a point of arrival but a point of departure towards the full appreciation of the translational approach of a paradigm that favours the transfer of knowledge from the experimental laboratory to clinical practice.

These contributions to public health and well-being maximize decision-making, which is key to preserve a healthy population and guarantee the quality of life in this complex world of patients.

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Preface

Primary immunodeficiencies (PID) are rare diseases. PID constitute a rapidly expanding field. As of 2019 there are 430 distinct human inborn errors of immunity listed in the current International Union of Immunological Societies (IUIS) classification. The study of cellular primary immunodeficiencies has represented a very important model for the study of the functions of the human immune system. Within the last 20 years, hundreds of genetic defects underlying PID have been identified. Patients with cellular primary immunodeficiencies may have different clinical presentations, such as severe infections, neoplasias, and autoimmune diseases.

The clinical and molecular aspects of cellular PID are here described in detail, while the universe of Severe Combined Immunodeficiencies will be the focus of a dedicated work soon to be published in this same series. This volume provides an overview of systemic and mucosal immune responses, as well as those of the immune synapse, and describes how dysfunctional immune synapses can impinge on the pathogenesis of cellular primary immunodeficiencies.

The prevalence of PID in western countries is around 50 patients per 100,000 inhabitants. The awareness of PID has highly increased in the past years in the community thanks to the creation of international registries and associations devoted to PID, such as the European Societies for Immunodeficiencies (ESID), the Foundation for Primary Immunodeficiency (FPID), the International Patient Organisation for Primary Immunodeficiencies (IPOPI), the Latin America Society for Immunodeficiencies (LASID), the Australasian Society of Clinical Immunology and Allergy PID Register, the Immune Deficiencies Foundation Australia (IFSA), the Japanese Society for Immunodeficiency and Autoinflammatory Diseases (JSIAD), the Asia Pacific Society of Immunodeficiency (APSID), and the African Society For Immunodeficiencies (ASID), due to both patient advocacy and research discoveries.

Chapter 6 describes combined immunodeficiencies (CID), a heterogeneous and expanding group of primary immunodeficiencies, associated with T and B cell impaired immunity that are due to several genetic variants.

Chapter 7 focuses on defects of phagocytes; these phagocytes form the first line of defense against invading pathogens. A common hallmark of all congenital defects of phagocytes is an increased susceptibility to severe bacterial and fungal infections. Typical sites of infection are the skin, the oral mucosa and gingiva, lymph nodes, the lungs, and other internal organs.

The defects in intrinsic and innate immunity are depicted in detail in Chap. 8. This group is artificially divided into four subgroups depending on the microorganism to which patients manifest susceptibility, such as pyogenic bacteria, mycobacteria, virus, or fungus. In this chapter, however, we will follow the phenotypic approach, which we believe is more useful for clinicians when approaching a patient with a suspected PID.

Inborn errors of immune regulation are a heterogeneous group of genetic disorders with variable clinical manifestations, including lymphoproliferation, autoimmunity, and increased susceptibility to infections, which are described in Chap. 9. Regulatory T cells (Tregs) play an essential role in controlling immune responses, and mutations affecting the transcription factor FOXP3 cause immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome. Chapter 10 focuses on IPEX syndrome and IPEX-related disorders. According to the latest classification from the IUIS, there are almost 40 diseases being classified as autoinflammatory and these are described in-depth in Chap. 11. Almost any organ can be affected in cellular PID. The main clinical and epidemiological features associated with lung involvement in cellular PID can be found in Chap. 12. Additionally, gastrointestinal manifestations are present in 5–50% of patients with primary immunodeficiencies and are described thoroughly in Chap. 13. Malignancies are major causes of morbidity and mortality in patients with Cellular PID and are detailed in Chap. 14. Clinician awareness of malignancy risks, regular screening, and early investigation of symptoms are imperative. A history of previous malignancy in a patient with a cellular immunodeficiency or risk of future malignant disease development should also prompt consideration of definitive therapies such as allogeneic hematopoietic stem cell transplantation or gene therapy.

Around 25% of patients with PID present one or more autoimmune diseases with organ- or non-organ-specific manifestations, and these are dealt with in Chap. 15. A clinically differential, immunological, and genetic diagnostic workup is required for the majority of cellular PID patients, and the best practices are illustrated in Chap. 16, whereas Chap. 17 highlights the general strategies and tools in the clinical management of cellular PID.

The aim of Chap. 18 is to issue recommendations based on published scientific literature and practical experience: it evaluates how and when vaccines can be used in primary cellular immune deficiencies, in order to facilitate physician decisions and to ensure the best immune protection with the lowest risk for patient health.

Chapter 19 focuses on the use of gene therapy (GT) in cellular PID and outlines its role in the management of cellular primary immunodeficiencies. GT for cellular PID has been developed over the last 30 years, and while several setbacks have been encountered along the way, there is now a licensed GT product for adenosine deaminase-deficient severe combined immunodeficiency, and promising results from phase I/II clinical trials have demonstrated that GT may offer high clinical efficacy in several cellular PID.

The perception of cellular immune responses as a complex identity and the identification of specific defects leading to that phenotype have brought major contributions to the understanding of the human immune system in the past years. The discovery of genetic defects has also supported the identification of new therapeutic targets and the forecasting of adverse events that may occur with target therapies.

We hope that this book will boost the curiosity of young students approaching immunology for the first time, as well as be of interest to MD specialists in clinical immunology, pediatricians, hematologists, and researchers all over the world.

27 May 2021
Firenze, Italy — Mario Milco D'Elios
Siena, Italy — Cosima Tatiana Baldari
Firenze, Italy — Francesco Annunziato

Contents

Primary Immunodeficiencies

1

Lorenzo Cosmi, Boaz Palterer, and Francesco Annunziato

Abstract

Primary immunodeficiency diseases (PID) are a part of the growing field of inborn errors of immunity (IEI), sharing a raised susceptibility to infectious diseases and often associated to other features such as autoimmunity, autoinflammation, and lymphoproliferation and susceptibility to neoplastic disease and atopic diseases.

Keywords

Primary immunodeficiencies · Humoral immunity · Cellular immunity Antibodies · T cells · Dendritic cells

Primary immunodeficiency diseases are a relatively young field of medicine. Patients with primary immunodeficiencies may have different clinical presentations, such as severe infections and/or neoplasia and/or autoimmune diseases (Fig. 1.1). The development of medical practices, such as respiratory support, rehydration therapy, microbiology diagnostics, vaccination, and antibiotics, greatly reduced the mortality and morbidity of infectious disease in the general population and paved the way to the description of the first PIDs.

There were a few reports describing peculiar clinical syndromes before World War II that have been years later characterized as PIDs, such as ataxia-telangiectasia syndrome and severe congenital neutropenia [1]. However, Colonel Ogden Bruton is credited with the formal discovery of the first PID in 1952, Bruton's agammaglobulinemia, when he serendipitously found that a boy with a history of 18 pneumonias had an absent gamma fraction on protein electrophoresis [2].

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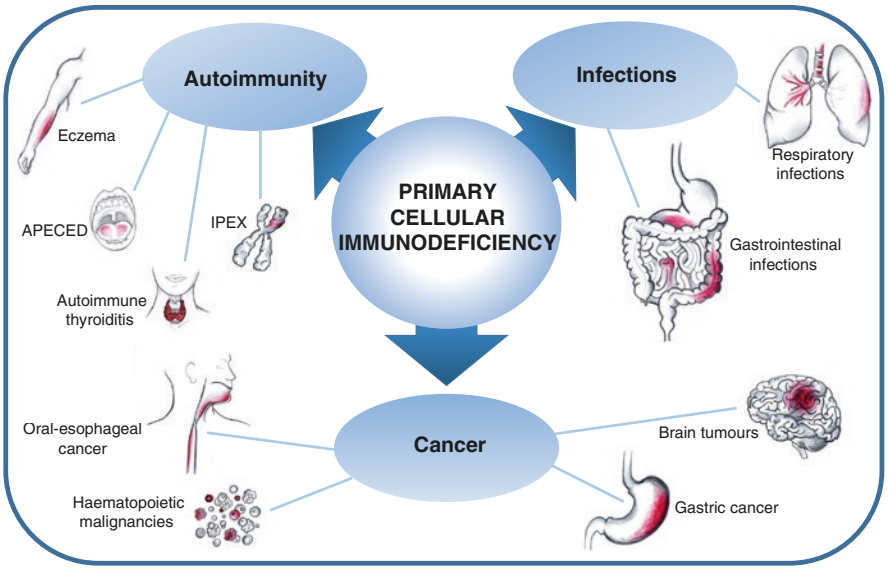

Fig. 1.1 *Schematic representation of clinical outcomes in patients with primary cellular immunodeficiencies.* Patients with primary cellular immunodeficiencies may have different clinical presentations, such as severe infections (e.g., respiratory or gastrointestinal infections) and/or neoplasia (e.g., gastric cancer, brain tumors, oral-esophageal cancers, hematopoietic malignancies) and/or autoimmune diseases (e.g., autoimmune thyroiditis or diabetes, eczema, autoimmune polyendocrinopathy candidiasis and ectodermal dystrophy (APECED), immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome)

The description of patients with X-linked hyper-IgM syndromes contributed to the understanding of the B-T interaction [3] and culminated in the discovery of mutations of the CD40-CD40L system in the early 90s. The study of autosomal recessive hyper-IgM syndromes elucidated the functions of activation-induced cytidine deaminase (AID) and uracil-N glycosylase (UNG) in class-switch recombination and ATM and PMS2 in DNA repair [4].

Many PIDs represent inestimable human models for the study of the function of the immune system, with implications beyond the clinical immunology field, such as cancer, autoimmune disease, transplantation, and infectious disease.

Defects in immune regulation have been associated with hyper-inflammation, lymphoproliferation, and autoimmunity.

The paradoxical co-occurrence of immunodeficiency with autoimmunity puzzled researchers for many years. The naïve definitions of autoimmunity as an "excess of immunity" and immunodeficiency as "lack of immunity" were incompatible with the clinical evidence [5].

The study of patients with autoimmune polyendocrinopathy candidiasis and ectodermal dystrophy (APECED) and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome contributed to the discovery of regulatory T

cells, and their master regulator FOXP3, and the thymus mechanisms for purging autoreactive T cells, by presenting self-antigen expressed under the regulation of AIRE.

CTLA-4 deficiency and defects in CTLA-4 recycling (LRBA and DEF6) present as PID with prominent autoimmunity. In 2018 James Allison and Tasuku Honjo received the Nobel Prize for cancer immunotherapy with checkpoint inhibitors, such as anti-CTLA-4, and interestingly the main side effects of these medications are immune-related adverse events (IRAEs) [6].

In the last decade, next-generation sequencing (NGS) led to the collapse of sequencing time and costs and brought a revolution in the PID field. The discovery of the molecular mechanisms behind PID is helping the development and application of personalized and targeted therapies [7].

The genomic revolution brought an unprecedented rate of discoveries, helping dissect the complexity of the genetic heterogeneity and pleiotropy of IEI. The last International Union of Immunological Societies (IUIS) IEI classification, published in 2019, includes over 430 separate entities [8].

A pivotal contribution of the IEI field has been the discovery of the mechanisms underlying immune redundancy and how some defects lead to susceptibility to a restricted spectrum of pathogens. These efforts brought recognition of IL-17 as a key player in mucosal immunity against fungi [9], the TLR-3 pathway in herpes encephalitis [10], and the IL-12/IFNγ axis in disseminated mycobacteria infections [11].

The translation of the study of these rare genetic diseases in large-scale genomic efforts enabled the description of the first common genetic polymorphism causing predisposition to mycobacterial disease. The TYK2 p.P1104A variant was shown to abrogate IL-23 signaling but not IL-12 signaling [12].

Moreover, since the immune response is under strong pressure by natural selection, the study of variants in the immune system can help to distinguish genes that are essential, redundant, or advantageous for human survival. These studies can provide information about the evolution of the immune system and the history of past epidemics [13].

Similarly, in the current SARS-CoV-2 pandemic, defects of type I interferon production and signaling were found to underlie life-threatening COVID-19 pneumonia in previously healthy patients [14].

The efforts to treat PIDs helped lay the foundations for hematopoietic stem cell transplantation. The first inhuman gene therapy was carried out in a boy with adenosine deaminase deficiency severe combined immunodeficiency (ADA-SCID) [15].

Going into the future, IEI are at the forefront of immunology and medical research.

References

1. Ochs HD, Hitzig WH (2012) History of primary immunodeficiency diseases. Curr Opin Allergy Clin Immunol 12:577–587
2. Bruton OC (1952) Agammaglobulinemia. Pediatrics 9(6):722–728
3. Rosen FS, Kevy SV, Merler E, Janeway CA, Gitlin D (1961) Dysgammaglobulinemia and recurrent bacterial infection. Lancet 277(7179):700
4. Durandy A, Peron S, Fischer A (2006) Hyper-IgM syndromes. Curr Opin Rheumatol 18(4):369–376
5. Bacchetta R, Notarangelo LD (2013) Immunodeficiency with autoimmunity: beyond the paradox. Front Immunol 4:77. http://journal.frontiersin.org/article/10.3389/fimmu.2013.00077/abstract
6. Fritz JM, Lenardo MJ (2019) Development of immune checkpoint therapy for cancer. J Exp Med 216(6):1244–1254
7. Meyts I, Bosch B, Bolze A et al (2016) Exome and genome sequencing for inborn errors of immunity. J Allergy Clin Immunol 138(4):957–969
8. Bousfiha A, Jeddane L, Picard C et al (2020) Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. J Clin Immunol 40(1):66–81
9. Puel A (2020) Human inborn errors of immunity underlying superficial or invasive candidiasis. Hum Genet 139(6–7):1011–1022
10. Jouanguy E, Béziat V, Mogensen TH, Casanova J-L, Tangye SG, Zhang S-Y (2020) Human inborn errors of immunity to herpes viruses. Curr Opin Immunol 62:106–122
11. Rosain J, Kong X-F, Martinez-Barricarte R et al (2019) Mendelian susceptibility to mycobacterial disease: 2014-2018 update. Immunol Cell Biol 97(4):360–367
12. Boisson-Dupuis S, Ramirez-Alejo N, Li Z et al (2018) Tuberculosis and impaired IL-23–dependent IFN-γ immunity in humans homozygous for a common *TYK2* missense variant. Sci Immunol 3(30):eaau8714
13. Barreiro LB, Quintana-Murci L (2020) Evolutionary and population (epi)genetics of immunity to infection. Hum Genet 139(6–7):723–732
14. Zhang Q, Bastard P, Liu Z et al (2020) Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science 370:eabd4570
15. Ferrua F, Aiuti A (2017) Twenty-five years of gene therapy for ADA-SCID: from bubble babies to an approved drug. Hum Gene Ther 28(11):972–981

Genetics of Cellular Immunodeficiencies

2

Natalie Frede

Abstract

Primary immunodeficiencies (PIDs) constitute a rapidly expanding field. As of 2019 there are 430 distinct human inborn errors of immunity listed in the current IUIS classification (International Union of Immunological Societies) (Tangye et al., J Clin Immunol 40:24–64, 2020).

Within the last 20 years, hundreds of genetic defects underlying PID have been identified. Historically, these were classified into humoral immunodeficiencies, on the one hand, and cellular immunodeficiencies, on the other hand. However, as our understanding has been evolving, these categories have become increasingly complex: genetic defects, for example, can affect multiple cell populations simultaneously, may affect communication between different cell types, may constitute failure of the bone marrow or other compartments to support and regulate certain cell populations, or may be associated with syndromic features. Within this chapter, we aim to provide an overview over the basic concepts of genetics as well as genetics of cellular immunodeficiencies.

Keywords

Genetics · Gene defect · Mutation · Variant · Sequencing · Primary immunodeficiencies · Cellular immunodeficiencies

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2.1 Introduction

Primary immunodeficiencies (PIDs) constitute a rapidly expanding field. As of 2019 there are 430 distinct human inborn errors of immunity listed in the current IUIS classification (International Union of Immunological Societies) [1].

Within the last 20 years, hundreds of genetic defects underlying PID have been identified. Historically, these were classified into humoral immunodeficiencies, on the one hand, and cellular immunodeficiencies, on the other hand. However, as our understanding has been evolving, these categories have become increasingly complex: genetic defects, for example, can affect multiple cell populations simultaneously, may affect communication between different cell types, may constitute failure of the bone marrow or other compartments to support and regulate certain cell populations, or may be associated with syndromic features. Within the IUIS classification, this is illustrated by the notion to classify PIDs into ten general categories, each with numerous distinct subcategories [1].

2.2 Importance of Establishing a Genetic Diagnosis

Establishing a genetic diagnosis is essential for confirming a suspected diagnosis and counseling PID patients and their family members regarding treatment options, prognosis, as well as family planning. The in-depth molecular characterization of underlying genetic defects and involved pathways has greatly advanced our knowledge of immunology, in general, and furthered our understanding of specific molecular principles of infection control but also of the termination of immune responses once the infection is controlled. For invasive therapies, such as hematopoietic stem cell transplantation or gene therapy approaches, the prior identification of a molecular defect is an essential prerequisite. A better understanding of defective or involved pathways may furthermore help to identify novel therapeutic targets, both for aiding patients with primary immunodeficiencies and for the development of novel immunosuppressive or antiproliferative drugs used in autoimmune diseases and malignancies, as experienced with JAK inhibitors or the BTK antagonist ibrutinib [2].

2.3 A Brief History of Genetics

The origins of genetics can be found within Augustinian monk Gregor Mendel's experiments on plant hybridization published in 1866, in which he described his observations on the heritability of different traits in peas [3]. However, it took until the early 1900s for the chromosomal theory of heredity to develop, combining Mendel's laws with the idea of chromosomes as the carriers of hereditary information [4]. Nucleic acid was first discovered in 1869 by the Swiss doctor Friedrich Miescher, though its significance was unclear at the time. It took until 1944 for Avery, McLeod, and McCarthy to show that, in fact, DNA constitutes the genetic material of a cell [5]. In 1953 Watson and Crick famously discovered the structure

of DNA and are since often hailed as the founders of modern genetics [6]. Meanwhile the notion of gene mutations as local alterations of a chromosome was derived in the 1920s from Hermann J. Muller's mutagenesis experiments on drosophila. The first description of a human mutation is generally attributed to V.M. Ingram, who described an amino acid exchange as a cause for sickle cell anemia in 1956 [7]. The first molecular defect underlying a primary immunodeficiency was identified by E.R. Giblett with ADA-SCID in 1972 [8], when she described two young girls with recurrent infections without measurable adenosine deaminase enzyme activity in their erythrocytes while their parents had approximately half-normal levels, from which an autosomal recessive inheritance pattern was correctly assumed.

Initial sequencing approaches constituted protein sequencing methods, allowing for the identification of the amino acid sequence of proteins from the 1950s on. At this time the exact mechanisms of transcription and translation had not yet been identified. Subsequently early RNA sequencing methods were developed, while the classical Sanger DNA sequencing method of dideoxy-chain termination was finally published in 1977 by Fred Sanger [9]. The era of PID genetics finally started in the early 1990s with the discovery of BTK mutations as the genetic cause of X-linked agammaglobulinemia more than 40 years after Bruton's first report of the disease in 1952 [10, 11]. Since then, advances in technology including automated sequencing and next-generation sequencing have enabled a rapid growth of the field with numerous exciting discoveries.

2.4 Patterns of Inheritance

Human inborn errors of immunity constitute by definition germline monogenic defects and thus follow Mendelian rules of inheritance. Specifically, monogenic defects can follow an autosomal dominant, autosomal recessive, or X-linked pattern of inheritance.

In the case of an autosomal dominant mode of inheritance, only one affected allele is sufficient to cause disease (i.e., a heterozygous mutation). Males and females will be affected at equal frequency, and the disease will not skip any generations. Autosomal dominant mutations can affect the expression and function of the gene product in different ways, namely, may cause haploinsufficiency, may result in gain of function, or may have a dominant negative effect. Haploinsufficiency arises when a single wild-type allele will not lead to sufficient expression of the gene product, usually a protein, and thus leads to a phenotypic effect. Gain-of-function mutations lead to an increased level of activity, novel function, or prolonged life span of the gene product, thus leading to a gain of function. Gain-of-function mutations are generally less common than loss-of-function mutations. Dominant negative mutations lead to a gene product, which will act antagonistically to the wild-type gene product within the same cell, also referred to as antimorphic mutations. Dominant negative mutations will thus reduce effective expression or function by greater than 50%. A dominant negative effect commonly arises in proteins, which form polymeric structures.

In autosomal recessive inheritance, two copies of an affected allele are required for an individual to express the disease phenotype (i.e., a homozygous or compound heterozygous mutation). Males and females will be similarly affected. Typically, both parents are heterozygous carriers, and statistically one-fourth of their children will be affected. In consanguineous unions, conditions with autosomal recessive inheritance will appear with an increased frequency due to common ancestry alleles. Autosomal recessive mutations generally result in loss of function.

In the case of X-linked inheritance usually only males are affected as they only possess one gene copy. Transmission occurs through female carriers, who are mostly phenotypically unaffected (no father-to-son transmission). The disease often occurs in multiple generations.

Whereas human inborn errors of immunity are generally defined through an underlying germline monogenic defect, some primary immunodeficiencies seem to have a complex pattern of inheritance, also referred to as polygenic inheritance. In particular, IgA deficiency and some forms of common variable immunodeficiency, both, however, not constituting cellular immunodeficiencies, seem to follow a complex inheritance pattern with an increased incidence in some families. The genetics of antibody deficiency was described in detail in the first book of this series, Humoral Primary Immunodeficiencies [12]. A list with explanations of genetic terms can be found in (Table 2.1).

Table 2.1 Genetic defects leading to cellular immunodeficiency, modified from Tangye et al. [1] (Springer OA BY CC License 4.0)

	Defect	Gene	Inheritance
Severe combined immunodeficiencies (see Chap. 6 of this book)			
T-B+ SCID	Gamma chain deficiency	*IL2RG*	XL
	JAK3 deficiency	*JAK3*	AR
	IL7Ra deficiency	*IL7R*	AR
	CD45 deficiency	*PTPRC*	AR
	CD3D deficiency	*CD3D*	AR
	CD3E deficiency	*CD3E*	AR
	CD3Z deficiency	*CD3Z*	AR
	Coronin 1A deficiency	*CORO1A*	AR
	LAT deficiency	*LAT*	AR
T-B− SCID	RAG1 deficiency	*RAG1*	AR
	RAG2 deficiency	*RAG2*	AR
	Artemis deficiency	*DCLRE1C*	AR
	DNA PKcs deficiency	*PRKDC*	AR
	Cernunnos/XLF deficiency	*NHEJ1*	AR
	DNA ligase 4 deficiency	*LIG4*	AR
	Adenosine deaminase (ADA) deficiency	*ADA*	AR
	AK2 defect	*AK2*	AR
	Activated RAC2 defect	*RAC2*	AD
Combined immunodeficiencies less severe than SCID (see Chap. 7 of this book)			

Table 2.1 (continued)

	Defect	Gene	Inheritance
	CD40 ligand deficiency	*CD40LG*	XL
	CD40 deficiency	*CD40*	AR
	ICOS deficiency	*ICOS*	AR
	ICOSL deficiency	*ICOSLG*	AR
	CD3γ deficiency	*CD3G*	AR
	CD8 deficiency	*CD8A*	AR
	ZAP-70 deficiency	*ZAP70*	AR (LOF or GOF)
	MHC class I deficiency	*TAP1*	AR
		TAP2	AR
		TAPBP	AR
		B2M	AR
	MHC class II deficiency	*CIITA*	AR
		RFXANK	AR
		RFX5	AR
		RFXAP	AR
	IKAROS deficiency	*IKZF1*	AD
	DOCK8 deficiency	*DOCK8*	AR
	DOCK2 deficiency	*DOCK2*	AR
	Polymerase D deficiency	*POLD1*	AR
		POLD2	AR
	RHOH deficiency	*RHOH*	AR
	STK4 deficiency	*STK4*	AR
	TCRα deficiency	*TRAC*	AR
	LCK deficiency	*LCK*	AR
	ITK deficiency	*ITK*	AR
	MALT1 deficiency	*MALT1*	AR
	CARD11 deficiency	*CARD11*	AR
	BCL10 deficiency	*BCL10*	AR
	IL-21 deficiency	*IL21*	AR
	IL-21 receptor deficiency	*IL21R*	AR
	OX40 deficiency	*TNFRSF4*	AR
	IKBKB deficiency	*IKBKB*	AR
	NIK deficiency	*MAP3K14*	AR
	RelB deficiency	*RELB*	AR
	RelA haploinsufficiency	*RELA*	AD
	Moesin deficiency	*MSN*	XL
	TFRC deficiency	*TFRC*	AR
	c-Rel deficiency	*REL*	AR
	FCHO1 deficiency	*FCHO1*	AR

(continued)

Table 2.1 (continued)

	Defect	Gene	Inheritance
Combined immunodeficiencies with associated or syndromic features (see Chap. 8 of this book)			
Immunodeficiency with congenital thrombocytopenia	Wiskott-Aldrich syndrome	*WAS*	XL
	WIP deficiency	*WIPF1*	AR
	Arp2/3-mediated filament branching defect	*ARPC1B*	AR
Other DNA repair defects	Ataxia-telangiectasia	*ATM*	AR
	Nijmegen breakage syndrome	*NBS1*	AR
	Bloom syndrome	*BLM*	AR
	Immunodeficiency with centromeric instability and facial anomalies	*DNMT3B*	AR
		ZBTB24	AR
		CDCA7	AR
		HELLS	AR
	PMS2 deficiency	*PMS2*	AR
	RIDDLE syndrome/RNF168 deficiency	*RNF168*	AR
	MCM4 deficiency	*MCM4*	AR
	Polymerase E subunit 1 deficiency (FILS syndrome)	*POLE1*	AR
	Polymerase E subunit 2 deficiency	*POLE2*	AR
	Ligase I deficiency	*LIG1*	AR
	NSMCE3 deficiency	*NSMCE3*	AR
	ERCC6L2/Hebo deficiency	*ERCC6L2*	AR
	GINS1 deficiency	*GINS1*	AR
Thymic defects with additional congenital anomalies	DiGeorge/chromosome 22q11.2 deletion syndrome	*Deletion in 22q11.2 (typically spanning TBX1)*	AD
	TBX1 deficiency	*TBX1*	AD
	CHARGE syndrome	*CHD7*	AD
		SEMA3E	AD
	Winged helix nude FOXN1 deficiency/haploinsufficiency	*FOXN1*	AR/AD
	Chromosome 10p13-p14 deletion syndrome	*Del10p13-p14*	AD
	Chromosome 11q deletion syndrome (Jacobsen syndrome)	*11q23del*	AD
Immuno-osseous dysplasias	Cartilage hair hypoplasia	*RMRP*	AR
	Schimke immuno-osseous dysplasia	*SMARCAL1*	AR
	MYSM1 deficiency	*MYSM1*	AR
	MOPD1 deficiency (Roifman syndrome)	*RNU4ATAC*	AR
	Immunoskeletal dysplasia with neurodevelopmental abnormalities (EXTL3 deficiency)	*EXTL3*	AR

Table 2.1 (continued)

	Defect	Gene	Inheritance
Hyper-IgE syndromes (HIES)	STAT3 deficiency (job syndrome)	*STAT3*	AD
	IL6 receptor deficiency	*IL6R*	AR
	IL6 signal transducer (IL6ST) deficiency	*IL6ST*	AR
	ZNF341 deficiency	*ZNF341*	AR
	ERBIN deficiency	*ERBB2IP*	AD
	Loeys-Dietz syndrome (TGFBR deficiency)	*TGFBR1*	AD
		TGFBR2	AD
	Comel-Netherton syndrome	*SPINK5*	AR
	PGM3 deficiency	*PGM3*	AR
	CARD11 deficiency	*CARD11*	AD
Defects of vitamin B12 and folate metabolism	Transcobalamin 2 deficiency	*TCN2*	AR
	SLC46A1/PCFT deficiency	*SLC46A1*	AR
	Methylene-tetrahydrofolate dehydrogenase 1 (MTHFD1) deficiency	*MTHFD1*	AR
Anhidrotic ectodermal dysplasia with immunodeficiency	NEMO/IKBKG deficiency	*IKBKG*	XL
	IKBA GOF mutation	*NFKBIA*	AD GOF
	IKBKB GOF mutation	*IKBKB*	AD GOF
Calcium channel defects	ORAI-1 deficiency	*ORAI1*	AR
	STIM1 deficiency	*STIM1*	AR
Other defects	Purine nucleoside phosphorylase (PNP) deficiency	*PNP*	AR
	Immunodeficiency with multiple intestinal atresias	*TTC7A*	AR
	Tricho-hepato-enteric syndrome	*TTC37*	AR
		SKIV2L	AR
	Hepatic veno-occlusive disease with immunodeficiency (VODI)	*SP110*	AR
	BCL11B deficiency	*BCL11B*	AD
	EPG5 deficiency (Vici syndrome)	*EPG5*	AR
	HOIL1 deficiency	*RBCK1*	AR
	HOIP deficiency	*RNF31*	AR
	Hennekam lymphangiectasia-lymphedema syndrome	*CCBE1*	AR
		FAT4	AR
	Activating de novo mutations in nuclear factor, erythroid 2 like (NFE2L2)	*NFE2L2*	AD
	STAT5b deficiency	*STAT5B*	AR/AD
	Kabuki syndrome	*KMT2D*	AD
		KDM6A	XL
	KMT2A deficiency (Wiedemann-Steiner syndrome)	*KMT2A*	AD

(continued)

Table 2.1 (continued)

	Defect	Gene	Inheritance
Congenital defects of phagocytes (see Chap. 9 of this book)			
Congenital neutropenias	Elastase deficiency/severe congenital neutropenia 1(SCN1)	*ELANE*	AD
	GFI 1 deficiency (SCN2)	*GFI1*	AD
	HAX1 deficiency (Kostmann disease) (SCN3)	*HAX1*	AR
	G6PC3 deficiency (SCN4)	*G6PC3*	AR
	VPS45 deficiency (SCN5)	*VPS45*	AR
	Glycogen storage disease type 1b	*G6PT1*	AR
	X-linked neutropenia/ myelodysplasia	*WAS*	XL GOF
	P14/LAMTOR2 deficiency	*LAMTOR2*	AR
	Barth syndrome (3-methylglutaconic aciduria type II)	*TAZ*	XL
	Cohen syndrome	*VPS13B*	AR
	Clericuzio syndrome (Poikiloderma with neutropenia)	*USB1*	AR
	JAGN1 deficiency	*JAGN1*	AR
	3-Methylglutaconic aciduria	*CLPB*	AR
	G-CSF receptor deficiency	*CSF3R*	AR
	SMARCD2 deficiency	*SMARCD2*	AR
	Specific granule deficiency	*CEBPE*	AR
	Shwachman-diamond syndrome	*SBDS*	AR
		DNAJC21	AR
		EFL1	AR
	HYOU1 deficiency	*HYOU1*	AR
	SRP54 deficiency	*SRP54*	AD
Defects of motility	Leukocyte adhesion deficiency type 1 (LAD1)	*ITGB2*	AR
	Leukocyte adhesion deficiency type 2 (LAD2)	*SLC35C1*	AR
	Leukocyte adhesion deficiency type 3 (LAD3)	*FERMT3*	AR
	Rac2 deficiency	*RAC2*	AD LOF
	β actin deficiency	*ACTB*	AD
	Localized juvenile periodontitis	*FPR1*	AR
	Papillon-Lefèvre syndrome	*CTSC*	AR
	WDR1 deficiency	*WDR1*	AR
	Cystic fibrosis	*CFTR*	AR
	MKL1 deficiency	*MKL1*	AR
Defects of respiratory burst	X-linked chronic granulomatous disease (CGD), gp91phox	*CYBB*	XL
	Autosomal recessive CGD	*CYBA*	AR
		CYBC1	AR
		NCF1	AR
		NCF2	AR
		NCF4	AR
	G6PD deficiency class I	*G6PD*	XL

Table 2.1 (continued)

	Defect	Gene	Inheritance
Other nonlymphoid defects	GATA2 deficiency	*GATA2*	AD
	Pulmonary alveolar proteinosis	*CSF2RA*	XL
		CSFR2B	AR
Defects of innate and intrinsic immunology (see Chap. 10 of this book)			
Mendelian susceptibility to mycobacterial disease	IL-12 and IL-23 receptor β1 chain deficiency	IL12RB1	AR
	IL-12p40 (IL-12 and IL-23) deficiency	IL12B	AR
	IL-12Rβ2 deficiency	IL12RB2	AR
	IL-23R deficiency	IL23R	AR
	IFN-γ receptor 1 deficiency	IFNGR1	AR/AD
	IFN-γ receptor 2 deficiency	IFNGR2	AR
	STAT1 deficiency	STAT1	AD LOF
	Macrophage gp91 phox deficiency	CYBB	XL
	IRF8 deficiency	IRF8	AD
	SPPL2a deficiency	SPPL2A	AR
	Tyk2 deficiency	TYK2	AR
	ISG15 deficiency	ISG15	AR
	RORγt deficiency	RORC	AR
	JAK1 deficiency	JAK1	AR
Epidermodysplasia verruciformis (HPV)	EVER1 deficiency	TMC6	AR
	EVER2 deficiency	TMC8	
	CIB1 deficiency	CIB1	
	WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome	CXCR4	AD GOF
Predisposition to severe viral infection	STAT1 deficiency	STAT1	AR LOF
	STAT2 deficiency	STAT2	AR
	IRF9 deficiency	IRF9	AR
	IRF7 deficiency	IRF7	AR
	IFNAR1 deficiency	IFNAR1	AR
	IFNAR2 deficiency	IFNAR2	AR
	CD16 deficiency	FCGR3A	AR
	MDA5 deficiency	IFIH1	AR
	RNA polymerase III deficiency	POLR3A	AD
		POLR3C	AD
		POLR3F	AD
Predisposition to herpes simplex encephalitis	TLR3 deficiency	TLR3	AR/AD
	UNC93B1 deficiency	UNC93B1	AR
	TRAF3 deficiency	TRAF3	AD
	TRIF deficiency	TICAM1	AD/AR
	TBK1 deficiency	TBK1	AD
	IRF3 deficiency	IRF3	AD
	DBR1 deficiency	DBR1	AR

(continued)

Table 2.1 (continued)

	Defect	Gene	Inheritance
Predisposition to recurrent fungal infections	CARD9 deficiency	CARD9	AR
	IL-17RA deficiency	IL17RA	AR
	IL-17RC deficiency	IL17RC	AR
	IL-17F deficiency	IL17F	AD
	STAT1 GOF	STAT1	AD GOF
	ACT1 deficiency	TRAF3IP2	AR
TLR signaling pathway deficiency with bacterial susceptibility	IRAK4 deficiency	IRAK4	AR
	MyD88 deficiency	MYD88	AR
	IRAK1 deficiency	IRAK1	XL
	TIRAP deficiency	TIRAP	AR
Other inborn errors of immunity related to non-hematopoietic tissues	Isolated congenital asplenia (ICA)	RPSA	AD
		HMOX	AR
	Trypanosomiasis	APOL1	AD
	Acute liver failure due to NBAS deficiency	NBAS	AR
	Acute necrotizing encephalopathy	RANBP2	AR
	Osteopetrosis	CLCN7	AR
		SNX10	AR
		OSTM1	AR
		PLEKHM1	AR
		TCIRG1	AR
		TNFRSF11A	AR
		TNFSF11	AR
	Hidradenitis suppurativa	NCSTN	AD
		PSEN	AD
		PSENEN	AD
Other inborn errors of immunity related to leukocytes	IRF4 haploinsufficiency	IRF4	AD
	IL-18BP deficiency	IL18BP	AR

Abbreviations: *XL* X-linked, *AR* Autosomal recessive, *AD* Autosomal dominant, *GOF* Gain of function, *LOF* Loss of function

2.5 Germline Versus Somatic Mutations

A **germline** mutation is defined as a heritable mutation, which occurred originally in a germ cell or the zygote at single-cell stage, and thus will be present in all cells in the offspring, including the germ cells. Thus, a germline mutation can be passed on from generation to generation.

In contrast to germline mutations, somatic mutations occur when a mutation arises postzygotically, which will lead to mosaicism. The phenotype of mosaicism depends upon the developmental stage at which the mutation arises. A mutation in early embryonic development will likely affect many different tissues. If only

somatic cells are affected, this will be called somatic mosaicism. Somatic mosaicism will not be transmitted to the next generation. If both somatic cells and germ cells are affected, this is defined as gonosomal mosaicism, which will cause symptoms depending on the affected somatic tissues and may be transmitted on to the next generation. In contrast, gonadal mosaicism affects only the germ cells and, thus, typically has no phenotype in the carrier but will be transmitted to the offspring as a germline mutation. Somatic mutations are generally considered phenocopies of PID within the IUIS classification, although we want to point out that mosaicism may in theory cause a phenotype indistinguishable from germline mutations and, if including the germ cells, can lead to full germline mutations in the offspring. Mosaicism should be suspected in case of marked intergenerational phenotypical differences, unexpected intrafamilial reoccurrence in children of seemingly healthy parents without mutation in Sanger sequencing, or unequal height/intensity of sequencing peaks in Sanger chromatograms [13].

2.6 Types of Mutations

Mutations can be classified either by their impact on DNA or by their impact on the respective protein.

Regarding the impact of a mutation on the DNA, a mutation can further be classified as a substitution, i.e., one or multiple bases are substituted by an equal number of other bases; an insertion, where additional bases are gained; or a deletion, i.e., a loss of bases. Inversions and translocations may also be listed in this category and constitute structural rearrangements of DNA. An inversion is defined as an end-to-end reversion of a piece of DNA, whereas a translocation describes the integration of a piece of DNA or a piece of a chromosome at another position. Translocations can be balanced, i.e., an even exchange of DNA, or unbalanced, thus leading to loss or gain of genetic material in daughter cells.

Regarding the impact on the resulting protein, mutations can be classified as silent mutations, missense mutations, or frameshift mutations. A silent mutation is a mutation which does not lead to a change in the amino acid sequence of the protein. A missense mutation is defined as an exchange of a single amino acid within the protein sequence. A nonsense mutation constitutes a change of one amino acid within the protein into a premature stop codon. In contrast, a readthrough mutation constitutes the change of a stop codon into an amino acid. A frameshift mutation arises from a deletion or insertion of bases in the DNA sequence of a number not divisible by three, thus changing the reading frame as the genetic code is organized in base triplets, i.e., codons, each coding for a specific amino acid. Also mutations outside of the coding DNA sequence may have an impact on the protein. Splice site mutations have long been recognized as disease causing; however, deep intronic mutations may have an impact on the protein sequence or expression through inclusion of pseudo-exons due to creation of alternative splice sites or may affect expression when located within regulatory regions, such as promoter or enhancer sequences [14].

2.7 Pleiotropy of PIDs

Many different genetic defects have overlapping phenotypes. Genetic heterogeneity can generally be classified into allelic heterogeneity and locus heterogeneity. Allelic heterogeneity is defined as different mutations (alleles) within the same gene producing a similar phenotype. In contrast, locus heterogeneity implies that a similar phenotype may be caused by mutations in different genes. Similarly, the term genocopy refers to a genotype or mutation resulting in a similar phenotype to another genotype or mutation at a different locus. In contrast, a phenocopy is defined as environmental factors producing the same phenotype as a specific genetic mutation, thus mimicking the phenotype [15]. A phenocopy by definition is not a genetic trait and thus is not hereditary in a strict Mendelian sense, though epigenetic changes may count as phenocopies and can be passed on to daughter cells.

Differences in phenotype despite the same genotype may be caused by a variable expressivity of a trait or phenotype. Expressivity thus constitutes a measure for the extent of phenotypic expression. In contrast, penetrance refers to the proportion of individuals with a certain genotype, who exhibit the associated phenotype. With complete penetrance all individuals with a certain genotype, i.e., a certain mutation, show the associated symptoms/trait, whereas reduced penetrance means that some individuals who carry a genetic defect may in fact be phenotypically healthy.

2.8 Sequencing Technologies

In the past Sanger sequencing constituted the gold standard for genetic diagnostics. Depending on the clinical phenotype, the most likely candidate genes needed to be identified and sequenced sequentially exon by exon. However, this constituted a laborious time- and resource-consuming process and often did not lead to success due to atypical presentations and obvious limitations due to being a hypothesis-driven approach (i.e., the candidate gene needed to be known). In recent years, great advances have been made with the help of next-generation sequencing techniques, which have substituted Sanger sequencing in the diagnostic workup process of PIDs in many places.

Next-generation sequencing technologies allow for the simultaneous massive parallel sequencing of thousands of genes at dramatically reduced costs. With many novel sequencing techniques, several patients can be multiplexed and thus sequenced in the same sequencing run. The introduction of next-generation sequencing methods thus has greatly facilitated the identification of novel genetic defects, which is reflected in the rising number of novel defects described every year. In general, next-generation sequencing methods consist of the following three steps: The first step is the preparation of a library, in which the DNA is fragmented (usually through either restriction enzymes or sonication) and fragments are ligated with custom linkers or sequencing adapters. In panel and exome sequencing, there is an amplification step relying on clonal amplification/PCR, whereas whole genome sequencing, in general, does not necessitate amplification. Lastly, the fragments are

sequenced. Depending on the sequencer, different technologies are employed for this step.

Next-generation sequencing approaches can be divided into panel sequencing approaches, whole exome sequencing, and whole genome sequencing, each with their distinct advantages and disadvantages.

Gene panel sequencing provides a high coverage of sequenced regions, which is one of its distinct advantages over whole exome and especially whole genome sequencing and is crucial to reduce errors, particularly in a diagnostic setting. Since less regions are sequenced, panel sequencing results in less variants of unknown significance. Limitations include that panel sequencing is a biased approach (variants can only be detected in sequenced regions, i.e., in identified target genes), preassembled panels are rigid and may not contain all genes of interest, and continuous redesigning and revalidation of the panel may be necessary.

Whole exome sequencing constitutes an unbiased approach, allowing the identification of novel defects. However, in practice, complete coverage of all coding exons is impracticable, and a significant proportion of regions will have a low read depth, necessitating resequencing for use in a clinical context.

Whole genome sequencing is the only method which also allows for the identification of deep intronic variants. However, data interpretation is still difficult and hampered by detection of vast numbers of variants of unknown significance.

All next-generation sequencing approaches necessitate bioinformatic analysis in order to align the sequenced fragments correctly to the reference genome and identify variants. Detected variants subsequently need to be compared with reference databases and evaluated for harmfulness through either prediction tools or experimental validation.

2.9 Interpretation of Sequencing Results

A drawback of the novel sequencing technologies is the detection of numerous variants of unclear significance. Sometimes it may be difficult to establish whether a variant is in fact disease causing or not, which may pose clinical as well as at times ethical and legal challenges.

Sequence variants are common, often benign, and the source of genetic variation. In fact, each genome is thought to have approximately 4 million sequence variants, which mostly constitute single-nucleotide polymorphisms (SNP, by definition frequency above 1%) but may also encompass, e.g., structural variants [16]. The relative occurrence of sequence variants varies between regions and genes, with important functional domains of genes often showing evolutionary conservation through many different species.

To establish whether a variant may be benign or disease causing, several methods can be employed. Firstly, to establish whether a variant has been reported before or is listed as a SNP, bioinformatic analysis including queries of population databases can

be employed; useful resources include, e.g., dbSNP, Human Gene Mutation Database (HGMD), ClinVar, and gnomAD (formerly ExAc) [17–20]. If the variant has not been reported as disease associated before, in silico prediction tools, such as CADD, PolyPhen2, or SIFT, may provide helpful insights [21–23]. Additionally, genetic analysis of affected and unaffected family members as well as functional testing including cloning of the mutation may be performed but constitute laborious processes.

To facilitate the classification and interpretation of sequencing results, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology have published recommendations [24]. In particular, they recommend to classify variants into the following categories: (i) pathogenic, (ii) likely pathogenic, (iii) uncertain significance, (iv) likely benign, or (v) benign. Furthermore, they advise to use a standardized nomenclature of variants, as published and regularly updated by the Human Genome Variation Society (HGVS) [25].

As a general rule, variants should always be described at the most basic level possible, which will normally be the DNA level (e.g., c.1650C>T, where ">" is used to label a base substitution and "del," "ins," and "dup" label deletions, insertions, and duplications, respectively). However, in the case of, e.g., frameshifts, the protein sequence may be used, such as p.R908Kfs*15, indicating that the variant leads to an amino acid exchange from R to K in position 908 with a subsequent frameshift (fs) and termination after 15 amino acids. Importantly, a reference sequence or transcript should always be provided. The online tool Mutalyzer (https://mutalyzer.nl) provides both a name generator producing a valid HGVS variant description and a syntax checker to assess existing variant descriptions regarding compatibility with HGVS nomenclature [26].

Furthermore, variants described in only a single patient should be taken with caution. Casanova et al. published criteria aiding the decision whether data establish a causal relationship between phenotype and genotype for this case [27]. In brief, the variant in question should not occur in healthy individuals, there need to be experimental data indicating that the gene product is altered in function or expression by the variant, and a causal relationship between genotype and phenotype must be confirmed in an animal model or a relevant cellular phenotype.

2.10 Genetics of Combined Immunodeficiencies

Combined immunodeficiencies can be classified into severe combined immunodeficiencies (SCID) and combined immunodeficiencies less severe than SCID (CID). In total, the current IUIS classification lists a total number of 50 disorders caused by 58 distinct genetic defects.

Clinically, SCID defects can be classified by presence or absence of T, B, and NK cells. Genetically, SCID defects can be grouped by pathogenic mechanism into VDJ recombination and T cell receptor defects, cytokine signaling defects, defects with toxic metabolite accumulation, and defects with defective survival of hematopoietic precursors. SCID usually follows an autosomal recessive or X-linked pattern of inheritance.

VDJ recombination defects constitute mainly enzymatic defects, which are inherited in an autosomal recessive pattern and include RAG1/RAG2 deficiency, DNA cross-link repair enzyme 1c (DCLRE1C)/Artemis deficiency, nonhomologous end-joining enzyme (NHEJ1)/Cernunnos deficiency, DNA ligase IV (LIG4) deficiency, and DNA PKcs deficiency (PRKDC). Recombination-activating proteins (RAG) 1 and 2 initiate recombination of immunoglobulin and T cell receptor genes in B and T cells to diversify the repertoire through rearrangement of variable (V), diversity (D), and joining (J) segments. Failure to induce VDJ recombination will lead to apoptosis. It is essential that the induced double-strand breaks subsequently are repaired correctly to produce a recombined continuous DNA strand. Defects in nonhomologous end-joining DNA repair mechanisms, such as mutations in DCLRE1C, DNA PKcs, NHEJ1, and LIG4, will thus lead to SCID with increased radiosensitivity as DNA damage cannot be sufficiently repaired. Other DNA repair defects leading to radiosensitivity and immunodeficiency regularly include syndromic features and thus belong to syndromic combined immunodeficiencies.

T cell receptor defects affecting CD3D, CD3E, and CD3Z lead to T-B+NK+ SCID, whereas CD3G deficiency leads to combined immunodeficiency, generally less profound than SCID. Also, CD45 deficiency may be counted toward the T cell receptor defects in that CD45 encodes a receptor-associated tyrosine phosphatase essential for the activation of T cells through the T cell receptor, its deficiency also leads to T-B + NK+ SCID. T cell receptor defects generally follow an autosomal recessive inheritance pattern.

Cytokine signaling defects causing SCID include common gamma chain deficiency (IL2RG), JAK3 deficiency, and IL7Rα deficiency (IL7RA). The common gamma chain is a receptor chain shared by the receptors of interleukins IL2, IL4, IL7, IL9, IL15, and IL21. As IL7 and IL15 are essential in the development of T and NK cells, common gamma chain deficiency leads to T-B + NK- SCID. Since the *IL2RG* gene is located on the X chromosome, common gamma chain deficiency follows an X-linked inheritance pattern and thus is also referred to as X-SCID. The Janus kinase 3 mediates the signal transduction downstream of common gamma chain cytokines; therefore, deficiency also leads to T-B+NK− SCID, however, following an autosomal recessive inheritance pattern. IL7 signaling is essential in the early development of lymphocytes but also proliferation and survival of T cells peripherally; thus, deficiency of the IL7 receptor alpha chain also causes T-B+NK+ SCID. Multiple other cytokine and signaling defects lead to less severe primary immunodeficiencies.

Defects with toxic metabolite accumulation include ADA and PNP deficiency. The adenosine deaminase (ADA) is an essential enzyme of the purine salvage pathway, mediating the deamination of adenosine and 2-deoxyadenosine into inosine and deoxyinosine. Deficiency of ADA leads to toxic accumulation of these metabolites, which results in lymphocyte apoptosis (T-B−NK− SCID). The purine nucleoside phosphorylase (PNP) constitutes another key enzyme within the purine salvage pathway downstream of ADA, deribosylating inosine to hypoxanthine and guanosine to guanine. PNP deficiency leads to toxic accumulation of deoxyguanosine and deoxyguanosine triphosphate, leading to apoptosis, mainly affecting the T cells

(T-B+NK− SCID). As PNP deficiency leads to neurological symptoms and autoimmunity, the IUIS classification lists it as a combined immunodeficiency with associated features.

Hypomorphic mutations in any of these SCID genes may lead to less severe phenotypes of leaky SCID/combined immunodeficiency. Detailed descriptions of severe combined immunodeficiencies and combined immunodeficiencies may be found in Chaps. 6 and 7 of this book, whereas Table 2.2 gives an overview of genetic defects associated with cellular immunodeficiencies (Table 2.2).

Table 2.2 Definitions of genetic terms

Patterns of inheritance	
Autosomal recessive inheritance	Two copies of an affected allele are required for an individual to express the disease phenotype (i.e., a homozygous or compound heterozygous mutation); may occur if gene is located on autosomal chromosome
Autosomal dominant inheritance	Only one affected allele is sufficient to cause disease (i.e., a heterozygous mutation); may occur if gene is located on autosomal chromosome
X-linked inheritance	Usually only males are affected as they only possess one gene copy. Transmission occurs through female carriers, who are mostly phenotypically unaffected; only occurs in genes located on X chromosome
Zygosity	
Homozygosity	Cell possesses two identical alleles of a particular gene, one inherited from each parent
Heterozygosity	Cell possesses two different alleles (one wild-type allele and one variant allele) of a particular gene
Compound heterozygosity	Cell possesses two different variant alleles but no wild-type allele of a particular gene
Hemizygosity	Cell possesses only one copy of a particular gene, either through location of that gene on sex chromosome (X,Y) or through loss of homologous chromosome
Types of mutations	
Substitution	Mutation, in which one or multiple bases are replaced with an equal number of other bases
Deletion	Mutation which leads to loss of bases
Insertion	Mutation which leads to gain of additional bases
Inversion	End-to-end reversion of a piece of DNA
Translocation	Integration of a piece of DNA or piece of a chromosome at another position
Silent mutation	Mutation which does not change the amino acid sequence of the protein
Missense mutation	Mutation which leads to exchange of a single amino acid within a protein sequence
Nonsense mutation	Mutation which leads to formation of premature stop codon
Readthrough mutation	Mutation which changes the stop codon into an amino acid
Frameshift mutation	Deletion or insertion of a base number not divisible by three, thus changing the reading frame

Table 2.2 (continued)

Patterns of inheritance	
Mosaicism and germline mutations	
Germline mutation	Heritable mutation, which occurred originally in a germ cell or zygote at single-cell stage and thus will be present in all cells in the offspring
Somatic mutation	Postzygotic mutation, which will lead to mosaicism of one of the three types listed below:
Somatic mosaicism	Affects only somatic cells, thus not transmitted to the next generation; will produce phenotype depending on affected cells/tissues
Gonosomal mosaicism	Affects both somatic cells and germ cells, may thus cause symptoms depending on affected somatic tissues, and may be transmitted to the next generation
Gonadal mosaicism	Affects only germ cells, thus, typically has no phenotype in the carrier but may be transmitted to the offspring as a germline mutation
Pleiotropy and heterogeneity	
Allelic heterogeneity	Different mutations (alleles) within the same gene produce a similar phenotype
Locus heterogeneity	Mutations in different genes produce a similar phenotype
Genocopy	Genotype or mutation, which produces a similar phenotype to another genotype or mutation at a different locus
Phenocopy	Environmental factors produce the same phenotype as a specific genetic mutation, thus mimicking the phenotype
Expressivity	Measure for the extent of phenotypic expression
Penetrance	Proportion of individuals with a certain genotype, who exhibit the associated phenotype

2.11 Genetics of Combined Immunodeficiencies with Associated or Syndromic Features

In most primary immunodeficiencies, the immunodeficiency is the most prominent clinical finding. In contrast, syndromic immunodeficiencies are characterized by associated syndromes or clinical findings taking a front role. Associated features may commonly affect the skeletal, nervous, or ectodermal development or function but may include almost any organ system. In contrast to most other classes of immunodeficiencies, not all syndromic immunodeficiencies are typically caused by a genetic defect in a single gene but may result from underlying cytogenetic abnormalities, i.e., abnormalities of chromosomal number or structure. DiGeorge syndrome caused by 22q11 deletions is the most well-known example. Cytogenetic abnormalities may be detected by karyotyping or fluorescence in situ hybridization (FISH).

As of 2019, there are a total number of 58 combined immunodeficiencies with associated or syndromic features comprising 62 distinct genetic defects [1]. These include immunodeficiencies with congenital thrombocytopenia (*WAS, WIPF1, ARPC1B*), other DNA repair defects (*ATM, NBS1, BLM, DNMT3B, CDCA7, HELLS, PMS2, RNF168, MCM4, POLE1, POLE2, LIG1, NSMCE3, ERCC6L2,*

GINS1), thymic defects with congenital abnormalities (22q11.2DS, TBX1, CHD7, SEMA3E, FOXN1, 10p13-p14DS, 11q23del), immuno-osseous dysplasias (*RMRP, SMARCAL1, MYSM1, RNU4ATAC, EXTL3*), hyper-IgE syndromes (*STAT3, IL6R, IL6ST, ZNF341, ERBB2IP, TGFBR1, TGFBR2, SPINK5, PGM3, CARD11*), defects in vitamin B12 and folate metabolism (*TCN2, SLC46A1, MTHFD1*), anhidrotic ectodermal dysplasia with immunodeficiency (*IKBKG, NFKBIA, IKBKB*), calcium channel defects (*ORAI1, STIM1*), and other defects (*PNP, TTC7A, TTC37, SKIV2L, SP110, BCL11B, EPG5, RBCK1, RNF31, CCBE1, FAT4, NFE2L2, STAT5B, KMT2D, KDM6A, KMT2A*). A detailed description of combined immunodeficiencies with associated or syndromic features may be found in Chap. 8 of this book.

2.12 Genetics of Defects in Intrinsic and Innate Immunity

Defects of innate immunity comprise multiple heterogeneous groups of defects, out of which some can be counted toward cellular immunodeficiency, others constitute defects of soluble factors such as complement factors, and yet other defects derive from a defective barrier function, which are defects of nonimmune cells, however, predisposing to infection. Furthermore, defects of innate immunity may have an impact on the adaptive immune system through impaired (co-)stimulation or antigen presentation.

In recent years many novel inborn errors of innate immunity have been described, often leading to an increased susceptibility to a narrow range of pathogens. These include Mendelian susceptibility to mycobacterial disease, predisposition to chronic mucocutaneous candidiasis or invasive fungal infections, predisposition to herpes simplex encephalitis, and other severe viral infections. A detailed description of defects of intrinsic and innate immunity may be found in Chap. 10 of this book.

A major subgroup within the defects of innate immunity are the congenital defects of phagocytes; thus, they are often listed separately, and also this book dedicated a separate chapter to them (for congenital defects of phagocytes, see Chap. 9). Congenital defects of phagocytes comprise congenital neutropenias (i.e., defects with reduced neutrophil numbers); defects of neutrophil function including motility, chemotaxis, and adhesion; defects of respiratory burst (chronic granulomatous disease); and other nonlymphoid defects. As of 2019, the IUIS recognizes 41 distinct phagocyte defects [1].

2.13 Outlook

While so far more than 430 genetic defects causing primary immunodeficiencies have been identified, there may be many more novel defects left to discover. In theory, the number of potential human inborn errors of immunity is only limited by the number of genes related to the immune system. The gene ontology (GO) database lists 2782 genes within the category “immune system process”; thus, there may be many discoveries of novel primary immunodeficiencies in the years to come.

As our understanding of molecular as well as regulatory processes evolves, phenocopies of PID with somatic mutations, polygenic traits, and also epigenetic changes, all not constituting PID in the narrower sense, might gain further importance, which may ultimately lead to changes in our understanding of the concept of what constitutes a primary immunodeficiency or an inborn error of immunity.

References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40(1):24–64
2. Herman SE, Gordon AL, Hertlein E, Ramanunni A, Zhang X, Jaglowski S et al (2011) Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. Blood 117(23):6287–6296
3. Mendel G (1996) Experiments in plant hybridization (1865). Verhandlungen des naturforschenden Vereins Brünn) Available online
4. Morgan TH, Sturtevant AH, Bridges CB, Muller HJ (1915) The mechanism of Mendelian heredity. H. Holt, New York
5. Avery OT, Macleod CM, McCarty M (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types: induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J Exp Med 79(2):137–158
6. Watson JD, Crick FH (1953) Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature 171(4356):737–738
7. Ingram VM (1956) A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. Nature 178(4537):792–794
8. Giblett ER, Anderson JE, Cohen F, Pollara B, Meuwissen HJ (1972) Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 2(7786):1067–1069
9. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74(12):5463–5467
10. Vetrie D, Vorechovský I, Sideras P, Holland J, Davies A, Flinter F et al (1993) The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. Nature 361(6409):226–233
11. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I et al (1993) Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell 72(2):279–290
12. D'Elios MM, Rizzi M (2019) Humoral Primary Immunodeficiencies. Springer, Cham
13. Mensa-Vilaró A, Bravo García-Morato M, de la Calle-Martin O, Franco-Jarava C, Martínez-Saavedra MT, González-Granado LI et al (2019) Unexpected relevant role of gene mosaicism in patients with primary immunodeficiency diseases. J Allergy Clin Immunol 143(1):359–368
14. Vaz-Drago R, Custódio N, Carmo-Fonseca M (2017) Deep intronic mutations and human disease. Hum Genet 136(9):1093–1111
15. Goldschmidt R (1935) Gen und Außeneigenschaft. Z Indukt Abstamm Vererbungsl 69(1):38–69
16. Marian AJ (2010) DNA sequence variants and the practice of medicine. Curr Opin Cardiol 25(3):182–185
17. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM et al (2001) dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29(1):308–311
18. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S et al (2018) ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 46(D1):D1062–D10D7
19. Karczewski KJ, Weisburd B, Thomas B, Solomonson M, Ruderfer DM, Kavanagh D et al (2017) The ExAC browser: displaying reference data information from over 60 000 exomes. Nucleic Acids Res 45(D1):D840–D8D5

20. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q et al (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581(7809):434–443
21. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J (2014) A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46(3):310–315
22. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P et al (2010) A method and server for predicting damaging missense mutations. Nat Methods 7(4):248–249
23. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4(7):1073–1081
24. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424
25. den Dunnen JT, Dalgleish R, Maglott DR, Hart RK, Greenblatt MS, McGowan-Jordan J et al (2016) HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat 37(6):564–569
26. Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PE (2008) Improving sequence variant descriptions in mutation databases and literature using the Mutalyzer sequence variation nomenclature checker. Hum Mutat 29(1):6–13
27. Casanova JL, Conley ME, Seligman SJ, Abel L, Notarangelo LD (2014) Guidelines for genetic studies in single patients: lessons from primary immunodeficiencies. J Exp Med 211(11):2137–2149

Phenocopies of Primary Immunodeficiency Diseases

3

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Abstract

The term "phenocopies of primary immunodeficiency diseases" refers to a group of diseases mimicking the phenotype of primary immunodeficiencies; however, they are caused by somatic mutations or autoantibodies against cytokines rather than germline monogenic defects. They are classified as a separate group by the International Union of Immunological Societies (IUIS).

Keywords

Fas · Fas ligand · TNF · TNF receptor · Autoimmune lymphoproliferative syndrome · Ras · GTPase · Autoantibodies anti-IL-17 · Autoantibodies anti-IL-22 Autoantibodies anti-IFN-γ · Autoantibodies anti-IL-6 · Autoantibodies anti-IL-6 Autoantibodies anti-GM-CSF · Autoantibodies anti-IFN-α · Autoantibodies anti-L-12p70

3.1 Introduction

The phenocopies of primary immunodeficiency diseases have been characterized during the last decades and manifest as a clinical phenocopy to patients with genomic mutations affecting the same biological pathway. In this chapter

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we will provide an updated description of the clinical manifestations, diagnosis, and treatment.

3.2 Phenocopies of PID Caused by Somatic Mutations

The phenocopies of primary immunodeficiency diseases manifest as a clinical phenocopy to patients with genomic mutations affecting the same biological pathway (Fig. 3.1) [1].

The traditional definition of a mosaic is any pattern or image made from multiple pieces; its individual elements can be recognized just by close inspection. In biological organisms, mosaicism denotes an individual with more than one genetically distinct cell population [2]. It might be imperceptible unless closely analyzed. If it takes place during embryonic development, germline and somatic cells will be affected. Otherwise, only somatic cells will be affected. Mosaicism can be caused by DNA mutations, epigenetic factors, and chromosomal abnormalities [3].

Somatic variants require high-throughput sequencing techniques to be detected. During data analysis specific algorithms are fundamental, as these mutations have

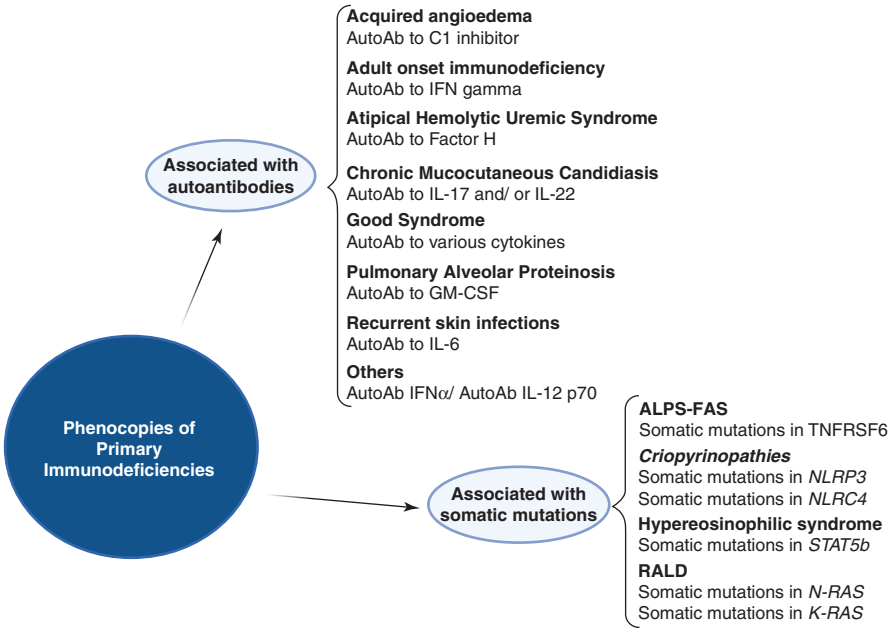

Fig. 3.1 *Classification of phenocopies of primary immunodeficiency diseases.* Phenocopies of PID are a group of diseases caused by somatic mutations or autoantibodies against various cytokines. Clinical manifestations can mimic those of other PIDs. The diseases belonging to each group are shown in the figure

very low allele frequencies in the population. High deep reads are recommended in order to increase the accuracy [4]. Single-cell sequencing has enabled us to detect somatic mutations as heterozygous variants that occur in a subset of cells [5].

Below, we describe several conditions characterized by somatic mutations and mimicking primary immunodeficiency diseases (PID) (Table 3.1).

Table 3.1 Phenocopies of PID. Immunophenotype and clinical characteristics associated with their similar genetic counterpart

Disease	Immunophenotype	Similar features to primary immunodeficiencies
Associated with somatic mutations		
ALPS *TNFRSF6*	Increased DNT cells	Autoimmune cytopenias, defective lymphocyte apoptosis, splenomegaly, lymphadenopathy
RALD *KRAS*	B cells elevated	Autoimmune cytopenias, granulocytosis, monocytosis, splenomegaly, lymphadenopathy
NRAS	Increased DNT cells, B cells elevated	Splenomegaly, lymphadenopathy, autoantibodies
Cryopyrinopathies		
NLRP3	Neutrophilic leukocytosis	Fever, arthropathy, chronic aseptic meningitis, urticarial rash
NLRC4	Neutrophilic leukocytosis	Urticarial rash, chronic meningitis, and arthropathy
Hypereosinophilic syndrome *STAT5b*	Eosinophilia	Persistent eosinophilia with organ involvement atopic dermatitis, urticarial rash, diarrhea
Associated with autoantibodies		
Autoantibodies to IL-17, **Autoantibodies to IL-22**	Normal	Recurrent candida infections of the mucosal surfaces, nails, and skin. Infections may become resistant to antifungals Thymoma
Autoantibodies to IFN gamma	Naïve T cells decreased Hypergammaglobulinemia	Chronic infections with intracellular pathogens, particularly lymphadenitis, skin, soft tissue, and bone infections; constitutional symptoms. Thymoma
Autoantibodies to IL-6	Normal	Recurrent staphylococcal skin infections
Autoantibodies to GM-CSF	Normal	Pulmonary alveolar proteinosis. Progressive respiratory failure. Cryptococcal meningitis
Autoantibodies to IFNα	Normal	Varicella zoster disseminated
Autoantibodies to IL-12p70	Normal	Thymoma, myasthenia gravis

ALPS Autoimmune lymphoproliferative syndrome, *DNT* Double-negative T cells, *RALD* Ras-associated autoimmune leukoproliferative disease

3.2.1 Autoimmune Lymphoproliferative Syndrome (ALPS) Caused by Somatic Mutation in *TNFRSF6* Gene

Autoimmune lymphoproliferative syndrome is a condition of impaired lymphocyte homeostasis, resulting from mutations in genes involved in the Fas pathway. Clinical manifestations include lymphadenopathy, splenomegaly and autoimmune cytopenias. Patients have a predisposition to malignancy, especially lymphomas [6, 7].

FAS (CD95/Apo1) is a cell receptor that belongs to the tumor necrosis factor receptor (TNFR) superfamily. It is codified by the gene *TNFRSF6*. Upon binding to its ligand (Fas ligand), Fas starts a series of events leading to apoptosis to maintain lymphocyte homeostasis [8]. Its role was initially identified in mouse models with a germline mutation in *TNFRSF6* that manifest with autoimmunity [9]. In humans, patients develop a syndrome known as autoimmune lymphoproliferative syndrome (ALPS) [10–12].

If the mutation is clear, ALPS can be categorized as ALPS type Ia (*FAS/TNFRSF6*), ALPS type Ib (FAS ligand), and ALPS type II (caspase 8 or 10 genes). ALPS type III is caused by somatic mutations, and it is the second most common type of ALPS. Somatic mutations have been described in patients without germline mutations but a clinical phenotype similar to other types of ALPS: lymphadenopathy, splenomegaly, hepatomegaly, autoimmunity, elevated DNT cells, increased serum FAS ligand, and elevated levels of IL-10 and vitamin B12 [13–16]. The number of reported cases due to somatic mutations has increased over the last years.

Patients with ALPS type III have a later onset, and the symptoms remain mild for a long period and hence lead to diagnostic delay [17]. The clinical phenotype can suggest a somatic mutation, but this is not enough to make the diagnosis. In vitro studies in cells of these patients have shown Fas-mediated apoptosis, with a higher degree compared to patients with ALPS type Ia [14].

Diagnosis is challenging; all patients with ALPS phenotype, elevated serum biomarkers, and no germline mutation should be evaluated for somatic mutations. The identification of somatic mutations is established by sequencing FAS on double-negative T cells (DNT) [13]. DNT cells seem to be originated from activated peripheral single-positive T cells that received a death-inducing signal but cannot go to apoptosis as they harbor a Fas defect [18].

Treatment is similar to ALPS patients with germline mutations. It focuses on treatment of disease manifestations such as lymphoproliferation and autoimmune cytopenias [19]. Patients require steroid therapy and more than 50% immunosuppressive drugs to control autoimmunity. Malignancy can be treated with conventional protocols. As secondary options, intravenous gammaglobulin, plasmapheresis, and bortezomib should be considered [20]. Hematopoietic stem cell transplantation (HSCT) has been used for refractory patients [21].

3.2.2 RALD: Ras-Associated Autoimmune Leukoproliferative Disease (ALPS like)

Ras-associated autoimmune leukoproliferative disorder (RALD) is characterized by autoimmune manifestations, persistent monocytosis, leukocytosis, and non-malignant lymphoproliferation. Clinical and laboratory features overlap with those of juvenile myelomonocytic leukemia (JMML) and chronic myelomonocytic leukemia (CMML) [22]. The somatic mutations affect genes of the Ras family, *KRAS*, *NRAS*, and *RAS*, involved in myeloid and lymphoid lineages [23]. Mutations found in RALD patients are also reported in around 25% of JMML patients, suggesting a shared molecular etiology [24]. The presence of autoimmunity supports RALD diagnosis, but these patients can have malignant cell transformation and evolve to JMML [25].

RAS (named for their role in forming rat sarcomas) encodes for GTPases important in cell division, cell differentiation, and apoptosis. Opposite to ALPS, DNT cells or serum vitamin B12 levels are not always increased, and there is no defect in Fas-mediated apoptosis. A key feature of RALD is persistent absolute or relative monocytosis [23, 26]. The autoimmune manifestations can mimic lupus with low complement levels and elevated autoantibodies (dsDNA) [27]. Patients with mutations in *NRAS* may have DNT cells elevation [28]. Restricted clonal expansion of TCR and BCR in one patient has been reported; this might explain the reduce lymphocyte repertoire and immunodeficient state in this disease [29].

There are some reported cases with cutaneous involvement known as RALD cutis. Patients present with panniculitis-like erythematous plaques and sweet syndrome. Usually, they have a benign course [30, 31].

Management is based on corticosteroid therapy and other immunomodulatory agents for the autoimmunity. Rituximab has been published as an effective option in patients with refractory cytopenias [32].

3.2.3 Cryopyrinopathies

NLRP3 auto-inflammatory disorders (*NLRP3*-AIDs) were previously known as cryopyrin-associated periodic syndromes (CAPSs), including overlapping entities with increasing severity: familial cold auto-inflammatory syndrome (FCAS); Muckle-Wells syndrome (MWS); chronic infantile neurological, cutaneous, and articular syndrome (CINCA); and neonatal-onset multisystem inflammatory disease (NOMID) [33].

NLRP3-AIDs are autosomal dominant disorders caused by germline mutations in *NLRP3*. The gene encodes for cryopyrin, which leads to hyperactivation of IL-1β [33]. Somatic mutations have been described. Patients present a late onset of the disease and milder symptoms [34–40]. Clinical manifestations include fever, joint involvement, and skin rash. Laboratory workup reveals neutrophilic leukocytosis, elevated C-reactive protein, and erythrocyte sedimentation rate.

A prompt molecular diagnosis is critical; it requires high-deep next-generation sequencing techniques and specific pipelines [41].

Treatment targets IL-1β, and anti-IL1 (anakinra, rilonacept, and canakinumab) are generally effective [42].

A somatic mutation in *NLRC4*, the caspase recruitment domain-containing 4 gene, was found in a Japanese male child with auto-inflammatory symptoms compatible with neonatal-onset multisystem inflammatory disease. The patient had complete response to anakinra [43].

3.2.4 Hypereosinophilic Syndrome Due to Somatic Mutations in *STAT5b* Gene (*STAT5b* Gain-of-Function Mutation)

Somatic mutations in STAT5b have been described in hematologic malignancies [44–46]. Recently, a somatic mutation in STAT5b was found in two patients with eosinophilia, atopic dermatitis, and urticarial rash. The first one, a 3-year-old girl, presented autoimmunity manifestations (alopecia *totalis*). She had history of one event of pneumonia and measles-like illness 10 days after MMR vaccination. The other patient had a severe clinical presentation with recurrent events of bronchiolitis, worsening eosinophilia, failure to thrive, and delayed speech. Gut biopsy revealed eosinophilic infiltrates. She underwent umbilical cord stem cell transplant but died later. Functional tests in CD3-CD4+ T cells showed increase STAT5B responsiveness [47]. Management was based on steroid therapy.

3.3 Phenocopies of PIDs Caused by Autoantibodies against Various Cytokines

Autoantibodies can be found in healthy individuals; they are mainly IgM and have moderate affinity for self-antigens contributing to the homeostasis of the immune system [48]. In contrast, high-affinity and high-titer autoantibodies reflect the loss of balance in effector functions of the immune system. Clinical presentation is correlated with the affected cytokine pathway. These diseases present as a clinical phenocopy of patients with germline mutations in the same associated pathway [49]. Here, we review current knowledge focusing on diseases with increased susceptibility to infections.

3.3.1 Autoantibodies against IL-17 and/or IL-22

Chronic mucocutaneous candidiasis (CMC) is a disorder characterized by recurrent or persistent candida infections involving the skin, nails, and mucous membrane [50]. When the disease is associated with autoimmune hypoparathyroidism and

primary adrenocortical insufficiency is named APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy). APECED is caused by a loss-of-function mutation in *AIRE*, an essential gene in central immune tolerance [51]. These conditions occur in association with impaired IL-17 and IL-22 immunity [50, 52].

Several genetic mutations lead to impair production of IL-17 and IL-22: *IL17F*, *IL17RA*, IL-*17RC*, and *TRAF3IP2/ACT1* [53–55]. Autoantibodies against IL-17F, IL-17A, and IL-22 have been found in patients with CMC [56]. In one study, high titers of autoantibodies against IL-17A, IL-17F, and/or IL-22 were found in 33 APECED patients; from those 29/33 developed CMC [57]. Along with these findings, autoantibodies against IL-17A (41%), IL-17F (75%), and/ or IL-22 (91%) were detected in more than 150 APECED patients, mainly among the CMC group. Remarkably, in this study, autoantibodies were also found in patients with thymoma who later developed CMC [51].

Diagnosis is based on the detection of autoantibodies by techniques such as western blotting and enzyme-linked immunosorbent assay (ELISA). Management includes antifungal therapy and treatment of associated endocrine and infectious manifestations. Members of the azole family are usually effective for the treatment of CMC [58, 59].

3.3.2 Autoantibodies Against IL12p70

IL-12p70 is a heterodimeric molecule consisting of IL-12p35 and IL-12p40 subunits; it signals through a heterodimeric receptor complex of IL-12Rβ1 and IL-12Rβ2 [60]. The signaling pathway IL-12p40-STAT4-IFNγ is involved in protection against intracellular pathogens, such as mycobacterium [61].

There is just one case of anti-IL-12p70 autoantibodies detected in one Cambodian patient. She presented with severe recurrent *Burkholderia gladioli* lymphadenitis and was demonstrated to have isolated neutralizing anti-IL-12p70 autoantibodies as the only immune defect [62]. Interestingly, patients with myasthenia gravis or thymoma have high titers of autoantibodies against IL12p40, but they do not develop infections [63].

3.3.3 Autoantibodies to Interferon-α (IFN-α)

Type I IFNs include IFN-α, IFN-β, and IFN-ω. IFN-α is involved in the transcription of type 1 interferon genes and acts via phosphorylation of STAT1/2 [64, 65]. Autoantibodies to type I IFNs have been detected in healthy donors [66], autoimmune diseases [67–69], malignancy [70], APECED [71], and thymoma [72, 73]. Infections have been reported in a patient with dermatomal varicella zoster reactivation. IFN-α was given as treatment with good response [74].

3.3.4 Autoantibodies to IFN-γ and Susceptibility to Intracellular Pathogens

Interferon-gamma (IFN-γ) is produced by type 1 helper T lymphocytes and NK cells and is crucial for immunity against intracellular pathogens. The IFN-γ receptor is composed of two subunits IFNγR1 and IFNγR2, which combine in duplicate, to form a tetramer, and bind IFN-γ. IFN-γ downstream signaling is mainly through the JAK-STAT1 pathway [52, 75]. Autoantibodies against IFN-γ may impair the production of IL-12. Mendelian susceptibility to mycobacterial disease (MSMD) is a condition that predisposes to infections by low pathogenicity mycobacteria such as nontuberculous mycobacteria (NTM) or bacille Calmette-Guérin (BCG) [76]. Patients are also susceptible to *Salmonella*, *Candida*, and *Mycobacterium tuberculosis*. Genetic defects along these pathways confer similar infection susceptibility [77].

The first cases reporting an association between anti-IFN-γ autoantibodies with severe atypical NMT infection were published in 2004 [78, 79]. Supporting these data, several cases are published, including one study in which 85 patients were enrolled [78–88]. Other opportunistic infections have been reported, including *Salmonella*, *Burkholderia*, *Penicillium*, *Histoplasma*, *Cryptococcus*, and viruses, in particular *varicella zoster virus (VZV)* [89]; these infections resemble those observed in patients with germline mutations in the IFN-γ-IL12 axis.

A high prevalence rate among patients from South East Asia was observed; this was later explained by the discovery of a strong HLA association: HLA-DQB1*05:01/05:02 and DRB1*15:02/16:02. In addition, a major epitope, P12-131, located at the C-terminus of IFN-γ was identified [90, 91].

Patients with opportunistic infections and neutrophilic dermatosis (Sweet syndrome) were reported to have anti-IFN-γ autoantibodies [92]. Lymph nodes are the main site of involvement [93], and 80% of patients have skin manifestations such as reactive dermatoses, erythema nodosum, pustular psoriasis, and exanthematous pustulosis [89, 94].

Laboratory workup reveals features of chronic inflammation including anemia, leukocytosis, elevated erythrocyte sedimentation rate, polyclonal hypergammaglobulinemia, and elevated C-reactive protein (CRP) and/or β2-microglobulin. Other immunological parameters are normal [89]. Undetectable levels or low levels of IFN-γ suggest the presence of autoantibodies. Autoantibodies can be measured using particle-based technology or ELISA [95, 96]. For screening, QuantiFERON-TB Gold In-Tube (QFT-GIT) test can be useful [97].

Management is based on antimicrobial therapy. NTM are usually refractory to first-line therapy and often require second-line drugs for months to years. If the response is poor, immunomodulatory agents can help to decrease autoantibody production. Rituximab has been used in four cases; all patients had a decrease in anti-IFN-γ autoantibody levels. Use of rituximab was reported in a series of four cases, all of which responded clinically, with commensurate decrease in neutralizing capacity [98]. Plasmapheresis and cyclophosphamide were used in one patient [99].

3.3.5 Autoantibodies Against Granulocyte Macrophage Colony Stimulation Factor (GM-CSF)

Granulocyte macrophage colony stimulation factor (GM-CSF) is a growth factor which promotes the immune activation, proliferation, and differentiation of neutrophils, dendritic cells, erythrocyte progenitors, macrophages, and megakaryocytes [100]. In the lung, it is essential for function and differentiation of alveolar macrophages. GM-CSF induces phosphorylation of STAT5, nuclear translocation, and induction of transcription factor PU.1. Together, GM-CSF and PU.1 are essential for surfactant catabolism in the pulmonary alveoli [101–104].

High titers of neutralizing autoantibodies against GM-CSF are associated with pulmonary alveolar proteinosis (PAP) [105]. PAP is a disease linked to congenital or acquired defects in the GM-CSF signaling pathway, causing the impairment of GM-CSF-dependent catabolism of surfactant and leading to accumulation in pulmonary alveoli [106]. PAP is classified in different types according to the underlying pathogenesis: primary PAP characterized by the disruption of GM-CSF signaling which can be autoimmune [107] or hereditary (mutations in CSF2RA or CSF2RB) [108, 109], secondary PAP in patients on immunosuppressive therapy or malignancies [110], and congenital PAP caused by mutations in genes involved in surfactant production [86–88]. Histopathological findings are alveolar filling with acellular periodic acid-Schiff (PAS)-positive proteinaceous material [111].

Autoimmune PAP is the most common, representing approximately 90% of cases [112]. Autoimmune PAP can cause respiratory failure, and it presents between 20 and 50 years of age. The presentation is heterogeneous; it can range from asymptomatic to progressive respiratory failure. Autoantibodies can be detected in the bronchoalveolar lavage (BAL) fluid [113]. It has been suggested its levels may correlate with disease severity and predict the need for additional treatment.

Patients with autoimmune PAP can present defects in neutrophil functions, manifesting as infections by *Nocardia* [114, 115], *nontuberculous mycobacteria (NMT)* [116], *Histoplasma* [117], and *Cryptococcus* [118]. Pulmonary and extrapulmonary infections do not always develop in the same patient. To date, it remains unknown why some patients have just PAP and others just infections.

Useful tools for the diagnostic are pulmonary function tests, which may reveal a restrictive pattern [119]; high-resolution computed tomography (HRCT) of the lungs, which could show a “crazy paving” pattern [120]; and levels of autoantibodies in BAL lavage [121].

The first-line treatment in PAP are whole-lung lavage to remove the proteinaceous material contained in the alveoli and long-term antimicrobial agents for patients with infections [122]. Inhaled and subcutaneous GM-CSF were effective in some studies [123–125]. Rituximab has been used in a small number of patients [126, 127].

3.3.6 Antibodies to Interleukin-6

IL-6 is a cytokine involved in the acute-phase response and in chronic inflammation. It is produced by B and T lymphocytes, macrophages, endothelial cells, hepatocytes, and synovial cells. It regulates the acute phase response in the liver with induction of serum C-reactive protein (CPR) and elevated erythrocyte sedimentation rate [128–130].

Autoantibodies to IL-6 have been found in healthy controls [131, 132] and in four patients associated with severe bacterial infections. The first patient was a 4-year-old boy with a history of recurrent staphylococcal cellulitis and abscesses [133]. The second case was detected in a 20-month-old female with severe septic shock [134]. The third was a 67-year-old man with fatal thoracic empyema by *Escherichia coli* and *Streptococcus intermedius*, and the fourth was a 56-year-old woman with multiple abscesses by *Staphylococcus aureus* [135]. Management included supportive care and antibiotic treatment.

All patients had undetectable levels of CRP despite severity of infections, suggesting impaired IL-6 activity. Functional assays with plasma of patients showed block of activity of IL-6 in vitro. However, IL-6 production from peripheral blood monocytes was normal. Hence, patients with autoantibodies against IL-6 have increased susceptibility to staphylococcal infections; a hint toward the diagnosis is low levels of CRP, despite severity of infection.

3.3.7 Autoantibodies in Good Syndrome

Good syndrome is defined as the triad of thymoma, immunodeficiency, and hypogammaglobulinemia [136]. Clinical manifestations are increased susceptibility to bacterial infections with encapsulated organisms and opportunistic viral and fungal infections. Patients have combined B and T cell immunodeficiency [137, 138]. Anti-cytokine autoantibodies have been identified in these patients and are a potential cause of immunodeficiency [139, 140]. This disorder should be treated by resection of the thymoma and immunoglobulin replacement to maintain adequate trough IgG values. Anti-cytokine autoantibodies have been also associated with infection in patients with thymoma [63]. These need to be further studied.

References

1. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W et al (2018) The 2017 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol 38:129–143
2. Youssoufian H, Pyeritz RE (2002) Mechanisms and consequences of somatic mosaicism in humans. Nat Rev Genet 3(10):748–758. https://doi.org/10.1038/nrg906
3. Gottlieb B, Beitel LK, Trifiro MA (2001) Somatic mosaicism and variable expressivity. Trends Genet 17(2):79–82. https://doi.org/10.1016/S0168-9525(00)02178-8
4. King DA, Sifrim A, Fitzgerald TW et al (2017) Detection of structural mosaicism from targeted and whole-genome sequencing data. Genome Res 27(10):1704–1714. https://doi.org/10.1101/gr.212373.116

5. Dou Y, Gold HD, Luquette LJ, Park PJ (2018) Detecting somatic mutations in Normal cells. Trends Genet 34(7):545–557. https://doi.org/10.1016/j.tig.2018.04.003
6. George LA, Teachey DT (2016) Optimal management of autoimmune lymphoproliferative syndrome in children. Pediatr Drugs 18(4):261–272. https://doi.org/10.1007/s40272-016-0175-3
7. DeNicola DB (2011) Autoimmune lymphoproliferative syndrome: new approaches to diagnosis and management. Top Companion Anim Med 26(2):52–61. https://doi.org/10.1053/j.tcam.2011.02.001
8. Arends MJ, Wyllie AH, Schwartz LM et al (1995) The fas death factor. Science 267(5203):1449–1456
9. Nagata S, Suda T (1995) Fas and Fas ligand: Ipr and gld mutations. Immunol Today 16(1):39–43
10. Fisher GH, Rosenberg FJ, Straus SE et al (1995) Dominant interfering fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 81(6):935–946. https://doi.org/10.1016/0092-8674(95)90013-6
11. Le Deist F, Emile J, Rieux-laucat F et al (1996) Clinical, immunological, and pathological consequences of Fas-deficient conditions. Lancet 348:719–723
12. Sneller BMC, Wang J, Dale JK et al (1997) Clinical, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis. Blood 89:1341–1348
13. Holzelova E, Vonarbourg C, Stolzenberg MC et al (2004) Autoimmune lymphoproliferative syndrome with somatic Fas mutations. N Engl J Med 351(14):1409–1418. https://doi.org/10.1056/NEJMoa040036
14. Dowdell KC, Niemela JE, Price S et al (2010) Somatic FAS mutations are common in patients with genetically undefined autoimmune lymphoproliferative syndrome. Blood 115(25):5164–5169. https://doi.org/10.1182/blood-2010-01-263145
15. Martínez-Feito A, Melero J, Mora-Díaz S et al (2016) Autoimmune lymphoproliferative syndrome due to somatic FAS mutation (ALPS-sFAS) combined with a germline caspase-10 (CASP10) variation. Immunobiology 221(1):40–47. https://doi.org/10.1016/j.imbio.2015.08.004
16. Neven B, Magerus-Chatinet A, Florkin B et al (2011) Asurvey of 90 patients with autoimmune lymphoproliferative syndrome related to TNFRSF6 mutation. Blood 118(18):4798–4807. https://doi.org/10.1182/blood-2011-04-347641
17. García García GM, Bureo Dacal JC, Pineda SS-V, Izaguirre RE (2015) Adult onset autoimmune lymphoproliferative syndrome due to somatic FAS mutation. Intern Med J 45(4):461–462. https://doi.org/10.1111/imj.12711
18. Bleesing JJH, Brown MR, Dale JK et al (2001) TcR-α/β+ CD4-CD8- T cells in humans with the autoimmune lymphoproliferative syndrome express a novel CD45 isoform that is analogous to murine B220 and represents a marker of altered O-glycan biosynthesis. Clin Immunol 100(3):314–324. https://doi.org/10.1006/clim.2001.5069
19. Teachey DT, Lambert MP (2013) Diagnosis and management of autoimmune cytopenias in childhood. Pediatr Clin N Am 60(6):1489–1511. https://doi.org/10.1016/j.pcl.2013.08.009
20. Rao VK (2015) Approaches to managing autoimmune cytopenias in novel immunological disorders with genetic underpinnings like autoimmune lymphoproliferative syndrome. Front Pediatr 3(July):1–6. https://doi.org/10.3389/fped.2015.00065
21. Sleight BJ, Prasad VS, DeLaat C et al (1998) Correction of autoimmune lymphoproliferative syndrome by bone marrow transplantation. Bone Marrow Transplant 22(4):375–380. https://doi.org/10.1038/sj.bmt.1701306
22. Calvo KR, Price S, Braylan RC et al (2015) JMML and RALD (Ras-associated autoimmune leukoproliferative disorder): common genetic etiology yet clinically distinct entities. Blood 125(18):2753–2758. https://doi.org/10.1182/blood-2014-11-567917
23. Niemela JE, Lu L, Fleisher TA et al (2011) Somatic KRAS mutations associated with a human nonmalignant syndrome of autoimmunity and abnormal leukocyte homeostasis. Blood 117(10):2883–2886. https://doi.org/10.1182/blood-2010-07-295501

24. Miyauchi BJ, Asada M, Sasaki M, Tsunematsu Y, Kojima S, Mizutani S (1994) Mutations of the N-ras gene in juvenile chronic myelogenous leukemia. Blood 83(8):2248–2254
25. Herman SEM, Butchar JP, Cheney C, Johnson AJ, Byrd JC (2019) RAS-associated lymphoproliferative disease evolves into severe juvenile myelo-monocytic. Blood 123(12):2–4
26. Oliveira JB, Bidère N, Niemela JE et al (2007) NRAS mutation causes a human autoimmune lymphoproliferative syndrome. Proc Natl Acad Sci U S A 104(21):8953–8958. https://doi.org/10.1073/pnas.0702975104
27. Wang W, Zhou Y, Zhong L et al (2019) RAS-associated autoimmune Leukoproliferative disease (RALD) manifested with early-onset SLE-like syndrome: a case series of RALD in Chinese children. Pediatr Rheumatol 17(1):1–8. https://doi.org/10.1186/s12969-019-0346-1
28. Picard C, Bobby Gaspar H, Al-Herz W et al (2018) International Union of Immunological Societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. J Clin Immunol 38(1):96–128. https://doi.org/10.1007/s10875-017-0464-9
29. Levy-Mendelovich S, Lev A, Rechavi E et al (2017) T and B cell clonal expansion in Ras-associated lymphoproliferative disease (RALD) as revealed by next-generation sequencing. Clin Exp Immunol 189(3):310–317. https://doi.org/10.1111/cei.12986
30. Tran TAN, Grow C-CC WB (2019) Superficial and deep cutaneous involvement by RAS-associated autoimmune Leukoproliferative disease (RALD cutis): a histologic mimicker of Histiocytoid sweet syndrome. Am J Dermatopathol 41(8):606–610
31. Giacaman A, Bauzá Alonso A, Salinas Sanz JA et al (2018) Cutaneous involvement in an 8-year-old boy with Ras-associated autoimmune leucoproliferative disorder (RALD). Clin Exp Dermatol 43(8):913–916. https://doi.org/10.1111/ced.13668
32. Toyoda H, Deguchi T, Iwamoto S et al (2018) Weekly rituximab followed by monthly rituximab treatment for autoimmune disease associated with RAS-associated autoimmune leukoproliferative disease. J Pediatr Hematol Oncol 40(8):e516–e518. https://doi.org/10.1097/MPH.0000000000001276
33. Neven B, Prieur AM, dit Maire PQ (2008) Cryopyrinopathies: update on pathogenesis and treatment. Nat Clin Pract Rheumatol 4(9):481–489. https://doi.org/10.1038/ncprheum0874
34. Rowczenio DM, Gomes SM, Aróstegui JI et al (2017) Late-onset cryopyrin-associated periodic syndromes caused by somatic NLRP3 mosaicism-UK single center experience. Front Immunol 8(OCT). https://doi.org/10.3389/fimmu.2017.01410
35. Lasigliè D, Mensa-Vilaro A, Ferrera D et al (2017) Cryopyrin-associated periodic syndromes in Italian patients: evaluation of the rate of somatic NLRP3 mosaicism and phenotypic characterization. J Rheumatol 44(11):1667–1673. https://doi.org/10.3899/jrheum.170041
36. Zhou Q, Aksentijevich I, Wood GM et al (2015) Late-onset Cryopyrin-associated periodic syndrome due to myeloid-restricted somatic NLRP3 mosaicism. Arthritis Rheum 67(9):2482–2486
37. Omoyinmi E, Gomes SM, Standing A et al (2014) Whole-exome sequencing revealing somatic NLRP3 mosaicism in a patient with chronic infantile neurologic, cutaneous, articular syndrome. Arthritis Rheumatol 66(1):197–202. https://doi.org/10.1002/art.38217
38. Tanaka N, Izawa K, Saito MK et al (2011) High incidence of NLRP3 somatic mosaicism in patients with chronic infantile neurologic, cutaneous, articular syndrome: results of an international multicenter collaborative study. Arthritis Rheum 63(11):3625–3632. https://doi.org/10.1002/art.30512
39. Aróstegui JI, Saldaña MDL, Pascal M et al (2010) A somatic NLRP3 mutation as a cause of a sporadic case of chronic infantile neurologic, cutaneous, articular syndrome/neonatal-onset multisystem inflammatory disease novel: evidence of the role of low-level mosaicism as the pathophysiologic mechanism unde. Arthritis Rheum 62(4):1158–1166. https://doi.org/10.1002/art.27342
40. Nakagawa K, Gonzalez-Roca E, Souto A et al (2015) Somatic NLRP3 mosaicism in muckle-Wells syndrome. A genetic mechanism shared by different phenotypes of cryopyrin-associated periodic syndromes. Ann Rheum Dis 74(3):603–610. https://doi.org/10.1136/annrheumdis-2013-204361

41. Louvrier C, Assrawi E, El Khouri E et al (2020) NLRP3-associated autoinflammatory diseases: phenotypic and molecular characteristics of germline versus somatic mutations. J Allergy Clin Immunol 145(4):1254–1261. https://doi.org/10.1016/j.jaci.2019.11.035
42. Sag E, Bilginer Y, Ozen S (2017) Autoinflammatory diseases with periodic fevers. Curr Rheumatol Rep 19(7):1–10. https://doi.org/10.1007/s11926-017-0670-8
43. Kawasaki Y, Oda H, Ito J et al (2017) Identification of a high-frequency somatic NLRC4 mutation as a cause of autoinflammation by pluripotent cell–based phenotype dissection. Arthritis Rheumatol 69(2):447–459. https://doi.org/10.1002/art.39960
44. Kiel MJ, Velusamy T, Rolland D et al (2014) Integrated genomic sequencing reveals mutational landscape of T-cell prolymphocytic leukemia. Blood 124(9):1460–1472. https://doi.org/10.1182/blood-2014-03-559542
45. Kontro M, Kuusanmäki H, Eldfors S et al (2014) Novel activating STAT5B mutations as putative drivers of T-cell acute lymphoblastic leukemia. Leukemia 28(8):1738–1742. https://doi.org/10.1038/leu.2014.89
46. Rajala HLM, Eldfors S, Kuusanmäki H et al (2013) Discovery of somatic STAT5b mutations in large granular lymphocytic leukemia. Blood 121(22):4541–4550. https://doi.org/10.1182/blood-2012-12-474577
47. Lee LY, Hernandez D, Rajkhowa T, Smith SC, Raman JR, Nguyen B (2017) Somatic STAT5b gain-of-function mutations in early onset nonclonal eosinophilia, urticaria, dermatitis, and diarrhea. Blood 129(2):257–261
48. Elkon KCP (2008) Nature and functions of autoantibodies. Nat Clin Pract Rheumatol 4(9):491–498. https://doi.org/10.1038/jid.2014.371
49. Ludwig RJ, Vanhoorelbeke K, Leypoldt F et al (2017) Mechanisms of autoantibody-induced pathology. Front Immunol 8(MAY). https://doi.org/10.3389/fimmu.2017.00603
50. Okada S, Puel A, Casanova J-L, Kobayashi M (2016) Chronic mucocutaneous candidiasis disease associated with inborn errors of IL-17 immunity. Clin Transl Immunol 5(12):e114. https://doi.org/10.1038/cti.2016.71
51. Kisand K, Bøe Wolff AS, Podkrajšek KT et al (2010) Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 207(2):299–308. https://doi.org/10.1084/jem.20091669
52. Browne SK, Holland SM (2010) Anticytokine autoantibodies in infectious diseases: pathogenesis and mechanisms. Lancet Infect Dis 10(12):875–885. https://doi.org/10.1016/S1473-3099(10)70196-1
53. Lim HK, Migaud M, Israel L et al (2011) Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science 332:65–69
54. Boisson B, Wang C, Pedergnana V et al (2013) An ACT1 mutation selectively abolishes interleukin-17 responses in humans with chronic mucocutaneous candidiasis. Immunity 39(4):676–686. https://doi.org/10.1016/j.immuni.2013.09.002
55. Ling Y, Cypowyj S, Aytekin C, Galicchio M, Camcioglu YNS et al (2015) Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis. J Exp Med 212:619–631
56. Sarkadi AK, Taskó S, Csorba G, Tóth B, Erdos M, Maródi L (2014) Autoantibodies to IL-17A may be correlated with the severity of mucocutaneous candidiasis in APECED patients. J Clin Immunol 34(2):181–193. https://doi.org/10.1007/s10875-014-9987-5
57. Puel A, Döffinger R, Natividad A et al (2010) Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 207(2):291–297. https://doi.org/10.1084/jem.20091983
58. Horsburgh CR, Kirkpatrick CH (1983) Long-term therapy of chronic mucocutaneous candidiasis with ketoconazole: Experience with twenty-one patients. Am J Med 74(1 PART 2):23–29. https://doi.org/10.1016/0002-9343(83)90511-9
59. Levo Y (2001) Chronic mucocutaneous candidiasis. Pediatr Infect Dis J 20:197–206. https://doi.org/10.1136/bmj.2.5914.334
60. Presky DH, Yang H, Minetti LJ et al (1996) A functional interleukin 12 receptor complex is composed of two β-type cytokine receptor subunits. Proc Natl Acad Sci U S A 93(24):14,002–14,007. https://doi.org/10.1073/pnas.93.24.14002

61. Hölscher C, Atkinson RA, Arendse B et al (2001) A protective and agonistic function of IL-12p40 in mycobacterial infection. J Immunol 167(12):6957–6966. https://doi.org/10.4049/jimmunol.167.12.6957
62. Sim BTT, Browne SK, Vigliani M et al (2013) Recurrent burkholderia gladioli suppurative lymphadenitis associated with neutralizing anti-IL-12p70 autoantibodies. J Clin Immunol 33(6):1057–1061. https://doi.org/10.1007/s10875-013-9908-z
63. Burbelo PD, Browne SK, Sampaio EP et al (2010) Anti-cytokine autoantibodies are associated with opportunistic infection in patients with thymic neoplasia. Blood 116(23):4848–4858. https://doi.org/10.1182/blood-2010-05-286161
64. Lavoie TB, Kalie E, Crisafulli-Cabatu S et al (2011) Binding and activity of all human alpha interferon subtypes. Cytokine 56(2):282–289. https://doi.org/10.1016/j.cyto.2011.07.019
65. DA Szabo G (2008) The role of plasmacytoid dendritic cell–derived IFNα in antiviral immunity. Crit Rev Immunol 28(1):61–94
66. Ross C, Hansen MB, Schyberg T, Berg K (2008) Autoantibodies to crude human leucocyte interferon (IFN), native human IFN, recombinant human IFN-alpha 2b and human IFN-gamma in healthy blood donors. Clin Exp Immunol 82(1):57–62. https://doi.org/10.1111/j.1365-2249.1990.tb05403.x
67. Morimoto AM, Flesher DT, Yang J et al (2011) Association of endogenous anti-interferon-α autoantibodies with decreased interferon-pathway and disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 63(8):2407–2415. https://doi.org/10.1002/art.30399
68. Baechler EC, Batliwalla FM, Karypis G et al (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A 100(5):2610–2615. https://doi.org/10.1073/pnas.0337679100
69. Slavikova M, Schmeisser H, Kontsekova E, Mateička F, Borecky L, Kontsek P (2003) Incidence of autoantibodies against type I and type II interferons in a cohort of systemic lupus erythematosus patients in Slovakia. J Interf Cytokine Res 23(3):143–147. https://doi.org/10.1089/107999003321532475
70. Trown PW, Dennin RA, Kramer MJ, Connell EV, Palleroni AV, Gutterman JU (1983) Antibodies to human leucocyte interferons in cancer patients. Lancet 8316(1):81–84
71. Meager A, Visvalingam K, Peterson P et al (2006) Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med 3(7):1152–1164. https://doi.org/10.1371/journal.pmed.0030289
72. Meager A, Wadhwa M, Dilger P, Bird C, Thorpe R et al (2003) Anti-cytokine autoantibodies in autoimmunity preponderance. Clin Exp Immunol 132:128–136
73. Shiono H, Wong YL, Matthews I et al (2003) Spontaneous production of anti-IFN-α and anti-IL-12 autoantibodies by thymoma cells from myasthenia gravis patients suggests autoimmunization in the tumor. Int Immunol 15(8):903–913. https://doi.org/10.1093/intimm/dxg088
74. Pozzetto B, Mogensen KE, Tovey MG, Gresser I (1984) Characteristics of autoantibodies to human interferon in a patient with varicella-zoster disease. J Infect Dis 150(5):707–713. https://doi.org/10.1093/infdis/150.5.707
75. Bhat MY, Solanki HS, Advani J, Khan AA, Prasad TSK (2018) Comprehensive network map of interferon gamma signaling. J Cell Commun Signal 12(4):745–751
76. Bustamante J, Boisson-dupuis S (2014) Mendelian susceptibility to mycobacterial disease: Genetic, immunological, and clinical features of inborn errors of IFN-gamma immunity. Semin Immunol 26(6):454–470. https://doi.org/10.1016/j.smim.2014.09.008
77. Al-muhsen S, Casanova J, Arabia S (2008) The genetic heterogeneity of Mendelian susceptibility to mycobacterial diseases. J Allergy Clin Immunol 122(6):1043–1051. https://doi.org/10.1016/j.jaci.2008.10.037
78. Doffinger R, Helbert MR, Barcenas-Morales G, Yang K, Dupuis S et al (2004) Autoantibodies to interferon-gamma in a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity. Clin Infect Dis 38(1):e10–e14
79. Hoflich C, Sabat R, Rosseau S, Temmesfeld B, Slevogt H et al (2004) Naturally occurring anti—IFN-gamma autoantibody and severe infections with Mycobacterium chelo-

neae and Burkholderia cocovenenans. Blood 103(2):673–675. https://doi.org/10.1182/blood-2003-04-1065.Reprints
80. Browne SK, Zaman R, Sampaio EP et al (2012) Anti-CD20 (rituximab) therapy for anti-IFN-γ autoantibody-associated nontuberculous mycobacterial infection. Blood 119(17):3933–3939. https://doi.org/10.1182/blood-2011-12-395707
81. Chi CY, Chu CC, Liu JP, Lin CH, Ho MW et al (2012) Anti-interferon-γ autoantibodies in adults with disseminated nontuberculous mycobacterial infections are associated with HLA-DRB1*16:02 and DQB1*05:02 and the reactivation of latent varicella-zoster virus infection. Blood 121:1357–1366
82. Kampitak T, Suwanpimolkul G, Browne S, Suankratav C (2010) Anti-interferon-γ autoantibody and opportunistic infections case series and review of the literature. Infection 39:65–71
83. Kampmann B, Hemingway C, Stephens A, Davidson R, Goodsall A et al (2005) Acquired predisposition to mycobacterial disease due to autoantibodies to IFN-γ. J Clin Investig 115:2480–2488
84. Koya T, Tsubata C, Kagamu H et al (2009) Anti-interferon- g autoantibody in a patient with disseminated Mycobacterium avium complex. J Infect Chemother 15(2):118–122. https://doi.org/10.1007/s10156-008-0662-8
85. Patel SY, Ding L, Brown MR, Lantz L, Gay T et al (2005) Anti-IFN-γ autoantibodies in disseminated nontuberculous mycobacterial infections. J Immunol 175:4769–4776
86. Poulin S, Corbeil C, Nguyen M et al (2013) Fatal Mycobacterium colombiense/cytomegalovirus coinfection associated with acquired immunodeficiency due to autoantibodies against interferon gamma: a case report. BMC Infect Dis:1–6. Published online. https://doi.org/10.1186/1471-2334-13-24
87. Tanaka Y, Hori T, Ito K, Fujita T, Ishikawa T et al (2007) Disseminated Mycobacterium avium complex infection in a patient with autoantibody to interferon-γ. Intern Med 46:1005–1009
88. Tang BS, Chan JF, Chen M et al (2010) Disseminated penicilliosis, recurrent bacteremic nontyphoidal salmonellosis, and burkholderiosis associated with acquired immunodeficiency due to autoantibody against gamma interferon. Clin Vaccine Immuno 17(7):1132–1138. https://doi.org/10.1128/CVI.00053-10
89. Browne SK, Burbelo PD, Chetchotisakd P et al (2012) Adult-onset immunodeficiency in Thailand and Taiwan. N Engl J Med 367(8):725–734. https://doi.org/10.1056/NEJMoa1111160
90. Lin C-H, Chi C-Y, Shih H-P, Ding J-Y, Lo C-C, Wang S-Y, Kuo C-Y, Yeh C-F, Tu K-H, Liu S-H, Chen H-K, Ho C-H, Ho M-W, Lee C-H, Lai H-C, Ku C-L (2016) Identification of a major epitope by anti-interferon-γ autoantibodies in patients with mycobacterial disease. Nat Med 22:994–1001. https://www.nature.com/articles/nm.4158
91. Ku C-L, Lin C-H, Chang S-W, Chu C-C, Chan JFW, Kong X-F, Lee C-H, Rosen EA, Ding J-Y, Lee W-I, Bustamante J, Witte T, Shih H-P, Kuo C-Y, Chetchotisakd P, Kiertiburanakul S, Suputtamongkol Y, Yuen K-Y, Casa C-Y (2016) Anti-IFN-γ autoantibodies are strongly associated with HLA-DR*15:02/16:02 and HLA-DQ*05:01/05:02 across Southeast Asia. J Allergy Clin Immunol 137:945–948
92. Chan JFW, Trendell-Smith NJ, Chan JCY et al (2013) Reactive and infective dermatoses associated with adult-onset immunodeficiency due to anti-interferon-gamma autoantibody: Sweet's syndrome and beyond. Dermatology 226(2):157–166. https://doi.org/10.1159/000347112
93. Wongkulab P, Wipasa J, Chaiwarith R, Supparatpinyo K (2013) Autoantibody to interferon-gamma associated with adult-onset immunodeficiency in non-HIV individuals in northern Thailand. PLoS One 8(9):e76371. https://doi.org/10.1371/journal.pone.0076371
94. Jutivorakool K, Sittiwattanawong P, Kantikosum K, Hurst CP, Kumtornrut C, Asawanonda P, Klaewsongkram J, Rerknimitr P (2018) Skin manifestations in patients with adult-onset immunodeficiency due to anti-interferon-gamma autoantibody: a relationship with systemic infections. Acta Derm Venereol 98(8):742–747. https://doi.org/10.2340/00015555-2959
95. Ding L, Mo A, Jutivorakool K, Pancholi M, Holland SM, Browne SK (2012) Determination of human anticytokine autoantibody profiles using a particle-based approach. J Clin Immunol 32(2):238–245. https://doi.org/10.1007/s10875-011-9621-8

96. Browne SK (2014) Anticytokine autoantibody–associated immunodeficiency. Annu Rev Immunol 32(1):635–657. https://doi.org/10.1146/annurev-immunol-032713-120222
97. Wu UI, Chuang YC, Sheng WH et al (2018) Use of QuantiFERON-TB gold in-tube assay in screening for neutralizing anti-interferon-γ autoantibodies in patients with disseminated nontuberculous mycobacterial infection. Clin Microbiol Infect 24(2):159–165. https://doi.org/10.1016/j.cmi.2017.06.029
98. Browne SK, Zaman R, Sampaio EP, Jutivorakool KRL (2012) Anti-CD20 (rituximab) therapy for anti-interferon-γ autoantibody-associated nontuberculous mycobacterial infection. Blood 119:3933–3939. https://www.annualreviews.org/servlet/linkout?suffix=B68&dbid=16&doi=10.1146%2Fannurev-immunol-032713-120222&key=10.1086%2F605581
99. Baerlecken N, Jacobs R, Stoll M, Schmidt REWT (2009) Recurrent, multifocal Mycobacterium avium-intercellulare infection in a patient with interferon-γ autoantibody. Clin Infect Dis 49:e76–e78
100. Becher B, Tugues S, Greter M (2016) Review GM-CSF: from growth factor to central mediator of tissue inflammation. Immunity 45(5):963–973. https://doi.org/10.1016/j.immuni.2016.10.026
101. Akagawa KS, Kamoshita KTT (1988) Effects of granulocyte-macrophage colony-stimulating factor and colony-stimulating factor-1 on the proliferation and differentiation of murine alveolar macrophages. J Immunol:3383–3390. Published online
102. Shibata Y, Berclaz P, Chroneos ZC, Yoshida M, Whitsett JA, Trapnell BC (2001) GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU. 1. Immunity 15:557–567
103. Bonfield TL, Raychaudhuri B, Malur A et al (2003) PU.1 regulation of human alveolar macrophage differentiation requires granulocyte-macrophage colony-stimulating factor. Am J Physiol Lung Cell Mol Physiol 285:1132–1136. https://doi.org/10.1152/ajplung.00216.2003
104. Chen BD, Mueller M, Chou T (1988) Role of granulocyte/macrophage colony-stimulating factor in the regulation of murine alveolar macrophage proliferation and differentiation. J Immunol 141:139–144. Information about subscribing to The Journal of Immunology is online at the regulation of murine alveolar macr
105. Factor GMC, Kitamura BT, Tanaka N, Watanabe J (1999) Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against. J Exp Med 190(6):875–880
106. Trapnell BC, Whitsett JA, Nakata K (2003) Pulmonary alveolar proteinosis. N Engl J Med 349(26):2527–2539
107. Seymour JF, Presneill JJ (2002) Pulmonary alveolar proteinosis: progress in the first 44 years. Am J Respir Crit Care Med 166:7–9. https://doi.org/10.1164/rccm.2109105
108. Suzuki T, Sakagami T, Young LR et al (2010) Hereditary pulmonary alveolar proteinosis: pathogenesis, presentation, diagnosis, and therapy. Am J Respir Crit Care Med 182:1292–1304. https://doi.org/10.1164/rccm.201002-0271OC
109. Tanaka T, Motoi N, Tsuchihashi Y et al (2011) Adult-onset hereditary pulmonary alveolar proteinosis caused by a single-base deletion in CSF2RB. J Med Genet 48(3):205–209. https://doi.org/10.1136/jmg.2010.082586
110. Fieury-feith J (1996) Secondary alveolar proteinosis in cancer patients. Support Care Cancer 4(6):420–426
111. Carey B, Trapnell BC (2010) The molecular basis of pulmonary alveolar proteinosis. Clin Immunol 135(2):223–235. https://doi.org/10.1016/j.clim.2010.02.017
112. Inoue Y, Trapnell BC, Tazawa R, Arai T, Takada T, Hizawa N (2008) Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. Am J Respir Crit Care Med 177:752–762. https://doi.org/10.1164/rccm.200708-1271OC
113. Kitamura T, Uchida K, Tanaka N et al (2000) Serological diagnosis of idiopathic pulmonary alveolar proteinosis. Am J Respir Crit Care Med 162:658–662. https://doi.org/10.1164/ajrccm.162.2.9910032
114. Wgh O, Enh J, Rl P (1998) Primary EMMM. Primary cerebellar nocardiosis and alveolar proteinosis. Acta Neurol Scand:138–141. Published online

115. Andersen BR, Eclund REKW (1960) Pulmonary alveolar proteinosis with systemic nocardiosis. JAMA 3(174):28–31
116. Witty LA, Tapson VFPC (1994) Isolation of mycobacteria in patients with pulmonary alveolar proteinosis. Medicine (Baltimore) 73:103–109
117. Hartung MSK (1975) Pulmonary alveolar proteinosis and histoplasmosis: report of three cases. Virchows Arch A Pathol Anat Histol 368(4):281–287
118. Rosen LB, Freeman AF, Yang LM et al (2013) Anti–GM-CSF autoantibodies in patients with cryptococcal meningitis. J Immunol 190(8):3959–3966. https://doi.org/10.4049/jimmunol.1202526
119. Borie R, Danel C, Debray M, Taille C, Dombret M, Aubier M (2011) Pulmonary alveolar proteinosis. Eur Respir Rev 20(120):98–107. https://doi.org/10.1183/09059180.00001311
120. Holbert JM, Costello P, Hoffman RM, Rogers RM (2001) CT features of pulmonary alveolar. Am J Roentgenol 176:1287–1294
121. Campo I, Kadija Z, Mariani F et al (2012) Pulmonary alveolar proteinosis: diagnostic and therapeutic challenges. Multidiscip Respir Med 7:4
122. Griese M (2017) Pulmonary alveolar proteinosis: a comprehensive clinical perspective. Pediatrics 140(2):e20170610
123. Kavuru MS, Sullivan EJ, Piccin R, Thomassen MJ, Stoller JK (2000) Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis. Am J Respir Crit Care Med Care Med 161:1143–1148
124. Seymour JF, Presneill JJ, Schoch OD et al (2001) Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. Am J Respir Crit Care Med 163:524–531
125. Tazawa R, Trapnell BC, Inoue Y et al (2010) Inhaled granulocyte/macrophage—colony stimulating factor as therapy for pulmonary alveolar proteinosis. Am J Respir Crit Care Med 181:1345–1354. https://doi.org/10.1164/rccm.200906-0978OC
126. Kavuru MS, Malur A, Marshall I et al (2011) An open-label trial of rituximab therapy in pulmonary alveolar proteinosis. Eur Respir J 38(6):1361–1367. https://doi.org/10.1183/09031936.00197710
127. Borie R, Debray MP, Laine C, Aubier M, Crestani B (2009) Rituximab therapy in autoimmune pulmonary alveolar proteinosis. Eur Respir J 33:1503–1506. https://doi.org/10.1183/09031936.00160908
128. Jones SA (2005) Directing transition from innate to acquired immunity: defining a role for IL-6. J Immunol 175:3463–3468. https://doi.org/10.4049/jimmunol.175.6.3463
129. Majello B, Arcone R, Toniatti C (1990) Constitutive and IL-6-induced nuclear factors that interact with the human C-reactive protein promoter. EMBO 9(2):457–465
130. Heinrich PC, Castellt JV (1990) Interleukin-6 and the acute phase response. Biochem J 265:621–636
131. Galle P, Svenson M, Bendtzen K, Hansen MB (2004) High levels of neutralizing IL-6 autoantibodies in 0.1% of apparently healthy blood donors. Eur J Immunol 34:3267–3275. https://doi.org/10.1002/eji.200425268
132. Hansen MB, Diamant M, Svenson M, Bendtzen K (1991) Anti-interleukin-6 antibodies in normal human serum. Scand J Immunol 33:777–781
133. Puel A, Picard C, Lorrot M et al (2008) Recurrent staphylococcal cellulitis and subcutaneous abscesses in a child with autoantibodies against IL-6. J Immunol 180:647–654. https://doi.org/10.4049/jimmunol.180.1.647
134. Bloomfield M, Parackova Z, Cabelova T, Pospisilova I (2019) Anti-IL6 autoantibodies in an infant with CRP-less septic shock. Front Immunol 10:1–6. https://doi.org/10.3389/fimmu.2019.02629
135. Nanki T, Onoue I, Nagasaka K, Takayasu A, Ebisawa M et al (2013) Suppression of elevations in serum C reactive protein levels by anti-IL-6 autoantibodies in two patients with severe bacterial infections. Rheum Dis 72(6):1100–1102
136. Kelleher P, Misbah SA (2003) What is Good's syndrome? Immunological abnormalities in patients with thymoma. J Clin Pathol 56:12–16

137. Trober WAS, Undles CHCU, Ucey DARL (2001) Infections in patients with immunodeficiency with thymoma (Good Syndrome). Report of 5 cases and review of the literature. Medicine (Baltimore) 80(2):123–133
138. Agarwal S, Cunningham-Rundles C (2007) Thymoma and immunodeficiency (good syndrome): a report of 2 unusual cases and review of the literature. Ann Allergy Asthma Immunol 98(2):185–190. https://doi.org/10.1016/S1081-1206(10)60695-3.Thymoma
139. Meager A, Vincent A, Willcox N (1997) Spontaneous neutralising antibodies to interferon--alpha and interleukin-12 in thymoma-associated autoimmune disease. Lancet 350:1996–1997
140. Jansen A, Van Deuren M, Miller J, Litzman J, De Gracia J, Sáenz-cuesta M (2016) Prognosis of Good syndrome: mortality and morbidity of thymoma associated immunodeficiency in perspective. Clin Immunol 171:12–17. https://doi.org/10.1016/j.clim.2016.07.025

Dysfunctional Immune Synapses in T Cell Immunodeficiencies

4

Nagaja Capitani, Laura Patrussi, Mario Milco D'Elios, and Cosima Tatiana Baldari

Abstract

Adaptive immune responses take place in the T cell area of secondary lymphoid organs, where, on encounter of antigen-presenting cells (APCs) bearing specific MHC-associated antigen, T lymphocytes assemble a highly organized intercellular junction, referred to as the immune synapse, on which T cell activation, proliferation, and differentiation crucially depend. Immune synapse assembly and function are impaired in a subgroup of human immunodeficiencies, characterized by altered adhesion or defective cross talk between the two synaptic partners. In this chapter, we will provide an overview of the immune synapse and describe how dysfunctional immune synapses can impinge on the pathogenesis of T cell immunodeficiencies, highlighting the reciprocal contribution of alterations at either side of the IS.

Keywords

Immune synapse · T cell immunodeficiencies · F-actin dynamics · Adhesion Chemotaxis

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4.1 Introduction

Efficient immune responses are dependent on a finely tuned communication network among immune cells themselves and with other cells of the surrounding tissue, which is mediated by specialized, transient cell-cell junctions. The paradigm of this mode of communication is represented by T lymphocytes, whose activation on encounter of a cognate antigen-presenting cell (APC) is accompanied by rapid architectural changes that result in the assembly of a specialized interface known as the immune synapse (IS).

All T cells express a unique T cell receptor (TCR), which is dedicated to recognizing peptides presented by the major histocompatibility complex (MHC) on the surface of APCs. Engagement of cognate peptide-MHC (pMHC) by the TCR induces robust proliferative, transcriptional, and secretory responses that are generally associated with T cell activation [1]. Early after antigen recognition, the T cell/APC contact area rapidly reorganizes to assemble an immune synapse [1, 2], where the cellular partners communicate via membrane-bound receptors and secreted mediators. Within the IS, tight membrane apposition between T cell and APC is essential for ligand-dependent receptor activation and downstream signaling and is also a prerequisite for the polarized secretion of soluble mediators in the intercellular milieu [3]. Already in the early 1990s, different research groups contributed to define the IS as a "small space between the two interacting cells," to describe the confined area where polarized cytokine secretion takes place [4–6]. Twenty-five years later, the many molecular steps required to establish this extremely complex platform—initial exploration, contact formation and eventual stabilization—have been in part elucidated. Once a stable interaction is established, the IS matures to become a highly organized signaling platform, where both the intensity and the duration of the signals emanating from the TCR and co-engaged receptors determine cell fate.

The importance of the signaling pathways elicited at IS is witnessed by a subset of human pathologies, known as T cell immunodeficiencies, whose etiology is related to mutations of genes controlling these pathways. Here, we describe the structure and functions of the IS and analyze the outcome of IS defects on the immune response in the context of T cell immunodeficiencies.

4.2 The Immune Synapse

4.2.1 Architecture of the Immune Synapse

During IS formation receptors, adhesion molecules, cytoskeletal components, and organelles polarize toward the T cell contact area with the cognate APC [1, 7–9]. The canonical organization of the mature IS was described as a bull's eye with a central region, defined as central supramolecular activation complex (cSMAC), surrounded by a peripheral SMAC (pSMAC) and a distal SMAC (dSMAC). The cSMAC is characterized by the presence of TCRs, together with other

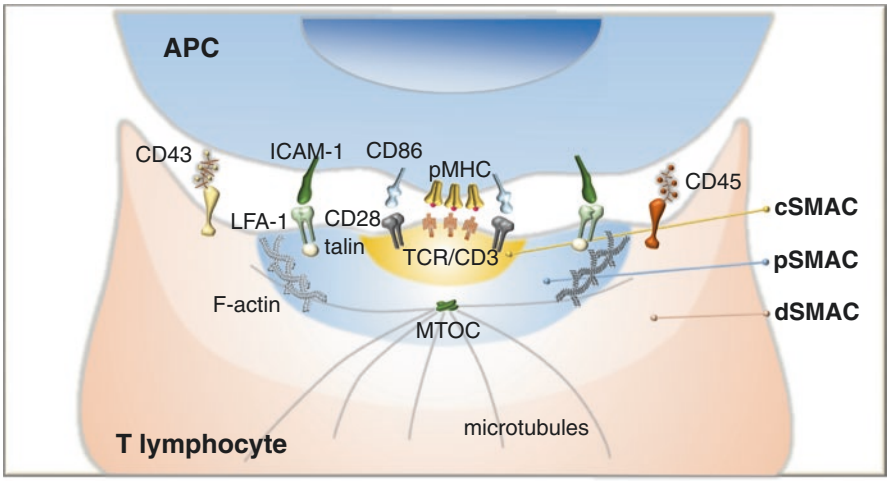

Fig. 4.1 Organization of the mature immune synapse. The highly organized structure of the mature immune synapse (IS), with a central supramolecular activation complex (cSMAC) enriched in TCR and CD28, a peripheral SMAC (pSMAC) enriched in LFA-1, and a distal SMAC (dSMAC), enriched in actin filaments (F-actin) coupled to integrins by the adaptor talin. The microtubule-organizing center (MTOC) reorients toward the T lymphocyte-APC contact zone, together with the intracellular secretory machinery

co-receptors and co-stimulatory molecules such as CD28, CD4, CD8, and CD2 and their associated signaling proteins. The pSMAC, highly enriched in LFA-1 and in the cytoskeletal linker talin, describes a ring of tight adhesion between interacting cells [10, 11]. Beyond the pSMAC, an additional domain forms, known as dSMAC, consisting of a circular array of filamentous actin (F-actin), which contains molecules that are not specifically recruited to the cSMAC or pSMAC or selectively excluded because either the large size of their ectodomain (e.g., CD43) or their function, as negative regulators of signaling (e.g., the transmembrane tyrosine phosphatase CD45), can hamper the assembly and maintenance of a functional IS [12]. A hallmark of the IS is the TCR-dependent reorientation of the microtubule-organizing center (MTOC) toward the APC contact area, which is accompanied by the polarization of the intracellular secretory machinery [13, 14] (Fig. 4.1).

4.2.2 Signaling Pathways at the Immune Synapse

Following TCR engagement, the immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic domains of the TCR complex subunits are phosphorylated by the combined action of the Src family kinase Lck and the Syk family kinase ZAP-70 [15]. These phosphorylation events prime a signaling cascade that leads to the phosphorylation of the transmembrane adapter protein linker of activated T cells

(LAT) and the recruitment of the phospholipase Cγ (PLCγ). Once fully activated, PLCγ induces sustained Ca^{2+} signaling and calcineurin activation, leading to dephosphorylation and nuclear translocation of the nuclear factor of activated T cells NFAT [16]. The transcription factors AP-1 (activation protein 1) and nuclear factor-κB (NF-κB), which together with NFAT initiate the transcriptional cascade, leading to the clonal proliferation of the activated T cell and its subsequent differentiation to an effector or memory cell, are activated through different pathways. AP-1 activation is mediated by the combined action of the adapter protein Grb2-guanine nucleotide exchange factor (GEF) Sos-Ras GTPase-Erk1/2 pathway and the GEF Vav1-Rac GTPase-Jun pathway. NF-κB activation is coordinated by two interconnected pathways, the serine/threonine kinase PKCθ-IKK (IκB kinase)-IκB (inhibitor of κB) pathway [17] and the pathway involving the molecular scaffold CARMA1-Bcl10-MALT1 protein complex (commonly known as the CBM complex), which is initiated by PKCθ-mediated phosphorylation of CARMA1 [18, 19] (Fig. 4.2).

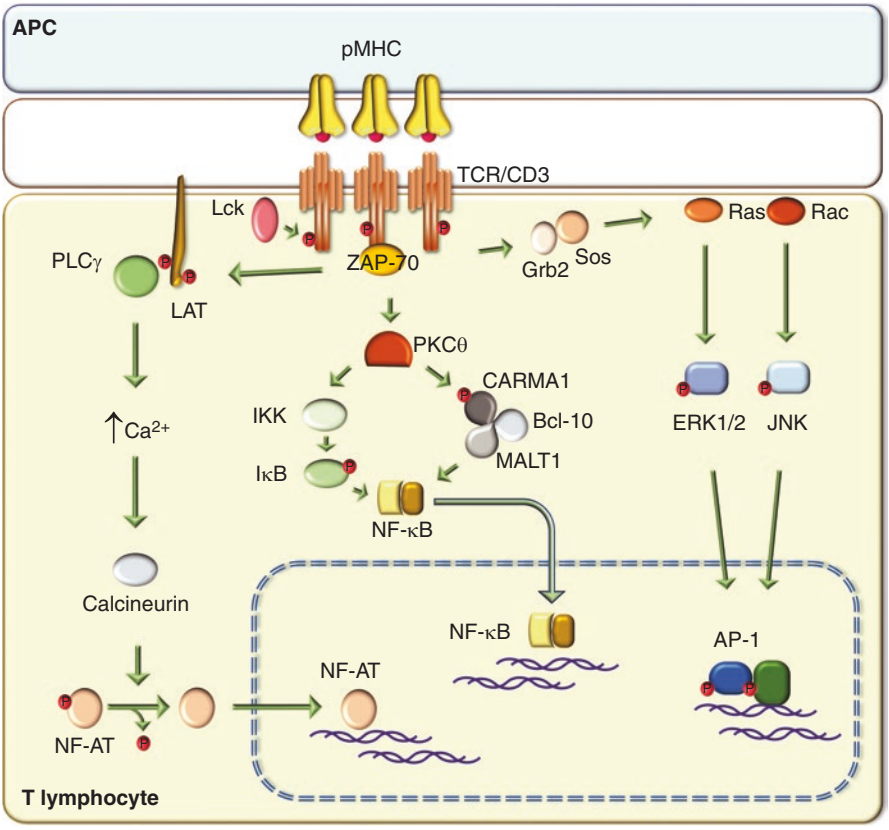

Fig. 4.2 Transcription factors activated by TCR/CD3 at the IS. Distinct signaling pathways, elicited by TCR/CD3 binding to cognate peptide-MHC (pMHC), trigger nuclear translocation and/or activation of the transcription factors NFAT, NF-κB, and AP-1 in T lymphocytes

TCR triggering also eventually results in the recruitment and/or activation of phosphatases and ubiquitin ligases to timely turn off signaling at the IS. Among the phosphatases, SHP-1 is a major player. Following its SH2 domain-mediated interaction with the immunoreceptor tyrosine-based inhibitory motifs (ITIM) in the cytoplasmic domains of inhibitory receptors, such as CTLA-4, PD-1, or LAIR, SHP-1 effectively counteracts tyrosine kinase signaling, leading to signal termination [20].

Because of the high local TCR concentration, the cSMAC was initially considered the synaptic domain, where sustained signaling occurs [8, 21]. However, the development of sophisticated methods for the study of IS assembly in live cells has added another layer of complexity to this process. It is now clear that signaling occurs at TCR microclusters that form at the pSMAC and move centripetally toward the cSMAC, powered by the actomyosin cytoskeleton, to eventually become signaling-incompetent as they accumulate at the cSMAC [22, 23]. There, exhausted TCRs are internalized and undergo ubiquitylation, which leads to their degradation both at lysosomes and via the proteasome [24]. Ubiquitylated TCRs are also released as ectosomes, a process coordinated by the endosomal sorting complex ESCRT-I [25]. Interestingly, internalization of exhausted TCRs is counterbalanced by fresh supplies of TCRs from an intracellular pool associated with recycling endosomes that undergoes polarized recycling to the IS [26].

Cell–cell adhesion is a prerequisite for T cells to probe APCs for the presence of cognate pMHC as well as for the subsequent establishment of a stable IS. Integrins are α/β heterodimeric receptors that play a critical role during IS formation. Upon TCR engagement, LFA-1, the most important integrin in IS formation and maintenance [27, 28], rapidly polarizes to the synaptic area, where it becomes activated. A panel of molecules were identified as key players in the pathway linking TCR activation to LFA-1 clustering, a process known as inside-out signaling. These include the small Rho GTPases and their GEF Vav1 [29, 30] and the small GTPase Rap1 and its regulators [31], the adaptors ADAP [32, 33] and SKAP55 [34–36], and the GEF RIAM [37–40]. Inside-out signaling induces a conformational change in LFA-1, leading to enhanced LFA-1-/ICAM-1-mediated cell aggregation [41, 42]. LFA-1 clustering and activation are further enhanced by Vav1-/Arp2/3-mediated actin reorganization and association with the actin-binding protein talin [40]. Once in its fully active conformation, LFA-1 engages in a high-affinity interaction with its ligand ICAM-1 on the surface of the APC, which stabilizes the T cell-APC interaction, allowing for sustained TCR signaling and T cell activation [43]. In addition to promoting efficient TCR clustering, LFA-1 binding to ICAM-1 also activates the Erk/MAPK pathway, which contributes to the reorientation of the MTOC toward the IS [44].

Chemokines produced by APCs have been implicated in regulating the duration of the T cell-APC interaction at the IS [45]. Chemokine signaling induces indeed a rapid increase in the affinity of LFA-1 for ICAM-1, thereby contributing to stabilize T cell-APC conjugates [46]. This is exemplified by the chemokine-dependent recruitment and accumulation of the chemokine receptors CCR5 and CXCR4 at the IS, which results in stronger T cell-APC attraction, reduction of T cell

responsiveness to chemotactic gradients, and higher proliferative responses and cytokine production by the T cell [47]. Similarly, the chemokine receptor CCR7 has been recently reported to accumulate to the IS, enhancing TCR-dependent LFA-1 activation to promote cell adhesion [48].

4.2.3 Actin Dynamics at the Immune Synapse

The actin cytoskeleton plays a central role in the formation and maintenance of the IS [49]. Initially, a uniform sheet of F-actin spreads radial symmetric protrusions over the surface of the APC. During IS stabilization, cortical F-actin reorganizes in a peripheral ring at the dSMAC. Continuous retrograde flow within the dSMAC promotes adhesion by clustering LFA-1 at the pSMAC, thereby contributing to control TCR signaling and to maintain IS symmetry. F-actin depletion from the center of the IS eventually generates an "actin hypodense" region in correspondence to the subsynaptic localization of the MTOC that plays a key role in vesicular trafficking to and from the plasma membrane and hence in T cell activation and effector functions. This is most clearly exemplified by the lytic synapse formed by cytotoxic T cells, where actin depletion is a prerequisite for granule release [50].

T cell actin dynamics are regulated by different classes of molecules. Among these, a key role is played by type I and type II nucleation-promoting factors (NPFs) [51, 52]. Type I NPFs induce robust actin polymerization by recruiting and activating the Arp2/3 complex, driving the growth of branched F-actin arrays [52]. The two predominant class I NPFs in T cells are the Wiskott-Aldrich syndrome protein (WASp) and the WASp family verprolin-homologous protein 2 (WAVE2) [49]. Both WASp and WAVE2 are coupled to upstream TCR-triggered signals by members of the Rho family of GTPases, which function as master regulators of the actin cytoskeleton in the majority of cell types. Type II NPFs include cortactin and the leukocyte-specific cortactin homolog HS1. Similar to WASp and WAVE2, HS1 is able to recruit and activate the Arp2/3 complex [53]. HS1 is not required for early TCR activation events but is essential for Ca^{2+} influx and IL-2 gene transcription. Phosphorylated HS1 also couples to multiple signaling proteins, including Lck, PLCγ1, and Vav1, and is essential for the stable recruitment of Vav1 to the IS [54].

The Rho family small GTPases are central players in F-actin dynamics. These molecules are activated by GEFs, which promote the exchange of GTP to GDP within their GTPase active site. Among GEFs, Vav1 is considered as the major regulator of actin polymerization in TCR signaling [55]. Actin accumulation is also regulated by actin-binding proteins, such as α-actinin and filamin, that accumulate at the IS and are required for T cell activation [56, 57]. These proteins directly bind to the cytoplasmic tail of β integrins [58, 59], accounting for integrin accumulation at the IS. Notably, the structurally defined filamin-binding site of β integrins overlaps with that of the integrin-regulator talin, suggesting that filamin and talin compete for binding to the integrin tails [60].

Together with F-actin reorganization, IS formation is accompanied by a profound remodeling of the T cell microtubule cytoskeleton. Following antigen

recognition, the MTOC moves beneath the plasma membrane of the cSMAC [61], and a polarity axis is established within the T cell through microtubule orientation toward the center of the IS (Fig. 4.1). Moreover, the Golgi apparatus, recycling endosomes and mitochondria, polarize toward the IS [62, 63]. Synaptic F-actin ring formation occurs just prior to MTOC docking at the center of the IS and is required for this process [62].

4.3 Immune Synapse Dysfunctions in T Cell Immunodeficiency

T cell immunodeficiencies represent a heterogeneous group of disorders, characterized by an incomplete reduction in T cell number or activity associated with autoimmunity, inflammation, and high immunoglobulin E (IgE) production. T cell immunodeficiencies develop either as primary disorders or secondary to chronic infections, illness, or drug therapy. The highly variable clinical presentation of these diseases mainly depends on the presence or absence of concomitant syndromic pathologies. In this chapter, we discuss a subgroup of immunodeficiencies whose associated genetic defects (Table 4.1) lead to impaired or aberrant IS formation, focusing on the T-side of the medal and mentioning some relevant features of the APC-side.

4.3.1 Actin-Related Immunodeficiencies

As described above (see Sect. 4.2.3), actin dynamics, which is essential to the establishment of a mature IS, is tightly controlled by several different NPFs, in turn activated by multiple signaling pathways [64]. Loss-of-function mutations in the gene encoding WASp, localized in the short arm of the X chromosome (Xp11.23) [65], are associated to the *Wiskott-Aldrich syndrome* (WAS) [66], a X-linked disorder characterized by thrombocytopenia, eczema, and immunodeficiency [67]. In T cells, WASp promotes TCR-dependent rearrangements of the cortical actin cytoskeleton, which in turn favor IS formation and stability [68]. Early during IS formation, WASp recruitment is coordinated by the adaptor protein SLP76, which is recruited to LAT and functions as a scaffold, bringing another adaptor protein, Nck, and WASP in proximity to Vav1 and the GTPase Cdc42 [69]. Following binding to GTP-bound Cdc42, WASp becomes phosphorylated acquiring an active conformation, which allows to recruit the Arp2/3 complex and induce the nucleation of new actin filaments [70].

Loss of function of WASp in WAS T cells causes alterations during the early phases of IS formation, resulting from actin cytoskeletal defects (Fig. 4.3). T cells from WAS patients assemble immune synapses with an abnormal architecture, with dispersed phosphotyrosine signaling at the cSMAC and defective MTOC polarization [71]. Downstream events in T cell activation, including calcium flux, IL-2 production, and T cell proliferation, are also affected by WASp deficiency in these

Table 4.1 Human immunodeficiency syndromes with defective IS formation

Disease	Gene mutated	Protein affected	IS defect	Refs
Wiskott-Aldrich syndrome (WAS)	*WASp* (~350 mutations)	WASp	Actin cytoskeletal defects, dispersed phosphotyrosine signaling at the cSMAC, defective MTOC polarization	[64–73]
WIP deficiency	*WIPF1* (S434Ter)	WIP	WAS-related defects, defective actin filaments formation and stabilization, defective lytic granule polarization and secretion	[74–76]
DOCK8 immunodeficiency syndrome	*DOCK8* (~130 mutations)	DOCK8	Defective LFA-1 polarization at the IS	[77–80]
Common variable immunodeficiency with T cell defects (T-CVID)	*VAV1* (single case: Deletion exons 2–27)	VAV1	Defective Vav1/Rac pathway controlling F-actin dynamics	[81–83]
CRAC channelopathies	*STIM1, ORAI1* Mutations or deletions	STIM1, ORAI1	Defective trafficking of the CRAC channels to the IS, defective Ca^{2+} signaling	[84–86]
WHIM syndrome	*CXCR4* (most common heterozygous mutations: R334X, S339fs342X, E343X, G335X)	CXCR4	Aberrantly prolonged CXCR4-dependent signaling	[91–96]
Leukocyte adhesion deficiency (LAD)	*LAD I: ITGB2* (mutations or deletions) *LAD II: SLC35C1* (R147C, T308R, E31Ter, 3-BP DEL, 501CTT) *LAD III: FERMT3* (R509X W16X and splice site mutations)	LAD I: β2 subunit of integrin (CD18) LAD II: GDP-fucose transporter 1 LAD III: Kindlin-3	*LAD I:* Deficiency of LFA-1, mac-1, or p150/95 *LAD II:* Defective glycosylation of proteins required for IS stabilization *LAD III:* Defect in LFA1-ICAM-1 interaction	[97–101]
X-linked lymphoproliferative disease (XLP)	*SH2D1A* (mutations or deletion)	SAP	Defective B-T cell interactions, impaired tyrosine phosphorylation pattern, and MTOC reorientation	[105–109]

Table 4.1 (continued)

Disease	Gene mutated	Protein affected	IS defect	Refs
Hermansky-Pudlak syndrome type 2 (HPS2)	*AP3B1* (mutations or deletion)	B3A subunit of adaptor protein 3	Impaired adhesion to target cells, impaired polarization	[118]
Chediak-Higashi syndrome (CHS)	*LYST* (mutations or deletion)	LYST	Impaired exocytosis of lytic granules	[126, 127]
Griscelli syndrome type 2 (GS2)	*RAB27A* (mutations or deletion)	RAB27A	Impaired granule transport from MTOC to plasma membrane	[128]

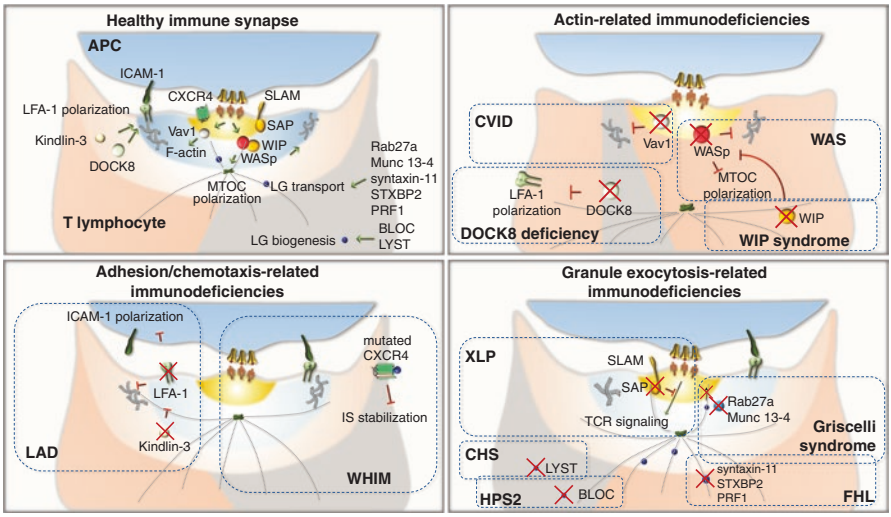

Fig. 4.3 Defective immune synapses in immunodeficiencies. Molecular defects associated to defective immune synapse organization, stability, or function in immunodeficiencies. LG: lytic granules

patients [72]. The abnormalities in actin polymerization in WAS T cells impact not only on their ability to spread but also on their homing to lymphoid organs [67]. Since these are the sites where T cells are primed to become activated by cognate APCs, the homing defects contribute to the defective T cell responses in WAS patients. In addition to the well-known T cell pathology associated with WAS, many of the actin-related functional defects described above also affect APCs. In particular, WAS dendritic cells are unable to migrate from the original site of antigen uptake to lymph nodes as a consequence of defective adhesion to ICAM-1, polarization, and migratory responses to chemokine gradients [73].

Recently, a new actin cytoskeleton-related immunodeficiency has been characterized, which is associated to mutations in the WAS protein-interacting protein (WIP) [74]. *WIP deficiency* is a recessive disorder that leads to premature

degradation of WASp. WIP controls indeed WASp stability and activity [75], and its silencing due to stop codon mutations within the protein coding sequence results in almost undetectable WASp levels and to clinical features similar to WAS [74]. Interestingly, WIP exerts other functions independently of WASp, such as directly binding to actin and promoting the formation and stabilization of actin filaments, or controlling lytic granule polarization and secretion in NK cells [76] (Fig. 4.3).

A further example of immunodeficiency related to actin cytoskeleton abnormalities that affect IS formation is *DOCK8 deficiency*. Also known as "DOCK8 immunodeficiency syndrome," this disease is the autosomal recessive form of hyper-IgE syndrome, a genetic disorder characterized by increased susceptibility to skin viral infections, T cell lymphopenia, and impaired antibody response [77]. DOCK8, one of the GEFs for Rho and Rac GTPases, controls the activation of actin regulatory proteins, such as talin and WASp [78]. DOCK8 mutant or deficient T cells show defective LFA-1 polarization at the IS, which results in impaired T cell proliferation and survival [79]. Moreover, a key role of DOCK8 at the APC-side of the IS has been reported. DOCK8-deficient B cells lack normal recruitment of ICAM-1 to the B cell contact with the T cell, are unable to form marginal zone B cells, and have reduced persistence and maturation of germinal center B cells [80] (Fig. 4.3).

T cell immunodeficiencies related to defects in actin dynamics at the IS include *common variable immunodeficiency* (CVID). This primary immune disorder is mainly characterized by recurrent infections and low antibody levels, accounting for increased susceptibility to bacterial infections and a trend to develop autoimmune disorders and cancer [81]. Although B cell dysfunctions account for a major proportion of CVID, B cells from a significant proportion of patients are functional in vitro but are not activated in vivo into mature antibody-secreting plasma cells because of defective T cell help. T cells from this subgroup of CVID patients (T-CVID) are characterized by defective TCR-dependent signaling pathways, which are accompanied in some patients by defects in the Vav1/Rac pathway controlling F-actin dynamics [82]. We have identified in one T-CVID patient a heterozygous deletion in the *Vav1* gene spanning exons 2–27, which strongly affects Vav1 expression, highlighting *Vav1* as an autosomal dominant disease gene associated with CVID with defective T cell function [83] (Fig. 4.3).

CRAC channelopathies are a subset of recently identified immunodeficiencies caused by inherited null or loss-of-function mutations in the Ca^{2+} channel ORAI1 and its activator stromal interaction molecule 1 (STIM1). These patients lack calcium release-activated Ca^{2+} channel (CRAC) function and store-operated Ca^{2+} entry (SOCE) and are characterized by severe combined immunodeficiency (SCID)-like disease, autoimmunity, muscular hypotonia, and ectodermal dysplasia [84, 85]. Voros and colleagues recently demonstrated that trafficking of the CRAC channels to the IS, which is crucial for efficient pMHC-triggered Ca^{2+} signaling, is mainly regulated by the actin-regulatory adaptor protein HS1 [86]. Although these data support the potential involvement of actin cytoskeleton remodeling in CRAC channelopathies, no direct involvement of either HS1 or actin rearrangements in these immunodeficiencies has been demonstrated to date.

FCHO1 deficiency is related to T cell deficiency and loss-of-function mutations in FCH domain only 1 (*FCHO1*) gene. FCHO1 participates in the early stages of clathrin-mediated endocytosis. Patients with FCHO1 deficiency show T and B cell lymphopenia and are unresponsive to TCR triggering [87]. While the role of FCHO1 deficiency in IS assembly has not been investigated as yet, it is plausible that TCR trafficking to the IS via recycling endosomes may be affected in these patients.

4.3.2 Immunodeficiencies Associated to Adhesion and Chemotactic Molecules

In addition to their well-known involvement in regulating trafficking of T cells and APCs to secondary lymphoid organs in both homeostatic and inflammatory conditions [88, 89], chemokine-chemokine receptor axes are also strongly implicated in tuning the strength of T cell-APC interaction, thereby contributing to adhesion and IS formation [90]. Not surprisingly, a subset of immunodeficiencies is characterized by IS defects, caused by mutations affecting the expression or function of either chemokines or chemokine receptors.

A paradigm of this class of diseases is represented by the *WHIM syndrome*, which is an acronym for some of the characteristic symptoms of the disorder: (w) arts, (h)ypogammaglobulinemia, (i)nfections, and (m)yelokathexis [91]. WHIM syndrome is associated with dominant mutations in the chemokine receptor CXCR4 that lead to truncation of its carboxy-terminal domain. The truncated version of CXCR4 is unable to undergo internalization after binding to the cognate ligand CXCL12, resulting in aberrantly prolonged CXCR4-dependent signaling and enhanced migration after chemokine stimulation [92–94]. Interestingly, WHIM-mutant CXCR4 is normally recruited to the IS but is readily removed therefrom following CXCL12 binding. This results in T cell motility rather than formation of a stable IS, eventually leading to aberrant T cell activation [95, 96] (Fig. 4.3).

Leukocyte Adhesion Deficiency (LAD) disorders also belong to the subclass of immunodeficiencies characterized by recurrent infections, impaired cytokine production, maturation, and migratory responses in myeloid cells, impaired extravasation of blood effector cells, and severe spontaneous bleedings. The IS defects harbored by LAD patients are mainly related to failure in integrin/integrin receptor axes. LAD has been subgrouped into three classes which share similar disease-causing molecular defects. LAD I syndromes are caused by mutations that impair either expression or function of β2 integrins (CD11/CD18 integrins, or leukocyte β2 integrins) [97]. This rare inherited autosomal recessive disorder is characterized by a deficiency of the integrin LFA-1, the complement receptor Mac-1, or the p150/95 glycoprotein of leukocyte α/β heterodimers (CD11c/CD18) due to the expression of an aberrantly small, nonfunctional form of the β subunit common to the three proteins. This abnormality is caused by loss-of-function mutations or deletions in the respective gene [98, 99] (Fig. 4.3). Although experimental evidence is missing, the integrin defects harbored by LAD I patients are likely to affect both IS formation and IS stability.

The focal adhesion protein Kindlin-3 is a key LFA-1 coactivator deleted in LAD III syndrome [100]. While Kindlin-3-null primary T cells from LAD III patients engage normal TCR signaling, TCR activation fails to trigger robust LFA-1-mediated T cell spreading on ICAM-1-expressing DCs. Additionally, Kindlin-3 was found to be essential for the anchorage of the LFA-1 heterodimer to the actin cytoskeleton and for the generation of LFA-1 microclusters within focal dots of TCR-stimulated lymphocytes when plated on ICAM-1 [101], supporting the key contribution of Kindlin-3 to the stabilization of LFA-1/ICAM-1 interactions (Fig. 4.3).

LAD II patients harbor clinical features similar to LAD I/III patients, but in the presence of intact leukocyte integrin expression and function [102]. The molecular basis for LAD II is the defective glycosylation of ligands recognized by the selectin family of adhesion molecules expressed by leukocytes. The defect has been ascribed to mutations in a fucose transporter localized at the Golgi apparatus [103]. Since glycosylated proteins, such as CD2, CD43 and CD45, contribute to the stabilization of IS [104], we can hypothesize that IS organization may be affected in LAD II patients, although further studies are needed to elucidate this issue.

4.3.3 Other Immunodeficiency Diseases Related to Impaired Cytotoxic Function

X-linked lymphoproliferative disease (XLP) is a lymphoproliferative disorder characterized by the inability to mount an immune response to the Epstein-Barr virus (EBV), which often leads to death caused by bone marrow failure, irreversible hepatitis, and malignant lymphoma [105, 106]. It is caused by loss-of-function mutations in signaling lymphocyte activation molecule-associated protein (SAP), an adaptor protein which links SLAM family surface receptors to downstream signaling by recruiting the Src family kinase Fyn and preventing recruitment of the inhibitory phosphatases SHP-1, SHP-2, and SHIP-1 [105, 107]. Interestingly, this defect is selective for B-T cell interactions, as witnessed by the finding that DC-T cell interactions are normal in XLP patients, accounting for a selective role for the SAP family in controlling adhesive mechanisms required to stabilize T cell-B cell conjugates and in delivering signals to support B cell proliferation [108, 109] (Fig. 4.3).

SAP-deficient T cells exhibit impaired cytotoxic IS formation. The absence of SAP results indeed in increased recruitment of the phosphatase SHP-1 to SLAM family receptors, thereby hampering the activation of Src family kinases and impairing general tyrosine phosphorylation pattern and MTOC reorientation at the IS, which results in defective cytotoxic effector T cell functions [109] (Fig. 4.3). Interestingly, the impaired IS formation, exhibited by SAP-deficient T cells, is reversed by downregulation of diacylglycerol kinase α (DGKα) [110, 111], a negative regulator of TCR signaling which lowers the levels of diacylglycerol, an important second messenger in IS assembly and TCR signaling [112, 113]. Silencing or inhibition of DGKα activity in SAP-deficient T cells restores indeed the correct level of diacylglycerol and its downstream effectors at the IS and recovers MTOC

polarization [111], highlighting DGKα as an important modulator of SAP signaling at the IS.

During IS formation, cytotoxic lymphocytes polarize their secretory machinery to allow fusion with the IS membrane of specialized lysosomes known as "lytic granules" that contain the pore-forming protein perforin and serine protease granzymes, resulting in the release of their content into the synaptic space, a process which strictly depends on MTOC reorientation at the IS [114, 115]. Failure to deliver lytic granules to the IS leads to impaired clearance of target cells and manifests in often fatal, hyper-inflammatory syndromes [116]. Patients with defective killing of infected cells develop systemic inflammation due to persistent stimulation of the innate immune system. These defects are associated with hypersecretion of multiple pro-inflammatory cytokines and chemokines (IFNα/γ, IL-2, TNFα, CCL2, CCL8, and RANTES) through an abnormally long-lived IS, characterized by impaired trafficking, docking, or exocytosis of cytotoxic granules, that eventually fails to kill [117].

Several different components of the secretory machinery that controls the biogenesis of lytic granules and their trafficking to and fusion with the plasma membrane at the lytic IS have been identified to date. Lytic granules, generated at the trans-Golgi network, pass through a series of maturation stages, assisted by the biogenesis of lysosome-related organelles complexes (BLOC) 1, 2, and 3 [118] and by the lysosomal trafficking regulator protein (LYST) [119], and are eventually delivered, docked, and fused with the plasma membrane of the IS [120, 121]. Exocytosis of lytic granules into the synaptic cleft, promoted by MTOC reorientation close to the plasma membrane of the IS, requires the combined action of the molecular motors, dynein and kinesin-1, and, during the very late phase, of the small GTPase Rab27a, which, through its interaction with the vesicle tether Munc13–4 [122, 123], mediates granule movement from the MTOC to the plasma membrane [121, 124]. Granule fusion and secretion is finally assisted by the bridging activity of specific SNARE and SNARE-associated proteins. Among these, the binding partners syntaxin-11 and Munc18-2 have been found to localize to the plasma membrane of cytotoxic cells and cooperate to drive the final steps of granule fusion [125].

Mutations within genes encoding members of this complex biogenesis/maturation/exocytic machinery lead to defective cytotoxic cell function and result in often lethal immune disorders. Impaired secretory granule maturation results in reduced cytotoxic activity in *Hermansky-Pudlak syndrome type 2* (HPS2), characterized by inactivating mutations in any of the components of BLOC-1, -2, and -3 complexes [118], and *Chediak-Higashi syndrome* (CHS), a rare autosomal recessive defect in the LYST protein [118, 126, 127].

Loss-of-function mutations in Rab27a impair granule transport from the MTOC to the plasma membrane and underlie *Griscelli syndrome type 2* (GS2) [128]. Mutations in Munc13–4 [129], syntaxin-11 [130], syntaxin-binding protein 2 (STXBP2, Munc18-2) [131], and perforin 1 (PRF1) [132] cause defective cytolytic granule exocytosis in *familial hemophagocytic lymphohistiocytosis* patients [133] (Fig. 4.3).

4.4 Conclusions and Perspectives

Although much is known about the processes that occur during IS formation, we continue to discover new molecules and mechanisms that participate in the regulation of the architecture and function of this specialized intercellular junction. This complexity is reflected in T cell-related immunodeficiency disorders, which, while sharing several features in their disease presentation, show extremely variable etiologies related to the specific causative molecular defects. Additionally, the etiology of many immunodeficiency disorders, including T cell deficiencies (such as T cell-related CVIDs), is as yet unknown. This poses a major hamper to the development of IS-targeted therapies. In this era of personalized medicine, molecular-targeted and disease-specific pharmacological treatments able to restore IS formation might help in the treatment of these still incurable diseases. This is the case of SAP-deficient XLP patients, for whom DGKα inhibitors have been recently designed, which reverse the inhibitory effects of DGKα, highlighting an interesting new frontier for their treatment [134].

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References

1. Fooksman DR, Vardhana S, Vasiliver-Shamis G, Liese J, Blair DA, Waite J et al (2010) Functional anatomy of T cell activation and synapse formation. Annu Rev Immunol 28:79–105
2. Dustin ML, Long EO (2010) Cytotoxic immunological synapses. Immunol Rev 235:24–34
3. Dustin ML, Choudhuri K (2016) Signaling and polarized communication across the T cell immunological synapse. Annu Rev Cell Dev Biol 32:303–325
4. Poo WJ, Conrad L, Janeway CA (1988) Receptor-directed focusing of lymphokine release by helper T cells. Nature. https://doi.org/10.1038/332378a0
5. Kupfer A, Mosmann TR, Kupfer H (1991) Polarized expression of cytokines in cell conjugates of helper T cells and splenic B cells. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.88.3.775
6. Kupfer H, Monks CR, Kupfer A (1994) Small splenic B cells that bind to antigen-specific T helper (Th) cells and face the site of cytokine production in the Th cells selectively proliferate: immunofluorescence microscopic studies of Th-B antigen-presenting cell interactions. J Exp Med 179:1507–1515
7. Dustin ML (1998) Making a little affinity go a long way: a topological view of LFA-1 regulation. Cell Adhes Commun 6:255–262
8. Monks CRF, Freiberg BA, Kupfer H, Sciaky N, Kupfer A (1998) Three-dimensional segregation of supramolecular activation clusters in T cells. Nature. https://doi.org/10.1038/25764
9. Grakoui A, VanDyk LF, Dowdy SF, Allen PM (1998) Molecular basis for the lack of T cell proliferation induced by an altered peptide ligand. Int Immunol. https://doi.org/10.1093/intimm/10.7.969
10. Johnson KG, Bromley SK, Dustin ML, Thomas ML (2000) A supramolecular basis for CD45 tyrosine phosphatase regulation in sustained T cell activation. Proc Natl Acad Sci 97:10138–10143

11. Freiberg BA, Kupfer H, Maslanik W, Delli J, Kappler J, Zaller DM et al (2002) Staging and resetting T cell activation in SMACs. Nat Immunol. https://doi.org/10.1038/ni836
12. Alarcón B, Mestre D, Martínez-Martín N (2011) The immunological synapse: a cause or consequence of T-cell receptor triggering? Immunology. https://doi.org/10.1111/j.1365-2567.2011.03458.x
13. Soares H, Lasserre R, Alcover A (2013) Orchestrating cytoskeleton and intracellular vesicle traffic to build functional immunological synapses. Immunol Rev. https://doi.org/10.1111/imr.12110
14. Bustos-Morán E, Blas-Rus N, Martín-Cófreces NB, Sánchez-Madrid F (2016) Orchestrating lymphocyte polarity in cognate immune cell–cell interactions. Int Rev Cell Mol Biol 327:195–261. https://doi.org/10.1016/bs.ircmb.2016.06.004
15. Iwashima M, Irving BA, Van Oers NSC, Chan AC, Weiss A (2014) Sequential interactions of the TCR with two distinct cytoplasmic tyrosine kinases. J Immunol. https://doi.org/10.1126/science.7509083
16. Avni O, Rao A (2000) T cell differentiation: a mechanistic view. Curr Opin Immunol 12:654–659. https://doi.org/10.1016/S0952-7915(00)00158-8
17. Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhl L et al (2000) PKC-θ is required for TCR-induced NF-κB activation in mature but not immature T lymphocytes. Nature. https://doi.org/10.1038/35006090
18. Matsumoto R, Wang D, Blonska M, Li H, Kobayashi M, Pappu B et al (2005) Phosphorylation of CARMA1 plays a critical role in T cell receptor-mediated NF-κB activation. Immunity. https://doi.org/10.1016/j.immuni.2005.10.007
19. Blonska M, Lin X (2011) NF-κB signaling pathways regulated by CARMA family of scaffold proteins. Cell Res. https://doi.org/10.1038/cr.2010.182
20. Castro-Sánchez P, Aguilar-Sopeña O, Alegre-Gómez S, Ramirez-Munoz R, Roda-Navarro P (2019) Regulation of CD4+ T cell signaling and immunological synapse by protein tyrosine phosphatases: molecular mechanisms in autoimmunity. Front Immunol. https://doi.org/10.3389/fimmu.2019.01447
21. Grakoui A (1999) The immunological synapse: a molecular machine controlling T cell activation. Science 285:221–227
22. Billadeau DD, Burkhardt JK (2006) Regulation of cytoskeletal dynamics at the immune synapse: new stars join the actin troupe. Traffic. https://doi.org/10.1111/j.1600-0854.2006.00491.x
23. Yokosuka T, Saito T (2010) The immunological synapse, TCR microclusters, and T cell activation. Curr Top Microbiol Immunol. https://doi.org/10.1007/978-3-642-03858-7_5
24. Balagopalan L, Barr VA, Samelson LE (2009) Endocytic events in TCR signaling: focus on adapters in microclusters. Immunol Rev. https://doi.org/10.1111/j.1600-065X.2009.00840.x
25. Choudhuri K, Llodrá J, Roth EW, Tsai J, Gordo S, Wucherpfennig KW et al (2014) Polarized release of T-cell-receptor-enriched microvesicles at the immunological synapse. Nature 507:118–123
26. Das V, Nal B, Dujeancourt A, Thoulouze MI, Galli T, Roux P et al (2004) Activation-induced polarized recycling targets T cell antigen receptors to the immunological synapse: involvement of SNARE complexes. Immunity. https://doi.org/10.1016/S1074-7613(04)00106-2
27. Burkhardt JK, Carrizosa E, Shaffer MH (2008) The actin cytoskeleton in T cell activation. Annu Rev Immunol. https://doi.org/10.1146/annurev.immunol.26.021607.090347
28. Springer TA, Dustin ML (2012) Integrin inside-out signaling and the immunological synapse. Curr Opin Cell Biol 24:107–115
29. Ardouin L, Bracke M, Mathiot A, Pagakis SN, Norton T, Hogg N et al (2003) Vav1 transduces TCR signals required for LFA-1 function and cell polarization at the immunological synapse. Eur J Immunol. https://doi.org/10.1002/eji.200323858
30. Krawczyk C, Oliveira-Dos-Santos A, Sasaki T, Griffiths E, Ohashi PS, Snapper S et al (2002) Vav1 controls integrin clustering and MHC/peptide-specific cell adhesion to antigen-presenting cells. Immunity. https://doi.org/10.1016/S1074-7613(02)00291-1

31. Ménasché G, Kliche S, Bezman N, Schraven B (2007) Regulation of T-cell antigen receptor-mediated inside-out signaling by cytosolic adapter proteins and Rap1 effector molecules. Immunol Rev. https://doi.org/10.1111/j.1600-065X.2007.00543.x
32. Kliche S, Breitling D, Togni M, Pusch R, Heuer K, Wang X et al (2006) The ADAP/SKAP55 signaling module regulates T-cell receptor-mediated integrin activation through plasma membrane targeting of Rap1. Mol Cell Biol. https://doi.org/10.1128/mcb.00331-06
33. Kuropka B, Schraven B, Kliche S, Krause E, Freund C (2016) Tyrosine-phosphorylation of the scaffold protein ADAP and its role in T cell signaling. Expert Rev Proteomics. https://doi.org/10.1080/14789450.2016.1187565
34. Wang H, Moon EY, Azouz A, Wu X, Smith A, Schneider H et al (2003) SKAP-55 regulates integrin adhesion and formation of T cell-APC conjugates. Nat Immunol. https://doi.org/10.1038/ni913
35. Wang H, Liu H, Lu Y, Lovatt M, Wei B, Rudd CE (2007) Functional defects of SKAP-55-deficient T cells identify a regulatory role for the adaptor in LFA-1 adhesion. Mol Cell Biol. https://doi.org/10.1128/mcb.00556-07
36. Wang H, Rudd CE (2008) SKAP-55, SKAP-55-related and ADAP adaptors modulate integrin-mediated immune-cell adhesion. Trends Cell Biol. https://doi.org/10.1016/j.tcb.2008.07.005
37. Peterson EJ (2003) The TCR ADAPts to integrin-mediated cell adhesion. Immunol Rev. https://doi.org/10.1034/j.1600-065X.2003.00026.x
38. Mor A, Dustin ML, Philips MR (2007) Small GTPases and LFA-1 reciprocally modulate adhesion and signaling. Immunol Rev 218:114–125
39. Menasche G, Kliche S, Chen EJH, Stradal TEB, Schraven B, Koretzky G (2007) RIAM links the ADAP/SKAP-55 signaling module to Rap1, facilitating T-cell-receptor-mediated integrin activation. Mol Cell Biol. https://doi.org/10.1128/mcb.02011-06
40. Zhang Y, Wang H (2012) Integrin signalling and function in immune cells. Immunology. https://doi.org/10.1111/j.1365-2567.2011.03549.x
41. Katagiri K, Hattori M, Minato N, Irie S-k, Takatsu K, Kinashi T (2000) Rap1 is a potent activation signal for leukocyte function-associated antigen 1 distinct from protein kinase C and phosphatidylinositol-3-OH kinase. Mol Cell Biol. https://doi.org/10.1128/mcb.20.6.1956-1969.2000
42. Sebzda E, Bracke M, Tugal T, Hogg N, Cantrell DA (2002) Rap 1A positively regulates T cells via integrin activation rather than inhibiting lymphocyte signaling. Nat Immunol. https://doi.org/10.1038/ni765
43. Scholer A, Hugues S, Boissonnas A, Fetler L, Amigorena S (2008) Intercellular adhesion molecule-1-dependent stable interactions between T cells and dendritic cells determine CD8+ T cell memory. Immunity. https://doi.org/10.1016/j.immuni.2007.12.016
44. Li D, Molldrem JJ, Ma Q (2009) LFA-1 regulates CD8+ T cell activation via T cell receptor-mediated and LFA-1-mediated Erk1/2 signal pathways. J Biol Chem. https://doi.org/10.1074/jbc.M109.002865
45. Wong MM, Fish EN (2003) Chemokines: attractive mediators of the immune response. Semin Immunol. https://doi.org/10.1016/S1044-5323(02)00123-9
46. Tybulewicz VLJ (2002) Chemokines and the immunological synapse. Immunology. https://doi.org/10.1046/j.1365-2567.2002.01467.x
47. Molon B, Gri G, Bettella M, Gómez-Moutón C, Lanzavecchia A, Martínez-A C et al (2005) T cell costimulation by chemokine receptors. Nat Immunol. https://doi.org/10.1038/ni1191
48. Laufer JM, Kindinger I, Artinger M, Pauli A, Legler DF (2019) CCR7 is recruited to the immunological synapse, acts as co-stimulatory molecule and drives LFA-1 clustering for efficient T cell adhesion through ZAP70. Front Immunol. https://doi.org/10.3389/fimmu.2018.03115
49. Gomez TS, Billadeau DD (2008) T cell activation and the cytoskeleton: you can't have one without the other. Adv Immunol. https://doi.org/10.1016/S0065-2776(08)00001-1
50. Ritter AT, Kapnick SM, Murugesan S, Schwartzberg PL, Griffiths GM, Lippincott-Schwartz J (2017) Cortical actin recovery at the immunological synapse leads to termination of lytic granule secretion in cytotoxic T lymphocytes. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1710751114

51. Derivery E, Gautreau A (2010) Assaying WAVE and WASH complex constitutive activities toward the Arp2/3 complex. Methods Enzymol. https://doi.org/10.1016/B978-0-12-381298-8.00033-2
52. Goley ED, Welch MD (2006) The ARP2/3 complex: an actin nucleator comes of age. Nat Rev Mol Cell Biol. https://doi.org/10.1038/nrm2026
53. Hao JJ, Zhu J, Zhou K, Smith N, Zhan X (2005) The coiled-coil domain is required for HS1 to bind to F-actin and activate Arp2/3 complex. J Biol Chem. https://doi.org/10.1074/jbc.M504552200
54. Gomez TS, McCarney SD, Carrizosa E, Labno CM, Comiskey EO, Nolz JC et al (2006) HS1 functions as an essential actin-regulatory adaptor protein at the immune synapse. Immunity. https://doi.org/10.1016/j.immuni.2006.03.022
55. Fischer KD, Kong YY, Nishina H, Tedford K, Marengère LEM, Kozieradzki I et al (1998) Vav is a regulator of cytoskeletal reorganization mediated by the T-cell receptor. Curr Biol. https://doi.org/10.1016/s0960-9822(98)70224-6
56. Gordón-Alonso M, Sala-Valdés M, Rocha-Perugini V, Pérez-Hernández D, López-Martín S, Ursa A et al (2012) EWI-2 association with α-actinin regulates T cell immune synapses and HIV viral infection. J Immunol. https://doi.org/10.4049/jimmunol.1103708
57. Hayashi K, Altman A (2006) Filamin A is required for T cell activation mediated by protein kinase C-θ. J Immunol. https://doi.org/10.4049/jimmunol.177.3.1721
58. Loo DT, Kanner SB, Aruffo A (1998) Filamin binds to the cytoplasmic domain of the β1-integrin: identification of amino acids responsible for this interaction. J Biol Chem. https://doi.org/10.1074/jbc.273.36.23304
59. Otey CA, Vasquez GB, Burridge K, Erickson BW (1993) Mapping of the α-actinin binding site within the β1 integrin cytoplasmic domain. J Biol Chem
60. Kiema T, Lad Y, Jiang P, Oxley CL, Baldassarre M, Wegener KL et al (2006) The molecular basis of filamin binding to integrins and competition with Talin. Mol Cell. https://doi.org/10.1016/j.molcel.2006.01.011
61. Huse M, Le Floc'h A, Liu X (2013) From lipid second messengers to molecular motors: microtubule-organizing center reorientation in T cells. Immunol Rev. https://doi.org/10.1111/imr.12116
62. Stinchcombe JC, Salio M, Cerundolo V, Pende D, Arico M, Griffiths GM (2011) Centriole polarisation to the immunological synapse directs secretion from cytolytic cells of both the innate and adaptive immune systems. BMC Biol. https://doi.org/10.1186/1741-7007-9-45
63. Torralba D, Martín-Cófreces NB, Sanchez-Madrid F (2019) Mechanisms of polarized cell-cell communication of T lymphocytes. Immunol Lett. https://doi.org/10.1016/j.imlet.2019.03.009
64. Wurzer H, Hoffmann C, Al Absi A, Thomas C. Actin cytoskeleton straddling the immunological synapse between cytotoxic lymphocytes and cancer cells. Cells 2019. doi:https://doi.org/10.3390/cells8050463
65. Jin Y, Mazza C, Christie JR, Giliani S, Fiorini M, Mella P et al (2004) Mutations of the Wiskott-Aldrich syndrome protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. Blood. https://doi.org/10.1182/blood-2003-05-1592
66. Derry JMJ, Ochs HD, Francke U (1994) Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. Cell. https://doi.org/10.1016/0092-8674(94)90528-2
67. Thrasher AJ, Burns SO (2010) WASP: A key immunological multitasker. Nat Rev Immunol. https://doi.org/10.1038/nri2724
68. Malinova D, Fritzsche M, Nowosad CR, Armer H, Munro PMG, Blundell MP et al (2016) WASp-dependent actin cytoskeleton stability at the dendritic cell immunological synapse is required for extensive, functional T cell contacts. J Leukoc Biol. https://doi.org/10.1189/jlb.2a0215-050rr
69. Zeng R, Cannon JL, Abraham RT, Way M, Billadeau DD, Bubeck-Wardenberg J et al (2003) SLP-76 coordinates Nck-dependent Wiskott-Aldrich syndrome protein recruitment with Vav-1/Cdc42-dependent Wiskott-Aldrich syndrome protein activation at the T cell-APC contact site. J Immunol. https://doi.org/10.4049/jimmunol.171.3.1360

70. Higgs HN, Pollard TD (2001) Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. Annu Rev Biochem. https://doi.org/10.1146/annurev.biochem.70.1.649
71. Calvez R, Lafouresse F, de Meester J, Galy A, Valitutti S, Dupré L (2011) The Wiskott-Aldrich syndrome protein permits assembly of a focused immunological synapse enabling sustained T-cell receptor signaling. Haematologica. https://doi.org/10.3324/haematol.2011.040204
72. Cannon JL, Burkhardt JK (2004) Differential roles for Wiskott-Aldrich syndrome protein in immune synapse formation and IL-2 production. J Immunol. https://doi.org/10.4049/jimmunol.173.3.1658
73. De Noronha S, Hardy S, Sinclair J, Blundell MP, Strid J, Schulz O et al (2005) Impaired dendritic-cell homing in vivo in the absence of Wiskott-Aldrich syndrome protein. Blood. https://doi.org/10.1182/blood-2004-06-2332
74. Lanzi G, Moratto D, Vairo D, Masneri S, Delmonte O, Paganini T et al (2012) A novel primary human immunodeficiency due to deficiency in the WASP-interacting protein WIP. J Exp Med. https://doi.org/10.1084/jem.20110896
75. De La Fuente MA, Sasahara Y, Calamito M, Antón IM, Elkhal A, Gallego MD et al (2007) WIP is a chaperone for Wiskott-Aldrich syndrome protein (WASP). Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.0610275104
76. Fried S, Matalon O, Noy E, Barda-Saad M (2014) WIP: more than a WASp-interacting protein. J Leukoc Biol. https://doi.org/10.1189/jlb.2ru0314-162r
77. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ et al (2009) Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med. https://doi.org/10.1056/NEJMoa0905506
78. Ham H, Guerrier S, Kim J, Schoon RA, Anderson EL, Hamann MJ et al (2013) Dedicator of cytokinesis 8 interacts with Talin and Wiskott-Aldrich syndrome protein to regulate NK cell cytotoxicity. J Immunol. https://doi.org/10.4049/jimmunol.1202792
79. Randall KL, Lambe T, Johnson AL, Johnson A, Treanor B, Kucharska E et al (2009) Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. Nat Immunol 10:1283–1291
80. Randall KL, Lambe T, Johnson A, Treanor B, Kucharska E, Domaschenz H et al (2009) Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. Nat Immunol. https://doi.org/10.1038/ni.1820
81. Park MA, Li JT, Hagan JB, Maddox DE, Abraham RS (2008) Common variable immunodeficiency: a new look at an old disease. Lancet. https://doi.org/10.1016/S0140-6736(08)61199-X
82. Paccani SR, Boncristiano M, Patrussi L, Ulivieri C, Wack A, Valensin S et al (2005) Defective Vav expression and impaired F-actin reorganization in a subset of patients with common variable immunodeficiency characterized by T-cell defects. Blood. https://doi.org/10.1182/blood-2004-05-2051
83. Capitani N, Ariani F, Amedep A, Pezzicoli A, Matucci A, Vultaggio A et al (2012) Vav1 haploinsufficiency in a common variable immunodeficiency patient with defective t-cell function. Int J Immunopathol Pharmacol. https://doi.org/10.1177/039463201202500332
84. Feske S (2010) CRAC channelopathies. Pflugers Arch Eur J Physiol. https://doi.org/10.1007/s00424-009-0777-5
85. Feske S (2019) CRAC channels and disease—from human CRAC channelopathies and animal models to novel drugs. Cell Calcium. https://doi.org/10.1016/j.ceca.2019.03.004
86. Vörös O, Panyi G, Hajdu P (2019) Molecular background of Orai1 accumulation in the immunological synapse. J Immunol 202:184.3
87. Łyszkiewicz M, Ziętara N, Frey L, Pannicke U, Stern M, Liu Y et al (2020) Human FCHO1 deficiency reveals role for clathrin-mediated endocytosis in development and function of T cells. Nat Commun. https://doi.org/10.1038/s41467-020-14809-9
88. Schulz O, Hammerschmidt SI, Moschovakis GL, Förster R (2016) Chemokines and chemokine receptors in lymphoid tissue dynamics. Annu Rev Immunol. https://doi.org/10.1146/annurev-immunol-041015-055649
89. Thelen M, Stein JV (2008) How chemokines invite leukocytes to dance. Nat Immunol 9:953–959

90. Bromley SK, Dustin ML (2002) Stimulation of naive T-cell adhesion and immunological synapse formation by chemokine-dependent and -independent mechanisms. Immunology 106:289–298
91. Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F et al (2003) Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet. https://doi.org/10.1038/ng1149
92. Kawai T, Choi U, Whiting-Theobald NL, Linton GF, Brenner S, Sechler JMG et al (2005) Enhanced function with decreased internalization of carboxy-terminus truncated CXCR4 responsible for WHIM syndrome. Exp Hematol. https://doi.org/10.1016/j.exphem.2005.01.001
93. Balabanian K, Lagane B, Pablos JL, Laurent L, Planchenault T, Verola O et al (2005) WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12. Blood. https://doi.org/10.1182/blood-2004-06-2289
94. Kawai T, Malech HL (2009) WHIM syndrome: congenital immune deficiency disease. Curr Opin Hematol. https://doi.org/10.1097/MOH.0b013e32831ac557
95. Kallikourdis M, Trovato AE, Anselmi F, Sarukhan A, Roselli G, Tassone L et al (2013) The CXCR4 mutations in WHIM syndrome impair the stability of the T-cell immunologic synapse. Blood. https://doi.org/10.1182/blood-2012-10-461830
96. Kallikourdis M, Viola A, Benvenuti F (2015) Human immunodeficiencies related to defective APC/T cell interaction. Front Immunol. https://doi.org/10.3389/fimmu.2015.00433
97. van de Vijver E, Maddalena A, Sanal Ö, Holland SM, Uzel G, Madkaikar M et al (2012) Hematologically important mutations: leukocyte adhesion deficiency (first update). Blood Cells Mol Dis. https://doi.org/10.1016/j.bcmd.2011.10.004
98. Kishimoto TK, O'Connor K, Springer TA (1989) Leukocyte adhesion deficiency. Aberrant splicing of a conserved integrin sequence causes a moderate deficiency phenotype. J Biol Chem 264:3588–3595
99. Bunting M, Harris ES, McIntyre TM, Prescott SM, Zimmerman GA (2002) Leukocyte adhesion deficiency syndromes: adhesion and tethering defects involving β2 integrins and selectin ligands. Curr Opin Hematol. https://doi.org/10.1097/00062752-200201000-00006
100. Rognoni E, Ruppert R, Fässler R (2016) The kindlin family: functions, signaling properties and implications for human disease. J Cell Sci. https://doi.org/10.1242/jcs.161190
101. Feigelson SW, Grabovsky V, Manevich-Mendelson E, Pasvolsky R, Shulman Z, Shinder V et al (2011) Kindlin-3 is required for the stabilization of TCR-stimulated LFA-1:ICAM-1 bonds critical for lymphocyte arrest and spreading on dendritic cells. Blood. https://doi.org/10.1182/blood-2010-12-322859
102. Harris ES, Smith TL, Springett GM, Weyrich AS, Zimmerman GA (2012) Leukocyte adhesion deficiency-I variant syndrome (LAD-iv, LAD-III): molecular characterization of the defect in an index family. Am J Hematol. https://doi.org/10.1002/ajh.22253
103. Sturla L, Etzioni A, Bisso A, Zanardi D, De Flora G, Silengo L et al (1998) Defective intracellular activity of GDP-D-mannose-4,6-dehydratase in leukocyte adhesion deficiency type II syndrome. FEBS Lett. https://doi.org/10.1016/S0014-5793(98)00615-2
104. Maller SM, Pinto NA, Morosi LG, Rabinovich GA (2017) Regulatory circuits mediated by lectin-glycan interactions in immune and vascular signaling programs: implications in the design of novel therapeutic strategies in autoimmune diseases and cancer. Rev Quim Viva 16. http://www.quimicaviva.qb.fcen.uba.ar/v16n1/E0060.html
105. Panchal N, Booth C, Cannons JL, Schwartzberg PL (2018) X-linked lymphoproliferative disease type 1: a clinical and molecular perspective. Front Immunol. https://doi.org/10.3389/fimmu.2018.00666
106. Morra M, Howie D, Grande MS, Sayos J, Wang N, Wu C et al (2001) X-linked lymphoproliferative disease: a progressive immunodeficiency. Annu Rev Immunol. https://doi.org/10.1146/annurev.immunol.19.1.657
107. Wu N, Veillette A (2016) SLAM family receptors in normal immunity and immune pathologies. Curr Opin Immunol. https://doi.org/10.1016/j.coi.2015.11.003

108. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN (2008) SAP-controlled T-B cell interactions underlie germinal centre formation. Nature. https://doi.org/10.1038/nature07345
109. Zhao F, Cannons JL, Dutta M, Griffiths GM, Schwartzberg PL (2012) Positive and negative signaling through SLAM receptors regulate synapse organization and thresholds of cytolysis. Immunity. https://doi.org/10.1016/j.immuni.2012.05.017
110. Baldanzi G, Pighini A, Bettio V, Rainero E, Traini S, Chianale F et al (2011) SAP-mediated inhibition of diacylglycerol kinase α regulates TCR-induced diacylglycerol signaling. J Immunol 187. https://doi.org/10.4049/jimmunol.1002476
111. Ruffo E, Malacarne V, Larsen SE, Das R, Patrussi L, Wülfing C et al (2016) Inhibition of diacylglycerol kinase α restores restimulation-induced cell death and reduces immunopathology in XLP-1. Sci Transl Med 8. https://doi.org/10.1126/scitranslmed.aad1565
112. Baldanzi G, Bettio V, Malacarne V, Graziani A (2016) Diacylglycerol kinases: shaping diacylglycerol and phosphatidic acid gradients to control cell polarity. Front Cell Dev Biol 4. https://doi.org/10.3389/fcell.2016.00140
113. Gawden-Bone C, Griffiths GM (2019) Phospholipids: pulling back the actin curtain for granule delivery to the immune synapse. Front Immunol. https://doi.org/10.3389/fimmu.2019.00700
114. Kabanova A, Zurli V, Baldari CT (2018) Signals controlling lytic granule polarization at the cytotoxic immune synapse. Front Immunol. https://doi.org/10.3389/fimmu.2018.00307
115. De La Roche M, Asano Y, Griffiths GM (2016) Origins of the cytolytic synapse. Nat Rev Immunol. https://doi.org/10.1038/nri.2016.54
116. Voskoboinik I, Trapani JA (2013) Perforinopathy: A spectrum of human immune disease caused by defective perforin delivery or function. Front Immunol. https://doi.org/10.3389/fimmu.2013.00441
117. Krzewski K, Coligan JE (2012) Human NK cell lytic granules and regulation of their exocytosis. Front Immunol 3. https://doi.org/10.3389/fimmu.2012.00335
118. Bowman SL, Bi-Karchin J, Le L, Marks MS (2019) The road to lysosome-related organelles: insights from Hermansky-Pudlak syndrome and other rare diseases. Traffic 20:404–435
119. Sepulveda FE, Burgess A, Heiligenstein X, Goudin N, Ménager MM, Romao M et al (2015) LYST controls the biogenesis of the endosomal compartment required for secretory lysosome function. Traffic. https://doi.org/10.1111/tra.12244
120. Pattu V, Halimani M, Ming M, Schirra C, Hahn U, Bzeih H et al (2013) In the crosshairs: investigating lytic granules by high-resolution microscopy and electrophysiology. Front Immunol. https://doi.org/10.3389/fimmu.2013.00411
121. Luzio JP, Hackmann Y, Dieckmann NMG, Griffiths GM (2014) The biogenesis of lysosomes and lysosome-related organelles. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a016840
122. Gholam C, Grigoriadou S, Gilmour KC, Gaspar HB (2011) Familial haemophagocytic lymphohistiocytosis: advances in the genetic basis, diagnosis and management. Clin Exp Immunol. https://doi.org/10.1111/j.1365-2249.2010.04302.x
123. Elstak ED, Neeft M, Nehme NT, Voortman J, Cheung M, Goodarzifard M et al (2011) The munc13-4–rab27 complex is specifically required for tethering secretory lysosomes at the plasma membrane. Blood 118:1570–1578
124. Kurowska M, Goudin N, Nehme NT, Court M, Garin J, Fischer A et al (2012) Terminal transport of lytic granules to the immune synapse is mediated by the kinesin-1/Slp3/Rab27a complex. Blood. https://doi.org/10.1182/blood-2011-09-382556
125. Dieckmann NMG, Hackmann Y, Aricò M, Griffiths GM (2015) Munc18-2 is required for Syntaxin 11 localization on the plasma membrane in cytotoxic T-lymphocytes. Traffic. https://doi.org/10.1111/tra.12337
126. Rodina YA, Matveev VE, Balashov DN, Dubrovina ME, Shcherbina AY (2016) Chediak-Higashi syndrome. Pediatr Hematol Immunopathol. https://doi.org/10.20953/1726-1708-2016-1-27-33
127. Jin Y, Zhang L, Wang S, Chen F, Gu Y, Hong E et al (2017) Whole genome sequencing identifies novel compound heterozygous lysosomal trafficking regulator gene mutations associ-

ated with autosomal recessive Chediak-Higashi syndrome. Sci Rep. https://doi.org/10.1038/srep41308
128. Ménasché G, Pastural E, Feldmann J, Certain S, Ersoy F, Dupuis S et al (2000) Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. Nat Genet 25:173–176
129. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C et al (2003) Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell. https://doi.org/10.1016/S0092-8674(03)00855-9
130. zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter JI et al (2005) Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet. https://doi.org/10.1093/hmg/ddi076
131. Jain R, Puliyel M, Moses PD, Sieni E (2012) Novel STXBP2 mutation causing familial hemophagocytic lymphohistiocytosis. Indian Pediatr. https://doi.org/10.1007/s13312-012-0094-5
132. Bordbar MR, Modarresi F, Farazi Fard MA, Dastsooz H, Shakib Azad N, Faghihi MA (2017) A case report of novel mutation in PRF1 gene, which causes familial autosomal recessive hemophagocytic lymphohistiocytosis. BMC Med Genet. https://doi.org/10.1186/s12881-017-0404-9
133. Janka GE (2012) Familial and acquired hemophagocytic lymphohistiocytosis. Annu Rev Med. https://doi.org/10.1146/annurev-med-041610-134208
134. Velnati S, Ruffo E, Massarotti A, Talmon M, Varma KSS, Gesu A et al (2019) Identification of a novel DGKα inhibitor for XLP-1 therapy by virtual screening. Eur J Med Chem. https://doi.org/10.1016/j.ejmech.2018.12.061

Mucosal Immunity in Primary Immunodeficiencies

5

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Abstract

The epithelia covering the gastrointestinal, respiratory, urogenital, conjunctiva, and inner ear are integral parts of the immune system. Through mechanical and chemical means, they prevent invasion by pathogens. The mucosal immune system consists of an innate and acquired system that interact with each other in a complex way. The mucosal immune system also has the delicate task of differentiating between pathogens and non-pathogens. The T and B lymphocytes present in the mucous membranes are specific to these sites, differing from those we can find in the peripheral circle, and produce specific responses, such as local IgA secretion. In this chapter, we will discuss in a non-exhaustive way the main components and mechanisms of innate and adaptive mucosal immunity and how this can be compromised in primary immunodeficiencies.

Keywords

T cell development · Mucosal immunity · T helper 1 · T helper 2 · T helper 17 · T regulatory cells · Primary immunodeficiency · Intestinal immunity · Bronchial immunity · Infections · Allergy

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5.1 The MALT

The mucosa-associated lymphoid tissue (MALT) is a secondary lymphoid organ that includes more or less organized lymphoid structures [1]. It includes multiple subregions, the most important being the gut-associated lymphoid tissue (GALT), the nasal-associated lymphoid tissue (NALT), and the bronchus-associated lymphoid tissue (BALT). In the human airway of adults, evidence of an organized lymphoid tissue is still lacking in health, while an induced BALT (iBALT) is present in pathological conditions. The MALT is characterized by region-specific inductive and effector sites. Systemic and mucosal immune systems have distinct functional structures and can be activated and regulated in an independent manner [2, 3]. Common characteristics of the MALT are the presence of different cell types, such as B cells, T cells, dendritic cells (DC), as well as innate immune cells, that together contribute to an efficient immune response. Other common characteristics of the MALT are the lack of afferent lymphatics as well as the presence of high endothelial regions [1, 4, 5].

5.2 Epithelia and Innate Mucosal Immunity in Respiratory Tract and Gut

Mucosal epithelia are constantly exposed to external antigens and, in combination with several cell types, facilitate the protection of the gastrointestinal and respiratory tract [6]. The secretion and composition of saliva, including mucus, secretory immunoglobulin A (SIgA), and humoral innate immune proteins, such as lactoferrin, lysozyme, and the defensins, are regulated by the airway epithelium, which provides a physical and chemical barrier that prevents infections as well as chronic inflammatory processes potentially occurring in response to the constant exposure to antigens. The mucosal epithelium, in combination with localized antigen-presenting cells, has a crucial role in connecting the innate and adaptive immune system via the production of cytokines and chemokines to initiate inflammation in case of infection. However, specialized lymphoid cells and immune mechanisms are present at the mucosal sites to exert an immunosuppressive function on adaptive immune processes to tightly modulate and control inflammatory responses.

L-selectin, a receptor molecule prominent in the head and neck mucosa and in the lymph nodes, regulates the trafficking of lymphocytes to these sites. α-defensins regulate the mucosal immune system of the gut and prevent microbial invasion at the epithelial surface and modulate the activity of different T cell subpopulations for further adaptive responses [7, 8]. Increasing data suggest that at mucosal level, innate immunity is the main regulator of the immune response [9].

Congenital defects in the epithelium can lead to very early-onset IBD [10]. The epithelium has an important regulatory function of innate immunity. Severe colitis was described in two children with biallelic LOF mutation in *ALPI* gene, coding for the intestinal phosphatase alkaline, a brush border metalloenzyme that hydrolyzes phosphate from the lipid A moiety of lipopolysaccharides and thereby drastically reduces Toll-like receptor 4 agonist activity [11].

Mutations in TTC7A, an epithelial protein, can result in various phenotypes that may or may not be associated with combined immunodeficiency (CID), including multiple intestinal atresia (MIA) and very early-onset inflammatory bowel disease [12].

Antimicrobial peptide expression by the intestinal epithelium is believed to have an important function in controlling the number of bacteria around epithelial cells and has recently been suggested to have a principal role in the pathogenesis of inflammatory bowel disease. An important role in the regulation of antimicrobial peptide expression is played by the NF-κB signaling pathway [13].

A role of lack of defensins was suggested in the development of colitis in NEMO hypomorphic mutation. In $NEMO^{IEC\text{-}KO}$ mice, the expression of beta-defensin-3 (homologous to human beta-defensin-2, which predisposes to colonic Crohn's disease in humans) [14] was significantly downregulated [15].

5.3 Mucosal T Cells

Conventional T cells develop in the thymus from double-negative (CD4−CD8−) progenitors. After TCRβ expression, CD4−CD8− cells enter a double-positive (CD4+CD8+) stage. The strongly self-reactive cells are eliminated by negative selection, while T cells that present low affinity to self-antigens develop into single-positive CD4+ (via interaction with MHC II) and single-positive CD8+ (by interaction with MHC I) [16].

After leaving the thymus, naïve CD4+ T and CD8αβ+ T cells migrate through the circulation to the gut-associated lymphoid tissues (GALTs), such as the mesenteric lymph nodes and Peyer's patches. Here, they are primed by the antigen-presenting cells (APCs) and by the upregulation of gut-homing molecules, such as CCR9, CD44, integrin α4β7, LFA-1, and VLA-4, that are able to home to different mucosal sites guided by the presence of their specific ligands. The APCs and the intestinal epithelial cells (IEC) regulate differentiation of CD4+ T cells into Th1, Th2, Th17, and intestinal Treg (iTreg) in response to the various food or microbial antigens present at the site [17]. This intestinal T cells mainly migrate to the lamina propria and present an effector memory phenotype.

One small population of thymocytes does not undergo the selection in the thymus, lacks the so-called "conventional" T cell coreceptors (CD4 and CD8αβ), and expresses either TCRγδ or TCRαβ and CD8αα homodimers and are called unconventional T cells. These cells mainly exert regulatory functions and are mainly located between the gut lumen and enterocytes as intraepithelial lymphocytes (IELs) [18–21].

γδ T cell were also described in the lung during respiratory infections, where they contribute to clearance of intracellular and extracellular bacteria. During active pulmonary tuberculosis circulating, γδ T cells are an important source of IL17 [22, 23].

Conventional and nonconventional T cells both concur to provide protection against pathogens and, at the same time, to maintain immune tolerance to commensals and antigens derived by food, contributing to intestinal homeostasis.

IELs play an important role in maintaining the barrier function. A homeostasis in the gut mucosa is depending from a balance between T cells with effector function, which rapidly mount an immune response against pathogens, and regulatory T cells, as well as IL-10-producing CD4+ T cells. A disbalance between these specialized players of the adaptive immune system can lead to autoimmune enteropathy.

IEL were also described in the lung. In biopsies from healthy volunteers, 20 bronchial IEL/100 epithelial cell nuclei were found, mostly expressing ab T cell receptors [24].

Intestinal tolerance to commensal microorganisms and food is mainly mediated by FOXP3+ Treg cells. From the total of CD4+ T cells in the intestine, around 30% are localized in the colon and approximately 20% of those in the small intestine. Gut microbiota affect the number and function of Treg cells. In a mouse model, the number of Treg cells in the small intestine was found to be significantly reduced in germ-free mice, suggesting that a microbiota-independent induction occurs in the small intestine, but not in the colon [25].

Also, the airway mucosa contains specialized lymphoid cells able to regulate and modulate inflammatory responses. It was shown that inducible Tregs ($foxp3^{+}helios^{-}$) in the airways contained the highest frequency of IL-17-producing cells of the $CD4^{+}$ T cell subsets. A higher percentage of $foxp3^{-}CD4^{+}$ T cells produced IL-10 than peripheral blood [26]. The higher frequency of inducible Treg-producing IL-17 may be important for the transport of SIgA, through the induction of T-helper (Th) 17 cells required for T cell-dependent immunoglobulin A production as shown in Peyer's patches [27].

Other T cell subsets considered as nonconventional T cells are MAIT cells. When these nonconventional cells emerge from the thymus, they are already able to act as effector cells. Their TCR are anyway not able to recognize a wide variety of antigens. These characteristics suggest for these cells a role between innate and adaptive immune system.

MAIT cells are found in mucosal tissues, like the intestine and the lung as well as in the liver. These cells can recognize only conserved nonpeptide antigens presented by the MHC class I-like protein MR1 and when activated produce TNF-a and IFN-g, controlling bacterial intracellular infections. These cells are present in the lung and seem to be an important role in respiratory infections. Circulating MAITS were reduced in patients with tuberculosis and almost absent in patients with active tuberculosis, probably due to their recruitment in the lung. Here, they were shown, in murine models of respiratory bacterial infections, to expand and produce IFN-g, TNF-a, and IL-17. Mice lacking MAIT cells showed reduced and delayed response to BCG and *F. tularensis* infection [28, 29]. MAIT cell alterations were recently found in CVID patients that resulted reduced in number and frequency [30]. The remaining cells expressed activation markers and as well as a reduced IFN-g response when challenged in vitro with *E. coli*, similarly to patients affected by

chronic infections, like HIV HTLV1 and HCV [31–34], and to patients with cystic fibrosis [35]. Lower blood MAIT cells were also observed in patients with chronic *H. pylori* or mycobacterial infections [36, 37] (Fig. 5.1).

5.3.1 Autoimmune Enteropathy, Regulatory T Cells, and IL-17 Production

Autoimmune enteropathy (AIE) is a rare disease, clinically manifesting with chronic diarrhea, and malabsorption, that can be associated with autoimmune comorbidities [38].

A disbalance between Tregs and effector T cell activation is one important factor in the development of AIE.

Patients with immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), an X-linked disease caused by mutations in FOXP3, typically present with AIE. The role of Foxp3 is crucial for the development and function of regulatory T cells (CD4+CD25+) [39]. Non-IPEX adult-onset cases have been also reported [40]. FOXP3 expression, as well as CD4+CD25+ Treg expression, is reduced in IPEX, which may explain the intestinal inflammation and villous atrophy due to uncontrolled activation of gut-associated lymphoid tissue. Biopsies from the duodenal mucosa in these patients showed CD4+CD8+ T cell infiltrates [41].

A phenotypic and functional analysis in a severe case of AIE in a non-IPEX adult patient demonstrated production of IFN-γ and IL-17 by intraepithelial T lymphocytes (IEL) in the duodenal mucosa. These findings were absent in samples from Crohn's and celiac disease or healthy controls. In this study, it was shown that TCR-activated IL-17 production has different cytokine and transforming growth factor-β (TGF-β) requirement in the lamina propria and intraepithelial CD4+ and CD8+ lymphocytes. TGF-β in its active form was found in the intestinal mucosa of AIE patients. Tregs with low expression of FOXP3 maintain the ability to produce TGF-β and increase IL-17 production by IEL CD8+ T cells [42]. Remarkably, it was shown in mice models that Treg cells are able to suppress $CD8\alpha^+$ T cell receptor (TCR)$\gamma\delta^+$ T cells, including an interleukin-17 (IL-17)-expressing population, responsible for inflammatory colitis [43].

Regulatory T cell defects are at the basis of gastrointestinal involvement in immune dysregulation syndromes. Gastrointestinal involvement in IPEX syndrome, CD25 deficiency, and CTLA4 insufficiency is described in Chap. 15 of this volume. LRBA deficiency is discussed in the second book of this series, Humoral Primary Immunodeficiencies [1].

Increased and uncontrolled function of effector T cell can as well lead to inflammatory entheropathy.

A disbalance in Treg/ effector T cell activation is at the basis of autoimmune entheropathy in other complex immune deficiencies, like MALT1 deficiency and DOCK 8 deficiency, where a reduced activity of Tregs was described, as well as in STAT1 GOF [44] and STAT3 GOF mutations [45] and in the recently described JAK1 GOF [46].

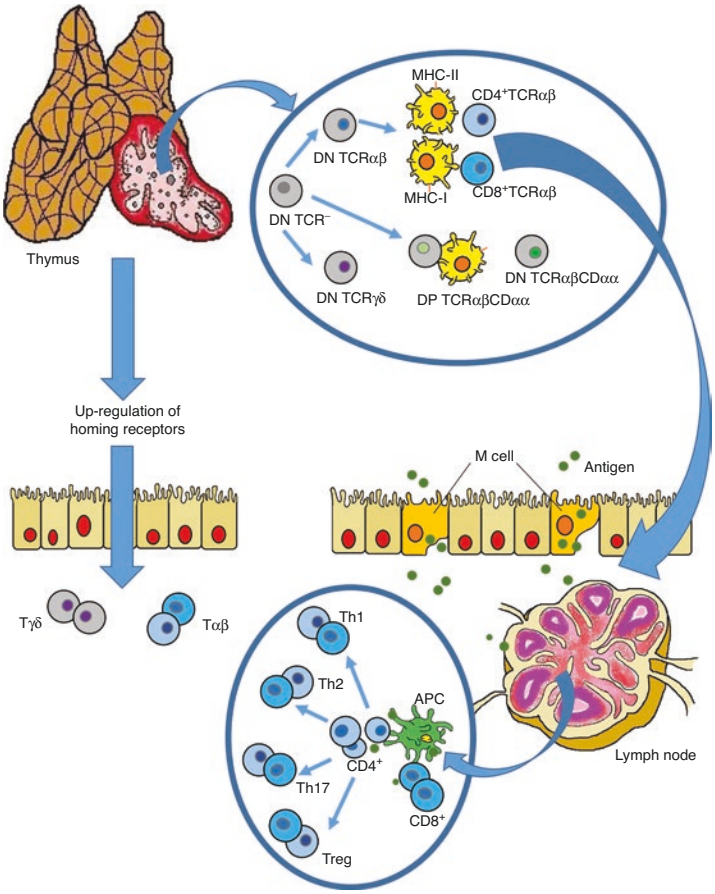

Fig. 5.1 *Origin and development of T lymphocyte lineage subsets.* Conventional T cells develop in the thymus from double-negative (DN) (CD4−CD8−) progenitors. After TCRαβ expression, CD4−CD8− cells enter a double-positive (DP) (CD4+CD8+) stage. The strongly self-reactive cells are eliminated by negative selection, while T cells that present low affinity to self-antigens develop into single-positive CD4+ (via interaction with MHC II) and single-positive CD8+ (by interaction with MHC I). After leaving the thymus, naïve CD4+αβ+ T and CD8+αβ+ T cells migrate through the circulation to the gut-associated lymphoid tissues, such as the mesenteric lymph nodes and Peyer's patches. Here, they are primed by antigen-presenting cells (APCs) and by upregulation of gut-homing molecules, such as CCR9, CD44, integrin α4β7, LFA-1, and VLA-4, that are able to home to different mucosal sites guided by the presence of their specific ligands. The APCs and the intestinal epithelial cells (IEC) regulate differentiation of CD4+ T cells into Th1-producing IFN-γ, Th2-producing IL-4, Th17-producing IL-17, and intestinal T regulatory cells (Treg) in response to the various microbial or food antigens present at the site. These mucosal T cells mainly migrate to the lamina propria and present an effector memory phenotype. One small population of thymocytes does not undergo the selection in the thymus, lacks the so-called "conventional" T cell coreceptors (CD4 and CD8αβ) and express either TCRγδ or TCRαβ and CD8αα homodimers and are called unconventional T cells. These cells mainly exert regulatory functions and are mainly located between the gut lumen and enterocytes as intraepithelial lymphocytes. Υδ+T cells have been also described in the lung during respiratory infections, where they contribute to clearance of intracellular and extracellular bacteria. Conventional and nonconventional T cells both concur to provide protection against pathogens and, at the same time, to maintain immune tolerance to commensals and antigens derived by food, contributing to intestinal homeostasis

5.3.2 CD4+ T Cell Depletion in Gut Mucosal and Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency affecting phagocytes. Patients affected by this condition harbor mutations in NADPH oxidase, leading to impaired reactive oxygen species (ROS) production by neutrophils and monocytes, defects in microorganism clearance, and chronic inflammation [47, 48]. T cell alterations have been previously reported [49]. The underlying mechanisms related to the T cell compartment alterations remain unclear and need further investigation.

Progressive CD4+ lymphopenia, with reduction of naive cells, lymphocyte activation, and expansion of interleukin (IL)-17-producing CD4 T cells, was shown in an adult CGD patient. At the age of 34, the patient presented persistent diarrhea with watery stool, without blood or mucous, associated to hypoalbuminemia without a microbial cause or malabsorption being identified. An endoscopy with biopsies was performed. Lymphoid aggregates and inflammatory infiltrates were reported. Cell suspensions from sigmoid biopsies were analyzed by flow cytometry, showing reduced number of CD4+ T cells, compared to control at the intestinal mucosa level (with decreased CD4+/CD8+ ratio) [50].

The pathogenesis of T cell alterations in the mucosal compartment seems to be related to immunosenescence and needs further investigation.

References

1. D'Elios MM, Rizzi M (2019) Humoral primary immunodeficiencies. Springer, Cham
2. Mei HE, Yoshida T, Sime W, Hiepe F, Thiele K, Manz RA et al (2009) Blood-borne human plasma cells in steady state are derived from mucosal immune responses. Blood 113(11):2461–2469
3. Weitkamp JH, Kallewaard NL, Bowen AL, Lafleur BJ, Greenberg HB, Crowe JE Jr (2005) VH1-46 is the dominant immunoglobulin heavy chain gene segment in rotavirus-specific memory B cells expressing the intestinal homing receptor alpha4beta7. J Immunol 174(6):3454–3460
4. Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P (2008) The immune geography of IgA induction and function. Mucosal Immunol 1(1):11–22
5. Dwivedy A, Aich P (2011) Importance of innate mucosal immunity and the promises it holds. Int J General Med 4:299–311. https://doi.org/10.2147/IJGM.S17525
6. Weitnauer M, Mijošek V, Dalpke A (2016) Control of local immunity by airway epithelial cells. Mucosal Immunol 9:287–298. https://doi.org/10.1038/mi.2015.126
7. Wilson CL, Ouellette AJ, Satchell DP et al (1999) Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science 286(5437):113–117. https://doi.org/10.1126/science.286.5437.113
8. Heather LC, Cole MA, Lygate CA et al (2006) Fatty acid transporter levels and palmitate oxidation rate correlate with ejection fraction in the infarcted rat heart. Cardiovasc Res 72:430–437
9. Heritage PL, Brook MA, Underdown BJ et al (1998) Intranasal immunization with polymer-grafted microparticles activates the nasal-associated lymphoid tissue and draining lymph nodes. Immunology 93:249–256

10. Parlato M, Charbit-Henrion F et al (2017) First identification of biallelic inherited duox2 inactivating mutations as a cause of very early onset inflammatory Bowel disease. Gastroenterology 153:609–611.e3
11. Parlato M, Charbit-Henrion F, Pan J et al (2018) Human ALPI deficiency causes inflammatory bowel disease and highlights a key mechanism of gut homeostasis. EMBO Mol Med 10:e8483
12. Jardine S, Dhingani N, Muise AM (2019) TTC7A: Steward of intestinal health. Cell Mol Gastroenterol Hepatol 7(3):555–557
13. Wehkamp J, Fellermann K, Herrlinger KR, Bevins CL, Stange EF (2005) Mechanisms of disease: defensins in gastrointestinal diseases. Nature Clin Pract Gastroenterol Hepatol 2:406–415
14. Fellermann K et al (2006) A chromosome 8 gene-cluster polymorphism with low human β-defensin 2 gene copy number predisposes to Crohn disease of the colon. Am J Hum Genet 79:439–448
15. Nenci A, Becker C, Wullaert A et al (2007) Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature 446:557–561. https://doi.org/10.1038/nature05698
16. Hayday A, Gibbons D (2008) Brokering the peace: the origin of intestinal T cells. Mucosal Immunol 1(3):172–174. https://doi.org/10.1038/mi.2008.8.Brokering
17. Ma H, Tao W, Zhu S (2019) T lymphocytes in the intestinal mucosa: defense and tolerance. Cell Mol Immunol 16(3):216–224. https://doi.org/10.1038/s41423-019-0208-2
18. Baldwin TA, Hogquist KA, Jameson SC (2004) The fourth way? Harnessing aggressive tendencies in the Thymus. J Immunol 173(11):6515–6520. https://doi.org/10.4049/jimmunol.173.11.6515
19. Chen Y, Chou K, Fuchs E, Havran WL, Boismenu R (2002) Protection of the intestinal mucosa by intraepithelial γδ T cells. Proc Natl Acad Sci U S A 99(22):14338–14343. https://doi.org/10.1073/pnas.212290499
20. Darlington D, Rogers AW (1966) Epithelial lymphocytes in the small intestine of the mouse. J Anat 100(Pt 4):813–830
21. Zeitz M, Schieferdecker HL, Ullrich R, Jahn HU, James SP, Riecken EO (1991) Phenotype and function of lamina propria T lymphocytes. Immunol Res 10(3–4):199–206. https://doi.org/10.1007/BF02919693
22. Wands JM, Roark CL, Aydintug MK, Jin N, Hahn YS et al (2005) Distribution and leukocyte contacts of gammadelta T cells in the lung. J Leukoc Biol 78:1086–1096
23. Peng MY, Wang ZH, Yao CY, Jiang LN, Jin QL et al (2008) Interleukin 17-producing gamma delta T cells increased in patients with active pulmonary tuberculosis. Cell Mol Immunol 5:203–208
24. Fournier M, Lebargy F, Le Roy F, Ladurie LE, Pariente R (1989) Intraepithelial T-lymphocyte subsets in the airways of normal subjects and of patients with chronic bronchitis. Am Rev Respir Dis 140:4737–4742
25. Bilate AM, Lafaille JJ (2012) Induced CD4 + Foxp3 + regulatory T cells in immune tolerance. Annu Rev Immunol 30(1):733–758. https://doi.org/10.1146/annurev-immunol-020711-075043
26. Ballke C, Gran E, Baekkevold ES et al (2016) Characterization of regulatory T-cell markers in CD4+ T cells of the upper airway mucosa. PLoS One 11:e0148826. https://doi.org/10.1371/journal.pone.0148826
27. Hirota K, Turner J-E, Villa M et al (2013) Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. Nat Immunol 14:372–379. https://doi.org/10.1038/ni.2552
28. Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM et al (2010) Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol 8:e1000407
29. Meierovics A, Yankelevich WJ, Cowley SC (2013) MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. Proc Natl Acad Sci U S A 110:E3119–E3128

30. Paquin-Proulx D, Santos BAN et al (2017) Loss of circulating mucosal-associated invariant T cells in common variable immunodeficiency is associated with immune activation and loss of eomes and PLZF. ImmunoHorizons 1(7):142–155. https://doi.org/10.4049/immunohorizons.1700039
31. Leeansyah E, Ganesh A, Quigley MF et al (2013) Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. Blood 121:1124–1135
32. Cosgrove C, Ussher JE, Rauch A et al (2013) Early and nonreversible decrease of CD161++ / MAIT cells in HIV infection. Blood 121:951–961
33. Paquin-Proulx D, Greenspun BC, Costa EA et al (2017) MAIT cells are reduced in frequency and functionally impaired in human T lymphotropic virus type 1 infection: potential clinical implications. PLoS One 12:e0175345
34. Hengst J, Strunz B, Deterding K et al (2016) Nonreversible MAIT cell-dysfunction in chronic hepatitis C virus infection despite successful interferon-free therapy. Eur J Immunol 46:2204–2210
35. Smith DJ, Hill GR, Bell SC et al (2014) Reduced mucosal associated invariant T-cells are associated with increased disease severity and Pseudomonas aeruginosa infection in cystic fibrosis. PLoS One 9:e109891
36. Booth JS, Salerno-Goncalves R, Blanchard TG et al (2015) Mucosal-associated invariant T cells in the human gastric mucosa and blood: role in Helicobacter pylori infection. Front Immunol 6:466
37. Kwon YS, Cho YN, Kim MJ et al (2015) Mucosal-associated invariant T cells are numerically and functionally deficient in patients with mycobacterial infection and reflect disease activity. Tuberculosis 95:267–274
38. Gentile NM, Murray JA, Pardi DS (2012) Autoimmune enteropathy: a review and update of clinical management. Curr Gastroenterol Rep 14(5):380–385. https://doi.org/10.1007/s11894-012-0276-2
39. Barzaghi F, Passerini L, Bacchetta R (2012) Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: A paradigm of immunodeficiency with autoimmunity. Front Immunol 3:1–25. https://doi.org/10.3389/fimmu.2012.00211
40. Moes N, Rieuxlaucat F, Begue B et al (2010) Reduced expression of FOXP3 and regulatory T-cell function in severe forms of early-onset autoimmune enteropathy. Gastroenterology 139(3):770–778. https://doi.org/10.1053/j.gastro.2010.06.006
41. Ruemmele FM, Brousse N, Goulet O (2004) Autoimmune enteropathy: molecular concepts. Curr Opin Gastroenterol 20(6):587–591. https://doi.org/10.1097/00001574-200411000-00014
42. Paroni M, Magarotto A, Tartari S et al (2016) Uncontrolled IL-17 production by intraepithelial lymphocytes in a case of non-IPEX autoimmune enteropathy. Clin Transl Gastroenterol 7(7):e182–e111. https://doi.org/10.1038/ctg.2016.41
43. Park SG, Mathur R, Long M et al (2010) T regulatory cells maintain intestinal homeostasis by suppressing γδ T cells. Immunity 33(5):791–803. https://doi.org/10.1016/j.immuni.2010.10.014
44. Toubiana J, Okada S, Hiller J et al (2016) Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. Blood 127(25):3154–3164. https://doi.org/10.1182/blood-2015-11-679902
45. Milner JD, Vogel TP, Forbes L et al (2015) Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. Blood 125(4):591–599. https://doi.org/10.1182/blood-2014-09-602763
46. Gruber CN, Calis JJA, Buta S et al (2020) Complex autoinflammatory syndrome unveils fundamental principles of JAK1 kinase transcriptional and biochemical function. Immunity 53(3):672–684.e11. https://doi.org/10.1016/j.immuni.2020.07.006
47. Di R (2016) Chronic granulomatous disease. Br Med Bull 118(1):50–63. https://doi.org/10.1093/bmb/ldw009

48. Holland SM (2013) Chronic granulomatous disease. Hematol Oncol Clin North Am 27(1):89–99. https://doi.org/10.1016/j.hoc.2012.11.002.Chronic
49. Heltzer M, Jawad AF, Rae J, Curnutte JT, Sullivan KE (2002) Diminished T cell numbers in patients with chronic granulomatous disease. Clin Immunol 105(3):273–278. https://doi.org/10.1006/clim.2002.5291
50. Albuquerque AS, Fernandes SM, Tendeiro R et al (2017) Major CD4 T-cell depletion and immune senescence in a patient with chronic granulomatous disease. Front Immunol 8:4–11. https://doi.org/10.3389/fimmu.2017.00543

Combined Immunodeficiencies

6

Francesco Liotta and Lorenzo Salvati

Abstract

Combined immunodeficiencies (CID) are a heterogeneous and expanding group of primary immunodeficiencies associated with T and B cell impaired immunity due to several genetic variants. In contrast to severe combined immunodeficiencies (SCID), CID are typically milder diseases and can have a delayed onset. Patients with CID may present with recurrent, often severe, viral, bacterial, mycobacterial, fungal, and protozoan infections, mainly affecting the respiratory and gastrointestinal tract, immune dysregulation (autoimmunity, inflammatory bowel disease, severe dermatitis, lymphoproliferation, granulomas, vasculitis), and malignancies. On laboratory evaluation, lymphocyte numbers and phenotype and humoral assessment can help to orientate the diagnosis. Genetic analysis is essential for CID classification. Most CID have an autosomal recessive mode of inheritance. The prognosis varies according to the disease and the time of diagnosis. The treatment of patients with CID is individualized, but generally it comprises supportive therapy (immunoglobulin replacement therapy and antimicrobial treatment or prophylaxis), as well as allogenic hematopoietic stem cell transplantation in selected cases.

Keywords

Primary immunodeficiency · Inborn errors of immunity · Lymphopenia · T cell impairment · Poor T cell proliferation · Opportunistic infections · Immune dysregulation

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"When you hear hoofbeats, think of zebras too!"

6.1 Definition

Combined immunodeficiencies (CID) are primary immunodeficiencies resulting from several genetic mutations that determine impairment of T cells, but B cells can also be affected as a result of intrinsic defects or altered T helper cell function. CID are a large and expanding group of monogenic diseases that are characterized by T cell deficiency, frequent immune dysregulation, and variable capacity of adaptive cellular response. They differ from severe combined immunodeficiencies (SCID) mainly because the disease onset may occur later in life, even in adults, and there is not profound T cell deficiency [1, 2]. Apart from the definition of atypical SCID (300–1500 CD3 cells/μL with residual—10–50% the lower limit of normal—capacity to proliferate to phytohemagglutinin [PHA]) as less severe disease than SCID (<300 CD3 cells/μL with less than 10% of lower limit of normal proliferation to PHA) by the Primary Immune Deficiency Treatment Consortium (PIDTC) of North America[3], the European Society for Immunodeficiency (ESID) developed a set of criteria for a working definition for clinical diagnosis of CID. In patients without HIV infection or other conditions that are syndromic disease in general more severe than CID (e.g., ataxia-telangiectasia, dyskeratosis congenita, congenital hair hyperplasia), the following criteria must be met: at least one clinical criteria (severe infection, immune dysregulation, malignancy, affected family member) and two of four laboratory criteria (low CD3 or CD4 or CD8 T cells, low naïve CD4 and/or CD8 T cells, expansion of TCR γδ T cells, reduced proliferation to mitogens or TCR stimulation) [4] (Table 6.1). A subset of common variable immunodeficiencies (CVID) with severe T cell defect has been reclassified as late-onset combined immunodeficiencies (LOCID) [5]. In the French DEFI study, 9% of patients with CVID had LOCID, as defined by the occurrence of an opportunistic infection and/or a CD4 T

Table 6.1 Clinical criteria for a probable diagnosis of combined immunodeficiency (CID) according to the European Society for Immunodeficiencies Registry [4, 25]

At least one of the following: • At least one severe infection (requiring hospitalization) • One manifestation of immune dysregulation (autoimmunity, inflammatory bowel disease, severe eczema, lymphoproliferation, granuloma) • Malignancy • Affected family member
AND two of four T cell criteria fulfilled: • Reduced CD3 or CD4 or CD8 T cells (using age-related reference values) • Reduced naïve CD4 and/or CD8 T cells • Elevated TCR γδ T cells • Reduced proliferation to mitogen or TCR stimulation
AND HIV excluded
AND exclusion of a clinical diagnosis associated with CID (e.g., defined syndromic diseases, ataxia-telangiectasia, dyskeratosis congenita, cartilage hair hypoplasia)

cell count <200 cells/μL [6]. Patients with LOCID had higher prevalence of gastrointestinal disease, splenomegaly, granulomatous disease, and lymphomas and required more frequent antibiotic therapy and hospitalization than other patients with CVID [6]. The LOCID definition has then been modified by classifying patients with opportunistic infections or a naïve CD4 T cell count <20 cells/μL [5]. It has been recently shown that the relative reduction of naïve CD4 T cells below 10% is the most sensitive indicator of LOCID for all adult CVID patients without a clear diagnostic feature of CID; however, none of the current clinical definitions is sufficient to distinguish CID from CVID patients [7].

6.2 Genetics

The number of CID has rapidly increased in the last few decades, as a result of improved awareness and the use of next-generation sequencing that has led to the identification of novel genetic mutations as well as the description of new disorders [8, 9]. The 2019 updated classification of primary immunodeficiencies from the International Union of Immunological Societies (IUIS) Expert Committee listed 40 genetic defects underlying different inborn errors of immunity, collectively defined as CID less profound than SCID and recently the 2021 Interim Update has added newly identified genetic variants [9, 10] (Table 6.2). These monogenic germline mutations cause variable immune defects of cellular and humoral immunity and may led to more severe conditions depending upon the penetrance or the functional consequences of the specific mutation. Indeed, some patients have "leaky" defects in the same genes, in which amorphic mutations cause typical SCID [11]. Hypomorphic mutations resulting in reduced production of a protein, or in a protein with reduced function, are associated with a wide spectrum of clinical phenotypes. For instance, a group of patients presenting later in childhood or even in young adulthood with CID associated with granulomatous disease and/or autoimmunity is compound heterozygote for mutations in combination activating gene 1 or 2 (*RAG1* or *RAG2*) [12, 13]. The majority of CID is inherited in an autosomal recessive pattern, while CD40L deficiency and moesin deficiency are X-linked disorders, IKAROS deficiency and RelA haploinsufficiency are autosomal dominant disorders. More specifically, dominant-negative *IKZF1* mutations underlie the IKAROS deficiency, an early-onset CID [14]. Compared to the previous classification [8], the 2019 updated version has included seven new inborn errors of immunity (*ICOSLG*, *IKZF1*, *POLD1*, *POLD2*, *RELA*, *REL*, *FCHO1*) among CID less profound than SCID (Table 6.2) and classified BCL11B deficiency in the group of CID with associated or syndromic features. This latter category of disorders includes, among others, the purine nucleoside phosphorylase deficiency and the calcium channel defects (ORAI-1 deficiency and STIM1 deficiency). The 2021 Interim Update of IUIS classification added four novel inborn errors of immunity, classifying variants in CTNNBL1, TNFSF13 (APRIL), NOS2, and NCKAP1L (HEM1) genes [10, 15–19] (Table 6.2). Next-generation sequencing diagnostics is contributing to distinguish the clinical phenotype of patients with CID that may often overlap with CVID [20–24]; a study of the ESID Registry found that 7.4% of patients, initially diagnosed as CVID after genetic analysis, were reclassified as CID [25].

Table 6.2 Genetic defects, clinical presentation, and laboratory features of CID classified by the International Union of Immunological Societies Expert Committee (modified from [9, 10]) (Springer OA BY CC License 4.0)

Disease	Gene	Clinical presentation	Laboratory features
CD40 ligand deficiency (CD154)	*CD40LG*	Severe and opportunistic infections, liver and biliary tract disease (hepatitis, cholangitis, cholangiocarcinoma), *Cryptosporidium* infections, peripheral ectodermal tumors	Normal to low T cells; idiopathic neutropenia, thrombocytopenia, hemolytic anemia; decreased antigen-specific responses; reduced memory B cells, absent switched memory B cells; IgM normal or high, IgG, IgA, and IgE low
CD40 deficiency	*CD40*	Opportunistic infections, gastrointestinal, liver and biliary tract disease, *Cryptosporidium* infections	Normal T cells; neutropenia; decreased antigen-specific responses; reduced memory B cells, absent switched memory B cells; IgM normal or high, IgG, IgA, and IgE low
ICOS deficiency	*ICOS*	Recurrent infections, autoimmunity, gastroenteritis, granulomas	Normal T and B cells, low Ig levels
ICOSL deficiency	*ICOSLG*	Recurrent bacterial and viral infections	Low T cells, low B cells, low Ig levels; neutropenia
CD3γ deficiency	*CD3G*	Immune dysregulation of variable severity	Normal T cell number with low TCR expression, normal B cells, normal Ig levels
CD8 deficiency	*CD8A*	Recurrent viral respiratory tract infections; can be asymptomatic	Absent CD8 cells, normal CD4 cells, normal T cell proliferation, normal B cells, normal Ig levels
ZAP70 deficiency (ZAP70 LOF)	*ZAP70*	May have immune dysregulation, autoimmunity	Low CD8, normal CD4 cells number, but poor T cell function; normal B cells, normal Ig levels
ZAP70 combined hypomorphic and activating mutations	*ZAP70*	Severe autoimmunity (bullous pemphigoid, inflammatory colitis)	Low CD8, normal or low CD4 cells; normal or low B cells, normal IgA, low IgM, low/normal IgG; protective antibody responses to vaccines
MHC class I deficiency	*TAP1* *TAP2* *TAPBP*	Vasculitis, pyoderma gangrenosum	Low CD8, normal CD4 number, absent MHC I on lymphocytes; normal B cells, normal Ig levels
	B2M	Sinopulmonary infections, cutaneous granulomas	Low CD8, normal CD4 number, absent MHCI on lymphocytes; normal B cells, normal Ig levels. Absent β2m-associated proteins MHCI, CD1a, CD1b, CD1c

Table 6.2 (continued)

Disease	Gene	Clinical presentation	Laboratory features
MHC class II deficiency group A, B, C, D	*CIITA* *RFXANK* *RFX5* *RFXAP*	Respiratory and gastrointestinal infections, biliary tract and liver disease, failure to thrive, diarrhea	Low CD4 cells; reduced/absent MHCII expression on lymphocytes; normal B cells and normal to low Ig levels
IKAROS deficiency	*IKZF1*	*Pneumocystis jirovecii* pneumonia, severe bacterial or viral respiratory infections, early CID onset (<2 years), T cell acute lymphoblastic leukemia	No memory T cells, no memory B cells, low Ig levels
DOCK2 deficiency	*DOCK2*	Early invasive HSV and bacterial infections	Low T cells, normal B cells, IgG normal or low, poor antibody responses, normal NK cells but impaired function; poor interferon responses in hematopoietic and non-hematopoietic cells
DOCK8 deficiency	*DOCK8*	Recurrent staphylococcal, viral and fungal skin infections; mucocutaneous candidiasis, severe atopy and allergic disease, cancer diathesis	Low T cell number, low naÏve CD8 cells, increased CD8 T effector memory cells, elevated γδ T cells, poor T cell proliferation; few, poorly functioning Treg; low NK cells with poor function; eosinophilia; increased total B cells, low CD27+ memory B cells and poor peripheral B tolerance; low IgM, normal-high IgG and IgA, very high IgE, poor antibody response
Polymerase δ deficiency	*POLD1* *POLD2*	Recurrent respiratory tract infections, skin infections, warts and molluscum, short stature, intellectual disability	Low CD4 cells, low B cells but normal maturation, low IgG levels
RHOH deficiency	*RHOH*	HPV infection, lung granulomas, molluscum contagiosum, lymphoma	Normal T cell number, low naÏve T cells, restricted repertoire, poor T cell proliferation to CD3; normal B cells and normal Ig levels
STK4 deficiency	*STK4*	Bacterial, viral (HPV, EBV, molluscum), candidal infections, lymphoproliferation, lymphoma, congenital heart disease	Low CD4 cells, low naÏve T cells, increased TEM and TEMRA; poor T cell proliferation; low B cells; low IgM, high IgG, IgA, and IgE levels; intermittent neutropenia, autoimmune cytopenias

(continued)

Table 6.2 (continued)

Disease	Gene	Clinical presentation	Laboratory features
TCRα deficiency	*TRAC*	Recurrent bacterial and fungal infections, immune dysregulation and autoimmunity, diarrhea	Absent TCRαβ (except for a minor $CD3^{dim}$ TCRαβ population, all T cells are γδ), poor T cell proliferation; normal B cells and normal Ig levels
LCK deficiency	*LCK*	Recurrent infections, immune dysregulation, autoimmunity	Low CD4 cells, low Treg, restricted T cell repertoire, poor TCR signaling; normal B cells; normal IgG and IgA, high IgM
ITK deficiency	*ITK*	EBV-associated B cell lymphoproliferation, lymphoma, immune dysregulation	Progressive low CD4 T cells, reduced T cell activation; normal B cells, normal to low Ig levels
MALT1 deficiency	*MALT1*	Bacterial, fungal, and viral infections	Normal T cells number, poor T cell proliferation; normal B cells; normal Ig levels, poor specific antibody response
CARD11 deficiency	*CARD11*	*Pneumocystis jirovecii* pneumonia, bacterial and viral infections	Normal T cell number, predominant naïve T cells, poor T cell proliferation; normal, transitional B cell predominance; absent or low Ig levels
BCL10 deficiency	*BCL10*	Recurrent bacterial and viral infections, candidiasis, gastroenteritis	Normal T cell number, low memory and Treg cells, poor antigen and anti-CD3 proliferation; normal B cell number, low memory and switched B cells; low Ig levels
IL-21 deficiency	*IL21*	Severe early-onset colitis, recurrent sinopulmonary infections	Normal T cell number, normal to low T cell function; low B cells, low memory and switched B cells; low IgG levels, high IgE, poor specific antibody response
IL-21R deficiency	*IL21R*	Recurrent infections, *Pneumocystis jirovecii*, Cryptosporidium infections and liver disease	Normal T cell number; low cytokine production; poor antigen proliferation; normal B cells; normal Ig levels, poor specific antibody responses
OX40 deficiency	*TNFRSF4*	Impaired immunity to HHV8, Kaposi's sarcoma	Normal T cell number, low antigen-specific memory CD4, normal B cell number, low memory B cells, normal Ig levels

Table 6.2 (continued)

Disease	Gene	Clinical presentation	Laboratory features
IKBKB deficiency	*IKBKB*	Recurrent bacterial, viral, and fungal infections, opportunistic infections	Normal T cell number, absent Treg and γδ T cells, impaired TCR activation; normal B cell number, poor B cell function; low Ig levels
NIK deficiency	*MAP3K14*	Recurrent bacterial, viral, and *Cryptosporidium* infections	Normal T cell number, poor T cell proliferation to antigen; low B cells number, low switched memory B cells, low Ig levels; low NK cell number and function
RelB deficiency	*RELB*	Recurrent infections	Normal T cell number, poor T cell diversity, reduced proliferation to mitogens, no response to antigen; high B cell number; normal Ig levels, impaired specific antibody response
RelA haploinsufficiency	*RELA*	Chronic mucocutaneous ulceration	Normal/high T cells, normal B cells, normal Ig levels; impaired NF-κB activation, reduced production of inflammatory cytokines
Moesin deficiency	*MSN*	Recurrent bacterial and VZV infections	Normal T cell number, defective T cell migration and proliferation; neutropenia; low B cell number; low Ig levels over time
TFRC deficiency	*TFRC*	Recurrent infections	Normal T cell number, poor T cell proliferation; neutropenia, thrombocytopenia; normal B cell number, low memory B cells; low Ig levels
c-Rel deficiency	*REL*	Recurrent infections (bacteria, mycobacteria, salmonella, opportunistic organisms)	Normal T cells, low memory CD4, poor T cell proliferation; low B cells, mostly naÏve B cells, low switched memory B cells, impaired B cell proliferation; low Ig levels, poor antibody specific response; defective innate immunity

(continued)

Table 6.2 (continued)

Disease	Gene	Clinical presentation	Laboratory features
FCHO1 deficiency	*FCHO1*	Recurrent viral, mycobacterial, bacterial and fungal infections, lymphoproliferation, failure to thrive	Low T cells, poor T cell proliferation; normal B cells; normal Ig levels; increased activation-induced T cell death; defective clathrin-mediated endocytosis
CTNNBL1 deficiency	CTNNBL1	CVID, autoimmune cytopenias, recurrent infections, hyperplastic germinal centers on lymph node biopsy	Low T cells; reduced memory B cells; impaired CSR, SHM; progressive severe low Ig levels
TNFSF13 (*APRIL*) deficiency	TNFSF13 (*APRIL*)	CVID, chronic but mild infections	Normal T cells; normal NK cells; normal total B cell counts with increased IgM+ marginal zone, reduced switched memory B cells, low plasmablasts; low Ig levels
NOS2 deficiency	NOS2	Severe susceptibility to CMV-induced disease; Pneumocystis jirovecii pneumonia secondary to CMV	Low CD4 cells, normal CD8 cells; low NK cells; low B cells; normal Ig levels
NCKAP1L (HEM1) deficiency	NCKAP1L (HEM1)	Recurrent upper respiratory tract infectinos, skin rashes/abscesses, ulcers; SLE-like, lymphadenopathy, fever, HLH-like; failure to thrive; atopy, lymphoproliferation and hyperinflammation	Normal T cell numbers, increased TCM; normal B cells and naïve/memory subsets, increased CD21lo cells; normal/high Ig levels; reduced T cell proliferation; anti-dsDNA antibodies

APRIL A proliferation-inducing ligand, *B2M* β-2-microglobulin, *BCL10* B cell CLL/lymphoma 10, *CARD11* Caspase recruitment domain family member 11, *CIITA* Class II major histocompatibility complex transactivator, *CMV* Cytomegalovirus, *CTNNBL1* β-catenin–like protein 1, *CSR* Class swith recombination, *CVID* common variable immunodeficiency, *DOCK2* Dedicator of cytokinesis 2, *DOCK8* Dedicator of cytokinesis 8, *EBV* Epstein-Barr virus, *FCHO1* F-BAR domain only protein 1, *HEM1* Hematopoietic protein 1, *HLH* Hemophagocytic lymphohistiocytosis, *HPV* Human papilloma virus, *ICOS* Inducible T cell costimulator, *ICOSLG* Inducible T cell costimulator ligand, *IKBKB* Inhibitor of nuclear factor-κB kinase subunit β, *IKZF1* IKAROS family zinc finger 1, *IL-21* Interleukin 21, *IL-21R* Interleukin 21 receptor, *ITK* IL-2-inducible T cell kinase, *LCK* LCK proto-oncogene, Src family tyrosine kinase, *LOF* Loss-of-function, *MALT1* MALT1 paracaspase, *MAP3K14* Mitogen-activated protein kinase kinase kinase 14, *MHC* Major histocompatibility complex, *MSN* Moesin, *NCKAP1L* NCK associated protein 1 like, *NF-κB* Nuclear factor-κB, NIK Nuclear factor-κB-inducing kinase, *NOS2* Nitric oxide synthase 2, *POLD1* DNA polymerase δ 1, catalytic subunit, *POLD2* DNA polymerase δ 1, accessory subunit, *RFX5* Regulatory factor X5, *RFXANK* Regulatory factor X-associated ankyrin-containing protein, *RFXAP* Regulatory factor X-associated protein, *RELA* RELA proto-oncogene, NF-κB subunit, *RELB* RELB proto-oncogene, NF-κB subunit, *RHOH* ras homolog family member H, *SHM* Somatic hypermutation, *SLE* Systemic lupus erythematosus, *STK4* Serine/threonine kinase 4, *TAP1* Transporter 1, ATP-binding cassette subfamily B member, *TAP2* Transporter 2, ATP-binding cassette subfamily B member, *TAPBP* TAP-binding protein, *TCR* T cell receptor, *TEMRA* Terminally effector memory, *TFRC* Transferrin receptor, *TNFRSF4* TNF receptor superfamily member 4, *TNFSF13* TNF superfamily member 13, *TRAC* T cell receptor α constant, *Treg* Regulatory T cell, *VZV* Varicella zoster virus, *ZAP70* ζ chain of T cell receptor-associated protein kinase 7. *Note that additional genetic variants causing novel inborn errors of immunity could have been identified from the publication of this table.*

6.3 Pathogenesis

CID are in some ways the living representation of the immune system redundancy [26]. Causal mutations affecting the expression of molecules required for T and B cell activation, function, and maturation result in an impaired immune response that phenotypically causes increased vulnerability to infections and/or immunopathology, including allergy, autoimmunity, autoinflammation, and lymphoproliferation [2]. Figure 6.1 shows the gene defects involved in CID according to the 2019 IUIS classification and 2021 interim update [9, 10]. Many genetic variants in CID affect the T cell receptor (TCR) signaling, which is essential to lymphocyte function [27]. The antigen receptor of MHC-restricted CD4 and CD8 cells is a heterodimer made of two transmembrane polypeptide chains (α/β or γ/δ) associated with the CD3 signal transduction chains (γ, δ, ε, and ζ). Upon TCR engagement, the first molecule to be recruited to the TCR-CD3 complex is the SRC family kinase member LCK, which is released from inhibition by a transmembrane phosphatase, CD45, and then phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3γ chain, δ chain, ε chain, and ζ chains [28, 29]. Phosphorylation of the ITAMs enables the recruitment of ζ chain-associated protein kinase of 70 kDa (ZAP70), which becomes phosphorylated by LCK and consequently activated [28, 30]. Activated ZAP70 phosphorylates linker for the activation of T cells (LAT)

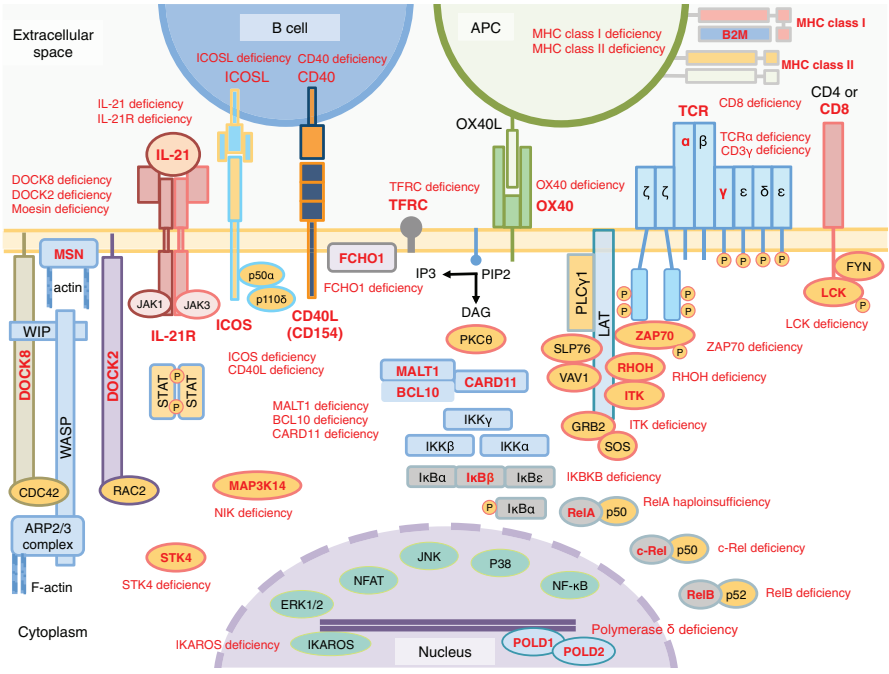

Fig. 6.1 Schematic critical steps in T lymphocyte signalling leading to combined immunodeficiencies

which, in turn, recruits numerous signaling molecules, including phospholipase Cγ1 (PLCγ1), growth factor receptor-bound protein 2 (GRB2), GRB2-related adaptor protein GADS, SH2 domain-containing leukocyte protein of 76 kDa (SLP76), adhesion- and degranulation-promoting adaptor protein (ADAP), interleukin-2-inducible T cell kinase (ITK), NCK1, and VAV1, to form a multiprotein complex, termed the LAT signalosome [28, 30]. PLCγ1 is responsible for the calcium-dependent signaling, VAV1 activates the p38 and JNK transcription factors, while GRB2 associates with the SOS protein to activate ERK1 transcription factor. All these proteins are able to recruit and activate NCK, which contributes to coordinate WASP and ARP-2/3, in order to change the actin cytoskeleton state and structure that is an essential factor for lymphocyte cell activation. On the other hand, dedicator of cytokinesis 8 (DOCK8) is important for the activation of CDC42, while dedicator of cytokinesis 2 (DOCK2) is important in the activation and RAC2 [31]. Once activated, CDC42 is crucial, together with WASp, for the activation of the ARP2/3 complex and nucleation of actin filaments and branching. RAC2 is involved in downstream F-actin formation, while moesin (MSN) connects actin filaments to the membrane [32]. PLCγ1 cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). IP3 induces Ca^{2+} release from the endoplasmic reticulum vesicles, whose depletion induces clustering of the STIM1 protein, leading to the induction of a multicenter complexes with ORAI protein in the plasma membrane. Ca^{2+} favors calmodulin detachment from protein kinase C (PKC) members, so that DAG can bind and activate PKC. In T and B cells, PKCθ and PKCβ activate the CARD11/BCL10/MALT1 complex (CBM complex) [33, 34]. The activation of the CBM complex in turn activates IkB kinase through caspase-8, responsible for the nuclear translocation of nuclear factor-κB (NF-κB). The NF-κB transcription factor family consists of five Rel proteins, (p50/p105, p52/p100, RelA, RelB, and c-Rel), which dimerize with each other and activate or inhibit gene expression in the nucleus. Typically, NF-κB pathway is stimulated by microbial products or by pro-inflammatory cytokines, such as IL-1β and TNF; its activation is subordinated to degradation of NF-κB inhibitor α (IκBα) through phosphorylation and ubiquitination. IkBα phosphorylation is mediated by the inhibitor of κB kinase (IKK) complex, including IKKα and IKKβ and the regulatory protein called NF-κB essential modulator (NEMO) or IKKγ. This leads to the formation of heterodimers with RelA, RelB, and c-Rel able to enter the nucleus and drive transcription of pro-inflammatory genes [35].

With regard to lymphocyte development, B cells develop and maturate in the bone marrow, while precursors of T and of NK cells are derived from the bone marrow but are early recruited in the thymus, where they become mature cells [36]. The IkZF family of transcription factors comprises a series of five proteins: Ikaros (encoded by the gene IKZF1), Helios (IKZF2), Aiolos (IKZF3), Eos (IKZF 4), and Pegasus (IKZF 5) [37]. Ikaros is a transcription factor that regulates cytokine signaling pathways and CD4 cell differentiation [37]. T cell maturation requires major histocompatibility complex (MHC) class I and II molecules to be expressed on thymic stromal cells to provide adequate antigen presentation and also antigen receptor selective processes. Antigenic activation of lymphocytes leads to new

transcriptional programs responsible for the driving of the immune response. Therefore, the transcription factors and regulatory proteins, such as serine/threonine kinase STK4 (MST1), are critical for lymphocytes' activation [38]. IL-21 receptor transduces activating signals via JAK-STAT pathway [39]. The interaction between the T cell effector molecule CD40 ligand (CD154, expressed by CD4+ T cells, upon antigen activation) and its receptor CD40 (expressed by B cells but also by macrophage and by dendritic cells) plays an essential role in T cell-dependent B cell activation and, in general, for the activation of all antigen-presenting cells (APCs) [40, 41]. OX40 is also expressed by activated T cells and OX40L by APC; this cross talk is important in T cell-B cell costimulatory signaling as well as for macrophage and by dendritic cell costimulation [42]. Clathrin-mediated endocytosis is a receptor-mediated process responsible for the uptake of cell-surface cargo proteins and extracellular molecules, including metabolites, hormones, proteins, and molecules involved in cell signaling [43]. The FCH domain only 1 and 2 (FCHO1/FCHO2) proteins are crucial for the early phases of clathrin-mediated endocytosis being involved in the maturation of clathrin-coated pit formation. Deficiency of FCHO1/FCHO2 function has been recently reported as correlated to primary immunodeficiency in humans, leading to variable in B and T cell numbers and functional T cell alterations, including cell activation impairment upon T cell receptor stimulation [43, 44].

6.4 Clinical Features

Although distinctive clinical phenotypes may characterize some monogenic disorders [45], patients with CID typically present with recurrent respiratory and gastrointestinal tract infections that are caused by a broad spectrum of pathogens: viruses, bacteria, mycetes, protozoa, and helminths [9, 10]. At the same time, patients may have manifestations of immune dysregulation: severe eczema, allergy, autoimmune disease, autoimmune cytopenia, vasculitis, granulomatous disease, lymphoproliferation, and inflammatory bowel disease. The main clinical features of each monogenic disorder are summarized in Table 6.2. The disease onset is commonly delayed compared to SCID (>1 year of age) and less severe because of residual T cell function. In addition, patients with milder illness can present later in childhood or even in early adulthood. CID should be suspected in children with failure to thrive; chronic or recurrent respiratory tract infections, which corresponds to more than eight upper respiratory tract infections (rhinosinusitis, pharyngitis) per year or more than one lower respiratory tract infection (pneumonia) per year; persistent viral systemic infections; invasive bacterial infections; opportunistic infections; chronic diarrhea; autoimmunity and other manifestations of immune dysregulation; EBV-positive lymphoproliferative disease; a family history of immunodeficiency; and chronic lymphopenia (total lymphocyte count <1500 cells/μL in children over 5 years of age, <2500 cells/μL in younger children) [46]. In general, in children with severe infections, a diagnosis of CID should be excluded [47]. Many of these clinical features are similar in adults that can also present with unexplained weight loss,

onset of an autoimmune disease, development or worsening of lymphopenia, severe acute or chronic infections, opportunistic infections, granulomatous disease, lymphoproliferative disorders, and autoinflammatory disease [48]. As a result of T cell dysfunction, viral infections are particularly relevant in all the patients with CID and mainly involve the upper and lower respiratory tract, the gastrointestinal tract, and the skin. All viruses can account for infection in CID patients, especially herpesviruses, as HSV-1 (causing recurrent stomatitis), HSV-2, cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), and human herpesvirus 8 (HHV-8) that cause childhood-onset classic Kaposi's sarcoma in OX40 deficiency [49], as well as respiratory viruses (respiratory syncytial virus, adenoviruses, influenza virus, parainfluenza virus type 3) that variably determine bronchiolitis, bronchitis and pneumonia, norovirus and rotavirus that cause gastroenteritis, human papillomavirus (HPV) that depending on the type may cause warts or carcinomas as in DOCK8 deficiency and RHOH deficiency [50], molluscum contagiosum virus, JC virus that causes progressive multifocal leukoencephalopathy, and tick-borne viruses such as dengue virus causing dengue fever [51]. In patients with CID, SARS-CoV-2 infection results in variable COVID-19 clinical course, severity, complications, and outcomes [52–54]. As a general rule, opportunistic and chronic infections may underlie CID. Among bacterial infections, the following pathogens are usually reported in CID patients: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Neisseria meningitidis*, *Mycoplasma pneumoniae*, *Salmonella typhi*, *Listeria monocytogenes*, enteric flora, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and nontuberculous mycobacteria [55]. Considering fungal infections, the following pathogens are commonly reported: *Candida* species, *Aspergillus* species, *Cryptococcus neoformans*, and *Histoplasma capsulatum*. Among protozoan infections, *Pneumocystis jirovecii*, *Toxoplasma gondii*, *Cryptosporidium parvum* that causes acute enteritis, *Giardia lamblia, Leishmania species, Trypanosoma species, and Plasmodium species* can be involved [55]. Schistosomiasis, filariasis, echinococcosis, and onchocerciasis can be diagnosed in patients with CID [56]. Chronic respiratory infection may result in bronchiectasis formation and consequent reduced pulmonary function together with increased susceptibility to new pulmonary infections. Chronic diarrhea is a common symptom and has a wide differential diagnosis, including infectious and noninfectious causes. Within the first year after the initial presentation, manifestations of immune dysregulation and infections are the most common events in CID patients [57].

Some conditions have distinctive clinical features [58]: severe atopy, eosinophilia, hyper-IgE, low IgM, and skin viral and bacterial infections in DOCK8 deficiency[59]; recurrent respiratory tract infections, viral infections, and severe atopic disease in CARD11 deficiency [60]; hyper-IgM, neutropenia, thrombocytopenia, and opportunistic infections in CD40L and CD40 deficiency [61]; EBV-associated recurrent nonmalignant lymphoproliferative disorder or malignant B cell lymphoproliferation in ITK deficiency; HPV infection and lack of naïve T cells in RHOH

deficiency [50]; cytopenias, absent B and NK cells, nonfunctional T cells in IKAROS deficiency [14]; viral infections, autoimmunity, and only γδ TCR T cells in TRAC deficiency [62]; vasculitis and pyoderma gangrenosum in MHC class I deficiency [63]; classic Kaposi's sarcoma in OX40 deficiency [49]; chronic mucocutaneous ulceration in RelA haploinsufficiency [64]; and bullous pemphigoid in ZAP70 combined hypomorphic and activating mutations [65]. On the contrary, all the other CID lack characteristic-associated clinical features; however, some laboratory clues can help in the diagnosis. When CD8 cells are very low, it orientates toward CD8 deficiency, and when the TCR is low, toward CD3γ deficiency. Reduced CD4 cells with the absence or very low HLA-DR expression on lymphocytes are characteristic of MHC class II deficiency, while if CD4 cells are low and the TCR repertoire is restricted, it orientates toward LCK deficiency [58]. In ZAP70 deficiency, the lymphocyte count can be normal or even elevated but CD8 cells are very low (<5%), T cell receptor excision circles progressively decline during the first year of life, and notably T cell proliferative responses to mitogens in vitro are absent, which is consistent with its more severe infectious susceptibility compared to CD8 deficiency [66].

*Patients with CID have an increased risk to develop autoimmunity and malignancy. Autoimmune disease*s can be diagnosed particularly since early childhood [57]. Patients can present with autoimmune cytopenias, such as autoimmune hemolytic anemia and autoimmune thrombocytopenia [67, 68]. Organ-specific autoimmunity can also develop, such as autoimmune thyroiditis, vitiligo, alopecia, bullous pemphigoid, enteropathy, inflammatory bowel disease, vasculitis, and granulomatous lymphocytic interstitial lung disease [69]. Other characteristic presentations are granulomatous disease affecting mostly the skin, but any organ can be involved, and lymphoproliferation occurring with lymphadenopathy and splenomegaly.

Approximately 5% of patients diagnosed with CID have been reported to have a malignancy in the United States Immune Deficiency Network (USDIN) Registry [70]. *Malignancies* in CID are generally due to defective viral immunosurveillance and consequent uncontrolled viral infection [71]. Patients especially develop EBV-driven lymphoma and HPV-associated squamous cell carcinoma [70]. Regarding lymphomas, patients can present with classic Hodgkin's lymphoma and non-Hodgkin lymphomas (Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, T cell lymphoblastic lymphoma); EBV is involved in most cases, but EBV-negative lymphomas can also occur [72, 73]. Lymphoma can manifest with diffuse lymphadenopathy and splenomegaly and must be distinguished from polyclonal EBV-positive lymphoproliferative disorder [74]. For some CID susceptibility to EBV infection, lymphoproliferative conditions, and lymphoma are the main presenting features, such as ITK deficiency [75, 76]. Malignancy in patients with CD40LG deficiency is commonly reported involving the gastrointestinal tract including the bile ducts (biliary tract tumors) and frequently classified as neuroendocrine tumors (peripheral primitive neuroectodermal tumor) [77]. Leukemia is not common in CID patients, and it associates with DNA repair defects [78].

6.5 Diagnostics

First of all, to diagnose a CID, one must think about it. The motto of the Immune Deficiency Foundation is "Think Zebra!" and it is based on an old medical saying "when you hear hoof beats, think horses, not zebras." However, in order to make unlikely diagnosis and direct appropriate treatment, even uncommon diseases must be included in the differential diagnosis. Remarkably, delayed CID recognition results in a worse outcome [57]. A potential approach to CID diagnosis is exemplified in Fig. 6.2. A precise collection of patient's history (comprehensive of a detailed family history, as well as travel and exposure history) and a thorough physical examination are fundamental in suspecting a diagnosis of CID. It is important to note that testing for T cell receptor excision circless (TRECs), which is used as SCID newborn screening, may not identify CID if thymic output is only mildly or moderately depressed [79–81]. As abovementioned, the clinical phenotype may help to discern CID that are characterized by distinctive clinical features, but even in these cases and in general, patients with CID have no unique signs and symptoms. Consequently, a patient suspected of having CID requires complete evaluation of humoral and cellular immunity [46, 82]. Physicians should start with blood cell count with differential, serum protein electrophoresis, measurement of serum total protein, immunoglobulin levels, and specific antibody titers. These laboratory tests should be followed by flow cytometry, in order to enumerate (absolute numbers and percentages) CD4 and CD8 T cells, B cells, and NK cells, and by assessment of T cell function [83]. Advanced tests include the following: flow cytometry, to enumerate B cell subsets and T cell subsets, and in vitro proliferative response to mitogens, including PHA and anti-CD3 monoclonal antibodies, and also to antigens. Moreover, T cell cytotoxicity, surface and intracellular marker expression, and cytokine production, in response to polyclonal in vitro stimulation, are important [83]. After immunological tests, genetic analysis must be performed [84]. According to the robustness of the clinical hypothesis, sequencing of candidate genes or a diagnostic gene panel (next-generation sequencing and/or whole-exome sequencing) can be used to identify the genetic defect [85, 86]. For any novel suspected disease-causing variant, the causal relationship between genotype and phenotype must be validated [84, 87]. The mode of inheritance is a key factor when determining the relevance of a genotype for phenotype [2, 88]. Functional analysis, by evaluating whether the detected variant destroys, impairs, or alters the expression of the gene product, can assess if it causes loss-of-function or gain-of-function effect [86, 87]. For many CID, such as CD40L deficiency, DOCK8 deficiency, MHC class I and II deficiency, or TCRα deficiency, it is possible to evaluate protein expression by flow cytometry. Finally, for full validation, the cellular phenotype must be rescued. Nonetheless, a great proportion of CID gene defects is still unknown, as we are unable to identify with the current tools which disease-causing gene is involved. In summary, when approaching a patient with suspected CID, physicians should consider the clinical phenotype and, on the basis of laboratory tests, orientate the diagnosis; then, genetic analysis and functional testing are needed to correlate with

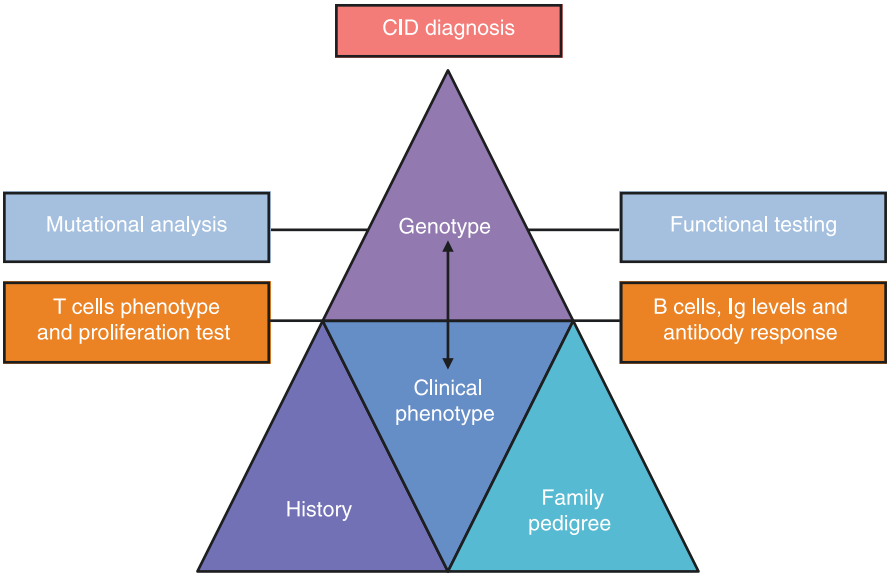

Fig. 6.2 Diagnostic approach to combined immunodeficiencies

the genotype (Fig. 6.2). This diagnostic process must be fully accomplished, because the most specific diagnosis is essential for the most accurate prognosis, therapy, and genetic counseling [46, 84].

6.6 Management and Prognosis

Clinical monitoring differs for each patient, but it generally comprises routine laboratory tests, examination of lung status through pulmonary function tests and computed tomography scan of the chest, evaluation of hepatorenal function, and examination of the intestine, skin, and endocrine organs status. Monitoring chronic infections, such as EBV or CMV infection, is also important in the follow-up schedule, as well as cancer surveillance, particularly for lymphoma and squamous cell carcinoma, and early diagnosis of immune dysregulation manifestations (autoimmunity, allergy, autoinflammation, vasculitis, granulomatous disease, lymphoproliferation) [89, 90]. Clinical management is based on preventive measures, supportive therapy, and, in selected cases, hematopoietic stem cell transplantation (HSCT) or gene therapy. Patients with CID and hypogammaglobulinemia receive intravenous or subcutaneous immunoglobulin replacement therapy. Supportive therapy also includes administration of trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* pneumonia prophylaxis; azithromycin for *Mycobacterium avium* complex prophylaxis; acyclovir, famciclovir, or valacyclovir for HSV and VZV prophylaxis; and fluconazole for *Candida* prophylaxis, as indicated in those patients that are at

increased risk for opportunistic infections or other infections. Aggressive antimycobacterial therapy and sometimes interferon gamma are used in patients with increased susceptibility to mycobacterial infections. Palivizumab, a humanized monoclonal antibody against respiratory syncytial virus (RSV), may be considered in severely immunodeficient children, especially those younger than 24 months of age, during RSV season [91, 92]. Live vaccines and nonirradiated blood transfusions should be avoided in patients with CID [93, 94]. Unless there is low or no capacity of humoral response, HPV vaccine should be routinely used, and nonviable influenza vaccine and pneumococcal vaccine should be administered annually in all patients [95]. The choice of treatment depends upon the type and the severity of the disorder, but prompt and aggressive therapy of infections, immune suppression if autoimmune manifestations occur, adequate nutritional support, and prompt diagnosis and treatment of malignancies must be pursued. Given the variable disease course of some CID, decisions regarding the opportunity and the timing of hematopoietic stem cell transplantation (HSCT) can be difficult, because the natural history of the disease is often unknown. Approximately 40% of patients with profound CID is transplanted [96]. Historically, the outcome of HSCT in patients with CID is suboptimal for various reasons [97, 98]. In CID T cells are generally present, and consequently chemotherapy and immune suppression are needed before transplantation [99]. There are different conditioning regimens with various myeloablation and immune suppression intensity/toxicity [100]. Notably, the conditioning regimen before HSCT is patient-tailored, and it depends on different factors: presence of active infections and/or immune dysregulation (i.e., overactive immune system), preexistence of organ dysfunction at the time of transplant, and pathophysiology of the disorder, on which it is based the need of full or mixed chimerism to correct the CID phenotype. Experience in CD40L deficiency showed better outcome in HSCT performed before the development of organ damage and in children less than 10 years old at the time of transplantation [101, 102]. Patients with CD40L deficiency undergoing HSCT at less than 5 years of age had almost 90% overall survival at 2 and 5 years after transplantation, while patients older than 10 years had 38% overall survival at 5 years [101]. In the majority of patients, HSCT resulted in complete or partial donor chimerism; among those who discontinued immunoglobulin replacement therapy, T cell chimerism was 50% or greater donor, in 85% of the subjects [101]. In patients with CD40 deficiency, early HSCT ($\leq$2 years) from diagnosis and the use of myeloablative regimens resulted in improved survival, while reduced intensity and nonmyeloablative conditioning were associated with poor donor cell engraftment [101]. Mortality, which mostly occurred within 6 months of HSCT, was mainly related to transplantation-associated complications, including infections and graft rejection [101]. Patients with DOCK8 deficiency, if left untreated, have a dismal prognosis, but allogenic HSCT can be curative; particularly, the use of a reduced-toxicity regimen may offer the best chance for survival [103]. Lymphoma can be treated and it represent an indication to proceed to HSCT [72]. In patients with CID, the optimal management strategy may be hard to define, because many disorders are extremely rare and limited data are nowadays available on the efficacy of different therapeutic options. Moreover, there is no general

treatment that applies to all forms of CID. For this reason, the network and collaboration between specialists plays a crucial role. Societies and organizations, like the European Society for Immunodeficiencies (ESID), the Primary Immune Deficiency Treatment Consortium (PIDTC) of North America, the Clinical Immunology Society (CIS), the Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation (IEWP-EBMT), the Italian Primary Immunodeficiency Network (IPINET) and many others, are a reliable source for specialists. For instance, the Clinical Immunological Society has gathered a group of physicians, expert in primary immunodeficiencies, that exchange information on treatment protocols via the mailing service CIS-PIDD [104] available as open online archive since 2015 [105].

The overall frequency of severe clinical events requiring hospitalization in CID patients is 1.4% per year [96]. More precisely, 51% of these events are manifestations of immune dysregulation (a third of which are episodes of autoimmune cytopenia), while 49% are bacterial and viral infections and chronic lung disease [96]. CID are heterogeneous conditions: some genetic defects affect mainly T cell number and other T cell function; moreover, in some disorders other immune cells are also affected (Table 6.2). Therefore, CID patients have a variable prognosis according to the underling genetic/biological alteration and to the severity of the clinical phenotype.

References

1. Fischer A, Notarangelo LD, Neven B et al (2015) Severe combined immunodeficiencies and related disorders. Nat Rev Dis Primers 1:15061
2. Notarangelo LD, Bacchetta R, Casanova JL, Su HC (2020) Human inborn errors of immunity: an expanding universe. Sci Immunol 5(49):eabb1662
3. Shearer WT, Dunn E, Notarangelo LD et al (2014) Establishing diagnostic criteria for severe combined immunodeficiency disease (SCID), leaky SCID, and Omenn syndrome: the Primary Immune Deficiency Treatment Consortium experience. J Allergy Clin Immunol 133(4):1092–1098
4. European Society for Immunodeficiencies (2019). European Society for Immunodeficiencies website. ESID registry—working definitions for clinical diagnosis of primary immunodeficiencies/inborn errors of immunity. https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria. Accessed 3 Mar 2021
5. Bertinchamp R, Gérard L, Boutboul D et al (2016) Exclusion of patients with a severe T-cell defect improves the definition of common variable immunodeficiency. J Allergy Clin Immunol Pract 4(6):1147–1157
6. Malphettes M, Gérard L, Carmagnat M et al (2009) Late-onset combined immune deficiency: a subset of common variable immunodeficiency with severe T cell defect. Clin Infect Dis 49(9):1329–1338
7. von Spee-Mayer C, Koemm V, Wehr C et al (2019) Evaluating laboratory criteria for combined immunodeficiency in adult patients diagnosed with common variable immunodeficiency. Clin Immunol 203:59–62
8. Picard C, Bobby Gaspar H et al (2018) International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee report on inborn errors of immunity. J Clin Immunol 38(1):96–128

9. Tangye SG, Al-Herz W, Bousfiha A et al (2020) Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40(1):24–64
10. Tangye SG, Al-Herz W, Bousfiha A et al (2021) The ever-increasing array of novel inborn errors of immunity: an interim update by the IUIS committee. J Clin Immunol 41(3):666–679
11. Puck JM (2012) Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles. J Allergy Clin Immunol 129(3):607–616
12. Schuetz C, Huck K, Gudowius S et al (2008) An immunodeficiency disease with RAG mutations and granulomas. N Engl J Med 358(19):2030–2038
13. Notarangelo LD, Kim MS, Walter JE, Lee YN (2016) Human RAG mutations: biochemistry and clinical implications. Nat Rev Immunol 16(4):234–246
14. Boutboul D, Kuehn HS, Van de Wyngaert Z et al (2018) Dominant-negative IKZF1 mutations cause a T, B, and myeloid cell combined immunodeficiency. J Clin Invest 128(7):3071–3087
15. Kuhny M, Forbes LR, Çakan E et al (2020) Disease-associated CTNNBL1 mutation impairs somatic hypermutation by decreasing nuclear AID. J Clin Invest 130(8):4411–4422
16. Tangye SG (2020) It's that time of year-APRIL promotes humoral immunity in humans. J Allergy Clin Immunol 146(5):1013–1015
17. Drutman SB, Mansouri D, Mahdaviani SA et al (2020) Fatal cytomegalovirus infection in an adult with inherited NOS2 deficiency. N Engl J Med 382(5):437–445
18. Cook SA, Comrie WA, Poli MC et al (2020) HEM1 deficiency disrupts mTORC2 and F-actin control in inherited immunodysregulatory disease. Science 369(6500):202–207
19. Castro CN, Rosenzwajg M, Carapito R et al (2020) NCKAP1L defects lead to a novel syndrome combining immunodeficiency, lymphoproliferation, and hyperinflammation. J Exp Med 217(12):e20192275
20. Thaventhiran JED, Lango Allen H, Burren OS et al (2020) Whole-genome sequencing of a sporadic primary immunodeficiency cohort. Nature 583(7814):90–95
21. Okano T, Imai K, Naruto T et al (2020) Whole-exome sequencing-based approach for germline mutations in patients with inborn errors of immunity. J Clin Immunol 40(5):729–740
22. Maffucci P, Filion CA, Boisson B et al (2016) Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. Front Immunol 7:220
23. de Valles-Ibáñez G, Esteve-Solé A, Piquer M et al (2018) Evaluating the genetics of common variable immunodeficiency: monogenetic model and beyond. Front Immunol 9:636
24. Fusaro M, Rosain J, Grandin V et al (2021) Improving the diagnostic efficiency of primary immunodeficiencies with targeted next-generation sequencing. J Allergy Clin Immunol 147(2):734–737
25. Seidel MG, Kindle G, Gathmann B et al (2019) The European Society for Immunodeficiencies (ESID) registry working definitions for the clinical diagnosis of inborn errors of immunity. J Allergy Clin Immunol Pract 7(6):1763–1770
26. Fischer A, Rausell A (2016) Primary immunodeficiencies suggest redundancy within the human immune system. Sci Immunol 1(6):pii:eaah5861
27. Notarangelo LD (2014) Combined immunodeficiencies with nonfunctional T lymphocytes. Adv Immunol 121:121–190
28. Brownlie RJ, Zamoyska R (2013) T cell receptor signalling networks: branched, diversified and bounded. Nat Rev Immunol 13(4):257–269
29. Fu G, Rybakin V, Brzostek J et al (2014) Fine-tuning T cell receptor signaling to control T cell development. Trends Immunol 35(7):311–318
30. Smith-Garvin JE, Koretzky GA, Jordan MS (2009) T cell activation. Annu Rev Immunol 27:591–619
31. Moens L, Gouwy M, Bosch B et al (2019) Human DOCK2 deficiency: report of a novel mutation and evidence for neutrophil dysfunction. J Clin Immunol 39(3):298–308
32. Janssen E, Geha RS (2019) Primary immunodeficiencies caused by mutations in actin regulatory proteins. Immunol Rev 287(1):121–134

33. Bedsaul JR, Carter NM, Deibel KE et al (2018) Mechanisms of regulated and dysregulated CARD11 signaling in adaptive immunity and disease. Front Immunol 9:2105
34. Lu HY, Bauman BM, Arjunaraja S et al (2018) The CBM-opathies-a rapidly expanding spectrum of human inborn errors of immunity caused by mutations in the CARD11-BCL10-MALT1 complex. Front Immunol 9:2078
35. Kaustio M, Haapaniemi E, Göös H et al (2017) Damaging heterozygous mutations in NFKB1 lead to diverse immunologic phenotypes. J Allergy Clin Immunol 140(3):782–796
36. Blom B, Spits H (2006) Development of human lymphoid cells. Annu Rev Immunol 24:287–320
37. Powell MD, Read KA, Sreekumar BK, Oestreich KJ (2019) Ikaros zinc finger transcription factors: regulators of cytokine signaling pathways and CD4(+) T helper cell differentiation. Front Immunol 10:1299
38. Abdollahpour H, Appaswamy G, Kotlarz D et al (2012) The phenotype of human STK4 deficiency. Blood 119(15):3450–3457
39. Spolski R, Leonard WJ (2008) Interleukin-21: basic biology and implications for cancer and autoimmunity. Annu Rev Immunol 26:57–79
40. Quezada SA, Jarvinen LZ, Lind EF, Noelle RJ (2004) CD40/CD154 interactions at the interface of tolerance and immunity. Annu Rev Immunol 22:307–328
41. Elgueta R, Benson MJ, de Vries VC et al (2009) Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev 229(1):152–172
42. Croft M, So T, Duan W, Soroosh P (2009) The significance of OX40 and OX40L to T-cell biology and immune disease. Immunol Rev 229(1):173–191
43. Calzoni E, Platt CD, Keles S et al (2019) F-BAR domain only protein 1 (FCHO1) deficiency is a novel cause of combined immune deficiency in human subjects. J Allergy Clin Immunol 143(6):2317–2321.e12
44. Lyszkiewicz M, Ziętara N et al (2020) Human FCHO1 deficiency reveals role for clathrin-mediated endocytosis in development and function of T cells. Nat Commun 11(1):1031
45. Bousfiha A, Jeddane L, Picard C et al (2018) The 2017 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol 38(1):129–143
46. Notarangelo L (2019) Combined immunodeficiencies. In: Orange JS, TePas E (eds) UpToDate. https://www.uptodate.com/contents/combined-immunodeficiencies. Accessed 24 Feb 2020
47. Casanova JL (2015) Severe infectious diseases of childhood as monogenic inborn errors of immunity. Proc Natl Acad Sci U S A 112(51):E7128–E7137
48. Mauracher AA, Gujer E, Bachmann LM et al (2021) Patterns of immune dysregulation in primary immunodeficiencies: a systematic review. J Allergy Clin Immunol Pract 9(2):792–802.e10
49. Byun M, Ma CS, Akçay A et al (2013) Inherited human OX40 deficiency underlying classic Kaposi sarcoma of childhood. J Exp Med 210(9):1743–1759
50. Crequer A, Troeger A, Patin E et al (2012) Human RHOH deficiency causes T cell defects and susceptibility to EV-HPV infections. J Clin Invest 122(9):3239–3247
51. Ruffner MA, Sullivan KE, Henrickson SE (2017) Recurrent and sustained viral infections in primary immunodeficiencies. Front Immunol 8:665
52. Meyts I, Bucciol G, Quinti I et al (2021) Coronavirus disease 2019 in patients with inborn errors of immunity: an international study. J Allergy Clin Immunol 147(2):520–531
53. Shields AM, Burns SO, Savic S et al (2021) COVID-19 in patients with primary and secondary immunodeficiency: the United Kingdom experience. J Allergy Clin Immunol 147(3):870–875.e1
54. Delavari S, Abolhassani H, Abolnezhadian F et al (2021) Impact of SARS-CoV-2 pandemic on patients with primary immunodeficiency. J Clin Immunol 41(2):345–355
55. Notarangelo LD (2010) Primary immunodeficiencies. J Allergy Clin Immunol 125(2 Suppl 2):S182–S194
56. Grencis RK (2015) Immunity to helminths: resistance, regulation, and susceptibility to gastrointestinal nematodes. Annu Rev Immunol 33:201–225

57. Fischer A, Provot J, Jais JP et al (2017) Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. J Allergy Clin Immunol 140(5):1388–1393.e8
58. Bousfiha A, Jeddane L, Ailal F et al (2013) A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. J Clin Immunol 33(6):1078–1087
59. Biggs CM, Keles S, Chatila TA (2017) DOCK8 deficiency: insights into pathophysiology, clinical features and management. Clin Immunol 181:75–82
60. Dorjbal B, Stinson JR, Ma CA et al (2019) Hypomorphic caspase activation and recruitment domain 11 (CARD11) mutations associated with diverse immunologic phenotypes with or without atopic disease. J Allergy Clin Immunol 143(4):1482–1495
61. França TT, Barreiros LA, Al-Ramadi BK et al (2019) CD40 ligand deficiency: treatment strategies and novel therapeutic perspectives. Expert Rev Clin Immunol 15(5):529–540
62. Morgan NV, Goddard S, Cardno TS et al (2011) Mutation in the TCRα subunit constant gene (TRAC) leads to a human immunodeficiency disorder characterized by a lack of TCRαβ+ T cells. J Clin Invest 121(2):695–702
63. Hanna S, Etzioni A (2014) MHC class I and II deficiencies. J Allergy Clin Immunol 134(2):269–275
64. Badran YR, Dedeoglu F, Leyva Castillo JM et al (2017) Human RELA haploinsufficiency results in autosomal-dominant chronic mucocutaneous ulceration. J Exp Med 214(7):1937–1947
65. Chan AY, Punwani D, Kadlecek TA et al (2016) A novel human autoimmune syndrome caused by combined hypomorphic and activating mutations in ZAP-70. J Exp Med 213(2):155–165
66. Roifman CM, Dadi H, Somech R et al (2010) Characterization of ζ-associated protein, 70 kd (ZAP70)-deficient human lymphocytes. J Allergy Clin Immunol 126(6):1226–1233.e1
67. Notarangelo LD (2009) Primary immunodeficiencies (PIDs) presenting with cytopenias. Hematology Am Soc Hematol Educ Program:139–143
68. Seidel MG (2014) Autoimmune and other cytopenias in primary immunodeficiencies: pathomechanisms, novel differential diagnoses, and treatment. Blood 124(15):2337–2344
69. Grimbacher B, Warnatz K, Yong PFK et al (2016) The crossroads of autoimmunity and immunodeficiency: lessons from polygenic traits and monogenic defects. J Allergy Clin Immunol 137(1):3–17
70. Mayor PC, Eng KH, Singel KL et al (2018) Cancer in primary immunodeficiency diseases: cancer incidence in the United States Immune Deficiency Network Registry. J Allergy Clin Immunol 141(3):1028–1035
71. Hauck F, Voss R, Urban C, Seidel MG (2018) Intrinsic and extrinsic causes of malignancies in patients with primary immunodeficiency disorders. J Allergy Clin Immunol 141(1):59–68.e4
72. Herber M, Mertz P, Dieudonné Y, Guffroy B et al (2020) Primary immunodeficiencies and lymphoma: a systematic review of literature. Leuk Lymphoma 61(2):274–284
73. Riaz IB, Faridi W, Patnaik MM, Abraham RS (2019) A systematic review on predisposition to lymphoid (B and T cell) neoplasias in patients with primary immunodeficiencies and immune dysregulatory disorders (inborn errors of immunity). Front Immunol 10:777
74. Sawada A, Inoue M (2018) Hematopoietic stem cell transplantation for the treatment of Epstein-Barr virus-associated T- or NK-cell lymphoproliferative diseases and associated disorders. Front Pediatr 6:334
75. Tangye SG (2020) Genetic susceptibility to EBV infection: insights from inborn errors of immunity. Hum Genet 139:885–901
76. Latour S, Winter S (2018) Inherited immunodeficiencies with high predisposition to Epstein-Barr Virus-driven lymphoproliferative diseases. Front Immunol 9:1103
77. de la Morena MT (2016) Clinical phenotypes of hyper-IgM syndromes. J Allergy Clin Immunol Pract 4(6):1023–1036
78. Haas OA (2019) Primary immunodeficiency and cancer predisposition revisited: embedding two closely related concepts into an integrative conceptual framework. Front Immunol 9:3136
79. Puck JM (2019) Newborn screening for severe combined immunodeficiency and T-cell lymphopenia. Immunol Rev 287(1):241–252

80. Amatuni GS, Currier RJ, Church JA et al (2019) Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California, 2010-2017. Pediatrics 143(2):pii:e20182300
81. Currier R, Puck JM (2021) SCID newborn screening: what we've learned. J Allergy Clin Immunol 147(2):417–426
82. Hausmann O, Warnatz K (2014) Immunodeficiency in adults: a practical guide for the allergist. Allergo J Int 23:261–268
83. Bonilla FA, Khan DA, Ballas ZK et al (2015) Practice parameter for the diagnosis and management of primary immunodeficiency. J Allergy Clin Immunol 136(5):1186–205.e1-78
84. Abraham RS, Butte MJ (2021) The new "Wholly Trinity" in the diagnosis and management of inborn errors of immunity. J Allergy Clin Immunol Pract 9(2):613–625
85. Conley ME, Casanova JL (2014) Discovery of single-gene inborn errors of immunity by next generation sequencing. Curr Opin Immunol 30:17–23
86. Stray-Pedersen A, Sorte HS, Samarakoon P et al (2017) Primary immunodeficiency diseases: Genomic approaches delineate heterogeneous Mendelian disorders. J Allergy Clin Immunol 139(1):232–245
87. Sullivan KE (2021) The scary world of variants of uncertain significance (VUS): a hitchhiker's guide to interpretation. J Allergy Clin Immunol 147(2):492–494
88. Itan Y, Casanova JL (2015) Can the impact of human genetic variations be predicted? Proc Natl Acad Sci U S A 112(37):11426–11427
89. Mahlaoui N, Warnatz K, Jones A et al (2017) Advances in the care of primary immunodeficiencies (PIDs): from birth to adulthood. J Clin Immunol 37(5):452–460
90. van de Ven AA, Warnatz K (2015) The autoimmune conundrum in common variable immunodeficiency disorders. Curr Opin Allergy Clin Immunol 15(6):514–524
91. American Academy of Pediatrics Infectious Diseases and Bronchiolitis Guidelines Committee. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. Pediatrics 2014;134:415-420
92. Olchanski N, Hansen RN, Pope E, et al. Palivizumab prophylaxis for respiratory syncytial virus: examining the evidence around value. Open Forum Infect Dis. 2018;5(3):ofy031.
93. Bonilla FA (2018) Update: vaccines in primary immunodeficiency. J Allergy Clin Immunol 141(2):474–481
94. Medical Advisory Committee of the Immune Deficiency Foundation, Shearer WT, Fleisher TA et al (2014) Recommendations for live viral and bacterial vaccines in immunodeficient patients and their close contacts. J Allergy Clin Immunol 133(4):961–966
95. Sobh A, Bonilla FA (2016) Vaccination in primary immunodeficiency disorders. J Allergy Clin Immunol Pract 4(6):1066–1075
96. Speckmann C, Doerken S, Aiuti A et al (2017) A prospective study on the natural history of patients with profound combined immunodeficiency: An interim analysis. J Allergy Clin Immunol 139(4):1302–1310.e4
97. Gennery AR, Slatter MA, Grandin L et al (2010) Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? J Allergy Clin Immunol 126(3):602–10.e1-11
98. Gennery AR, Albert MH, Slatter MA, Lankester A (2019) Hematopoietic stem cell transplantation for primary immunodeficiencies. Front Pediatr 7:445
99. Neven B, Ferrua F (2020) Hematopoietic stem cell transplantation for combined immunodeficiencies, on behalf of IEWP-EBMT. Front Pediatr 7:552
100. European Society for Blood and Marrow Transplantation (2017) EBMT website. EBMT/ESID guidelines for haematopoietic stem cell transplantation for primary immunodeficiencies. https://www.ebmt.org/sites/default/files/migration_legacy_files/document/Inborn%20Errors%20Working%20Party%20ESID%20EBMT%20HSCT%20Guidelines%202017.pdf. Accessed 3 Mar 2021

101. Ferrua F, Galimberti S, Courteille V et al (2019) Hematopoietic stem cell transplantation for CD40 ligand deficiency: results from an EBMT/ESID-IEWP-SCETIDE-PIDTC study. J Allergy Clin Immunol 143(6):2238–2253
102. de la Morena MT, Leonard D, Torgerson TR et al (2017) Long-term outcomes of 176 patients with X-linked hyper-IgM syndrome treated with or without hematopoietic cell transplantation. J Allergy Clin Immunol 139(4):1282–1292
103. Aydin SE, Freeman AF, Al-Herz W et al (2019) Hematopoietic stem cell transplantation as treatment for patients with DOCK8 deficiency. J Allergy Clin Immunol Pract 7(3):848–855
104. Clinical Immunology Society Clinical Immunology Society website. Application for list service access. https://cis.execinc.com/edibo/Lists. Accessed 3 Mar 2021
105. Clinical Immunology Society Clinical Immunology Society website. CIS-PIDD forum. http://mindbender.dundee.net/read/?forum=cis-pidd. Accessed 3 Mar 2021

Congenital Defects of Phagocytes

7

Delfien J. Bogaert and Filomeen Haerynck

Abstract

Phagocytes form the first line of defense against invading pathogens and play a key role in wound healing following tissue injury. A broad range of monogenic defects affecting phagocyte numbers and/or function have been described. A common hallmark of all congenital defects of phagocytes is an increased susceptibility to severe bacterial and fungal infections. Typical sites of infection are the skin, the oral mucosa and gingiva, lymph nodes, the lungs, and other internal organs. Furthermore, many congenital phagocyte disorders are associated with an increased risk of inflammatory manifestations and/or hematological malignancies. In the 1950s, survival of the first described disorders was dramatically poor with most patients dying from infectious complications in the first years of life. However, in the past decades, advances in diagnosis and treatment strategies have significantly improved patient outcome. In addition, the increased understanding of the molecular disease mechanisms has paved the way for

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gene therapy as a promising new treatment option. This chapter outlines the current knowledge on congenital defects of phagocytes, categorized according to their major underlying defect.

Keywords

Phagocytes · Neutrophils · Congenital neutropenia · Chemotaxis · Actin polymerization · Leukocyte adhesion defect · Chronic granulomatous disease Respiratory burst

7.1 Introduction

7.1.1 The Role of Phagocytes in the Immune System

Phagocytes (neutrophils, monocytes, macrophages, and dendritic cells (DCs)) are essential components of the innate immune system. They play a key role in the body's first line of defense against invading pathogens and the removal of damaged tissues. At time of infection or injury, myelopoiesis in the bone marrow is increased and large numbers of phagocytes are recruited to the site of inflammation by a gradient of cytokines and chemokines (i.e., chemotaxis). There, the cells adhere to the endothelium of local blood vessels, after which they extravasate into the affected tissue (Fig. 7.1) [1]. The primary function of neutrophils, monocytes, and macrophages is to phagocytize and destroy pathogens and damaged cells. Phagocytes have three main antimicrobial mechanisms to clear intracellular pathogens. First, neutrophils and, to a lesser extent, macrophages use nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to convert molecular oxygen (O_2) into reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), a process called respiratory burst or oxidative burst (Fig. 7.1) [1]. Second, macrophages generate nitric oxide (NO) by the action of inducible NO synthase (iNOS). NO then combines with O_2^- and H_2O_2 to produce highly reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$), which are particularly important for defense against mycobacteria and *Salmonella* species [1]. Third, neutrophils and macrophages can also destroy microorganisms by releasing lytic enzymes and bactericidal peptides from intracellular granules (Fig. 7.1) [1]. Furthermore, neutrophils defend against extracellular microbes by releasing inflammatory mediators in the extracellular space and by forming neutrophil extracellular traps (NETs). NETs are large fibril networks composed of decondensed chromatin and cytosolic and granule proteins of apoptotic neutrophils. As the name suggests, NETs trap extracellular pathogens, thereby facilitating their neutralization [2]. Finally, phagocytes enhance and guide subsequent innate and adaptive immune responses through direct contact with other immune cells and the secretion of cytokines and chemokines [1].

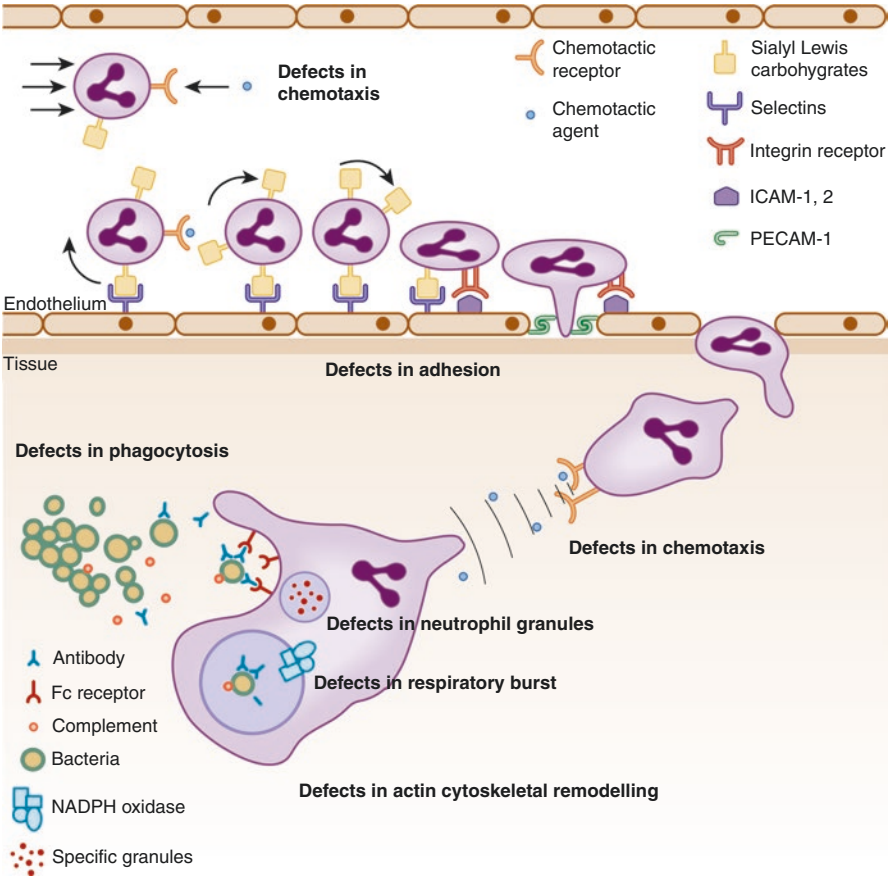


Fig. 7.1 *Steps in the response of circulating neutrophils to infection.* The adhesion molecule E-selectin is upregulated on endothelial cells in response to inflammatory mediators such as interleukin-1 (IL-1), endotoxin, and tumor necrosis factor-α. E-selectin interacts with sialyl Lewis carbohydrates on the surface of neutrophils, resulting in rolling attachment of neutrophils on the endothelium. Chemoattractants such as IL-8 cause upregulation of neutrophil integrins that, in turn, mediate tight adhesion to ICAM-1, 2 and PECAM-1 on endothelial cells. Activated neutrophils can detect as little as a 2% change in the chemoattractant gradient and move to the site of infection. In the infected tissue, neutrophils phagocytize bacteria that are opsonized by antibodies and complement. Both oxidative and non-oxidative antimicrobial mechanisms are then used to kill the engulfed bacteria. Dynamic rearrangements of the actin cytoskeleton are needed for neutrophil migration, phagocytosis, intracellular vesicle-mediated transport, and degranulation. Inborn defects can occur in each of the above steps, giving rise to congenital phagocyte disorders. *ICAM* Intercellular adhesion molecule, *NADPH* Nicotinamide adenine dinucleotide phosphate, *PECAM* Platelet endothelial cell adhesion molecule. (Modified with permission from Ref. [329])

7.1.2 Clinical Features of Congenital Phagocyte Defects

In the 1950s, publications on chronic granulomatous disease (CGD) [3] and Kostmann disease [4] reported the first patients with congenital defects of phagocytes. Since then, our molecular understanding of phagocyte defects has known tremendous progress. Currently, multiple disease-causing genes associated with disorders of phagocyte number and/or function have been identified [5]. Although each disorder has its unique immunological and non-immunological features, the common hallmark of phagocyte defects is recurrent, severe, and/or unusual bacterial and fungal infections. The common sites of infection are the skin, mucosae, lymph nodes, lungs, and other internal organs. Patients commonly present with cellulitis, abscesses of the skin and internal organs, gingivitis, and aphthous stomatitis. In addition, congenital phagocyte disorders can be accompanied by autoinflammatory manifestations, autoimmunity, and/or hematological malignancies. Most patients are recognized in the first years of life, but some may go undiagnosed until adulthood [6].

7.1.3 Principles of Treatment of Congenital Phagocyte Defects

Several general health measures can be applied to prevent infections [7]. First, patients should receive all routine immunizations in accordance with (inter) national recommendations, including live-attenuated viral vaccines and the yearly influenza vaccine. However, live-attenuated bacterial vaccines (e.g., BCG, oral *Salmonella typhi*) are contraindicated because of the risk of vaccine-strain infection. Furthermore, skin abrasions and wounds should be thoroughly washed with water and soap, and a topical antiseptic applied (e.g., 2% H_2O_2 or Betadine). Patients with recurrent skin infections are recommended to bathe with an antiseptic two to three times a week to reduce the microbial load, followed by application of an emollient to prevent dry skin. Constipation and rectal manipulations (e.g., suppositories) should be avoided because it increases the risk of anorectal fissures and abscesses. Early anal infections can be treated with soaking in antiseptic baths. Proper dental care, the use of antibacterial mouthwashes (e.g., chlorhexidine 0.2%), and regular dental visits help prevent gingivitis and periodontitis. Finally, to prevent pulmonary infections, patients are advised not to smoke, avoid indoor humidifiers, and stay away from sources of *Aspergillus* spores (e.g., construction sites, animal stables, and decaying plant materials such as hay, wood chips, mulch, and compost piles) [7].

Patients with congenital neutropenia are often treated with granulocyte colony-stimulating factor (G-CSF) (e.g., filgrastim). G-CSF stimulates the production of granulocytes in the bone marrow, thereby increasing the number of neutrophils in the blood and reducing the frequency and severity of infections [6].

Patients are commonly treated with long-term antimicrobial prophylaxis, especially those with severe disorders of phagocyte function. Trimethoprim-sulfamethoxazole (TMP-SMX) is commonly used as antibacterial prophylactic drug. In case of recurrent periodontitis, prophylactic antibiotics with activity against oral microflora should be administrated, such as metronidazole, a combination of metronidazole and amoxicillin, or amoxicillin/clavulanic. The drug of choice for antifungal prophylaxis is usually itraconazole. Fluconazole is generally not recommended because it has a poor activity against *Aspergillus* spp. The prophylactic dose of TMP-SMX is 5 mg/kg/day (with a maximum of 320 mg/day) of trimethoprim in two divided doses daily, although some centers use different schemes such as three times per week on consecutive or alternate days. Itraconazole prophylaxis is prescribed at 5 mg/kg of the oral solution (better bioavailability than capsules) once daily, with a maximum dose of 200 mg/day. Liver function tests should be monitored in patients receiving TMP-SMX and/or itraconazole [6, 7].

Early diagnosis and aggressive treatment of acute infections is essential. Reasonable attempts should be made to obtain microbiological specimens prior to the start of therapy. Empiric treatment with parenteral antibiotic and/or antifungal drugs should be started as quickly as possible and cover the major pathogens associated with the specific disease. Abscesses and deep-seated infections may require surgical drainage or excision [6].

Inflammatory and autoimmune manifestations are generally treated with steroids and other immunosuppressive drugs, but their use should be limited because of the associated infection risk [6].

Currently, the only curative option for congenital phagocyte disorders is allogeneic hematopoietic stem cell transplantation (HSCT). Given the serious risks associated with HSCT, this treatment modality is generally reserved for cases with a poor prognosis. Still, HSCT is unable to correct non-hematological features [8]. Gene therapy, albeit still in development, holds great promise to further improve outcome [9].

7.1.4 Classification of Congenital Disorders of Phagocytes

The classification of congenital defects of phagocytes can be approached in different ways [10]. However, no classification is absolute since some disease entities will always fit in multiple groups. In Table 7.1, congenital disorders of phagocytes are categorized according to their major defect, which is largely based on the most recent classification of the International Union of Immunological Societies (IUIS 2019) [11].

Table 7.1 Overview of congenital disorders of phagocytes

Disorder	Disease-causing genes	Inheritance	Associated distinctive features
Congenital neutropenia			
Severe congenital neutropenia, generally non-syndromic	*ELANE*	AD	Susceptibility to MDS/AML
	GFI1	AD	T and B lymphopenia
	SRP54	AD	Features reminiscent of Shwachman-Diamond syndrome in some patients. Poor response to G-CSF therapy in some patients
	HAX1	AR	Also called Kostmann disease. Susceptibility to MDS/AML. Neurological features in some patients, including developmental delay, convulsions
	JAGN1	AR	Susceptibility to MDS/AML. Poor response to G-CSF therapy in some patients. Extra-hematopoietic features in some patients, such as short stature, skeletal and dental abnormalities, neurological disease, and pancreatic insufficiency
	VPS45A	AR	Myelofibrosis. Extramedullary hematopoiesis causing hepatosplenomegaly and nephromegaly. No response to G-CSF therapy
	CSF3R	AR	Susceptibility to MDS/AML. No response to G-CSF therapy
	WAS (GOF)	XR	Monocytopenia. T, B, and NK lymphopenia. Susceptibility to MDS/AML
Cyclic neutropenia	*ELANE*	AD	Very regular periodic neutropenia with reciprocal monocytosis. Predictable recurrence of symptoms
p14 deficiency	*LAMTOR2*	AR	Partial oculocutaneous albinism, short stature, antibody deficiency, reduced $CD8^+$ T cell cytotoxicity
Shwachman-Diamond syndrome	*SBDS*	AR	Bone marrow failure, exocrine pancreatic insufficiency, skeletal abnormalities. Susceptibility to MDS/AML.
	DNAJC21	AR	Bone marrow failure. Exocrine pancreatic insufficiency and skeletal abnormalities in some patients. Susceptibility to MDS/AML
	EFL1	AR	Bone marrow failure, exocrine pancreatic insufficiency, skeletal abnormalities
	SRP54	AD	Bone marrow failure. Exocrine pancreatic insufficiency and skeletal abnormalities in some patients. Poor response to G-CSF therapy in some patients
3-Methylglutaconic aciduria type II (Barth syndrome)	*TAZ*	XR	Cardiomyopathy, skeletal myopathy, delayed motor development, short stature. Neutropenia of variable severity, may be intermittent
3-Methylglutaconic aciduria type VII	*CLPB*	AR	Progressive brain atrophy, developmental delay, hypotonia, movement disorder, cataract. Neutropenia of variable severity, may be intermittent

Glycogen storage disease type 1b		*SLC37A4*	AR	Fasting hypoglycemia, lactic acidosis, hyperlipidemia, hepatomegaly, IBD, doll-like facies, short stature
G6PC3 deficiency		*G6PC3*	AR	Congenital heart defects, prominent superficial veins, urogenital abnormalities, growth retardation, IBD, intermittent thrombocytopenia, T and/or B lymphopenia. Susceptibility to MDS/AML
Cohen syndrome		*VPS13B*	AR	Developmental delay, intellectual disability, typical facial dysmorphism, pigmentary retinopathy
Poikiloderma with neutropenia (Clericuzio syndrome)		*USB1*	AR	Poikiloderma, palmoplantar hyperkeratosis, pachyonychia, short stature. Increased susceptibility to MDS/AML and squamous cell carcinoma
HYOU1 deficiency		*HYOU1*	AR	Recurrent oral herpes virus infections, recurrent hypoglycemia, inflammatory complications
Defects in neutrophil migration				
Leukocyte adhesion deficiency	Type I	*ITGB2*	AR	Lack of pus, delayed separation of the umbilical cord, omphalitis, poor wound healing, skin ulcers, chronic periodontitis, persistent neutrophilia
	Type II	*SLC35C1*	AR	Lack of pus, poor wound healing, chronic periodontitis, persistent neutrophilia, Bombay (hh) blood group, growth retardation, developmental delay, intellectual disability
	Type III	*FERMT3*	AR	Lack of pus, delayed separation of the umbilical cord, omphalitis, poor wound healing, skin ulcers, chronic periodontitis, persistent neutrophilia, severe Glanzmann-like bleeding tendency, osteopetrosis
Dominant-negative Rac2 deficiency		*RAC2*	AD	Lack of pus, delayed separation of the umbilical cord, omphalitis, poor wound healing, persistent neutrophilia, mild lymphopenia mild hypogammaglobulinemia
β-Actin deficiency		*ACTB*	AD	Mental retardation, short stature, photosensitivity, leukopenia, thrombocytopenia
WDR1 deficiency		*WDR1*	AR	Poor wound healing, skin ulcers, chronic stomatitis with oral stenosis, autoinflammatory disease, mild neutropenia, variable T and B cell defects
MKL1 deficiency		*MKL1*	AR	Intermittent mild thrombocytopenia
Papillon-Lefèvre syndrome		*CTSC*	AR	Aggressive periodontitis, palmoplantar hyperkeratosis
Localized juvenile periodontitis		*FPR1*, *CTSC*[a]	AR	Aggressive periodontitis only

(continued)

Table 7.1 (continued)

Disorder	Disease-causing genes	Inheritance	Associated distinctive features
Defects in respiratory burst			
Chronic granulomatous disease	*CYBB*	XR	Invasive bacterial and fungal infections, inflammatory features such as granulomata and IBD. McLeod syndrome (contiguous deletion into Kell locus on X chromosome). Symptomatic X-linked female carriers (oral ulcers, gingivitis, discoid lupus erythematosus)
	CYBA	AR	Invasive bacterial and fungal infections, inflammatory features such as granulomata and IBD
	NCF1	AR	Invasive bacterial and fungal infections, inflammatory features such as granulomata and IBD
	NCF2	AR	Invasive bacterial and fungal infections, inflammatory features such as granulomata and IBD
	NCF4	AR	Invasive bacterial and fungal infections, inflammatory features such as granulomata and IBD
	CYBC1	AR	Invasive bacterial and fungal infections, inflammatory features such as granulomata and IBD
Myeloperoxidase deficiency	*MPO*	AR	Asymptomatic, recurrent *Candida* infections
Glucose-6-phosphate dehydrogenase deficiency	*G6PD*	XR	Invasive bacterial and fungal infections in class I disease. Hemolytic anemia upon oxidative stress
Glutathione synthetase deficiency	*GSS*	AR	Bacterial infections in severe forms. Hemolytic anemia upon oxidative stress, metabolic acidosis, neurological features
Defects in neutrophil granules			
Specific granule deficiency	*CEBPE*	AR	Neutrophils with bilobed nuclei and reduced granules.
	SMARCD2	AR	Neutropenia, neutrophils with hyposegmented nuclei and reduced granules
Other defects			
GATA2 deficiency	*GATA2*	AD	Cytopenias in neutrophils, monocytes, dendritic cells, and B and NK lymphocytes. Mycobacterial infections, human papillomavirus infections, pulmonary alveolar proteinosis, lymphedema. Susceptibility to MDS/AML
Hereditary pulmonary alveolar proteinosis	*CSF2RA*	AR[b]	Pulmonary alveolar proteinosis
	CSF2RB	AR	Pulmonary alveolar proteinosis

All disorders listed in the table are caused by loss-of-function mutations in their associated disease-causing genes, except for the X-linked form of severe congenital neutropenia that is caused by gain-of-function (GOF) mutations in *WAS*. *AD* Autosomal dominant, *AML* Acute myeloid leukemia, *AR* Autosomal recessive, *G-CSF* Granulocyte colony-stimulating factor, *IBD* Inflammatory bowel disease, *MDS* Myelodysplastic syndrome, *XR* X-linked recessive. [Modified with permission from *Primary Immunodeficiency Diseases: Definition, Diagnosis, and Management*, 2nd edition, Uwe Wintergerst, Taco W. Kuijpers, Sergio D. Rosenzweig, Steven M. Holland, Mario Abinun, Harry L. Malech, and Nima Rezaei, *Phagocytes Defects*, Chapter 4, Pages 245–294, Copyright Springer-Verlag Berlin Heidelberg, 2017.]

[a]The genetic cause of localized juvenile periodontitis is unknown in the majority of patients

[b]*CSF2RA* is located in a pseudoautosomal region on the X chromosome and exhibits an AR inheritance pattern

7.2 Congenital Neutropenia

7.2.1 Main Characteristics and Classification of Congenital Neutropenia

Neutrophils constitute 50–70% of circulating leukocytes. Neutropenia is defined as a significant reduction in absolute neutrophil counts (ANCs) in the peripheral blood. An ANC between 1.0 and 1.5 × 10^9/L (1000–1500/mm^3) is designated as mild neutropenia, between 0.5 and 1.0 × 10^9/L (500–1000/mm^3) as moderate neutropenia, and below 0.5 × 10^9/L (<500/mm^3) as severe neutropenia. The majority of cases with neutropenia have an acquired cause secondary to, for example, infection, (allo-/auto-) immune disorders and drugs. Note that primary autoimmune neutropenia is difficult to diagnose because the detection of antineutrophil antibodies is trying. Congenital neutropenia, i.e., neutropenia caused by germline mutations, is far less frequent with an estimated prevalence of 10 per million people worldwide. Although multiple disease-causing genes have been identified, the underlying genetic defect remains unknown in approximately 25% of cases. Most patients with congenital neutropenia have a sustained neutropenia throughout life, whereas some only show intermittent reductions in ANCs. Moreover, the neutropenia can be an isolated feature or can be associated with other clinical and laboratory abnormalities called syndromic diseases [12–14].

Patients with congenital neutropenia are susceptible to bacterial and fungal infections, predominantly of the skin, mucosal surfaces, and respiratory tract. The frequency and severity of infections are directly correlated with the degree of ANC reduction. Skin abscesses, omphalitis, aphthous stomatitis, gingivitis, periodontitis, pneumonia, otitis media, and septicemia are typically encountered. The major pathogens are staphylococci and streptococci. Fungal infections are usually caused by *Candida* and *Aspergillus* species. In contrast to other congenital phagocyte disorders or post-chemotherapy neutropenia, severe fungal infections are infrequently reported in patients with inherited neutropenia. Furthermore, many forms of congenital neutropenia are associated with an increased risk of developing hematological malignancies, predominantly myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In syndromic forms of congenital neutropenia, other organ systems such as the central nervous system, heart, and pancreas are also affected. In combined immunodeficiencies, defects in the adaptive immune system cause an increased susceptibility to additional types to infections. Overall, the three leading causes of mortality in congenital neutropenia disorders are sepsis, hematological malignancies, and organ failure (e.g., heart dysfunction in Barth syndrome) [13–15].

Regarding infections, the cornerstones of management are long-term G-CSF treatment, antibiotic prophylaxis (e.g., TMP-SMX), and aggressive management of acute infections (see also Sect. 7.1.3) [12, 14]. In general, dosing of G-CSF is started at 5 mcg/kg/day and then titrated until there is a good response (i.e., ANCs >1.0 x 10^9/L). Most patients receive 3 to 10 mcg/kg/day. Intermittent instead of daily administration is also possible. A minority of patients does not respond to G-CSF or require very high doses. Adverse effects of G-CSF therapy include bone pain, skin rash, splenomegaly, thrombocytopenia, osteopenia and osteoporosis, and an increased risk of malignant

transformation to MDS/AML. During prolonged treatment of G-CSF, bone density should be monitored regularly to screen for osteoporosis [14].

The only curative treatment of congenital neutropenia is allogeneic HSCT. HSCT is indicated in patients who fail to respond to conservative therapy with antimicrobial drugs and/or G-CSF and those who develop MDS/AML. However, posttransplant survival is significantly better if HSCT is performed before the onset of life-threatening infections or malignancy. Therefore, early HSCT should be considered in patients with genetic defects that are known to have a poor prognosis [14, 16].

Disorders associated with congenital neutropenia have been classified in different ways. The flowchart shown in Fig. 7.2, separating non-syndromic from syndromic subtypes and other primary immunodeficiencies (PIDs), can serve as a diagnostic tool [14]. Note that this flowchart does not aim to be exhaustive, and that a myriad of PIDs and non-immunological inherited diseases can be associated with some degree of neutropenia [11, 17].

PID subtypes associated with neutropenia include Chédiak-Higashi syndrome, Griscelli syndrome type II, Hermansky-Pudlak syndrome, dyskeratosis congenita, GATA2 deficiency, cartilage hair hypoplasia, MIRAGE syndrome (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy), X-linked agammaglobulinemia (Bruton disease) and other antibody deficiencies, several combined immunodeficiencies including some SCID subtypes, WHIM syndrome (warts, hypogammaglobulinemia, infections, and myelokathexis), and Wiskott-Aldrich syndrome (Fig. 7.2). Since inborn phagocyte abnormalities are not the main defect in these PIDs, they are not reviewed in this chapter. Of note, SMARCD2 deficiency is discussed in Sect. 7.5.1.

7.2.2 Severe Congenital Neutropenia

7.2.2.1 Etiology

This section comprises subtypes of severe congenital neutropenia (SCN) that are generally non-syndromic. The exact prevalence of SCN is unclear, but is estimated to be about 1–2 cases per million. SCN is genetically heterogeneous and can show different inheritance modes: sporadic, autosomal dominant, autosomal recessive, and X-linked recessive. The genetic defects underlying SCN as well as the mechanisms of malignant transformation in SCN are reviewed.

Genes Associated with SCN

The disease-causing genes associated with SCN are listed in Table 7.2. Despite their diverse functions, all these genetic defects ultimately result in an increased apoptosis of myeloid progenitor cells in the bone marrow and/or neutrophils in the peripheral blood [12, 18, 19].

Heterozygous mutations in *ELANE* (formerly called *ELA2*) account for more than 50% of autosomal dominant and sporadic forms of SCN [20, 21]. *ELANE* encodes for neutrophil elastase, a granule serine protease produced during the

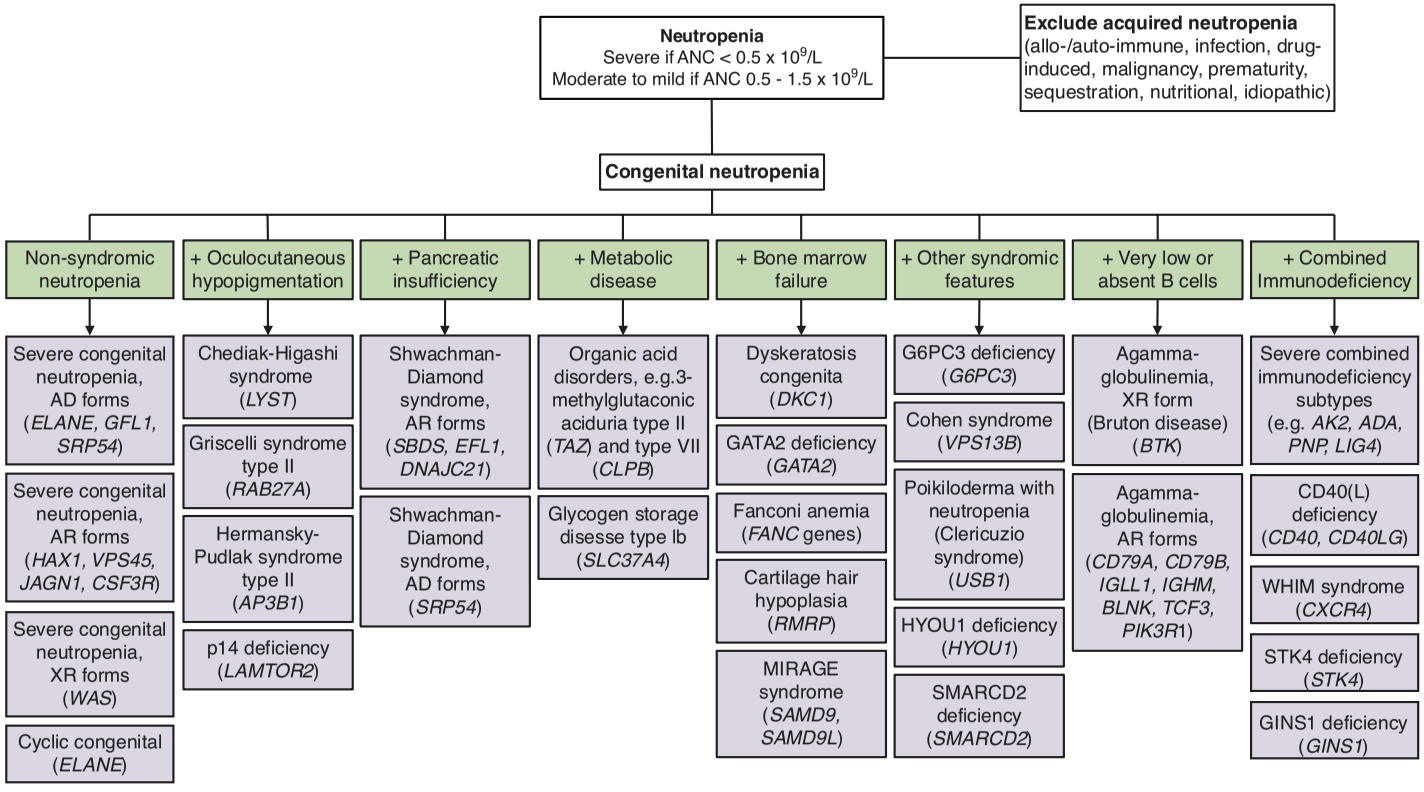


Fig. 7.2 Diagnostic approach to congenital neutropenia. The corresponding disease-causing genes are indicated between brackets. *AD* Autosomal dominant, *ANC* Absolute neutrophil count, *AR* Autosomal recessive, *MIRAGE* Myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy, *WHIM* Warts, hypogammaglobulinemia, infections, and myelokathexis, *XR* X-linked recessive. (Modified with permission from reference [14])

Table 7.2 Genes associated with severe congenital neutropenia

Gene, OMIM no.	Gene locus	Inheritance	Protein	Cause of SCN
ELANE, *130130	19p13.3	AD	Neutrophil elastase	Maturation arrest at the promyelocyte/myelocyte stage due to premature apoptosis
GFI1, *600871	1p22.1	AD	Growth factor independent 1 (GFI1)	Early block in granulopoiesis
TCIRG1, *604592	11q13.2	AD	T cell immune regulator 1	*Causality in SCN not yet confirmed (see text)*
SRP54, *604857	14q130.2	AD	Signal recognition particle 54 kDa protein	Maturation arrest at the promyelocyte/myelocyte stage Due to premature apoptosis
HAX1, *605998	1q21.3	AR	HS-1-associated protein X-1 (HAX1)	Maturation arrest at the promyelocyte/myelocyte stage Due to premature apoptosis
JAGN1, *616012	3p25.3	AR	Jagunal homolog 1 (JAGN1)	Maturation arrest at the promyelocyte/myelocyte stage Due to premature apoptosis
VPS45, *610035	1q21.2	AR	Vacuolar protein sorting-associated protein 45 (VPS45)	Increased apoptosis of bone marrow myeloid cells and blood neutrophils
CSF3R, *138971	1p34.3	AR	Granulocyte colony-stimulating factor (G-CSF) receptor	Reduced survival of mature neutrophils
WAS (GOF), *300392	Xp11.23	XR	Wiskott-Aldrich syndrome protein (WASP)	Maturation arrest at the promyelocyte/myelocyte stage due to premature apoptosis

AD Autosomal dominant, *AR* Autosomal recessive, *GOF* Gain-of-function, *SCN* Severe congenital neutropenia *XR* X-linked recessive.

promyelocyte stage. Over 200 *ELANE* mutations have been described, involving all exons as well as introns 3 and 4 [21]. Mutant neutrophil elastase accumulates in the endoplasmic reticulum (ER) of promyelocytes, resulting in ER stress and the activation of the unfolded protein response (UPR). As the UPR fails to eliminate the mutant protein, prolonged ER stress induces apoptosis of the promyelocytes, followed by a developmental block at that stage [22–24]. Another form of autosomal dominant SCN is caused by mutations in *GFI1*, the gene encoding growth factor independent 1. GFI1 is a transcriptional repressor that plays a role in the regulation of normal hematopoiesis. In particular, GFI1 functions as a rate-limiting molecular switch for granulopoiesis. Defects in GFI1 result in a severe granulocyte maturation arrest; the development of other cell lines may be variable disturbed [25, 26]. In 2014, a heterozygous variant in *TCIRG1* was found in a large pedigree with autosomal dominant SCN, segregating perfectly with the disease. However, no functional tests were performed to confirm its contribution to SCN [27]. In 2016, nine rare heterozygous missense variants in *TCIRG1* were associated with reduced ANCs in a cohort of more than 1000 individuals [28]. Further studies are needed to verify the role of TCIRG1 in the pathogenesis of congenital neutropenia [27, 28]. Heterozygous mutations in *SRP54*, resulting in SRP54 deficiency, were initially reported in patients with a phenotype resembling Shwachman-Diamond syndrome [29].

However, shortly after that first report, it was demonstrated that *SRP54* mutations can also cause an isolated form of SCN without extra-hematopoietic manifestations [30]. Moreover, *SRP54* was the second most common genetic defect after *ELANE* identified in the French Chronic Neutropenia Registry cohort [30]. SRP54 deficiency is discussed further in Sect. 7.2.5.

In 1956, Rolf Kostmann described the first SCN, referred to as Kostmann disease, in a Swedish family [4]. In 2007, *HAX1* was identified as the underlying autosomal recessive genetic defect in this kindred [31]. HAX1 is found primarily in the mitochondria of almost all tissues and is especially important in maintaining homeostasis of myeloid and neuronal cells. HAX1 deficiency leads to premature apoptosis, with a specific genotype-phenotype correlation. Mutations that only cause deficiency of isoform A manifest as isolated SCN, whereas mutations affecting both isoforms A and B additionally result in neurological disease ranging from mild intellectual disability to severe developmental delay and convulsions [32]. Homozygous mutations in *JAGN1* cause a maturation arrest at the promyelocyte/myelocyte stage, similar to *ELANE* and *HAX1* mutations [33]. JAGN1, a ubiquitously expressed ER protein, is crucial in the development of myeloid progenitor cells. JAGN1-deficient neutrophils demonstrate enlarged ER, absence of granules, and an increased tendency to apoptosis [33]. In some patients with JAGN1 deficiency, extra-hematopoietic symptoms have been described, including facial dysmorphism, short stature, skeletal and dental abnormalities, developmental delay, convulsions, and pancreatic insufficiency [33, 34]. Another subtype of autosomal recessive SCN is caused by biallelic mutations in *VPS45* [35, 36]. VPS45 is highly expressed in hematopoietic cells and contributes to the assembly of the SNARE complex, essential for vesicle-mediated trafficking of intracellular molecules. In neutrophils, VPS45 deficiency disrupts the release of antimicrobial peptides, proteolytic enzymes, inflammatory mediators, and proteins involved in neutrophil adhesion and chemotaxis. Neutrophils and bone marrow myeloid cells in VPS45-deficient patients show accelerated apoptosis, whereas lymphocytes do not [35]. Patients typically present with primary myelofibrosis, progressive bone marrow failure with transfusion-dependent anemia and thrombocytopenia, and hepatosplenomegaly and nephromegaly due to extramedullary hematopoiesis. Neurological symptoms and facial dysmorphism have occasionally been reported [36, 37]. It is important to mention that all patients with JAGN1 deficiency or VPS45 deficiency currently reported descend from consanguineous parents, and that other recessive alleles may thus be responsible for the extra-hematopoietic features seen in some of these patients [38]. Biallelic germline mutations in *CSF3R* can also cause autosomal recessive SCN [39]. *CSF3R* encodes the receptor for G-CSF (also called CSF3), which stimulates granulopoiesis as well as survival and function of mature neutrophils. In contrast to other forms of SCN, patients with G-CSF receptor deficiency display full maturation of myeloid cells in the bone marrow [39].

The only X-linked form of SCN described thus far is caused by a gain-of-function (GOF) mutation in the *WAS* gene, encoding Wiskott-Aldrich syndrome protein (WASP) [40]. Note that *WAS* mutations have also been associated with two other phenotypes: partial loss-of-function mutations result in X-linked

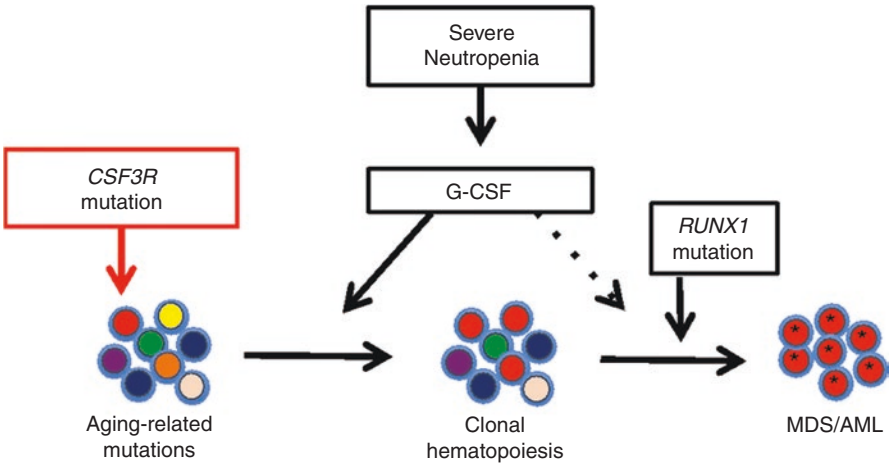


Fig. 7.3 *Model of leukemic transformation in severe congenital neutropenia (SCN).* Aging-related mutations, which occur during the replication of hematopoietic stem cells (HSCs), result in the production of a genetically heterogeneous pool of HSCs. In SCN, the persistently high levels of granulocyte colony-stimulating factor (G-CSF) result in the selection of HSCs that carry somatic truncation mutations in *CSF3R* (encoding the G-CSF receptor). Additional somatic mutations, most commonly in *RUNX1*, are required for transformation to myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML). [Reprinted with permission from reference [42]]

thrombocytopenia, and a complete loss of WASP expression in classic Wiskott-Aldrich syndrome [14]. WASP plays a role in the regulation of actin cytoskeletal rearrangements in myeloid and lymphoid lineage cells. A constitutively activating mutation in the GTPase-binding domain of WASP enhances actin polymerization, causing disruption in cell division, increased apoptosis, and genomic instability [41]. Next to severe neutropenia, patients also have monocytopenia; T, B, and NK lymphopenia; and an increased risk of MDS/AML [40, 41].

Leukemic Transformation in SCN

SCN is considered a preleukemic condition whereby a significant number of patients develop myelodysplastic syndrome (MDS) and leukemia, predominantly acute myeloid leukemia (AML) but also chronic myelomonocytic leukemia (CMML) and acute lymphoblastic leukemia (ALL). MDS and leukemia have been reported in SCN caused by mutations in *ELANE*, *HAX1*, *JAGN1*, *CSF3R*, and *WAS* [16, 42]. The cumulative risk of malignant transformation in patients with SCN is at least 10–20%. Patients with a poor response to G-CSF, or receiving a high dose of G-CSF, have an increased incidence of MDS/AML development [42]. Somatic (acquired) mutations in *CSF3R* that truncate the cytoplasmic domain of the G-CSF receptor are considered a major risk factor for leukemogenesis in patients with SCN. These somatic *CSF3R* mutations are found in approximately 80% of SCN patients with MDS/AML, compared to 30–35% of those without leukemic transformation. The mutant truncated receptors have a prolonged half-life due to impaired internalization, resulting in enhanced signaling and increased proliferation of myeloid progenitor cells [42, 43]. It is hypothesized that high levels of G-CSF drive the clonal

expansion of hematopoietic progenitor cells carrying somatic *CSF3R* truncation mutations (Fig. 7.3) [42]. Next to the contribution of G-CSF and somatic *CSF3R* mutations to leukemogenesis, additional somatic mutations need to be acquired to induce leukemic progression in patients with SCN, which often includes somatic mutations in *RUNX1* and alterations of chromosome 7 (Fig. 7.3) [42].

7.2.2.2 Clinical Features

Patients with SCN present in infancy with persistent severe neutropenia (ANC usually below 0.2×10^9/L) and severe recurrent bacterial infections (see also Sect. 7.2.1). Signature infections of SCN are abscesses, aphthous stomatitis, gingivitis, and periodontitis. Furthermore, patients with SCN are at considerable risk of developing MDS and leukemia (mainly AML) [21]. Associated manifestations related to specific genetic defects have been described above.

Prior to the introduction of G-CSF therapy, the mortality rate due to bacterial infections was more than 80%, with 50% of patients dying in the first year of life [21]. Currently, the overall survival of patients with SCN, including those who develop malignancy, is estimated to be more than 80%. About 10% of patients still die from infectious complications, which mainly concerns G-CSF nonresponders [21].

7.2.2.3 Diagnosis

The diagnosis is suspected based on the presence of severe neutropenia in association with early-onset severe infections. Bone marrow aspiration usually shows an early block in myeloid cell development (Table 7.2). Genetic analysis can confirm the diagnosis and help predict the response to treatment and the risk of malignant progression [14].

7.2.2.4 Treatment

Regular G-CSF administration is the treatment of choice in all patients, and dosing should be titrated to achieve ANCs $>1.0 \times 10^9$/L (see also Sect. 7.2.1). More than 90% of patients with SCN show a good response [21]. However, SCN due to *VPS45* and *CSF3R* mutations does not respond to G-CSF therapy [35, 39]. Several patients with mutations in *SRP54* and *JAGN1* were also described to have a poor response to G-CSF [30, 33]. Some patients may require long-term antibiotic prophylaxis. Acute infections should be diagnosed early and treated promptly, given the high risk of mortality (see also Sect. 7.1.3).

There have been several reports on successful allogeneic HSCT in patients with SCN [16]. Both reduced-intensity and myeloablative conditioning regimes have been used. HLA-matched related or unrelated donors are preferred over haploidentical donors. The 3-year overall survival is about 80%, and the outcome appears to be better in patients transplanted before 10 years of age [16, 44]. In general, HSCT is indicated in patients who (1) are unresponsive to G-CSF or require more than 8 μg/kg/day of G-CSF to maintain adequate neutrophils counts, (2) suffer from recurrent severe infections despite maximum conservative management, or (3) develop MDS or leukemia. To date, there are no guidelines if and when SCN patients with genetic subtypes predisposing to malignancy should undergo preemptive HSCT [16].

7.2.3 Cyclic Neutropenia

7.2.3.1 Etiology

Cyclic neutropenia (CyN) is characterized by the cyclic recurrence of severe neutropenia approximately every 3 weeks and lasting for several days. In between periods of neutropenia, the ANC returns to normal levels. The prevalence of CyN is estimated at one per million people [45]. The pathophysiology of CyN is incompletely understood, but there seems to be a periodic alteration in the production of hematopoietic precursor cells [46]. In most patients with CyN, there are concurrent oscillations in the levels of other hematopoietic cells. Most often, a decrease in ANCs is accompanied by a rise in monocyte counts. Fluctuations in lymphocytes, eosinophils, reticulocytes, and platelets have also been reported [45].

In at least 95% of cases, CyN is caused by heterozygous mutations in *ELANE*, one of the genes associated with autosomal dominant SCN (Table 7.2) [20, 47]. Intriguingly, the same *ELANE* mutations are found in both SCN and CyN patients. It is believed that the final phenotype is determined by differences in genetic background, such as the function of proteins that regulate the extent of the UPR (see Sect. 7.2.2, Etiology) [14]. A biallelic mutation in *HAX1*, one of the genes associated with autosomal recessive SCN (Table 7.2), was recently reported in a child with cyclic variations in ANC and recurrent oral ulcerations during periods of severe neutropenia. After a few years, the child's phenotype evolved to a persistent severe neutropenia, and the diagnosis was changed to SCN [48].

7.2.3.2 Clinical Features

CyN generally has a milder disease course than SCN since symptoms only occur during periods of severe neutropenia. Most patients present in early childhood with recurrent episodes of fever, malaise, pharyngitis, aphthous stomatitis, gingivitis, periodontitis, cellulitis, and abscesses [45, 46]. Severe bacterial infections and mortality from sepsis have been occasionally described. Noteworthy, patients with CyN are at risk of developing intestinal ulcerations and typhlitis (neutropenic enterocolitis), which can rapidly progress to acute bowel perforation and life-threatening clostridial or gram-negative sepsis. Therefore, abdominal discomfort in a patient with CyN should always be urgently evaluated [49]. The cycle period and duration of neutropenia within a cycle are quite consistent for each patient, resulting in a predictable recurrence of symptoms. Between these episodes, patients are usually asymptomatic [45]. By adult age, the phenotype can progress to a mild chronic neutropenia without distinct cycles [49]. In contrast to SCN, CyN is not associated with malignant transformation [42, 50].

7.2.3.3 Diagnosis

CyN is diagnosed by documenting very regular cyclic oscillations in the ANC, from normal levels ($>1.5 \times 10^9$/L) to severe neutropenic levels ($<0.5 \times 10^9$/L). The monocyte counts concurrently cycle in an opposite fashion, with monocytosis at time of severe neutropenia and vice versa [45, 46]. The diagnosis is established by performing a complete blood count with differential two to three times a week for 6 to 9 weeks. One cycle is about 21 days in most patients, but can range from 14 to

35 days. The neutropenia lasts 3–6 days [45, 46]. The administration of G-CSF may significantly alter the cycle pattern. Bone marrow examination is less useful as the findings differ depending on when in the cycle the sample is taken. During a period of neutropenia, a maturation arrest in early myeloid precursors is seen [46]. Genetic analysis of *ELANE* is recommended to help confirm the diagnosis [45]. An important differential diagnosis is acquired adult-onset cyclic neutropenia, which has an autoimmune etiology [51].

7.2.3.4 Treatment

Like for SCN, regular G-CSF administration is the treatment of choice in all patients with CyN (see also Sect. 7.2.1) [49]. G-CSF therapy has been shown to be very effective in preventing infections and reducing gingival and dental complications. The dosing of G-CSF is based on the ANC (target level $>0.5 \times 10^9$/L) and symptom control, and is usually lower than that needed for SCN. G-CSF may be discontinued when there is a spontaneous improvement of the disease at older age [49]. In addition, proper dental care and the use of antibacterial mouthwashes (e.g., chlorhexidine 0.2%) are recommended to reduce gingivitis and periodontitis. Acute febrile infections should be treated aggressively, and antibiotics should cover gram-negative and clostridial species when typhlitis is suspected (see also Sect. 7.1.3) [46, 49].

7.2.4 p14 Deficiency

7.2.4.1 Etiology

p14, or MAPBP-interacting protein (MAPBPIP), is an endosomal adaptor protein that is ubiquitously expressed. It is involved in endosome rearrangements during MAP kinase signal transduction. p14 is encoded by *LAMTOR2* (OMIM *610389), located at chromosome 1q22. So far, only one family (four patients) with p14 deficiency has been reported [52]. These patients had a homozygous mutation in the 3′ untranslated region (UTR) of *LAMTOR2*, leading to disrupted mRNA processing and decreased protein levels of p14 [52, 53]. p14 deficiency was found to be associated with an aberrant maturation and function of specialized lysosomes in melanocytes, neutrophils, and cytotoxic T cells, causing a combined phenotype of hypopigmentation and immunodeficiency [52]. The mechanism of neutropenia in p14 deficient patients remains to be elucidated. Unlike patients with SNC, myeloid maturation in bone marrow was intact and neutrophils had no increased susceptibility to apoptosis [52].

7.2.4.2 Clinical Features

p14 deficiency is characterized by partial oculocutaneous albinism, short stature, coarse facial features, and recurrent lower respiratory tract infections mainly caused by *Streptococcus pneumoniae*. Consistent laboratory findings include severe neutropenia (ANC $<0.5 \times 10^9$/L), decreased cytotoxic activity of $CD8^+$ T cells, reduced memory B cells, decreased serum IgM levels, and inadequate antibody responses to vaccination. Two patients also demonstrated a progressive decline of serum IgG levels [52]. So far, hemophagocytic lymphohistiocytosis (HLH) has not been reported in these patients.

7.2.4.3 Diagnosis

The diagnosis is suspected based on the combination of oculocutaneous albinism, severe neutropenia, and recurrent infections. Genetic analysis of *LAMTOR2* should be performed to confirm the diagnosis [52]. Other PIDs associated with oculocutaneous hypopigmentation should be excluded (Fig. 7.2).

7.2.4.4 Treatment

G-CSF therapy may be considered to treat neutropenia (see Sect. 7.2.1). Immunoglobulin replacement therapy may be indicated in case of antibody deficiency [52]. Acute infections should be treated aggressively as explained in Sect. 7.1.3. The identification of additional patients is required to define the complete phenotype and the most adequate treatment.

7.2.5 Shwachman-Diamond Syndrome

7.2.5.1 Etiology

Shwachman-Diamond syndrome (SDS), also called Shwachman-Bodian-Diamond syndrome, is a ribosomopathy characterized by a triad of exocrine pancreas insufficiency, bone marrow failure, and metaphyseal chondrodysplasia [54, 55]. SDS is a genetically heterogeneous disorder with an estimated incidence between 1 in 168,000 and 1 in 77,000 live births (Table 7.3) [56, 57]. All genes associated with SDS are ubiquitously expressed and involved in protein synthesis, either through ribosome biogenesis or protein trafficking to the ER (Fig. 7.4) [58, 59]. Approximately 90% of SDS patients have biallelic mutations in *SBDS* (*Shwachman-Bodian-Diamond syndrome*), which was the first gene found to be associated with SDS [60]. The protein encoded by *SBDS* plays a role in multiple fundamental processes, including the maturation of the large 60S ribosomal subunit, stabilization of the mitotic spindle during cell division, mitochondrial function, actin polymerization, DNA repair, and organization of the stromal microenvironment in the bone marrow [59]. Different types of mutations have been identified in the *SBDS* gene,

Table 7.3 Genes associated with Shwachman-Diamond syndrome

Gene, OMIM no.	Gene locus	Inheritance	Protein	Estimated frequency in SDS
SBDS, *607444	7q11.21	AR	Ribosome maturation protein SBDS	~92%
DNAJC21, *617048	5p13.2	AR	DnaJ homolog subfamily C member 21	<1%
EFL1, *617538	15q25.2	AR	Elongation factor-like GTPase 1	<1%
SRP54, *604857	14q13.2	AD	Signal recognition particle 54 kDa protein	<1%

AD Autosomal dominant, *AR* Autosomal recessive, *SDS* Shwachman-Diamond syndrome

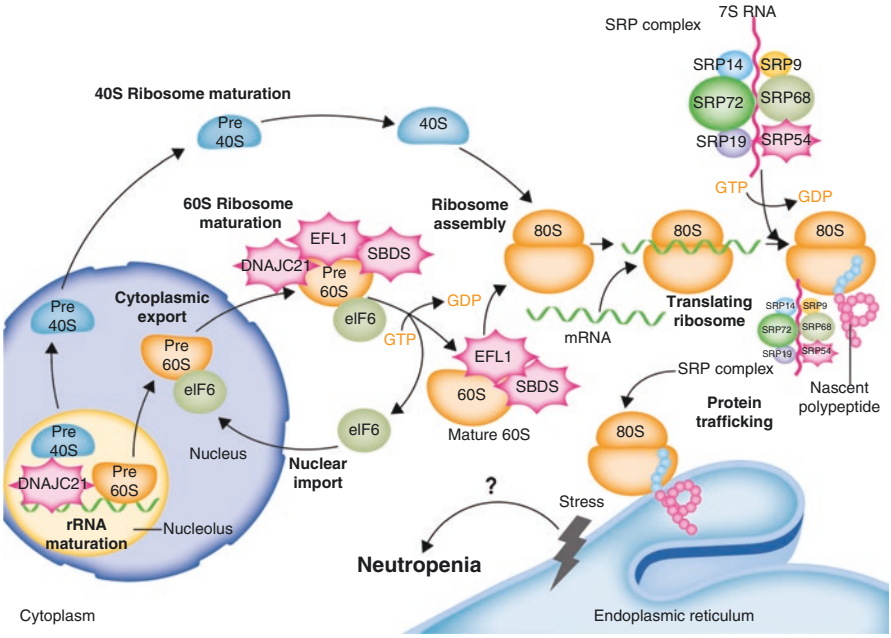


Fig. 7.4 *The proteins implicated in Shwachman-Diamond syndrome are important for ribosome biogenesis or protein trafficking to the endoplasmic reticulum (ER).* Association of the large 60S and small 40S ribosomal subunits is blocked by eukaryotic initiation factor 6 (eIF6). SBDS interacts with EFL1 on the pre-60S ribosome to displace eIF6, allowing the assembly of the 40S and 60S subunits and formation of the translationally active 80S ribosome. DNAJC21 is implicated in ribosomal RNA biogenesis and late cytoplasmic maturation of the 60S subunit. SRP54 is one of the six proteins of the signal recognition particle (SRP) ribonucleoprotein complex, which escorts the nascent polypeptide coming off the 80S ribosome to the ER to complete translation and possible posttranslational modification. Defects in ribosome biogenesis and protein synthesis result in cellular stress, which may induce increased apoptosis in hematopoietic stem cells. *GDP* Guanosine diphosphate, *GTP* Guanosine triphosphate. (Reprinted with permission from reference [58])

including intragenic deletions and splice site mutations [59]. All *SBDS*-mutant patients display a marked reduction in protein activity. Complete loss of SBDS protein function in humans has not been described and is believed to be incompatible with life [57, 61]. More recently, biallelic mutations in *DNAJC21* and *EFL1* disrupting protein function have been reported in a handful of patients with an SDS-like phenotype [62, 63]. DNAJC21 is a chaperone protein that plays a role in ribosome RNA biogenesis and the maturation of the large 60S ribosomal subunit. The EFL1 protein interacts with the SBDS protein and is involved in the formation of mature ribosomes (Fig. 7.4) [59]. Biallelic *DNAJC21* mutations have also been described in patients with bone marrow failure, short stature, and dental abnormalities, but without pancreatic dysfunction [64, 65]. Therefore, it has been suggested that *DNAJC21*-related disease should be considered a distinct inherited bone marrow failure syndrome rather than an SDS subtype [65]. Lastly, heterozygous mutations in *SRP54* have been associated with an autosomal dominant SDS-like disorder

[29, 30]. The SRP54 protein is part of the SRP ribonucleoprotein complex that facilitates trafficking of the newly synthesized protein to the ER (Fig. 7.4). Mutant SRP54 impairs the synthesis of secreted and membrane-bound proteins, leading to ER stress [30]. In *SRP54*-mutant patients, bone marrow examination showed dysgranulopoiesis and a maturation arrest at the promyelocyte stage. However, of all patients with heterozygous *SRP54* mutations reported thus far, only some have extra-hematopoietic features reminiscent of SDS, while the majority presents isolated SCN (see also Sect. 7.2.2) [29, 30]. In less than 10% of patients with SDS, the genetic defect is unknown [57].

How defects in ribosome biogenesis and protein synthesis contribute to the phenotype observed in SDS patients has been recently reviewed by Bezzerri et al. [59]. However, the pathophysiological mechanisms remain incompletely understood. Most patients with SDS have a hypocellular bone marrow showing reduced $CD34^+$ hematopoietic stem cells (HSCs) and an impaired granulopoiesis. There is evidence that cellular stress caused by impaired ribosome biogenesis or protein synthesis induces p53 signaling, which leads to cell cycle arrest and apoptosis [30, 42, 59]. Furthermore, neutrophil chemotaxis is often impaired, which may be due to dysregulated actin polymerization [59]. Similar to SCN, patients with SDS have a significantly increased risk of malignant transformation to MDS/AML, with a cumulative incidence of up to 36% [66]. SDS-related MDS/AML is characterized by distinct molecular features, including somatic mutations in *TP53* and abnormalities in chromosome 7 [42]. A recent study demonstrated that somatic *TP53* mutations were present in all cases of SDS-related MDS/AML, suggesting that they are required to overcome the cell cycle arrest in HSCs. Continued ribosome biogenesis stress favors the selection of HSCs carrying *TP53* mutations, facilitating clonal expansion and leukemic progression [42].

7.2.5.2 Clinical Features

SDS is a syndromic disorder characterized by bone marrow failure and hematological manifestations, exocrine pancreatic insufficiency, skeletal abnormalities, and other variable features including neurodevelopmental problems, endocrine dysfunction, congenital heart defects, and sensorineural hearing loss [57]. Infants with SDS commonly present with failure to thrive and steatorrhea due to exocrine pancreatic insufficiency, and recurrent bacterial infections due to neutropenia and/or impaired neutrophil chemotaxis [57]. Almost all patients with SDS show neutropenia (i.e., ANC $< 1.5 \times 10^9$/L) of variable severity. Even more, about one third of cases have persistent neutropenia, and two thirds show intermittent neutropenia. About 50% of patients also develop anemia and/or thrombocytopenia, which may be transfusion-dependent [57]. As discussed above, patients with SDS are at considerable risk of developing MDS and AML, with a reported cumulative incidence of up to 36% [66]. So far, MDS/AML development has not been documented in any of the patients with mutations in *EFL1* and *SRP54* [29, 30, 63, 67, 68]. Note that bone marrow failure is the major cause of morbidity and mortality in SDS, and patients with MDS/AML have a particularly poor prognosis. In one cohort of 36 SDS patients, the overall 3-year survival was 11% for patients with leukemia and 51%

for those with MDS [66]. Malabsorption due to exocrine pancreatic insufficiency may cause a state of malnutrition, deficiencies in fat-soluble vitamins, and development of osteoporosis if not treated properly. However, in about half of patients, pancreatic insufficiency spontaneously improves over time, eliminating the need for pancreatic enzyme therapy [57]. Other gastrointestinal features encountered in SDS patients are enteropathy that may contribute to vitamin and mineral deficiencies and hepatic dysfunction, resulting in elevated liver enzymes, hepatomegaly, cholestasis, and in some cases even hepatic failure [57]. In addition, patients with SDS have characteristic skeletal abnormalities, such as short stature, progressive metaphyseal dysplasia, thoracic dystrophy, and generalized osteopenia. Endocrinological manifestations seen in SDS patients include delayed onset of puberty and growth hormone deficiency. Finally, many patients display a variable degree of cognitive and behavioral problems [57].

7.2.5.3 Diagnosis

SDS should be suspected in patients with symptoms of bone marrow failure and exocrine pancreatic insufficiency. Diagnostic criteria for SDS were established in

Table 7.4 Diagnostic criteria of Shwachman-Diamond syndrome

Molecular (genetic) diagnosis
Biallelic *SBDS* mutations known or predicted to be pathogenic, or mutations in other SDS-associated genes *DNAJC21* (AR), *ELF1* (AR), *SRP54* (AD)
Clinical diagnosis
1. Hematologic features (at least one of the following) (a) Neutropenia (ANC < 1.5×10^9/L) on at least two occasions (b) Anemia or macrocytosis on at least two occasions (unexplained by other causes, such as iron/vitamin B12 deficiency) (c) Thrombocytopenia (platelet count <150,000/μL) on at least two occasions (d) Bone marrow findings • Hypocellularity for age • Myelodysplasia • Leukemia • Cytogenetic abnormalities
2. Features of exocrine pancreatic insufficiency (at least one of the following) (a) Reduced levels of pancreatic enzyme relevant to age • Serum trypsinogen (age <3 years) • Serum isoamylase (age ≥3 years) (b) Low levels of fecal elastase (c) Supportive features • Abnormal pancreatic imaging (ultrasound, MRI) with lipomatosis • Elevated fecal fat excretion >72 h
3. Additional supportive features (at least one of the following) (a) Skeletal abnormalities, e.g., metaphyseal dysplasia and thoracic dystrophy (b) Neurocognitive or behavioral problems (c) Unexplained height less than third percentile (d) First-degree family member with Shwachman-Diamond syndrome

AD Autosomal dominant, *ANC* Absolute neutrophil count, *AR* Autosomal recessive, *MRI* Magnetic resonance imaging. (Modified with permission from reference [57])

2002 and updated in 2011 and 2018 [57, 69, 70]. The most recent consensus criteria are provided in Table 7.4. Genetic testing is recommended to confirm the diagnosis, although in about 10% of patients no mutations are found in the SDS-associated genes [57]. Other causes of bone marrow failure and exocrine pancreatic insufficiency should be considered in the differential diagnosis, especially cystic fibrosis, dyskeratosis congenita, Fanconi anemia, and Pearson syndrome [14, 71].

7.2.5.4 Treatment

Regular screening for known complications of SDS should be performed (Table 7.5) [57]. G-CSF therapy can be considered when neutropenia is associated with recurrent severe infections or ANCs are persistently below 0.5 x 10^9/L (see also Sect. 7.2.1). However, prolonged administration of G-CSF may increase the risk of malignant transformation, and therefore its use should be based on an individual benefit-risk assessment [57]. Patients with pancreatic insufficiency are treated with pancreatic enzyme replacement therapy and fat-soluble vitamin supplementation.

Table 7.5 Follow-up of patients with Shwachman-Diamond syndrome

Parameter	Frequency of evaluation
Hematology	
CBC	At diagnosis, every 3–6 months or as indicated
Bone marrow aspirate and biopsy	At diagnosis, every 1–3 years or as indicated
Iron, folate, vitamin B12	At diagnosis, as clinically indicated
Immunoglobulins and lymphocyte subpopulations	At diagnosis, as clinically indicated
HLA testing	As clinically indicated, impending HSCT
Gastroenterology	
Pancreatic enzyme measurement	At diagnosis, as clinically indicated
Fat-soluble vitamins and prothrombin time	At diagnosis, 1 month after start of pancreatic enzyme therapy, then every 6–12 months
Hepatic profile	At diagnosis, every year or as clinically indicated
Pancreatic imaging (ultrasound, MRI)	At diagnosis
Endoscopy	As clinically indicated
Skeletal system	
Growth evaluation: Height, weight, head circumference	At diagnosis, every 6–12 months until fully grown (more frequently if on GH replacement)
Bone densitometry	Before puberty, during puberty, then as clinically indicated
X-rays of hips and knees	At diagnosis, at times of rapid growth or as clinically indicated
Other	
Neuropsychological testing	At diagnosis, reassessment at age 6–8 years, 11–13 years, and 15–17 years
Endocrine evaluation (e.g., TSH, GH)	As clinically indicated
Auditory testing	As clinically indicated

CBC Complete blood count, *GH* Growth hormone, *HLA* Human leukocyte antigen, *HSCT* Hematopoietic stem cell transplantation, *MRI* Magnetic resonance imaging, *TSH* Thyroid-stimulating hormone. (Modified with permission from reference [57])

Acute infections should be treated promptly (see also Sect. 7.1.3). Prophylactic antibiotics may be required during complex dental and surgical procedures. Transfusions with erythrocytes and/or platelets are given in case of severe anemia, respectively thrombocytopenia [57]. Allogeneic HSCT is indicated in patients with SDS, who develop bone marrow failure (i.e., transfusion-dependent anemia, increased bleeding tendency, and recurrent severe infections), or MDS/AML [16]. It has been shown that HSCT can successfully correct the hematopoietic manifestations in SDS patients. However, the overall posttransplant survival is only about 60–70%. The outcome is worse in patients who are transplanted following the development of MDS/AML, with an overall survival of 40–50%. Therefore, regular bone marrow examinations with screening for cytogenetic alterations are advised (Table 7.5), although there are no guidelines on when to proceed to HSCT if premalignant alterations are detected [16].

7.2.6 3-Methylglutaconic Aciduria Type II and Type VII

Organic acid disorders are inherited metabolic diseases that cause an accumulation of organic acid intermediates situated upstream of the specific enzyme deficiency. These patients commonly present with hematological abnormalities, including neutropenia. The neutropenia can be of variable severity and may fluctuate within the same patient [72]. Here, 3-methylglutaconic aciduria types II and VII are discussed in more detail.

7.2.6.1 Etiology

Methylglutaconic aciduria type II (also called Barth syndrome) is an X-linked disease caused by defects in tafazzin, encoded by *TAZ* (OMIM *300394) at chromosome Xq28. Tafazzin is involved in the remodeling of cardiolipin, which is the main component of the inner mitochondrial membrane and needed for normal functioning of the respiratory chain [73].

Methylglutaconic aciduria type VII (or CLBP deficiency) is an autosomal recessive disorder caused by biallelic mutations in *CLPB* (OMIM *616254), located at chromosome 11q13.4. CLBP is a mitochondrial ATP-dependent chaperone, believed to be involved in the refolding of misfolded proteins [74].

7.2.6.2 Clinical Features

Barth syndrome is characterized by (mainly dilated) cardiomyopathy, (intermittent) neutropenia, facial dysmorphism, skeletal myopathy, delayed motor development, and short stature. Cardiac failure is the main cause of mortality. Neutropenia is usually associated with recurrent aphthous stomatitis and severe bacterial infections such as pneumonia and sepsis [73, 75].

Patients with methylglutaconic aciduria type VII present with neurologic manifestations (e.g., progressive brain atrophy, developmental delay, hypotonia, movement disorder, epilepsy), cataract, and neutropenia. Depending on the severity of the underlying defect, the neutropenia ranges from severe persistent to mild intermittent [74].

7.2.6.3 Diagnosis

The diagnosis is suspected based on the typical clinical findings and an elevated urinary excretion of 3-methylglutaconic acid. However, urinary 3-methylglutaconic acid may be normal even in severe cases. Genetic testing is recommended to establish a definite diagnosis [73, 74].

7.2.6.4 Treatment

These disorders require a multidisciplinary approach encompassing symptom management and supportive care. There is no curative treatment. In case of recurrent infections, G-CSF therapy and/or prophylactic antibiotics can be used (see also Sects. 7.1.3 and 7.2.1) [75].

7.2.7 Glycogen Storage Disease Type 1b

7.2.7.1 Etiology

Glycogen storage disease type 1b (GSD-1b) is an autosomal recessive metabolic disorder caused by biallelic mutations in *SLC37A4* (OMIM *602671), located at chromosome 11q23.3. This gene encodes glucose-6-phosphate translocase (G6PT), which is involved in the transport of glucose into the ER. G6PT forms a complex with glucose 6 phosphate catalytic subunit (G6PC) enzymes and is important for maintaining interprandial glucose homeostasis [76]. Congenital neutropenia associated with G6PC3 deficiency is discussed in Sect. 7.2.8. Neutrophils, in which the G6PC3/G6PT complex is defective, show impaired chemotaxis and respiratory burst and enhanced apoptosis [77, 78].

7.2.7.2 Clinical Features

GSD-1b is characterized by fasting hypoglycemia, lactic acidosis, hepatomegaly, nephromegaly, hypertriglyceridemia, hypercholesterolemia, hyperuricemia, osteopenia, a doll-like facies, and short stature. Most patients also have (intermittent) severe neutropenia and neutrophil dysfunction predisposing to recurrent oral ulcers, recurrent bacterial infections such as abscesses, and inflammatory bowel disease (IBD) [79, 80].

7.2.7.3 Diagnosis

If the clinical phenotype is suggestive of GSD-1b, mutation analysis of *SLC37A4* should be done to confirm the diagnosis. If genetic testing is inconclusive, enzyme assays (e.g., glucose-6-phosphate activity) performed on liver biopsy specimens can help establish the diagnosis [79].

7.2.7.4 Treatment

Patients with GSD-1b should receive dietary advice to achieve optimal metabolic control [79]. In severe cases, liver transplantation may be required to control glucose metabolism and can also improve neutrophil function, although transplant-related mortality is high [81]. Prophylactic antibiotics and G-CSF therapy are

recommended in patients with recurrent infections (see also Sects. 7.1.3 and 7.2.1). G-CSF therapy may also improve IBD-related symptoms [80, 82]. Allogeneic HSCT has been sporadically performed in patients with severe infections and IBD [83]. Gene therapy is being studied [84].

7.2.8 G6PC3 Deficiency

7.2.8.1 Etiology

Glucose-6 phosphatase catalytic subunit 3 (G6PC3) is one of the three G6PC enzymes that catalyzes the final step of glycogenolysis, and is particularly important for the function and survival of neutrophils (see also Sect. 7.2.7). G6PC3 deficiency, caused by biallelic mutations in *G6PC3* (OMIM *611045) at chromosome 17q21.31, is therefore characterized by an impaired chemotaxis and respiratory burst and enhanced apoptosis of neutrophils [77, 85].

7.2.8.2 Clinical Features

Patients with G6PC3 deficiency commonly present with severe neutropenia, recurrent bacterial infections from the first months of life, congenital heart defects, prominent superficial veins, urogenital abnormalities, facial dysmorphism, and failure to thrive. Additional features include intermittent thrombocytopenia, moderate to severe T and/or B lymphopenia, IBD, developmental delay, osteopenia/osteoporosis, and endocrine disorders (growth hormone deficiency, delayed puberty) [85, 86]. Bone marrow examination may show dysplastic changes in all three lineages, and patients are at risk of developing MDS/AML [86]. Rarely, G6PC3-deficient patients only have isolated SCN [87].

7.2.8.3 Diagnosis

In patients with a suggestive phenotype, a definite diagnosis is established by genetic analysis of *G6PC3* [85].

7.2.8.4 Treatment

Patients with G6PC3 deficiency should be managed multidisciplinary. Regarding the neutropenia, G-CSF therapy is the mainstay of treatment (see also Sect. 7.2.1). In mild cases, infection control can be achieved with prophylactic antibiotics (see also Sect. 7.1.3). Endocarditis prophylaxis may be required in patients with congenital heart defects. Allogeneic HSCT can be considered in patients who do not respond to G-CSF therapy or develop MDS/AML [85, 86].

7.2.9 Cohen Syndrome

7.2.9.1 Etiology

Cohen syndrome is a rare developmental disorder associated with variable degrees of neutropenia. It is caused by biallelic mutations in *VPS13B* (OMIM *607817),

located at chromosome 8q22.2 [88]. The function of the VPS13B (or COH1) protein is incompletely understood. It was shown that VPS13B is a Golgi-associated peripheral membrane protein essential for the integrity and function of the Golgi complex, which is the organelle where most posttranslational glycosylation reactions occur [89]. Serum proteins of patients show an abnormal glycosylation pattern, supporting the hypothesis of a Golgi complex dysfunction as the pathophysiological mechanism in Cohen syndrome [90].

7.2.9.2 Clinical Features

Cohen syndrome is characterized by developmental delay, intellectual disability, microcephaly, typical facial features, truncal obesity, hypotonia, pigmentary retinopathy, and neutropenia. Neutropenia is generally mild to moderate and can be intermittent. Patients may have recurrent aphthous stomatitis, gingivitis, and bacterial infections. Severe infections are uncommon in Cohen syndrome [88].

7.2.9.3 Diagnosis

Chandler et al. determined the diagnostic criteria for Cohen syndrome as having intellectual disability in combination with at least two of the following features: characteristic facial dysmorphism, pigmentary retinopathy, or neutropenia [91]. Genetic confirmation of the diagnosis is recommended. Importantly, there is a high prevalence of intragenic deletions and duplications in the *VPS13B* gene that could be missed by traditional sequencing techniques [92].

7.2.9.4 Treatment

As all syndromic disorders, Cohen syndrome requires a multidisciplinary approach. Severe neutropenia and/or recurrent infections can be treated with G-CSF therapy (see also Sect. 7.2.1) [88].

7.2.10 Poikiloderma with Neutropenia (Clericuzio Syndrome)

7.2.10.1 Etiology

Poikiloderma with neutropenia (PN), or Clericuzio syndrome, is a rare dermatological disorder associated with congenital neutropenia. It is caused by mutations in the autosomal recessive gene *USB1* (OMIM *613276) at chromosome 16q21 [93]. USB1 is a phosphodiesterase that plays a role in RNA splicing. In PN, the splicing of genes important for myeloid development is disrupted, resulting in a maturation arrest and neutropenia [94, 95]. Moreover, the remaining neutrophils are dysfunctional as evidenced by a reduced respiratory burst [96].

7.2.10.2 Clinical Features

During the first year of life, patients develop an inflammatory papulo-erythematous rash that starts at the extremities and gradually spreads toward the trunk and face. Afterward, the skin condition progresses to post-inflammatory poikiloderma, consisting of areas of skin hypo- and hyperpigmentation, telangiectasia, and atrophy

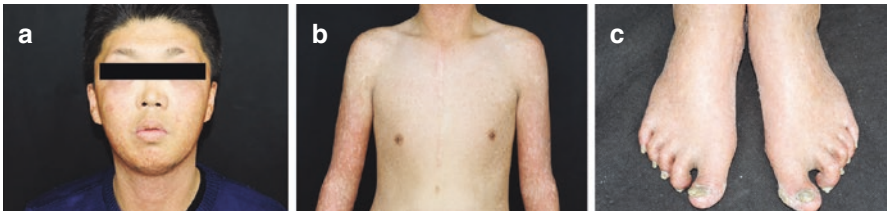


Fig. 7.5 *Characteristic ectodermal findings in an adult patient with poikiloderma with neutropenia.* (**a**, **b**) Reticulated hyper- and hypopigmentation with erythema overlying the body. (**c**) Pachyonychia of the toenails. (Reprinted with permission from reference [97])

(Fig. 7.5a, b). Other typical dermatologic manifestations are pruritic palmoplantar hyperkeratosis and toenail pachyonychia (i.e., hyperkeratotic nails) (Fig. 7.5c). Patients also have persistent, moderate to severe neutropenia resulting in recurrent bacterial infections from early childhood, predominantly affecting the respiratory tract. Other findings include reactive airway disease, bronchiectasis, transient anemia and/or thrombocytopenia, short stature, facial dysmorphism, dental abnormalities, skeletal abnormalities, and delayed puberty. Finally, patients with PN have an increased susceptibility to developing MDS/AML and squamous cell carcinoma [98, 99].

7.2.10.3 Diagnosis

The diagnosis is suspected based on the characteristic combination of poikiloderma, congenital neutropenia, and recurrent infections. Genetic testing of *USB1* should be done as confirmation [98]. Two disorders that are important to consider in the differential diagnosis of PN are dyskeratosis congenita and Rothmund-Thomson syndrome [96, 98].

7.2.10.4 Treatment

Management of PN consists of symptomatic and supportive treatment. Intensive topical therapy of the skin lesions and strict sun-protective measures are required. G-CSF therapy can be used to increase neutrophil counts, but it is unclear if it significantly reduces the frequency of infections (see also Sect. 7.2.1) [98, 99].

7.2.11 HYOU1 Deficiency

7.2.11.1 Etiology

HYOU1 is a chaperone protein that localizes to both the ER and mitochondria. It plays a role in cellular stress responses, including oxidative stress and ER stress with the activation of the UPR (see also Sect. 7.2.2) [100]. Currently, only one patient with HYOU1 deficiency, caused by biallelic mutations in *HYOU1* (OMIM *601746) at chromosome 11q23.3, has been reported [100]. HYOU1 deficiency is associated with disruption of the UPR, mitochondrial function, and expression of proteins involved in oxidative metabolism [100].

7.2.11.2 Clinical Features

HYOU1 deficiency is characterized by a combined immunodeficiency and an impaired glucose metabolism. The patient presented in infancy with recurrent bacterial infections of skin, mucous membranes, and respiratory tract, recurrent herpetic gingivostomatitis, recurrent episodes of stress-induced hypoglycemia, and growth failure. At adult age, the subject suffered from herpetic encephalitis, recurrent condylomata, and relapsing Takayasu arteritis [100]. Blood analysis revealed severe persistent neutropenia, anemia, fluctuating thrombocytopenia, B cell and dendritic cell deficiency, and absent antibody responses to polysaccharide antigens. Bone marrow examination showed a maturation arrest in myeloid progenitor cells [100].

7.2.11.3 Diagnosis

If HYOU1 deficiency is suspected, genetic analysis is necessary to establish the diagnosis [100].

7.2.11.4 Treatment

G-CSF therapy may be used in the treatment of neutropenia (see also Sect. 7.2.1). Immunoglobulin replacement therapy may be indicated for polysaccharide antibody deficiency. Inflammatory complications may be treated with corticosteroids and/or steroid-sparing immunosuppressive drugs such as rituximab. Maintenance immunosuppression may be considered in case of frequent relapses [100]. Additional patients will need to be identified to determine the full phenotypical spectrum and most appropriate management.

7.3 Defects in Neutrophil Migration

7.3.1 Leukocyte Adhesion Deficiency

Leukocyte adhesion deficiency (LAD) is caused by a defect in the adhesion of leukocytes (especially neutrophils) to endothelial cells, which severely impairs leukocyte migration from the bloodstream into infected or inflamed tissues. Currently, three subtypes of LAD have been defined (i.e., LAD-I, -II and -III). All LAD patients present with neutrophilia, severe bacterial infections, and absent pus formation. A delayed separation of the umbilical cord is seen in patients with severe disease. In addition, LAD-II patients demonstrate developmental problems and the so-called Bombay (hh) blood group, and LAD-III patients have an increased bleeding tendency due to platelet dysfunction [101].

The cascade of leukocyte-endothelial adhesion during inflammation is briefly reviewed, followed by a detailed discussion of the three LAD subtypes.

7.3.1.1 The Leukocyte Adhesion Cascade

During inflammation, chemokines and lipid chemoattractants are generated from the inflamed tissue, local complement activation, and/or the pathogens themselves. These chemoattractants diffuse into the local blood vessels forming a gradient along

the site of inflammation, which is used to recruit leukocytes in a process called chemotaxis (Fig. 7.1). To enter the affected tissue, leukocytes need to traverse the endothelium lining the vessel walls. This is achieved by a complex interplay between adhesion molecules expressed on leukocytes and endothelial cells, historically divided into three steps (Fig. 7.6) [102, 103].

The first step is mediated by the adhesion molecules L-selectin (CD62L) on leukocytes and P-selectin (CD62P) and E-selectin (CD62E) on endothelial cells. The low-avidity interaction of selectins with their corresponding ligands on the opposite cells causes the leukocytes to slow down and begin rolling along the blood vessel wall (Fig. 7.6). Selectin ligands are only functional when glycosylated correctly by fucosyltransferase VII and a sialyltransferase. In particular, the sialyl Lewis^X (sLeX,

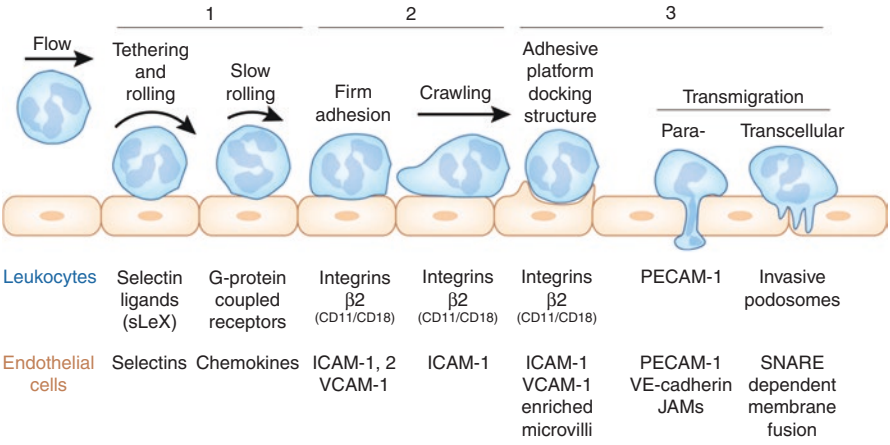


Fig. 7.6 *Leukocyte adhesion and extravasation.* The classic view of leukocyte interactions with activated endothelium is a three-step process. Step 1 entails tethering and rolling involving selectins. Proinflammatory cytokines induce the expression of endothelial selectins, able to interact with sLeX(CD15a)-carrying selectin ligands on leukocytes. Selectin engagement mediates rolling and, together with chemokines induced on the endothelial surface, initiates the "inside-out" activation of leukocyte integrins (mainly β_2 integrins, also called CD11/CD18 integrins). The interaction of partly activated integrins first leads to leukocytes slow rolling on endothelial cells. Step 2 entails firm attachment via integrins. The level of intracellular calcium rises, leading to full integrin activation and firm arrest on endothelial cells. Chemokines trigger the polarization of leukocytes, with the formation of leading and trailing edges. The activation of integrins at the leading edge promotes intravascular crawling on the luminal surface to the point of transmigration. Two ways of transendothelial migration or diapedesis can be distinguished. As shown in step 3, the paracellular migration through endothelial junctions involves homotypic PECAM-1 and JAM-A interactions, resulting in unzipping of the endothelial cell junctions. In the transcellular migration, high density of ICAM-1 and VCAM-1 on specialized docking structures (migratory cup) captures crawling leukocytes and facilitates their way through the endothelial cells. *CD* Cluster of differentiation, *ICAM* Intercellular adhesion molecule, *JAMs* Junctional adhesion molecules, *PECAM* Platelet/endothelial-cell adhesion molecule, *sLeX* sialyl Lewis^X carbohydrate group, *SNARE* Soluble N-ethylmaleimide-sensitive factor attachment protein receptor, *VCAM* Vascular cell-adhesion molecule, *VE-cadherin* Vascular endothelial-cadherin. (Modified with permission from reference [102])

also known as CD15a) tetrasaccharide group serves as a minimal recognition motif for all selectins [103].

Step two entails firm adhesion dependent on the interaction between (mainly β_2) integrins on leukocytes, such as lymphocyte function-associated antigen-1 (LFA-1, or $\alpha_L\beta_2$-integrin, or CD11a/CD18), and their ligands on endothelial cells, such as intercellular adhesion molecule-1 (ICAM-1) (Fig. 7.6). Leukocyte integrins are capable of transducing bidirectional signaling across the plasma membrane. When leukocytes are activated by endothelial-derived selectins and chemokines (step 1), intracellular signaling subsequently induces conformational changes in the integrins that increase their ligand affinity (referred to as inside-out signaling). Clustering of integrins further enhances ligand avidity. Interaction of integrins with their ligands activates downstream signaling cascades involved in adhesion stabilization, cell motility, proliferation, and apoptosis (outside-in signaling). Talin and kindlin-3 are two adaptor proteins that bind to the cytoplasmic tail of integrins and play an important role in the inside-out and outside-in signaling cascades [101–103].

After firm adhesion, leukocytes are guided by chemotactic gradients to crawl along the endothelial surface to the preferred site of transmigration (Fig. 7.6). During leukocyte crawling, continuous modulation of the actin cytoskeleton and the ligand-binding capacity of integrins are essential for the formation of leukocyte leading and trailing edges [103].

In the third and last step, the leukocytes move in an ameboid fashion across the endothelial barrier to enter the inflamed tissue by a process known as diapedesis or transmigration. Leukocytes usually migrate through endothelial-cell junctions (paracellular route), or in some cases through the endothelial cell itself (transcellular route) (Fig. 7.6) [103].

7.3.1.2 Leukocyte Adhesion Deficiency Type I

Etiology

Leukocyte adhesion deficiency type I (LAD-I) is a rare autosomal recessive disorder caused by mutations in the gene encoding CD18, the common β chain of the leukocyte β_2 integrins. The β_2 integrin family includes CD11a/CD18 (LFA-1; $\alpha_L\beta_2$), CD11b/CD18 (macrophage antigen-1 (Mac-1); complement receptor 3 (CR3); $\alpha_M\beta_2$), CD11c/CD18 (glycoprotein 150/95 (gp150/95); $\alpha_X\beta_2$), and CD11d/CD18 ($\alpha_D\beta_2$; only expressed on macrophages). Both the α and β chain of the heterodimeric integrins are required for successful surface expression. Therefore, defects in CD18 result in a deficiency of all β_2 integrin members; impairing migration and chemotaxis of all leukocytes (Fig. 7.6) [104]. In lymphocytes, however, it is believed that other adhesion proteins (e.g., β_1 integrins) can at least partially compensate for the loss of β_2 integrins [103, 105]. Since CD11b/CD18 (CR3) is the predominant phagocyte receptor for the complement factor C3b, phagocytosis and subsequent intracellular killing of C3b-opsonized pathogens are also defective in LAD-I patients [106].

CD18 is encoded by the *ITGB2* gene (OMIM *600065), located on chromosome 21q22.3. Most mutations lead to markedly decreased to absent protein levels, but some mutations impair protein function without affecting expression. To date, a few

hundred patients have been reported. The estimated incidence is one in a million live births per year worldwide [106–108].

Clinical Features

The severity of the clinical presentation in patients with LAD-I is correlated with the degree of CD18 deficiency. LAD-I patients with less than 2% residual CD18 expression develop a severe phenotype characterized by recurrent, life-threating infections from birth onward, and a very high mortality rate (60–75%) in the first 2 years of life in the absence of allogeneic HSCT. In contrast, LAD-I patients with 2–30% residual CD18 expression develop a moderate form of the disease with less severe infections and survival into adulthood [106].

The hallmark features of LAD-I are persistent neutrophilia, recurrent bacterial infections mostly of the skin and mucosal surfaces, and absence of pus formation due to impaired neutrophil migration into infected tissues. Classic presentations are recurrent cold skin abscesses, omphalitis with delayed separation of the umbilical cord (i.e., later than 3 weeks), impaired wound healing, perirectal cellulitis, severe gingivitis, ulcerative stomatitis, and septicemia. Skin and soft tissue infections can become necrotizing and progress to ulceration. Infections of the respiratory and gastrointestinal tracts also occur. The major pathogens associated with LAD-I are *Staphylococcus aureus* and enterobacteriaceae. Some cases may present with fungal infections, particularly by *Candida albicans* and *Aspergillus* spp. [101, 106]. In between infections, patients often have a moderate neutrophilia. During infection, a very high white blood cell count (up to 150,000/mm^3), with a marked neutrophilia and mild lymphocytosis, is usually observed in the lab workup, due to the impaired extravasation of leukocyte into tissues [106].

Among LAD-I patients who survive early childhood, chronic periodontitis is a serious complication. By adolescent age, many patients will have lost their permanent teeth [109, 110]. Recently, dysregulation of the IL-23/IL-17 axis was reported to play a key role in the chronic inflammation and bone loss in LAD-I-associated periodontitis. The authors postulated that in the absence of tissue neutrophils, oral microbes are allowed to endure in the gingiva were they continually trigger an inflammatory response driven by IL-23 and IL-17 [111].

A handful of LAD-I patients were reported with autoimmune manifestations, such as Crohn disease, autoimmune cytopenia, and juvenile idiopathic arthritis [112].

Of note, the mean time of umbilical cord separation is between 7 and 15 days after birth, though cord separation at 3 weeks or later is seen in up to 10% of healthy infants. An infant with delayed cord separation, who is healthy and has a normal neutrophil count, is very unlikely to have LAD-I, and alternative diagnoses (e.g., urachal cyst) should be considered [113, 114].

Diagnosis

The diagnosis of LAD-I should be suspected in any child with recurrent non-purulent infections of soft tissues and persistent leukocytosis. Since LAD-I is in most cases a life-threatening condition, rapid identification of patients is essential [106].

The diagnosis is made by flow cytometric measurement of CD18 and CD11a/b/c expression on the surface of neutrophils, using commercially available antibodies. The sole assessment of CD18 expression is discouraged as this may be misleading (e.g., normal expression of nonfunctional CD18). A recent study showed that CD11a expression was consistently low in all LAD-I patients regardless of their CD18 levels. Therefore, a combined flow cytometric analysis of CD18 and CD11a expression is recommended [115]. In most cases, flow cytometry can differentiate between severe (<2% CD18) and moderate (2–30% CD18) forms of LAD-I. Genetic analysis of *ITGB2* can confirm the diagnosis and provide prognostic information [106].

In vitro chemotaxis assays, which measure neutrophil migration across a chemoattractant gradient, are no longer used in a routine setting because they are highly prone to artifacts arising from the neutrophil purification procedures and the assay protocol itself [116].

Treatment

Treatment of LAD-I depends on the clinical severity of the phenotype. Patients with a moderate form of LAD-I may survive into adulthood with conservative therapy. They should be advised in the general preventative health measures discussed in Sect. 7.1.3. In case of acute infections, early recognition and aggressive treatment with systemic antibiotics are required. Prophylactic antibiotics, such as TMP-SMX, are sometimes used (see also Sect. 7.1.3) [101, 106]. Ustekinumab, a monoclonal antibody directed against the common p40 subunit of IL-12 and IL-23, was able to resolve refractory periodontitis and a nonhealing sacral ulcer in a 19-year-old male with moderate LAD-I. This supports the hypothesis of a dysregulated IL-17/IL-23 axis in LAD-I-associated chronic inflammation (see section on "Clinical Features") [111]. G-CSF therapy is not useful in LAD patients, as this does not overcome the leukocyte adhesion defect.

In patients with severe LAD-I and in those with moderate disease who do not respond to conservative therapy, allogeneic HSCT is the treatment of choice. HSCT is the only curative treatment for LAD-I and has a good success rate. In one series of 36 patients, the overall survival rate was 75% at a median follow-up of 5 years posttransplant [117]. Both reduced-intensity and myeloablative conditioning regimens are used. HLA-matched (un)related donors are preferred over haploidentical donors [106, 117, 118]. Gene therapy for LAD-I is being investigated but has so far been unsuccessful [119].

7.3.1.3 Leukocyte Adhesion Deficiency Type II

Etiology

Leukocyte adhesion deficiency type II (LAD-II) is a very rare disorder caused by a defect in the fucosylation (i.e., a type of glycosylation) of various cell surface glycoproteins, including selectin ligands [120–122]. Approximately 10 cases have been published to date. In LAD-II patients, selectin ligands on leukocytes lack the

fucosylated sLeX (CD15a) motif and are unable to bind to their corresponding selectin receptors on endothelial cells (Fig. 7.6). Consequently, selectin-mediated rolling of leukocytes along endothelial cells is impaired, severely impeding leukocyte extravasation at sites of inflammation. However, integrin-mediated adhesion and transmigration is intact and can achieve some leukocyte migration to inflamed tissues (Fig. 7.6) [101, 123]. Besides leukocyte adhesion, fucosylation is also important for other functions such as neurological development and the formation of several blood group antigens [120–122].

The fucosylation defect in LAD-II is caused by a deficiency in the Golgi guanosine diphosphate (GDP)-fucose transport protein (GFTP), encoded by *SLC35C1* (OMIM *605881) at chromosome 11p11.2. Mutations in *SLC35C1* are inherited in an autosomal recessive manner. Because the molecular defect is situated in the glycosylation metabolism, LAD-II was also classified as congenital disorder of glycosylation (CDG) type IIc [124, 125].

Clinical Features

The infectious phenotype of LAD-II is similar to that of LAD-I, but is generally not as severe since there is still some level of neutrophil defense in tissues. Most patients present in the first years of life with persistent neutrophilia and recurrent bacterial infections of the skin, mucus membranes, and/or lungs. Pus formation at the site of infection is impaired. Chronic periodontitis is seen at older age. In contrast to LAD-I, skin and soft tissue infections do not become necrotic or progress to ulceration, and separation of the umbilical cord is not delayed. In many LAD-II patients, the frequency of infections and the neutrophil counts decrease significantly with aging [101, 120–122].

Furthermore, red blood cells of LAD-II patients are deficient of the fucosylated A, B, H, and Lewis blood group antigens. The absence of A, B, and H antigens on erythrocytes is called the Bombay (hh) blood group, which is very rare [121, 122]. Additional features observed in patients with LAD-II include intrauterine growth retardation, failure to thrive, short stature, facial dysmorphism, microcephaly, mental retardation, developmental delay, convulsions, cerebral atrophy, and autism [122, 126].

It appears that the dominant clinical manifestations in LAD-II patients shift with age, from immunodeficiency and recurrent infections in early childhood to the metabolic consequences (i.e., developmental delay, growth retardation) at later age [122].

Diagnosis

LAD-II should be considered in patients with a combination of recurrent mild-moderate infections, persistent leukocytosis, the Bombay (hh) blood group, and developmental delay. The diagnosis is made by use of flow cytometry, demonstrating the absence of sLeX (CD15a) expression on leukocytes, with normal CD11/CD18 expression. Mutation analysis of *SLC35C1* should be done to genetically confirm the diagnosis [101, 122].

Treatment

Similar to LAD-I, general preventative health measures and prompt recognition and treatment of acute infections are recommended. Young children with frequent infections may benefit from prophylactic antibiotics (see also Sect. 7.1.3) [101, 122].

A trial of high-dose oral supplementation of L-fucose is recommended in all LAD-II patients. Fucose supplementation was shown to be successful in some LAD-II patients, evidenced by increased expression of sLeX, normalization of neutrophil counts, reduced infections, and even improvement in psychomotor functions, if supplementation is started before irreversible neurological damage occurs [127]. However, in other patients, fucose supplementation only partially resolved the clinical symptoms or had no effect at all. This variable effect is probably due to differences in the underlying mutations and biochemical mechanisms. For example, administration of excess fucose could overcome reduced substrate binding of the GDP-fucose transporter, but is futile in case of absent expression of the transport protein [127–130].

In contrast to LAD-I, most patients with LAD-II survive the first years of life using conservative therapy. Thereafter, their prognosis is mainly determined by the neurometabolic complications, which cannot be remedied by HSCT [101].

7.3.1.4 Leukocyte Adhesion Deficiency Type III

Etiology

Leukocyte adhesion deficiency type III (LAD-II) is a very rare, autosomal recessive disorder caused by a deficiency in kindlin-3, which is encoded by *FERMT3* (OMIM *607901) at chromosome 11q13.1 [131–133]. Approximately 35 cases have been reported so far. Kindlin-3 binds to the cytoplasmic domain of integrins in all blood cells and regulates integrin activation and function (see also Sect. 7.3.1.1) [134]. Since β1/β2 integrin signaling is imperative for the migration of leukocytes into infected tissues (Fig. 7.6), LAD-III is characterized by a LAD-I-like immunodeficiency (see Sect. 7.3.1.2) [135]. Beside the adhesion defect, the activation of natural killer cells is also impaired in LAD-III [136]. In addition, patients with LAD-III have a severe bleeding tendency resembling Glanzmann thrombasthenia (a β3 integrin-related disorder), indicating the importance of kindlin-3 in β3 integrin signaling during platelet aggregation [137]. This combination of leukocyte and platelet defects was initially termed LAD-I/variant (LAD-I/v), but was later renamed LAD-III [135].

Earlier studies suggested that mutations in *RASGRP2*, encoding CalDAG-GEF1 (a guanine nucleotide exchange factor for RAP1, involved in integrin activation), were responsible for the development of LAD-III [134, 138, 139]. However, it was later shown that CalDAG-GEF1 deficiency in humans only impairs hemostasis and does not affect leukocyte adhesion [133, 140, 141].

Clinical Features

The infectious phenotype of LAD-III is very similar to that of LAD-I, although LAD-III-associated infections are not often life-threatening. Patients with LAD-III suffer from severe, recurrent, bacterial infections, mainly of the skin, mucosal

surfaces, and lungs. There is no pus formation. Wound healing is impaired, resulting in necrotic lesions and ulceration. Infants present with delayed separation of the umbilical cord (i.e., later than 3 weeks), which can be complicated by omphalitis. Chronic periodontitis is usually seen later in life. Fungal infections have been described in some patients. As in LAD-I, there is a persistent neutrophilia that augments further during acute infections [101, 142, 143].

Furthermore, LAD-III patients have a severe Glanzmann-like bleeding tendency due to dysfunctional platelet aggregation, which is usually apparent from birth. Common bleeding complications include intracranial hemorrhage, petechial/purpuric lesions of the skin and mucosa, epistaxis, gingival bleeding, gastrointestinal bleeding, pulmonary bleeding, and hematuria [137, 142].

Some LAD-III patients also have osteopetrosis-like bone defects. This is probably explained by an impaired integrin-mediated activation of osteoclasts, the cells required for bone resorption. However, it is unclear why other patients to not develop osteopetrosis [144].

Diagnosis

The diagnosis should be suspected in patients with recurrent infections, delayed umbilical cord separation, severe bleeding tendency, and marked leukocytosis. Unlike for LAD-I and -II, there is no routine immunological assay to confirm the diagnosis of LAD-III. Testing for impaired integrin activation is only performed in few expert laboratories. CD11 and CD18 expression on leukocytes is normal (see also Sect. 7.3.1.2). The diagnosis should be confirmed by genetic analysis of *FERMT3* [142].

Treatment

Regarding infections, patients with LAD-II require antibiotic prophylaxis (e.g., TMP-SMX) with or without antifungal prophylaxis (e.g., itraconazole). Acute infections should be treated promptly with systemic antimicrobial drugs (see also Sect. 7.1.3) [145].

Regarding the bleeding tendency, the majority of patients receive repeated transfusions with erythrocytes and platelets. The transfusion need differs between patients, but may be extremely high [143]. It was recently shown that the use of recombinant factor VIIa (rFVIIa) in a child with LAD-III could effectively treat and prevent bleeding. In this patient, rFVIIa was administrated as first-line treatment for severe bleeding and as preventative treatment in high-risk situations. Long-term prophylaxis with tranexamic acid has been occasionally used in LAD-III patients and was also given to the patient treated with rFVIIa [145].

Allogeneic HSCT is the only curative treatment for LAD-III. The disease has a high mortality rate in childhood, as a result of bleeding or infectious complications. It is estimated that about half of non-transplanted patients survive into adulthood. HSCT has, therefore, been recommended to be performed as early in life as possible [146]. However, a recent study showed that the mortality rate among transplanted patients was 22% and could be directly attributed to HSCT-associated complications. Overall, severe complications were seen in 48% of patients who underwent HSCT [145].

7.3.2 Dominant-Negative Rac2 Deficiency

7.3.2.1 Etiology

Ras-related C3 botulinum toxin substrate 2 (Rac2) is a hematopoietic-specific Rho-GTPase. Rho GTPases are a family of small GTP-binding proteins that act as molecular switches controlling various signal transduction pathways. In neutrophils, Rac2 plays a role in multiple important functions, including regulation of actin cytoskeletal remodeling; release of antimicrobial peptides, proteolytic enzymes, adhesion molecules, and chemotactic receptors from neutrophil granules (i.e., degranulation); phagocytosis; generation of ROS during the respiratory burst; and expression of L-selectin (CD62L) involved in selectin-mediated leukocyte rolling (see Sect. 7.3.1.1) (Fig. 7.7) [147, 148].

Rac2 deficiency, also called neutrophil immunodeficiency syndrome, is caused by heterozygous dominant-negative mutations in *RAC2* (OMIM *602049), located on chromosome 22q13.1 [149, 150]. Currently, only 4 cases have been reported in literature. The disorder is characterized by a global neutrophil dysfunction, combining features of LAD (see Sect. 7.3.1), chronic granulomatous disease (see Sect. 7.4.1), specific granule deficiency (see Sect. 7.5.1), and β-actin deficiency (see Sect. 7.3.3) [150–153].

Of note, a homozygous *RAC2* mutation that completely abolished protein expression was identified in two siblings diagnosed with common variable immunodeficiency, showing progressive B cell lymphopenia and hypogammaglobulinemia, urticaria, and auto-immunity. In contrast to patients with dominant-negative Rac2 deficiency, these siblings had considerably less severe defects in neutrophil function. It is possible that dominant-negative Rac2 mutants also compromise the function of Rac1, the other major Rac GTPase in neutrophils, resulting in a more profound neutrophil dysfunction [154, 155]. Finally, heterozygous gain-of-function mutations in *RAC2* were recently described to cause a combined T and B cell immunodeficiency, with neutrophils showing excessive ROS production but impaired N-formylmethionyl-leucyl-phenylalanine (fMLP)-directed chemotaxis [156, 157].

7.3.2.2 Clinical Features

Patients with Rac2 deficiency present in infancy with a severe clinical picture reminiscent of LAD-I: delayed separation of the umbilical cord, omphalitis, severe recurrent infections of the skin and mucus membranes, perirectal and periumbilical abscesses, poor wound healing, and lack of pus formation at sites of infection. Infections can be life-threatening. Importantly, wound biopsies of Rac2-deficient patients show numerous neutrophils, whereas those of LAD-I patients are completely devoid of neutrophils. Upon laboratory workup, patients demonstrate neutrophilia, mild to severe lymphopenia (mostly T lymphopenia), mild to moderate hypogammaglobulinemia, an impaired chemotaxis, and a decreased respiratory burst [150–153].

One patient was picked up during newborn SCID screening, because of very low TREC numbers. The latter was discrepant with the relatively mild lymphopenia and normal in vitro T cell proliferation in this patient. The cause of the low TREC levels

is unclear, but it was suggested to involve impairment of integrin-dependent functions of thymocytes [153].

7.3.2.3 Diagnosis

Neutrophils of Rac2-deficient patients show impaired responses upon stimulation with the chemoattractant fMLP, such as a decreased chemotaxis, decreased degranulation, deficient polarization, and a reduced production of ROS (respiratory burst) (Fig. 7.7) [151]. These assays, however, are only available in a few specialized laboratories. Genetic analysis of *RAC2* should be done to establish the diagnosis.

LAD and other neutrophil migration disorders should be considered in the differential diagnosis. LAD can be excluded by normal expression of CD11, CD18, and CD15a on leukocytes and the presence of neutrophils on wound biopsies, though LAD-III may be challenging to rule out without genetic testing (see Sect. 7.3.1).

7.3.2.4 Treatment

Similar to LAD, patients with dominant-negative Rac2 deficiency require antibiotic prophylaxis as well as aggressive treatment of acute infections (see also Sect. 7.1.3). Allogeneic HSCT, the only curative therapy, should be performed in infancy if possible [150–153].

7.3.3 β-Actin Deficiency

7.3.3.1 Etiology

The actin cytoskeleton is a dynamic network of polymeric actin filaments and actin-associated proteins and is involved in cell structural integrity, mitosis, motility, and intracellular signaling. β-actin deficiency is caused by a heterozygous mutation in *ACTB* (OMIM *102630), the gene encoding cytoplasmic β-actin, located at chromosome 7p22.1. The disorder comprises a defect in actin polymerization of neutrophils, resulting in impaired chemotaxis and phagocytosis. Only a single patient has been reported so far. In the original report, it was shown that binding of the mutant β-actin to the actin-regulatory protein profilin was decreased [158]. However, this could not be confirmed by other studies [159, 160].

Heterozygous *ACTB* mutations have also been identified as the culprit of Baraitser-Winter syndrome (BRWS), a condition characterized by a distinctive facial appearance, structural brain abnormalities, developmental delay, ocular colobomata, short stature, and abnormalities of the heart, urinary tract, and/or ears. However, patients with BRWS do not typically present with recurrent infections [161]. On the other hand, the patient with β-actin deficiency and neutrophil dysfunction did not have the characteristic BRWS appearance [158]. The broad phenotypic variability associated with heterozygous *ACTB* mutations could be explained by the fact that different mutations affect different functions of the actin structure. In a recent review, *ACTB* mutations were divided into two groups: those causing an abnormality in the dynamics of actin polymerization-depolymerization (associated

with BRWS) and those affecting the regulation of actin polymerization (associated with a more pleiotropic phenotype) [161].

7.3.3.2 Clinical Features

The patient presented with recurrent pyogenic infections, recurrent stomatitis, mental retardation, short stature, photosensitivity, polyarthralgia, cardiomegaly, hepatomegaly, hypothyroidism, leukopenia, and thrombocytopenia. The patient died of infectious complications at the age of 15 years [158].

7.3.3.3 Diagnosis

If the diagnosis is suspected, neutrophil chemotaxis and actin polymerization upon in vitro stimulation with fMLP can be assessed (Fig. 7.7) [158]. Since these tests are prone to artifacts and only performed in few specialized laboratories [116], a definite diagnosis should be obtained by mutation analysis of the *ACTB* gene [158]. LAD (see Sect. 7.3.1) and other neutrophil migration disorders should be excluded.

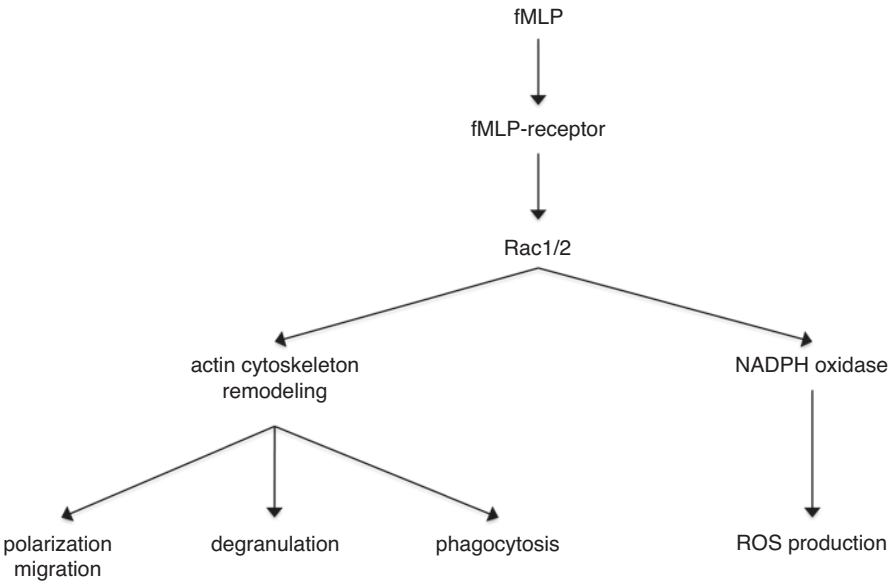


Fig. 7.7 *Simplified scheme of the function of Rac in neutrophils.* Neutrophil activation, for example, by the chemoattractant N-formylmethionyl-leucyl-phenylalanine (fMLP), results in cycling of Rac from the inactive GDP-bound form to the active GTP-bound form. Activated Rac translocates to the membrane to participate in the formation of the NADPH oxidase enzyme complex, with subsequent production of reactive oxygen species (ROS). Activated Rac also regulates rearrangements of the actin cytoskeleton, which is important for polarization, migration, degranulation, and phagocytosis. *NADPH* nicotinamide adenine dinucleotide phosphate; Rac, Ras-related C3 botulinum toxin substrate

7.3.3.4 Treatment

Management is similar to that of other leukocyte migration defects, encompassing antimicrobial prophylaxis and aggressive treatment of acute infections (see also Sect. 7.1.3). Early allogeneic HSCT is recommended, though this will likely not correct the non-hematological manifestations [162].

7.3.4 WDR1 Deficiency

7.3.4.1 Etiology

As discussed above, dynamic remodeling of the actin cytoskeleton is essential for normal neutrophil function and is regulated by various actin-associated proteins. WDR1 or actin-interacting protein 1 (Aip1), encoded by the gene *WDR1* (OMIM *604734) at chromosome 4p16.1, plays a key role in the depolymerization of actin filaments via cofilin, thereby regulating actin turnover. Next to neutrophils, WDR1 is also essential for the normal function of monocytes, megakaryocytes, and T and B lymphocytes. Biallelic loss-of-function mutations in *WDR1* have been recently described as the cause of three distinct immunological phenotypes: severe neutrophil dysfunction, auto-inflammatory disease, and combined immunodeficiency [163–165].

7.3.4.2 Clinical Features

WDR1 deficiency was first described in 2016 in four patients with a severe neutrophil dysfunction [163]. They had mild neutropenia, recurrent severe pyogenic infections, poor wound healing, and severe chronic stomatitis with oral stenosis impeding the opening of the mouth and oral feeding. The neutrophils of these patients showed distinctive nuclear lobe herniations, impaired migration and chemotaxis, and an increased respiratory burst [163].

Since the first report, eight additional cases with WDR1 deficiency have been published. Remarkably, two of these patients presented with a therapy-resistant autoinflammatory disease with periodic episodes of fever, elevated serum levels of IL-18, leukocytosis, and macrothrombocytopenia [164]. Similar to the first patient series, they also suffered from severe recurrent bacterial and fungal infections, severe chronic stomatitis with oral stenosis, and recurrent perianal ulceration. On in vitro analysis, however, the neutrophil defects appeared relatively mild with no significant alterations in migratory, phagocytic, or respiratory burst capacity [164].

The latest case series on six WDR1-deficient patients reports on a combined immunodeficiency phenotype encompassing defects in both myeloid and lymphoid cells. Similar to the previously published patients, they presented with severe recurrent infections involving the skin, mucosal surfaces, and the respiratory tract, skin ulceration, severe chronic stomatitis, and mental retardation. Neutrophils showed impaired migration and chemotaxis. In addition, there were severe aberrations in B cell development and differentiation, T cell activation, and the assembly of B and T cell immunological synapses [165].

Although the full phenotypic spectrum of WDR1 deficiency is still being unraveled, it is consistently found to be a severe disorder with a high risk of mortality. So far, 2 of the 12 reported patients succumbed to infectious or inflammatory complications before reaching adulthood (8 years and 14 years, respectively) [163, 164].

7.3.4.3 Diagnosis

Since the clinical and laboratory findings are quite variable, the diagnosis is confirmed by genetic testing of the *WDR1* gene [163–165]. LAD (see Sect. 7.3.1) and other neutrophil migration disorders should be excluded.

7.3.4.4 Treatment

Aggressive management with prophylactic and therapeutic antimicrobial drugs is required (see also Sect. 7.1.3) [163–165]. Patient with severe defects in the B cell compartment are likely to benefit from immunoglobulin replacement therapy [165]. Symptomatic treatment of autoinflammatory manifestations is very challenging, because corticosteroids, anakinra (IL-1 blockade), and other immunosuppressive drugs only seem to have a limited effect [164]. Given the severity of the condition, early allogeneic HSCT should be considered. So far, two patients have been successfully transplanted [163, 164].

7.3.5 MKL1 Deficiency

7.3.5.1 Etiology

Megakaryoblastic leukemia 1 (MKL1), also called myocardin-related transcription factor A (MRTFA), was first identified as part of an oncogenic fusion protein in acute megakaryocytic leukemia [166]. MKL1 is widely expressed and functions as a coactivator of the transcription factor serum response factor (SRF). In resting state, MLK1 is retained in the cytoplasm by monomeric actin units. Upon cellular stimulation, actin monomers are incorporated in polymeric actin filaments, thereby releasing MKL1. MKL1 subsequently translocates to the nucleus and interacts with SRF to induce the expression of cytoskeletal genes, including actin and actin-regulatory genes. In turn, increased actin expression provides feedback regulation by binding MKL1. Hence, the actin-MKL1-SRF loop is essential for the dynamic remodeling of the actin cytoskeleton [167]. MKL1 deficiency, due to biallelic mutations in *MKL1* (OMIM *606078) at chromosome 22q13.1–q13.2, has so far only been reported in one patient [168]. MKL1 deficiency results in reduced intracellular actin levels and severely disrupted cytoskeletal rearrangement in fibroblasts, lymphocytes, and myeloid lineage immune cells. Nonetheless, the phenotype of the reported patient is dominated by features of neutrophil defects, without manifesting lymphocyte defects. This finding was supported by in vitro data, which showed reduced phagocytosis and almost completely absent migration of the patient's neutrophils [168].

7.3.5.2 Clinical Features

The patient presented with severe to life-threating bacterial infections from the first year of life and was particularly prone to bacterial skin infections that healed poorly. BCG vaccination, administrated in the neonatal period, caused a large ulcerating abscess. Blood analysis revealed intermittent mild thrombocytopenia. Lymphocyte subsets, serum immunoglobulin levels, ANCs, and the respiratory burst all were within normal ranges [168].

7.3.5.3 Diagnosis

Patient-derived neutrophils show a severe migratory defect upon in vitro stimulation with fMLP. Genetic analysis of *MKL1* is required to confirm the diagnosis [168]. LAD (see Sect. 7.3.1) and other neutrophil migration disorders should be excluded.

7.3.5.4 Treatment

Maintenance treatment with prophylactic antibiotics and immunoglobulin replacement therapy can be considered. Acute infections should be treated aggressively with intravenous antibiotics followed by long-term oral therapy (see also Sect. 7.1.3) [168]. More patients need to be identified to fully uncover the phenotype and optimal management of this disease.

7.3.6 Papillon-Lefèvre Syndrome

7.3.6.1 Etiology

Papillon-Lefèvre syndrome (PLS) is a rare, autosomal recessive disorder characterized by palmoplantar hyperkeratosis and premature loss of both the deciduous (or primary) and permanent teeth. Worldwide, the prevalence of PLS is about 1 to 4 per million. The disorder is caused by loss-of-function mutations in the gene *CTSC* (OMIM, *602365) at chromosome 11q14.2, which encodes for cathepsin C [169, 170]. Cathepsin C is a lysosomal protease that functions by cleaving the N-terminal dipeptide, and is highly expressed in epithelial tissues, myeloid cells, and lymphocytes. In particular, cathepsin C plays a role in epidermal differentiation and desquamation and is important for the activation of granule serine proteases in phagocytes [171]. In PLS, mutations in *CTSC* result in (almost) complete absence of protein activity. The reduced chemotactic and phagocytic function of neutrophils is believed to impair local defense mechanisms of the gingiva against oral bacteria, resulting in severe gingivitis, chronic periodontal inflammation, and subsequent loss of dentition [172, 173]. Note most PLS patients do not present with a profound immunodeficiency, suggesting other mechanisms can compensate for the cathepsin C deficiency [171].

7.3.6.2 Clinical Features

Shortly after the eruption of the deciduous teeth, patients with PLS develop severe gingivitis that rapidly progresses to aggressive chronic periodontitis [171, 174]. The gingival sulci of PLS patients contain bacteria that are also found in neutropenic patients, such as *Actinobacillus actinomycetemcomitans*, *Fusobacterium*

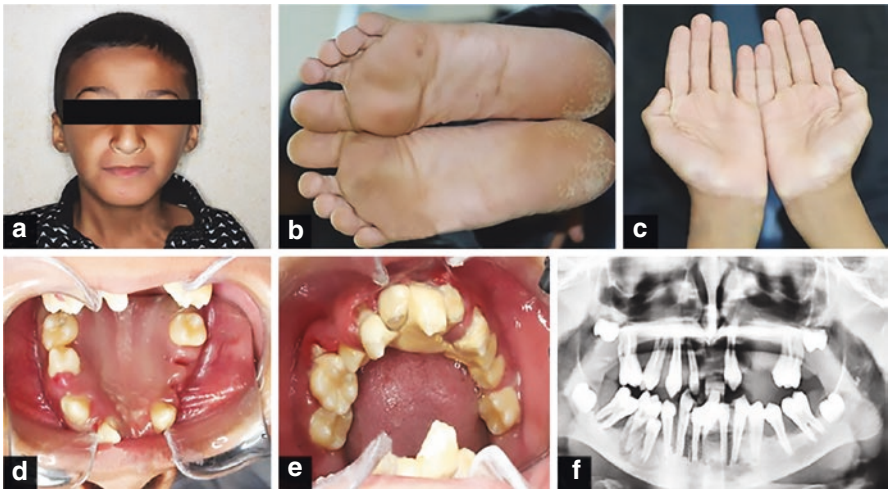


Fig. 7.8 *Characteristic clinical features of Papillon-Lefèvre syndrome.* (**a**) An 11-year-old male patient; (**b**) plantar hyperkeratosis; (**c**) palmar hyperkeratosis; (**d**) maxillary arch teeth; (**e**) mandibular arch teeth; (**f**) orthopantomogram showing loss of multiple teeth and alveolar bone loss around many present teeth. (Reprinted with permission from reference [174])

nucleatum, *Eikenella corrodens*, *Capnocytophaga* species, and *Aggregatibacter actinomycetemcomitans*. The chronic periodontal inflammation results in lysis of alveolar bone, by which the teeth come loose and fall out (Fig. 7.8d–f). In general, the deciduous teeth are lost by the age of 3 to 5 years. After the eruption of the permanent teeth, the periodontitis recurs and patients are usually edentulous by the age of 15 years [171, 175, 176]. Another major feature of PLS is hyperkeratosis of the palms and soles, which appears in the first years of life (Fig. 7.8b,c). Hyperkeratosis may also affect other sites, such as the ankles, knees, elbows, and dorsal side of fingers and toes [171, 174]. In addition, PLS patients have an increased susceptibility to pyogenic infections, especially superficial and deep skin infections, and abscesses of internal organs such as the liver, kidney, and brain. The most common causative pathogen of deep abscesses is *Staphylococcus aureus* [177–179]. Some patients may present with intracranial calcifications, mild mental retardation, and/or hyperhidrosis [171].

7.3.6.3 Diagnosis

The diagnosis is suspected based on the combination of early-onset palmoplantar hyperkeratosis and dental loss. In advanced stages, loss of the alveolar bone can be seen on an orthopantomogram (i.e., dental radiograph) (Fig. 7.8). Neutrophil chemotaxis on in vitro stimulation is impaired. Mutation analysis of *CTSC* gene can confirm the diagnosis [171, 174].

The majority of PLS patients lack urinary cathepsin C activity. Screening of urine in infants with severe gingivitis could identify the disorder at an early stage, at which prompt treatment could help limit periodontitis and loss of teeth [180].

7.3.6.4 Treatment

Patients are treated with antibiotics targeting the above-mentioned oral pathogens (e.g., metronidazole, amoxicillin/clavulanic). Furthermore, meticulous dental care and oral hygiene are pivotal (see also Sect. 7.1.3). If periodontal disease persists during deciduous dentition, all primary teeth should be extracted to preserve the non-erupted permanent teeth. After the eruption of the permanent teeth, moderate periodontitis can be controlled with antibiotics and daily use of an antiseptic mouthwash, but advanced periodontitis necessitates extraction of all teeth [181]. Under proper maintenance care, dental implants can be successfully used in PLS patients [182, 183]. However, peri-implantitis was described in some cases [184].

Hyperkeratotic skin lesions are treated with topical anti-inflammatory and keratolytic agents. Therapy with systemic retinoids has been reported to be effective for both skin lesions and periodontitis, but the success rate is variable and the side effects can be severe [171, 185].

7.3.7 Localized Juvenile Periodontitis

7.3.7.1 Etiology

Localized juvenile periodontitis (LJP) is a form of early-onset aggressive periodontitis characterized by severe periodontitis and alveolar bone resorption in children and adolescents who are otherwise healthy [186]. There is no hyperkeratosis. The periodontal inflammation in LJP is due to impaired immune responses against oral microflora and bacterial plaque formation. The exact prevalence of the disorder is unclear, with estimations varying from 0.1% to 15%. It is more frequently seen in individuals of African ethnicity [187]. The etiology of LJP is unknown, but it is believed to be a genetically heterogeneous disease. Polymorphisms in multiple genes, including formyl peptide receptor 1 (*FPR1*), have been associated with an increased susceptibility to the disorder [188]. Furthermore, a few patients with LJP were found to have mutations in *CTSC*, the disease-causing gene for Papillon-Lefèvre syndrome (see Sect. 7.3.6) [189].

7.3.7.2 Clinical Features

Patients commonly present with severe periodontitis around the time of puberty. Destruction of alveolar bone and subsequent loss of teeth involve the permanent incisors and first molars. Patients also show extensive dental plaques and calculus. The major pathogen in LJP is *Actinobacillus actinomycetemcomitans* [187].

7.3.7.3 Diagnosis

LJP is a clinical diagnosis based on the typical localization of teeth loss and the absence of systemic disease. In many patients, the chemotactic response of neutrophils on stimulation with fMLP is reduced [187, 190].

Note that many qualitative and quantitative congenital phagocyte defects are also associated with severe periodontal disease and should thus be excluded (e.g., congenital neutropenia, LAD, chronic granulomatous disease).

7.3.7.4 Treatment

Treatment of LJP encompasses meticulous dental care and oral hygiene and regular antibiotics to reduce plaque formation (see also Sect. 7.1.3) [187]. The choice of antibiotics depends on the pathogens isolated from cultures of oral samples. Frequently used antibiotics include tetracyclines, metronidazole, combination of metronidazole and amoxicillin, and combination of metronidazole and amoxicillin/clavulanic acid [191]. Affected teeth should be extracted. Periodontal surgery may be required in more severe cases [187].

7.4 Defects in Respiratory Burst

7.4.1 Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is a genetically heterogeneous disorder caused by a defective function of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex. NADPH oxidase initiates the phagocyte respiratory burst pathway leading to the generation of reactive oxygen species (ROS), which are critical in microbial killing (see also Sect. 7.1.1) [192]. Patients with CGD typically present with recurrent, severe to life-threatening bacterial and fungal infections, granuloma formation, and other inflammatory manifestations such as IBD. In the 1950s, this disease was initially termed "fatal granulomatous disease of childhood," reflecting its severity [3, 193]. Prognosis has substantially improved since then, with most patients now living into middle age [194–197]. The estimated incidence of CGD is between 1 in 200,000 and 1 in 250,000 live births per year [194, 196, 198]. There is a male predominance, given that about two thirds of genetic defects are located in the X-encoded subunit of the NAPDH oxidase complex [199].

7.4.1.1 Etiology

Respiratory Burst

The NADPH oxidase enzyme complex consists of six subunits. Five of these subunits are named in accordance to their molecular mass (kDa) with the designation *phox*, for *ph*agocyte *ox*idase. The subunits gp91^{phox} and p22^{phox} are located in the plasma membrane and in the membrane of intracellular granules of phagocytes, forming a heterodimer known as flavocytochrome b_{558}. The other four subunits are found in the cytosol: p47^{phox}, p67^{phox}, p40^{phox}, and the small G-protein Rac (predominantly Rac1 in macrophages and Rac2 in neutrophils; see also Sect. 7.3.2) (Fig. 7.9) [201]. Upon phagocyte activation by phagocytic or inflammatory stimuli, p47^{phox} and p67^{phox} are phosphorylated and bind to each other, followed by complete assembly of the NADPH oxidase complex. Activated NADPH oxidase catalyzes the transfer of an electron from NADPH to molecular oxygen (O_2), leading to the formation of superoxide (O_2^-). Superoxide is converted to hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase. In turn, H_2O_2 is metabolized in hypochlorous acid

(HOCl) by myeloperoxidase (MPO) (Fig. 7.9) [200, 201]. The names "respiratory burst" and "oxidative burst" thus refer to the consumption of oxygen in order to generate ROS. Note that O_2^- and H_2O_2 can also react with NO, generated by iNOS, to produce highly reactive nitrogen species (RNS) important for defense against mycobacteria and *Salmonella* species (see also Sect. 7.1.1). In addition to a direct microbiocidal effect, ROS also trigger the influx of potassium and protons into the phagolysosome, resulting in the activation of proteases (e.g., elastase, cathepsin G) that also destroy phagocytosed microbes [202]. Furthermore, ROS facilitate the formation of neutrophil extracellular traps (NETs). These are particularly important for defense against the hyphae of *Aspergillus* spp., which are too large for phagocytosis [2, 203].

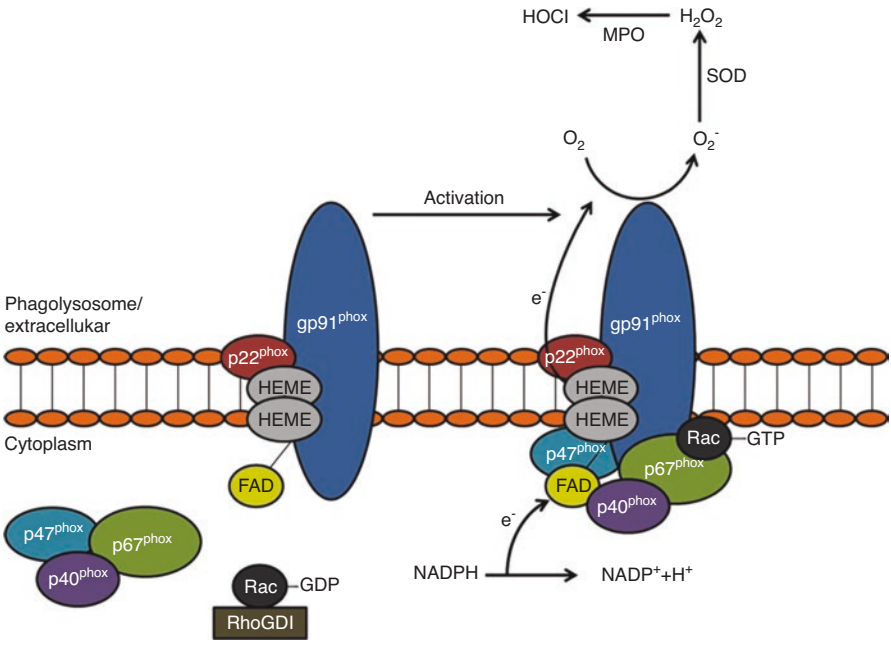


Fig. 7.9 *The phagocyte NADPH oxidase complex and respiratory burst.* The gp91^{phox} and p22^{phox} subunits of NADPH oxidase are located in the plasma membrane or membrane of secondary granules and phagolysosomes. This heterodimer, also called flavocytochrome b_{558}, is the redox center of the enzyme. The p47^{phox}, p67^{phox}, p40^{phox}, and Rac subunits are found in the cytosol. Upon cellular activation initiated by phagocytosis, p47^{phox} and p67^{phox} are phosphorylated and bind together. Afterward, they move to the membrane in association with p40^{phox} and GTP-bound Rac, and complete assembly of enzyme complex is established. Activated NADPH oxidase catalyzes the transfer of electrons (e^-) from cytosolic NADPH across the membrane via FAD and heme redox centers to molecular oxygen (O_2) forming superoxide anion (O_2^-) within the lumen of the phagolysosome or extracellularly. Superoxide anion can be further enzymatically converted to produce a range of toxic reactive oxygen species, including hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). *FAD* flavin adenine dinucleotide; *GDP* guanosine diphosphate; *GTP* guanosine triphosphate; *MPO* myeloperoxidase; *NADPH* nicotinamide adenine dinucleotide phosphate; *SOD* superoxide dismutase. (Modified with permission from reference [200])

Genetics

Mutations in five subunits of the NADPH oxidase complex are known to cause CGD: gp91^{phox} (encoded by *CYBB*, located on the X chromosome), p22^{phox} (encoded by *CYBA*), p47^{phox} (encoded by *NCF1*), p67^{phox} (encoded by *NCF2*), and p40^{phox} (encoded by *NCF4*) (Table 7.6) [192]. *CYBC1*, encoding the protein EROS (essential for ROS), was recently recognized as a sixth gene for CGD [204, 205]. EROS co-localizes with gp91^{phox} in the endoplasmic reticulum and is believed to act as a chaperone for the assembly of gp91^{phox} and p22^{phox} [204]. EROS-deficient patients had a decreased gp91^{phox} expression and an impaired respiratory burst. Interestingly, monocytes and macrophages seemed to be more severely affected than neutrophils in these patients, suggesting a partially redundant chaperone function in neutrophils [204].

Mutations in *CYBB*, causing X-linked CGD, account for about 65% of all cases worldwide. Approximately one third of *CYBB* mutations occur de novo. Defects in *NCF1* account for approximately 25% of patients. Mutations in the remaining four genes are far less frequent (Table 7.6) [192, 196, 206, 207]. In regions with high rates of consanguinity, autosomal recessive forms of CGD are more prevalent, and the overall incidence may be higher [208, 209].

The majority of the currently identified mutations in *CYBB*, *CYBA*, *NCF1*, and *NCF2* result in (nearly) complete absence of NADPH oxidase activity. Rarely, residual protein function is seen. Overall, patients with X-linked CGD (*CYBB* mutations) have a more severe phenotype with earlier onset than those with p47^{phox} deficiency (*NCF1* mutations). In contrast, mutations in *NCF4* and *CYBC1* reported thus far all caused a partial loss of enzyme activity [192, 196, 204, 205, 210–212]. Higher residual ROS production is associated with less severe infections and a better survival but does not affect the degree of CGD-related colitis [213]. CGD patients with *NCF4* mutations, for example, typically have a much milder infectious phenotype without invasive infections but suffer from severe colitis [211]. Historically, the protein levels associated with a specific mutation were indicated with the superscripts $^{+}$ (normal), $^{-}$ (decreased), and ^{0} (absent), e.g., X91^{+}, X91^{-}, and X91^{0} [212].

Table 7.6 Genes associated with CGD

Gene, OMIM no.	Gene locus	Inheritance	Protein	Estimated frequency in CGD
CYBB, *300481	Xp21.1	XR	gp91^{phox}	~65%
CYBA, *608508	16q24.3	AR	p22^{phox}	~5%
NCF1, *608512	7q11.23	AR	p47^{phox}	~25%
NCF2, *608515	1q25.3	AR	p67^{phox}	~5%
NCF4, *601488	22q13.1	AR	p40^{phox}	<1%
CYBC1, *618334	17q25.3	AR	EROS	<1%

AR Autosomal recessive, *CGD* Chronic granulomatous disease, *XR* X-linked recessive

Since protein function is a more relevant parameter than protein levels [213], this nomenclature has become obsolete.

7.4.1.2 Clinical Features

Patients with CGD suffer from recurrent, severe to life-threatening bacterial and fungal infections as well as inflammatory complications such as granuloma formation and colitis. There is no increased incidence of viral infections. Other features seen in CGD include growth failure, abnormal wound healing, hepatomegaly, and splenomegaly. Patients with CGD do not appear to have an increased incidence of malignancy [196].

In about two thirds of CGD patients, onset of disease occurs in the first year of life with recurrent infections. The majority of patients are diagnosed before the age of 5 years [196]. Nonetheless, patients with CGD can present at any age from infancy to late adulthood. In some patients, the diagnosis is only made in late childhood or adulthood, such as in milder cases of autosomal recessive CGD [194]. Furthermore, CGD may not be recognized if patients present with common infections that respond well to the standard antimicrobial treatment, delaying diagnosis until they develop severe or unusual infections or inflammatory complications.

Infectious Manifestations

The most common sites of infection are the lungs, skin, liver, lymph nodes, and bones (Table 7.7). Pulmonary infections usually present as pneumonia, but lung abscesses, empyema, and hilar lymphadenopathy can also occur. Abscesses, with pus formation, are commonly located in the skin, perianal/perirectal, and liver. Gingivitis and stomatitis are also frequently seen [196, 212].

The most frequently encountered pathogens in CGD are catalase-positive organisms: *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia* complex, *Pseudomonas* spp., *Nocardia* spp., and *Aspergillus* spp. Since the introduction of TMP-SMX prophylaxis (see section on "Treatment"), staphylococcal infections are mostly confined to the skin, liver, and lymph nodes, while the lungs and bones are less affected. Other catalase-positive pathogens to which CGD patients are particularly susceptible include enteric gram-negative bacteria (especially *Salmonella* spp., *Klebsiella pneumoniae*, *Escherichia coli*), *Candida* spp., *Actinomyces, Granulibacter bethesdensis, Chromobacterium violaceum*, and *Penicillium piceum* [196, 222–226]. Furthermore, CGD patients are more prone to infections with *Mycobacterium tuberculosis* and bacillus Calmette-Guérin (BCG). In most patients, BCG complications range from none to localized BCGitis (skin and/or lymph node infection at the site of vaccination). Disseminated BCGosis is rarely seen [227–229].

Pneumonia is the most prevalent type of infection in CGD (Table 7.7) [223]. One of the most frequently isolated organisms from the respiratory tract is *Aspergillus* spp. [194]. Fungal pneumonias do not generally cavitate in CGD patients. In contrast, cavitation is seen in pneumonias due to *Nocardia*. *Burkholderia cepacia* complex is another common pathogen in CGD-related pneumonia, and patients can present with recurrent pulmonary infections caused by different *Burkholderia*

strains [230]. Lymphadenitis and abscesses are also very prevalent in CGD (Table 7.7) [223]. Lymph node excision or surgical drainage of abscesses is often required next to parenteral antibiotics. More recently, it was shown that simultaneous use of antibiotics and steroids can effectively treat liver abscesses, eliminating the need for surgery [231, 232]. Perirectal abscesses are very difficult to treat and can result in the formation of fistulae [196]. Bone infections are usually caused by *Serratia* spp. or *Aspergillus* spp. (Table 7.7). Osteomyelitis due to *Serratia marcescens* is particularly common in infants with CGD [233]. Osteomyelitis by *Aspergillus* spp. typically occurs in the ribs and vertebral bodies, arising from direct extension from the lung. *Aspergillus nidulans* is almost exclusively seen in CGD patients. Remarkably, *Aspergillus nidulans* has a more subtle, nonspecific clinical presentation and a higher mortality rate compared to *Aspergillus fumigatus* [234, 235]. In infants, recurrent severe impetigo should raise suspicion of CGD (Table 7.7). Septicemia is uncommon in CGD. If sepsis occurs, it is usually due to *Burkholderia cepacia* complex, *Serratia marcescens*, or *Chromobacterium violaceum* (Table 7.7) [196, 212].

Bacterial and *Nocardia* infections are usually symptomatic and associated with fever and increased inflammatory parameters (leukocytosis, elevated CRP and ESR).

Table 7.7 Infections in CGD

Type of infection	Estimated prevalence	Infectious organisms
Pneumonia	70–80%	*Aspergillus*, *Staphylococcus*, *Burkholderia cepacia*, *Pseudomonas*, *Nocardia*, *Mycobacterium* (including atypical), *Serratia*, *Candida*, *Klebsiella*, *Paecilomyces*
Lymphadenitis	50–70%	*Staphylococcus*, *Serratia*, *Candida*, *Klebsiella*, *Nocardia*
Cutaneous infections (cellulitis/impetigo or abscesses)	50–60%	*Staphylococcus*, *Serratia*, *Aspergillus*, *Klebsiella*, *Candida*, enteric gram-negative bacteria
Hepatic or perihepatic abscesses	20–30%	*Staphylococcus*, *Serratia*, *Streptococcus viridans*, *Nocardia*, *Aspergillus*
Osteomyelitis	20–30%	*Serratia*, *Aspergillus*, *Paecilomyces*, *Staphylococcus*, *Burkholderia cepacia*, *Pseudomonas*, *Nocardia*
Perirectal abscesses or fistulae	15–30%	Enteric gram-negative bacteria, *Staphylococcus*
Septicemia	10–20%	*Burkholderia cepacia*, *Serratia, Chromobacterium violaceum*, *Klebsiella*, *Pseudomonas*, *Salmonella*, *Staphylococcus*
Urinary tract infections or pyelonephritis	5–15%	Enteric gram-negative bacteria
Brain abscesses	<5%	*Aspergillus*, *Staphylococcus*
Meningitis	<5%	*Candida lusitaniae*, *Haemophilus influenzae*, *Burkholderia cepacia*

The estimated prevalence of the types of infections and the causative organisms were extracted from several large series of patients with chronic granulomatous disease (CGD) in the United States, Europe, Iran, and Japan ([196, 212, 214–221], unpublished United States CGD Registry). The infectious organisms are arranged in approximate order of frequency for each type of infection. (Modified with permission from Ref. [329])

In contrast, fungal infections often remain asymptomatic for a long time without fever or leukocytosis and are either detected during routine screening or at advanced stage. Although fungal infections are less prevalent than bacterial infections, they are the leading cause of mortality in CGD patients. *Burkholderia cepacia* complex infections are also associated with a high mortality rate. This organism is often multiresistant and associated with septicemia and hemophagocytic lymphohistiocytosis (HLH). The importance of a microbiological diagnosis cannot be underestimated in CGD patients, and diagnostic specimens should be obtained prior to the start of therapy. Especially the diagnosis of *Aspergillus* infections can be challenging. Histopathology and culture are complimentary in identifying the underlying pathogen and susceptibility testing. In case of pulmonary aspergillosis, lung biopsy increases the pathogen detection rate from 30% to 50% in CGD patients compared to bronchoalveolar lavage and fine-needle biopsy. In addition, biopsies of *Aspergillus*-induced osteomyelitis demonstrate clear histopathological differences between CGD and non-CGD patients. In CGD patients, bone biopsies show chronic inflammation with granuloma formation, multinucleated giant cells and histiocytes, and absence of granulation tissue and remodeled bone tissue [196, 222, 223, 233–237].

Inflammatory Manifestations

In contrast to infectious manifestations, the degree of residual ROS production does not correlate with the severity of inflammatory complications in CGD patients [238]. The precise mechanisms of the inflammatory manifestations in CGD are incompletely understood. In the absence of ROS production, higher levels of inflammatory cytokines and enhanced inflammasome activation are seen, suggesting dysregulated inflammatory responses. In addition, clearance of apoptotic inflammatory cells by phagocytes is impaired, which may contribute to granuloma formation [239–243]. Granulomata and excessive granulation tissue formation are distinctive hallmarks of CGD, which determined the name of the disease. They most often occur in the gastrointestinal and genitourinary tracts. Other organs and tissues, such as the retina, liver, lungs, and bones, may also be affected by granulomata [243].

Gastrointestinal inflammatory manifestations occur is 30–40% of CGD patients. All patients with confirmed IBD and granulomata on gut biopsies presented with symptoms of abdominal pain, diarrhea, colitis, proctitis, strictures, fistulae, and/or obstruction [244]. Involvement of the upper gastrointestinal tract has also been reported (e.g., esophageal stricture, gastric outlet obstruction) but is usually less severe than the colonic involvement [245]. Screening for CGD is recommended in all patients with an atypical presentation of Crohn disease. It is important to differentiate CGD patients with IBD from those with classical Crohn disease, because several CGD patients treated with the TNF inhibitor infliximab developed severe, sometimes fatal, infections by typical CGD pathogens [246].

In some reports, up to 40% of CGD patients have genitourinary tract abnormalities. Besides urinary tract infections and granulomata, these include ureteral and urethral strictures and altered renal function [247].

Chorioretinal lesions have been described in up to one fourth of patients with X-linked CGD. Most chorioretinal lesions are asymptomatic retinal scars associated

with pigment clumping along large retinal vessels [248]. Bacterial DNA was detected in some of these lesions. Since the latter were nonprogressive, even under treatment with immunosuppressive drugs, the role of infection in chorioretinal lesions remains unclear [249].

Hepatic abnormalities are frequently described in CGD. In an NIH cohort of 194 patients, 73% had increased liver enzymes on at least one occasion. Twenty-five percent had a persistent elevation of alkaline phosphatase. Liver abscesses, drug-induced hepatotoxicity, hepatomegaly, and splenomegaly were reported in, respectively, 35%, 15%, 34%, and 56% of cases. In patients who underwent liver biopsy, histology showed granulomata in 75% and lobular hepatitis in 90%. About 80% of patients had evidence of portal venopathy that was often associated with splenomegaly. Portal hypertension was identified as an important risk factor for mortality [250].

Chronic pulmonary complications due to recurrent infections and inflammation are common in CGD, especially in adult patients. These include fibrosis, bronchiectasis, obliterative bronchiolitis, pleural thickening, and pulmonary hypertension [251–253].

Oral manifestations in CGD encompass recurrent aphthous ulceration, periodontitis, gingivitis, and gingival hypertrophy [254, 255].

Noninfectious skin manifestations reported in CGD patients include photosensitivity, discoid lupus, granulomata, and vasculitis [254].

Up to 5% of CGD patients have autoimmune manifestations. Patients are at particular risk for development of lupus erythematous (both discoid and systemic), immune thrombocytopenia (ITP), and juvenile idiopathic arthritis (JIA) [196, 256, 257].

Other Features

Growth failure is frequently seen in CGD patients and is a common presentation in young children. Growth often improves in adolescence, and many patients reach their predicted height by adulthood [254, 258, 259].

The *XK* gene, encoding a membrane protein necessary for expression of the Kell blood group antigens, is located on the X chromosome adjacent to the *CYBB* gene (encoding gp91^{phox}). Patients with deletions in the X chromosome, affecting both *CYBB* and *XK* (contiguous gene disorder), present with X-linked CGD as well as McLeod syndrome. McLeod syndrome is characterized by variable acanthocytosis, anemia, elevated creatine phosphokinase, late-onset peripheral and central nervous system manifestations, and weak expression of Kell blood group antigens. Therefore, all X-linked CGD patients should be tested for Kell antigens, to avoid transfusion of Kell-positive blood productions into Kell-negative patients [260, 261].

X-Linked Carriers

Female carriers for the X-linked form of CGD (*CYBB*, encoding gp91^{phox}) may also present symptoms. In X-linked carriers of CGD, lyonization or X-inactivation results in two types of phagocytes: one with a normal and another with an impaired respiratory burst activity [262]. Therefore, X-CGD carriers display a typical mosaic pattern on respiratory burst testing of peripheral blood cells (see Sect. 7.4.1.3). X-linked

carriers with less than 20% residual respiratory burst activity, due to skewed X-inactivation in favor of the defective chromosome, suffer from mild to severe infections [262–264]. Noteworthy, progressive skewing of lyonization with age in previously healthy X-CGD carriers can cause late-onset manifestations of CGD [265]. Female X-CGD carriers with severely skewed X-inactivation should also be tested for Kell antigens, to avoid hemolytic transfusion reactions [260, 261].

Moreover, all X-CGD carriers are at risk for autoimmune and inflammatory manifestations, regardless of the degree of skewed X-inactivation [262]. About one fourth of X-linked carriers develop symptoms of discoid lupus erythematosus with discoid skin lesions and photosensitivity, which usually presents in the second decade of life. Aphthous stomatitis and/or gingivitis have been described in up to half of X-CGD carriers. X-linked carriers can also have CGD-related chorioretinal lesions, joint pain, and fatigue [266–268].

7.4.1.3 Diagnosis

If CGD is suspected based on the clinical presentation and/or a positive family history, the diagnosis can be made by functional assessment of the NADPH oxidase activity in neutrophils stimulated in vitro [269]. The nitroblue tetrazolium (NBT) reduction test has been used historically to measure superoxide production. In the NBT assay, neutrophils are stimulated with phorbol myristate acetate (PMA) in the presence of NBT dye. The generated superoxide reduces the yellow NBT to dark-blue/black formazan, which precipitates in the cell. The color change in the cells is visually analyzed by microscopy. Neutrophils that lack a functional NADPH oxidase complex will remain yellow. Note that cells with a small amount of residual NADPH oxidase function will also reduce the NBT and turn blue. The NBT assay is a simple and rapid method to determine NADPH oxidase activity, but the readout is only semiquantitative. In addition, the test does not always allow reliable identification of carriers of an X-linked or autosomal recessive CGD mutation, has a high rate of false-negative results, and requires significant operator experience [269].

Currently, the dihydrorhodamine (DHR)-123 oxidation assay is the preferred diagnostic test for CGD, since it overcomes most of the limitations encountered with the NBT test: it is more quantitative, more sensitive, easier to perform, and less dependent on operator experience and interpretation. In the DHR test, nonfluorescent DHR-123 is taken up by PMA-stimulated neutrophils and oxidized by hydrogen peroxide to the fluorescent rhodamine-123. The fluorescent signal of each cell is assessed by flow cytometry, providing a cell-by-cell distribution of NADPH oxidase activity. The mean fluorescence intensity of rhodamine-123 quantitatively correlates with the degree of residual ROS production, which is important in terms of infection risk and survival. The DHR assay can usually distinguish between X-linked CGD, autosomal recessive CGD, and X-linked CGD carriers (Fig. 7.10). In addition, the DHR assay can be used to determine the chimerism status in CGD patients who have undergone HSCT [269, 270]. Because the oxidation of DHR requires some MPO activity, MPO deficiency can give an abnormal DHR test (see also Sect. 7.4.2). MPO deficiency can be distinguished from CGD by a normal NBT test [269, 271].

In patients with an impaired NADPH oxidase function (DHR or NBT test), the diagnosis of CGD should be confirmed by genetic testing. Sequencing of all

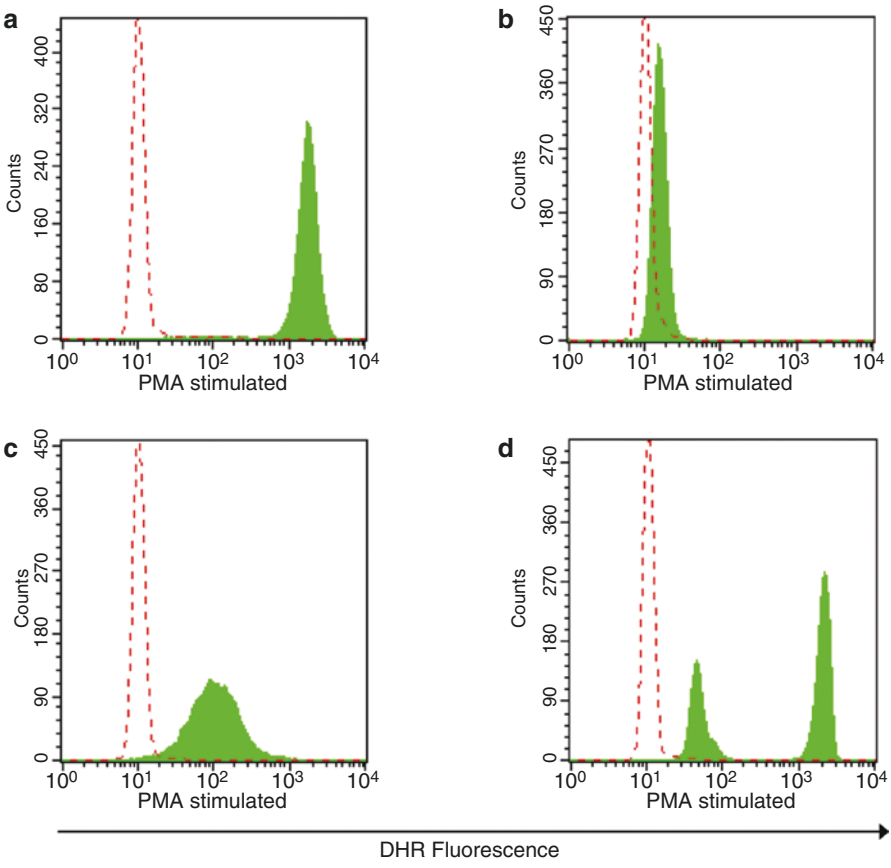


Fig. 7.10 *Flow cytometry-based dihydrorhodamine (DHR)-123 oxidation test.* (**a**) Normal neutrophil respiratory burst showing a complete shift in fluorescence after stimulation. (**b**) X-linked chronic granulomatous disease (CGD) (*gp91*^{phox}) histogram showing no significant change in neutrophil fluorescence after stimulation. (**c**) Autosomal recessive CGD (*p47*^{phox}) histogram showing a proportion of unchanged stimulated neutrophils overlapping with the background fluorescence and some neutrophils with significantly reduced fluorescence. (**d**) X-linked female carrier status histogram with a pattern of two peaks reflecting the two populations of abnormal and normal neutrophils that result from X chromosome inactivation. The dashed line indicates the background fluorescence of unstimulated neutrophils, and the green histogram indicates neutrophil fluorescence after phorbol myristate acetate (PMA) stimulation. (Reprinted with permission from reference [269])

CGD-related genes (Table 7.6) is recommended to pinpoint the exact molecular defect. The *NCF1* gene (encoding p47^{phox}) is flanked on each side by a pseudogene that closely resembles *NCF1* but does not encode an active protein. Interference of the pseudogene sequences hampers the identification of *NCF1* mutations. Moreover, the most common p47^{phox} defect is caused by pseudogene conversion and is often missed in routine sequencing studies [269].

If genetic testing is unavailable or inconclusive, immunoblotting or flow cytometry can be used to identify the deficient protein. However, immunoblotting cannot

distinguish between gp91^{phox} deficiency and p22^{phox} deficiency since the expression of these subunits is mutually codependent [212, 269].

Regardless of the functional or genetic test used, it is recommended that they are performed by experienced laboratories or referral centers on appropriately handled blood samples in order to avoid inconclusive or false results [269].

As explained above, patients with X-linked CGD should be screened for the McLeod red cell phenotype (absence of Kell antigens) (see Sect. 7.4.1.2, "Other Features").

7.4.1.4 Treatment

Management of CGD encompasses (1) lifelong antimicrobial prophylaxis with or without immunomodulatory prophylaxis; (2) early diagnosis and aggressive treatment of acute infections using invasive diagnostic procedures and (prolonged) parenteral antimicrobial drugs; and (3) rigorous management of inflammatory complications (see also Sect. 7.1.3) [7]. At present, the only curative treatment for CGD available in routine practice is allogeneic HSCT [272]. Gene therapy and novel targeted molecular treatments are being studied [273].

Lifelong antimicrobial prophylaxis has considerably improved prognosis in CGD patients and thus became the standard of care. Antimicrobial prophylaxis in CGD consists of antibacterial (e.g., TMP-SMX) and antifungal (e.g., itraconazole) therapy with or without immunomodulatory therapy (i.e., recombinant interferon-γ (rIFN-γ)). Dosages of TMP-SMX and itraconazole are discussed in Sect. 7.1.3. TMP-SMX prophylaxis is effective in reducing the incidence of bacterial infections without increasing the risk of fungal infections. If sulfonamides are contraindicated, suitable alternatives are trimethoprim as a single agent, dicloxacillin, an oral cephalosporine, or ciprofloxacin [220, 274]. Itraconazole, which has a good activity against *Aspergillus* spp., is highly effective as antifungal prophylaxis in CGD [274–276]. Fungal infections resistant to itraconazole do occur, but most are responsive to voriconazole or posaconazole [277, 278]. Immunomodulatory therapy with rIFN-γ is thought to improve phagocyte function and killing by non-oxidative mechanisms in CGD patients [7]. A multicenter, randomized, placebo-controlled trial in 128 CGD patients demonstrated that rIFN-γ substantially reduced the number and severity of infections, independent of patient age, genotype, or concomitant use of other prophylactic drugs [279]. However, a significant reduction in *Aspergillus* infections could not be demonstrated [279]. Open label studies confirmed the efficacy and tolerability of more prolonged courses of rIFN-γ, without an increase in inflammatory complications [221, 280]. The combination of prophylactic TMP-SMX, itraconazole, and rIFN-γ can significantly reduce severe infections from 1 per patient-year to almost 1 per 10 patient-years [7, 274, 275, 279, 280]. Nonetheless, rIFN-γ is only sporadically prescribed outside of the United States. In the earlier studies that demonstrated a significant effect of rIFN-γ prophylaxis, patients were not yet treated with prophylactic itraconazole [221, 279, 280]. Later, a prospective study found no significant change in the rate of severe infections when rIFN-γ was added to TMP-SMX and itraconazole [281]. Therefore, it is unclear if rIFN-γ provides an additional benefit beyond that of the combination of TMP-SMX and itraconazole. In addition, rIFN-γ is expensive, requires repeated subcutaneous injections, and causes side effects of

flu-like symptoms such as fever, headache, and myalgias [7]. If used, prophylactic rIFN-γ is administrated subcutaneously three times a week at a dose of 50 μg/m^2, or 1.5 μg/kg in children less than 0.5 m^2. The flu-like side effects can be minimized by administration before bedtime and simultaneous use of acetaminophen [7].

Early diagnosis and prompt treatment of acute infections is essential. Reasonable attempts should be made to find the source of infection and obtain microbiological specimens prior to the start of antimicrobial therapy (see also "Infectious Manifestations" above). CGD patients can sometimes present with high fever or an established infection (e.g., pneumonia). In contrast, some serious infections, especially fungal infections, can also be asymptomatic or only slightly symptomatic at time of presentation. Increases in CRP or ESR should, therefore, always initiate a search for an infectious focus. Empiric treatment with parenteral antibiotics should be started as soon as possible and at least cover *S. aureus* and enteric gram-negative organisms. If the initial antibiotic therapy fails, more aggressive diagnostic procedures (e.g., computed tomography, bone scan, or open biopsies) should be considered, and empiric antibiotic treatment should be broadened to cover *Burkholderia cepacia* complex. If the site of infection is located, the empiric treatment can be extended to cover for the most frequent causative pathogens (Table 7.7). If a fungal organism is isolated or suspected, parenteral voriconazole or posaconazole should be associated. In case of bacterial lymphadenitis and abscesses, surgical drainage or excision is often necessary in addition to prolonged parenteral antibiotics [7]. Staphylococcal liver abscesses also respond well to concomitant treatment with parenteral antibiotics and steroids (1 mg/kg/day for about 2 weeks and then tapered slowly), reducing the need for drainage [232]. The association of systemic steroids is also indicated in severe pulmonary infections to control the excessive inflammation, such as in *Nocardia* pneumonia and mulch pneumonitis. Mulch pneumonitis (or inhalational acute military pneumonia) is an acute fulminant fungal pneumonitis with hypoxia, often due to the inhalation of *Aspergillus* spp. from exposure to garden mulch, and is almost pathognomonic of CGD [282, 283]. Since many CGD-related infections respond slowly to antimicrobial treatment, intravenous therapy must be followed by long-term oral treatment, sometimes for several months [7]. Granulocyte transfusions have been successfully used as an adjunctive therapy in selected patients with life-threatening or treatment-refractory infections. However, their use remains controversial. A major concern is the risk of alloimmunization, which compromises the success of a future HSCT [284].

Inflammatory manifestations are generally treated with steroids or other immunosuppressive drugs, but their use should be limited because of the infection risk. IBD, granulomata, and obstructive lesions of the gastrointestinal and genitourinary tracts are effectively treated with oral steroids. Steroids are usually given at a dose of 1 mg/kg/day of prednisone for a brief period and then slowly tapered over several weeks to months. In case of frequent relapses, maintenance therapy with low-dose steroids (e.g., 0.1–0.2 mg/kg every other day) may be required and is relatively well tolerated with no increase in severe infections [7]. Steroid-sparing immunosuppressive drugs such as azathioprine and infliximab have also been used in CGD-related colitis, though infliximab was associated with an increased incidence of severe (and even fatal) infections [246]. Secondary macrophage activation syndrome

(MAS)–hemophagocytic lymphohistiocytosis (HLH) has been described in CGD. MAS-HLH should always be considered in CGD patients with fever and associated cytopenias, splenomegaly, and/or liver dysfunction, as specific therapy may be indicated [237, 285].

Allogeneic HSCT is the only established curative therapy for CGD thus far. Transplant outcomes have significantly improved over the past years. Currently, the event-free survival rate is more than 80%, and the overall survival rate about 90%. HLA-identical sibling donors and matched unrelated donors are preferred. The decision to proceed to HSCT is based on the individual clinical course and donor availability. HSCT can also be performed in patients with active treatment-refractory infections or inflammatory complications, but this should only be done in centers with experience in this procedure [272].

Gene therapy, aimed at correction of the defective gene in hematopoietic stem cells, is still in development but holds great promise as a curative option in CGD patients. Based on the experience in X-linked CGD carriers, restoration of normal oxidase activity in 10–20% of neutrophils would already significantly improve prognosis. Trials using newer methods, such as lentiviral vectors and novel gene-editing technologies, are ongoing or underway [273]. In parallel, several targeted molecular treatments are being studied. Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists (e.g., pioglitazone) may enhance ROS production in phagocytes, thereby facilitating the clearance of pathogens. The mTOR inhibitor rapamycin has been shown to induce autophagy and reduce the levels of inflammatory cytokines and may thus assist in the treatment of inflammatory complications. Prospective trials are needed to evaluate the effect of such targeted therapies in CGD patients [273].

7.4.1.5 Prognosis

Routine use of lifelong antimicrobial prophylaxis, the advent of azole antifungal drugs, and more aggressive diagnosis and treatment of acute infections have greatly improved the prognosis of CGD patients. Currently, the average survival is at least 40 years [194–197]. Patients with autosomal recessive forms of CGD have a higher survival rate than those with X-linked CGD. This can be explained by the fact that *CYBB* mutations are usually more deleterious with (almost) no residual ROS production compared to mutations in the autosomal recessive CGD genes [196]. Despite improvements in antimicrobial therapy, the main cause of death in CGD remains infections, especially pulmonary *Aspergillus* infections [222].

7.4.2 Myeloperoxidase Deficiency

7.4.2.1 Etiology

MPO is the most abundant enzyme found in primary (azurophilic) granules of neutrophils and monocytes. It catalyzes the conversion of hydrogen peroxide (H_2O_2) to hypochlorous acid (HOCl) and other reactive intermediates, amplifying the toxicity of the ROS produced during the respiratory burst (see Sect. 7.4.1.1) (Fig. 7.9). In addition to its role in bacterial and fungal killing, MPO has both pro- and

anti-inflammatory properties and is involved in various inflammatory and autoimmune diseases [286].

Primary myeloperoxidase (MPO) deficiency is the most frequent congenital disorder of phagocytes. It is caused by germline mutations in the *MPO* gene (OMIM *606989), which are inherited in an autosomal recessive manner. Some mutations affect the posttranslational processing of the MPO precursor protein, whereas mutations in the regulatory region of the gene cause pre-translational defects. Complete MPO deficiency is present in about 1 in 4000 individuals, and partial MPO deficiency in approximately 1 in 2000 individuals [287].

Less frequently, MPO deficiency can also be acquired or secondary in various conditions, including diabetes mellitus, pregnancy, lead poisoning, cytotoxic drugs, severe infections, neuronal lipofuscinosis, and hematopoietic malignancies (e.g., myeloid leukemia, myelodysplastic syndromes, Hodgkin lymphoma). In some hematopoietic malignancies, the *MPO* gene, located on chromosome 17q22–23, is involved in the breakpoint region of somatic chromosomal translocations. However, in most cases, the pathophysiological mechanism of secondary MPO deficiency is incompletely understood. Generally, secondary MPO deficiency is partial and resolves upon improvement of the underlying condition [287].

7.4.2.2 Clinical Features

Although studies in MPO-deficient mice and in isolated MPO-deficient human neutrophils have shown a markedly impaired killing of *Candida* spp., *Aspergillus* spp., and bacteria, the vast majority (>95%) of patients with primary MPO deficiency are asymptomatic [287–289]. Patients with primary MPO deficiency and associated diabetes mellitus or cancer are more likely to be symptomatic [290, 291].

Symptomatic patients usually present with recurrent infections due to *Candida* spp. Both mucocutaneous candidiasis and more invasive *Candida* infections (e.g., meningitis, osteomyelitis, sepsis) have been reported [292–296]. Patients do not appear to have an increased risk of *Aspergillus* infections.

Patients with primary MPO deficiency have an increased risk for inflammatory and autoimmune disorders, such as polyarthritis, lupus nephritis, and diabetes mellitus [286]. In contrast, MPO deficiency may also protect against inflammation-induced tissue damage, like in cardiovascular disease and chronic kidney disease [297–300].

7.4.2.3 Diagnosis

MPO deficiency should be considered in patients with recurrent and/or invasive *Candida* infections. The diagnosis is usually established by histochemical staining for MPO, which is absent in neutrophils but normal in eosinophils. Genetic confirmation can be done. Since most individuals with primary MPO deficiency are asymptomatic, it is important that symptomatic patients are evaluated for associated immune-compromising conditions such as diabetes mellitus [287, 290, 291].

The differential diagnosis of recurrent and/or invasive *Candida* infections also includes other primary immunodeficiencies with qualitative and/or quantitative neutrophil defects (e.g., CGD, congenital neutropenia), primary T cell immunodeficiencies, and primary defects in CARD9- and IL-17-mediated immunity (e.g., autosomal

dominant STAT3 deficiency, autosomal recessive CARD9 deficiency, autosomal dominant STAT1 gain-of-function). Most of these disorders will cause additional clinical manifestations [301, 302]. Remember that MPO deficiency can give an abnormal DHR test, complicating the differential diagnosis with CGD (see Sect. 7.4.1.3) [271].

7.4.2.4 Treatment

In symptomatic patients, aggressive treatment of acute infections and long-term antifungal prophylaxis with fluconazole or itraconazole are indicated (see also Sect. 7.1.3). Prophylaxis is not required in asymptomatic individuals. Immunosuppressive drugs and prolonged use of antibiotics should be avoided, because of the increased risk of fungal infections [287].

7.4.3 Glucose-6-Phosphate Dehydrogenase Deficiency

7.4.3.1 Etiology

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most prevalent enzymopathy worldwide and affects about 400 million people. G6PD is the rate-limiting enzyme in the pentose phosphate pathway, through which NADPH is produced (Fig. 7.11). NADPH is required for the protection of cells against oxidative stress and for the generation of ROS in the phagocyte respiratory burst (Fig. 7.9). G6PD deficiency is caused by mutations in the *G6PD* gene (OMIM *305900), located on chromosome Xq28. Mutations in *G6PD* are divided in five classes based on their associated degree of deficiency and clinical manifestations, with class I being the most severe. Since the disease is X-linked, it mainly affects males. Females may be symptomatic if they are homozygous or if they have skewed X-inactivation in favor of the defective chromosome [304, 305].

7.4.3.2 Clinical Features

Most patients with G6PD deficiency are asymptomatic throughout life. The most frequent clinical presentation is acute hemolytic anemia due to oxidative damage to red blood cells, precipitated by infection and certain drugs, chemicals, and food (e.g., antimalarials, naphthalene, fava beans) [304]. Only patients with G6PD deficiency class I (less than 10% of residual enzyme activity) have a severely attenuated respiratory burst, causing an increased susceptibility to infections similar to CGD (see Sect. 7.4.1). Typical are invasive bacterial infections and an increased risk of sepsis; *Aspergillus* infections are less common [305–307].

7.4.3.3 Diagnosis

The diagnosis of G6PD deficiency is made by measuring G6PD enzyme activity in red blood cells (e.g., semiquantitative fluorescent spot screening assay, quantitative spectrophotometric assay) and can be confirmed genetically [304].

7.4.3.4 Treatment

Treatment of G6PD-deficient patients with recurrent infections includes lifelong antibiotic prophylaxis, with or without antifungal prophylaxis, and an aggressive approach of acute infections (see also Sect. 7.1.3) [306]. Note that some commonly

used antibiotics, such as nitrofurantoin, ciprofloxacin, and TMP-SMX, can cause a hemolytic crisis and should be avoided [304].

7.4.4 Disorders of Glutathione Metabolism

7.4.4.1 Etiology

Glutathione (GSH) is involved in various fundamental cellular functions, including protection from the harmful effects of ROS. In phagocytes, GSH is important for preserving the activity of NADPH oxidase. Oxidation of GSH, catalyzed by glutathione peroxidase, facilitates the reduction of hydrogen peroxide (H_2O_2) into water (H_2O). Oxidized glutathione (GSSG) is converted back into its reduced form by glutathione reductase, thereby maintaining intracellular GSH levels. In addition, GSH is synthesized de novo by glutathione synthetase (Fig. 7.11) [303].

Deficiencies in glutathione synthetase, glutathione reductase, and glutathione peroxidase are extremely rare. These disorders are caused by mutations in the genes *GSS* (OMIM *601002), *GSR* (OMIM *138300), and *GPX1* (OMIM *138320),

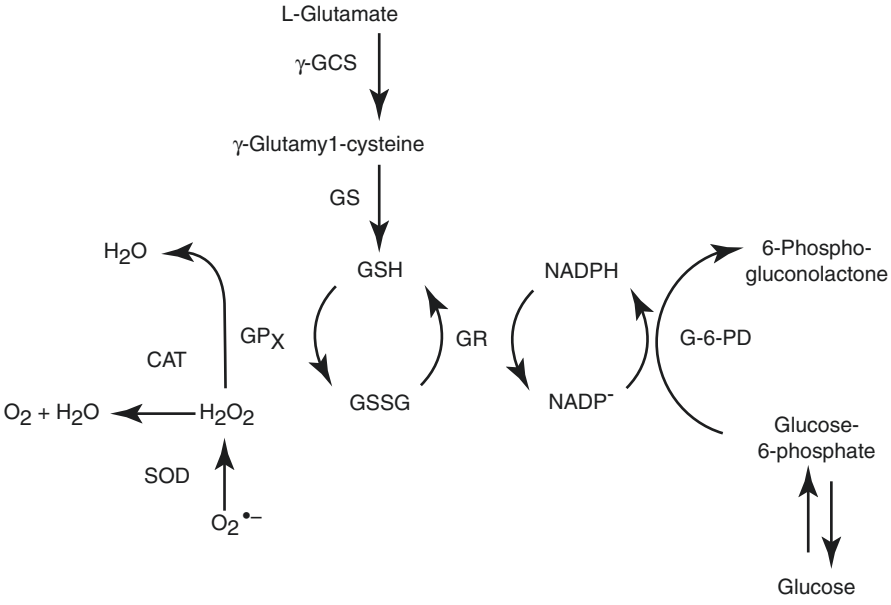


Fig. 7.11 *Metabolism and antioxidant properties of glutathione.* To prevent excessive damage to phagocytes, reactive oxygen species are neutralized by antioxidant enzymes. Glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide (H_2O_2) to water (H_2O), resulting in the oxidation of glutathione (GSH). Oxidized glutathione (GSSG) is converted back into its reduced form GSH by glutathione reductase (GR). Intracellular levels of GSH are also maintained through *de novo* synthesis by glutathione synthetase (GS). Glutathione metabolism is dependent on several cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is produced from the pentose phosphate pathway, of which glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme. *CAT* Catalase, *O2*$^-$ Superoxide anion, *SOD* Superoxide dismutase. (Reprinted with permission from reference [303])

respectively, and inherited in an autosomal recessive manner. Glutathione synthetase, glutathione reductase, and glutathione peroxidase deficiencies are typically associated with hemolytic crises upon oxidative stress triggered by infection and certain drugs. So far, an increased susceptibility to infections has only been reported in patients with glutathione synthetase deficiency, which is discussed further [308].

7.4.4.2 Clinical Features

In addition to hemolytic anemia, glutathione synthetase deficiency is characterized by metabolic acidosis due to accumulation of the metabolic intermediate 5-oxoproline, and progressive central nervous system damage. Disease severity varies from mild to severe, which is partially dependent on the type of mutation [309]. Recurrent bacterial infections have been described in patients with severe disease and may be due to impairments in phagocytosis and bacterial killing mechanisms [310, 311].

7.4.4.3 Diagnosis

The diagnosis of glutathione synthetase deficiency can be investigated by specialized laboratory tests: increased 5-oxoprolinuria in urine, low GSH levels in red blood cells (colorimetric assay), and low GS activity in cultured fibroblasts. Genetic analysis of *GSS* can also be performed [308, 309].

7.4.4.4 Treatment

Data on optimal treatment of glutathione synthetase deficiency is limited. Metabolic acidosis should be corrected, and acute infections should be managed aggressively (see also Sect. 7.1.3). Early supplementation with vitamin E (400 IU/day) was found to reduce the infection rate in these patients. Furthermore, it is recommended to avoid drugs and chemicals known to precipitate hemolytic crises in G6PD deficiency (see also Sect. 7.4.3) [308, 312].

7.5 Defects in Neutrophil Granules

Mature neutrophils have three types of intracellular granules, each containing a different set of antimicrobial peptides and proteolytic enzymes. Based on their sequential appearance during myelopoiesis, they are called primary, secondary, and tertiary granules. Upon neutrophil activation, intracellular granules fuse with phagosomes as well as the plasma membrane, enhancing the destruction of phagocytized and extracellular pathogens respectively [313]. The two major disorders of neutrophil granules are Chédiak-Higashi syndrome and specific granule deficiency (SGD). Chédiak-Higashi syndrome is discussed in Chap. 11.

7.5.1 Specific Granule Deficiency

7.5.1.1 Etiology

SGD (previously called lactoferrin deficiency) is an extremely rare disorder. The neutrophils of these patients show atypical bilobed nuclei, absence of secondary (or specific) granules, and deficiencies in multiple secondary and tertiary granule

proteins (e.g., lactoferrin, gelatinase B). Primary (or azurophilic) granules are present and have normal amounts of MPO but are deficient of defensins. Besides a marked decrease in oxygen-independent bactericidal activity, neutrophils of SGD patients also have an impaired chemotaxis. This is due to a lack of leukocyte adhesion molecules and chemotactic receptors, which are also stored within specific granules [313–315]. Furthermore, eosinophils have deficiencies in eosinophil-specific granule proteins (e.g., eosinophil cationic protein), platelets display abnormalities in α granules, and monocytes have morphological abnormalities and a decrease of certain proteins. Therefore, these cell types are also believed to be functionally impaired in patients with SGD [316–318]. To date, two disease-causing genes have been associated with SGD: *CEBPE* located on chromosome 14q11.2 (OMIM *600749) and *SMARCD2* on chromosome 17q23.3 (OMIM *601736) [319, 320]. However, there are still SGD patients in whom the causative genetic defect has not yet been identified. Mutations in *CEBPE* and *SMARCD2* are inherited autosomal recessively. *CEPBE* encodes C/EBP-ε, a myeloid-specific transcription factor that regulates the synthesis of specific granule proteins, and is required for the differentiation of promyelocytes to myelocytes [319]. In vitro studies demonstrated that SMARCD2 interacts with C/EBP-ε and controls expression of specific granule proteins. In addition, SMARCD2 is a key regulator of early myelopoiesis and has been mainly studied for its role in leukemia [320, 321].

7.5.1.2 Clinical Features

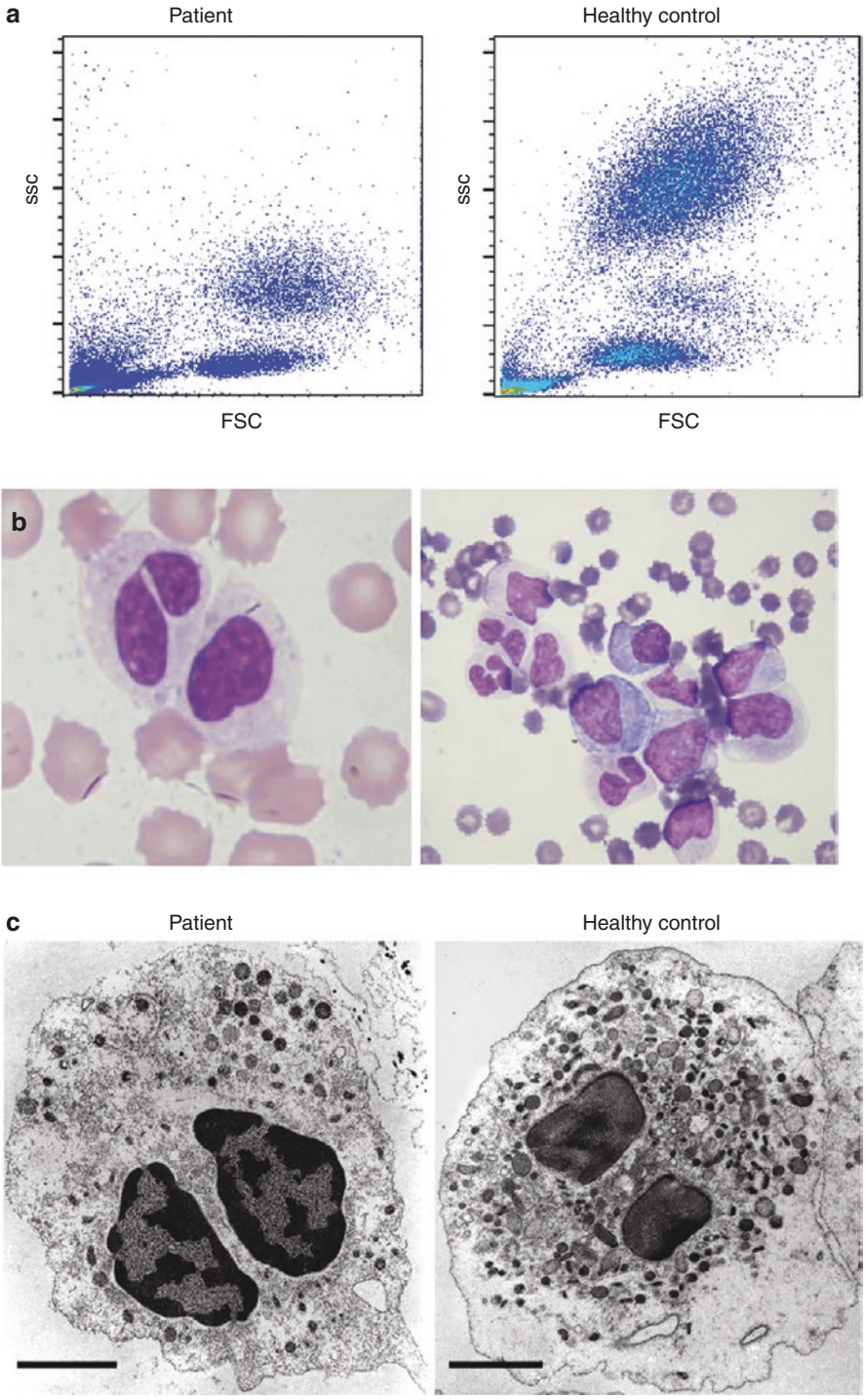
Patients with SGD present with recurrent bacterial and fungal infections from infancy or early childhood. They typically have recurrent pneumonias, lung abscesses, middle ear infections, and mastoiditis. Large, smoldering, ulcerative infections of the skin and mucus membranes are also commonly seen. The major pathogens causing infections in SGD are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, and *Candida albicans* [314, 315].

The few SGD patients identified with *SMARCD2* mutations additionally had neutropenia, developmental delay, facial dysmorphism, skeletal anomalies, and an increased risk of myelodysplastic syndrome [320].

7.5.1.3 Diagnosis

Due to the severe reduction of neutrophil granularity in SGD patients, the neutrophil population will cluster with monocytes in the forward/sideward scatter plot on flow cytometric analysis of whole blood (Fig. 7.12a). The diagnosis is confirmed microscopically: hematoxylin/eosin staining of neutrophils shows bilobed or hyposegmented nuclei resembling the Pelger-Huet anomaly (Fig. 7.12b), and transmission electron microscopy of neutrophils demonstrates paucity or absence of specific granules (Fig. 7.12c) [314, 315]. Additional laboratory findings may include an

Fig. 7.12 *Characteristic neutrophil abnormalities in specific granule deficiency caused by mutations in CEBPE.* (**a**) Flow cytometric forward/sideward scatter plot of whole blood in a patient (left) and a healthy control (right). (**b**) Hematoxylin/eosin staining of peripheral blood smear (left) and bone marrow aspirate (right) of a patient. (**c**) Transmission electron microscopy of neutrophils in a patient (left) and a healthy control (right); scale bar: 2 μm. (Modified with permission from [314])


a
Patient
Healthy control
SSC
FSC
SSC
FSC
b
c
Patient
Healthy control

abnormal neutrophil chemotaxis, an impaired respiratory burst, and a prolonged bleeding time due to platelet dysfunction [314, 315, 318]. Although the underlying genetic causes are not all accounted for, mutation analysis of *CEBPE* and *SMARCD2* can in some cases establish a definite molecular diagnosis [319, 320].

7.5.1.4 Treatment

Treatment of SGD is similar to that of other congenital defects of phagocytes. Lifelong antibiotic prophylaxis (e.g., TMP-SMX) is recommended. Acute infections should be diagnosed and treated promptly, with antibiotics covering at least for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella* spp. Large cutaneous and soft tissue infections may sometimes require surgical debridement (see also Sect. 7.1.3) [314, 315, 319]. A limited number of patients with SGD have been successfully treated with allogeneic HSCT [320, 322]. With adequate antimicrobial treatment patients may survive into adulthood, though the rarity of the disease makes it difficult to predict the long-term outcome.

7.6 Pulmonary Alveolar Proteinosis

Pulmonary alveolar proteinosis (PAP) is a rare disease characterized by a progressive accumulation of surfactant in the pulmonary alveoli due to an abnormal production or impaired clearance of surfactant. The main clinical manifestations are progressive dyspnea, hypoxemia, secondary infections, pulmonary fibrosis, and respiratory failure [323]. Based on the underlying etiology, PAP is divided into different subtypes. Congenital PAP is caused by mutations in genes involved in surfactant production. In primary PAP, granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling is disrupted by either GM-CSF autoantibodies (autoimmune PAP) or mutations in the GM-CSF receptor (hereditary PAP), resulting in dysfunction of the alveolar macrophages and impaired clearance of surfactant. Autoimmune PAP accounts for about 90% of all patients with PAP. In secondary PAP, the number and/or function of alveolar macrophages is reduced secondary to an underlying disease (e.g., hematologic malignancy, immune defects), drugs (e.g., immunosuppressive drugs), or external exposures (e.g., infection, fume inhalation) [323]. Secondary PAP has also been described in GATA2 deficiencies, which is discussed in Chap. 8.

7.6.1 Hereditary Pulmonary Alveolar Proteinosis

7.6.1.1 Etiology

GM-CSF signaling is important in the differentiation of alveolar macrophages. The GM-CSF receptor is composed of two subunits. The binding α chain is encoded by *CSF2RA* (OMIM *306250), located in a pseudoautosomal region on the X chromosome (Xp22.33). The common β chain is also found in the receptors for IL-3 and IL-5 and is encoded by *CSF2RB* (OMIM *138981) at chromosome 22q12.3.

Hereditary PAP can be caused by autosomal recessive mutations in either subunit, reducing GM-CSF receptor expression [324–326]. Disease severity is variable, even across family members with the same mutations, suggesting that other factors (genetic and/or environmental) are involved [323].

7.6.1.2 Clinical Features

The clinical picture can vary from asymptomatic to progressive respiratory failure. Symptomatic patients usually present in late infancy or childhood. The onset of disease is insidious. Patients demonstrate progressive dyspnea with or without hypoxemia, cough, production of white sputum, fatigue, and/or weight loss. They also have an increased susceptibly to secondary pulmonary and extrapulmonary infections. Severe cases evolve to pulmonary fibrosis and respiratory failure, needing bilateral lung transplantation [323].

7.6.1.3 Diagnosis

Diagnostic evaluation includes imaging of the lungs. Chest radiography typically shows symmetrical infiltrates in the perihilar regions. Bilateral ground-glass opacities and septal thickening are characteristic findings on high-resolution CT. Bronchoalveolar lavage fluid is milky, counts an excess of macrophages, and contains large amounts of sediment that stains positive with periodic acid-Schiff. The diagnosis is confirmed through genetic testing of *CSF2RA* and *CSF2RB*. The presence of GM-CSF autoantibodies should be excluded [323].

7.6.1.4 Treatment

Patients with moderate symptoms can be treated using whole-lung lavage (WLL), an invasive procedure to physically remove surfactant from the lungs [323]. In severe cases of hereditary PAP, allogeneic HSCT should be considered, although the success rate has been limited [323, 327]. The outcome of bilateral lung transplantation is also variable, and the disease may recur due to repopulation of the transplanted lungs with recipient-origin macrophages [328]. GM-CSF administration is not useful given the GM-CSF receptor deficiency [323].

References

1. Abbas AK, Lichtman AH, Pillai S (2017) Cellular and molecular immunology, 9th edn. Elsevier Saunders, Philadelphia
2. Papayannopoulos V (2018) Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 18(2):134–147
3. Janeway CA, Craig J, Davison M, Doroney W, Gitlin D, Sullivan JC (1954) Hypergammaglobulinemia associated with severe, recurrent, and chronic non-specific infection. Am J Dis Child 88:388–392
4. Kostmann R (1956) Infantile genetic agranulocytosis. A new recessive lethal disease in man. Acta Paediatr Scand 45:1–78
5. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T et al (2018) International Union of Immunological Societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. J Clin Immunol 38(1):96–128

6. Lanini LL, Prader S, Siler U, Reichenbach J (2017) Modern management of phagocyte defects. Pediatr Allergy Immunol 28(2):124–134
7. Seger RA (2008) Modern management of chronic granulomatous disease. Br J Haematol 140(3):255–266
8. Gennery AR, Albert MH, Slatter MA, Lankester A (2019) Hematopoietic stem cell transplantation for primary immunodeficiencies. Front Pediatr 7:445
9. Mukherjee S, Thrasher AJ (2013) Gene therapy for PIDs: progress, pitfalls and prospects. Gene 525(2):174–181
10. Samarghitean C, Ortutay C, Vihinen M (2009) Systematic classification of primary immunodeficiencies based on clinical, pathological, and laboratory parameters. J Immunol 183(11):7569–7575
11. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. In: J Clin Immunol
12. Donadieu J, Fenneteau O, Beaupain B, Mahlaoui N, Chantelot CB (2011) Congenital neutropenia: diagnosis, molecular bases and patient management. Orphanet J Rare Dis 6:26
13. Donadieu J, Beaupain B, Mahlaoui N, Bellanne-Chantelot C (2013) Epidemiology of congenital neutropenia. Hematol Oncol Clin North Am 27(1):1–17, vii
14. Spoor J, Farajifard H, Rezaei N (2019) Congenital neutropenia and primary immunodeficiency diseases. Crit Rev Oncol Hematol 133:149–162
15. Donadieu J, Beaupain B, Fenneteau O, Bellanne-Chantelot C (2017) Congenital neutropenia in the era of genomics: classification, diagnosis, and natural history. Br J Haematol 179(4):557–574
16. Bakhtiar S, Shadur B, Stepensky P (2019) The evidence for allogeneic hematopoietic stem cell transplantation for congenital neutrophil disorders: a comprehensive review by the inborn errors working party group of the EBMT. Front Pediatr 7:436
17. Sullivan KE (2019) Neutropenia as a sign of immunodeficiency. J Allergy Clin Immunol 143(1):96–100
18. Klein C (2011) Genetic defects in severe congenital neutropenia: emerging insights into life and death of human neutrophil granulocytes. Annu Rev Immunol 29:399–413
19. Xia J, Link DC (2008) Severe congenital neutropenia and the unfolded protein response. Curr Opin Hematol 15(1):1–7
20. Dale DC, Person RE, Bolyard AA, Aprikyan AG, Bos C, Bonilla MA et al (2000) Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. Blood 96(7):2317–2322
21. Skokowa J, Dale DC, Touw IP, Zeidler C, Welte K (2017) Severe congenital neutropenias. Nat Rev Dis Primers 3:17032
22. Kollner I, Sodeik B, Schreek S, Heyn H, von Neuhoff N, Germeshausen M et al (2006) Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response. Blood 108(2):493–500
23. Nanua S, Murakami M, Xia J, Grenda DS, Woloszynek J, Strand M et al (2011) Activation of the unfolded protein response is associated with impaired granulopoiesis in transgenic mice expressing mutant Elane. Blood 117(13):3539–3547
24. Nayak RC, Trump LR, Aronow BJ, Myers K, Mehta P, Kalfa T et al (2015) Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem cells. J Clin Invest 125(8):3103–3116
25. Anguita E, Candel FJ, Chaparro A, Roldan-Etcheverry JJ (2017) Transcription factor GFI1B in health and disease. Front Oncol 7:54
26. Zarebski A, Velu CS, Baktula AM, Bourdeau T, Horman SR, Basu S et al (2008) Mutations in growth factor independent-1 associated with human neutropenia block murine granulopoiesis through colony stimulating factor-1. Immunity 28(3):370–380
27. Makaryan V, Rosenthal EA, Bolyard AA, Kelley ML, Below JE, Bamshad MJ et al (2014) TCIRG1-associated congenital neutropenia. Hum Mutat 35(7):824–827

28. Rosenthal EA, Makaryan V, Burt AA, Crosslin DR, Kim DS, Smith JD et al (2016) Association between absolute neutrophil count and variation at TCIRG1: the NHLBI exome sequencing project. Genet Epidemiol 40(6):470–474
29. Carapito R, Konantz M, Paillard C, Miao Z, Pichot A, Leduc MS et al (2017) Mutations in signal recognition particle SRP54 cause syndromic neutropenia with Shwachman-Diamond-like features. J Clin Invest 127(11):4090–4103
30. Bellanne-Chantelot C, Schmaltz-Panneau B, Marty C, Fenneteau O, Callebaut I, Clauin S et al (2018) Mutations in the SRP54 gene cause severe congenital neutropenia as well as Shwachman-Diamond-like syndrome. Blood 132(12):1318–1331
31. Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schaffer AA et al (2007) HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nat Genet 39(1):86–92
32. Klein C (2017) Kostmann's disease and HCLS1-associated protein X-1 (HAX1). J Clin Immunol 37(2):117–122
33. Boztug K, Jarvinen PM, Salzer E, Racek T, Monch S, Garncarz W et al (2014) JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. Nat Genet 46(9):1021–1027
34. Baris S, Karakoc-Aydiner E, Ozen A, Delil K, Kiykim A, Ogulur I et al (2015) JAGN1 deficient severe congenital neutropenia: two cases from the same family. J Clin Immunol 35(4):339–343
35. Stepensky P, Saada A, Cowan M, Tabib A, Fischer U, Berkun Y et al (2013) The Thr224Asn mutation in the VPS45 gene is associated with the congenital neutropenia and primary myelofibrosis of infancy. Blood 121(25):5078–5087
36. Vilboux T, Lev A, Malicdan MC, Simon AJ, Jarvinen P, Racek T et al (2013) A congenital neutrophil defect syndrome associated with mutations in VPS45. N Engl J Med 369(1):54–65
37. Meerschaut I, Bordon V, Dhooge C, Delbeke P, Vanlander AV, Simon A et al (2015) Severe congenital neutropenia with neurological impairment due to a homozygous VPS45 p.E238K mutation: a case report suggesting a genotype-phenotype correlation. Am J Med Genet A 167a(12):3214–3218
38. Shadur B, Asherie N, Newburger PE, Stepensky P (2019) How we approach: severe congenital neutropenia and myelofibrosis due to mutations in VPS45. Pediatr Blood Cancer 66(1):e27473
39. Triot A, Jarvinen PM, Arostegui JI, Murugan D, Kohistani N, Dapena Diaz JL et al (2014) Inherited biallelic CSF3R mutations in severe congenital neutropenia. Blood 123(24):3811–3817
40. Devriendt K, Kim AS, Mathijs G, Frints SG, Schwartz M, Van Den Oord JJ et al (2001) Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet 27(3):313–317
41. Westerberg LS, Meelu P, Baptista M, Eston MA, Adamovich DA, Cotta-de-Almeida V et al (2010) Activating WASP mutations associated with X-linked neutropenia result in enhanced actin polymerization, altered cytoskeletal responses, and genomic instability in lymphocytes. J Exp Med 207(6):1145–1152
42. Link DC (2019) Mechanisms of leukemic transformation in congenital neutropenia. Curr Opin Hematol 26(1):34–40
43. Touw IP, van de Geijn GJ (2007) Granulocyte colony-stimulating factor and its receptor in normal myeloid cell development, leukemia and related blood cell disorders. Front Biosci 12:800–815
44. Fioredda F, Iacobelli S, van Biezen A, Gaspar B, Ancliff P, Donadieu J et al (2015) Stem cell transplantation in severe congenital neutropenia: an analysis from the European Society for Blood and Marrow Transplantation. Blood 126(16):1885–1892; quiz 1970
45. Dale DC, Bolyard AA, Aprikyan A (2002) Cyclic neutropenia. Semin Hematol 39(2):89–94
46. Rezaei N, Farhoudi A, Pourpak Z, Aghamohammadi A, Ramyar A, Moin M et al (2004) Clinical and laboratory findings in Iranian children with cyclic neutropenia. Iran J Allergy Asthma Immunol 3(1):37–40

47. Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC (1999) Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. Nat Genet 23(4):433–436
48. Cipe FE, Celiksoy MH, Erturk B, Aydogmus C (2018) Cyclic manner of neutropenia in a patient with HAX-1 mutation. Pediatr Hematol Oncol 35(3):181–185
49. Palmer SE, Stephens K, Dale DC (1996) Genetics, phenotype, and natural history of autosomal dominant cyclic hematopoiesis. Am J Med Genet 66(4):413–422
50. Dale DC, Bolyard A, Marrero T, Makaryan V, Bonilla M, Link DC et al (2017) Long-term effects of G-CSF therapy in cyclic neutropenia. N Engl J Med 377(23):2290–2292
51. Loughran TP Jr, WPt H (1986) Adult-onset cyclic neutropenia is a benign neoplasm associated with clonal proliferation of large granular lymphocytes. J Exp Med 164(6):2089–2094
52. Bohn G, Allroth A, Brandes G, Thiel J, Glocker E, Schaffer AA et al (2007) A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein p14. Nat Med 13(1):38–45
53. Langemeier J, Schrom EM, Rabner A, Radtke M, Zychlinski D, Saborowski A et al (2012) A complex immunodeficiency is based on U1 snRNP-mediated poly(A) site suppression. EMBO J 31(20):4035–4044
54. Bodian M, Sheldon W, Lightwood R (1964) Congenital hypoplasia of the exocrine pancreas. Acta Paediatr 53:282–293
55. Shwachman H, Diamond LK, Oski FA, Khaw KT (1964) The syndrome of pancreatic insufficiency and bone marrow dysfunction. J Pediatr 65:645–663
56. Minelli A, Nicolis E, Cannioto Z, Longoni D, Perobelli S, Pasquali F et al (2012) Incidence of Shwachman-Diamond syndrome. Pediatr Blood Cancer 59(7):1334–1335
57. Nelson AS, Myers KC (2018) Diagnosis, treatment, and molecular pathology of Shwachman-Diamond Syndrome. Hematol Oncol Clin North Am 32(4):687–700
58. Oyarbide U, Corey SJ (2018) SRP54 and a need for a new neutropenia nosology. Blood 132(12):1220–1222
59. Bezzerri V, Cipolli M (2019) Shwachman-Diamond Syndrome: molecular mechanisms and current perspectives. Mol Diagn Ther 23(2):281–290
60. Boocock GR, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR et al (2003) Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet 33(1):97–101
61. Zhang S, Shi M, Hui CC, Rommens JM (2006) Loss of the mouse ortholog of the shwachman-diamond syndrome gene (Sbds) results in early embryonic lethality. Mol Cell Biol 26(17):6656–6663
62. Dhanraj S, Matveev A, Li H, Lauhasurayotin S, Jardine L, Cada M et al (2017) Biallelic mutations in DNAJC21 cause Shwachman-Diamond syndrome. Blood 129(11):1557–1562
63. Stepensky P, Chacon-Flores M, Kim KH, Abuzaitoun O, Bautista-Santos A, Simanovsky N et al (2017) Mutations in EFL1, an SBDS partner, are associated with infantile pancytopenia, exocrine pancreatic insufficiency and skeletal anomalies in aShwachman-Diamond like syndrome. J Med Genet 54(8):558–566
64. Tummala H, Walne AJ, Williams M, Bockett N, Collopy L, Cardoso S et al (2016) DNAJC21 mutations link a cancer-prone bone marrow failure syndrome to corruption in 60S ribosome subunit maturation. Am J Hum Genet 99(1):115–124
65. D'Amours G, Lopes F, Gauthier J, Saillour V, Nassif C, Wynn R et al (2018) Refining the phenotype associated with biallelic DNAJC21 mutations. Clin Genet 94(2):252–258
66. Myers KC, Furutani E, Weller E, Siegele B, Galvin A, Arsenault V et al (2019) Clinical features and outcomes of patients with Shwachman-Diamond syndrome and myelodysplastic syndrome or acute myeloid leukaemia: a multicentre, retrospective, cohort study. Lancet Haematol 7:e238
67. Tan QK, Cope H, Spillmann RC, Stong N, Jiang YH, McDonald MT et al (2018) Further evidence for the involvement of EFL1 in a Shwachman-Diamond-like syndrome and expansion of the phenotypic features. Cold Spring Harb Mol Case Stud 4(5)
68. Tan S, Kermasson L, Hoslin A, Jaako P, Faille A, Acevedo-Arozena A et al (2019) EFL1 mutations impair eIF6 release to cause Shwachman-Diamond syndrome. Blood 134(3):277–290

69. Rothbaum R, Perrault J, Vlachos A, Cipolli M, Alter BP, Burroughs S et al (2002) Shwachman-Diamond syndrome: report from an international conference. J Pediatr 141(2):266–270
70. Dror Y, Donadieu J, Koglmeier J, Dodge J, Toiviainen-Salo S, Makitie O et al (2011) Draft consensus guidelines for diagnosis and treatment of Shwachman-Diamond syndrome. Ann N Y Acad Sci 1242:40–55
71. Hashmi SK, Allen C, Klaassen R, Fernandez CV, Yanofsky R, Shereck E et al (2011) Comparative analysis of Shwachman-Diamond syndrome to other inherited bone marrow failure syndromes and genotype-phenotype correlation. Clin Genet 79(5):448–458
72. Ramsay J, Morton J, Norris M, Kanungo S (2018) Organic acid disorders. Ann Transl Med 6(24):472
73. Saric A, Andreau K, Armand AS, Moller IM, Petit PX (2015) Barth syndrome: from mitochondrial dysfunctions associated with aberrant production of reactive oxygen species to pluripotent stem cell studies. Front Genet 6:359
74. Wortmann SB, Zietkiewicz S, Kousi M, Szklarczyk R, Haack TB, Gersting SW et al (2015) CLPB mutations cause 3-methylglutaconic aciduria, progressive brain atrophy, intellectual disability, congenital neutropenia, cataracts, movement disorder. Am J Hum Genet 96(2):245–257
75. Steward CG, Groves SJ, Taylor CT, Maisenbacher MK, Versluys B, Newbury-Ecob RA et al (2019) Neutropenia in Barth syndrome: characteristics, risks, and management. Curr Opin Hematol 26(1):6–15
76. Chou JY, Jun HS, Mansfield BC (2015) Type I glycogen storage diseases: disorders of the glucose-6-phosphatase/glucose-6-phosphate transporter complexes. J Inherit Metab Dis 38(3):511–519
77. Chou JY, Jun HS, Mansfield BC (2010) Neutropenia in type Ib glycogen storage disease. Curr Opin Hematol 17(1):36–42
78. Kim GY, Lee YM, Kwon JH, Jun HS, Chou J (2017) Glycogen storage disease type Ib neutrophils exhibit impaired cell adhesion and migration. Biochem Biophys Res Commun 482(4):569–574
79. Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GP (2002) Guidelines for management of glycogen storage disease type I—European study on glycogen storage disease type I (ESGSD I). Eur J Pediatr 161(Suppl 1):S112–S119
80. Visser G, Rake JP, Labrune P, Leonard JV, Moses S, Ullrich K et al (2002) Consensus guidelines for management of glycogen storage disease type 1b - European study on glycogen storage disease type 1. Eur J Pediatr 161(Suppl 1):S120–S123
81. Adachi M, Shinkai M, Ohhama Y, Tachibana K, Kuratsuji T, Saji H et al (2004) Improved neutrophil function in a glycogen storage disease type 1b patient after liver transplantation. Eur J Pediatr 163(4–5):202–206
82. Dale DC, Bolyard AA, Marrero T, Kelley ML, Makaryan V, Tran E et al (2019) Neutropenia in glycogen storage disease Ib: outcomes for patients treated with granulocyte colony-stimulating factor. Curr Opin Hematol 26(1):16–21
83. Pierre G, Chakupurakal G, McKiernan P, Hendriksz C, Lawson S, Chakrapani A (2008) Bone marrow transplantation in glycogen storage disease type 1b. J Pediatr 152(2):286–288
84. Chou JY, Cho JH, Kim GY, Mansfield BC (2018) Molecular biology and gene therapy for glycogen storage disease type Ib. J Inherit Metab Dis 41(6):1007–1014
85. Boztug K, Rosenberg PS, Dorda M, Banka S, Moulton T, Curtin J et al (2012) Extended spectrum of human glucose-6-phosphatase catalytic subunit 3 deficiency: novel genotypes and phenotypic variability in severe congenital neutropenia. J Pediatr 160(4):679–683.e672
86. Desplantes C, Fremond ML, Beaupain B, Harousseau JL, Buzyn A, Pellier I et al (2014) Clinical spectrum and long-term follow-up of 14 cases with G6PC3 mutations from the French severe congenital neutropenia registry. Orphanet J Rare Dis 9:183
87. Banka S, Wynn R, Byers H, Arkwright PD, Newman WG (2013) G6PC3 mutations cause non-syndromic severe congenital neutropenia. Mol Genet Metab 108(2):138–141
88. Rodrigues JM, Fernandes HD, Caruthers C, Braddock SR, Knutsen AP (2018) Cohen syndrome: review of the literature. Cureus 10(9):e3330

89. Seifert W, Kuhnisch J, Maritzen T, Horn D, Haucke V, Hennies HC (2011) Cohen syndrome-associated protein, COH1, is a novel, giant Golgi matrix protein required for Golgi integrity. J Biol Chem 286(43):37665–37675
90. Duplomb L, Duvet S, Picot D, Jego G, El Chehadeh-Djebbar S, Marle N et al (2014) Cohen syndrome is associated with major glycosylation defects. Hum Mol Genet 23(9):2391–2399
91. Chandler KE, Kidd A, Al-Gazali L, Kolehmainen J, Lehesjoki AE, Black GC et al (2003) Diagnostic criteria, clinical characteristics, and natural history of Cohen syndrome. J Med Genet 40(4):233–241
92. Parri V, Katzaki E, Uliana V, Scionti F, Tita R, Artuso R et al (2010) High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome. Eur J Hum Genet 18(10):1133–1140
93. Volpi L, Roversi G, Colombo EA, Leijsten N, Concolino D, Calabria A et al (2010) Targeted next-generation sequencing appoints c16orf57 as clericuzio-type poikiloderma with neutropenia gene. Am J Hum Genet 86(1):72–76
94. Colombo EA, Carra S, Fontana L, Bresciani E, Cotelli F, Larizza L (2015) A zebrafish model of Poikiloderma with neutropenia recapitulates the human syndrome hallmarks and traces back neutropenia to the myeloid progenitor. Sci Rep 5:15814
95. Patil P, Uechi T, Kenmochi N (2015) Incomplete splicing of neutrophil-specific genes affects neutrophil development in a zebrafish model of poikiloderma with neutropenia. RNA Biol 12(4):426–434
96. Van Hove JL, Jaeken J, Proesmans M, Boeck KD, Minner K, Matthijs G et al (2005) Clericuzio type poikiloderma with neutropenia is distinct from Rothmund-Thomson syndrome. Am J Med Genet A 132a(2):152–158
97. Chen X, Yang Y, Lin Z (2019) Image gallery: Poikiloderma with neutropenia. Br J Dermatol 180(1):e6
98. Koparir A, Gezdirici A, Koparir E, Ulucan H, Yilmaz M, Erdemir A et al (2014) Poikiloderma with neutropenia: genotype-ethnic origin correlation, expanding phenotype and literature review. Am J Med Genet A 164a(10):2535–2540
99. Farruggia P, Indaco S, Dufour C, Lanza T, Mosa C, Macaluso A et al (2014) Poikiloderma with neutropenia: a case report and review of the literature. J Pediatr Hematol Oncol 36(4):297–300
100. Haapaniemi EM, Fogarty CL, Keskitalo S, Katayama S, Vihinen H, Ilander M et al (2017) Combined immunodeficiency and hypoglycemia associated with mutations in hypoxia upregulated 1. J Allergy Clin Immunol 139(4):1391–1393.e1311
101. van de Vijver E, van den Berg TK, Kuijpers TW (2013) Leukocyte adhesion deficiencies. Hematol Oncol Clin North Am 27(1):101–116, viii
102. Halbwachs L, Lesavre P (2012) Endothelium-neutrophil interactions in ANCA-associated diseases. J Am Soc Nephrol 23(9):1449–1461
103. Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 7(9):678–689
104. Schmidt S, Moser M, Sperandio M (2013) The molecular basis of leukocyte recruitment and its deficiencies. Mol Immunol 55(1):49–58
105. Ferreira CP, Cariste LM, Santos Virgilio FD, Moraschi BF, Monteiro CB, Vieira Machado AM et al (2017) LFA-1 mediates cytotoxicity and tissue migration of specific CD8(+) T cells after heterologous prime-boost vaccination against Trypanosoma cruzi infection. Front Immunol 8:1291
106. Almarza Novoa E, Kasbekar S, Thrasher AJ, Kohn DB, Sevilla J, Nguyen T et al (2018) Leukocyte adhesion deficiency-I: A comprehensive review of all published cases. J Allergy Clin Immunol Pract 6(4):1418–1420.e1410
107. van de Vijver E, Maddalena A, Sanal O, Holland SM, Uzel G, Madkaikar M et al (2012) Hematologically important mutations: leukocyte adhesion deficiency (first update). Blood Cells Mol Dis 48(1):53–61

108. Cabanillas D, Regairaz L, Deswarte C, Garcia M, Richard ME, Casanova JL et al (2016) Leukocyte adhesion deficiency type 1 (LAD1) with expressed but nonfunctional CD11/CD18. J Clin Immunol 36(7):627–630
109. Dababneh R, Al-Wahadneh AM, Hamadneh S, Khouri A, Bissada NF (2008) Periodontal manifestation of leukocyte adhesion deficiency type I. J Periodontol 79(4):764–768
110. Cox DP, Weathers DR (2008) Leukocyte adhesion deficiency type 1: an important consideration in the clinical differential diagnosis of prepubertal periodontitis. A case report and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 105(1):86–90
111. Moutsopoulos NM, Konkel J, Sarmadi M, Eskan MA, Wild T, Dutzan N et al (2014) Defective neutrophil recruitment in leukocyte adhesion deficiency type I disease causes local IL-17-driven inflammatory bone loss. Sci Transl Med 6(229):229ra240
112. De Rose DU, Giliani S, Notarangelo LD, Lougaris V, Lanfranchi A, Moratto D et al (2018) Long term outcome of eight patients with type 1 leukocyte adhesion deficiency (LAD-1): not only infections, but high risk of autoimmune complications. Clin Immunol 191:75–80
113. Razvi S, Murphy R, Shlasko E, Cunningham-Rundles C (2001) Delayed separation of the umbilical cord attributable to urachal anomalies. Pediatrics 108(2):493–494
114. Shigeoka A (1999) Delayed umbilical cord separation is NOT commonly associated with leukocyte adhesion deficiency. Pediatr Res 45(12)
115. Levy-Mendelovich S, Rechavi E, Abuzaitoun O, Vernitsky H, Simon AJ, Lev A et al (2016) Highlighting the problematic reliance on CD18 for diagnosing leukocyte adhesion deficiency type 1. Immunol Res 64(2):476–482
116. Cano PM, Vargas A, Lavoie JP (2016) A real-time assay for neutrophil chemotaxis. BioTechniques 60(5):245–251
117. Qasim W, Cavazzana-Calvo M, Davies EG, Davis J, Duval M, Eames G et al (2009) Allogeneic hematopoietic stem-cell transplantation for leukocyte adhesion deficiency. Pediatrics 123(3):836–840
118. Hamidieh AA, Pourpak Z, Hosseinzadeh M, Fazlollahi MR, Alimoghaddam K, Movahedi M et al (2012) Reduced-intensity conditioning hematopoietic SCT for pediatric patients with LAD-1: clinical efficacy and importance of chimerism. Bone Marrow Transplant 47(5):646–650
119. Bauer TR Jr, Hickstein DD (2000) Gene therapy for leukocyte adhesion deficiency. Curr Opin Mol Ther 2(4):383–388
120. Etzioni A, Frydman M, Pollack S, Avidor I, Phillips ML, Paulson JC et al (1992) Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency. N Engl J Med 327(25):1789–1792
121. Marquardt T, Brune T, Luhn K, Zimmer KP, Korner C, Fabritz L et al (1999) Leukocyte adhesion deficiency II syndrome, a generalized defect in fucose metabolism. J Pediatr 134(6):681–688
122. Gazit Y, Mory A, Etzioni A, Frydman M, Scheuerman O, Gershoni-Baruch R et al (2010) Leukocyte adhesion deficiency type II: long-term follow-up and review of the literature. J Clin Immunol 30(2):308–313
123. Phillips ML, Schwartz BR, Etzioni A, Bayer R, Ochs HD, Paulson JC et al (1995) Neutrophil adhesion in leukocyte adhesion deficiency syndrome type 2. J Clin Invest 96(6):2898–2906
124. Luhn K, Wild MK, Eckhardt M, Gerardy-Schahn R, Vestweber D (2001) The gene defective in leukocyte adhesion deficiency II encodes a putative GDP-fucose transporter. Nat Genet 28(1):69–72
125. Lubke T, Marquardt T, Etzioni A, Hartmann E, von Figura K, Korner C (2001) Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. Nat Genet 28(1):73–76
126. Cagdas D, Yilmaz M, Kandemir N, Tezcan I, Etzioni A, Sanal O (2014) A novel mutation in leukocyte adhesion deficiency type II/CDGIIc. J Clin Immunol 34(8):1009–1014
127. Marquardt T, Luhn K, Srikrishna G, Freeze HH, Harms E, Vestweber D (1999) Correction of leukocyte adhesion deficiency type II with oral fucose. Blood 94(12):3976–3985

128. Hidalgo A, Ma S, Peired AJ, Weiss LA, Cunningham-Rundles C, Frenette PS (2003) Insights into leukocyte adhesion deficiency type 2 from a novel mutation in the GDP-fucose transporter gene. Blood 101(5):1705–1712
129. Luhn K, Marquardt T, Harms E, Vestweber D (2001) Discontinuation of fucose therapy in LADII causes rapid loss of selectin ligands and rise of leukocyte counts. Blood 97(1):330–332
130. Wild MK, Luhn K, Marquardt T, Vestweber D (2002) Leukocyte adhesion deficiency II: therapy and genetic defect. Cells Tissues Organs 172(3):161–173
131. Kuijpers TW, van de Vijver E, Weterman MA, de Boer M, Tool AT, van den Berg TK et al (2009) LAD-1/variant syndrome is caused by mutations in FERMT3. Blood 113(19):4740–4746
132. Malinin NL, Zhang L, Choi J, Ciocea A, Razorenova O, Ma YQ et al (2009) A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans. Nat Med 15(3):313–318
133. Svensson L, Howarth K, McDowall A, Patzak I, Evans R, Ussar S et al (2009) Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. Nat Med 15(3):306–312
134. Malinin NL, Plow EF, Byzova TV (2010) Kindlins in FERM adhesion. Blood 115(20):4011–4017
135. Kuijpers TW, Van Lier RA, Hamann D, de Boer M, Thung LY, Weening RS et al (1997) Leukocyte adhesion deficiency type 1 (LAD-1)/variant. A novel immunodeficiency syndrome characterized by dysfunctional beta2 integrins. J Clin Invest 100(7):1725–1733
136. Gruda R, Brown AC, Grabovsky V, Mizrahi S, Gur C, Feigelson SW et al (2012) Loss of kindlin-3 alters the threshold for NK cell activation in human leukocyte adhesion deficiency-III. Blood 120(19):3915–3924
137. van de Vijver E, De Cuyper IM, Gerrits AJ, Verhoeven AJ, Seeger K, Gutierrez L et al (2012) Defects in Glanzmann thrombasthenia and LAD-III (LAD-1/v) syndrome: the role of integrin beta1 and beta3 in platelet adhesion to collagen. Blood 119(2):583–586
138. Bergmeier W, Goerge T, Wang HW, Crittenden JR, Baldwin AC, Cifuni SM et al (2007) Mice lacking the signaling molecule CalDAG-GEFI represent a model for leukocyte adhesion deficiency type III. J Clin Invest 117(6):1699–1707
139. Pasvolsky R, Feigelson SW, Kilic SS, Simon AJ, Tal-Lapidot G, Grabovsky V et al (2007) A LAD-III syndrome is associated with defective expression of the Rap-1 activator CalDAG-GEFI in lymphocytes, neutrophils, and platelets. J Exp Med 204(7):1571–1582
140. Canault M, Ghalloussi D, Grosdidier C, Guinier M, Perret C, Chelghoum N et al (2014) Human CalDAG-GEFI gene (RASGRP2) mutation affects platelet function and causes severe bleeding. J Exp Med 211(7):1349–1362
141. Kato H, Nakazawa Y, Kurokawa Y, Kashiwagi H, Morikawa Y, Morita D et al (2016) Human CalDAG-GEFI deficiency increases bleeding and delays alphaIIbbeta3 activation. Blood 128(23):2729–2733
142. Etzioni A (2014) Leukocyte adhesion deficiency III—when integrins activation fails. J Clin Immunol 34(8):900–903
143. Kuijpers TW, van Bruggen R, Kamerbeek N, Tool AT, Hicsonmez G, Gurgey A et al (2007) Natural history and early diagnosis of LAD-1/variant syndrome. Blood 109(8):3529–3537
144. Schmidt S, Nakchbandi I, Ruppert R, Kawelke N, Hess MW, Pfaller K et al (2011) Kindlin-3-mediated signaling from multiple integrin classes is required for osteoclast-mediated bone resorption. J Cell Biol 192(5):883–897
145. Saultier P, Szepetowski S, Canault M, Falaise C, Poggi M, Suchon P et al (2018) Long-term management of leukocyte adhesion deficiency type III without hematopoietic stem cell transplantation. Haematologica 103(6):e264–e267
146. Stepensky PY, Wolach B, Gavrieli R, Rousso S, Ben Ami T, Goldman V et al (2015) Leukocyte adhesion deficiency type III: clinical features and treatment with stem cell transplantation. J Pediatr Hematol Oncol 37(4):264–268
147. Troeger A, Williams DA (2013) Hematopoietic-specific rho GTPases Rac2 and RhoH and human blood disorders. Exp Cell Res 319(15):2375–2383

148. Roberts AW, Kim C, Zhen L, Lowe JB, Kapur R, Petryniak B et al (1999) Deficiency of the hematopoietic cell-specific Rho family GTPase Rac2 is characterized by abnormalities in neutrophil function and host defense. Immunity 10(2):183–196
149. Gu Y, Jia B, Yang FC, D'Souza M, Harris CE, Derrow CW et al (2001) Biochemical and biological characterization of a human Rac2 GTPase mutant associated with phagocytic immunodeficiency. J Biol Chem 276(19):15929–15938
150. Williams DA, Tao W, Yang F, Kim C, Gu Y, Mansfield P et al (2000) Dominant negative mutation of the hematopoietic-specific Rho GTPase, Rac2, is associated with a human phagocyte immunodeficiency. Blood 96(5):1646–1654
151. Ambruso DR, Knall C, Abell AN, Panepinto J, Kurkchubasche A, Thurman G et al (2000) Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. Proc Natl Acad Sci U S A 97(9):4654–4659
152. Kurkchubasche AG, Panepinto JA, Tracy TF Jr, Thurman GW, Ambruso DR (2001) Clinical features of a human Rac2 mutation: a complex neutrophil dysfunction disease. J Pediatr 139(1):141–147
153. Accetta D, Syverson G, Bonacci B, Reddy S, Bengtson C, Surfus J et al (2011) Human phagocyte defect caused by a Rac2 mutation detected by means of neonatal screening for T-cell lymphopenia. J Allergy Clin Immunol 127(2):535. –538.e531–532
154. Bogaert DJ, Dullaers M, Lambrecht BN, Vermaelen KY, De Baere E, Haerynck F (2016) Genes associated with common variable immunodeficiency: one diagnosis to rule them all? J Med Genet 53(9):575–590
155. Alkhairy OK, Rezaei N, Graham RR, Abolhassani H, Borte S, Hultenby K et al (2015) RAC2 loss-of-function mutation in 2 siblings with characteristics of common variable immunodeficiency. J Allergy Clin Immunol 135(5):1380. –1384.e1381–1385
156. Lougaris V, Chou J, Beano A, Wallace JG, Baronio M, Gazzurelli L et al (2019) A monoallelic activating mutation in RAC2 resulting in a combined immunodeficiency. J Allergy Clin Immunol 143(4):1649–1653.e1643
157. Hsu AP, Donko A, Arrington ME, Swamydas M, Fink D, Das A et al (2019) Dominant activating RAC2 mutation with lymphopenia, immunodeficiency, and cytoskeletal defects. Blood 133(18):1977–1988
158. Nunoi H, Yamazaki T, Tsuchiya H, Kato S, Malech HL, Matsuda I et al (1999) A heterozygous mutation of beta-actin associated with neutrophil dysfunction and recurrent infection. Proc Natl Acad Sci U S A 96(15):8693–8698
159. Hundt N, Preller M, Swolski O, Ang AM, Mannherz HG, Manstein DJ et al (2014) Molecular mechanisms of disease-related human beta-actin mutations p.R183W and p.E364K. FEBS J 281(23):5279–5291
160. Rommelaere H, Waterschoot D, Neirynck K, Vandekerckhove J, Ampe C (2004) A method for rapidly screening functionality of actin mutants and tagged actins. Biol Proced Online 6:235–249
161. Sandestig A, Green A, Jonasson J, Vogt H, Wahlstrom J, Pepler A et al (2019) Could dissimilar phenotypic effects of ACTB missense mutations reflect the actin conformational change? Two novel mutations and literature review. Mol Syndromol 9(5):259–265
162. Nunoi H, Yamazaki T, Kanegasaki S (2001) Neutrophil cytoskeletal disease. Int J Hematol 74(2):119–124
163. Kuhns DB, Fink DL, Choi U, Sweeney C, Lau K, Priel DL et al (2016) Cytoskeletal abnormalities and neutrophil dysfunction in WDR1 deficiency. Blood 128(17):2135–2143
164. Standing AS, Malinova D, Hong Y, Record J, Moulding D, Blundell MP et al (2017) Autoinflammatory periodic fever, immunodeficiency, and thrombocytopenia (PFIT) caused by mutation in actin-regulatory gene WDR1. J Exp Med 214(1):59–71
165. Pfajfer L, Mair NK, Jimenez-Heredia R, Genel F, Gulez N, Ardeniz O et al (2018) Mutations affecting the actin regulator WD repeat-containing protein 1 lead to aberrant lymphoid immunity. J Allergy Clin Immunol 142(5):1589–1604.e1511

166. Ma Z, Morris SW, Valentine V, Li M, Herbrick JA, Cui X et al (2001) Fusion of two novel genes, RBM15 and MKL1, in the t(1;22)(p13;q13) of acute megakaryoblastic leukemia. Nat Genet 28(3):220–221
167. Filippi MD (2015) Neutrophil actin regulation: MKL1 is in control. Blood 126(13):1519–1520
168. Record J, Malinova D, Zenner HL, Plagnol V, Nowak K, Syed F et al (2015) Immunodeficiency and severe susceptibility to bacterial infection associated with a loss-of-function homozygous mutation of MKL1. Blood 126(13):1527–1535
169. Toomes C, James J, Wood AJ, Wu CL, McCormick D, Lench N et al (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. Nat Genet 23(4):421–424
170. Hart TC, Hart PS, Michalec MD, Zhang Y, Marazita ML, Cooper M et al (2000) Localisation of a gene for prepubertal periodontitis to chromosome 11q14 and identification of a cathepsin C gene mutation. J Med Genet 37(2):95–101
171. Dalgic B, Bukulmez A, Sari S (2011) Eponym: Papillon-Lefevre syndrome. Eur J Pediatr 170(6):689–691
172. de Haar SF, Hiemstra PS, van Steenbergen MT, Everts V, Beertsen W (2006) Role of polymorphonuclear leukocyte-derived serine proteinases in defense against Actinobacillus actinomycetemcomitans. Infect Immun 74(9):5284–5291
173. Ghaffer KA, Zahran FM, Fahmy HM, Brown RS (1999) Papillon-Lefevre syndrome: neutrophil function in 15 cases fron 4 families in Egypt. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 88(3):320–325
174. Fageeh HN (2018) Papillon-Lefevre Syndrome: a rare case report of two brothers and review of the literature. Int J Clin Pediatr Dent 11(4):352–355
175. Wiebe CB, Hakkinen L, Putnins EE, Walsh P, Larjava HS (2001) Successful periodontal maintenance of a case with Papillon-Lefevre syndrome: 12-year follow-up and review of the literature. J Periodontol 72(6):824–830
176. Albandar JM, Khattab R, Monem F, Barbuto SM, Paster BJ (2012) The subgingival microbiota of Papillon-Lefevre syndrome. J Periodontol 83(7):902–908
177. Dhanawade SS, Shah SD, Kakade GM (2009) Papillon-lefevre syndrome with liver abscess. Indian Pediatr 46(8):723–725
178. Kanthimathinathan HK, Browne F, Ramirez R, McKaig S, Debelle G, Martin J et al (2013) Multiple cerebral abscesses in Papillon-Lefevre syndrome. Childs Nerv Syst 29(8):1227–1229
179. Morgan RD, Hannon E, Lakhoo K (2011) Renal abscess in Papillion-Lefevre syndrome. Pediatr Surg Int 27(12):1381–1383
180. Hamon Y, Legowska M, Fergelot P, Dallet-Choisy S, Newell L, Vanderlynden L et al (2016) Analysis of urinary cathepsin C for diagnosing Papillon-Lefevre syndrome. FEBS J 283(3):498–509
181. Tinanoff N, Tempro P, Maderazo EG (1995) Dental treatment of Papillon-Lefevre syndrome: 15-year follow-up. J Clin Periodontol 22(8):609–612
182. Etoz OA, Ulu M, Kesim B (2010) Treatment of patient with Papillon-Lefevre syndrome with short dental implants: a case report. Implant Dent 19(5):394–399
183. Toygar HU, Kircelli C, Firat E, Guzeldemir E (2007) Combined therapy in a patient with Papillon-Lefevre syndrome: a 13-year follow-up. J Periodontol 78(9):1819–1824
184. Nickles K, Schacher B, Ratka-Kruger P, Krebs M, Eickholz P (2013) Long-term results after treatment of periodontitis in patients with Papillon-Lefevre syndrome: success and failure. J Clin Periodontol 40(8):789–798
185. Kressin S, Herforth A, Preis S, Wahn V, Lenard HG (1995) Papillon-Lefevre syndrome—successful treatment with a combination of retinoid and concurrent systematic periodontal therapy: case reports. Quintessence Int 26(11):795–803
186. Fine DH, Patil AG, Loos BG (2018) Classification and diagnosis of aggressive periodontitis. J Periodontol 89(Suppl 1):S103–s119
187. Oh TJ, Eber R, Wang HL (2002) Periodontal diseases in the child and adolescent. J Clin Periodontol 29(5):400–410

188. Goncalves PF, Harris TH, Elmariah T, Aukhil I, Wallace MR, Shaddox LM (2018) Genetic polymorphisms and periodontal disease in populations of African descent: a review. J Periodontal Res 53(2):164–173
189. Hewitt C, McCormick D, Linden G, Turk D, Stern I, Wallace I et al (2004) The role of cathepsin C in Papillon-Lefevre syndrome, prepubertal periodontitis, and aggressive periodontitis. Hum Mutat 23(3):222–228
190. Seifert R, Wenzel-Seifert K (2001) Defective Gi protein coupling in two formyl peptide receptor mutants associated with localized juvenile periodontitis. J Biol Chem 276(45):42043–42049
191. Seymour RA, Heasman PA (1995) Pharmacological control of periodontal disease. II. Antimicrobial agents. J Dent 23(1):5–14
192. Roos D (2019) Chronic granulomatous disease. Methods Mol Biol 1982:531–542
193. Bridges RA, Berendes H, Good RA (1959) A fatal granulomatous disease of childhood; the clinical, pathological, and laboratory features of a new syndrome. Am J Dis Child 97(4):387–408
194. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L et al (2009) Chronic granulomatous disease: the European experience. PLoS One 4(4):e5234
195. Liese J, Kloos S, Jendrossek V, Petropoulou T, Wintergerst U, Notheis G et al (2000) Long-term follow-up and outcome of 39 patients with chronic granulomatous disease. J Pediatr 137(5):687–693
196. Winkelstein JA, Marino MC, Johnston RB Jr, Boyle J, Curnutte J, Gallin JI et al (2000) Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore) 79(3):155–169
197. Cale CM, Jones AM, Goldblatt D (2000) Follow up of patients with chronic granulomatous disease diagnosed since 1990. Clin Exp Immunol 120(2):351–355
198. de Oliveira-Junior EB, Zurro NB, Prando C, Cabral-Marques O, Pereira PV, Schimke LF et al (2015) Clinical and genotypic spectrum of chronic granulomatous disease in 71 Latin American patients: first report from the LASID registry. Pediatr Blood Cancer 62(12):2101–2107
199. Roos D, Kuhns DB, Maddalena A, Roesler J, Lopez JA, Ariga T et al (2010) Hematologically important mutations: X-linked chronic granulomatous disease (third update). Blood Cells Mol Dis 45(3):246–265
200. Singel KL, Segal BH (2016) NOX2-dependent regulation of inflammation. Clin Sci (Lond) 130(7):479–490
201. Thomas DC (2017) The phagocyte respiratory burst: historical perspectives and recent advances. Immunol Lett 192:88–96
202. Reeves EP, Lu H, Jacobs HL, Messina CG, Bolsover S, Gabella G et al (2002) Killing activity of neutrophils is mediated through activation of proteases by K+ flux. Nature 416(6878):291–297
203. Bianchi M, Hakkim A, Brinkmann V, Siler U, Seger RA, Zychlinsky A et al (2009) Restoration of NET formation by gene therapy in CGD controls aspergillosis. Blood 114(13):2619–2622
204. Arnadottir GA, Norddahl GL, Gudmundsdottir S, Agustsdottir AB, Sigurdsson S, Jensson BO et al (2018) A homozygous loss-of-function mutation leading to CYBC1 deficiency causes chronic granulomatous disease. Nat Commun 9(1):4447
205. Thomas DC, Charbonnier LM, Schejtman A, Aldhekri H, Coomber EL, Dufficy ER et al (2019) EROS/CYBC1 mutations: Decreased NADPH oxidase function and chronic granulomatous disease. J Allergy Clin Immunol 143(2):782–785.e781
206. Ariga T, Furuta H, Cho K, Sakiyama Y (1998) Genetic analysis of 13 families with X-linked chronic granulomatous disease reveals a low proportion of sporadic patients and a high proportion of sporadic carriers. Pediatr Res 44(1):85–92
207. Roos D, de Boer M, Kuribayashi F, Meischl C, Weening RS, Segal AW et al (1996) Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. Blood 87(5):1663–1681

208. Wolach B, Gavrieli R, de Boer M, Gottesman G, Ben-Ari J, Rottem M et al (2008) Chronic granulomatous disease in Israel: clinical, functional and molecular studies of 38 patients. Clin Immunol 129(1):103–114
209. Kutukculer N, Aykut A, Karaca NE, Durmaz A, Aksu G, Genel F et al (2019) Chronic granulamatous disease: two decades of experience from a paediatric immunology unit in a country with high rate of consangineous marriages. Scand J Immunol 89(2):e12737
210. Matute JD, Arias AA, Wright NA, Wrobel I, Waterhouse CC, Li XJ et al (2009) A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40 phox and selective defects in neutrophil NADPH oxidase activity. Blood 114(15):3309–3315
211. van de Geer A, Nieto-Patlan A, Kuhns DB, Tool AT, Arias AA, Bouaziz M et al (2018) Inherited p40phox deficiency differs from classic chronic granulomatous disease. J Clin Invest 128(9):3957–3975
212. Segal BH, Leto TL, Gallin JI, Malech HL, Holland SM (2000) Genetic, biochemical, and clinical features of chronic granulomatous disease. Medicine (Baltimore) 79(3):170–200
213. Kuhns DB, Alvord WG, Heller T, Feld JJ, Pike KM, Marciano BE et al (2010) Residual NADPH oxidase and survival in chronic granulomatous disease. N Engl J Med 363(27):2600–2610
214. Johnston RB, Newman SL (1977) Chronic granulomatous disease. Pediatr Clin N Am 24(2):365–376
215. Cohen MS, Isturiz RE, Malech HL, Root RK, Wilfert CM, Gutman L et al (1981) Fungal infection in chronic granulomatous disease. The importance of the phagocyte in defense against fungi. Am J Med 71(1):59–66
216. Hitzig WH, Seger RA (1983) Chronic granulomatous disease, a heterogeneous syndrome. Hum Genet 64(3):207–215
217. Tauber AI, Borregaard N, Simons E, Wright J (1983) Chronic granulomatous disease: a syndrome of phagocyte oxidase deficiencies. Medicine (Baltimore) 62(5):286–309
218. Hayakawa H, Kobayashi N, Yata J (1985) Chronic granulomatous disease in Japan: a summary of the clinical features of 84 registered patients. Acta Paediatr Jpn 27(501)
219. Forrest CB, Forehand JR, Axtell RA, Roberts RL, Johnston RB Jr (1988) Clinical features and current management of chronic granulomatous disease. Hematol Oncol Clin North Am 2(2):253–266
220. Mouy R, Fischer A, Vilmer E, Seger R, Griscelli C (1989) Incidence, severity, and prevention of infections in chronic granulomatous disease. J Pediatr 114(4 Pt 1):555–560
221. Bemiller LS, Roberts DH, Starko KM, Curnutte JT (1995) Safety and effectiveness of long-term interferon gamma therapy in patients with chronic granulomatous disease. Blood Cells Mol Dis 21(3):239–247
222. Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S et al (2015) Common severe infections in chronic granulomatous disease. Clin Infect Dis 60(8):1176–1183
223. Bennett N, Maglione PJ, Wright BL, Zerbe C (2018) Infectious complications in patients with chronic granulomatous disease. J Pediatric Infect Dis Soc 7(suppl_1):S12–s17
224. Greenberg DE, Shoffner AR, Zelazny AM, Fenster ME, Zarember KA, Stock F et al (2010) Recurrent Granulibacter bethesdensis infections and chronic granulomatous disease. Emerg Infect Dis 16(9):1341–1348
225. Meher-Homji Z, Mangalore RP, Johnson DR, Chua YL (2017) Chromobacterium violaceum infection in chronic granulomatous disease: a case report and review of the literature. JMM Case Rep 4(1):e005084
226. Reichenbach J, Lopatin U, Mahlaoui N, Beovic B, Siler U, Zbinden R et al (2009) Actinomyces in chronic granulomatous disease: an emerging and unanticipated pathogen. Clin Infect Dis 49(11):1703–1710
227. Bustamante J, Aksu G, Vogt G, de Beaucoudrey L, Genel F, Chapgier A et al (2007) BCG-osis and tuberculosis in a child with chronic granulomatous disease. J Allergy Clin Immunol 120(1):32–38
228. Conti F, Lugo-Reyes SO, Blancas Galicia L, He J, Aksu G, Borges de Oliveira E Jr et al (2016) Mycobacterial disease in patients with chronic granulomatous disease: a retrospective analysis of 71 cases. J Allergy Clin Immunol 138(1):241–248.e243

229. Zhou Q, Hui X, Ying W, Hou J, Wang W, Liu D et al (2018) A cohort of 169 chronic granulomatous disease patients exposed to BCG vaccination: a retrospective study from a single center in Shanghai, China (2004-2017). J Clin Immunol 38(3):260–272
230. Greenberg DE, Goldberg JB, Stock F, Murray PR, Holland SM, Lipuma JJ (2009) Recurrent Burkholderia infection in patients with chronic granulomatous disease: 11-year experience at a large referral center. Clin Infect Dis 48(11):1577–1579
231. Lublin M, Bartlett DL, Danforth DN, Kauffman H, Gallin JI, Malech HL et al (2002) Hepatic abscess in patients with chronic granulomatous disease. Ann Surg 235(3):383–391
232. Leiding JW, Freeman AF, Marciano BE, Anderson VL, Uzel G, Malech HL et al (2012) Corticosteroid therapy for liver abscess in chronic granulomatous disease. Clin Infect Dis 54(5):694–700
233. Galluzzo ML, Hernandez C, Davila MT, Perez L, Oleastro M, Zelazko M et al (2008) Clinical and histopathological features and a unique spectrum of organisms significantly associated with chronic granulomatous disease osteomyelitis during childhood. Clin Infect Dis 46(5):745–749
234. Segal BH, DeCarlo ES, Kwon-Chung KJ, Malech HL, Gallin JI, Holland SM (1998) Aspergillus nidulans infection in chronic granulomatous disease. Medicine (Baltimore) 77(5):345–354
235. King J, Henriet SSV, Warris A (2016) Aspergillosis in chronic granulomatous disease. J Fungi (Basel) 2(2)
236. Dorman SE, Guide SV, Conville PS, DeCarlo ES, Malech HL, Gallin JI et al (2002) Nocardia infection in chronic granulomatous disease. Clin Infect Dis 35(4):390–394
237. Alvarez-Cardona A, Rodriguez-Lozano AL, Blancas-Galicia L, Rivas-Larrauri FE, Yamazaki-Nakashimada MA (2012) Intravenous immunoglobulin treatment for macrophage activation syndrome complicating chronic granulomatous disease. J Clin Immunol 32(2):207–211
238. Schappi MG, Jaquet V, Belli DC, Krause KH (2008) Hyperinflammation in chronic granulomatous disease and anti-inflammatory role of the phagocyte NADPH oxidase. Semin Immunopathol 30(3):255–271
239. Brown KL, Bylund J, MacDonald KL, Song-Zhao GX, Elliott MR, Falsafi R et al (2008) ROS-deficient monocytes have aberrant gene expression that correlates with inflammatory disorders of chronic granulomatous disease. Clin Immunol 129(1):90–102
240. Meissner F, Seger RA, Moshous D, Fischer A, Reichenbach J, Zychlinsky A (2010) Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. Blood 116(9):1570–1573
241. Fernandez-Boyanapalli RF, Frasch SC, McPhillips K, Vandivier RW, Harry BL, Riches DW et al (2009) Impaired apoptotic cell clearance in CGD due to altered macrophage programming is reversed by phosphatidylserine-dependent production of IL-4. Blood 113(9):2047–2055
242. Sanmun D, Witasp E, Jitkaew S, Tyurina YY, Kagan VE, Ahlin A et al (2009) Involvement of a functional NADPH oxidase in neutrophils and macrophages during programmed cell clearance: implications for chronic granulomatous disease. Am J Physiol Cell Physiol 297(3):C621–C631
243. Rosenzweig SD (2008) Inflammatory manifestations in chronic granulomatous disease (CGD). J Clin Immunol 28(Suppl 1):S67–S72
244. Marciano BE, Rosenzweig SD, Kleiner DE, Anderson VL, Darnell DN, Anaya-O'Brien S et al (2004) Gastrointestinal involvement in chronic granulomatous disease. Pediatrics 114(2):462–468
245. Khangura SK, Kamal N, Ho N, Quezado M, Zhao X, Marciano B et al (2016) Gastrointestinal features of chronic granulomatous disease found during endoscopy. Clin Gastroenterol Hepatol 14(3):395–402.e395
246. Uzel G, Orange JS, Poliak N, Marciano BE, Heller T, Holland SM (2010) Complications of tumor necrosis factor-alpha blockade in chronic granulomatous disease-related colitis. Clin Infect Dis 51(12):1429–1434
247. Walther MM, Malech H, Berman A, Choyke P, Venzon DJ, Linehan WM et al (1992) The urological manifestations of chronic granulomatous disease. J Urol 147(5):1314–1318

248. Goldblatt D, Butcher J, Thrasher AJ, Russell-Eggitt I (1999) Chorioretinal lesions in patients and carriers of chronic granulomatous disease. J Pediatr 134(6):780–783
249. Wang Y, Marciano BE, Shen D, Bishop RJ, Park S, Holland SM et al (2013) Molecular identification of bacterial DNA in the chorioretinal scars of chronic granulomatous disease. J Clin Immunol 33(5):917–924
250. Hussain N, Feld JJ, Kleiner DE, Hoofnagle JH, Garcia-Eulate R, Ahlawat S et al (2007) Hepatic abnormalities in patients with chronic granulomatous disease. Hepatology 45(3):675–683
251. Godoy MC, Vos PM, Cooperberg PL, Lydell CP, Phillips P, Muller NL (2008) Chest radiographic and CT manifestations of chronic granulomatous disease in adults. AJR Am J Roentgenol 191(5):1570–1575
252. Khanna G, Kao SC, Kirby P, Sato Y (2005) Imaging of chronic granulomatous disease in children. Radiographics 25(5):1183–1195
253. Salvator H, Mahlaoui N, Catherinot E, Rivaud E, Pilmis B, Borie R et al (2015) Pulmonary manifestations in adult patients with chronic granulomatous disease. Eur Respir J 45(6):1613–1623
254. Jones LB, McGrogan P, Flood TJ, Gennery AR, Morton L, Thrasher A et al (2008) Special article: chronic granulomatous disease in the United Kingdom and Ireland: a comprehensive national patient-based registry. Clin Exp Immunol 152(2):211–218
255. Scully C (1981) Orofacial manifestations of chronic granulomatous disease of childhood. Oral Surg Oral Med Oral Pathol 51(2):148–151
256. Manzi S, Urbach AH, McCune AB, Altman HA, Kaplan SS, Medsger TA Jr et al (1991) Systemic lupus erythematosus in a boy with chronic granulomatous disease: case report and review of the literature. Arthritis Rheum 34(1):101–105
257. De Ravin SS, Naumann N, Cowen EW, Friend J, Hilligoss D, Marquesen M et al (2008) Chronic granulomatous disease as a risk factor for autoimmune disease. J Allergy Clin Immunol 122(6):1097–1103
258. Kobayashi S, Murayama S, Takanashi S, Takahashi K, Miyatsuka S, Fujita T et al (2008) Clinical features and prognoses of 23 patients with chronic granulomatous disease followed for 21 years by a single hospital in Japan. Eur J Pediatr 167(12):1389–1394
259. Buescher ES, Gallin JI (1984) Stature and weight in chronic granulomatous disease. J Pediatr 104(6):911–913
260. Marsh WL, Oyen R, Nichols ME, Allen FH Jr (1975) Chronic granulomatous disease and the Kell blood groups. Br J Haematol 29(2):247–262
261. Frey D, Machler M, Seger R, Schmid W, Orkin SH (1988) Gene deletion in a patient with chronic granulomatous disease and McLeod syndrome: fine mapping of the Xk gene locus. Blood 71(1):252–255
262. Marciano BE, Zerbe CS, Falcone EL, Ding L, DeRavin SS, Daub J et al (2018) X-linked carriers of chronic granulomatous disease: illness, lyonization, and stability. J Allergy Clin Immunol 141(1):365–371
263. Anderson-Cohen M, Holland SM, Kuhns DB, Fleisher TA, Ding L, Brenner S et al (2003) Severe phenotype of chronic granulomatous disease presenting in a female with a de novo mutation in gp91-phox and a non familial, extremely skewed X chromosome inactivation. Clin Immunol 109(3):308–317
264. Lewis EM, Singla M, Sergeant S, Koty PP, McPhail LC (2008) X-linked chronic granulomatous disease secondary to skewed X chromosome inactivation in a female with a novel CYBB mutation and late presentation. Clin Immunol 129(2):372–380
265. Rosen-Wolff A, Soldan W, Heyne K, Bickhardt J, Gahr M, Roesler J (2001) Increased susceptibility of a carrier of X-linked chronic granulomatous disease (CGD) to Aspergillus fumigatus infection associated with age-related skewing of lyonization. Ann Hematol 80(2):113–115
266. Cale CM, Morton L, Goldblatt D (2007) Cutaneous and other lupus-like symptoms in carriers of X-linked chronic granulomatous disease: incidence and autoimmune serology. Clin Exp Immunol 148(1):79–84

267. Brandrup F, Koch C, Petri M, Schiodt M, Johansen KS (1981) Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease. Br J Dermatol 104(5):495–505
268. Kragballe K, Borregaard N, Brandrup F, Koch C, Staehrjohansen K (1981) Relation of monocyte and neutrophil oxidative metabolism to skin and oral lesions in carriers of chronic granulomatous disease. Clin Exp Immunol 43(2):390–398
269. Yu JE, Azar AE, Chong HJ, Jongco AM 3rd, Prince BT (2018) Considerations in the diagnosis of chronic granulomatous disease. J Pediatric Infect Dis Soc 7(suppl_1):S6–s11
270. Kim HY, Kim HJ, Ki CS, Kim DW, Yoo KH, Kang ES (2013) Rapid determination of chimerism status using dihydrorhodamine assay in a patient with X-linked chronic granulomatous disease following hematopoietic stem cell transplantation. Ann Lab Med 33(4):288–292
271. Mauch L, Lun A, O'Gorman MR, Harris JS, Schulze I, Zychlinsky A et al (2007) Chronic granulomatous disease (CGD) and complete myeloperoxidase deficiency both yield strongly reduced dihydrorhodamine 123 test signals but can be easily discerned in routine testing for CGD. Clin Chem 53(5):890–896
272. Connelly JA, Marsh R, Parikh S, Talano JA (2018) Allogeneic hematopoietic cell transplantation for chronic granulomatous disease: controversies and state of the art. J Pediatr Infect Dis Soc 7(suppl_1):S31–s39
273. Keller MD, Notarangelo LD, Malech HL (2018) Future of care for patients with chronic granulomatous disease: gene therapy and targeted molecular medicine. J Pediatr Infect Dis Soc 7(suppl_1):S40–s44
274. Margolis DM, Melnick DA, Alling DW, Gallin JI (1990) Trimethoprim-sulfamethoxazole prophylaxis in the management of chronic granulomatous disease. J Infect Dis 162(3):723–726
275. Gallin JI, Alling DW, Malech HL, Wesley R, Koziol D, Marciano B et al (2003) Itraconazole to prevent fungal infections in chronic granulomatous disease. N Engl J Med 348(24):2416–2422
276. Mouy R, Veber F, Blanche S, Donadieu J, Brauner R, Levron JC et al (1994) Long-term itraconazole prophylaxis against Aspergillus infections in thirty-two patients with chronic granulomatous disease. J Pediatr 125(6 Pt 1):998–1003
277. Alsultan A, Williams MS, Lubner S, Goldman FD (2006) Chronic granulomatous disease presenting with disseminated intracranial aspergillosis. Pediatr Blood Cancer 47(1):107–110
278. Segal BH, Barnhart LA, Anderson VL, Walsh TJ, Malech HL, Holland SM (2005) Posaconazole as salvage therapy in patients with chronic granulomatous disease and invasive filamentous fungal infection. Clin Infect Dis 40(11):1684–1688
279. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease (1991) The international chronic granulomatous disease cooperative study group. N Engl J Med 324(8):509–516
280. Marciano BE, Wesley R, De Carlo ES, Anderson VL, Barnhart LA, Darnell D et al (2004) Long-term interferon-gamma therapy for patients with chronic granulomatous disease. Clin Infect Dis 39(5):692–699
281. Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A et al (2008) Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: an Italian multicenter study. Clin Immunol 126(2):155–164
282. Freeman AF, Marciano BE, Anderson VL, Uzel G, Costas C, Holland SM (2011) Corticosteroids in the treatment of severe nocardia pneumonia in chronic granulomatous disease. Pediatr Infect Dis J 30(9):806–808
283. Siddiqui S, Anderson VL, Hilligoss DM, Abinun M, Kuijpers TW, Masur H et al (2007) Fulminant mulch pneumonitis: an emergency presentation of chronic granulomatous disease. Clin Infect Dis 45(6):673–681
284. Marciano BE, Allen ES, Conry-Cantilena C, Kristosturyan E, Klein HG, Fleisher TA et al (2017) Granulocyte transfusions in patients with chronic granulomatous disease and refractory infections: the NIH experience. J Allergy Clin Immunol 140(2):622–625

285. Parekh C, Hofstra T, Church JA, Coates TD (2011) Hemophagocytic lymphohistiocytosis in children with chronic granulomatous disease. Pediatr Blood Cancer 56(3):460–462
286. Odobasic D, Kitching AR, Holdsworth SR (2016) Neutrophil-mediated regulation of innate and adaptive immunity: the role of Myeloperoxidase. J Immunol Res 2016:2349817
287. Pahwa R, Jialal I (2019) Myeloperoxidase deficiency. StatPearls. StatPearls Publishing LLC, Treasure Island, FL
288. Gazendam RP, van Hamme JL, Tool AT, Hoogenboezem M, van den Berg JM, Prins JM et al (2016) Human neutrophils use different mechanisms to kill Aspergillus fumigatus conidia and hyphae: evidence from phagocyte defects. J Immunol 196(3):1272–1283
289. Homme M, Tateno N, Miura N, Ohno N, Aratani Y (2013) Myeloperoxidase deficiency in mice exacerbates lung inflammation induced by nonviable Candida albicans. Inflamm Res 62(11):981–990
290. Cech P, Stalder H, Widmann JJ, Rohner A, Miescher PA (1979) Leukocyte myeloperoxidase deficiency and diabetes mellitus associated with Candida albicans liver abscess. Am J Med 66(1):149–153
291. Parry MF, Root RK, Metcalf JA, Delaney KK, Kaplow LS, Richar WJ (1981) Myeloperoxidase deficiency: prevalence and clinical significance. Ann Intern Med 95(3):293–301
292. Weber ML, Abela A, de Repentigny L, Garel L, Lapointe N (1987) Myeloperoxidase deficiency with extensive candidal osteomyelitis of the base of the skull. Pediatrics 80(6):876–879
293. Okuda T, Yasuoka T, Oka N (1991) Myeloperoxidase deficiency as a predisposing factor for deep mucocutaneous candidiasis: a case report. J Oral Maxillofac Surg 49(2):183–186
294. Nguyen C, Katner HP (1997) Myeloperoxidase deficiency manifesting as pustular candidal dermatitis. Clin Infect Dis 24(2):258–260
295. Ludviksson BR, Thorarensen O, Gudnason T, Halldorsson S (1993) Candida albicans meningitis in a child with myeloperoxidase deficiency. Pediatr Infect Dis J 12(2):162–164
296. Chiang AK, Chan GC, Ma SK, Ng YK, Ha SY, Lau YL (2000) Disseminated fungal infection associated with myeloperoxidase deficiency in a premature neonate. Pediatr Infect Dis J 19(10):1027–1029
297. Pattison DI, Davies MJ (2006) Reactions of myeloperoxidase-derived oxidants with biological substrates: gaining chemical insight into human inflammatory diseases. Curr Med Chem 13(27):3271–3290
298. Ndrepepa G (2019) Myeloperoxidase—a bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta 493:36–51
299. Lehners A, Lange S, Niemann G, Rosendahl A, Meyer-Schwesinger C, Oh J et al (2014) Myeloperoxidase deficiency ameliorates progression of chronic kidney disease in mice. Am J Physiol Renal Physiol 307(4):F407–F417
300. Kisic B, Miric D, Dragojevic I, Rasic J, Popovic L (2016) Role of Myeloperoxidase in patients with chronic kidney disease. Oxidative Med Cell Longev 2016:1069743
301. Vinh DC (2011) Insights into human antifungal immunity from primary immunodeficiencies. Lancet Infect Dis 11(10):780–792
302. Li J, Vinh DC, Casanova JL, Puel A (2017) Inborn errors of immunity underlying fungal diseases in otherwise healthy individuals. Curr Opin Microbiol 40:46–57
303. Weydert CJ, Cullen JJ (2010) Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 5(1):51–66
304. Luzzatto L, Nannelli C, Notaro R (2016) Glucose-6-phosphate dehydrogenase deficiency. Hematol Oncol Clin North Am 30(2):373–393
305. Roos D, van Zwieten R, Wijnen JT, Gomez-Gallego F, de Boer M, Stevens D et al (1999) Molecular basis and enzymatic properties of glucose 6-phosphate dehydrogenase volendam, leading to chronic nonspherocytic anemia, granulocyte dysfunction, and increased susceptibility to infections. Blood 94(9):2955–2962
306. Siler U, Romao S, Tejera E, Pastukhov O, Kuzmenko E, Valencia RG et al (2017) Severe glucose-6-phosphate dehydrogenase deficiency leads to susceptibility to infection and absent NETosis. J Allergy Clin Immunol 139(1):212–219.e213

307. Khan TA, Mazhar H, Nawaz M, Kalsoom K, Ishfaq M, Asif H et al (2017) Expanding the clinical and genetic spectrum of G6PD deficiency: the occurrence of BCGitis and novel missense mutation. Microb Pathog 102:160–165
308. Ristoff E, Mayatepek E, Larsson A (2001) Long-term clinical outcome in patients with glutathione synthetase deficiency. J Pediatr 139(1):79–84
309. Njalsson R, Ristoff E, Carlsson K, Winkler A, Larsson A, Norgren S (2005) Genotype, enzyme activity, glutathione level, and clinical phenotype in patients with glutathione synthetase deficiency. Hum Genet 116(5):384–389
310. Spielberg SP, Boxer LA, Oliver JM, Butler EJ, Schulman JD (1978) Altered phagocytosis and microtubule function in leukocytes from a patient with severe glutathione synthase deficiency (5-oxoprolinuria). Monogr Hum Genet 9:90–94
311. Baehner RL, Boxer LA (1979) Role of membrane vitamin E and cytoplasmic glutathione in the regulation of phagocytic functions of neutrophils and monocytes. Am J Pediatr Hematol Oncol 1(1):71–76
312. Boxer LA, Oliver JM, Spielberg SP, Allen JM, Schulman JD (1979) Protection of granulocytes by vitamin E in glutathione synthetase deficiency. N Engl J Med 301(17):901–905
313. Cowland JB, Borregaard N (2016) Granulopoiesis and granules of human neutrophils. Immunol Rev 273(1):11–28
314. Serwas NK, Huemer J, Dieckmann R, Mejstrikova E, Garncarz W, Litzman J et al (2018) CEBPE-mutant specific granule deficiency correlates with aberrant granule organization and substantial proteome alterations in neutrophils. Front Immunol 9:588
315. McIlwaine L, Parker A, Sandilands G, Gallipoli P, Leach M (2013) Neutrophil-specific granule deficiency. Br J Haematol 160(6):735
316. Shiohara M, Gombart AF, Sekiguchi Y, Hidaka E, Ito S, Yamazaki T et al (2004) Phenotypic and functional alterations of peripheral blood monocytes in neutrophil-specific granule deficiency. J Leukoc Biol 75(2):190–197
317. Rosenberg HF, Gallin JI (1993) Neutrophil-specific granule deficiency includes eosinophils. Blood 82(1):268–273
318. Parker RI, McKeown LP, Gallin JI, Gralnick HR (1992) Absence of the largest platelet-von Willebrand multimers in a patient with lactoferrin deficiency and a bleeding tendency. Thromb Haemost 67(3):320–324
319. Lekstrom-Himes JA, Dorman SE, Kopar P, Holland SM, Gallin JI (1999) Neutrophil-specific granule deficiency results from a novel mutation with loss of function of the transcription factor CCAAT/enhancer binding protein epsilon. J Exp Med 189(11):1847–1852
320. Witzel M, Petersheim D, Fan Y, Bahrami E, Racek T, Rohlfs M et al (2017) Chromatin-remodeling factor SMARCD2 regulates transcriptional networks controlling differentiation of neutrophil granulocytes. Nat Genet 49(5):742–752
321. Priam P, Krasteva V, Rousseau P, D'Angelo G, Gaboury L, Sauvageau G et al (2017) SMARCD2 subunit of SWI/SNF chromatin-remodeling complexes mediates granulopoiesis through a CEBPvarepsilon dependent mechanism. Nat Genet 49(5):753–764
322. Wynn RF, Sood M, Theilgaard-Monch K, Jones CJ, Gombart AF, Gharib M et al (2006) Intractable diarrhoea of infancy caused by neutrophil specific granule deficiency and cured by stem cell transplantation. Gut 55(2):292–293
323. Trapnell BC, Nakata K, Bonella F, Campo I, Griese M, Hamilton J et al (2019) Pulmonary alveolar proteinosis. Nat Rev Dis Primers 5(1):16
324. Martinez-Moczygemba M, Doan ML, Elidemir O, Fan LL, Cheung SW, Lei JT et al (2008) Pulmonary alveolar proteinosis caused by deletion of the GM-CSFRalpha gene in the X chromosome pseudoautosomal region 1. J Exp Med 205(12):2711–2716
325. Suzuki T, Sakagami T, Rubin BK, Nogee LM, Wood RE, Zimmerman SL et al (2008) Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. J Exp Med 205(12):2703–2710
326. Suzuki T, Maranda B, Sakagami T, Catellier P, Couture CY, Carey BC et al (2011) Hereditary pulmonary alveolar proteinosis caused by recessive CSF2RB mutations. Eur Respir J 37(1):201–204

327. Fremond ML, Hadchouel A, Schweitzer C, Berteloot L, Bruneau J, Bonnet C et al (2018) Successful haematopoietisssssc stem cell transplantation in a case of pulmonary alveolar proteinosis due to GM-CSF receptor deficiency. Thorax 73(6):590–592
328. Takaki M, Tanaka T, Komohara Y, Tsuchihashi Y, Mori D, Hayashi K et al (2016) Recurrence of pulmonary alveolar proteinosis after bilateral lung transplantation in a patient with a non-sense mutation in CSF2RB. Respir Med Case Rep 19:89–93
329. Dinauer MC, Coates TD (2018) Disorders of phagocyte function, chapter 50. In: Hoffman R et al (eds) Hematology: basic principles and practice, 7th edn. Elsevier, pp 691–709, Copyright Elsevier, 2018

Defects in Intrinsic and Innate Immunity

8

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Abstract

The defects in intrinsic and innate immunity are a group of monogenic diseases in which there is a numeric and/or functional defect of the cellular components of innate immunity, not included in phagocyte defects or complement defects. It is a very diverse group of primary immunodeficiencies (PID) or inborn errors of immunity (IEI), both immunologically and clinically, but all share that (1) microbial susceptibility is usually very selective and from an early age (infant), and (2) commonly used immunological studies to discard a PID (lymphocyte studies, immunoglobulin dosage, protein vaccine responses) are usually normal; thus, innate immune PID's diagnosis will require specific immunological tests.

These deficiencies are encompassed in group VI of PID classification of the International Union of Immunological Societies expert committee (now called Inborn Errors of Immunity Committee) (Tangye et al., J Clin Immunol 40:24-64; 2020). They represent 1.5% of all PIDs (Modell et al., Immunol Res 66:367–80; 2018). This group is artificially divided into four subgroups depending on the microorganism to which patients manifest susceptibility (pyogenic bacteria, mycobacteria, virus, or fungus). In this chapter, we will follow this phenotypic approach (Bousfiha et al., J Clin Immunol 38:129–43; 2018), which we believe is more useful for clinicians when approaching a patient with a suspected PID.

Keywords

Primary immunodeficiencies · Innate immunity · Toll-IL-1 · IFN-γ · Mendelian susceptibility to mycobacterial disease · Mucocutaneous candidiasis · Herpes simplex encephalitis · TLR3 pathway · Asplenia · Inborn errors of immunity

8.1 Introduction

The defects in intrinsic and innate immunity are a group of monogenic diseases in which there is a numeric and/or functional defect of the cellular components of innate immunity, not included in phagocyte defects or complement defects. It is a very diverse group of primary immunodeficiencies (PID) or inborn errors of immunity (IEI), both immunologically and clinically, but all share that (1) microbial susceptibility is usually very selective and from an early age (infant), and (2) commonly used immunological studies to discard a PID (lymphocyte studies, immunoglobulin dosage, protein vaccine responses) are usually normal; thus, innate immune PID's diagnosis will require specific immunological tests.

These deficiencies are encompassed in group VI of PID classification of the International Union of Immunological Societies expert committee (now called Inborn Errors of Immunity Committee) [1]. They represent 1.5% of all PIDs [2]. This group is artificially divided into four subgroups depending on the microorganism to which patients manifest susceptibility (pyogenic bacteria, mycobacteria, virus, or fungus). In this chapter, we will follow this phenotypic approach [3], which we believe is more useful for clinicians when approaching a patient with a suspected PID. Accordingly, the chapter will be divided into four different sections: (1) **predisposition to mycobacterial diseases** (Mendelian susceptibility to mycobacterial

disease, MSMD), (2) **predisposition to pyogenic diseases** (deficiencies in the Toll-IL1R pathway and congenital asplenia), (3) **predisposition to viral diseases** (susceptibility to HPV: epidermodysplasia verruciformis (HPV) and others; predisposition to severe viral infection: herpes simplex encephalitis (HSE)), and (4) p**redisposition to fungal diseases** (predisposition to invasive fungal diseases and predisposition to mucocutaneous candidiasis).

8.2 Section 1: Predisposition to Mycobacterial Diseases

8.2.1 Defects in the IFN-γ Circuit

8.2.1.1 Introduction

Adverse events after bacille Calmette-Guerin vaccination, in the form of localized (BCGitis) [4, 5] or disseminated (BCGosis) [6] infections, some of them with a fatal outcome, were first reported in the 50s of the past century. The first report of BCGosis, which was suggested to be the first description of Mendelian susceptibility to mycobacterial disease (MSMD), was published during the 50s [7], and a fatal environmental mycobacteria (EM) infection in three relatives was reported in 1964 [8]. However, it was not until 1996 that the first genetic etiology of MSMD, autosomal recessive (AR) IFN-γR1 deficiency, was described in children with severe BCG or EM infection [9, 10].

8.2.1.2 Physiopathology and Genetics

Studies deciphering the genetic basis of MSMD have revealed the central role of IFN-γ-mediated immunity in the defense against mycobacteria (Fig. 8.1 and Table 8.1). Until date, mutations in 16 genes have been found to cause isolated MSMD or syndromic MSMD (Table 8.1). These genes are involved in IFN-γ production (*IL12RB1* [11–13], *IL12B* [14, 15], *IL12RB2* [16], *IL23R* [16], *ISG15* [17, 18], *SPPL2A* [19, 20], *TYK2* [21, 22], *RORC* [23], and *IFNG* [24]), the cellular responses to IFN-γ (*IFNGR1* [9, 11, 25–28], *IFNGR2* [29, 30], *STAT1* [31–33], *JAK1* [34], and *CYBB* [35–37]), or both (*NEMO* [36] and *IRF8* [38]). Patients with syndromic MSMD, in contraposition to isolated MSMD, manifest a more complex clinical phenotype, with a predisposition to infection by other microorganisms or to other manifestations. Depending on the impact of the mutation (null or hypomorphic, resulting in complete or partial deficiency), the inheritance of the disease, the expression of the mutant allele (absent or detectable), or the mechanism responsible for the impaired function of the mutated protein, at least 30 different genetic etiologies of MSMD have been identified so far [20, 39]; IL-12Rβ1 deficiency and autosomal dominant (AD) IFN-γR1 deficiency are the first and second, respectively, most common defects [20, 24, 39, 40]. Mutations in *STAT1* [41], *IRF8* [38], and *TYK2* [21, 22] cause isolated or syndromic MSMD depending of the pattern of inheritance and the functional impact of the mutation, and mutations in *ISG15* [17, 18], *RORC* [23], and *JAzK1* [34] were described only in patients with syndromic MSMD. With so many forms, the clinical boundaries of MSMD, particularly of some of the less frequent etiologies, are not fully defined, and at present, the genetic etiology remains unknown in about half of the patients.

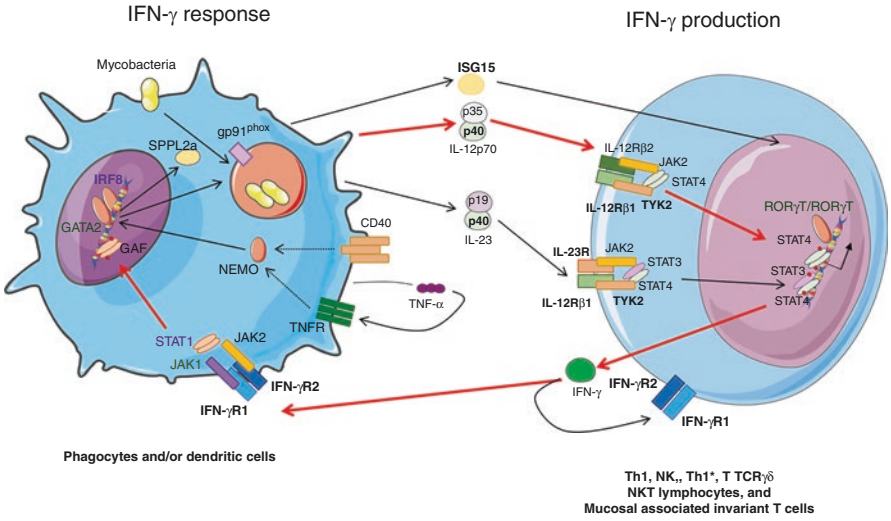

Fig. 8.1 *Summary of molecules implicated in IFN-γ-mediated immunity.* Molecules represented with bold characters are known to be cause of MSMD. Genes causing nonsyndromic forms are displayed in black, genes causing both syndromic and nonsyndromic forms in purple, and genes causing only syndromic forms in green

Recognition and phagocytosis of the bacilli by antigen-presenting cells (APC) and macrophages induce their activation and the production of an array of cytokines and chemokines, particularly tumor necrosis factor (TNF)-α, interferon-stimulated gene (ISG) 15, interleukin (IL)-12p70, and IL-23, which induce IFN-γ production by T, NK, and NKT cells. IL-12p40 (encoded by the IL12B gene) is common to IL-12p70 and IL-23. The IL-12Rβ1 molecule is shared by both the IL-12 and IL-23 receptor heterodimer, whereas IL-12Rβ2 and IL23R are unique to the IL-12 and IL-23 receptors, respectively. Tyk2 is a tyrosine kinase involved in IL-12R- and IL-23R-mediated signaling. ISG15 is secreted by many cell types, including myeloid cells and neutrophils, and it acts as a very potent IFN-γ-inducing cytokine in lymphocytes. ISG15 also encodes an intracellular interferon-induced ubiquitin-like protein that acts as a negative regulator of IFN-α/β, resulting in enhanced IFN-α/β immunity. The RORC gene encodes two protein isoforms that act as transcription factors: The nuclear orphan receptor γ (RORγ) is ubiquitously expressed, whereas the expression of RORγT is restricted to leukocytes. Patients with RORγ/RORγT deficiency show low numbers of ILC3, MAIT, and NKT cells and normal IFN-γ secretion by naïve or memory CD4+ T cells but strongly impaired IFN-γ production by Th1* cells and γδ T cells.

IFN-γ binding to the IFN-γ receptor, a heterodimer of IFN-γR1 and IFN-γR2, leads to activation of the tyrosine kinases Jak1 and Jak2 and to serine and tyrosine phosphorylation of the signal transducer and activator of transcription 1 (STAT1). Phosphorylated STAT1 forms a homodimer termed IFN-gamma-activated factor (GAF), which migrates into the nucleus and binds to the IFN gamma-activated sequence (GAS) to drive the expression of the target genes. Interferon regulatory factor 8 (IRF8) is a transcription factor induced by IFN-s, expressed in macrophages and dendritic cells. IRF8 binds to IFN-stimulated response elements (ISRE) and regulate the expression of many genes. SPPL2A encodes the signal peptide peptidase-like 2 A (SPPL2a), a protease with multiple substrates. A binding site for IRF8 has been identified in the Sppl2a promoter in mouse macrophages. Patients with SPPL2a deficiency have a deficit of conventional type 2 dendritic cells (cDC2). Both AD IRF8 and AR SPPL2a deficiencies confer a defect of IFN-γ production by mycobacterium-specific Th1* cells. NEMO encodes the nuclear factor-kappa B (NF-κB) essential modulator, which mediates signaling in the NF-κB pathway, required, among other signaling pathways, for TNF receptor- and CD40L-mediated activation. gp91phox, encoded by CYBB, is a major component of the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (PHOX) complex, required for effective intracellular killing of microorganisms.

8.2.1.3 Clinical Features

Mendelian susceptibility to mycobacterial diseases is a rare inherited condition characterized by a narrow vulnerability to poorly virulent mycobacteria, such as bacillus Calmette-Guerin (BCG) vaccines and environmental mycobacteria (EM), in otherwise healthy individuals. Tuberculosis, disseminated, extrapulmonary, or pulmonary, caused by the more virulent *M. tuberculosis* has been documented in some patients, particularly in deficiencies of IL-12Rβ1 [39, 42, 43], IFN-γR1 [26, 39], STAT1 [31, 39], or IL-12p40 [15, 44]. Besides the susceptibility to mycobacteria, some patients with isolated MSMD may also be susceptible to other intramacrophagic microorganisms: About half of the patients suffer from salmonellosis (frequently extra gastrointestinal), particularly nontyphoidal, and more rarely typhoidal; rare cases of infections caused by intramacrophagic fungi (histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, candidiasis), parasites (*Leishmaniasis*, *Toxoplasmosis*), and bacteria (listeriosis, nocardiosis, klebsiellosis) have also been reported. Complete deficiencies of IFN-γR1 and IFN-γR2 rarely predispose to viral disease, particularly by herpes viruses [39]. In addition, since IL-12Rβ1 is also part of the IL-23 receptor and IL-12p40 is also a subunit of IL-23,

Table 8.1 Genetic etiologies of isolated and syndromic Mendelian susceptibility to mycobacterial disease based on the mutated gene, the mode of inheritance, and the functional impact of the mutation*

Isolated MSMD		
Gene	Inheritance	Functional defect
IL12RB1	AR	C
IL12B	AR	C
IL12RB2	AR	C
IL23R	AR	C
IRF8[a]	AD	P
SPPL2A	AR	C
IFNG	AR	C
IFNGR1	AR	C
	AR	P
	AD	P
IFNGR2	AR	C
	AR	P
	AD	P
STAT1[b]	AD	P
NEMO (IKBKG)[c]	XR	P
CYBB[d]	XR	P
TYK2 (p.P1104A)[e]	AR	P
Syndromic MSMD		
IRF8[a]	AR	C
STAT1[b]	AR	C
	AR	P

(continued)

Table 8.1 (continued)

Isolated MSMD		
Gene	Inheritance	Functional defect
JAK1	AR	C
TYK2[e]	AR	C
RORC	AR	C
ISG15	AR	C

AR Autosomal recessive, *AD* Autosomal dominant, *XR* X-linked recessive, *C* Complete deficiency, *P* Partial deficiency. Depending on whether the mutated protein is expressed or not, up to 30 genetic etiologies can be classified

[a]IRF8. Patients with AD IRF8 deficiency lack the main subset of human blood myeloid DCs (DR+, CD11c+, CD1c+, or MDC1), which are potent producers of IL-12, and they are susceptible to mycobacterial infections. AR complete IRF8 deficiency is characterized by a complete absence of CD14+ and CD16+ circulating monocytes, CD11c+ conventional dendritic cells (DC), and CD11c+/CD123+ plasmacytoid DCs, and they suffer from multiple infections

[b]AD STAT1 deficiency, caused by loss-of-function or hypomorphic mutations, exerts a dominant negative effect, due to haploinsufficiency, on IFN-γ responses but not on IFN-α/β responses (which involves STAT1, STAT2, and IRF9), and patients present with isolated MSMD. AR complete STAT1 deficiency is characterized by the absence of STAT1 protein expression. As a consequence, patient's cells show abolished cellular responses to IFN-γ and the antiviral IFN-α/β and IFNλ, and patients have a life-threatening susceptibility to both mycobacteria and viruses. PR STAT1 deficiency, caused by hypomorphic mutations of STAT1, results in impaired but not abolished to IFN-γ and IFN-α, and patients are susceptible to both intracellular mycobacteria, salmonella, and viruses. Patients with AD gain-of-function mutations (GOF) in STAT1 are particularly susceptible to chronic mucocutaneous candidiasis; this inborn error of immunity is characterized by strong responses to IFN-γ, IFN-α, and IL-27, and it is unknown why some patients are prone to mycobacterial infections, although some patients develop a combined immunodeficiency

[c]Most hypomorphic mutations in NEMO cause ectodermal anhidrosis dysplasia and immunodeficiency, whereas null mutations cause incontinentia pigmenti

[d]Null mutations in CYBB underlie chronic granulomatous disease

[e]Tyk2 is involved in signal transduction by the receptors for IL-12, IL-23, IFN-α/β, and IL-10. Peripheral blood leukocytes from patients with complete AR Tyk2 deficiency have impaired responses to IL-12 and IL-23 as well as to IFN-α/β, which cause MSMD and susceptibility to viral infections, respectively; patients with complete AR Tyk2 deficiency and isolated MSMD have been also reported. Responses to IL-10 of these patients are poor, but not abolished, and therefore they are not associated with early-onset colitis. Partial AR Tyk2 deficiency is due to a common missense variant of TYK2, p.P1104A (around 1/600 individual of European descent are homozygous for this variant). Cells from patients with partial AR Tyk2 deficiency show low, but not abolished, responses to IL-23, and the patients are predisposed to EM and tuberculosis, although the clinical penetrance is very low; cellular responses to IL-12, IFN-α/β, and IL-10 are intact, and they are not particularly prone to viral infections

patients with IL-12Rβ1 and IL-12p40 deficiencies are prone to mild forms of chronic mucocutaneous candidiasis (CMC) [15, 45]. Patients usually have a normal resistance to other microorganisms.

Patients can present with a wide range of clinical manifestations of mycobacterial disease, from local BCGitis to disseminated, invasive, and lethal infections. Disease onset is usually in childhood, but diagnosis in adolescence and adulthood has been reported. Usually, the most severe forms (for instance, AR complete IFN-γR1 or AR complete IFN-γR2 deficiency) show an early onset, and infections

tend to be persistent and life-threatening in spite of antimycobacterial treatment. By contrast, the least severe forms (for instance, the partial -AR or AD- deficiencies of IFN-γR1 and IFN-γR2 or deficiencies of the IL-12/IL-23 receptors) can have a late onset and can improve with age, and the infections can be relatively circumscribed. In this context, osteomyelitis, even multifocal, by EM is common in patients with partial (AR or AD) IFN-γR1 and partial AD deficiency of STAT1 (and at a lesser extent in those with AR partial IFN-γR2 deficiency), whereas disseminated infections are classically observed in complete AR deficiencies of IFN-γR1, IFN-γR2, and STAT1 [20, 33, 39, 46–52]. Patients that survive to a mycobacterial disease may suffer from another mycobacterial infection or remain healthy; likewise, mycobacterial infections may or may not recur.

Clinical penetrance of MSMD may be incomplete, and it usually correlates with the extent of IFN-γ-mediated immunity. Complete AR IFN-γR1 and IFN-γR2 deficiencies are fully penetrant, and they are always lethal in the absence of hematopoietic stem cell transplantation (HSCT). By contrast, clinical penetrance is partial, and even very low, in patients with deficiencies of the IL-12 and/or IL-23 receptors. Only 50–70% of adults with IL-12Rβ1 deficiency, who have abolished IL-12- and IL-23-mediated responses, are symptomatic by the age of 40 years [13, 20, 39]. However, the clinical penetrance of IL-12Rβ2 deficiency, which does not affect IL-23-mediated responses, and of IL-23R and of partial AR Tyk2 deficiencies, which show normal IL-12-mediated responses, is very low (about 0.5%), and most patients remain asymptomatic in adulthood [16, 20, 22]. Therefore, some of the genetic etiologies of MSMD do not segregate as a bona fide Mendelian trait.

Some of these disorders predispose to syndromic MSMD due to involvement of the mutated proteins in signaling pathways other than the IFN-γ circuit. The etiopathogenesis of syndromic STAT1, IRF8, and Tyk2 deficiencies is discussed in Table 8.1 [20, 39]. Jak1 is involved in the cellular responses to numerous cytokines, including IFN-γ, IFNs-I/III, IL-4, IL-7, IL-9, IL-15, IL-21, and IL-6, and the only reported patient with JAK1 deficiency suffered from atypical mycobacterial disease, and a history of viral, fungal, and parasitic skin infection was documented [42]. Patients with biallelic *RORC* mutations display impaired IL-17A/F secretion by T cells, predisposing patients to CMC [23]. The absence of intracellular ISG15 leads to enhanced IFN-α/β immunity, and ISG15 deficiency also results in autoinflammation characterized by intracranial calcifications and epileptic seizures, resembling Aicardi-Goutieres syndrome and spondyloenchondromatosis [20, 43]. A few cases of carcinogenesis, even at young ages, have been described in patients with isolated MSMD [53, 54], and the patient with JAK1 deficiency died from urothelial carcinoma at the age of 22 years [34]. Macrophage activation syndrome or vasculitis was also reported [20, 39, 43].

8.2.1.4 Diagnosis and Immunological and Molecular Tests

Before the diagnosis of MSMD, acquired and inherited immunodeficiencies predisposing to mycobacterial diseases must first be excluded [43]. Several acquired immunodeficiencies predispose to mycobacterial infections: immunosuppressive drugs for solid organ transplantation, HSCT, leukemia, during chemotherapy, or

following HSCT and biologicals, particularly those against TNF-α-mediated immunity or human immunodeficiency virus infection (HIV; BCG vaccination is contraindicated in HIV-infected individuals). Environmental mycobacteria (EM) and *M. tuberculosis* infections are increasingly being reported in patients with congenital lung defects such as primary ciliary dyskinesia, pulmonary alveolar proteinosis, and cystic fibrosis.

Several PID or IEI need to be discarded, since they predispose to mycobacterial infections, albeit usually in patients with other infectious and immunological phenotypes [43], including (i) patients with PID involving defects in the number and/or function of T cells (severe combined immunodeficiencies and combined immunodeficiencies), (ii) chronic granulomatous disease (particularly susceptible to BCG and *M. tuberculosis*), and (iii) GATA2 deficiency, predisposing to disseminated EM infections, and less frequently to tuberculosis, which may be the first clinical presentation even in otherwise healthy adults, although they can also occur during childhood. GATA2 patients have a broader clinical spectra, including viral infections, particularly warts; hematological disorders (myelodysplastic syndrome/leukemia); pulmonary alveolar proteinosis; and other non-immunological anomalies [55–57]. Patients with GATA2 deficiency have characteristically monocytopenia and a deficiency of DC, and low numbers of B cells and NK cell as well as neutropenia are also characteristic, although this PID is progressive and these leukocyte populations are variably affected [55]. Patients with AD gain-of-function mutations in *STAT1* usually suffer from CMC, although disseminated EM infections and other opportunistic infections were reported [58].

Children or adults with recurrent or severe/disseminated mycobacterial infectious disease caused by BCG, EM, *Mtb,* or *Salmonella*, and in whom other inborn or acquired conditions predisposing to mycobacterial infection have been excluded, should be suspected of having MSMD. MSMD should be also suspected in patients with severe infections by other intramacrophagic microorganisms. Routine hematological and immunological analysis for PID used to be normal in patients with MSMD, although monocytopenia and DC deficiency, like in patients with GATA2 deficiency, can be detected in patients with IRF8 and SPPL2a deficiency. MSMD diagnosis comprises complex functional tests that need to be performed in specialized immunology laboratories [59].

Evaluation of cytokine production, developed by Feinberg et al. [11], is the gold standard for study of IFN-γ circuit integrity. This assay is based on the measurement of IL-12p40, IL-12p70, and IFN-γ after whole blood or, less frequently, peripheral blood mononuclear cells (PBMCs) stimulation. Stimulation conditions comprise incubation with live BCG with or without hrIL-12p70 or hr-IFN-γ co-stimulation for 18 h (for IL-12 measurement) or 48 h (for IFN-γ and IL-12 measurements). Although powerful, this technique has several limitations: (1) the intrinsic variability observed yet in healthy controls that hampers interpretation of results; (2) if fresh whole blood is used, it should be performed during the first 48h after extraction; and (3) the use of BCG stimulation can be limiting in diagnostic laboratories following ISO 15189 regulations. In an attempt to solve limitations, different strategies have been developed, including the performance of the test in cryopreserved

cells to eliminate time-from-extraction limitation and the use of phytohemagglutinin and lipopolysaccharide as stimuli to avoid the use of BCG.

Quantitation of IFN-γ levels in plasma is a fast and easy technique for detection of IFN-γR deficiencies since high levels of this cytokine are characteristic in these defects, especially in complete AR deficiencies [60]. Cytometric evaluation of the presence of the IFN-γR1, IFN-γR2, and IL-12Rβ1 receptors is also a very useful tool [13, 27, 39]. However, normal expression does not exclude a defect since there are forms (especially in IFN-γR deficiencies) in which normal (AR forms) or even high (AD forms) nonfunctional proteins are expressed [13, 25, 27, 28, 30, 39, 61, 62]. Finally, cytometric evaluation of receptors' downstream signaling after specific stimulation (STAT1 phosphorylation after IFN-γ and IFN-α stimulation [29, 32, 33, 47, 61, 63–65] and STAT4 phosphorylation after IL-12 stimulation [44, 62]) can help detect defects of IFN-γR/STAT1- and IL-12R-mediated activation [32, 33, 47, 65]. Other studies, such as IFN-γ production after IL-23 and ISG15 co-stimulation, may be useful for the characterization of the functional deficiency.

Genetic confirmation of the diagnosis of MSMD is of outmost importance for treatment and genetic counseling. When functional defects suggest a specific defect, Sanger sequencing is the option of choice. NGS technology, both in the form of gene panels or whole exome sequencing (WES), can be also useful to screen for all genetic etiologies simultaneously. In a more research-like setting, whole genome sequencing (WGS) is used to detect new disease-causing variations in nonprotein-coding regions of the genome.

8.2.1.5 Treatment

The clinical spectrum of MSMD ranges from mild forms to severe life-threatening disease. Complete AR deficiencies of IFN-γR1 and IFN-γR2, and the rare cases of complete AR deficiencies of STAT1 and IRF8, are lethal in the absence of HSCT in spite of antimycobacterial treatment [20, 39, 41, 66–73]. Milder cases (partial deficiencies of IFNγ-R1, deficiencies of IL-12p40, IL-12Rβ1, IL-12Rβ2, IL-23R, or ISG15) have a more favorable outcome and may respond well to appropriate antibiotic therapy [13, 15, 20, 39, 43, 68]. Subcutaneous IFN-γ therapy, in combination with antibiotics, should be considered in those patients able to mount cellular responses, even residual, to the cytokine. Prophylactic antimycobacterial antibiotics are usually not required in the less severe forms of MSMD, although it should be evaluated individually, particularly in patients with recurrent infections. Likewise, rare patients require prophylaxis against salmonella. Accurate genetic diagnosis and the functional distinction between complete and partial defects, as well as a careful characterization of the immunological phenotype, are of the utmost importance to ensure the best possible management of MSMD patients.

8.2.1.6 Autoantibodies Against IFN-γ. A Phenocopy of Inborn Errors of IFN-γ

In 2004 and 2005, the first reports of the existence of neutralizing autoantibodies against IFN-γ in patients with disseminated mycobacterial diseases were published, and numerous cases have been described so far [43, 74–77]. This condition is not

considered MSMD, but it is included in this section because it is classified as a phenocopy of inborn errors of IFN-γ. This disorder affects predominantly, but not exclusively, adults of Asian descent [74–76]. Most patients suffer from infections by EM, but *M. tuberculosis* was documented in some cases [43]. This condition is frequently associated with infections by other intramacrophagic microorganisms such as Salmonella, *Cryptococcus neoformans*, *Histoplasma capsulatum*, or *Penicillium marneffei*. The most direct approach for detecting IFN-γ autoantibodies is by using an ELISA system and by observing IFN-γ level recovery after the addition of exogenous IFN-γ to patient serum [78]. It should be included in the differential diagnosis of MSMD. Treatment of patients with anti-IFN-γ autoantibodies is complex and requires the control of the infection with long-term antibiotics and reducing the titer of autoantibodies; for the last, rituximab and cyclophosphamide have been used [75, 79, 80].

8.3 Section 2: Predisposition to Pyogenic Diseases

8.3.1 Deficiencies of the Toll-IL1R Pathway

In humans, Toll-like receptors (TLRs) are upon the most important receptors of infectious agents on myeloid leukocytes, particularly monocytes, macrophages, and dendritic cells (DCs). Certain TLRs are also found on lymphocytes and nonhematopoietic cells, such as fibroblasts, oligodendrocytes, and epithelial cells.

Each of the 10 TLRs is able to sense molecular patterns derived from bacteria, mycobacteria, viruses, fungi, and parasites. TLR1/2/4/5/6/10 are surface receptors while TLR3/7/8/9 are intracellular. Except for TLR3, upon TLR1-9 ligation, the cascade downstream TLRs will activate, through NF-kB, the transcription of a proinflammatory program including IL-6, IL-1b, and TNF-α to control the infection.

Most TLRs (except TLR3, and partially TLR4) and IL-1 receptors (IL1-R) (responsible for the response to IL-1, IL-18) share a common cytoplasmic domain, named TIR domain and a common downstream cascade, in which MyD88 is a key adaptor. IRAK family members (such as IRAK-4 and IRAK-1) are selectively recruited to TLRs and IL-1Rs by MyD88 (Fig. 8.2). TLR3, signals independently of MyD88 through TRIF, and TLR4, can signal both via TIRAP/MyD88 or TRIF. TLR10 uniquely inhibits both MyD88-dependent and -independent pathway [81].

8.3.1.1 Deficiencies in IRAK-4 and MyD88

IRAK-4 deficiency was first described in 2003 [82] and MyD88 deficiency in 2008 [83]).

Clinical Features

The central clinical feature of IRAK-4 (OMIM #607676) and MyD88 (OMIM #612260) deficiencies is the high susceptibility to invasive and noninvasive

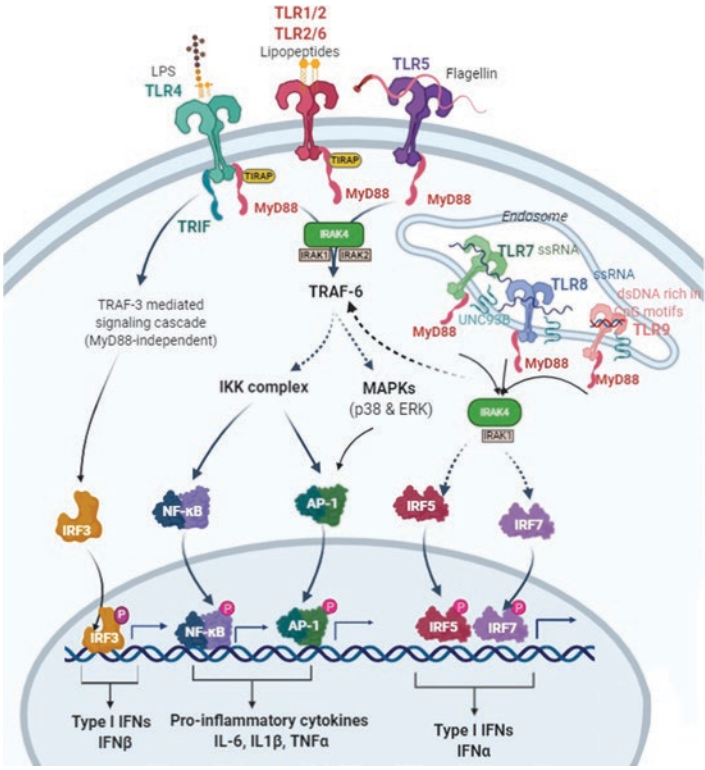

Fig. 8.2 Simplified schematic representation of Toll-IL1 pathway. Created with permission from BioRender.com

infections with only a few Gram-positive and Gram-negative bacteria with absent or delayed signs of inflammation (local or systemic, including C-reactive protein elevation, fever) in the course of infections [84, 85]. Clinically, IRAK-4 and MyD88 deficiencies are indistinguishable (also named phenocopies) [86]. Infections usually have an acute course. During infection, some patients develop neutropenia, which is transient.

Invasive infections include sepsis, meningitis, arthritis, osteomyelitis, and deep inner organs/tissue abscesses. They are mainly caused by *S. pneumoniae* (70% of cases) and, to a lesser extent, by *S. aureus* and *P. aeruginosa*. Most patients suffer from their first invasive bacterial infection before the age of 2 years (85% of IRAK-4 deficiency patients and 92% of MyD88 deficiency patients, and before the age of 6 months in 42% of patients). One of the major causes of death is pneumococcal sepsis-meningitis at that early ages. The combination of invasive pneumococcal and

staphylococcal disease is highly suggestive of a TIR deficiency. No invasive infections have been described after adolescence, suggesting that the MyD88-/IRAK-4-dependent TIR pathway becomes redundant once acquired immunity is fully functional and can ensure protection.

Noninvasive infections (cellulitis, furunculosis, folliculitis, lymphadenitis, usually necrotizing, and infections of the respiratory tract) are caused by *S. aureus* and, to a lesser extent, by pneumococcus and *P. aeruginosa*.

Infections by other Gram-positive and Gram-negative bacteria have also been observed, but IRAK-4- and MyD88-deficient patients are resistant to mycobacteria, viruses, fungi, and parasites.

Late umbilical cord separation (>2 weeks of age) has also been described in patients with IRAK-4 deficiency.

Immunological and Molecular Studies

Screening Tests

Evaluation of phagocyte respiratory oxidative burst using dihydrorhodamine test (DHR test), which is usually performed in the context of pyogenic infections to discard another primary immunodeficiency named chronic granulomatous disease (see Chap. 9), can raise the suspicion of deficiencies in IRAK-4/MyD88 because most patients display a specific pattern of responses, characterized by strongly diminished DHR responses to *E. coli* in the presence of normal DHR responses to phorbol myristate acetate (Alsina L, Vlagea A, manuscript in preparation).

Specific Tests

- *Shedding of CD62L* in granulocytes after stimulation with specific agonists for different TLRs. Upon activation, granulocytes will cleave the ectodomain of a significant fraction of the CD62L present on the plasma membrane (Fig. 8.3).
- *Quantification of IL-6 and/or TNF-α production by whole blood or PBMC* after stimulation with specific agonists of different TLRs. Responses across all TLRs (except for TLR3 and partially for TLR4), and the response to IL-1, IL-18, and IL-33, are abolished or diminished in IRAK-4 and MyD88 deficiencies [87, 88] (Fig. 8.4).

These tests cannot differentiate an IRAK-4 and MyD88 deficiency. Only genetics will confirm the mutation in *IRAK-4* or *MyD88*.

Other Tests

- There may be an elevation of IgE and IgG4 with normal levels of IgG, IgA, and IgM in one-third and two-thirds of patients, respectively. Production of specific antibodies against nonconjugated pneumococcal polysaccharides and isohemagglutinin levels are both diminished in one-third of patients [84].
- Globally, no overt abnormalities in leukocyte subsets are observed in patients with IRAK-4 or MyD88 deficiencies except for a modest impact on IgM-

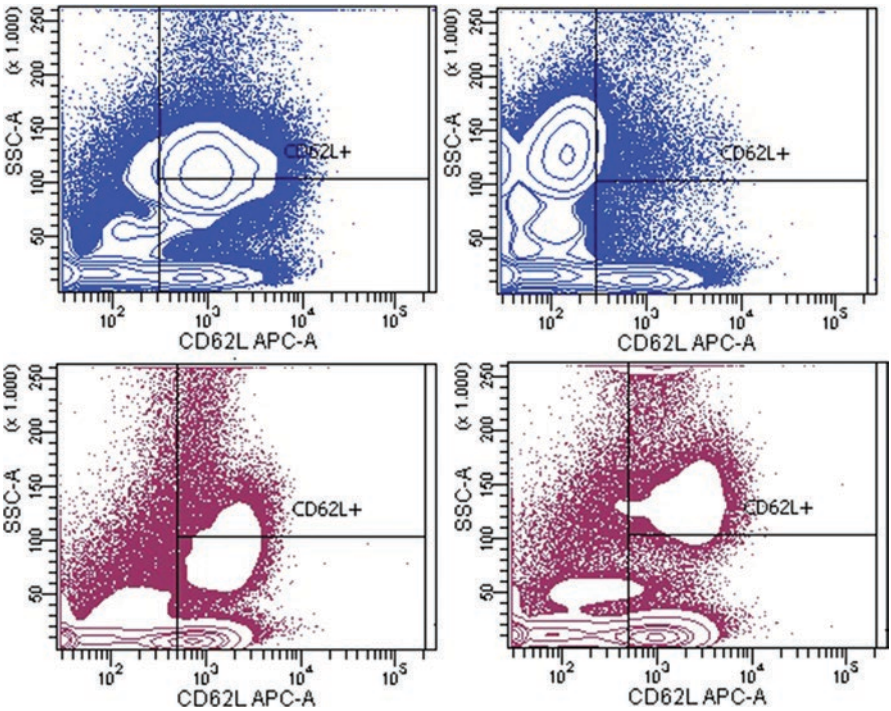

Fig. 8.3 CD62L shedding for the study of Toll-IL-1 pathway deficiencies

dependent B-cell immunity, delaying its maturation: A diminished level of $IgM^+IgD^+CD27^+$ B cells is observed, while $IgM^-IgD^+CD27^+$-switched B cells are largely normal [89].

- Genetics. Both diseases are autosomal recessive and show complete penetrance. A founder effect of *MyD88* in "*zingaros*" (E402X/del) has been described (Rodriguez-Gallego C, manuscript in preparation).

Treatment and Prognosis

Antibiotic prophylaxis (daily trimethoprim-sulfamethoxazole and/or amoxicillin depending on the pattern of resistance to *S. pneumoniae*) is recommended, also intensive vaccination with conjugated and nonconjugated bacterial vaccines, including against pneumococcus, meningococcus, and *H. influenza*e (there is no vaccine contraindication) [90]. Immunoglobulin replacement therapy (IRT) is usually recommended. IRT and antibiotic prophylaxis are usually recommended up to at least 10–14 years old. In TIR defects, after 8 years of age, mortality associated with invasive infection is rare. Patients must be instructed to seek for medical attention upon any sign of infection.

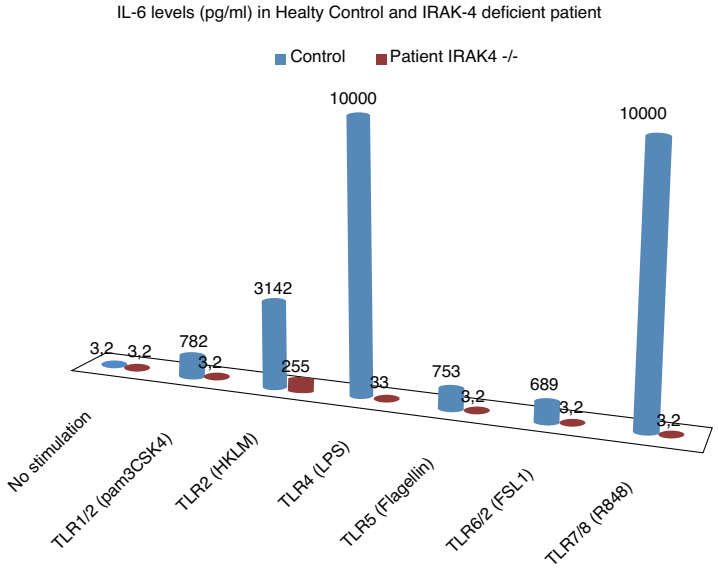

Fig. 8.4 Whole blood culture for cytokine analysis to study Toll-IL-1 pathway deficiencies

8.3.1.2 Deficiency in IRAK-1

Clinical Features

Single patient described in 2017, with X-linked recessive complete IRAK-1 deficiency [91]. He presented a urinary tract infection by *Klebsiella pneumoniae* and two episodes of aspiration pneumonia before his death at 7 months old due to respiratory failure, probably ascribed to his severe congenital encephalopathy, which was later confirmed to be caused by MECP2 deficiency, in the context of an intrachromosomal deletion of about 112 kb on the long arm of the X chromosome (Xq28), which encompasses *MECP2* and *IRAK1*.

With this single case, it is not possible to attribute a particular clinical phenotype to the lack of IRAK-1 since severe respiratory failure and pulmonary infections are commonly seen in patients with isolated MECP2 deficiency.

Immunological and Molecular Studies

-Fibroblasts from the IRAK-1- and MECP2-deficient patient responded poorly to all of the relevant TLR agonists tested. In contrast, IRAK-1- and MECP2-deficient fibroblasts from the patient displayed little or no impairment of IL-1R responses, by contrast to the results obtained for IRAK-4- or MyD88-deficient fibroblasts.

8.3.1.3 Deficiency in TIRAP

Clinical Features

Description in 2017 of a single family [92]. Autosomal recessive. Seven of the family members with the same genetic defect were healthy. The proband suffered from a pneumonia and sepsis due to *S. aureus* at 3 months of age. The difference in penetrance within family members was ascribed to the inability of the proband to develop antibodies against LTA (lipoteichoic acid), abundant in *S. aureus*, showing that human adaptive immunity had been able to rescue an inborn error of innate immunity in all family members, except for the proband, unable to produce specific antibodies.

Immunological and Molecular Studies

Responses to all TLR1/2, TLR2/6, and TLR4 agonists were impaired in the fibroblasts and leukocytes of all TIRAP-deficient individuals. However, the whole blood response to the TLR2/6 agonist staphylococcal lipoteichoic acid (LTA) was abolished only in the index case, the only family member lacking LTA-specific Abs. This defective response was reversed in the patient by anti-LTA mAb.

8.3.2 Isolated Congenital Asplenia

8.3.2.1 Clinical Features

Isolated congenital asplenia (ICA) is characterized by the absence of a spleen at birth without any other developmental defect. ICA predisposes individuals to life-threatening invasive infections early in childhood, caused by encapsulated bacteria, typically *Streptococcus pneumoniae* but occasionally *Neisseria meningitidis* and *Haemophilus influenzae b* [93].

A retrospective study in France showed that ICA affects at least 0.51 per 1 million newborns per year, but the incidence is probably higher (estimated 1 in 600,000) as individuals may not manifest until adulthood.

Asplenia can be suspected by the detection of Howell-Jolly bodies on a blood smear, which are the hallmark of the existence of a defect of spleen phagocytic function. Then, imaging tests can be performed to confirm the absence of spleen, such as ultrasound (US) or computed tomography (CT) scans of the abdomen, or even more sensitive and specific tests, such as selective spleen scintigraphy (SSS), which is performed using denatured erythrocytes labeled with Technetium-99m (Tc99m).

8.3.2.2 Immunological and Molecular Studies

RPSA gene is estimated to be responsible for 30–40% of ICA cases: 18 patients have been described to date bearing protein and nonprotein-coding mutations in *RPSA*, among a worldwide cohort of 73 patients with ICA. Most cases of ICA are sporadic, but multiplex kindreds exist, and the main mode of inheritance of ICA seems to be autosomal dominant (AD).

RPSA encodes for the ribosomal protein SA, a core component of the small subunit of the ribosome.

8.3.2.3 Treatment

Vaccines against encapsulated bacteria represent a major arm for the management of ICA patients. Annual influenza vaccination is recommended as well. Asplenia itself does not contraindicate the use of live attenuated vaccines. Antibiotic prophylaxis with penicillin V is recommended (amoxicillin is an alternative); the optimal duration of this antibiotic prophylaxis is still being debated (until 5 years old versus lifelong). In the case of allergy to penicillin, cotrimoxazole could be a valid alternative [94].

8.4 Section 3: Predisposition to Viral Diseases

8.4.1 Herpes Simplex Virus Encephalitis (HSE). Deficiencies of the TLR3 Pathway

8.4.1.1 Clinical Features

The primary infection by herpes simplex virus 1 (HSV-1) usually leads to symptoms involving the mucosa and skin, or most commonly asymptomatic infection. Other forms of HSV-1 infection exist, including a cutaneous form and eye infections consisting of keratitis and conjunctivitis. HSV-1 seroprevalence is high, demonstrating the typically benign nature of the infection [95]. Rarely does HSV-1 infect the CNS causing herpes simplex virus encephalitis (HSE). HSE is the most common form of sporadic viral encephalitis in Western countries, where it is estimated to occur in approximately two to four per 1,000.000 individuals per year [95, 96]. Peaks of HSE incidence occur between the ages of 6 months to 3 years, during primary HSV-1 infection, and in individuals older than 50 years, probably due to viral reactivation from latency.

Patients with impaired TLR3 immunity are susceptible to HSE. These patients remain normally resistant to other common viruses, as shown by positive serologic results to at least ten viruses without the occurrence of acute events. The patients have been also immunized with live vaccines with no adverse effect. HSV-1 infection outside the CNS is not usually observed. One TLR3-deficient patient developed CVB3 myocarditis in adulthood, and a mutation conferring *TRAF3* deficiency was associated with development of multiple myeloma [97, 98].

8.4.1.2 Molecular Studies

Human defects in several components of TLR3 pathway (TLR3, TRIF, TRAF3, TBK1, IRF3, and UNC93B1) are known as genetic etiology of HSE, by impairing cortical neuron-intrinsic type I interferon (IFN) immunity to HSV-1 (Fig. 8.5), with incomplete clinical penetrance [99–106]. These patients have a similar cellular phenotype consisting in impaired TLR3 signaling in fibroblasts, which results in impaired antiviral IFN production, and enhanced viral replication and cell death

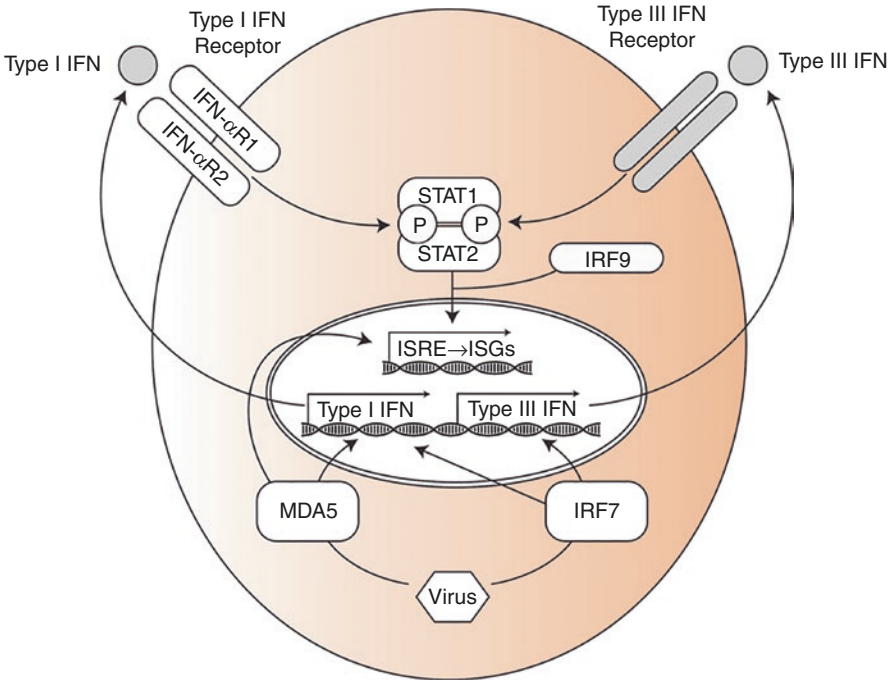

Fig. 8.5 Simplified schematic representation of the pathways in which mutations causing severe viral diseases have been identified

following infection with HSV-1 and vesicular stomatitis virus (VSV). Patients suffer from HSE in the course of primary infection, without detectable HSV-1 dissemination, and they do not suffer from multiple episodes. TLR3 is expressed and can stimulate IFN production in neurons, astrocytes, oligodendrocytes, and microglial cells [107]. The role of TLR3 immunity in host defense against HSV-1 in the CNS was tested in induced pluripotent stem cells derived from UNC-93B- and TLR3-deficient patients and from healthy controls [108]. UNC-93B-deficient and TLR3-deficient derived oligodendrocytes and neurons were much more susceptible to HSV-1 infection than control cells, whereas deficient, derived, neural stem cells and astrocytes were not. The increased susceptibility observed in oligodendrocytes and neurons was found to be associated with impaired IFN-β and IFN-λ1 production by these cell types in response to HSV infection.

No impaired responses to TLR3, or other TLR, agonists are usually observed in peripheral blood mononuclear cells (PBMC) or whole blood cells from patients with deficiencies of TLR3 pathway. At present, the best laboratory screening test for these PID is the analysis of antiviral IFN production after stimulation by TLR3 agonists in fibroblasts. It is worth mentioning that some patients do not display any TLR3-IFN-related phenotype in fibroblasts, so detection of antiviral IFN production, as well as viral replication and cell death rates, after incubation with HSV-1

and VSV is the laboratory test of choice for detection of PID associated with impaired TLR3-mediated immunity.

Finally, it has been described three unrelated children suffering from influenza A virus (IAV) infection manifesting as acute respiratory distress syndrome (IAV-ARDS) caused by two loss-of-function (LOF) TLR3 mutations, previously described in autosomal dominant (AD) TLR3 deficiency underlying HSE. AD TLR3-deficient leukocytes produce normal amount of IFN-α upon stimulation with TLR3 agonist, HSV-1 or IAV. Therefore, AD TLR3 deficiency, caused by the same type of TLR3 LOF variants and even by the same TLR3 variant, accounts for at least two sporadic infectious diseases, HSE and IAV-ARDS, affecting isolated organs such as the CNS and the lung, respectively, in otherwise healthy children [106]. Although theoretically both severe infections can hit the same individual with TLR3 deficiency, the coalescence of these infections has not yet been described.

8.4.1.3 Treatment and Prognosis

Untreated, HSE is fatal in up to 70% of cases. Treatment with acyclovir significantly decreases the mortality rate, but up to 60% of patients suffer from long-term neurological sequelae of varying severity [109–111].

Inherited defects of the TLR3 pathway should be considered in the differential diagnosis of children with HSE. HSE is uncommon in reported patients with PID, even in those with T, B, and/or NK cell deficiencies [3].

8.4.2 Susceptibility to HPV: Epidermodysplasia Verruciformis (HPV) and Others

8.4.2.1 Clinical Features

Epidermodysplasia verruciformis (EV) is a rare Mendelian genodermatosis presenting with persistent, disseminated, flat warts and pityriasis versicolor-like skin lesions induced by human β-papillomaviruses (β-HPVs) which are lacking E5 and E8 open reading frames (ORF). Some patients develop nonmelanoma skin cancer.

8.4.2.2 Molecular Studies

Biallelic null mutations in TMC6 or TMC8 encoding EVER1 and EVER2, respectively, account for half of EV cases [112]. Moreover, it has been described biallelic deleterious mutations in *CIB1,* encoding calcium- and integrin-binding protein 1, CIB1. The formation of a multimer consisting of CIB1, EVER1, and EVER2 is required for CIB1 stability [113, 114]. Therefore, the disruption of the IFN-independent CIB1-EVER1-EVER2-dependent keratinocyte-intrinsic immunity underlies the selective susceptibility to β-HPVs in EV patients. One of the CIB mutations has been identified as a *CIB1* splice-site founder mutation from Iranian origin with a common ancestor dating 650 years back [114].

8.4.2.3 Treatment and Prognosis

EV lesions are refractory to conventional therapies. Nonsurgical interventions with topical 5-fluorouracil, 5% imiquimod, tacalcitol, systemic retinoids combined with IFN-α, cimetidine, and topical 5-aminolevulinic acid photodynamic therapy yield inconsistent results. Approximately one-third of patients go on to develop malignancy with an average of 24 years between development of benign lesions and cancer. Invasive skin cancers are typically squamous cell carcinomas that often retain features of Bowen's carcinomas. They develop slowly and are locally destructive [115].

8.4.3 WHIM Syndrome

8.4.3.1 Clinical Features

WHIM syndrome (WHIM) is a congenital immunodeficiency with characteristic clinical features that include susceptibility to HPV infection-induced warts, condyloma acuminata, and carcinomas; neutropenia, B cell lymphopenia, and hypogammaglobulinemia-related recurrent infections; and bone marrow myelokathexis characterized by myeloid hyperplasia and apoptosis [116, 117].

8.4.3.2 Molecular Studies

Specific mutations identified in WHIM patients include heterozygous C-terminus deletional mutations of portions of the intracellular carboxy terminus of the chemokine receptor, CXC chemokine receptor 4 (CXCR4). WHIM leukocytes have enhanced responses to stromal cell-derived factor-1 (SDF-1), the cognate ligand of CXCR4. Enhanced activity of CXCR4 delays release of mature neutrophils from the bone marrow resulting in neutropenia and senescence with apoptosis of mature neutrophils retained in the marrow [116, 117].

8.4.3.3 Treatment and Prognosis

Treatment for WHIM patients is not standardized but aims at mitigating hematologic defects and clinical symptoms associated with the disease. It is controversial whether the main driver for susceptibility to infection is leukopenia versus hypogammaglobulinemia or the combination of the two. There are no pharmacologic agents that have a demonstrated ability to prevent or treat warts in WHIM patients; topical cidofovir has proven useful in particular cases. The HPV vaccine is limited to a small subset of the most highly cancer-associated strains. Successful treatment of warts in WHIM patients is typically restricted to destructive therapies. Current therapies for neutropenia and infections in WHIM patients include G-CSF, GM-CSF, intravenous immunoglobulin (IVIg), and the newly tested CXCR4 antagonists such as plerixafor. While both G-CSF and GM-CSF have been used to increase and maintain circulating neutrophil counts in the normal range, G-CSF is probably the

preferred and best tolerated agent. With hypogammaglobulinemia, administration of IVIg is effective at decreasing risk of infections. It has been reported that the hypogammaglobulinemia may improve following treatment with G-CSF. Use of prophylactic antibiotics in WHIM patients has not been evaluated statistically, but it is not unreasonable to extrapolate from studies in other primary immune deficiencies, neutropenias, or hypogammaglobulinemic states to support the use of antibiotic prophylaxis [116, 117].

The prognosis for WHIM patients depends in part on early recognition of the disorder, with aggressive medical intervention to reduce the frequency of recurrent bacterial infections and to detect and extirpate in the early stages any HPV lesions that appear to be dysplastic or malignant [116, 117].

8.4.4 Predisposition to Severe Virus Infections. The Critical Role of Type I and III Interferons

8.4.4.1 Clinical Features

Increasing numbers of patients suffering from severe viral diseases while remaining otherwise healthy have been reported in recent years. These patients routinely suffer from adverse reactions to live attenuated viral vaccines such as the yellow fever vaccine or the measles, mumps, and rubella (MMR) vaccine. Such patients often present with nonspecific symptoms, including rash in the site of inoculation, fevers, and lymphadenopathy. Besides these symptoms, the virus can disseminate and the patient may develop organ-specific features such as hepatitis, pneumonitis, hepatosplenomegaly, arthritis, or encephalitis, among others [118]. Additionally, this group of patients can also suffer from life-threatening diseases caused by viruses that, in most individuals, cause mild and self-limiting episodes, such as rhinovirus or influenza A. In these cases, patients present with fast-evolving lung infections that require hospitalization and can lead to acute respiratory distress syndrome (ARDS) [119, 120].

8.4.4.2 Molecular Studies

The genetic study of this group of patients has shown that deleterious mutations impairing immunity mediated by type I and III interferons are responsible for the disease (Fig. 8.1). Deficiency of MDA5 (encoded by *IFIH1*) that senses viral double-stranded RNAs (dsRNA) leads to impaired production of IFN-β and decreased induction of interferon-stimulated genes (ISG), hence reducing antiviral immunity. These defects are responsible for the severe disease that usually encompasses life-threatening rhinovirus and respiratory syncytial virus (RSV) infections [120–122]. Complete deficiency of the transcription factor IRF7 has been reported to cause severely reduced production of type I and type III interferons by leukocytes and plasmacytoid dendritic cells (pDCs) causing life-threatening infection by the influenza A virus H1N1 strain in addition to an adverse reaction to the MMR vaccine [119]. Deleterious biallelic mutations in the two chains of the receptor for type I interferons have been recently reported (IFN-αR1 and IFN-αR2) [118, 123]. These

mutations altogether abolish type I interferon signaling, causing the patients to suffer from complications following MMR vaccination. Besides, one of the patients reported with IFN-αR1 deficiency suffers from a viscerotropic disease caused by the vaccine strain of the yellow fever virus [118]. Following stimulation by type I and III interferons, STAT1 and STAT2 are phosphorylated. These two molecules heterodimerize and, after binding IRF9, travel to the cell nucleus to induce the transcription of ISGs by binding the ISRE sequence (Fig. 8.5). Deficiencies in these three components have also been described. Deficiencies of STAT1 and STAT2 that completely abolish signaling downstream of the type I and III IFN receptors cause susceptibility to severe diseases by different viruses. While, as described at the beginning of this chapter, STAT1-deficient patients display elevated susceptibility to multiple viral infections such as HSV-I, CMV, or HHV6, STAT2 deficiency results in severe adverse reactions to the MMR vaccine, such as disseminated vaccine strain measles [41, 124–130]. In addition, STAT1 complete deficiency also impairs type II interferon signaling (IFN-γ specifically) accounting for the mycobacterial disease observed in these patients [31, 124–127]. IRF9 complete deficiency impairs type I, and likely type III, interferon signaling, causing life-threatening influenza A infection in addition to an adverse reaction to the MMR vaccine [131]. CD16 deficiency underlies severe herpes virus infection independent of immunity mediated by type I or III interferons by a mechanism involving impairment of spontaneous NK cytotoxicity and reduced expression of CD2 in NK cells [132, 133].

8.4.4.3 Treatment and Prognosis

Given the limited number of patients with each genetic defect, no standardized treatment has been proposed to date. In most cases, treatment has been based on antiviral therapy according to the type of virus identified in each patient and, in the most severe cases, with admission to intensive care unit (ICU), supportive care, assisted respiration, mechanical ventilation, and/or extracorporeal membrane oxygenation [119, 120]. Hematopoietic stem cell transplantation (HSCT) has been attempted in 3 STAT1-deficient patients with mixed results [125]. One patient with STAT2 deficiency responded positively during infectious episodes to intravenous immunoglobulin (IVIG).

8.5 Section 4: Predisposition to Fungal Infections

8.5.1 Introduction

Saprophytic and commensal fungi infect billions of people each year [134]. Among all known fungal species (more than 100,000), only 300 are able to cause diseases in humans [135]; thus, only some of them are considered medically relevant, including yeast (*Candida* spp.), mold (*Aspergillus* spp.), atypical fungus (*Pneumocystis jirovecii)*, dimorphic fungi (*Coccioides*, *Paracoccioides,* and *Histoplasma* spp.), dermatophytes (*Trichophyton* spp.), and encapsulated fungi (*Cryptococcus* spp.).

The pathogenesis of the invasive and mucocutaneous fungal infections are largely different. While it seems that T-lymphocyte and IL-17 pathway determines the immune response involved in superficial infections, invasive infections are often seen related to quantitative or qualitative neutrophil disorders such as chronic granulomatous disease, autosomal recessive caspase recruitment domain-containing protein 9 (CARD9) deficiency, or neutropenic conditions [136].

8.5.2 Predisposition to Invasive Fungal Diseases

8.5.2.1 Definition and Epidemiology

Invasive fungal diseases (IFDs) are considered the major cause of morbidity and death among immunocompromised and hospitalized pediatric patients [137] with an incidence of around two million people worldwide [135] and with high rates of mortality (30–50%) [138]. The most common fungi involved in IFDs are *Candida, Aspergillus, Cryptococcus, and Pneumocystis* spp. [135].

Two main PID cause special susceptibility to suffer IFDs: chronic granulomatous disease (CDG; see Chap. 9) and CARD9 deficiency.

CARD9 Deficiency (OMIM 607212)

Clinical Features and Pathogenesis (Fig. 8.6)

CARD9 is an essential molecule of the innate immune system involved in the control of certain fungi identified by specific pathogen recognition receptors (such as Dectin-1, Dectin-2, mannose receptor, MINCLE), determining the myeloid and epithelial cell response [137, 139, 140]. Its impairment results in a poor production of specific inflammatory cytokines and chemokines (IL-6, IL-23, etc.), which are necessary for the differentiation of the IL-17-producing T cells [140]. Since CARD9 plays a specific role in antifungal immune response, these patients exhibit an extreme susceptibility to fungal infections but not to bacterial or viral infections [139].

The innate immunity to fungal infections relies on different mechanisms, including phagocytosis or production of reactive oxygen species, which are CARD9-independent. However, some fungi like unopsonized yeast cells (e.g., *Candida* spp) depend directly on the CARD9 pathway [139, 140]. This probably explains the specific clinical expression described in CARD9-deficient patients, which is characterized by the spontaneous development of *Candida* spp. infections, predominantly in oral mucosae, central nervous system (CNS), bone, and subcutaneous tissues. Infections due to dark-walled molds and yeast-like fungi (*Aspergillus, Exophiala,* and *Phialophora*) have been described in these patients too [137, 139, 140].

The clinical penetrance of this deficiency seems to be globally complete [141], and fungal disease can occur at any age, from early childhood to late adulthood; therefore, all children and adults with an unexplained IFD should be screened for CARD9 mutations [141].

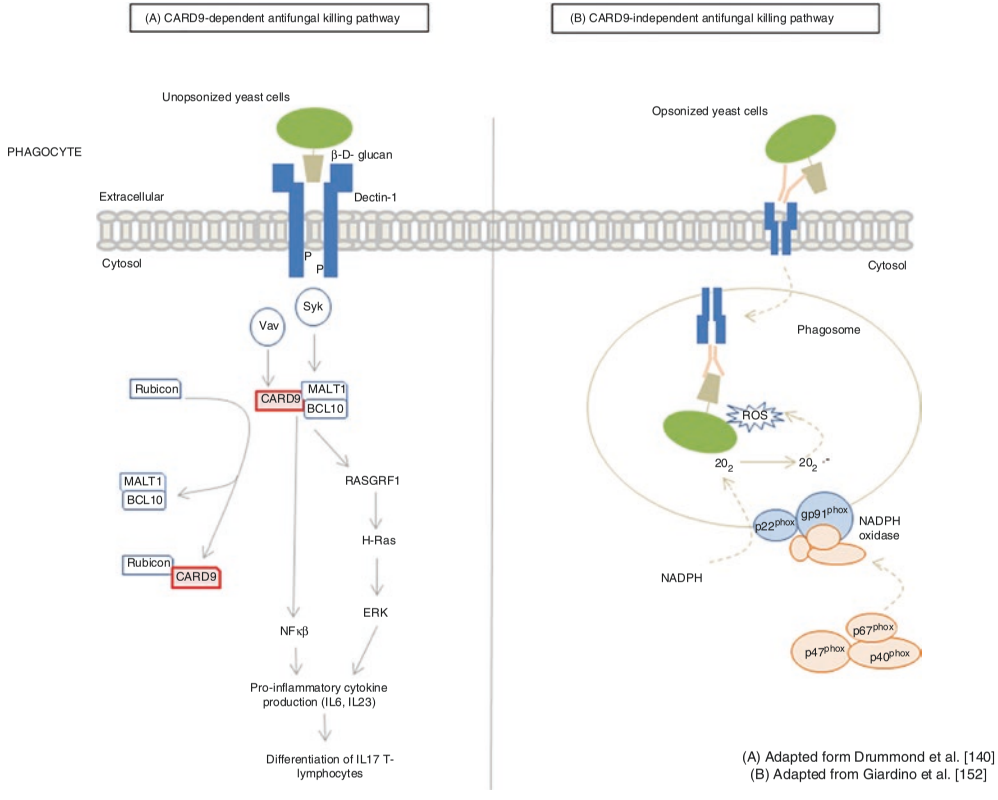

Fig. 8.6 Different phagocyte mechanisms involved in yeast cells innate immune response.
This figure depicts the two major mechanisms involved in antifungal innate responses in phagocytes, CARD9-dependent mechanisms (**a**) and CARD9-independent mechanisms (**b**)

Immunological and Molecular Studies

Until now, CARD9 deficiency has been reported only in patients with autosomal recessive mutations since none of the heterozygous patients has presented any unusual infection [141].

In the largest published cohort of analyzed CARD9 patients (31 patients), the basic immunological tests including cell count (neutrophils, T, B, and NK lymphocytes and monocytes) as well as the T and B lymphocytes subsets phenotyping, proliferative response to mitogens, or oxidative burst test were normal. Hypereosinophilia and high serum IgE levels had been observed in almost 50% of the patients without atopy or allergic symptoms [141]. The proportion of IL17-producing T cells was low in almost 2/3 of the patients and the production of IL-17A after stimulation with different fungal triggers (*C. albicans, P. verrucosa, E. spinifera*, etc.) was low only in 1/3 of the patients.

CARD9 protein expression could be assessed by western blotting in PBMCs, neutrophils, monocyte-derived dendritic cells, or monocytes-derived macrophages. However, since the protein expression varies depending on the consequences of the mutation on the protein, the detection of the protein is not sufficient to discard the diagnosis of CARD9 deficiency [141]. Although some more experimental tests are available to asses CARD9 pathway, in clinical practice, in front of a CARD9 suspicion, a genetic test should be performed to confirm this disease [141].

Treatment and Prognosis

The treatment options in these patients are limited [139]. Chronic mucocutaneous candidiasis (CMC) should be managed by long-life treatment with topic or azole agents as first-line treatments and with systemic therapy (azoles agents or echinocandins) in case of an extensive or uncontrolled disease. Treatment duration should be adapted to fungal species involved in the infection and to the site (CNS, skin, etc.) [141].

In some cases, adjuvant treatments should be added to antifungal treatment [141]. In this sense, good results using granulocyte-macrophage colony-stimulating [142] or granulocyte colony-stimulating factors [143] have been published, though this approach seems not effective in all cases. A recent case with successful hematopoietic stem cells transplant (HSCT) has been published [144], but the potential utility of HSCT in this disease remains unclear [141].

In conclusion, currently there are no defined guidelines for these patients: Long-term antifungal treatments are often needed with a fast relapse observed after withdrawal; besides, there is a lack of information regarding the role of HSCT in this disease [141].

8.5.3 Predisposition to Chronic Mucocutaneous Candidiasis

Candida spp. is commensal yeast that commonly colonizes mouth, colon, or vagina in healthy people. This asymptomatic colonization can turn to disease due to certain

acquired (broad-spectrum antibiotics, oral steroids) or inherited (PID) risk factors [136, 145].

Within inherited conditions, chronic mucocutaneous candidiasis (CMC) represents a phenotypic manifestation of an heterogenous group of PID characterized by increased susceptibility to chronic or recurrent superficial *Candida* spp. infections [146], mainly associated with IL-17-mediated impaired immunity (Fig. 8.7) including AD hyper-IgE syndrome, CARD9 deficiency (able to present CMC and invasive fungal infections), AD transducer and activator transcription 1 (STAT1 gain of function), mutations related to IL-17 signaling (IL-17 or IL-17 receptor mutations), IL-12 receptor β1 or PID with aberrant neutralizant autoantibodies *versus* TH-17-produced cytokines such as autoimmune polyendocrinopathy syndrome type I [136, 146].

In this chapter, we will focus on those inborn errors of innate immunity with special risk of CMC: *STAT1* GOF, *IL17F*, *IL17RA*, *IL17RF,* and *ACT1* mutations [1].

8.5.3.1 STAT1 Gain-of-Function Mutation (GOF) (OMIM 614162)

Autosomal dominant heterozygous missense mutations of *STAT1* have been identified in a growing number of patients since its description in 2011 [136, 147]. This mutation seems to be the major cause of CMC, detected in more than half of patients [136, 147].

Clinical Features

The clinical phenotype is variable, ranging from CMC to severe autoimmunity and life-threatening infections [147]. The symptoms usually start during childhood [147, 148].

In terms of infectious manifestations, CMC represents the main problem although patients can also develop viral (mainly herpes virus but also JC or varicella-zoster virus), mycobacterial, and bacterial (e.g., *St. aureus*) infections. CMC is described in the oral mucosae (most frequent), skin, esophageal, genital, or nails, and *Candida albicans* is the most frequent fungi involved. Despite this, other dermatophytic and mold fungi have been reported. In countries where other endemic mycosis exists, such as coccidiosis or histoplasmosis, severe disseminated infections can be observed [147]. Patients with STAT1 GOF can also develop bacterial infections, especially sinopulmonary that can evolve to bronchiectasis [147].

Other relevant manifestations are autoimmune and autoinflammatory events, described in 1/3 of the patients, including hypothyroidism, type 1 diabetes, autoimmune cytopenia, alopecia, or a complete IPEX-like phenotype [136, 148].

Less frequent but severe complications observed in these patients include vascular alterations, mainly intracranial aneurysms, conferring a risk of hemorrhage or death [147], and an increased cancer risk (currently 6%), being carcinomas of the upper gastrointestinal tract the most common [147].

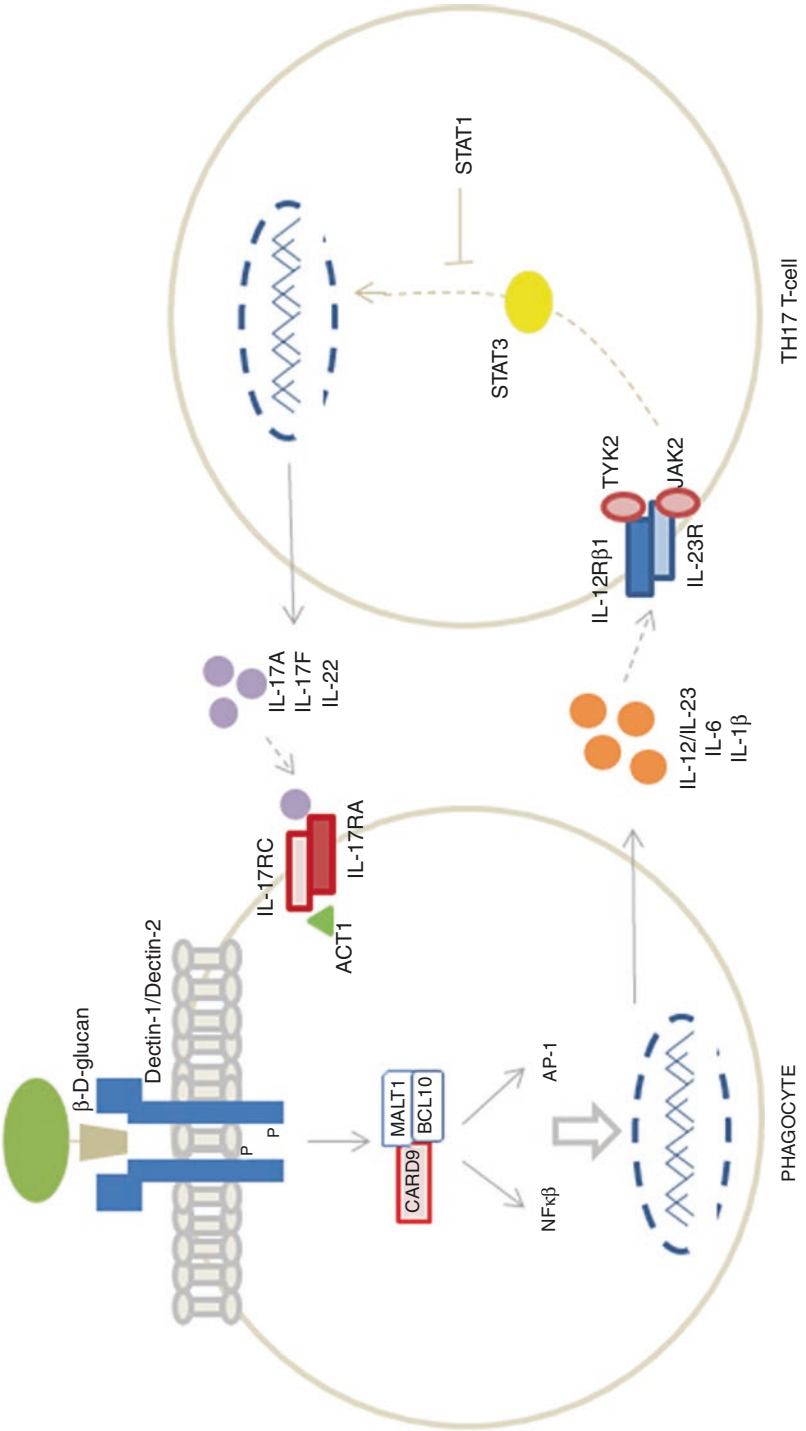

Fig. 8.7 Antifungal immunity involved in CMC (adapted from *Okada et al.* [136])

Immunological and Molecular Studies

The majority of patients show normal values for the initial immunologic screening, including immunoglobulins, immunoglobulin isotypes, and lymphocytes subpopulations (T, B, and NK). In some cases, low memory B cells and low IgG2 or IgG4 levels have been reported [136, 147]. On the other hand, TH17 count is commonly low (almost 80%) [147].

The STAT1 phosphorization/dephosphorization assay could be useful when STAT1 GOF mutation is suspected. An increased phosphorylation of tyrosine 701 after IFN-γ stimulation can be observed associated with impairment in dephosphorization in some patients, resulting in an increased of STAT1 phosphorylation in response to certain stimulations [136, 147]. Currently, the results of this assay are not conclusive, and normal result does not preclude the diagnosis [147].

Finally, a genetic test should be considered for patients with unexplained CMC. Until now, STAT1-GOF mutations described are located in coil-coil domain or DNA-binding domain (the latter might confer poor prognosis) [136, 147].

Treatment and Prognosis

Most of the affected patients have been successfully treated with long-term topical and/or systemic antifungal prophylaxis. Fluconazole is the main first-line oral therapy, while nystatin seems to be a good topical alternative [136, 147]. Antibacterial prophylaxis with trimethoprim-sulfamethoxazole should be considered in case of recurrent and uncontrolled bacterial infections [136] as well as IRT [147].

Janus-associated kinase (JAK), an upstream signal transducer for STAT1, inhibitors are being considered increasingly although only case reports are published with different rates of success. Recalcitrant CMC seems to respond successfully as well as certain autoimmunity events such as cytopenias [149] or alopecia. However, the effect seems to be transitory, and the treatment requires long-term administration. Infectious screening (mainly viral) is recommended during treatment, and in the absence of new evidence, it seems advisable considering antiviral prophylaxis during jak-inh therapy with acyclovir or valacyclovir [147].

HSCT could be considered as a curative-intention treatment, but currently the results are disappointing with approximately 50% of patient's survival. However, the use of JAK inhibitor as a bridge therapy followed by HSCT might improve the HSCT outcomes [147, 150].

Table 8.2 summarizes the clinical features, immunological studies, and treatment recommended in other PID related with IL-17 pathway.

Table 8.2 Other mutations involved in IL-17 pathway [136, 145]

Gene	Clinical features	Diagnosis	Treatment
AD IL-17F deficiency (OMIM 613956) Incomplete clinical penetrance	CMC Recurrent upper respiratory tract infections Furunculosis	– Absence of IL-17-expressing T cells – In vitro studies defective binding to IL-17RA on fibroblasts	– Antifungal prophylaxis: fluconazole (first-line oral drug) and nystatin (alternative topic treatment) – Antibacterial prophylaxis in case of Staph. infections: trimethoprim-sulfamethoxazole
AR IL-17RA deficiency (OMIM 613953) Complete clinical penetrance	CMC Recurrent upper respiratory tract infections Mild *St. aureus* infections	– Absence of protein expression with flow cytometry on the surface of the patient's fibroblasts and PBMCs – The fibroblasts do not respond to IL-17A and IL17F stimulation	
AR IL-17RC deficiency (OMIM 616445) Complete clinical penetrance	CMC No *St. aureus* infections	Lack of response to IL-17A and IL-17F stimulation but normal response to IL-17RC-independent signaling via IL-25	
AR ACT1 deficiency (OMIM 615527) Complete clinical penetrance	CMC Mild *St. aureus* infections	Impairment in response to IL-17A and IL-17F in fibroblasts and to IL-17E in leukocytes	

PBMCs Peripheral blood mononuclear cells

References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40:24–64
2. Modell V, Orange JS, Quinn J, Modell F (2018) Global report on primary immunodeficiencies: 2018 update from the Jeffrey Modell Centers Network on disease classification, regional trends, treatment modalities, and physician reported outcomes. Immunol Res 66(3):367–380
3. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W et al (2018) The 2017 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol 38(1):129–143
4. Blumenthal B (1954) A case of cutaneous tuberculosis: provoked by BCG-vaccination? Acta Derm Venereol 34(6):474–481
5. Groth-Petersen E (1952) Tuberculous abscess associated with BCG vaccination. Ugeskr Laeger 114(42):1503–1504
6. Wasz-Hockert O (1954) Bacterial meningitis in connection with BCG vaccination; report of a case. Ann Med Intern Fenn 43(1):77–80

7. Casanova J-L (2015) Severe infectious diseases of childhood as monogenic inborn errors of immunity. Proc Natl Acad Sci U S A 112(51):7128–7137
8. Engbaek HC (1964) Three cases in the same family of fatal infection with M. Avium. Acta Tuberc Pneumol Scand 45:105–117
9. Jouanguy E, Altare F, Lamhamedi S, Revy P, Emile JF, NEWPORT M et al (1996) Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. N Engl J Med 335(26):1956–1961
10. Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, Williamson R et al (1996) A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. N Engl J Med 335(26):1941–1949
11. Feinberg J, Fieschi C, Doffinger R, Feinberg M, Leclerc T, Boisson-Dupuis S et al (2004) Bacillus Calmette Guérin triggers the IL-12/IFN-γ axis by an IRAK-4- and NEMO-dependent, non-cognate interaction between monocytes, NK, and T lymphocytes. Eur J Immunol 34(11):3276–3284
12. van de Vosse E, Haverkamp MH, Ramirez-Alejo N, Martinez-Gallo M, Blancas-Galicia L, Metin A et al (2013) IL-12Rβ1 deficiency: mutation update and description of the IL12RB1 variation database. Hum Mutat 34(10):1329–1339
13. de Beaucoudrey L, Samarina A, Bustamante J, Cobat A, Boisson-Dupuis S, Feinberg J et al (2010) Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries. Medicine (Baltimore) 89(6):381–402
14. Altare F, Lammas D, Revy P, Jouanguy E, Döffinger R, Lamhamedi S et al (1998) Rapid publication inherited interleukin 12 deficiency in a child with Bacille Calmette-Guérin and Salmonella enteritidis disseminated infection. J Clin Invest 102(12):2035–2040
15. Prando C, Samarina A, Bustamante J, Boisson-Dupuis S, Cobat A, Picard C et al (2013) Inherited IL-12p40 deficiency: genetic, immunologic, and clinical features of 49 patients from 30 kindreds. Medicine (Baltimore) 92(2):109–122
16. Martínez-Barricarte R, Markle JG, Ma CS, Deenick EK, Ramírez-Alejo N, Mele F et al (2018) Human IFN-immunity to mycobacteria is governed by both IL-12 and IL-23. Sci Immunol 30(3)
17. Zhang X, Bogunovic D, Payelle-Brogard B, Francois-Newton V, Speer SD, Yuan C et al (2015) Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation. Nature 517(7532):89–93
18. Bogunovic D, Byun M, Durfee LA, Abhyankar A, Sanal O, Mansouri D et al (2012) Mycobacterial disease and impaired IFN-γ immunity in humans with inherited ISG15 deficiency. Science 337(6102):1684–1688
19. Kong X-F, Martinez-Barricarte R, Kennedy J, Mele F, Lazarov T, Deenick EK et al (2018) Disruption of an anti-mycobacterial circuit between dendritic and Th cells in human SPPL2a deficiency. Nat Immunol 19(9):973
20. Rosain J, Kong XF, Martinez-Barricarte R, Oleaga-Quintas C, Ramirez-Alejo N, Markle J et al (2019) Mendelian susceptibility to mycobacterial disease: 2014–2018 update. Immunol Cell Biol 97(4):360–367
21. Kreins AY, Ciancanelli MJ, Okada S, Kong X-F, Ramírez-Alejo N, Kilic SS et al (2015) Human TYK2 deficiency: mycobacterial and viral infections without hyper-IgE syndrome. J Exp Med 212(10):1641–1662
22. Kerner G, Ramirez-Alejo N, Seeleuthner Y, Yang R, Ogishi M, Cobat A et al (2019) Homozygosity for TYK2 P1104A underlies tuberculosis in about 1% of patients in a cohort of European ancestry. Proc Natl Acad Sci U S A 116(21):10430–10434
23. Okada S, Markle JG, Deenick EK, Mele F, Averbuch D, Lagos M et al (2015) Impairment of immunity to Candida and Mycobacterium in humans with bi-allelic RORC mutations. Science 349(6248):606–613
24. Kerner G, Guérin A, Oleaga-Quintas C, Rosain J, Rappaport F, Le Voyer T, et al. (2019) Mycobacterial disease due to inherited IFN-gamma deficiency. ESID 2019; Brussels.

25. Jouanguy E, Dupuis S, Pallier A, Döffinger R, Fondanèche M-C, Fieschi C et al (2000) In a novel form of IFN-γ receptor 1 deficiency, cell surface receptors fail to bind IFN-γ. J Clin Invest 105(10):1429–1436
26. Dorman SE, Picard C, Lammas D, Heyne K, van Dissel JT, Baretto R et al (2004) Clinical features of dominant and recessive interferon γ receptor 1 deficiencies. Lancet 364(9451):2113–2121
27. Sologuren I, Boisson-Dupuis S, Pestano J, Vincent QB, Fernández-Pérez L, Chapgier A et al (2011) Partial recessive IFN-γR1 deficiency: genetic, immunological and clinical features of 14 patients from 11 kindreds. Hum Mol Genet 20(8):1509–1523
28. Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondanèche MC, Dupuis S et al (1999) A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nat Genet 21(4):370–378
29. Rosenzweig SD, Schwartz OM, Brown MR, Leto TL, Holland SM (2004) Characterization of a dipeptide motif regulating IFN-receptor 2 plasma membrane accumulation and IFN-responsiveness. J Immunol 173(6):3991–3999
30. Moncada-Velez M, Martinez-Barricarte R, Bogunovic D, Kong X-F, Blancas-Galicia L, Tirpan C et al (2013) Partial IFN- R2 deficiency is due to protein misfolding and can be rescued by inhibitors of glycosylation. Blood 122(14):2390–2401
31. Chapgier A, Boisson-Dupuis S, Jouanguy E, Vogt G, Feinberg J, Prochnicka-Chalufour A et al (2006) Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. PLoS Genet 2(8):e131
32. Dupuis S (2001) Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. Science 293(5528):300–303
33. Hirata O, Okada S, Tsumura M, Kagawa R, Miki M, Kawaguchi H et al (2013) Heterozygosity for the Y701C STAT1 mutation in a multiplex kindred with multifocal osteomyelitis. Haematologica 98(10):1641–1649
34. Eletto D, Burns SO, Angulo I, Plagnol V, Gilmour KC, Henriquez F et al (2016) Biallelic JAK1 mutations in immunodeficient patient with mycobacterial infection. Nat Commun 7:13992
35. Bustamante J, Arias AA, Vogt G, Picard C, Galicia LB, Prando C et al (2011) Germline CYBB mutations that selectively affect macrophages in kindreds with X-linked predisposition to tuberculous mycobacterial disease. Nat Immunol 12(3):213–221
36. Bustamante J, Picard C, Boisson-Dupuis S, Abel L, J-LL C (2011) Genetic lessons learned from X-linked Mendelian susceptibility to mycobacterial diseases. Ann N Y Acad Sci 1246:92–101
37. Bustamante J, Picard C, Fieschi C, Filipe-Santos O, Feinberg J, Perronne C et al (2006) A novel X-linked recessive form of Mendelian susceptibility to mycobacterial disease. J Med Genet 44(2):e65–e65
38. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J et al (2011) IRF8 mutations and human dendritic-cell immunodeficiency. N Engl J Med 365(2):127–138
39. Bustamante J, Boisson-Dupuis SSS, Abel L, Casanova J-L (2014) Mendelian susceptibility to mycobacterial disease: Genetic, immunological, and clinical features of inborn errors of IFN gamma immunity. Semin Immunol 26(6):454–470
40. Filipe-Santos O, Bustamante J, Chapgier A, Vogt G, de Beaucoudrey L, Feinberg J et al (2006) Inborn errors of IL-12/23- and IFN-γ-mediated immunity: molecular, cellular, and clinical features. Semin Immunol 18(6):347–361
41. Chapgier A, Wynn RF, Jouanguy E, Filipe-Santos O, Zhang S, Feinberg J et al (2006) Human complete Stat-1 deficiency is associated with defective type I and II IFN responses in vitro but immunity to some low virulence viruses in vivo. J Immunol 176(8):5078–5083
42. Boisson-Dupuis S, El Baghdadi J, Parvaneh N, Bousfiha A, Bustamante J, Feinberg J et al (2011) IL-12Rβ1 deficiency in two of fifty children with severe tuberculosis from Iran, Morocco, and Turkey. Cardona P-J, editor. PLoS One 6(4):e18524
43. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, Camcioglu Y, Parvaneh N, El Azbaoui S et al (2015) Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. Immunol Rev 264(1):103–120

44. Fieschi C (2004) A novel form of complete IL-12/IL-23 receptor 1 deficiency with cell surface-expressed nonfunctional receptors. Blood 104(7):2095–2101
45. Ouederni M, Sanal O, Ikinciogullari A, Tezcan I, Dogu F, Sologuren I et al (2014) Clinical features of Candidiasis in patients with inherited interleukin 12 receptor β1 deficiency. Clin Infect Dis 58(2):204–213
46. Obinata K, Lee T, Niizuma T, Kinoshita K, Shimizu T, Hoshina T et al (2013) Two cases of partial dominant interferon-γ receptor 1 deficiency that presented with different clinical courses of bacille Calmette-Guérin multiple osteomyelitis. J Infect Chemother 19(4):757–760
47. Tsumura M, Okada S, Sakai H, Yasunaga S, Ohtsubo M, Murata T et al (2012) Dominant-negative STAT1 SH2 domain mutations in unrelated patients with Mendelian susceptibility to mycobacterial disease. Hum Mutat 33(9):1377–1387
48. Jirapongsananuruk O, Luangwedchakarn V, Niemela JE, Pacharn P, Visitsunthorn N, Thepthai C et al (2012) Cryptococcal osteomyelitis in a child with a novel compound mutation of the IL12RB1 gene. Asian Pac J Allergy Immunol 30(1):79–82
49. Boudjemaa S, Dainese L, Héritier S, Masserot C, Hachemane S, Casanova J-L et al (2016) Disseminated BCG osteomyelitis related to STAT 1 gene deficiency mimicking a metastatic neuroblastoma. Pediatr Dev Pathol 3:16-02-1778-CR.1
50. Shamriz O, Engelhard D, Rajs AP, Kaidar-Shwartz H, Casanova J-L, Averbuch D (2013) Mycobacterium szulgai chronic multifocal osteomyelitis in an adolescent with inherited STAT1 deficiency. Pediatr Infect Dis J 32(12):1345–1347
51. Sasaki Y, Nomura A, Kusuhara K, Takada H, Ahmed S, Obinata K et al (2002) Genetic basis of patients with bacille Calmette-Guerin osteomyelitis in Japan: identification of dominant partial interferon-gamma receptor 1 deficiency as a predominant type. J Infect Dis 185(5):706–709
52. Arend S (2001) Multifocal osteomyelitis caused by nontuberculous mycobacteria in patients with a genetic defect of the interferon-Î³ receptor. Neth J Med 59(3):140–151
53. Cardenes M, Angel-Moreno A, Fieschi C, Sologuren I, Colino E, Molines A et al (2010) Oesophageal squamous cell carcinoma in a young adult with IL-12R beta 1 deficiency. J Med Genet 47(9):635–637
54. Toyoda H, Ido M, Nakanishi K, Nakano T, Kamiya H, Matsumine A et al (2010) Multiple cutaneous squamous cell carcinomas in a patient with interferon gamma receptor 2 (IFN gamma R2) deficiency. J Med Genet 47(9):631–634
55. Donadieu J, Lamant M, Fieschi C, de Fontbrune FS, Caye A, Ouachee M et al (2018) Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients. Haematologica 103(8):1278–1287
56. Sologuren I, Martínez-Saavedra MT, Solé-Violán J, de Borges de Oliveira E, Betancor E, Casas I et al (2018) Lethal influenza in two related adults with inherited GATA2 deficiency. J Clin Immunol 38(4):513–526
57. Cohen JI, Dropulic L, Hsu AP, Zerbe CS, Krogmann T, Dowdell K et al (2016) Association of GATA2 deficiency with severe primary Epstein-Barr virus (EBV) infection and EBV-associated cancers. Clin Infect Dis 63(1):41–47
58. Toubiana J, Okada S, Hiller J, Oleastro M, Gomez ML, Becerra JCA et al (2016) Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. Blood 127(25):3154–3164
59. Esteve-Solé A, Sologuren I, Martínez-Saavedra MT, Deyà-Martínez À, Oleaga-Quintas C, Martinez-Barricarte R et al (2018) Laboratory evaluation of the IFN-γ circuit for the molecular diagnosis of Mendelian susceptibility to mycobacterial disease. Crit Rev Clin Lab Sci 55(3):184–204
60. Fieschi C, Dupuis S, Picard C, Smith CI, Holland SM, Casanova JL (2001) High levels of interferon gamma in the plasma of children with complete interferon gamma receptor deficiency. Pediatrics 107(4):E48
61. Kong X-FF, Vogt G, Itan Y, Macura-Biegun A, Szaflarska A, Kowalczyk D et al (2013) Haploinsufficiency at the human IFNGR2 locus contributes to mycobacterial disease. Hum Mol Genet 22(4):769–781

62. Ehlayel M, de Beaucoudrey L, Fike F, Nahas SA, Feinberg J, Casanova J-LL et al (2008) Simultaneous presentation of 2 rare hereditary immunodeficiencies: IL-12 receptor beta1 deficiency and ataxia-telangiectasia. J Allergy Clin Immunol 122(6):1217–1219
63. Rosenzweig SD, Dorman SE, Uzel G, Shaw S, Scurlock A, Brown MR et al (2004) A novel mutation in IFN-gamma receptor 2 with dominant negative activity: biological consequences of homozygous and heterozygous states. J Immunol 173(6):4000–4008
64. Kong X-FF, Vogt G, Chapgier A, Lamaze C, Bustamante J, Prando C et al (2010) A novel form of cell type-specific partial IFN-gammaR1 deficiency caused by a germ line mutation of the IFNGR1 initiation codon. Hum Mol Genet 19(3):434–444
65. Sampaio EP, Bax HI, Hsu AP, Kristosturyan E, Pechacek J, Chandrasekaran P et al (2012) A novel STAT1 mutation associated with disseminated mycobacterial disease. J Clin Immunol 32(4):681–689
66. Naviglio S, Soncini E, Vairo D, Lanfranchi A, Badolato R, Porta F (2017) Long-term survival after hematopoietic stem cell transplantation for complete STAT1 deficiency. J Clin Immunol 37(7):701–706
67. Salem S, Langlais D, Lefebvre F, Bourque G, Bigley V, Haniffa M et al (2014) Functional characterization of the human dendritic cell immunodeficiency associated with the IRF8(K108E) mutation. Blood 124(12):1894–1904
68. Ying W, Liu D, Dong X, Wang W, Hui X, Hou J et al (2019) Current status of the management of mendelian susceptibility to mycobacterial disease in mainland China. J Clin Immunol 39(6):600–610
69. Reed B, Dolen WK (2018) The child with recurrent mycobacterial disease. Curr Allergy Asthma Rep 18(8):44
70. Olbrich P, Martínez-Saavedra MT, Perez-Hurtado JM, Sanchez C, Sanchez B, Deswarte C et al (2015) Diagnostic and therapeutic challenges in a child with complete Interferon-γ Receptor 1 deficiency. Pediatr Blood Cancer 62(11):2036–2039
71. Martínez-Barricarte R, Megged O, Stepensky P, Casimir P, Moncada-Velez M, Averbuch D et al (2014) Mycobacterium simiae infection in two unrelated patients with different forms of inherited IFN-γR2 deficiency. J Clin Immunol 34(8):904–909
72. Bandari AK, Muthusamy B, Bhat S, Govindaraj P, Rajagopalan P, Dalvi A et al (2019) A novel splice site mutation in IFNGR2 in patients with primary immunodeficiency exhibiting susceptibility to mycobacterial diseases. Front Immunol 10:1964
73. Oleaga-Quintas C, Deswarte C, Moncada-Vélez M, Metin A, Krishna Rao I, Kanık-Yüksek S et al (2018) A purely quantitative form of partial recessive IFN-γR2 deficiency caused by mutations of the initiation or second codon. Hum Mol Genet 27(22):3919–3935
74. Phoompoung P, Ankasekwinai N, Pithukpakorn M, Foongladda S, Umrod P, Suktitipat B et al (2017) Factors associated with acquired anti IFN-γ autoantibody in patients with nontuberculous mycobacterial infection. PLoS One 12(4):1–10
75. Hong GH, Ortega-Villa AM, Hunsberger S, Chetchotisakd P, Anunnatsiri S, Mootsikapun P et al (2019) Natural history and evolution of anti-interferon-γ autoantibody-associated immunodeficiency syndrome in Thailand and the United States. Intergovernmental panel on climate change, editor. Clin Infect Dis 58(12):7250–7257
76. Liew WK, Thoon KC, Chong CY, Tan NWH, Cheng DT, Chan BSW et al (2019) Juvenile-onset immunodeficiency secondary to anti-interferon-gamma autoantibodies. J Clin Immunol 39(5):512–518
77. Doffinger R, Helbert MR, Barcenas-Morales G, Yang K, Dupuis S, Ceron-Gutierrez L et al (2004) Autoantibodies to interferon-γ in a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity. Clin Infect Dis 38(1):e10–e14
78. Chi CY, Chu CC, Liu JP, Lin CH, Ho MW, Lo WJ et al (2013) Anti-IFN-γ autoantibodies in adults with disseminated nontuberculous mycobacterial infections are associated with HLA-DRB1*16:02 and HLA-DQB1*05:02 and the reactivation of latent varicella-zoster virus infection. Blood 121(8):1357–1366

79. Patel SY, Ding L, Brown MR, Lantz L, Gay T, Cohen S et al (2005) Anti-IFN-gamma autoantibodies in disseminated nontuberculous mycobacterial infections. J Immunol 175(7):4769–4776
80. Chetchotisakd P, Anunnatsiri S, Nanagara R, Nithichanon A, Lertmemongkolchai G (2018) Intravenous cyclophosphamide therapy for anti-IFN-gamma autoantibody-associated Mycobacterium abscessus infection. J Immunol Res 2018:1–7
81. Jiang S, Li X, Hess NJ, Guan Y, Tapping RI (2016) TLR10 is a negative regulator of both MyD88-dependent and -independent TLR signaling. J Immunol 196(9):3834–3841
82. Picard C, Puel A, Bonnet M, Ku C-L, Bustamante J, Yang K et al (2003) Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science 299(5615):2076–2079
83. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku C-L et al (2008) Pyogenic bacterial infections in humans with MyD88 deficiency. Science 321(5889):691–696
84. Picard C, von Bernuth H, Ghandil P, Chrabieh M, Levy O, Arkwright PD et al (2010) Clinical features and outcome of patients with IRAK-4 and MyD88 deficiency. Medicine (Baltimore) 89(6):403–425
85. Picard C, Casanova JL, Puel A (2011) Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. Clin Microbiol Rev 24(3):490–497
86. Alsina L, Israelsson E, Altman MC, Dang KK, Ghandil P, Israel L et al (2014) A narrow repertoire of transcriptional modules responsive to pyogenic bacteria is impaired in patients carrying loss-of-function mutations in MYD88 or IRAK4. Nat Immunol 15(12): 1134–1142
87. Frans G, Meyts I, Picard C, Puel A, Zhang S-Y, Moens L et al (2014) Addressing diagnostic challenges in primary immunodeficiencies: laboratory evaluation of Toll-like receptor- and NF-κB-mediated immune responses. Crit Rev Clin Lab Sci 51(2):112–123
88. von Bernuth H, Ku C-L, Rodriguez-Gallego C, Zhang S, Garty B-Z, Maródi L et al (2006) A fast procedure for the detection of defects in Toll-like receptor signaling. Pediatrics 118(6):2498–2503
89. Weller S, Bonnet M, Delagreverie H, Israel L, Chrabieh M, Maródi L et al (2012) IgM+IgD+CD27+ B cells are markedly reduced in IRAK-4-, MyD88-, and TIRAP- but not UNC-93B-deficient patients. Blood 120(25):4992–5001
90. Routes J, Abinun M, Al-Herz W, Bustamante J, Condino-Neto A, De La Morena MT et al (2014) ICON: diagnóstico precoz de inmunodeficiencias congénitas. J Clin Immunol 34(4):398–424
91. Della Mina E, Borghesi A, Zhou H, Bougarn S, Boughorbel S, Israel L et al (2017) Inherited human IRAK-1 deficiency selectively impairs TLR signaling in fibroblasts. Proc Natl Acad Sci U S A 114(4):E514–E523
92. Israel L, Wang Y, Bulek K, Della Mina E, Zhang Z, Pedergnana V et al (2017) Human adaptive immunity rescues an inborn error of innate immunity. Cell 168(5):789–800.e10
93. Bolze A, Boisson B, Bosch B, Antipenko A, Bouaziz M, Sackstein P et al (2018) Incomplete penetrance for isolated congenital asplenia in humans with mutations in translated and untranslated RPSA exons. Proc Natl Acad Sci U S A 115(34):E8007–E8016
94. Aguilar C, Malphettes M, Donadieu J, Chandesris O, Coignard-Biehler H, Catherinot E et al (2014) Prevention of infections during primary immunodeficiency. Clin Infect Dis 59(10):1462–1470
95. Whitley RJ, Miller RL (2001) Review immunologic approach to herpes simplex virus. Viral Immunol 14(3):466–473. Mary Ann Liebert, Inc
96. Najioullah F, Bosshard S, Thouvenot D, Boibieux A, Menager B, Biron F et al (2000) Diagnosis and surveillance of herpes simplex virus infection of the central nervous system. J Med Virol 61(4):468–473
97. Annunziata CM, Davis RE, Demchenko Y, Bellamy W, Gabrea A, Zhan F et al (2007) Frequent engagement of the classical and alternative NF-κB pathways by diverse genetic abnormalities in multiple myeloma. Cancer Cell 12(2):115–130

98. Keats JJ, Fonseca R, Chesi M, Schop R, Baker A, Chng WJ et al (2007) Promiscuous mutations activate the noncanonical NF-κB pathway in multiple myeloma. Cancer Cell 12(2):131–144
99. Guo Y, Audry M, Ciancanelli M, Alsina L, Azevedo J, Herman M et al (2011) Herpes simplex virus encephalitis in a patient with complete TLR3 deficiency: TLR3 is otherwise redundant in protective immunity. J Exp Med 208(10):2083–2098
100. Zhang SY, Jouanguy E, Ugolini S, Smahi A, Elain G, Romero P et al (2007) TLR3 deficiency in patients with herpes simplex encephalitis. Science 317(5844):1522–1527
101. Pérez de Diego R, Sancho-Shimizu V, Lorenzo L, Puel A, Plancoulaine S, Picard C et al (2010) Human TRAF3 adaptor molecule deficiency leads to impaired toll-like receptor 3 response and susceptibility to herpes simplex encephalitis. Immunity 33(3):400–411
102. Sancho-Shimizu V, Pérez De Diego R, Lorenzo L, Halwani R, Alangari A, Israelsson E et al (2011) Herpes simplex encephalitis in children with autosomal recessive and dominant TRIF deficiency. J Clin Invest 121(12):4889–4902
103. Herman M, Ciancanelli M, Ou YH, Lorenzo L, Klaudel-Dreszler M, Pauwels E et al (2012) Heterozygous TBK1 mutations impair TLR3 immunity and underlie herpes simplex encephalitis of childhood. J Exp Med 209(9):1567–1582
104. Casrouge A, Zhang SY, Eidenschenk C, Jouanguy E, Puel A, Yang K et al (2006) Herpes simplex virus encephalitis in human UNC-93B deficiency. Science 314(5797):308–312
105. Andersen LL, Mørk N, Reinert LS, Kofod-Olsen E, Narita R, Jørgensen SE et al (2015) Functional IRF3 deficiency in a patient with herpes simplex encephalitis. J Exp Med 212(9):1371–1379
106. Lim HK, Huang SXL, Chen J, Kerner G, Gilliaux O, Bastard P et al (2019) Severe influenza pneumonitis in children with inherited TLR3 deficiency. J Exp Med 216(9):2038–2056
107. Zhang SY, Herman M, Ciancanelli MJ, Pérez de Diego R, Sancho-Shimizu V, Abel L et al (2013) TLR3 immunity to infection in mice and humans. Curr Opin Immunol 25:19–33
108. Lafaille FG, Pessach IM, Zhang SY, Ciancanelli MJ, Herman M, Abhyankar A et al (2012) Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. Nature 491(7426):769–773
109. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL et al (2008) The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 47(3):303–327
110. Abel L, Plancoulaine S, Jouanguy E, Zhang S-Y, Mahfoufi N, Nicolas N et al (2010) Age-dependent mendelian predisposition to herpes simplex virus type 1 encephalitis in childhood. J Pediatr 157(4):623–629.e1
111. Kneen R, Michael BD, Menson E, Mehta B, Easton A, Hemingway C et al (2012) Management of suspected viral encephalitis in children—Association of British Neurologists and British Paediatric Allergy, Immunology and Infection Group National Guidelines. J Infect 64(5):449–477
112. Ramoz N, Rueda LA, Bouadjar B, Montoya LS, Orth G, Favre M (2002) Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. Nat Genet 32(4):579–581
113. de Jong SJ, Créquer A, Matos I, Hum D, Gunasekharan V, Lorenzo L et al (2018) The human CIB1–EVER1–EVER2 complex governs keratinocyte-intrinsic immunity to β-papillomaviruses. J Exp Med 215(9):2289–2310
114. Vahidnezhad H, Youssefian L, Saeidian AH, Mansoori B, Jazayeri A, Azizpour A et al (2019) A CIB1 splice-site founder mutation in families with typical epidermodysplasia verruciformis. J Invest Dermatol 139(5):1195–1198
115. Leiding JW, Holland SM (2012) Warts and all: human papillomavirus in primary immunodeficiencies. J Allergy Clin Immunol 130:1030–1048. NIH Public Access
116. Heusinkveld LE, Yim E, Yang A, Azani AB, Liu Q, Gao JL et al (2017) Pathogenesis, diagnosis and therapeutic strategies in WHIM syndrome immunodeficiency. Expert Opin Orphan Drugs 5:813–825

117. Kawai T, Malech HL (2009) WHIM syndrome: congenital immune deficiency disease. Curr Opin Hematol 16:20–26
118. Hernandez N, Bucciol G, Moens L, Le Pen J, Shahrooei M, Goudouris E et al (2019) Inherited IFNAR1 deficiency in otherwise healthy patients with adverse reaction to measles and yellow fever live vaccines. J Exp Med 216(9):2057–2070
119. Ciancanelli MJ, Huang SX, Luthra P, Garner H, Itan Y, Volpi S et al (2015) Infectious disease. Life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. Science 348(6233):448–453
120. Lamborn IT, Jing H, Zhang Y, Drutman SB, Abbott JK, Munir S et al (2017) Recurrent rhinovirus infections in a child with inherited MDA5 deficiency. J Exp Med 214(7):1949–1972
121. Asgari S, Schlapbach LJ, Anchisi S, Hammer C, Bartha I, Junier T et al (2017) Severe viral respiratory infections in children with IFIH1 loss-of-function mutations. Proc Natl Acad Sci U S A 114(31):8342–8347
122. Zaki M, Thoenes M, Kawalia A, Nürnberg P, Kaiser R, Heller R et al (2017) Recurrent and prolonged infections in a child with a homozygous IFIH1 nonsense mutation. Front Genet 8:130
123. Duncan CJ, Mohamad SM, Young DF, Skelton AJ, Leahy TR, Munday DC et al (2015) Human IFNAR2 deficiency: lessons for antiviral immunity. Sci Transl Med 7(307):307ra154
124. Boisson-Dupuis S, Kong XF, Okada S, Cypowyj S, Puel A, Abel L et al (2012) Inborn errors of human STAT1: allelic heterogeneity governs the diversity of immunological and infectious phenotypes. Curr Opin Immunol 24(4):364–378
125. Burns C, Cheung A, Stark Z, Choo S, Downie L, White S et al (2016) A novel presentation of homozygous loss-of-function STAT-1 mutation in an infant with hyperinflammation-A case report and review of the literature. J Allergy Clin Immunol Pract 4(4):777–779
126. Dupuis S, Jouanguy E, Al-Hajjar S, Fieschi C, Al-Mohsen IZ, Al-Jumaah S et al (2003) Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. Nat Genet 33(3):388–391
127. Vairo D, Tassone L, Tabellini G, Tamassia N, Gasperini S, Bazzoni F et al (2011) Severe impairment of IFN-gamma and IFN-alpha responses in cells of a patient with a novel STAT1 splicing mutation. Blood 118(7):1806–1817
128. Hambleton S, Goodbourn S, Young DF, Dickinson P, Mohamad SM, Valappil M et al (2013) STAT2 deficiency and susceptibility to viral illness in humans. Proc Natl Acad Sci U S A 110(8):3053–3058
129. Moens L, Van Eyck L, Jochmans D, Mitera T, Frans G, Bossuyt X et al (2017) A novel kindred with inherited STAT2 deficiency and severe viral illness. J Allergy Clin Immunol 139(6):1995–1997.e9
130. Shahni R, Cale CM, Anderson G, Osellame LD, Hambleton S, Jacques TS et al (2015) Signal transducer and activator of transcription 2 deficiency is a novel disorder of mitochondrial fission. Brain 138(Pt 10):2834–2846
131. Hernandez N, Melki I, Jing H, Habib T, Huang SSY, Danielson J et al (2018) Life-threatening influenza pneumonitis in a child with inherited IRF9 deficiency. J Exp Med 215(10):2567–2585
132. de Vries E, Koene HR, Vossen JM, Gratama JW, von dem Borne AE, Waaijer JL et al (1996) Identification of an unusual Fc gamma receptor IIIa (CD16) on natural killer cells in a patient with recurrent infections. Blood 88(8):3022–3027
133. Grier JT, Forbes LR, Monaco-Shawver L, Oshinsky J, Atkinson TP, Moody C et al (2012) Human immunodeficiency-causing mutation defines CD16 in spontaneous NK cell cytotoxicity. J Clin Invest 122(10):3769–3780
134. Brown GD, Denning DW, Levitz SM (2012) Tackling human fungal infections. Science 336(6082):647
135. Schmiedel Y, Zimmerli S (2016) Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and Pneumocystis pneumonia. Swiss Med Wkly 146(February):w14281

136. Okada S, Puel A, Casanova J-L, Kobayashi M (2016) Chronic mucocutaneous candidiasis disease associated with inborn errors of IL-17 immunity. Clin Transl Immunol 5(12):e114
137. Pana ZD, Roilides E, Warris A, Groll AH, Zaoutis T (2017) Epidemiology of invasive fungal disease in children. J Pediatr Infect Dis Soc 6(1):S3–S11
138. Lanternier F, Cypowyj S, Picard C, Bustamante J, Lortholary O, Casanova JL et al (2013) Primary immunodeficiencies underlying fungal infections. Curr Opin Pediatr 25:736–747
139. Drummond RA, Franco LM, Lionakis MS (2018) Human CARD9: A critical molecule of fungal immune surveillance. Front Immunol 9(AUG):1–7
140. Alves de Medeiros AK, Lodewick E, Bogaert DJA, Haerynck F, Van daele S, Lambrecht B et al (2016) Chronic and invasive fungal infections in a family with CARD9 deficiency. J Clin Immunol 36(3):204–209
141. Corvilain E, Casanova JL, Puel A (2018) Inherited CARD9 deficiency: invasive disease caused by ascomycete fungi in previously healthy children and adults. J Clin Immunol 38(6):656–693
142. Gavino C, Cotter A, Lichtenstein D, Lejtenyi D, Fortin C, Legault C et al (2014) CARD9 deficiency and spontaneous central nervous system candidiasis: Complete clinical remission with GM-CSF therapy. Clin Infect Dis 59(1):81–84
143. Celmeli F, Oztoprak N, Turkkahraman D, Seyman D, Mutlu E, Frede N et al (2016) Successful granulocyte colony-stimulating factor treatment of relapsing Candida Albicans meningoencephalitis caused by CARD9 deficiency. Pediatr Infect Dis J 35(4):428–431
144. Queiroz-Telles F, Mercier T, Maertens J, Sola CBS, Bonfim C, Lortholary O et al (2019) Successful allogenic stem cell transplantation in patients with inherited CARD9 deficiency. J Clin Immunol 39(5):462–469
145. Huppler AR, Bishu S, Gaffen SL (2012) Mucocutaneous candidiasis: The IL-17 pathway and implications for targeted immunotherapy. Arthritis Res Ther 14(4)
146. Carey B, Lambourne J, Porter S, Hodgson T (2019) Chronic mucocutaneous candidiasis due to gain-of-function mutation in STAT1. Oral Dis 25(3):684–692
147. Olbrich P, Freeman AF (2018) STAT1 and STAT3 mutations: important lessons for clinical immunologists. Expert Rev Clin Immunol 14(12):1029–1041
148. Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC et al (2016) Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. Blood 127(25):3154–3164
149. Weinacht KG, Charbonnier LM, Alroqi F, Plant A, Qiao Q, Wu H et al (2017) Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. J Allergy Clin Immunol 139(5):1629–1640.e2
150. Leiding JW, Okada S, Hagin D, Abinun M, Shcherbina A, Balashov DN et al (2018) Hematopoietic stem cell transplantation in patients with gain-of-function signal transducer and activator of transcription 1 mutations. J Allergy Clin Immunol 141:704–717.e5

Diseases of Immune Dysregulation

9

Michele Proietti

Abstract

Inborn errors of immune regulation are a heterogeneous group of genetic disorders with variable clinical manifestations, including lymphoproliferation, autoimmunity, and increased susceptibility to infections. They are caused by defects in genes involved in controlling when and how the immune system must turn on, which and how much cytokines release, which antigens should be eliminated and which neglected, and why and when the immune response has to turn off. A defective regulation of one or more of these mechanisms leads to the recognition of self-antigen or to an excessive response to foreign antigens, and finally to and/or hyperinflammation that damage cells and organs. The treatment of these disorders is often challenging, as it often requires immunosuppression in the presence of increased risk of infections.

Keywords

Immune dysregulation · Immunodeficiency · Genetics · Autoimmunity · Infections

9.1 Introduction

The human immune system evolved to protect the host against invading pathogens. When the body is invaded, a coordinated response of innate and adaptive immunity is started aimed at eliminating the pathogen. However, raising an immune response always costs the host a price in terms that a certain degree of tissue and cell damage is unavoidable. Therefore, the mechanisms devoted at the immune protection must

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develop in equilibrium to others specifically aimed at preventing useless reactivity against self-antigens and at controlling the magnitude of the immune response. These mechanisms decide when and how the immune system must turn on, which and how much cytokines release, which antigens should be eliminated and which neglected, and why and when the immune response has to turn off. They contribute at maintaining and reseeding the homeostasis and preventing immune-mediated, potentially lethal damage, commonly named immunopathology. In relation to the kinetic of the immune response, they can be divided into mechanisms aimed at preventing an unneeded immune response and mechanisms aimed at controlling intensity and duration of an ongoing immune response. The main mechanisms identified so far are summarized in Fig. 9.1. They include central and peripheral deletion of autoreactive T and B cells, adaptive and innate immunological memory, tolerance and ignorance, control of immune cell activation, and control of proinflammatory and anti-inflammatory cytokines production [1–4]. Defects in one or more of these mechanisms lead to autoimmunity, inflammation, and tissue damage (Fig. 9.1b). Somatic recombination of TCR genes in immature T cells (Thymocytes) results in an enormous number of different TCR specificities (T cell repertoire), some useful, recognizing non-self-antigens but also some potentially self-reactive. The thymic selection operates to shape the T cell repertoire. Thymocytes with TCRs with low affinity for self-peptide–MHC complexes are positively selected, exit the thymus, and are ready to further differentiate and, if needed, take part of the immune

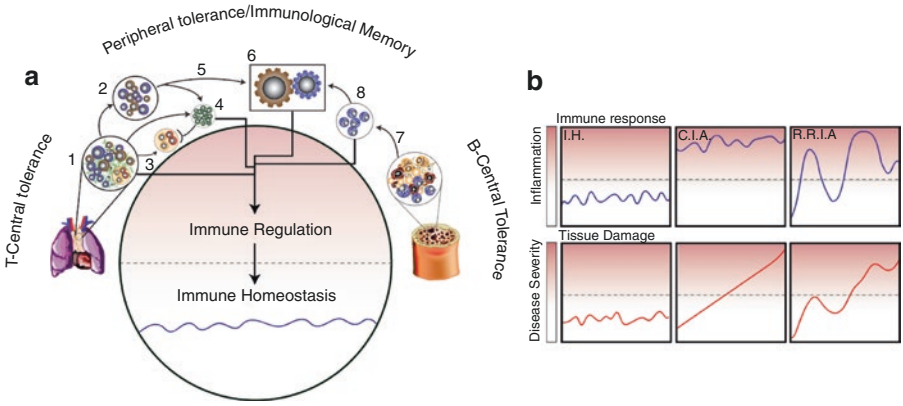

Fig. 9.1 Immunological mechanisms of immune regulation. (**a**) (1) Thymic refinement of TCR repertoire and generation of thymocytes with TCRs with low affinity for self-peptide–MHC complexes (2). (3) Inhibition of self-reactive T cells by regulator T cells (4). (5) Peripheral control of T cell activation and proliferation. (6) Cognate T-B interaction. (7) Deletion of autoreactive B cells in the bone marrow. (8) Control of peripheral B cell differentiation. (**b**) Defects in one or more of these mechanisms lead to uncontrolled activation of the immune response (chronic or relapsing remitting) which can damage tissues and organs

response. The remaining thymocytes, potentially self-reactive, can meet different destinies, can be deleted (clonal deletion), or can differentiate to regulatory T cells (clonal diversion) [3, 5, 6]. Medullary thymic epithelial cells (mTECs) play a crucial role in this process. They express a nuclear regulatory protein called autoimmune regulator (AIRE), which helps them to present a large number of tissue-specific self-antigens (TSAs) [7]. However, it is nowadays well recognized that the process of thymic selection fails to delete the totality of self-reactive T cells. Therefore further checkpoints are needed; they include peripheral tolerance and peripheral deletion [8, 9]. Peripheral tolerance describes a phenomenon where T cells, despite the engagement of the TCR, are not completely activated. Peripheral deletion indicates the removal of potentially self-reactive T cell clones that have escaped thymic deletion. A full activation of T cells needs a signal from the TCR, a costimulatory signal mediated by CD28 ligation, and the secretion of cytokines such as IL-2 [10, 11]. These signals converge into the complete activation of the PI3K/AKT-mTOR pathway and the consequent metabolic reprogramming of effector T cells [12, 13]. The immune system can finely tune each of these signals. TCR signaling is strongly influenced by the affinity for the recognized antigens; costimulatory pathways can also provide negative second signals that inhibit rather than stimulating the T cell responses, key among these are the programmed death 1 (PD-1) and CTLA-4 [14].

Regulatory T cells are also known to be involved in these processes; they express co-inhibitory molecules and can promote a hypoxic environment, which in turn induces anergy of effector T cell. Central and peripheral tolerance mechanisms likewise operate to select B cell populations neglecting self-antigens and responding to foreign antigens. A first deletion of autoreactive B cells happens in the bone marrow (central tolerance) where the B cells develop. Here, self-reactive cells are supposed to be censored by clonal deletion, clonal anergy, or by the replacement of the initial BCR with a new one with new specificity, a process called B cell receptor (BCR) editing. As for the T cell, this process is highly inefficient, leaving a number of B cells reaching the periphery. Peripheral B cell tolerance is also recognized to exist. Despite it is less well characterized than the central tolerance some checkpoints have been described. For example, while mature naive B cells contains few autoreactive, the number is high in immature transitional B cells recently emigrated from the bone marrow. This finding suggests that tolerance is shaped as transitional B cells differentiate into naive B cells [15].

However, autoreactive naive B cells that reach the periphery would need cognate autoreactive T cells to help it mature and eventually increase antigen affinity. Therefore it looks reasonable that major defects in T cell tolerance provoke loss of B cell tolerance. The abovementioned mechanisms involve mainly the adaptive immunity. However, also the innate immune response needs to be finely tuned. This is clearly shown by the existence of a group of genetic diseases, called autoinflammatory diseases, where the specific effector of damage is the innate immune-mediated inflammation. This group of diseases will not be addressed in this chapter.

9.2 Immunodysregulation Polyendocrinopathy Enteropathy X-Linked Syndrome (IPEX)

9.2.1 Definition, Clinical Manifestations, and Diagnosis

The immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a hemizygous disease, first recognized clinically in 1982 by Powell et al. [16], which usually manifests within the first years of life with severe enteropathy, chronic dermatitis, early onset type I diabetes mellitus (T1DM), hypoparathyroidism, cytopenias, and often other autoimmune manifestations [17–19]. Cases of antenatal occurrence and adult age of onset have rarely been reported. So far only affected males have been described; females carrying heterozygous Foxp3 mutations remain asymptomatic. The most common presentation of IPEX is a severe watery diarrhea that can be mucoid or bloody. This symptom usually appears in the early infancy and frequently worsens when the affected infant is switched from breastfeeding to formula. The diarrhea can also lead to severe dehydration and malabsorption, and consequent hypernatremia, metabolic acidosis, renal insufficiency, weight loss, and failure to thrive. The diarrhea is the consequence of an inflammatory enteropathy which typically involves the small intestine, although the colon may be also interested. Histologically it is characterized by villous atrophy, crypt hyperplasia or abscesses and extensive immune infiltrate. Acute urticaria, vomiting, or anaphylaxis can be the consequences of food allergies. Autoimmune hepatitis is often observed together with the enteropathy. During the course of the disease, the patients constantly develop autoimmune endocrinopathies. They include type I diabetes, thyroiditis, and hypoparathyroidism, and more rarely growth hormone deficiency and hypoadrenalism. Other typical and frequent signs of the disease are the skin manifestations. They include eczema, erythroderma, exfoliative dermatitis, and psoriasis-like dermatitis. About one half of the patients have hematological manifestations, most commonly coombs-positive hemolytic anemia, autoimmune thrombocytopenia, and autoimmune neutropenia. Increased susceptibility to infections is also frequent; in particular secondary to enterococcal and staphylococcal species, cytomegalovirus, and candida. Less frequently the kidney, nervous system, and lung can be affected. The disease is often fluctuant, with flares and remission. During flares it is frequent to observe worsening of diarrhea, hyperglycemia secondary to worsening of diabetes, dermatitis exacerbations, or appearance of new skin manifestations. Because of its clinical presentation and inheritance, IPEX should be suspected in any male infant with chronic diarrhea and failure to thrive in particular if associated with type 1 diabetes. The presence of dermatitis, autoimmune cytopenias, or thyroiditis further supports the diagnosis but should not be considered as required. An extensive laboratory evaluation is necessary in suspected cases. It should comprise complete blood count, direct and indirect Coombs test, measurement of anti-neutrophil and antiplatelet antibodies, monitoring of serum glucose, measurement of anti-islet

antibodies, assessment of thyroid function and anti-thyroid antibodies, and measurement of IgG, IgM, IgA, and IgE levels. In patients with recurrent dermatitis, serum measurement of allergen-specific IgE should be performed and preferred to skin prick testing. Immunological phenotype is also recommended and should include lymphocyte subsets and proliferation assays. Other exams can help the diagnosis but are not strictly necessary. These include intestinal endoscopy with biopsy and FoxP3 staining or skin biopsy and, if available, analysis of regulatory T cell phenotype and function. A definitive diagnosis requires the identification of a disease causing genetic variant in the gene *FOXP3* [20–22].

9.2.2 Molecular Genetics

IPEX is due to loss-of-function mutations in *FOXP3* resulting in quantitative reduction of protein expression or in functional impairment [23–25]. More than 70 distinct mutations have been reported so far; they spare the full length of the protein, indicating that all the domains of the molecule are important for its function. Despite a clear genotype/phenotype correlation has not been found, it is acknowledged that mutations that abrogate Foxp3 expression as the ones in the polyadenylation site tend to have a more severe clinical presentation [23–32].

9.2.3 Immunopathology

FOXP3 encodes the transcription factor forkhead box protein P3 (FOXP3). Foxp3 has been for long time considered the "master switch gene" of the Treg cell lineage being required for Treg cell phenotypic and functional differentiation [33–36]. Nowadays it is considered as an orchestrator of the Treg cell program, whose function appears dispensable for Treg differentiation, but essential for their effector functions [35, 37, 38]. In fact, recent studies have shown that in mice the development of thymic Treg cells is not affected by lack of Foxp3 and that Foxp3 consolidates pre-existing imprinting of Treg cell precursors to cement their phenotype rather than establish the Treg lineage [37, 38]. Indeed, Foxp3 activity was shown to amplify or prepress respectively regulatory (e.g., Il2ra, Ctla4, and Tnfrsf18) and effector T cells genes (e.g., IL-4, IFN-γ, IL-17, and IL-21 and the cyclic nucleotide phosphodiesterase 3B PDE3B). Accordingly, in humans, normal or even elevated TSDR demethylation, a parameter that correlates with the level of Treg lineage differentiation, can be measured in the peripheral blood of IPEX patients [39]. Along the same line, Foxp3 can act as a metabolic gatekeeper controlling Treg cells metabolic signature, which in turn sustains their proliferative and regulatory functions while constraining the establishment of T cells effector programs. Most IPEX patients carry a missense *FOXP3* mutation that allows protein expression but alters its function [40, 41], resulting in residual expression of Foxp3 but impaired Treg

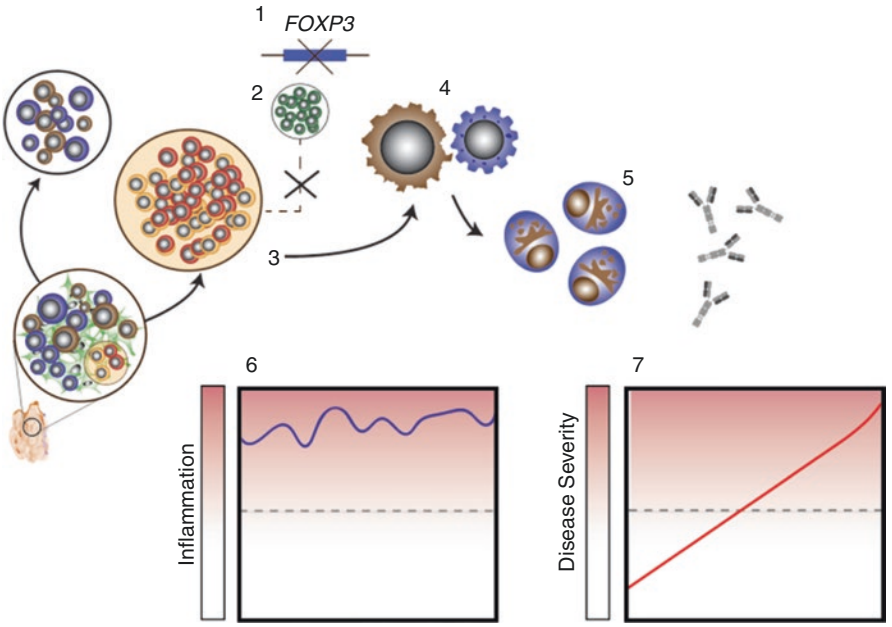

Fig. 9.2 Immunopathology of IPEX syndrome. Mutations in *FOXP3* (1) lead to defective regulatory T cells (2) that in turn results in the expansion of self-reactive T cells (3), defective control of T-cell mediated B cell help (4), production of autoantibodies (5) and finally autoimmunity and tissue damage (6, 7)

function [42, 43]. Treg cells from IPEX patients are unable to inhibit proliferation and cytokine production of autologous or allogenic Teff cells and, when homing an inflammatory environment, can convert to an effector phenotype. Defective regulatory T cells are constantly accompanied by multiple autoantibodies with different reactivity, often early before the autoimmune organs damage manifests [44–48]. Interestingly Kinnunen et al., measuring the reactivity of recombinant antibodies isolated from single B cells from IPEX patients, have found defective peripheral B cell tolerance, despite preserved central B cell tolerance [49]. These data conform recent experimental evidence showing a role of regulatory T cells in controlling the germinal center reaction and of consequence antibody production [50–52] (Fig. 9.2).

9.2.4 Management and Treatment

Without aggressive immunosuppression or bone marrow transplantation, the majority of affected males die within the first 1–2 years [16]; a few patients with hypomorphic *FOXP3* mutations can manifest with a milder phenotype and can survive into the second or third decade of life [53].

9.2.5 Management and Therapy

Treatment of IPEX can be divided into treatment of acute events and long-term treatment. The first group is characterized by prevention of infections, and treatment of invasive infections, supportive care, and intensive immune suppression aimed at stabilizing disease flares. Long-term treatment includes a combination of immune suppression and dietary modifications aimed at preventing food allergens and improving the nutritional state. Unfortunately, although controlled trials have not been performed, available evidence suggests that immune suppression, despite effective in ameliorating the symptoms, does not significantly modify the prognosis. In addition, its long-term use is associated with adverse side effects. The immune suppressive treatment is based on a combination of glucocorticoid therapy and a steroid-sparing agent, including calcineurin inhibitors and sirolimus. In 2018, Barzaghi et al. [54] have reported the results of a multicentric study that evaluated disease onset, progression, and long-term outcome of immunosuppression and HSCT in long-term IPEX survivors. Main conclusion of this important study was that rapamycin should be considered the preferred choice as immune-suppressive treatment, while HSCT should be considered in patients with low grade of organ involvement and stable clinical conditions. In addition, the authors reported a similar overall survival rates for individuals treated with immune suppression or that underwent HsCT, and higher mortality in the HSCT group in the peri-transplant period.

9.3 Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED)

9.3.1 Definition, Clinical Manifestations, and Diagnosis

Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED, OMIM 240300), also named Autoimmune polyendocrine syndrome type 1 (APS-1), is a rare autosomal recessive disorder, first described by Neufeld et al. as one of the three types of polyglandular autoimmune syndromes and manifesting with at least two between chronic mucocutaneous candidiasis, hypoparathyroidism, and primary adrenal insufficiency [55]. Several authors have subsequently reviewed the clinical findings of the disease emphasizing its broad clinical spectrum [56–61]. Together with the abovementioned symptoms, other APS-1-related symptoms include other autoimmune endocrinopathies (hypergonadotropic hypogonadism, insulin-dependent diabetes mellitus, autoimmune thyroid diseases, and pituitary defects), autoimmune or immuno-mediated gastrointestinal diseases (chronic atrophic gastritis, pernicious anemia, and malabsorption), chronic active hepatitis, autoimmune skin diseases (vitiligo and alopecia), ectodermal dystrophy, keratoconjunctivitis, photoreceptor degeneration, immunologic defects (cellular and humoral), asplenia,

and cholelithiasis. The first manifestations usually occur in childhood with two or more diseases of the classic triad developing in the first two decades of life. Candidiasis typically interests oral cavity or nails, more rarely the esophagus; usually it is not associated with increased susceptibility to other infections. Frequently the first damaged gland is the parathyroid, with consequent signs of hypoparathyroidism. Subsequently signs of aldosterone or cortisol deficiency can appear as a consequence of adrenocortical failure. Overall, the three main components of APECED occur in this chronologic order: candidiasis (usually before the age of 5 years), followed by hypoparathyroidism (usually before the age of 10 years), and later by Addison disease (usually before the age of 15 years). However, only about one-third to one-half of the cases has the full triade. Importantly, the earlier the first components appear, the higher is the probability to have multiple organs involvement. Information on longitudinal follow-up of APS1 patients is scarce. Meloni et al. reported that APS1 in Sardinia is characterized by severe phenotype, marked clinical heterogeneity, and relative genetic homogeneity. Bruserud et al. [62] in 2016 reported about a longitudinal follow-up of the 52 Norwegian patients. Because their results showed high clinical variability, the authors suggested that the diagnosis should be considered in all patients presenting one of the major clinical manifestations, especially if they appear early in childhood. In addition, because cases of late onset are possible, they also suggested testing all siblings of a patient even if with silent clinic.

9.3.2 Molecular Genetics

APECED is caused by biallelic mutations in the gene *AIRE* [63, 64]. More than 90 different mutations in this gene have been described as of today [65–67]. Mutations in AIRE cause the production of an abnormally short, nonfunctional protein. In rare cases, patients with certain monoallelic mutations, have some features of APECED, such as CMC, hypoparathyroidism, or vitamin B12 deficiency. These individuals usually have one similarly affected parent.

9.3.3 Immunopathology

The molecular basis for the pleomorphic spectrum of autoimmune manifestations of APS-1 has become clear in 1997 when Negamine et al. and the Finnish-German APECED consortium identified mutations in the *AIRE* gene [63, 64]. *AIRE* encodes for a protein which, based on its structure, acts as a transcription factor and controls the expression in the thymus and probably in secondary lymphoid organs of tissue-restricted antigens (TRA) [68–70]. Several studies indicate that transactivation by Aire necessitates interaction with other factors. Macedo et al. provided evidence that Aire cooperates with guanylate cyclase 2d (Gucy2d), which is connected to several tissue-restricted antigens (TRA) and then in controlling the transcription of promiscuous gene expression [71]. Abramson et al. identified four major functional

classes of protein that interacts with Aire: they include nuclear transport, chromatin binding/structure, transcription, and pre-mRNA processing [69]. Interestingly, one group of interactors was enriched on the pre-mRNA splicing and maturation machinery, possibly explaining the more effective processing of tissue-restricted antigens (TRA) transcripts in the presence of Aire. Aire mRNA and protein are expressed at highest levels within the thymus by medullary thymic epithelial cells (mTECs) [69]. The thymus is the organ where T cells, which originate in the bone marrow, migrate and maturate. Here the processes of positive and negative selection generate populations of T cell recognizing and responding to foreign antigens and conversely tolerant to self-antigens. The ability of Aire to mediate the ectopic expression of thousands of tissue-restricted antigens explains why, virtually, all tissues and organs may represent the target of the autoimmune attacks, therefore leading to the reported wide spectrum of clinical features. More recently it has become clear that AIRE is also expressed in peripheral lymphoid tissues and it is therefore likely that it contributes also to the process of peripheral T cell selection and T cell tolerance [72]. AIRE deficiency also affects B cell tolerance and APECED patients collectively display a broad repertoire of high titer autoantibodies, including some observed in some major autoimmune diseases [73–78]. Meyer et al. [79] have recently found that many APS1/APECED patients harbored extremely high-affinity, neutralizing autoantibodies. Their results suggest that the loss of B cell tolerance occurs during T cell dependent antibody affinity maturation. Of note, these antibodies were biologically active in vitro and in vivo, and those neutralizing type I interferons (IFNs) inversely correlated with type I diabetes, and thus their use could be of therapeutic utility. On the other side, autoantibody recognizing IL-17 [80], a cytokine important in the body's defense against Candida, by neutralizing its function are thought to contribute to the CMC susceptibility in APECED patients (Fig. 9.3).

9.3.4 Management and Treatment

Except for the treatment of candidiasis, treatment of APECED consists mostly on hormone replacement therapy. Only in patients with the most severe autoimmune manifestations (e.g., autoimmune hepatitis, interstitial nephritis and bronchiolitis) form, corticosteroid treatment in association with other immunosuppressive therapies is added. Recently, rituximab has been successfully used in a young patient with bronchiolitis [81].

9.4 Autoimmune Lymphoproliferative Syndrome (ALPS)

9.4.1 Definition, Clinical Manifestations, and Diagnosis

Autoimmune lymphoproliferative syndrome is a genetically heterogeneous syndrome characterized by lymphoproliferation and autoimmunity. The syndrome was

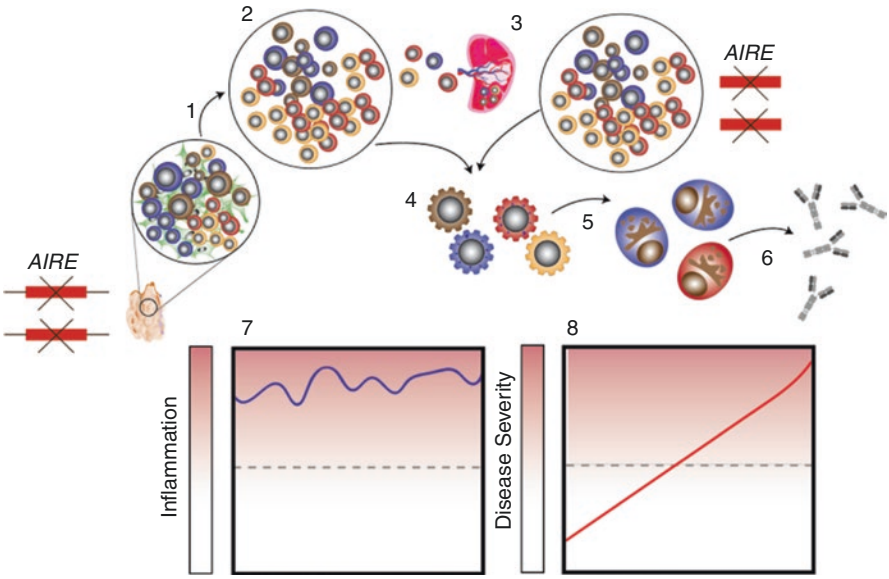

Fig. 9.3 Immunopathology of APECED syndrome. Mutations in AIRE (1) lead to defective thymic (2) and peripheral (3) deletion of auto-reactive T cells. This in turn results in defective T cell mediated B cell help (4), production of autoantibodies (5, 6) and autoimmunity and tissue damage (7, 8)

first described by Canale and Smith in 1967 [82]. They reported about five children, "with onset of symptoms between 1 month and 2 years of age: significant generalized lymphadenopathy; hepatosplenomegaly; deviations in immunological status with alterations in gamma globulins and manifestations of autoimmune disease; variable lymph node histology; response to immunosuppressive drugs, and chronic course." In 1992, Sneller et al. [83] reported that in two unrelated girls which also presented with lymphoproliferation and autoimmunity, peripheral blood analysis showed that both patients had increased numbers of mature CD3+, CD4−, CD8− double negative (DNT) T lymphocytes expressing alpha/beta T cell receptors. Different reports of limited number of patients with ALPS have been published after its first description [84]. These studies started to highlight the broad clinical phenotype of the disease, which has been further confirmed by two large cohort studies subsequently published. These studies involved patients with autoimmune lymphoproliferative syndrome secondary to mutations in FAS (ALPS type I OMIM: 601859, molecular genetic). In the first one, Neven et al. [85] performed a retrospective analysis of 90 ALPS patients monitored over a median period of 20.5 years. Their analysis indicated a trend toward spontaneous remission of lymphoproliferation in adulthood and mixed outcomes for autoimmune manifestations. They also observed a high risk of sepsis after splenectomy and a greater disease severity in males than in females. The authors could not observe any genotype-phenotype correlations. Subsequently, Price et al. [86] reported on the natural history over

2 decades of 150 ALPS patients and 63 healthy mutation-positive relatives. Their main findings were that FAS mutations have a clinical penetrance of less than 60%, that elevated serum vitamin B12 is an accurate biomarker of ALPS-FAS, and that postsplenectomy sepsis and development of lymphoma are the major causes of morbidity and mortality. From these studies appeared that ALPS can manifest with a very broad range of clinical manifestations including lymphadenopathy, hepatomegaly, splenomegaly, an increased risk of lymphoma, as well as signs of autoimmune disease, typically involving blood cells (e.g., autoimmune hemolytic anemia, autoimmune neutropenia, autoimmune thrombocytopenia) and less commonly other organs and tissues (e.g., glomerulonephritis, autoimmune hepatitis, uveitis, Guillain-Barre syndrome). Typically, the lymphoproliferation becomes apparent during childhood followed by the autoimmune manifestations. ALPS patients usually do not show increased susceptibility to infections. Diagnostic criteria for ALPS have been proposed in 2009. Chronic nonmalignant, noninfectious lymphadenopathy or splenomegaly or both Elevated CD3 + TCRαβ + CD4−CD8− DNT cells (1.5% of total lymphocytes or 2.5% of CD3 lymphocytes) in the setting of normal or elevated lymphocyte counts are considered required criteria. Accessory criteria have been also proposed; they include defective lymphocyte apoptosis (in two separate assays), somatic or germline pathogenic mutation in FAS, FASL, or CASP10, elevated plasma sFASL levels (>200 pg/mL) OR elevated plasma IL-10 levels (>20 pg/mL) OR elevated serum or plasma vitamin B12 levels (>1500 ng/L) OR elevated plasma IL-18 levels (>500 pg/mL), typical immunohistological findings as reviewed by an experienced hematopathologist, autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) AND elevated immunoglobulin G levels (polyclonal hypergammaglobulinemia), and family history of a nonmalignant/noninfectious lymphoproliferation with or without autoimmunity [87].

9.4.2 Molecular Genetics

TNFRSF6, the gene encoding FAS, was the first gene found to be mutated in ALPS (ALPS type IA) [88]. This finding was confirmed in 1996 by Drappa and colleagues [89]. ALPS type IA is caused by heterozygous mutations in CD95. In the majority of the affected patients, the mutation is found within the intracellular death domain. In the same year, Wu et al. [90] found a heterozygous mutation in the gene *TNFSF6* (encoding FAS ligand, ALPS type IB) in an Afro-American man with lupus-like phenotype and lymphadenopathy. Cases of ALPS caused by homozygous null mutations in *TNFRSF6* have been also reported (ALPS type 0). In 1997, Sneller et al. [83] found that 1 of 9 patients with ALPS did not have a mutation in either the *TNFRSF6* or *TNFSF6* gene. The authors proposed the designation ALPS type II. Today, globally four forms of ALPS in the absence of FAS or FASL mutations result querying OMIM for autoimmune lymphoproliferative syndrome; they are summarized in Table 9.1. This group includes ALPS type 2, an autosomal dominant disease caused by mutation in *CASP10* [91]; ALPS type 3, an autosomal recessive disease caused by mutations in *PRKCD* [92]; ALPS type 4, also called

Table 9.1 Classification of ALPS according to OMIM

Type	Full name	Gene	Inheritance	OMIM
IA	Autoimmune lymphoproliferative syndrome, type IA	*FAS/TNFRSF6*	AD	601859
IB	Autoimmune lymphoproliferative syndrome, type IB	*FASLG/TNFSF6*	AD	134638
IIA	Autoimmune lymphoproliferative syndrome, type IIA	*CASP10*	AD	603909
IIB	Autoimmune lymphoproliferative syndrome, type IIB	*CASP8*	AD	607271
III	Autoimmune lymphoproliferative syndrome, type III	*PRKCD*	AR	615559
IV	?RAS-associated autoimmune lymphoproliferative syndrome type IV, somatic	*NRAS*	Som	614470
	RAS-associated autoimmune leukoproliferative disorder	*KRAS*	AD	614470
0	?Autoimmune lymphoproliferative syndrome, type 0	*FAS*	AR	–

RAS-associated autoimmune leukoproliferative disorder (RALD), secondary to heterozygous mutations in *NRAS* [93] or somatic mutations in *KRAS* [94]; and finally ALPS type 5, caused by monoallelic mutations in *CTLA4* [95, 96]. Because the known pathogenic mechanisms of ALPS type 5 show only a limited overlap with the other forms of ALPS, hereby it has been preferred to describe this disease separately (see below CTLA4 haplo-insufficiency).

9.4.3 Immunopathology

Despite still not fully understood, the pathogenesis of ALPS can be ascribed to a defective control of lymphocyte differentiation, activation, and proliferation. Homeostasis of peripheral T cells is maintained by three mechanisms: unresponsiveness (anergy) of T cells, suppression by regulatory T cells, and activation-induced cell death (AICD). FAS and FAS ligand, the two molecules first found mutated in ALPS, regulate T cell death. During their thymic maturation, thymocytes that fail to rearrange correctly their T cell receptor (TCR) or those that recognize self-antigens undergo apoptosis. This process, called negative selection, is mediated by the interaction between FAS and its ligand [97]. Binding of FAS to its ligand FASL induces Fas trimerization [98], the formation of the death-inducing signaling complex (DISC) and the subsequent recruitment and activation of the proteases caspase 8 and 10 [99–101]. These caspases trigger a complex signal cascade that finally leads to cell death. FAS is also involved in the regulation of peripheral T cell tolerance [102]. Homeostasis of peripheral T cells is maintained by the following three mechanisms: unresponsiveness (anergy) of T cells, suppression by regulatory

T cells, and activation-induced cell death (AICD). FAS controls AICD in effector T cell population induced by repeated stimulation of the TCR. This mechanism contributes to limit the expansion of over-activated effector T cells. In agreement, mutations in FAS determine lymphocyte accumulation that leads to chronic organomegaly, in particular in the lymph nodes, liver, and spleen. Both B and T cells can accumulate; however, the most characteristic expanded population in ALPS patients is CD4−CD8− T cells expressing an α/β T cell receptor, commonly called double negative or DNT cells [83]. These cells are thought to be polyclonal activated mature T cells that have lost CD8 coreceptor expression. In agreement DNT cells markers *consistent with* resemble their linear differentiation from activated cytotoxic T cells (e.g., perforin) [103]. The fas pathway is known to control also reciprocal help during T-B interaction. The main mechanism is thought to be killing of Fas-expressing target cells, by B cell expressing FasL [104]; however it has been shown that Fas is highly expressed in germinal center (GC) B cells and that B cell-specific Fas deficiency is associated with the onset of autoimmunity [105]. These results suggest that Fas can directly control the expansion of self-reactive GC B cells. In addition, it is thought that DNT cells may also contribute to the production of autoantibodies secreting large amounts of IL-10 and other Th2 cytokines. Another gene involved in this pathway, found mutated in ALPS, is CASP10. However, to the best of our knowledge, only five mutations in CASP10 have been described so far. Two of them have been recognized as pathogenic (I406L and L258F), and others have been reported as variants of uncertain significance (V410l, Y446C) or polymorphisms (L522l). However, Miano et al. [106] have recently reported that the L522l, V410l, and Y446C variants are associated with impaired apoptosis similar to the pathogenic mutations. The authors speculated that, like Fas mediated ALPS or other monogenic disease of the immune system, their variable penetrance can be ascribed to other, still unknown, genetic and epigenetic factors.

9.4.4 Management and Therapy

As mentioned above onset and severity of ALPS is variable. Despite, many patients have a favorable prognosis with limited need for immunosuppressive therapy; the long-term follow-up of these patients is indicated, and it should include an assessment for lymphoma, in particular in patients with extensive lymphadenopathy. Its treatment is mostly based upon observational data and clinical experience since there are no randomized trials. Hematopoietic cell transplantation (HCT) is the only curative treatment. However, ALPS disease activity, potential consequences of long-term treatments, and risks of transplantation need to be carefully evaluated before HCT is performed. Examples of transplant indications include lymphoma, severe autoimmune cytopenias not responsive to therapy, development of severe immunodeficiency and recurrent severe invasive bacterial infection or sepsis post-splenectomy despite adequate antimicrobial prophylaxis and appropriate vaccinations, and "severe" genotype (e.g., patients with homozygous and compound heterozygous FAS defects).

9.5 Familial Hemophagocytic Lymphohistiocytosis (HLH)

9.5.1 Definition, Clinical Manifestations, and Diagnosis

Familial hemophagocytic lymphohistiocytosis (HLH) is a group of Mendelian disorders characterized by uncontrolled secretion of proinflammatory cytokines by activated macrophages (histiocytes) and lymphocytes. The syndrome was first reported in the literature by Scott & Robb-Smith and Anderson who described cases of adults with a fatal condition characterized by fever, progressive anemia, hepatomegaly, and lymphadenopathy [107]. The disorder was then recognized as familial by Farquhar and Claireaux in 1952 [108]. They reported about how two siblings presented at the same age of 9 weeks old with fever, diarrhea, "sunburnt skin," palpable liver and spleen edge, no identified infection, normochromic anemia, and "stained blood films consistently showing a high percentage of smear cells thought probably to be of lymphoid origin." FHL manifest in >70% of the cases in the first months of life [109]. An asymptomatic period of some days after birth is common. After this period the disease can manifest with a very broad number of signs and symptoms [110], including fever, organomegaly (e.g., splenomegaly, hepatomegaly, and lymphadenopathy), liver dysfunction, cytopenias, skin manifestations, coagulopathy, dermatologic abnormalities, and neurological dysfunction (irritability, seizures, hyper or hypotonia, or coma). If not treated, the disease has usually a progressive course leading to a lethal outcome [109, 111, 112]. A milder course has been observed in some patients with syntaxin 11 deficiency [113], while patients with missense variant in perforin 1 can have a late onset form [114, 115]. Despite the complete picture of HLH is rather characteristic, the first symptom is highly variable and none of the abovementioned symptoms is pathognomonic; therefore diagnosing HLH can be a challenge; Diagnostic criteria have been proposed [110, 116, 117] (see Table 9.2) . Five of the reported criteria must be present. Laboratory findings can help the diagnosis; they include anemia,

Table 9.2 Diagnostic criteria for hemophagocytic lymphohistiocytosis

Criteria
Fever ≥38.5 °C
Splenomegaly
Peripheral blood cytopenia, with at least two of the following: Hemoglobin <9 g/dL (for infants <4 weeks, hemoglobin <10 g/dL); platelets <100,000/μL; absolute neutrophil count <1000/μL
Hypertriglyceridemia (fasting triglycerides >265 mg/dL) and/or hypofibrinogenemia (fibrinogen <150 mg/dL)
Hemophagocytosis in the bone marrow, spleen, lymph node, or liver
Low or absent NK cell activity
Ferritin >500 ng/mL
Elevated soluble CD25 (soluble IL-2 receptor alpha) two standard deviations above age-adjusted values

thrombocytopenia, and leukopenia, elevated levels of transaminases, hyperbilirubinemia, and hypertriglyceridemia, coagulopathy, hyponatremia, and hypoproteinemia. Typically, HLH patients have raised levels of inflammatory markers, in particular the ferritinemia, that can reach values >500 ng/ml [118] and cytokines including TNF-α, IFN-γ, IL-6, and IL-1 [119] [120–122]. Immunological phenotyping usually shows decreased NK-mediated cytotoxic activity by NK cells and increased levels of activated CD8+ T cells [123–128]. Increased levels of soluble CD25 (sCD25, sIL-2R) [121] are also commonly measured and thought to reflect the massive macrophage, T cell, and NK cell activation. Pathologic findings can include the presence of hemophagocytosis, defined as the presence of macrophages engulfing erythrocytes, platelets, or white blood cells. This finding is observed in the majority of HLH patients in the bone marrow, spleen, liver, or lymph node, but can also be absent. Virtually constant is instead the organ (most frequently the spleen, liver, lymph nodes, bone marrow, and central nervous system (CNS)) infiltration of CD8 T cells and macrophages [129]. Despite we focus here on familial HLH, it is important to note that today this clinical entity is considered not an independent disease but rather a life-threatening clinical syndrome, consequence of a severe, uncontrolled hyperinflammatory reaction [130]. The syndrome is considered familial when a genetic defect can be identified and secondary otherwise. Until recently, age of onset was considered to roughly discriminate between familial and acquired secondary forms of HLH.

However, recent evidences argue against this assumption. Indeed, an increasing number of genetic cases are identified in adolescents and adults. In a recent study on 175 adult patients with HLH, hypomorphic monoallelic or biallelic mutations in genes of familial HLH (FHL) were found in 14% of the patients [131]. Secondary HLH form is usually triggered by infections, hematopoietic malignancies, or autoimmune disease. Since infections can also contribute to trigger familial HLH, it is mandatory a careful microbiological workup. This includes blood, urine, and cerebrospinal fluid (CSF) cultures and diagnostic evaluation for viral infections (Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, adenovirus, enterovirus, parvovirus, or human herpesvirus-6) and bacterial, fungal, or parasitic infections.

9.5.2 Molecular Genetics

Nowadays five forms of familial HLH are known. FHL-1 has been mapped to chromosome 9q21.3–22; however the exact genetic defect is still unknown [132]. FHL-2 is autosomal recessive disorder caused by biallelic mutation in PRF1 [125]. FHL3 is caused by homozygous or compound heterozygous mutation in the UNC13D gene [133], while FHL4 by homozygous mutation in the syntaxin-11 gene [134]. Finally, FHL5 is caused by homozygous or compound heterozygous mutation in the syntaxin-binding protein-2 gene [135]. In same cases the genetic defect cannot be identified.

9.5.3 Immunopathology

T lymphocytes, natural killer (NK) cells, and macrophages can release granules containing granzymes, a group of proteins triggering apoptosis of the target cells, and perforin, a protein that forms pores in the membrane of the target cell and facilitates the entrance of granzymes. The consequent death of the target cells contributes to recruit other immune cells, which release proinflammatory cytokines. This immune response is at risk of inducing tissue damage, if protracted or too intense. This is why the immune system of healthy individuals activates it only if necessary, with the proper intensity and for the needed time, for example, upon encountering virally infected cells or tumor cells. At the opposite in HLH patients, as a consequence of genetic defects (familial HLH) or as a consequence of an infectious, autoimmune, or malignant stimulus (secondary HLH) this process becomes uncontrolled and continuous, triggering a deleterious cycle leading to a progressive and cumulative tissue damage. It is thought that virtually all forms of HLH are due to defective cytotoxic T lymphocytes (CTLs) and NK cells functions. Perforin, the gene mutated in FHL2, is a cytolytic protein that creates pores in the target cells and therefore leads to their osmotic lysis [136]. The protein is also necessary for the transfer of granzymes in the target cells that will then accelerate cell death by protein degradation. This activity has the aim to remove the antigenic stimulation and terminate the inflammatory response. All the genetic defects lead to FHL described as of today or affect directly the perforin (FHL2) or dampen granule exocytosis (FHL3–5 and immunodeficiency syndromes). MUNC13–4 (FHL3) appears to have a role in vesicle maturation during exocytosis and is involved in regulation of cytolytic granules secretion [137]. Syntaxin 11 (FHL4) is a member of the syntaxin family. Syntaxins have been implicated in the targeting and fusion of intracellular transport vesicles. STXBP2 (FHL5) encodes a protein called syntaxin binding protein 2 that is involved in intracellular trafficking, control of SNARE (soluble NSF attachment protein receptor) complex assembly, and the release of cytotoxic granules by natural killer cells [138]. Genetic defects reducing the activity of one of these proteins as observed in familial HLH or other still unknown mechanisms as in secondary HLH can lead to the inability to clear the antigenic stimulus and thus to turn off the inflammatory response. This is what is currently considered to propagate the inflammatory response and to boost cytokine secretion. Hypercytokinemia is considered the main driver of the disease immunopathology [119, 139, 140]. Elevated levels of TNF-α, IFN-γ, and interleukin 18 are commonly detected. These cytokines are known to activate macrophages and stimulate tissue infiltration, therefore explaining hepatosplenomegaly and CNS infiltration. Activated macrophages secrete plasminogen activator that results in high plasmin levels, hyperfibrinolysis, and a decrease in fibrinogen. TNF-α and IFN-γ are known to inhibit lipoprotein lipase, leading to elevated triglycerides. IFN-γ is known to disrupt bone marrow homeostasis and therefore to affect lympho- and myelopoiesis. Interleukin 1 and

interleukin 6 produced by activated macrophages induce fever. Similar to FHL3, FHL4, and FHL5, impaired granule exocytosis can also be seen in some primary immunodeficiency syndromes, which are at high risk of developing HLH. They are characterized by defect in NK/T cell function and include Griscelli syndrome type 2 (GS-2) [141], Chédiak-Higashi syndrome (CHS) [142], and Hermansky-Pudlak syndrome (HPS-2) type 2 [143]. GS-2 is caused by mutations in *RAB27A*. This gene encodes a protein that regulates cytotoxic granule exocytosis in lymphocytes. CHS is thought to be secondary to mutation is in the *LYST* gene. This gene encodes for a protein involved in vesicle maturation and sorting. Finally, mutations in the AP3B1 gene cause HPS-2. AP3B1 encodes a protein implicated in vesicle maturation and protein transport.

9.5.4 Management and Therapy

The survival of patients with familial HLH without treatment is approx. 2 months [109, 112, 144]. The main goal of therapy is to terminate the inflammatory response by immunosuppression. In 1994, the Histiocyte Society designed the first treatment protocol for HLH (HLH-94) [118], which dramatically increased the survival rate to 54 percent with a median follow-up of 6 years. This protocol includes induction therapy of a series of weekly treatments with dexamethasone and etoposide (VP-16). In 2004, a new HLH protocol has been proposed (HLH-2004) [110]. The major modifications include the earlier use of cyclosporin (i.e., during the induction phase of therapy), and the addiction of intrathecal methotrexate and hydrocortisone to those patients with involvement of central nervous system. After the induction therapy, responders are weaned off of therapy; non-responders are put in continuation therapy, as a bridge to transplantation. Because infective agents can trigger the disease, a possible infection should be diagnosed as rapidly as possible and empiric antibiotic, antifungal, antiviral, or antiparasitic initiated. Treatment of the triggering condition alone can be an option in patients who are clinically stable and in whom a trigger (e.g., infection, rheumatologic condition) is identified. Because patients with HLH are severely ill, supportive care is also required. This includes appropriate transfusions, prevention and treatment of bleeding, and prevention and treatment of opportunistic infections. Hematopoietic cell transplant (HCT) is currently the only curative treatment. HCT is particularly indicated in patients with homozygous or compound heterozygous HLH gene mutations, lack of response to initial HLH therapy, central nervous system (CNS) involvement, and hematologic malignancy. Because HLH tends to be more severe when the age of onset is less than 2 years, the indications for HCT basically apply to almost all young children. Possible exceptions are when HLH is clearly triggered by a viral infection and it resolves with specific antiviral therapy or in case of complete remission lasting at least 6 months following the induction therapy. The success of HCT depends on the grade of control prior transplant.

9.6 Lymphoproliferative Syndrome, X-Linked

9.6.1 Definition, Clinical Manifestations, and Diagnosis

X-linked lymphoproliferative syndrome, or Duncan disease, is a primary immunodeficiency which manifests with severe immune dysregulation often as a consequence of viral infection, typically by Epstein-Barr virus (EBV). The disease was first reported by Purtilo et al., who described a family in which 6 males were affected by progressive combined immunodeficiency associated with fever, pharyngitis, lymphadenopathy, hepatosplenomegaly, and dysgammaglobulinemia [145]. EBV infection was detected in 3 out of 6 boys. The patients died between the ages of 2 and 19. Despite the disease is currently estimated to have an incidence of three in every million males, this value could be misjudged because males with lethal mononucleosis infection are not always evaluated for XLP. In 2011, Booth et al. [146] reported the results of a retrospective study on 91 patients. In this study, the median age of presentation was three and four, respectively, in patients without and with EBV infection. The three most frequent clinical presentations of XLP are fulminant infectious mononucleosis (FIM), dysgammaglobulinemia (22–31%) and lymphoproliferative disease, including lymphoma, usually of B cell origin (30%). Despite it has been for long time thought that XLP always occurred as a consequence of EBV infection, it is now well recognized that in particular FIM and dysgammaglobulinemia can occur also without EBV infection. For example, Brandau et al. did not find EBV infection in 18 of 82 [147] patients with lymphoma and XLP. Similarly, Sumegi et al. [148] reported on a cohort of 309 patients from 89 families and could not find evidence of EBV infection in 12 percent of patients who developed lymphoproliferative disease, dysgammaglobulinemia, or aplastic anemia. FIM is the most frequent and most severe clinical presentation of XLP. It manifests with rapidly progressive bone marrow and hepatic failure. FIM is frequently lethal; the patients who survive usually have permanent immune dysfunction, such as dysgammaglobulinemia or impaired NK cell functions, and may have fulminant hepatitis upon re-exposure to EBV. The second most common form of presentation of XLP is the dysgammaglobulinemia [149, 150]. It occurs in approximately one-third of the patients, it can be wrongly diagnosed with common variable immunodeficiency (CVID), and usually it manifests with decreased levels of IgG1 and IgG3, elevated levels of IgA and IgM, and marginal response to tetanus or diphtheria toxoids after immunization. A progression with involvement of all the subclasses is possible. The third form of XLP-associated clinical manifestations is lymphoma. Lymphoma occur in approximately one-third of the cases and are usually non-Hodgkin type B cell subtype [151]. They are often extranodal, in 75 percent of the cases localizing in the ileocecal region, more rarely in the central nervous system, liver, and kidneys. Histologically they are usually Burkitt lymphoma, immunoblastic lymphoma, small cleaved or mixed cell lymphoma, and unclassifiable lymphomas. Other rarer manifestations include lymphocytic vasculitis, aplastic anemia, and lymphomatoid granulomatosis. Testing for XLP should be considered in all males diagnosed with common variable immunodeficiency (CVID) or other hypogammaglobulinemia,

hemophagocytic lymphohistiocytosis (HLH) (especially if associated with Epstein-Barr virus [EBV] infection and/or early mortality), severe infectious mononucleosis, or lymphoma (especially B cell, non-Hodgkin lymphoma affecting extranodal sites). The demonstration of a gene mutation is the gold standard for diagnosing XLP; however gene sequencing may need time. Because a delayed diagnosis of XLP is detrimental to treatment, a flow cytometry screening of SAP protein expression is suggested because it is sensitive and specific enough [152]. In case of suspected XLP a complete blood count with differential, renal and liver profiles, and coagulation studies, fibrinogen, triglycerides, and lactate dehydrogenase (LDH) to monitor for HLH activity and liver involvement, qualitative and/or quantitative EBV-polymerase chain reaction (PCR) and EBV, serology soluble interleukin 2 (IL-2) receptor alpha (sIL2Ralpha), immunoglobulins, ferritin, and C-reactive protein (CRP) should be measured. If available, natural killer (NK) cell function and immunophenotyping of lymphocyte subpopulations can be evaluated.

9.6.2 Molecular Genetics

Mutations in the gene SH2D1A, the gene encoding for the signaling lymphocyte activation molecule (SLAM)-associated protein (SAP, also called Src homology 2 domain protein 1A [SH2D1A] or DSHP, MIM #300490), were first found in 1998 by Coffey et al. in nine unrelated patients with XLP1 [153]. Subsequently Brandau et al. [147] identified a deletion of exon 1 of the SH2D1A gene in two brothers with early onset non-Hodgkin lymphoma. The mutations usually lead to abnormal or absent SAP protein expression, and they include single nucleotide substitutions, deletions or insertions, splice-site variants and stop-gained. No genotype-phenotype correlation has been found.

9.6.3 Immunopathology

SH2D1A encodes for a molecule called signaling lymphocyte activation molecule (SLAM)-associated protein (SAP) mainly expressed in T cells, NK cells, invariant natural killer T (iNKT) cells, and some B cells [150]. SAP binds to the intracellular domains of the SLAM. SLAM, also called CD150, is a costimulatory receptor that helps T cell activation. SAP mediates the recruitment of Fyn (a Src-related protein tyrosine kinase) to SLAM and the subsequent Fyn activation. Mutations in SAP determine a significant reduced binding to SLAM [154]. SAP interacts also with other proteins, expressed in the immunologic synapse between T cells and antigen-presenting cells (APCs) and between NK cells and their target cells. They include CD84, CD229, CD244 (2B4), and NTBA (NK, T, and B cell antigen) [155]. The immunopathology of XLP is largely unknown. The lymphoproliferation (benign or malign) is thought to be the consequence of defective immune surveillance of infected cells (e.g., Epstein-Barr virus) due to defective T and natural killer (NK) cell interaction with B cells. This can be the consequence of different mechanisms;

SAP-deficient CD8+ CTLs can have reduced ability to lyse autologous EBV-infected B cells, in addition, because SAP enhances apoptosis in T and B cells, reduced apoptosis of CD8+ T cell during acute EBV infection could lead to uncontrolled cytokine production. However, as mentioned above, many clinical manifestations of XLP are not dependent on the presence of EBV. Finally, an abnormal SLAM-SAP signaling pathway can affect T cells activation and cytokines production, which in turn would affect T cell mediated B cell help and antibody production. In agreement, XLP patients have been reported to have reduced T follicular helper (Tfh) cells [156].

9.6.4 Management and Therapy

The overall survival has largely improved in the last two decades because of better treatment options. Currently, management of XLP focuses upon: treatment of acute manifestations, prevention of further sequelae, and curative therapy. Ablative B cell therapy with rituximab (anti-CD20) has been used successfully in some patients to control acute primary EBV infection. In addition, preemptive therapy with rituximab, in combination with immune globulin replacement therapy, should be considered for asymptomatic affected male relatives, in particular siblings of a symptomatic case. Aim of the preemptive therapy is protection from infections that can compromise the outcome of HCT and prevention of lethal disease manifestations, in particular fulminant infectious mononucleosis. Patients with lymphoma should be treated with standard chemotherapy. The only curative therapy currently available is HCT. Booth C. et al. have reported in 2011 about 43 XKP patients who underwent HCT between 1997 and 2009 [146]. They observed an overall survival of approximately 81 percent, with a higher rate when matched family donor was used. The insurgence of HLH reduced the survival to 50%.

Another form of X-linked Lymphoproliferative syndrome is known. It is called XLP2 and it manifests usually during the first years of life as a disease of immune regulation with hemophagocytic lymphohistiocytosis (HLH), often associated with chronic Epstein-Barr virus (EBV) infection, splenomegaly, fever, colitis or inflammatory bowel disease (IBD), and recurrent infections. XLP2 is due to hemizygous mutations of the XIAP gene encoding a protein that belongs to the "inhibitor of apoptosis protein" (IAP) gene family, which also includes HIAP1 (601721) and HIAP2 (601712). XIAP is known to inhibit apoptosis by directly inhibiting certain caspases.

9.7 CTLA4 Haplo-Insufficiency

9.7.1 Definition, Clinical Manifestations, and Diagnosis

CTAL4 haplo-insufficiency is an autosomal dominant disorder of immune regulation first described in 2014 by two independent groups. Kuehn et al. [96] reported

six patients from four families with a complex immune disorder that they called "CTLA4 haplo-insufficiency with autoimmune infiltration (CHAI)." The patients presented with autoimmune thrombocytopenia, hypogammaglobulinemia, and lymphopenia mostly affecting T cells as well as brain, enteropathy, and lung and brain lymphocytic infiltrates. In the same year Schubert et al. [95] reported 11 patients from six unrelated families which also presented with an immune dysregulation phenotype. The majority of these patients developed symptoms between the first decade and the third decade. The main common clinical features included diarrhea associated with lymphocytic enteropathy, recurrent respiratory infections, granulomatous lymphocytic interstitial lung disease, lymphocytic infiltration of organs, including the bone marrow, kidney, brain, and liver, lymphadenopathy and splenomegaly, and autoimmune cytopenias. Seven patients fulfilled the diagnosis of common variable immunodeficiency (CVID). Recently, Schwab et al. reported on the largest CTLA4-haplo-insufficiency cohort so far described [157]. They identified 133 subjects from 54 unrelated families. Median age of onset was 11 (<1 to 69 years). First manifestation was extremely variable and included autoimmune cytopenia, respiratory manifestations, enteropathy, type 1 diabetes, neurological symptoms, growth retardation, fever, skin manifestations, and Addison's disease. In agreement before the genetic diagnosis the patients of this cohort were diagnosed with a large number of different diseases including common variable immunodeficiency, inflammatory bowel disease, ALPS, and granulomatous lymphoproliferative interstitial lung disease (GLILD). Overall the majority of the patients present with hepatomegaly, lymphadenopathy, and splenomegaly. Lung and intestine are also commonly involved. Lung manifestations include granulomatous-lymphocytic interstitial lung disease (GLILD) and bronchiectasis. Gastrointestinal manifestations range from severe to mild diarrhea, T cell infiltrations in the submucosa, ulcerative lesions or inflammatory changes similar to Crohn's disease. Autoimmune cytopenia, including immune thrombocytopenia, autoimmune hemolytic anemia, pure red cell aplasia, or autoimmune neutropenia, is often severe and constitutes the main indication for hematopoietic stem cell transplantation (HSCT). Finally, the nervous system can also be involved. Recently Egg D [158]. et al. have reported a cancer prevalence of ~13% with 50% of the reported neoplasms found to be EBV associated.

9.7.2 Molecular Genetics

The disease is caused by monoallelic mutations in the gene *CTLA4*. So fa more than 50 different variants have been described; they include missense, frameshift, slice site, and stop-gain mutations [158]. The majority of mutations in CTLA4 affect the extracellular domain in particular the exon 2 coding for the ligand binding and dimerization domain. The majority of disease-associated mutations have been shown to affect CTLA4 expression. However, there is not a clear genotype–phenotype correlation and incomplete clinical penetrance has not been fully explained.

9.7.3 Immunopathology

The gene *CTLA4* encodes for a protein called Cytotoxic-T-lymphocyte-antigen-4 (CTLA4). The molecule is part of an integrated system composed of CD28, CD80, and CD86 [159, 160]. CTLA4 and CD28 are proteins expressed predominantly by regulator T cells and activated effector T lymphocytes, and interacting with CD80 and CD86 expressed by antigen-presenting cells. Despite both CD28 and CTLA4 can bind to CD80 and CD86, they mediate opposing effects. CD28 provides a costimulatory signal. When engaged it activates a signal cascade that is necessary for a full activation and proliferation of T lymphocytes [161]. Contrary CTLA4 is a negative regulator of T cell response [162, 163]. The full spectrum of mechanisms critical for CTLA4 function remain to be established; however it has been proposed that it can compete with CD28 for the binding of their ligand or mediate the transendocytosis of CD86 by regulatory T cells, therefore resulting in reduced expression of CD86 and the consequent CD28 stimulation [164]. As mentioned above it is now recognized that thymic selection fails to remove all self-reactive T cells, resulting in the existence of circulating, potentially autoimmune T cells. Therefore an additional level of peripheral control of the T cell response appears necessary to prevent the insurgence of immunopathology. It is currently thought that CTLA4 plays a major role at this level [165–168]. In agreement, it has been shown that a complete loss of CTLA4 expression, as seen in KO mice, determines fatal autoimmunity [169]. The same mechanism is thought to trigger the immunopathology of CTLA4 haplo-insufficiency in humans. It is however important to note that in humans the disease has an incomplete penetrance [169], then additional factors (additional genetic variations, epigenetic mechanisms, microbiome) are probable to play an important role.

9.7.4 Therapy and Management

The efficacy of CTLA-4 replacement by CTLA-4-Fc or inhibition of the CD28 signal cascade through mTOR inhibitors has been evaluated by Schwabb et al. [169]. In their cohort, 14 patients received the abatacept or belatacept; in 11 of them, the authors reported clinical improvement. In two, additional systemic immunosuppression could be reduced. In six, the treatment had to be discontinued: in three of them because they underwent HSCT, in two for an EBV reactivation, and in one for the development of severe respiratory infections, neutropenia, and agranulocytosis. In the same cohort, 13 patients were treated with sirolimus, an mTOR inhibitor. In eight of them, the authors registered a good response. Additional treatment options include corticosteroids, immunosuppressive steroid-sparing agents, and antibiotic prophylaxis when indicated.

9.8 LRBA Deficiency

9.8.1 Definition, Clinical Manifestations, and Diagnosis

LRBA deficiency was first described in 2012 by two independent groups. Lopez-Herrera et al. [170] described five patients from four unrelated consanguineous families with early-childhood onset of immunodeficiency and of autoimmunity. The immunodeficiency affected in particular the B cell compartment, manifesting with antibody deficiency, reduced memory B cells, and recurrent lung infection. The signs of autoimmunity are variable but overall included idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia, atrophic gastritis, colitis, hypothyroidism, and myasthenia gravis. In the same year, Alangari et al. [171] reported five patients from two branches of a consanguineous Saudi family. All the patients presented with enteropathy, some of them also with recurrent infections and autoimmunity. A report from 2016 by Gamez-Diaz et al. on a cohort of 22 patients confirmed that the disease manifests with immunodeficiency and variable signs of immune dysregulation often including enteropathy and blood manifestations. Subsequent studies have confirmed that the disease can manifest with a very broad range of clinical phenotype ranging from common variable immunodeficiency to inflammatory bowel disease [172, 173].

9.8.2 Molecular Genetics

The disease is caused by biallelic loss of function mutation in LRBA. More than 20 different mutations have been describe as of today; they include missense, splice-site, frameshift, and stop-gained. Disease-associated mutations have been shown to reduce protein expression [174, 175].

9.8.3 Immunopathology

Lo et al. [176] have recently described that LRBA regulates endosomal trafficking and therefore the expression of CTLA4 on the plasma membrane of FoxP3+ regulatory and activated conventional T cells. Therefore, explaining why on one side LRBA deficiency manifest with immune dysregulation similar to CTLA-4 deficiency, and on the other why Abatacept, a CTLA4 (cytotoxic T lymphocyte antigen-4)–immunoglobulin fusion, is a highly effective treatment. More recently, Alroqi et al. [177] have reported that LRBA-deficient circulatory follicular helper cells are biased toward a TH 1-like cell phenotype and support in vitro antibody production. In addition, the authors observed that LRBA-deficient regulatory T cells are unable to suppress in vitro T FH cell differentiation in a CTLA4-dependent manner. These findings suggest that LRBA is involved in the regulation of the germinal center reaction and therefore contribute to regulate production of autoantibodies.

9.8.4 Treatment and Management

Tesch et al. [178] have recently reported on the long-term outcome of 76 patients with LRBA deficiency differently treated. In this study, the authors also developed an immune deficiency and dysregulation activity (IDDA) score based on the sum and severity of organ involvement and infections, days of hospitalization, supportive care requirements, and performance indices. Twenty-four of 76 patients underwent HSCT. Overall survival in this group was 70.8% and all deaths were due to transplant-related mortality. Of 17 HSCT survivors, seven are in complete and five in good partial remission. Of the remaining 52 non-transplanted patients, 43 (82%) were alive and only five of them were without immunosuppression. The IDDA scores were lower in the HSCT group than in the one receiving immunosuppression. In the last group the score was better in the patients who received abatacept or sirolimus as compared to other conventional therapies, and in patients with residual LRBA expression.

References

1. Pelanda R, Piccirillo CA (2008) Tolerance, immune regulation, and autoimmunity: cells and cytokines that make a difference. Curr Opin Immunol 20:629–631
2. Kisielow P, Von Boehmer H (1995) Development and selection of T cells: facts and puzzles. Adv Immunol 58:87–209
3. Takaba H, Takayanagi H (2017) The mechanisms of T cell selection in the Thymus. Trends Immunol 8(11):805–816
4. Boraschi D, Italiani P (2018) Innate immune memory: time for adopting a correct terminology. Front Immunol 19;9:799
5. Koch U, Radtke F (2011) Mechanisms of t cell development and transformation. Annu Rev Cell Dev Biol 27:539–62
6. Hogquist KA, Baldwin TA, Jameson SC (2005) Central tolerance: learning self-control in the thymus. Nat Rev Immunol 5(10):772–82
7. Peterson P, Org T, Rebane A (2008) Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. Nat Rev Immunol 8(12):948–57
8. Mueller DL (2010) Mechanisms maintaining peripheral tolerance. Nat Immunol 11(1):21–7
9. Xing Y, Hogquist KA (2012) T-cell tolerance: central and peripheral. Cold Spring Harb Perspect Biol 4(6):1–15
10. Chen L, Flies DB (2013). Molecular mechanisms of T cell co-stimulation and co-inhibition. Nature Reviews Immunology. 13(4):227–42
11. Chambers CA, Allison JP (1997) Co-stimulation in T cell responses. Curr Opin Immunol 9(3):396–404
12. Yang K, Chi H (2012) MTOR and metabolic pathways in T cell quiescence and functional activation. Semin Immunol 24(6):421–8
13. Waickman AT, Powell JD (2012) mTOR, metabolism, and the regulation of T-cell differentiation and function. Immunol Rev 249(1):43–58
14. Wykes MN, Lewin SR (2018) Immune checkpoint blockade in infectious diseases. Nat Rev Immunol 18(2):91–104
15. Wardemann, Hedda; Yurasov, Sergey; Schaefer, Anne; Young, James W.; Meffre, Eric; Nussenzweig, Michel C. (2003-09-05). "Predominant autoantibody production by early human B cell precursors". Science. 301 (5638): 1374–1377
16. Powell BR, Buist NRM, Stenzel P (1982) An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. J Pediatr 100(5):731–7
17. Satake N, Nakanishi M, Okano M, Tomizawa K, Ishizaka A, Kojima K et al (1993) A Japanese family of X-linked auto-immune enteropathy with haemolytic anaemia and polyendocrinopathy. Eur J Pediatr 152(4):313–5

18. Wildin RS, Smyk-Pearson S, Filipovich AH (2002) Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet 39(8):537–45
19. Myers AK, Perroni L, Costigan C, Reardon W (2006) Clinical and molecular findings in IPEX syndrome. Arch Dis Child 91(1):63–4
20. Gambineri E, Torgerson TR, Ochs HD (2003) Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Curr Opin Rheumatol 15(4):430–5
21. Barzaghi F, Passerini L, Bacchetta R (2012) Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: a paradigm of immunodeficiency with autoimmunity. Front Immunol 3:211
22. Roberts J, Searle J (1995) Neonatal diabetes mellitus associated with severe diarrhea, hyperimmunoglobulin e syndrome, and absence of islets of langerhans. Fetal Pediatr Pathol 15(3):477–83
23. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C et al (2000) JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest 106(12):R75–81
24. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N et al (2001) X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 27(1):18–20
25. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L et al (2001) The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 27(1):20–1
26. Barzaghi F, Amaya Hernandez LC, Neven B, Ricci S, Kucuk ZY, Bleesing JJ et al (2018) Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: an international multicenter retrospective study. J Allergy Clin Immunol 141(3):1036–1049.e5
27. Park E, Chang HJ, Shin J, Lim BJ, Jeong HJ, Lee KB et al (2015) Familial IPEX syndrome: Different glomerulopathy in two siblings. Pediatr Int 57(2):e59–61
28. Baris S, Schulze I, Ozen A, Aydiner EK, Altuncu E, Karasu GT et al (2014) Clinical heterogeneity of immunodysregulation, polyendocrinopathy, enteropathy, X-linked: pulmonary involvement as a non-classical disease manifestation. J Clin Immunol 34(6):601–6
29. Nieves DS, Phipps RP, Pollock SJ, Ochs HD, Zhu Q, Scott GA et al (2004) Dermatologic and immunologic findings in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Arch Dermatol 140(4):466–72
30. Gambineri E, Perroni L, Passerini L, Bianchi L, Doglioni C, Meschi F et al (2008) Clinical and molecular profile of a new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: inconsistent correlation between forkhead box protein 3 expression and disease severity. J Allergy Clin Immunol 122(6):1105–1112.e1
31. Savova R, Arshinkova M, Houghton J, Konstantinova M, Gaydarova M, Georgieva E et al (2014) Clinical case of immune dysregulation, polyendocrinopaty, enteropathy, X-linked (IPEX) syndrome with severe immune deficiency and late onset of endocrinopathy and enteropathy. Case Rep Med 2014:564926
32. An YF, Xu F, Wang M, Zhang ZY, Zhao XD (2011) Clinical and molecular characteristics of immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome in China. Scand J Immunol 74(3):304–309
33. Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4(4):330–6
34. Hori S, Nomura T, Sakaguchi S (2017) Control of regulatory T cell development by the transcription factor Foxp3. J Immunol 198(3):981–985
35. Georgiev P, Charbonnier LM, Chatila TA (2019) Regulatory T cells: the many faces of Foxp3. J Clin Immunol 39(7):623–640
36. Stock P, Akbari O, Berry G, Freeman GJ, DeKruyff RH, Umetsu DT (2004) Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyperreactivity. Nat Immunol 5(11):1149–56

37. Lu L, Barbi J, Pan F The regulation of immune tolerance by FOXP3. Nat Rev Immunol 2017(17):703–717
38. Charbonnier LM, Cui Y, Stephen-Victor E, Harb H, Lopez D, Bleesing JJ et al (2019) Functional reprogramming of regulatory T cells in the absence of Foxp3. Nat Immunol 20(9):1208–1219
39. Barzaghi F, Passerini L, Gambineri E, Ciullini Mannurita S, Cornu T, Kang ES et al (2012) Demethylation analysis of the FOXP3 locus shows quantitative defects of regulatory T cells in IPEX-like syndrome. J Autoimmun 38(1):49–58
40. d'Hennezel E, Bin DK, Torgerson T, Piccirillo C (2012) The immunogenetics of immune dysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet 49(5):291–302
41. Ziegler SF (2006) FOXP3: of mice and men. Annu Rev Immunol 24:209–26
42. Bacchetta R, Barzaghi F, Roncarolo MG (2016) From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. Ann N Y Acad Sci 1417(1):5–22
43. Ochs HD, Gambineri E, Torgerson TR (2007) IPEX, FOXP3 and regulatory T-cells: a model for autoimmunity. Immunologic Research 38(1-3):112–21
44. Jones AD, Barnhard S (2017) Auto anti-e in a 2 month old male: A case of IPEX. Transfusion. 57: 122A
45. Hoshino A, Kanegane H, Nishi M, Tsuge I, Tokuda K, Kobayashi I et al (2019) Identification of autoantibodies using human proteome microarrays in patients with IPEX syndrome. Clin Immunol 203:9–13
46. Aschermann S, Lehmann CHK, Mihai S, Schett G, Dudziak D, Nimmerjahn F (2013) B cells are critical for autoimmune pathology in scurfy mice. Proc Natl Acad Sci U S A 110(47):19042–7
47. Kobayashi I, Kubota M, Yamada M, Tanaka H, Itoh S, Sasahara Y et al (2011) Autoantibodies to villin occur frequently in IPEX, a severe immune dysregulation, syndrome caused by mutation of FOXP3. Clin Immunol 41(1):83–9
48. Tsuda M, Torgerson TR, Selmi C, Gambineri E, Carneiro-Sampaio M, Mannurita SC et al (2010) The spectrum of autoantibodies in IPEX syndrome is broad and includes antimitochondrial autoantibodies. J Autoimmun 35(3):265–8
49. Kinnunen T, Chamberlain N, Morbach H, Choi J, Kim S, Craft J et al (2013) Accumulation of peripheral autoreactive B cells in the absence of functional human regulatory T cells. Blood 121(9):1595–1603
50. Wollenberg I, Agua-Doce A, Hernández A, Almeida C, Oliveira VG, Faro J et al (2011) Regulation of the germinal center reaction by Foxp3 + follicular regulatory T cells. J Immunol 187(9):4553–60
51. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF et al (2011) Foxp3+ follicular regulatory T cells control the germinal center response. Nat Med 17(8):975–82
52. Sage PT, Sharpe AH (2016) T follicular regulatory cells. Immunol Rev 271(1):246–59
53. Bakke AC, Purtzer MZ, Wildin RS (2004) Prospective immunological profiling in a case of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX). Clin Exp Immunol 137(2):373–8
54. Barzaghi F, Cristina Amaya Hernandez L, Neven B, Ricci S, Yesim Kucuk Z, Bleesing JJ et al (2018) Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: An international multicenter retrospective study on behalf of the Primary Immune Deficiency Treatment Consortium (PIDTC) and the Inborn Errors Working Party (IEWP) of the European Society for Blood and Marrow Transplantation (EBMT). J Allergy Clin Immunol 141:1036–1049.e5
55. Neufeld M, Maclaren N, Blizzard R (1980) Autoimmune polyglandular syndromes. Pediatr Ann 9(4):154–62
56. Capalbo D, Improda N, Esposito A, De Martino L, Barbieri F, Betterle C, et al. (2013) Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy from the pediatric perspective. Journal of Endocrinological Investigation. 36(10):903–12

57. Ferre EMN, Rose SR, Rosenzweig SD, Burbelo PD, Romito KR, Niemela JE et al (2016) Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathycandidiasis-ectodermal dystrophy. JCI Insight 1(13):e88782
58. Dominguez M, Crushell E, Ilmarinen T, McGovern E, Collins S, Chang B et al (2006) Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in the Irish population. J Pediatr Endocrinol Metab 19(11):1343–52
59. Mazza C, Buzi F, Ortolani F, Vitali A, LDLD N, Weber G et al (2011) Clinical heterogeneity and diagnostic delay of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome. Clin Immunol 139(1):6–11
60. Kisand K, Peterson P (2015) Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy. J Clin Immunol 35(5):463–78
61. Ahonen P, Myllärniemi S, Sipilä I, Perheentupa J (1990) Clinical variation of autoimmune Polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med 322(26):1829–36
62. Bruserud Ø, Oftedal BE, Landegren N, Erichsen MM, Bratland E, Lima K et al (2016) A longitudinal follow-up of autoimmune polyendocrine syndrome type 1. J Clin Endocrinol Metab 101(8):2975–2983
63. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M et al (1997) Positional cloning of the APECED gene. Nat Genet 17(4):393–8
64. Aaltonen J, Björses P, Perheentupa J, Horelli-Kuitunen N, Palotie A, Peltonen L et al (1997) An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet 17(4):399–403
65. Stolarski B, Pronicka E, Korniszewski L, Pollak A, Kostrzewa G, Rowińska E et al (2006) Molecular background of polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome in a polish population: novel AIRE mutations and an estimate of disease prevalence. Clin Genet 70(4):348–54
66. Halonen M, Eskelin P, Myhre AG, Perheentupa J, Husebye ES, Kämpe O et al (2002) AIRE mutations and human leukocyte antigen genotypes as determinants of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy phenotype. J Clin Endocrinol Metab 87(6):2568–74
67. Faiyaz-Ul-Haque M, Bin-Abbas B, Al-Abdullatif A, Abdullah Abalkhail H, Toulimat M, Al-Gazlan S et al (2009) Novel and recurrent mutations in the AIRE gene of autoimmune polyendocrinopathy syndrome type 1 (APS1) patients. Clin Genet 76(5):431–40
68. Giraud M, Yoshid H, Abramson J, Rahl PB, Young RA, Mathis D et al (2012) Aire unleashes stalled RNA polymerase to induce ectopic gene expression in thymic epithelial cells. Proc Natl Acad Sci U S A 109(2):535–40
69. Abramson J, Giraud M, Benoist C, Mathis D (2010) Aire's partners in the molecular control of immunological tolerance. Cell 140(1):123–135
70. Org T, Rebane A, Kisand K, Laan M, Haljasorg U, Andreson R et al (2009) AIRE activated tissue specific genes have histone modifications associated with inactive chromatin. Hum Mol Genet 18(24):4699–710
71. Macedo C, Evangelista AF, Magalhães DA, Fornari TA, Linhares LL, Junta CM et al (2009 Oct) Evidence for a network transcriptional control of promiscuous gene expression in medullary thymic epithelial cells. Mol Immunol 46(16):3240–3244
72. Gardner JM, DeVoss JJ, Friedman RS, Wong DJ, Tan YX, Zhou X et al (2008) Deletional tolerance mediated by extrathymic aire-expressing cells. Science 321(5890):843–7
73. Blizzard RM, Kyle M (1963) Studies of the adrenal antigens and antibodies in Addison's disease. J Clin Invest 42(10):1653–60
74. Söderbergh A, Gustafsson J, Ekwall O, Hallgren Å, Nilsson T, Kämpe O et al (2006) Autoantibodies linked to autoimmune polyendocrine syndrome type I are prevalent in down syndrome. Acta Paediatr Int J Paediatr 95(12):1657–60
75. Alimohammadi M, Björklund P, Hallgren Å, Pöntynen N, Szinnai G, Shikama N et al (2008) Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. N Engl J Med 358(10):1018–28

76. Hedstrand H, Ekwall O, Haavik J, Landgren E, Betterle C, Perheentupa J et al (2000) Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. Biochem Biophys Res Commun 267(1):456–61
77. Söderbergh A, Myhre AG, Ekwall O, Gebre-Medhin G, Hedstrand H, Landgren E et al (2004) Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. J Clin Endocrinol Metab 89(2):557–62
78. Krohn K, Uibo R, Aavik E, Peterson P, Savilahti K, Uibo R et al (1992) Identification by molecular cloning of an autoantigen associated with Addison's disease as steroid 17α-hydroxylase. Lancet 339(8796):770–3
79. Meyer S, Woodward M, Hertel C, Vlaicu P, Haque Y, Kärner J et al (2016) AIRE-deficient patients harbor unique high-affinity disease-ameliorating autoantibodies. Cell 166(3):582–595
80. Sarkadi AK, Taskó S, Csorba G, Tóth B, Erdos M, Maródi L (2014) Autoantibodies to IL-17A may be correlated with the severity of mucocutaneous candidiasis in APECED patients. J Clin Immunol 34(2):181–93
81. Popler J, Alimohammadi M, Kämpe O, Dalin F, Dishop MK, Barker JM et al (2012) Autoimmune polyendocrine syndrome type 1: utility of KCNRG autoantibodies as a marker of active pulmonary disease and successful treatment with rituximab. Pediatr Pulmonol 47(1):84–7
82. Canale VC, Smith CH (1967) Chronic lymphadenopathy simulating malignant lymphoma. J Pediatr 70(6):891–9
83. Sneller MC, Straus SE, Jaffe ES, Jaffe JS, Fleisher TA, Stetler-Stevenson M et al (1992) A novel lymphoproliferative/autoimmune syndrome resembling murine Ipr/gld disease. J Clin Invest 90(2):334–41
84. Sneller MC, Wang J, Dale JK, Strober W, Middelton LA, Choi Y et al (1997) Clinical, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis. Blood 89(4):1341–8
85. Neven B, Magerus-Chatinet A, Florkin B, Gobert D, Lambotte O, De Somer L et al (2011) Asurvey of 90 patients with autoimmune lymphoproliferative syndrome related to TNFRSF6 mutation. Blood 118(18):4798–807
86. Price S, Shaw PA, Seitz A, Joshi G, Davis J, Niemela JE et al (2014) Natural history of autoimmune lymphoproliferative syndrome associated with FAS gene mutations. Blood 123(13):1989–99
87. Oliveira JB, Bleesing JJ, Dianzani U, Fleisher TA, Jaffe ES, Lenardo MJ et al (2010) Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH international workshop. Blood 116(14):e35–e40
88. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middelton LA, Lin AY et al (1995) Dominant interfering fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 81(6):935–46
89. Drappa J, Vaishnaw AK, Sullivan KE, Chu JL, Elkon KB (1996) Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. N Engl J Med 335(22):1643–9
90. Wu J, Wilson J, He J, Xiang L, Schur PH, Mountz JD (1996) Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. J Clin Invest 98(5):1107–13
91. Wang J, Zheng L, Lobito A, Chan FKM, Dale J, Sneller M et al (1999) Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell 98(1):47–58
92. Salzer E, Santos-Valente E, Klaver S, Ban SA, Emminger W, Prengemann NK et al (2013) B-cell deficiency and severe autoimmunity caused by deficiency of protein kinase C δ. Blood 121(16):3112–6
93. Oliveira JB, Bidère N, Niemela JE, Zheng L, Sakai K, Nix CP et al (2007) NRAS mutation causes a human autoimmune lymphoproliferative syndrome. Proc Natl Acad Sci U S A 104(21):8953–8

94. Niemela JE, Lu L, Fleisher TA, Davis J, Caminha I, Natter M et al (2011) Somatic KRAS mutations associated with a human nonmalignant syndrome of autoimmunity and abnormal leukocyte homeostasis. Blood 117(10):2883–6
95. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A et al (2014) Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 20(12):1410–1416
96. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT et al (2014) Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 345(6204):1623–1627
97. Castro JE, Listman JA, Jacobson BA, Wang Y, Lopez PA, Ju S et al (1996) Fas modulation of apoptosis during negative selection of thymocytes. Immunity 5(6):617–27
98. Holler N, Tardivel A, Kovacsovics-Bankowski M, Hertig S, Gaide O, Martinon F et al (2003) Two adjacent trimeric fas ligands are required for fas signaling and formation of a death-inducing signaling complex. Mol Cell Biol 23(4):1428–40
99. Ashkenazi A, Dixit VM (1998) Death receptors: signaling and modulation. Science 281(5381):1305–8
100. Peter ME, Krammer PH (2003) The CD95(APO-1/Fas) DISC and beyond. Cell Death Differ 10(1):26–35
101. Scott FL, Stec B, Pop C, Dobaczewska MK, Lee JJ, Monosov E et al (2009) The Fas-FADD death domain complex structure unravels signalling by receptor clustering. Nature 457(7232):1019–22
102. Van Parijs L, Ibraghimov A, Abbas AK (1996) The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. Immunity 4(3):321–8
103. Hammond DM, Nagarkatti PS, Goté LR, Seth A, Hassuneh MR, Nagarkatti M (1993) Double-negative T cells from MRL-lpr/lpr mice mediate cytolytic activity when triggered through adhesion molecules and constitutively express perforin gene. J Exp Med 178(6):2225–30
104. Rathmell JC, Cooke MP, Ho WY, Grein J, Townsend SE, Davis MM et al (1995) CD95 (Fas)-dependent elimination of self-reactive B cells upon interaction with CD4 + T cells. Nature 376(6536):181–4
105. Hao Z, Duncan GS, Seagal J, Su YW, Hong C, Haight J et al (2008) Fas receptor expression in germinal-center B cells is essential for T and B lymphocyte homeostasis. Immunity 29(4):615–27
106. Miano M, Cappelli E, Pezzulla A, Venè R, Grossi A, Terranova P et al (2019) FAS-mediated apoptosis impairment in patients with ALPS/ALPS-like phenotype carrying variants on CASP10 gene. Br J Haematol 187(4):502–508
107. Bodley Scott R, AHT R-S (1939) Histiocytic medullary reticulosis. Lancet 234:194–198
108. Farquhar JW, Claireaux AE (1952) Familial haemophagocytic reticulosis. Arch Dis Child 27(136):519–525
109. Janka GE (2012) Familial and acquired Hemophagocytic Lymphohistiocytosis. Annu Rev Med 63:233–46
110. Bergsten E, Horne AC, Aricó M, Astigarraga I, Egeler RM, Filipovich AH et al (2017) Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. Blood 130(25):2728–2738
111. Janka GE (1983) Familial hemophagocytic lymphohistiocytosis. Eur J Pediatr 40(3):221–30
112. Henter JI, Elinder G, Soder O, Ost A (1991) Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. Acta Paediatr Scand 80(4):428–35
113. Rudd E, Göransdotter Ericson K, Zheng C, Uysal Z, Ozkan A, Gürgey A et al (2006) Spectrum and clinical implications of syntaxin 11 gene mutations in familial haemophagocytic lymphohistiocytosis: association with disease-free remissions and haematopoietic malignancies. J Med Genet 43(4):e14
114. Solomou EE, Gibellini F, Stewart B, Malide D, Berg M, Visconte V et al (2007) Perforin gene mutations in patients with acquired aplastic anemia. Blood 109(12):5234–7
115. Allen M, De Fusco C, Legrand F, Clementi R, Conter V, Danesino C, et al (2001). Familial hemophagocytic lymphohistiocytosis: how late can the onset be? Haematologica. 86(5):499–503

116. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL (2011) How I treat hemophagocytic lymphohistiocytosis. Blood 118(15): 4041–4052
117. Henter JI, Horne AC, Aricó M, Egeler RM, Filipovich AH, Imashuku S et al (2007) HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 48(2):124–31
118. Trottestam H, Horne AC, Aricò M, Egeler RM, Filipovich AH, Gadner H et al (2011) Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. Blood 118(17):4577–84
119. Henter JI, Elinder G, Soder O, Hansson M, Andersson B, Andersson U (1991) Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. Blood 78(11):2918–22
120. Tang Y, Xu X, Song H, Yang S, Shi S, Wei J et al (2008) Early diagnostic and prognostic significance of a specific Th1/Th2 cytokine pattern in children with haemophagocytic syndrome. Br J Haematol 143(1):84–91
121. Aricò M, Danesino C, Pende D, Moretta L (2001) Pathogenesis of haemophagocytic lymphohistiocytosis. Br J Haematol 114(4):761–9
122. Aricò M, Danesino C, Pende D, Moretta L (2001) Pathogenesis of haemophagocytic lymphohistiocytosis. Br J Haematol
123. Egeler RM, Shapiro R, Loechelt B, Filipovich A (1996) Characteristic immune abnormalities in hemophagocytic lymphohistiocytosis. J Pediatr Hematol Oncol 18(4):340–5
124. Eife R, Janka GE, Belohradsky BH, Holtmann H (1989) Natural killer cell function and interferon production in familial hemophagocyticlymphohistiocytosis. Pediatr Hematol Oncol 6(3):265–72
125. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA et al (1999) Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science 286(5446):1957–9
126. Ishii E, Ueda I, Shirakawa R, Yamamoto K, Horiuchi H, Ohga S et al (2005) Genetic subtypes of familial hemophagocytic lymphohistiocytosis: correlations with clinical features and cytotoxic T lymphocyte/natural killer cell functions. Blood 105(9):3442–8
127. Risma K, Jordan MB (2012) Hemophagocytic lymphohistiocytosis: updates and evolving concepts. Curr Opin Pediatr 24(1):9–15
128. Schmid JP, Côte M, Ménager MM, Burgess A, Nehme N, Ménasché G et al (2010) Inherited defects in lymphocyte cytotoxic activity. Immunol Rev 235(1):10–23
129. Rivière S, Galicier L, Coppo P, Marzac C, Aumont C, Lambotte O et al (2014) Reactive hemophagocytic syndrome in adults: a retrospective analysis of 162 patients. Am J Med 127(11):1118–1125
130. Janka GE, Lehmberg K et al (2013) Hematology Am Soc Hematol Educ Program 2013:605–611
131. Zhang K, Jordan MB, Marsh RA, Johnson JA, Kissell D, Meller J et al (2011) Hypomorphic mutations in PRF1, MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. Blood 118(22):5794–8
132. Ohadi M, MRA L, Sham P, Zhao J, Dearlove AM, Shiach C et al (1999) Localization of a gene for familial hemophagocytic lymphohistiocytosis at chromosome 9q21.3-22 by Homozygosity mapping. Am J Hum Genet 64(1):165–71
133. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C et al (2003) Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell 115(4):461–73
134. zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter JI et al (2005) Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet 14(6):827–34
135. zur Stadt U, Rohr J, Seifert W, Koch F, Grieve S, Pagel J et al (2009) Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in munc18-2 and impaired binding to syntaxin 11. Am J Hum Genet 85(4):482–92
136. Voskoboinik I, Whisstock JC, Trapani JA (2015) Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol 15(6):388–400

137. He J, Johnson JL, Monfregola J, Ramadass M, Pestonjamasp K, Napolitano G et al (2016) Munc13-4 interacts with syntaxin 7 and regulates late endosomal maturation, endosomal signaling, and TLR9-initiated cellular responses. Mol Biol Cell 27(3):572–87
138. Teng FYH, Wang Y, Tang BL (2001) The syntaxins. Genome Biol 2(11):REVIEWS3012
139. Osugi Y, Hara J, Tagawa S, Takai K, Hosoi G, Matsuda Y et al (1997) Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. Blood 89(11):4100–3
140. Filipovich A, McClain K, Grom A (2010) Histiocytic disorders: recent insights into pathophysiology and practical guidelines. Biol Blood Marrow Transplant 16(1 Suppl):S82–9
141. Ménasché G, Pastural E, Feldmann J, Certain S, Ersoy F, Dupuis S et al (2000) Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. Nat Genet 25(2):173–6
142. Karim MA, Nagle DL, Kandil HH, Bürger J, Moore KJ, Spritz RA (1997) Mutations in the Chediak-Higashi syndrome gene (CHS1) indicate requirement for the complete 3801 amino acid CHS protein. Hum Mol Genet 6(7):1087–9
143. Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS (1999) Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the β3A subunit of the AP-3 adaptor. Mol Cell 3(1):11–21
144. Janka GE (2007) Familial and acquired hemophagocytic lymphohistiocytosis. Eur J Pediatr
145. Purtilo DT, Cassel C, Yang JP (1974) Fatal infectious mononucleosis in familial lymphohistiocytosis. N Engl J Med 291(14):736
146. Booth C, Gilmour KC, Veys P, Gennery AR, Slatter MA, Chapel H et al (2011) X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease. Blood 117(1):53–62
147. Brandau O, Schuster V, Weiss M, Hellebrand H, Fink FM, Kreczy A et al (1999) Epstein-Barr virus-negative boys with non-Hodgkin lymphoma are mutated in the SH2D1A gene, as are patients with X-linked lymphoproliferative disease (XLP). Hum Mol Genet 8(13):2407–13
148. Sumegi J, Huang D, Lanyi A, Davis JD, Seemayer TA, Maeda A et al (2000) Correlation of mutations of the SH2D1A gene and Epstein-Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. Blood 96(9):3118–25
149. Seemayer TA, Gross TG, Egeler RM, Pirruccello SJ, Davis JR, Kelly CM et al (1995) X-linked lymphoproliferative disease: twenty-five years after the discovery. Pediatr Res 38(4):471–8
150. Engel P, Eck MJ, Terhorst C (2003) The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. Nat Rev Immunol 3(10):813–21
151. Gaspar HB, Sharifi R, Gilmour KC, Thrasher AJ (2002) X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. Br J Haematol 119(3):585–95
152. Gifford CE, Weingartner E, Villanueva J, Johnson J, Zhang K, Filipovich AH et al (2014) Clinical flow cytometric screening of SAP and XIAP expression accurately identifies patients with SH2D1A and XIAP/BIRC4 mutations. Cytom Part B Clin Cytom 86(4):263–71
153. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM et al (1998) Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet 20(2):129–35
154. Li C, Iosef C, Jia CYH, Gkourasas T, Han VKM, Li SSC (2003) Disease-causing SAP mutants are defective in ligand binding and protein folding. Biochemistry 42(50):14885–92
155. Rezaei N, Mahmoudi E, Aghamohammadi A, Das R, Nichols KE (2011) X-linked lymphoproliferative syndrome: a genetic condition typified by the triad of infection, immunodeficiency and lymphoma. Br J Haematol 152(1):13–30
156. Deenick EK, Chan A, Ma CS, Gatto D, Schwartzberg PL, Brink R et al (2010) Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. Immunity 33(2):241–53
157. Schwab C, Gabrysch A, Olbrich P, Patiño V, Warnatz K, Wolff D et al (2018) Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4–insufficient subjects. J Allergy Clin Immunol 142(6):1932–1946

158. Egg D, Schwab C, Gabrysch A, Arkwright PD, Cheesman E, Giulino-Roth L et al (2018) Increased risk for malignancies in 131 affected CTLA4 mutation carriers. Front Immunol 9:2012
159. Sansom DM (2000) CD28, CTLA-4 and their ligands: who does what and to whom? Immunology 01(2):169–77
160. Rudd CE (2008) The reverse stop-signal model for CTLA4 function. Nat Rev Immunol 8(2):153–60
161. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA (2016) CD28 Costimulation: from mechanism to therapy. Immunity 44(5):973–88
162. Walker LSK, Sansom DM (2011) The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. Nat Rev Immunol 11(12):852–63
163. Kavanagh B, O'Brien S, Lee D, Hou Y, Weinberg V, Rini B et al (2008) CTLA4 blockade expands FoxP3+ regulatory and activated effector CD4 + T cells in a dose-dependent fashion. Blood 112(4):1175–83
164. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM et al (2011) Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4. Science 332(6029):600–3
165. Abbas AK, Lohr J, Knoechel B, Nagabhushanam V (2004) T cell tolerance and autoimmunity. Autoimmun Rev 3(7-8):471–5
166. Eagar TN, Karandikar NJ, Bluestone JA, Miller SD (2002) The role of CTLA-4 in induction and maintenance of peripheral T cell tolerance. Eur J Immunol 32(4):972–81
167. Fife BT, Bluestone JA (2008) Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev 224:166–82
168. Greenwald RJ, Boussiotis VA, Lorsbach RB, Abbas AK, Sharpe AH (2001) CTLA-4 regulates induction of anergy in vivo. Immunity 14(2):145–55
169. Klocke K, Sakaguchi S, Holmdahl R, Wing K (2016) Induction of autoimmune disease by deletion of CTLA-4 in mice in adulthood. Proc Natl Acad Sci U S A 26;113(17)
170. Lopez-Herrera G, Tampella G, Pan-Hammarström Q, Herholz P, Trujillo-Vargas CM, Phadwal K et al (2012) Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet 90(6):986–1001
171. Alangari A, Alsultan A, Adly N, Massaad MJ, Kiani IS, Aljebreen A et al (2012) LPS-responsive beige-like anchor (LRBA) gene mutation in a family with inflammatory bowel disease and combined immunodeficiency. J Allergy Clin Immunol 130(2):481–8.e2
172. Serwas NK, Kansu A, Santos-Valente E, Kuloğlu Z, Demir A, Yaman A, Diaz LYG, Artan R, Sayar E, Ensari A, Grimbacher B, Boztug K (2015) Atypical manifestation of LRBA deficiency with predominant IBD-like phenotype. Inflamm Bowel Dis 21(1):40–7
173. Cagdas D, Halaçlı SO, Tan Ç, Lo B, Çetinkaya PG, Esenboğa S et al (2019) A Spectrum of clinical findings from ALPS to CVID: several novel LRBA defects. J Clin Immunol 39(7):726–738
174. Gámez-Díaz L, Sigmund EC, Reiser V, Vach W, Jung S, Grimbacher B (2018) Rapid flow cytometry-based test for the diagnosis of lipopolysaccharide responsive beige-like anchor (LRBA) deficiency. Front Immunol 9:720
175. Gámez-Díaz L, August D, Stepensky P, Revel-Vilk S, Seidel MG, Noriko M et al (2016) The extended phenotype of LPS-responsive beige-like anchor protein (LRBA) deficiency. J Allergy Clin Immunol 137(1):223–230
176. Lo B, Abdel-Motal UM (2017) Lessons from CTLA-4 deficiency and checkpoint inhibition. Curr Opin Immunol 49:14–19
177. Alroqi FJ, Charbonnier LM, Baris S, Kiykim A, Chou J, Platt CD et al (2018) Exaggerated follicular helper T-cell responses in patients with LRBA deficiency caused by failure of CTLA4-mediated regulation. J Allergy Clin Immunol 141(3):1050–1059
178. Tesch VK, Abolhassani H, Shadur B, Zobel J, Mareika Y, Sharapova S, Karakoc-Aydiner E, Rivière JG, Garcia-Prat M, Moes N, Haerynck F, Gonzales-Granado LI, Santos Pérez JL, Mukhina A, Shcherbina A, Aghamohammadi A, Hammarström L, Dogu F, Haskologlu S, İkinc SM (2019) Long-term outcome of LRBA deficiency in 76 patients after various treatment modalities as evaluated by the immune deficiency and dysregulation activity (IDDA) score. J Allergy Clin Immunol 145(5):1452–1463

IPEX Syndrome and IPEX-Related Disorders

10

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Abstract

Congenital immune disorders due to immune dysregulation usually present with multi-organ autoimmune manifestations, and recent genetic and molecular diagnosis techniques have deeply implemented the identification of new monogenic defects leading to an altered immune homeostasis. Regulatory T cells (Tregs) play an essential role in controlling immune response, and mutations affecting the transcription factor FOXP3 cause immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Strikingly, similar clinical phenotypes resemble IPEX but due to distinct molecular defects have been identified and classified as IPEX-related disorders. These are associated to altered expression of a plethora of factors, either pivotal for Tregs biology or Tregs unrelated, and acting at different levels during immune response regulation. The clinical similarities and differences between these inborn errors of immunity, along with their molecular cause, diagnosis, and treatment options, will be discussed.

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Keywords

Primary immunodeficiency diseases · IPEX syndrome · IPEX-like disorders
Immune dysregulation · Autoimmunity · Tregs

10.1 Introduction

Primary immunodeficiency disorders (PIDs), also defined as inborn errors of immunity, are a heterogeneous group of diseases characterized by an impaired immune response and by an altered homeostasis. They include more than 400 diseases, caused by monogenic germline mutations affecting gene expression, and associated clinical features range from increased susceptibility to infections to immune dysregulation, resulting in autoimmunity, allergy, inflammation, and/or malignancy [1]. The overall incidence is around 1:10.000, and they are more prevalent in children. PIDs clinical and molecular characterization is currently boosting the identification of previously unknown immune regulatory mechanisms, in light of a parallel technological improvement in genetic analysis approaches. The next-generation DNA sequencing (NGS) technology has heavily contributed to new disease-associated genes discovery, having a profound impact on diagnosis and development of targeted therapies.

The emerging prevalence, among PIDs, of immune dysregulation signs as autoimmunity has shifted the attention toward immune tolerance. While central tolerance depends on thymic epithelial cells and self-antigens expression driving T cells selection, peripheral tolerance is maintained by regulatory T cells (Tregs). This chapter will discuss about immune dysregulation-associated diseases—IPEX syndrome and IPEX-related disorders—and how these distinct and partially overlapping manifestations can be seen, depending on the disease-causing gene, from a "Tregs point of view," in a wider context of immune response pathways and specific cellular mechanisms.

10.2 IPEX Syndrome

The immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare disorder causing life-threatening systemic autoimmunity due to immune dysregulation and is the first human disease whose characterization highlighted Tregs pivotal role in immune homeostasis. The IPEX hallmark features comprise severe early-onset enteropathy, chronic dermatitis, elevated IgE levels, and autoimmune endocrinopathies, such as early-onset insulin-dependent type 1 diabetes mellitus (T1D) and/or thyroiditis. Patients develop symptoms early in infancy, and most die prematurely. IPEX syndrome is caused by mutations in *FOXP3* gene, located on X chromosome, coding for a key transcription factor critical for the development and function of $CD4^+$ $CD25^+$ Tregs and able to regulate the expression of multiple genes involved in T cell response, as it will be discussed later.

The classical IPEX syndrome presents with a triad of diarrhea or enteropathy, endocrinopathy (most commonly T1D), and eczema (Table 10.1, Fig. 10.1).

Table 10.1 IPEX-related disorders classification: genes, inheritance, and main manifestations are indicated (Table adapted from the IUIS classification 2017 [1, 6, 142, 143]) (All open access BY CC License 4.0)

Disease	IUIS Classification table	Genetic defect	Inheritance	T cells	B cells	Other features	Immunoglobulins	Associated features
CD25 deficiency	Table 4. Disease of immune dysregulation	IL-2RA	AR	Normal to decreased; no CD4+ CD25+ cells with impaired Tregs function				Lymphoproliferation, autoimmunity and impaired T cell proliferation in vitro
STAT5b deficiency	Table2. Combined immunodeficiency with associated or syndromic features.	STAT5B	AR	Modestly decreased, reduced Tregs number and function	Normal		Hypergammaglobulinemia, increased IgE	Growth-hormone insensitive dwarfism, dysmorphic features; eczema; lymphocytic interstitial pneumonitis; insulin like growth factor deficiency, prominent autoimmunity
CTLA-4 Haploinsufficiency	Table 4. Disease of immune dysregulation	CTLA4	AD	Reduced T cell and impaired Tregs function	Reduced B cell	Impaired Tregs function		Auto immune cytopenias, enteropathy, interstitial lung disease, extra lymphoid, lymphocytic tissue infiltration and recurrent infections
LRBA deficiency	Table 4. Disease of immune dysregulation	LRBA	AR	Normal or decreased CD4 + T cells and dysregulated T cells	Decreased or normal B cell		Reduced IgG and IgA	Inflammatory bowel disease, autoimmune cytopenias, enteropathy, interstitial lung disease, extra-lymphoid, lymphocytic infiltration, lymphoproliferation, autoimmune and recurrent infections

(continued)

Table 10.1 (continued)

Disease	IUIS Classification table	Genetic defect	Inheritance	T cells	B cells	Other features	Immunoglobulins	Associated features
STAT1 GOF	Table 6. Defects in intrinsic and innate immunity	STAT1	AD	T cells are affected mainly associated with impaired development of Th17 cells		B cells and monocytes may also be affected		CMC, various fungal, bacterial and viral (HSV) infections, autoimmunity (thyroiditis, diabetes, cytopenias), enteropathy
STAT3 GOF	Table 4. Disease of immune dysregulation	STAT3 GOF	AD	Decreased, decreased Tregs with impaired function	Decreased	Enhanced STAT3 signalling, leading to increased Th17 cell differentiation		Lymphoproliferation, solid organ auto immunity, recurrent infections
CD122 deficiency	Table 4. Disease of immune dysregulation	IL-2RB	AR	Increased memory CD8+ T cells, decreased Tregs	Increased memory B cells	Diminished IL-2RB expression, dysregulation signalling in response to IL-2 and IL-15, increased immature NK cells		Lymphoproliferation, lymphadenopathy, hepatosplenomegaly, autoimmune haemolytic anaemia, dermatitis, enteropathy, hypergammaglobulinemia, recurrent viral (EBV, CMV) infections

DOCK8 deficiency	Table 1. Immunodeficiencies affecting cellular and humoral immunity	DOCK8	AR	Reduced naïve CD8+ T cells, increased exhausted CD8+ TEM cells, reduced MAIT, NKT cells, increased γδ T cells; poor proliferation: few Tregs with poor function	Increased total B cells, reduced memory B cells and poor peripheral B cell tolerance	Low NK cells with poor function, eosinophilia	Low IgM, normal/high IgG and IgA, very high IgE, poor antibody responses	Recurrent infections, cutaneous viral, fungal and staphylococcal infections, severe atopy/allergic disease, cancer diathesis
Activated p110δ syndrome (APDS)	Table 3. Predominantly antibody deficiencies	PIK3CD GOF (APDS1)	AD		Reduced memory B cells, increased transitional B cells, decreased pro B cells		Normal of increased IgM, low IgA and IgG	Severe bacterial infections; EBV ± CMV viremia, lymphadenopathy/ splenomegaly, autoimmunity, lymphoproliferation, lymphoma
	Table 3. Predominantly antibody deficiencies	PIK3R1 (APDS2)	AD		Reduced memory B cells, increased transitional B cells			Severe bacterial infections, lymphadenopathy/ splenomegaly, lymphoproliferation, lymphoma; developmental delay

(continued)

Table 10.1 (continued)

Disease	IUIS Classification table	Genetic defect	Inheritance	T cells	B cells	Other features	Immunoglobulins	Associated features
Immunodeficiency with multiple intestinal atresias	Table 2. Combined immunodeficiencies with associated or syndromic features	TTC7A	AR	Variable; but may have low or absent TRECs on new born screening. Low T cells; may have SCID phenotype at birth	Normal or decreased	May have a SCID Phenotype at birth	Markedly low IgA, IgG, and IgM	Bacterial (sepsis), fungal, viral infections; multiple intestinal atresias, often with intrauterine polyhydramnios and early demise
Tricho-hepato-enteric syndrome	Table 2. Combined Immunodeficiencies with associated or syndromic features	TTC37 SKIV2L	AR	Impaired INFγ production	Variable decrease in numbers of switched memory B cells		Hypogammaglobulinemia with possible low antibody responses (anti pneumococcal)	Respiratory infections; recurrent bacterial and viral infections IUGR; facial dysmorphic features, wooly hair; early onset intractable diarrhea, liver cirrhosis; platelet abnormalities and growth restriction

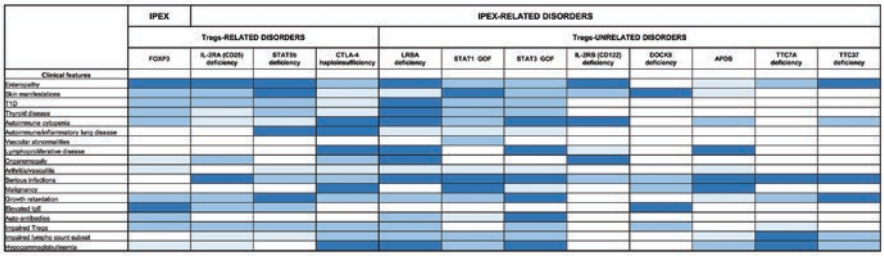

Fig 10.1 IPEX syndrome and IPEX-related disorders clinical features. (Modified from Ciullini Mannurita *et al.*, 2017)

Histopathological features of endoscopic biopsies done in IPEX patients included a heterogeneous array of findings, as pale fragile mucosa of upper gastrointestinal tract, duodenal villous atrophy, with varying signs of inflammation, ulceration in addition to severe colitis. Furthermore, such cases are associated with inflammation of the lamina propria with lymphocytic, macrophagic, eosinophilic, and plasmocyte infiltration in the duodenum, while patients with lower gastrointestinal tract involvement show predominant inflammatory cell infiltrates with lymphocytes and eosinophils [2]. Additionally, IPEX patients can present with T1D very soon after birth or, sometimes, congenital T1D can occur. Studies have demonstrated that early-onset insulin-requiring diabetes mellitus can sometimes be the only feature of IPEX in infants, especially in the absence of other known genetic causes [3]. Nonetheless, patients can also present with other endocrine disorders, most commonly thyroid disease and less commonly adrenal disease or adrenal insufficiency. The endocrine features of the disease are most entirely due to autoimmunity, with the presence of thyroid, glutamic acid decarboxylase (GAD), and islet cells autoantibodies.

Skin manifestations, and specifically dermatitis, represent a strong arm of the classical IPEX triad. Eczema is the most common presentation in IPEX; however, several reports have revealed that, in addition to eczematous dermatitis, other dermatological manifestations occurring in IPEX patients include erythroderma, psoriasiform dermatitis, alopecia, and bullous pemphigoid, with the latter two most prevalent in older individuals [4]. These manifestations are usually associated with the presence of autoantibodies versus different skin antigens.

In addition to diarrhea/enteropathy, multiple endocrinopathies, and dermatological diseases, patients with classical IPEX phenotype can present with acute, chronic, or recurrent autoimmune cytopenias, lymphoproliferation, and autoimmune hepatitis [5].

Autoimmune cytopenias frequently associated with IPEX syndrome are anemia, neutropenia, and thrombocytopenia. Interestingly, autoimmunity can present at varying degrees of systemic and/or organ-specific autoimmunity, which can include arthralgia/arthritis as well as renal and neurological manifestations, with the latter two mostly associated with vasculitis. Renal disease can present with different

patterns, and the most common manifestations described include glomerulonephritis, interstitial nephritis, hypertension, persistent proteinuria, or hematuria. Renal disease is usually either secondary to autoimmunity or secondary to side effects of immunosuppressive therapy [5]. Other serious autoimmune manifestations include pulmonary disease, which is associated with asthma, lymphadenopathy, and interstitial lung disease. Interestingly, many reports have described the different cardiovascular diseases commonly associated with IPEX and IPEX-like disorders: pericarditis, pericardial effusions, aneurysms, atrial flutter, and dilated aortic root. Furthermore, other IPEX-associated clinical features included neurological manifestations such seizures, developmental delay, and ventricular disease [6]. Most importantly, as IPEX and IPEX-like diseases are classified as primary immunodeficiency diseases, it is essential to mention that they can also present with recurrent severe infections, usually complicated by sepsis, such as meningitis, peritonitis, and pneumonia, with the varying causative organisms including *Staphylococcus aureus spp., Cytomegalovirus, and Candida spp.* Other serious infections associated with PIDs are due to *Pneumocystis jirovecii* infection and usually are due to the immunosuppressive therapy rather than to the primary disease [4–6].

10.3 IPEX-Related Disorders

Significantly, there is an emerging group of children presenting various clinical features typical of IPEX syndrome not associated with *FOXP3* mutations and whose clinical phenotype has been defined as "IPEX-like." Our recent study shows as, among a cohort of 173 patients, almost half of them have no *FOXP3* gene mutations, but they do display gene variants in other genes [6]. The IPEX-like clinical presentation can include other manifestations which can differ from the classical IPEX syndrome as patients usually present with a more severe systemic autoimmunity. Despite this, the common feature between these two clinical syndromes is enteropathy, usually evident as watery diarrhea [6]. Moreover, the severe persistent diarrhea usually leads to failure to thrive; although most commonly attributed to enteropathy, it can also be due to food allergy and malabsorption. Autoimmune enteropathy is a common pivotal feature of many inherited primary immune deficiencies, as IPEX and IPEX-like, although the severity and the clinical course might vary among patients with the same genotype. In this regard, the role of microbial diversity and composition is still unclear. The intestine functions as a major gateway for external environment and contains an extensive network of secondary lymphoid organs, and it is home to several lymphocytes including intestine-specific subpopulations. It has become evident that individual commensal species influence the makeup of T lymphocytes subsets, modulating a range of effector function with a general impact on immunity that reaches well beyond the intestinal lamina propria (Fig. 10.1).

Recently, the International Union of Immunological Society (IUIS) has updated PIDs classification, classifying IPEX-like disorders under different categories despite having similar clinical manifestations as IPEX syndrome. This is due to the

fact that these diseases are associated with other main clinical manifestations, which are considered primary features. Nevertheless, this doesn't deny that these disorders can commonly present with an IPEX-like picture, whose main manifestations are shown in Table 10.1 [6, 7].

Indeed, rapid development of next-generation DNA sequencing deeply impacted the discovery of new genetic aberrations affecting factors not previously associated to immune response regulatory mechanisms. In a cost-effective and time-efficient manner, sequencing of targeted gene panels, as well as whole exomes or whole genomes, allows efficient analysis of cohorts of patients whose disease is suspected to have a monogenic cause. Currently, IPEX-like disorders are defined as presenting with a similar phenotype to IPEX syndrome and are due to mutations in *CD25* (*IL-2RA*), *CD122* (*IL-2RB*), *STAT1*, *STAT3*, *STAT5B*, *PI3KCD*, *PI3KR1*, *CTLA4*, *LRBA*, *DOCK8*, *TTC7A,* and *TTC37* genes (Fig. 10.1). While these disorders are defined according to clinical manifestation, a parallel nomenclature according to the role played by impaired factors in these monogenic and partially overlapping disease can be applied by classifying regulatory mechanisms and their consequent dysfunction in light of their role (direct or indirect) in Tregs homeostasis.

10.4 Tregs or Not Tregs: When the Deficiency is Defining You

An efficient adaptive immune response is pivotal for survival, as well as self-reactivity control in the host, mediated by regulatory T cells (Tregs). Tregs are specialized T cells required for peripheral immune tolerance and immune response regulation in both human and mice. They are able to suppress many effector T cell (Teff) functions, such as proliferation, pro-inflammatory cytokines, and growth factors production [8]. The discovery of *FOXP3* gene deficiency as cause of IPEX has been followed by extensive Tregs biology dissection along with the characterization of IPEX-related disorders. As most patients with IPEX-like features have wild-type FOXP3 molecules, several biochemical studies indicated that FOXP3 may cooperate with multiple partners in a dynamic assembled supermolecular complex to modulate gene transcription and regulatory T cell function [9, 10]. Thus, it became evident that other genetic factors may be responsible for the immune dysregulation observed in IPEX-like patients.

Tregs constitutively express CD25, the subunit of the trimetric receptor for interleukin-2 (IL-2). The IL-2 plays a critical role in the maintenance of Tregs in vivo. The signaling cascade activated upon IL-2 binding to its receptor activates the signal transducer and activator of transcription 5b (STAT5b), which translocates to the nucleus and binds to a highly conserved STAT-binding site located within the first intron of the *FOXP3* gene, thus enhancing its transcription [8]. Patients with *STAT5b* or *CD25* (*IL-2RA*) mutations have been reported and showed decreased number and function of Tregs and a clinical phenotype similar to IPEX. The *IL-2R* transcription is upregulated by the complex formed by FOXP3 and the nuclear factor of activated T cells (NFAT), which binds to *IL-2* and *CD25* promoters, respectively suppressing and enhancing their transcription [8]. Moreover, the cytotoxic T-lymphocyte

antigen 4 (CTLA-4) protein is a key regulator of immune response involved in maintenance of peripheral tolerance by regulatory T cells. Its loss is causing fatal autoimmunity in mice, and it has been identified as a cause of a dysregulation syndrome with autosomal dominant inheritance characterized by hypogammaglobulinemia, recurrent infections, and autoimmune manifestations. In human, the *CTLA4* haploinsufficiency causes a dysregulation of FOXP3$^+$ Tregs, with cell hyperactivation and effector T lymphocyte infiltrates [8].

However, diseases of immune regulation are not exclusively caused by an altered regulatory T cells function. Other factors involved in alternative and/or overlapping mechanisms are found to be responsible for IPEX-like clinical phenotype. For instance, patients with IPEX-like phenotype due to monoallelic *STAT1* mutations have been described. They show a broad spectrum of clinical symptoms including a variety of infectious, in particular fungal infections, and autoimmune features, as well as carcinomas and aneurysms associated with a poor outcome [11]. In addition, germline gain-of-function (GOF) mutations in *STAT3* have also been associated with autoimmune disease, with involvement of the endocrine glands, skin, and hematologic compartment. The transcription factor STAT3 is involved in the regulation of STAT1 and STAT5, and *STAT3* GOF mutations cause a lack of STAT1 and STAT5 phosphorylation, resulting in Tregs dysfunction [8]. Lastly, patients with clinical phenotype resembling IPEX syndrome with mutation in the gene encoding the LPS-responsive beige-like anchor (LRBA) protein have been reported [12]. These patients are characterized by low expression of regulatory T cells markers such as FOXP3, CD25, and CTLA-4. All these factors, along with the phosphatidylinositol 3-kinase (PI3K) subunits, dedicator of cytokinesis 8 (DOCK8), and the tetratricopeptide repeat domain proteins (TTC7A and TTC37) recently identified, contribute along with Tregs to immune homeostasis, whose perturbation leads to the dysregulation-observed patients with IPEX-like clinical features.

In light of these evidences, single gene defects causative of IPEX syndrome or IPEX-like disorders can be classified as Tregs-related disorders when displaying a direct effect on regulatory T cell function, as occurs with mutations affecting *FOXP3*, *CD25*, *STAT5B,* and *CTLA4* gene expression; on the other hand, Tregs-unrelated disorders can be considered those having an indirect effect on immune homeostasis and Tregs biology, as observed for *CD122* (*IL-2RB*), *STAT1*, *STAT3*, *PI3KCD*, *PI3KR1*, *LRBA*, *DOCK8*, *TTC7A,* and *TTC37* genes defects. The altered molecular mechanisms, the clinical consequences of inefficient immune response, and diagnosis and therapy for these diseases will be discussed.

10.5 Tregs-Related Disorders

10.5.1 FOXP3 Deficiency

IPEX syndrome was first described by Powell et al. in 1982 in a family of 19 males. They clinically characterized eight male patients' cohort displaying variable clinical symptoms, as diarrhea, polyendocrinopathy, severe enteropathy,

T1D, and dermatitis. Most of the patients died in infancy, and in light of normal B cell function, T cell numbers, polymorphonuclear leukocytes chemotaxis, and complement system, they speculated a T cell defect of X-linked recessive inheritance [13]. In 2000, Chatila and collaborators identified the *JM2* genetic locus in patients suffering from early-onset T1D, chronic diarrhea, and food allergic reactions and displaying a skewed Th2 cells phenotype [14]. The *JM2* gene, which encodes a candidate transcription factor containing a forkhead homology domain, was later called *FOXP3* when Bennett et al. and Wildin et al. discovered additional mutations in seven IPEX patients [15, 16]. Interestingly, the X-linked *scurfy (sf)* mutation spontaneously arose in a mouse strain at the Oak Ridge National Laboratory in 1949 [17], and it is considered the IPEX mouse model. Scurfy male mice present with scaly and ruffled skin, reddened eyes, lymphadenopathy, and splenomegaly and undergo premature death. They also display dermis lymphohistiocytic infiltrates, anemia, elevated serum IgG and IgM, and positive direct Coomb's test, suggestive of an immune dysfunction/hyperactivity [18]. Molecular investigations conducted by Brunkow et al. showed a 2 bp insertion within the *sf* gene coding region, responsible for a frameshift leading to a truncated protein. By functional complementation of the *sf* mutation in transgenic mice, harboring gene copy number ranging between 3 and 70, they observed a complete rescue of scurfy defect and high expression of *scurfy* gene in thymus and spleen. They also observed smaller lymph nodes in transgenic animals due to a decrease in total T cell number [19].

The *FOXP3* gene is the human homolog of mouse gene *Foxp3*. This new evidence confirmed IPEX syndrome as human equivalent of the *scurfy* mouse phenotype [18]. It is located on the short arm of X chromosome (Xp11.23) and contains a 5′-untranslated region (exon-1) followed by 11 translated exons, encoding a protein of 431 amino acids; the mouse protein contains 429 amino acids and shares 86.5% amino acid sequence identity with human FOXP3. The gene is mainly expressed in lymphoid tissues (thymus, spleen, and lymph nodes) and, in particular, by $CD4^+$ $CD25^+$ Tregs, which play a pivotal role in peripheral tolerance to self and non-self-antigens by controlling reactive T cells [20]. IPEX patients harbor *FOXP3* mutations distributed throughout the gene, even if most of them are found within functional domains, as the repressor N-terminal domain, the leucine zipper, and the C-terminal forkhead domain [6].

FOXP3 is a member of the forkhead box (FOX) protein superfamily of transcriptional regulators, which play a role in cell proliferation, differentiation, survival, and apoptosis during embryonic development and homeostasis of adult tissues [21]. The FOXP3 protein displays multiple structural domains: a proline-rich domain at the N-terminus, interacting with factors involved in transcription regulation; central zinc finger and leucine zipper domains, required for oligomer formation; and at C-terminus, a conserved forkhead/winged helix domain (FKH) required for DNA binding and nuclear localization [22]. In humans, *FOXP3* transcripts alternative splicing leads to the expression of four isoforms: a full-length protein (FOXP3) and shortened isoforms, as a result of exclusion of either exon 2 (FOXP3Δ2), exon 7 (FOXP7), or both exons 2 and 7 (FOXP3Δ2Δ7) [23–25].

FOXP3 full-length and the FOXP3Δ2 isoform are the most abundantly expressed, the latter displaying mostly nuclear localization due to loss of nuclear export signal located within exon 2 [26].

FOXP3 is a master regulator of Treg lineage commitment [27], and studies conducted on murine T cell hybridomas provided insights into Foxp3-mediated transcriptional regulation, showing as its targets are those associated with the TCR signalling pathway, such as *Il2ra*, *Tnfrsf18* (GITR), *Nrp1,* and *Ccr4* [28]. Intriguingly, FOXP3 is able to interact with about 700 genes, and it acts as both activator or repressor to regulate Treg cell development, function, and homeostasis [28, 29]. Moreover, FOXP3 forms large protein complexes of 400–800 kDa and associates with more than 360 proteins [9, 10]. Many FOXP3 interactors are transcription factors, such as RUNX1, Eos, Helios, IRF4, RORγ, RORα, HIF1α, STAT3, TCF1, EZH2 [10], and, as already mentioned, NFAT [30].

Interestingly, genetic deletion of these transcription factors in mice does not induce a phenotype as severe as the one observed in *scurfy* mouse, whereas conditional deletion of posttranslational modifiers in Tregs, modulating FOXP3 transcriptional activity, results in more severe autoimmunity [31]. Strikingly, FOXP3 can be modulated by phosphorylation, O-GlcNAcylation, acetylation, ubiquitination, and methylation [32], and transcription regulation in Tregs is also dependent on its ability to shape chromatin remodelling at target gene loci. In particular, FOXP3 repression of IL-2 and IFNg genes upon TCR engagement is mediated by histone H3 deacetylation, while expression of GITR, TNFRSF18, CD25, and CTLA-4 occurs through histone acetylation [33].

IPEX syndrome classical hallmarks include severe early-onset enteropathy, dermatitis, and type 1 diabetes mellitus, and, due to its severity, it is considered fatal if not treated with immunosuppressive therapy and/or hematopoietic stem cell transplantation (HSCT) [34]. Nevertheless, patients with later onset and mild phenotype have been described by our team and others (Fig. 10.1) [6, 35]. Thus, FOXP3 can be considered the master regulator of Treg cell lineage identity [27] as retrovirus-mediated *Foxp3* expression in mouse-naïve $CD4^+$ T cells results in lower proliferation and IL-2, IFNg, IL-4, and IL-10 expression [36], and IPEX patients characterization heavily contributed to dissect the immune tolerance mechanisms as well as the consequences of an altered Tregs function.

10.5.2 CD25 (IL-2Rα) Deficiency

The interleukin-2 is a cytokine primarily produced by activated T cells, which plays a pivotal role in maintaining the immune system. The IL-2 receptor (IL-2R) is composed by three subunits: α (IL-2RA, CD25), β (IL-2RB, CD122), and γ common (IL-2RG, CD132), the latter shared by other five cytokine receptors (IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R). Two functional receptors for IL-2 are known: one is a heterodimeric complex formed by the β and γ chains, which binds IL-15 and IL-2 with intermediate affinity, and it is constitutively expressed on resting $CD8^+$ T cells and NK cells; the other is a trimeric membrane-spanning complex composed of the

α, β, and γ subunits, and it has a higher affinity for IL-2 than the former [37]. The IL-2-mediated signalling starts with formation of a quaternary IL-2-IL-2R complex, whose signal transduction is mediated by receptor-associated tyrosine kinases JAK1 and JAK3, associated with IL-2RB and IL-2RG, respectively. The phosphorylated tyrosines on IL-2RB subunit act as docking sites for signalling molecules, as the adaptor protein Shc, STAT5a, and STAT5b, thus leading to both STAT5 and MAPK pathway activation [38].

CD25 (IL-2Rα) is constitutively expressed at high levels by Treg cells—making them the first responders to IL-2 during immune response—and promotes *FOXP3* transcription by amplifying IL-2 signalling via STAT5b activation pathway [39, 40]. High expression of CD25 is considered as a Tregs marker [41], and this protein is essential for Tregs development and function [20]. Moreover, IL-2-mediated signalling controls antigen-specific peripheral T cell clonal deletion [42], displaying a dual role in lymphocyte homeostasis.

The human *CD25* gene is located on chromosome 10p15.1, and CD25 deficit, due to homozygous mutations, is described as associated with severe bacterial, viral, and fungal infections. Affected patients may also present with adenovirus gastroenteritis, chronic diarrhea, failure to thrive, lymphadenopathy, hepatosplenomegaly, autoimmunity, and dermatitis. Additionally, they have impaired Treg function and T cell proliferation in vitro (Table 10.1, Fig. 10.1) [1, 43–47]. Interestingly, the clinical phenotype due to a deficit of CD25 and STAT5b is partially overlapping, confirming as IPEX-related disease can be considered as "different shades" of a common and profound immune defect.

10.5.3 STAT5b Deficiency

The signal transducer and activator of transcription (STAT) pathway is a key signalling cascade able to mediate cell response to extracellular stimuli and regulate cell proliferation, differentiation, activation, and survival during immune and inflammatory responses. STATs are transcription factors located into the cytoplasm, activated by interferons, cytokines, and growth factors through Janus kinases (JAK)-mediated phosphorylation. Seven members have been identified within the STAT protein family: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. They display a common structure, consisting of a Src homology 2 (SH2) domain required for STATs homo- or heterodimerization; a coiled-coil domain, pivotal for nuclear localization; a DNA-binding domain, essential for transcription of target genes; and a transactivation domain required for coactivators recruitment [48]. STAT-mediated transcription regulation starts with extracellular stimuli, which interact with their surface-specific receptors. The JAK kinases (JAK1, JAK2, JAK3, and TYK2), constitutively associated with type I and type II receptors cytoplasmic tail, undergo transphosphorylation generating docking sites for cytoplasmic STATs binding. Thus, STATs phosphorylation on tyrosine residues and dimerization occurs, followed by nuclear translocation and binding to specific DNA sequences to activate or suppress gene transcription [48].

The JAK/STAT pathway shapes the fate of immune cells and, in particular, of helper T cells: STAT1 and STAT4 are key factors for Th1 cells, required for intracellular pathogens clearance, while Th2 cells—active in host defense against parasites—require STAT6 for signal transduction; STAT3 mediates RORγt expression, a Th17-specific transcription factor, thus contributing to immune response against extracellular bacterial and fungal infections; STAT5, instead, binds *FOXP3* promoter, shaping Tregs pool and host immune tolerance [48]. The plethora of factors involved at each step of these pathways showing as a tight orchestration—in terms of timing and specific molecules involved—of cellular response is required, resulting in specific transcriptional landscape able to shape immune cells' fate. Moreover, the discovery of *STAT1*, *STAT3,* and *STAT5b* germline mutations, leading to immune dysregulation and to an IPEX-like clinical profile as consequence of a GOF for the formers and a LOF for the latter, heavily contributed to dissect the exact role of STAT-mediated signalling during immune response. While STAT5b deficiency is considered as Tregs-related disease, thus discussed in this section, STAT1 and STAT3 GOF will be described in the following section, along with the Tregs-unrelated disorders.

STAT5b mediates signal transduction in response to IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-21, growth hormone (GH), erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor (G-CSF) [49]. Upon activation, STAT5a and/or STAT5b mostly form homo- or heterodimers and translocate to the nucleus, where they act as a transcriptional activator for *FOXP3*, *CD25*, *Bcl-2,* and insulin-like growth factor-I (*IGF-1)* target genes [50]. The *STAT5B* gene is located on chromosome 17q11.2, approximately 12 kb apart from *STAT5A* [51]; the encoded proteins are highly homologous, sharing more than 90% sequence similarity, and in humans they do not have redundant function [50].

STAT5b deficiency is a rare autosomal recessive disease, first described in 2003 and presenting with failure to thrive, chronic diarrhea, eczema, and recurrent pulmonary infections. Taking into account that FOXP3 and CD25 expression is pivotal for Tregs differentiation and maintenance [20, 52] and that impaired IL-2R signalling pathway also affects effector $CD4^+$ and $CD8^+$ T cells activation in response to IL-2, it is not surprising that altered STAT5b-mediated signal transduction leads to immune dysregulation. Patients affected by STAT5b deficiency present with hypergammaglobulinemia and T cells lymphopenia, in particular a Tregs defect, and the loss of immune homeostasis drives autoimmune manifestations [53]. Moreover, the GH-induced IGF-I expression promotes skeletal development and fat metabolism, and missed regulation through STAT5b results in growth delay [49]. Additionally, STAT5b deficit results in reduced STAT5 phosphorylation in response to GH or IFNg, whereas increased phosphorylation of STAT1 and STAT3 is observed (Fig. 10.1) [52, 54].

Clinical and molecular characterization of mutations affecting *STAT5B* expression—as well as *STAT1* and *STAT3* genes, as it will be described later—within an "IPEX-like scenario" strongly contributed to elucidate the role of these factors and the connections required for a proper immune response.

10.5.4 CTLA-4 Haploinsufficiency

Cytotoxic lymphocyte antigen 4 (CTLA-4, CD152) is an inhibitory receptor constitutively expressed by Treg cells and plays a key role in their suppressive function during immune response through inhibition of proliferation and cell cycle progression of antigen-stimulated T cells [55–57]. Additionally, CTLA-4 is also expressed by activated T cells and, upon binding of CD80 (B7-1) and CD86 (B7-2) molecules on APCs, competes with CD28 receptor for opposite regulatory functions [55, 58] as both CTLA-4 and CD28 share 30% protein identity [59]. Furthermore, CTLA-4 binds to CD80 and CD86 ligands with greater avidity [60, 61]. In fact, a CTLA-4 homodimer is able to bind two CD80 molecules, leading to a latticelike structure in the immunological synapse [62, 63], and in this configuration, the CD28-mediated co-stimulation and proteins assembly may be impaired. Moreover, upon CD80/CD86 binding, CTLA-4 is able to remove the ligands from cell surface via trans-endocytosis, with consequent T cells proliferation inhibition [64]. Being CTLA-4 a key factor in immune activation checkpoint, its localization is tightly regulated. In T cells, CTLA-4 is continuously internalized via endocytosis, and it localizes in intracellular compartments; however, after internalization, either it can be reexpressed on plasma membrane (recycling), or it can be targeted to lysosomes for degradation [65]. Despite the exact nature and signalling pathways related to CTLA-4-mediated immune response inhibition are still under debate, an effect on T cells motility—related to the limited contact between T cells and APCs—has also been proposed [66, 67].

The *CTLA4* gene is located on chromosome 2q33.2 in human and on chromosome 1 in mouse and harbors four exons: exon 1 encodes the signal peptide; exon 2, the dimerization and ligand-binding domains; exon 3, the transmembrane region; and exon 4, the cytoplasmic tail [68, 69]. Differential splicing of CTLA-4 transcript has been described, leading to the expression of different isoforms: a full-length transmembrane form, a soluble CTLA-4 form lacking exon 3, and a transcript encoding only for exons 1 and 4 are detected in humans, while mice also express a ligand-independent CTLA-4 isoform, lacking exon 2 [70]. CTLA-4 expression is regulated by NFAT and, in Tregs, also by FOXP3 [30, 71]. Regarding *CTLA4* mutations, more than 50 heterozygous gene variants have been identified in exons 1, 2, and 3: most of them are missense mutations, followed by insertions or deletions and several nonsense mutations [72].

CTLA-4 deficit has a profound impact on both mouse and human immune system. *Ctla4* knockout mice die prematurely within 1 month after birth from multiorgan inflammation [73, 74], while heterozygous mice appeared to be normal [56]. In humans, CTLA-4 haploinsufficiency leads to impaired Tregs function and loss of suppression, with hyperactivated immune response. Patients display immune dysregulation associated to a variable disease phenotype, not correlated with CTLA-4 protein expression, and the main clinical presentations include hypogammaglobulinemia, lymphoproliferation, cytopenia, and gastrointestinal complications (Table 10.1, Fig. 10.1) [75, 76]. Interestingly, both asymptomatic and symptomatic mutation carriers have been described as carrying lower CTLA-4 protein levels than

healthy controls, and further studies have confirmed the clinical variability observed in these cohorts, supporting the loss of correlation between genotype, phenotype, and penetrance [77, 78]. Additionally, SNPs on the human CTLA4 gene have been associated to increased susceptibility to autoimmune manifestations [79]. CTLA-4 reveals to be a key negative regulator of immune response, and its altered expression has a profound impact on immune defense.

10.6 Tregs-Unrelated Disorders

In addition to immune defects due to an inefficient Tregs-mediated regulation of response, genetic studies have evidenced other monogenic diseases leading to immune dysregulation and affecting molecular pathways indirectly related to Tregs, and their molecular and clinical characterization provides, once more, an excellent example of how immune response is based on overlapping and tightly orchestrated mechanisms.

10.6.1 LRBA Deficiency

Lipopolysaccharide-responsive and beige-like anchor (LRBA) is a cytosolic protein which interacts with CTLA-4 in recycling endosomes, favoring its expression on T cell surface [65]. The *LRBA* gene is located on human chromosome 4q31.3 and contains 57 exons, encoding a 2851 amino acids cytosolic protein expressed in different cell types, such as hematopoietic, neural, gastrointestinal, and endocrine cells [80]. Additionally, increased *LRBA* expression has been detected in several cancers [81]. LRBA harbors different functional domains, required for biological processes regulation. In particular, the PH-like domain and the BEACH domain, which precede the highly conserved WD40 domain at C-terminus, have been implicated in CTLA-4 regulation [82]. In B cells and macrophages, LRBA expression is mediated by LPS stimulation, and it has been shown that the protein localizes in trans-Golgi and endocytic vesicles, lysosomes, endoplasmic reticulum, and plasma membrane [83].

LRBA loss of function (LOF) due to biallelic mutations leads to immune dysregulation, whose manifestations are similar to those observed in presence of CTLA-4 haploinsufficiency, and the main clinical features observed are autoimmunity—as autoimmune cytopenia—enteropathy, lymphoproliferation, and humoral immunodeficiency [84]. In patients displaying LRBA deficit, a lower intracellular and cell surface CTLA-4 expression is detected in Tregs, which seems to be LRBA dose-dependent, whereas normal *CTLA4* mRNA levels found suggest a posttranslational regulation exerted by LRBA [82]. Strikingly, LRBA mutations identified are located throughout the protein, making a possible genotype/phenotype correlation unlikely [85]. Patients harboring biallelic LRBA mutations display a Treg cells defect (decreased numbers and FOXP3 and CD25 expression, reduced suppression activity), autoimmunity, and reduced number and function of B cells, unable to proliferate upon activation (Table 10.1, Fig. 10.1) [12]. If the regulatory T cells

defect is consistent with the role played by LRBA in CTLA-4 recycling, and consequent negative regulation of immune response, the humoral deficiency and the B cell compartment impairment seem to be associated with increased apoptosis and altered B cells autophagy in response to starvation [12].

Interestingly, *Lrba*-deficient mice display normal B and T cell development, as well as B cells proliferation, class switch recombination, and survival. In spite of reduced *Ctla4* expression in Tregs and activated T cells, which recapitulates what observed in *LRBA*-deficient patients, *Lrba*$^{-/-}$ mice do not show any clear sign of autoimmunity, neither any sign of abnormalities or pathologies, especially in the gastrointestinal tract [86, 87]. A similar discrepancy between human disease and mouse model, unable to recapitulate the clinical phenotype, is also observed for CTLA-4 haploinsufficiency [56], and the hypothesis of compensatory mechanisms occurring in mice and not in human has been made.

The recent identification of patients harboring LRBA mutations and the role played by this protein in multiple processes regulating immunological homeostasis—alone or in combination with CTLA-4—lead to many open questions about additional regulatory mechanisms which may occur in immune cells, and thus justify the highly variable clinical phenotype associated to its loss of function.

10.6.2 STAT1 Gain of Function

STAT1 plays an important role in antiviral and antimycobacterial defense by transducing signals from type I IFNs (IFNα/IFNβ) and IFNγ. During viral infection, IFNα and IFNβ activate JAK1/TYK2 kinases, leading to STAT1/STAT2 heterodimer formation, which is able to bind p48—a member of the IFN regulatory factor (IRF) family—and to form the IFN-stimulated gene factor 3 (ISGF3) complex. ISGF3 thus translocates to the nucleus and, upon binding to type I IFN-stimulated response element (ISRE), activates gene expression of viral replication-blocking enzymes, MHC class I and CD69 [48]. On the other hand, during mycobacterial infections, T cells and NK cells produce IFNγ, which through JAK1/2 induces STAT1 activation and homodimerization; thus, STAT1 homodimers act as IFNγ activation factor (GAF) and, into the nucleus, activate IFNγ-responsive genes such as *IRF8*, *GATA2,* and those coding for the NADPH oxidase components in macrophages [48].

The *STAT1* human gene is located on chromosome 2q32.2, and heterozygous GOF mutations, detected in the DNA-binding domain or coiled-coil domain, have been identified in patients presenting with IPEX-like clinical features, associated or not with chronic mucocutaneous candidiasis (CMC) [88–90]. In particular, patients harboring heterozygous STAT1-activating mutations present with various fungal, bacterial, and viral infections, especially herpesviruses infections, as well as autoimmunity (thyroiditis, diabetes, cytopenias), enteropathy, and vasculitis. Moreover, recurrent respiratory infections, lung and liver granulomas, bronchiectasis, and cerebral aneurysms can also be observed. Strikingly, most patients can have

different features, even if harboring the same mutation (Table 10.1, Fig. 10.1) [1, 11, 91]. The clinical phenotype associated to STAT1 GOF is related to an enhanced STAT1 activation, as observed in monocytes and $CD4^+$ T cells stimulated, respectively, with IFNα/IFNγ and IFNα/IL-27 [92], and recent evidence suggests that increased STAT1 protein expression, rather than a delayed dephosphorylation, may be responsible for its hyperactivation [93–95]. Moreover, increased STAT1 signalling exerts a negative effect on STAT3-dependent cytokine production, thus impairing IL-17-mediated immune response and favoring *Candida* infections predisposition [89, 90].

10.6.3 STAT3 Gain of Function

STAT3 is activated by many proinflammatory and anti-inflammatory cytokines, as IL-6, IL-10, IL-17, IL-21, IL-22, IL-23, IL-27, IFNα/IFNβ, and IFNγ, and is able to regulate many processes, such as cell growth, apoptosis, organogenesis, inflammation, infection, and oncogenesis [48]. JAKs engagement results in STAT3 phosphorylation and consequent dimerization, translocation to the nucleus, and activation of target genes transcription. Phosphorylated STAT3 forms mainly homodimers, but also heterodimers with STAT1, in response to IL-6-mediated activation, and STAT5, upon M-CSF stimulation, thus controlling different transcriptional programs [96].

The *STAT3* gene is located on chromosome 17q21.2, and GOF mutations, detected in the DNA binding, SH2, transactivation, or coiled-coil domain, lead to immune dysregulation associated with multiple clinical manifestations. Patients usually present with lymphoproliferation, organ-specific or solid organ autoimmunity, and recurrent infections and failure to thrive. Despite the main presentation, they can present with a clinical phenotype similar to IPEX as diabetes and enteropathy have also been observed; moreover, increased Th17 cells differentiation, secondary to enhanced STAT3 signalling, is also observed along with low Tregs number and function (Table 10.1, Fig. 10.1) [1, 6, 97–101]. Activating *STAT3* mutations are found throughout all the protein, and most of them are missense [102]. Strikingly, it has been showed as mutated STAT3 protein is not constitutively phosphorylated compared to wild-type, even if a delayed dephosphorylation has been reported, and that both resting and stimulated cells expressing *STAT3* GOF variant show higher transcriptional activity [97]. Moreover, some patients with *STAT3* GOF mutation show a reduced number of Treg cells, and decreased CD25 expression, maybe as consequence of SOCS3 upregulation [97].

10.6.4 CD122 (IL-2RB) Deficiency

The human CD122 (IL2RB) protein, whose coding gene is located on chromosome 22q12.3, beyond being a IL-2R subunit is also a subunit of IL-15 receptor (IL-15R), and homozygous recessive deficiency has been described [103, 104]. The main clinical features are gastroenteritis, dermatitis, severe diarrhea, and infections;

additionally, all patients who survived the neonatal period also had recurrent infections, as well as autoimmune disease (mainly autoimmune hemolytic anemia), leading to early death in most cases [103]. Moreover, lymphoproliferation, lymphadenopathy, hepatosplenomegaly, and elevated IgG levels have also been associated with CD122 deficiency (Table 10.1, Fig. 10.1) [1].

Mutation analysis revealed three mutations, located in the extracellular protein domain and leading to different outcomes: (a) the L77P missense mutation results in CD122 sequestration in the endoplasmic reticulum, with consequent abrogation of its surface expression and impaired IL-2 signalling in T cells, despite normal NK cells responsiveness to IL-2 and cytotoxic activity; (b) the patient harboring the S40L mutation shows decreased IL-2-mediated response in spite of CD122 surface expression, while (c) the Q96 stop-gain mutation is causative of a more severe phenotype due to complete absence of CD122 surface expression and IL-2 signalling [103]. Fernandez et al. also identified a CD122 mutation leading to reduced CD122 expression and altered downstream signalling, observing reduced Tregs frequency, lymphocyte populations skewing toward memory T cells, and tissues lymphocytic infiltration. Moreover, they detected increased numbers of less differentiated NK cells, as well as memory $CD8^+$ T cells and memory B cells, associated with increased IgG levels [104].

10.6.5 DOCK8 Deficiency

Dedicator of cytokinesis 8 (DOCK8) is a member of the DOCK180 superfamily of atypical guanine exchange factors (GEFs) involved in actin cytoskeleton regulation. DOCK proteins activate CDC42 and RAC, members of the Ras homolog gene family (Rho) of small guanine triphosphate binding proteins (GTPases), required for actin polymerization and cytoskeletal rearrangement regulation. DOCK8 is predominantly expressed in hematopoietic cells and plays a key role in both humoral and cellular immune responses [105]. In particular, DOCK8 has been shown to regulate cell differentiation, adhesion, and survival by activating CDC42, and through actin cytoskeleton remodelling, it allows spatial redistribution of signalling molecules at immunological synapses, as observed in T, B, and NK cells [106]. Additionally, DOCK8 is also important for lymphocyte subsets differentiation and survival: by acting on STAT3 nuclear translocation, it indirectly drives Th17 differentiation [107], and its absence leads to a memory $CD4^+$ T cells polarization toward a Th2 cytokine phenotype [108]. Furthermore, DOCK8 acts on memory B cells activation and antibody-mediated response [109, 110].

DOCK8 deficiency is a combined immunodeficiency first reported in 2009, when biallelic mutations in *DOCK8* gene were discovered in patients suffering from autosomal recessive hyper-IgE syndrome (AR-HIES) [111, 112]. So far, mutations identified are mainly large deletions even though splicing, frameshift, or nonsense mutations are also detected [113]. A deficit of DOCK8 leads to severe and recurrent mucocutaneous bacterial infections, as well as viral and fungal infections, eczema, and food allergies. Patients affected may display lymphopenia, normal/elevated IgG

and IgA levels, hyper-IgE, and decreased serum IgM; an impaired antigen-specific antibody responses may also be detected, and high incidence of malignancy is reported (Table 10.1, Fig. 10.1) [114].

10.6.6 Activated Phosphoinositide 3-Kinase D Syndrome (APDS): PIK3CD Gain of Function and PIK3R1 Loss of Function

Activated phosphoinositide 3-kinase δ syndrome (APDS) is a combined immunodeficiency disorder due to GOF mutations in the *PIK3CD*, encoding for the p110δ catalytic subunit of phosphatidylinositol 3-kinases (APDS1), to LOF mutations in *PIK3R1*, encoding for the p85α regulatory subunit of the kinase (APDS2) or due to LOF mutations in phosphatase and tensin homolog (PTEN) coding gene (APDS-L) [115].

The phosphatidylinositol 3-kinases (PI3Ks) are a family of heterodimeric lipid kinases, whose signalling pathway is involved in many cellular processes, such as growth, metabolism, differentiation, proliferation, and motility [115]. Within the PI3K protein family, class IA PI3Ks play a pivotal role in mammals' immune system, and they form heterodimers comprised of a catalytic subunit (p110α, p110β, or p110δ) and an Src homology 2 (SH2)-containing regulatory subunit [p85α, p55α, p50α, p85β, or p55γ]. The catalytic isoform p110δ is mainly expressed by immune cells, whereas p110α, p110β, and the regulatory isoforms p85α and p85β display ubiquitous expression and broad tissue distribution [115]. The p110δ protein, associated with the p85α regulatory subunit, forms PI3Kδ, which is mainly expressed in hematopoietic cells, and its activation is triggered by T and B cell antigen receptors (TCR and BCR), Toll-like receptors (TLRs), costimulatory molecules, and cytokine receptors in T, B, and myeloid cells. Active PI3Kδ catalyzes the addition of a phosphate group to the membrane phospholipid phosphatidylinositol (4,5)-bisphosphate ($PI(4,5)P_2$) to generate phosphoinositide-3,4,5-trisphosphate (PIP_3), which acts as a docking site to pleckstrin homology (PH) domain-containing intracellular signalling proteins. Signalling termination is mediated by PTEN, which dephosphorylates PIP_3 to PIP_2, or by SH2 domain-containing inositol 5-phosphatase (SHIP), which dephosphorylates PIP_3 to phosphatidylinositol (3,4)-bisphosphate ($PI(3,4)P_2$) [116]. Among the many PH domain-containing proteins interacting with PIP_3, the most characterized is the serine/threonine kinase AKT and the mTOR/FOXO1 signalling, which leads to glucose uptake and glycolysis, shifting cell metabolism toward growth, proliferation, and differentiation.

In B cells, PI3Kδ activation is mediated by BCR cross-linking, TLRs, chemokines (CXCR5), and cytokines (IL-4, IL-21, BAFF), and signalling through AKT leads to protein synthesis and cell growth. Additionally, this also results in FOXO1 phosphorylation and removal from the nucleus as FOXO1 is a transcription factor which regulates *RAG* genes, *IKAROS*, *CD62L*, and *AICDA* expression. In T cells, PI3Kδ activation is mainly mediated by TCR, ICOS, and IL-2R engagement and leads to *IL7RA* and *CD62L* downregulation, resulting in T cells mobility from

lymph nodes to circulation. Furthermore, PI3Kδ promotes T cell activation and effector phenotypes [115, 117].

In 2013 and 2014, heterozygous GOF mutations in the *PI3KCD* gene—encoding the p110δ protein—were identified, the most recurrent being the E1021K mutation, and affected patients were presenting with recurrent respiratory infections and progressive airway damage, e.g., bronchiectasis, lymphopenia, hyper-IgM, hypogammaglobulinemia, and impaired vaccine responses [118–121]. Additionally, patients can present with autoimmune features and diseases including cytopenias; thyroid, kidney, and liver disease; and enteropathy. Examination findings include lymphadenopathy and hepatosplenomegaly [122]. Significantly, hyperactivation of PI3K signalling has also been associated with malignant transformation, most commonly EBV-derived lymphomas [120–122]. The main laboratory findings in most patients with APDS include hypogammaglobulinemia and low $CD4^+$ T cell counts (Table 10.1, Fig. 10.1) [122].

Additionally, heterozygous LOF mutations in *PIK3R1* were reported as associated with a similar clinical phenotype, named APDS2 (Table 10.1, Fig. 10.1). These were heterozygous point mutations at splice donor site, resulting in skipping of exon 11 (coding exon 10), encoding amino acids 434 to 475 of p85a. This mutation affects all three proteins encoded by *PIK3R1* (p85α, p55α, and p50α regulatory subunits), thus leading to impaired p110δ inhibition [123, 124]. Recently, a novel heterozygous missense *PIK3R1* mutation, N564K, has been identified; it is predicted to influence binding to p110δ and is associated with APDS2 clinical features [125].

10.7 The Tetratricopeptide Repeat Domain Proteins

The tetratricopeptide repeat domain 7A (TTC7A) and tetratricopeptide repeat domain 37 (TTC37) are two factors associated with immune dysregulation, whose mutated genes we identified in a large cohort of IPEX-like patients, as they showed an overlapping clinical presentation [6].

10.7.1 TTC7A Deficiency

The tetratricopeptide repeat domain 7A (TTC7A) is a factor involved in multiple process regulating cell polarization, adhesion, and proliferation. The *TTC7A* gene is located on chromosome 2p21 and contains 20 exons, encoding an 858 amino acids protein, localized within the cytoplasm [126]. The encoded protein is expressed in many tissues and organs during development, such as brain, bone marrow, testis, pancreas, ovaries, liver, and blood, and is supposed to have some redundancy with its paralog, tetratricopeptide repeat domain 7B (TTC7B), as they share 49.47% sequence identity [126]. In thymus, TTC7A is expressed in thymic epithelial cells and, at a lower extent, is also detected in thymocytes [127]; in the gastrointestinal tract,

instead, TTC7A is strongly expressed in duodenum, ileum, and colon enterocytes, and this expression pattern is lost in patients harboring mutations predicted to reduce protein expression [128]. In gastric epithelial cells, TTC7A is proposed to regulate PI-4P synthesis and cells survival, cell polarity, apoptosis, cell adhesion, cytoskeletal homeostasis, cell motility, and barrier function, the latter resulting in bacteria translocation into lamina propria and inflammatory response triggering [126].

TTC7 proteins contain nine TPR domains, structurally conserved motifs which seem to be involved in multiprotein interactions [126]. Avitzur et al. described as the specific TTC7A partner phosphatidylinositol 4-kinase IIIa (PI4KIIIa), expressed by enterocytes and immune cells, is able to catalyze the production of phosphatidylinositol 4-phosphate (PI-4P) at plasma membrane and to regulate cell survival and polarity [128, 129]. Moreover, co-expression of TTC7A with the plasma membrane protein EFR3B is proposed to relocate TTC7A from cytosol to cell surface, where it acts as a scaffold leading to TTC7A/PI4KIIIa/EFR3 complex formation [126]. Additionally, TTC7A acts on cytoskeleton regulators within the RhoA/Rho-associated kinase (ROCK) pathway, thus influencing cell shape, polarization, and motility [130], although the exact regulatory mechanism is still not fully understood.

In human, biallelic mutations in the *TTC7A* gene are causative of hereditary multiple intestinal atresia (MIA), a rare cause of intestinal obstruction often associated with a profound combined immunodeficiency (MIA-CID) [127, 128, 130–133]. The immunological manifestations include severe hypogammaglobulinemia and lymphopenia, increased susceptibility to bacterial and opportunistic infections, and higher risk of graft-versus-host disease [134]. Furthermore, extraintestinal manifestations as integumentary hyperplasia and reduced hepatic function have been reported in TTC7A-deficient patients (Table 10.1, Fig. 10.1) [126].

Significantly, three spontaneous mouse models harboring Ttc7 mutations are known: a] the *Ttc7*^{fsn} (flaky skin) mouse, presenting with papulosquamous skin disease and multisystem defects, including anemia, testicular degeneration, imbalance of CD4/CD8 T cells, and apoptotic cecal intestinal epithelial cells; b] the *Hea* mouse model, presenting with hematological anomalies as severe anemia, abundant circulating erythroblasts, thymic atrophy, defective thymocytes differentiation, and increased number of apoptotic and necrotic cells; and c] the *Ttc7*$^{fsn\text{-}Jic}$ mouse, which displays low body weight, skin and hematological abnormalities, reduced white pulp in the spleen, and inflammatory infiltrates in the liver [134]. In spite of a loss of unique mouse model able to fully recapitulate the MIA-CID phenotype observed in patients, the important role played by TTC7A in functional regulation of both epithelial cells and the hematopoietic system becomes more evident [135].

10.7.2 TTC37 Deficiency

The RNA exosome is an evolutionarily conserved ribonuclease complex required for processing and degradation of different RNAs within the cell. It degrades RNA in 3′ to 5′ direction and consists of a barrel-like catalytic core and accessory

proteins, which recruit RNA substrates. The RNA exosome functional specificity depends on its cofactors as the multiprotein superkiller (SKI) complex, involved in cytosolic exosome-mediated RNA surveillance through regulation of normal mRNA and decay of nonfunctional mRNA, and which includes—among its proteins—the tetratricopeptide repeat domain 37 (TTC37) [136].

The human TTC37 protein contains 20 predicted TRP motifs, involved in protein-protein interaction, and its coding gene is located on chromosome 5q15 [136]. Mutations in the TTC37 encoding gene are associated with trichohepatoenteric syndrome (THES1), a rare autosomal recessive disorder presenting with growth restriction, severe infantile diarrhea, trichorrhexis nodosa-like hair morphology, hepatopathy, facial dysmorphism, and immunodeficiency [137–139]. The immunological features are represented by recurrent infections, hypogammaglobulinemia, and low vaccination response (Table 10.1, Fig. 10.1) [139, 140]. So far, no clear genotype/phenotype correlation has been made, most likely due to the broad spectrum of mutations identified. Interestingly, both TTC7A and TTC37 proteins harbor TPR domains, and little is known about protein function, in particular about the role of mutations in both of these TPR-domain-containing proteins resulting in immune system and gut dysfunction [126], even though recent data on Drosophila *ski3* mutant, ortholog of TTC37, propose a role in mitochondrial function and thus open new directions for future investigations [141].

10.8 Diagnosis of IPEX Syndrome and IPEX-Related Disorders

Diagnosis of IPEX and IPEX-related disorders mostly depends on strong suspicion based on clinical presentation. Usually, patients will have normal immunoglobulin levels, apart from increased IgE and, sometimes, increased IgA. Hypogammaglobulinemia, if present, is mostly associated with IPEX-like diseases, in particular with CTLA-4 haploinsufficiency, STAT3 GOF, and LRBA deficiency. Low immunoglobulin levels may also be seen if the patient has wasting syndrome. Lymphocyte counts are usually normal in IPEX, as well as lymphocyte proliferation in response to mitogens in vitro, and immunization responses, including those to protein antigens. However, lymphocyte impairment can be found in other diseases (i.e., DOCK8, CTLA-4 haploinsufficiency, CD25 deficiency, etc.). The $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells are usually present in IPEX, unless *FOXP3* mutations prevent or limit normal gene expression. Tregs levels are variable in IPEX-related disorders. Bacchetta et al. reported autoantibodies specific to IPEX, mainly anti-harmonin antibodies and anti-villin antibodies; however, their role in IPEX pathogenesis and diagnosis is yet to be revealed [4–6]. However, a first screening on Treg cells is recommended. Lack of CD25 expression by flow cytometry should highly raise the suspicion of *IL2Rα* mutation and address genetic testing appropriately to confirm the disease.

Patients without *FOXP3* mutation and clinical picture resembling IPEX can be screened according to the recent IUIS update for inborn errors of immunity, based on basic clinical, laboratory, and immune phenotyping features (Table 10.1). Thus,

patients can then be referred for further laboratory or genetic testing, if required. The IUIS classified IPEX in the "IV. Diseases of immune dysregulation: B. Syndromes of autoimmunity and others, with regulatory T cell defects." CTLA-4 haploinsufficiency, CD25 deficiency, STAT3 GOF and LRBA deficiency are included in the same classification. Other disorders with immune dysregulation as only part of the clinical picture were classified according to their main clinical features and immunological phenotype (e.g., DOCK8 deficiency, STAT5b deficiency, STAT1 GOF, TTC37, and TTC7A deficiency) [142].

The definitive diagnosis of IPEX and IPEX-like conditions is made through genetic and molecular analysis to identify possible causative mutations leading to these disorders. Methods applied are whole-exome sequencing (WES) or Sanger sequencing in cases of positive family history of IPEX or IPEX-like diseases.

In spite of IPEX being an X-linked disease, the study by Lin et al. showing as somatic and germline *FOXP3* mutations can occur concomitantly [144]. They reported a mosaicism of somatic and germline mutations in the mother of an affected patient; however, only one of the two reported mutations was vertically transmitted to her children, including a daughter and an affected son, as both daughter and son had the same de novo point mutation, with the healthy sister being a disease carrier [144]. Moreover, CTLA-4 haploinsufficiency often shows an incomplete clinical penetrance not fully understood. This makes diagnosis very challenging and further proves that all family members should be screened for mutations.

It would be appropriate to investigate patients presenting with an IPEX clinical picture for specific diseases according to a certain strategy based on their main presenting features and basic laboratory investigations findings [6]. Thereafter, if attainable, single gene sequencing can be attempted. Yet giving the wide clinical overlap of these conditions, with the recent advances in genetics, WES remains the most suitable and recommended method for definitive diagnosis [6].

10.9 Management and Treatment

Patients with suspected IPEX syndrome or IPEX-like manifestation must be monitored, followed up, and cared for by a multidisciplinary team of physicians, specialists, nurses, and nutritionists. Furthermore, this interdisciplinary patient management approach requires supportive therapy, immunosuppressive therapy, and—in most cases—hematopoietic stem cell transplantation (HSCT) as it is the current definitive treatment.

Most patients will present with failure to thrive, eczema, and skin manifestations, mostly consistent with atopic dermatitis. Hence, a combination of dermatological team follow-up, extensive nutritional guidance, and support is required, especially prior to confirming the diagnosis. The most used therapeutic approach includes induction of remission with steroids in a tapering dose, usually in combination of immunosuppressive medications, although patients sometimes tend to respond to a single immunosuppressive agent, and remission is usually maintained through a combination of at least two immunosuppressive medications. The most commonly

used therapeutic agent is rapamycin (Sirolimus), at a dose of 0.15 mg/kg/day, that is adjusted to maintain a serum Sirolimus level of 12–18 ng/mL [2, 5]. Rapamycin or Sirolimus Rapamycin has been proved to be extremely beneficial in resolving or improving IPEX-related autoimmune manifestations, and it was mostly used as a monotherapy [34]; it acts by inhibiting the mTOR pathway and it selectively inhibits effector T cell proliferation, while sparing rapamycin-resistant regulatory T cells, hence increasing Tregs number and function. Other immunosuppressive treatments used include calcineurin inhibitors, such as cyclosporine and tacrolimus, in addition to azathioprine and mycophenolate mofetil, methotrexate, or monoclonal antibodies as anti-TNF alpha antibodies, anti-CD20 mAbs, CTLA-4 infusion proteins, and more [34]. Barzaghi et al. [34] showed that a few patients in their cohort, harboring *FOXP3* mutations, have improved spontaneously without requiring immunosuppressive therapy or any other intervention. While one patient had improved under supportive therapy, only without requiring immunosuppressive medications, another patient with the same mutation remained asymptomatic throughout the period of the study [34]. However, this review pointed out that most patients will require immunosuppression with concomitant use of steroids. Calcineurin inhibitors as cyclosporine had some benefit in some patients (40%), and their conditions resolved with significant improvement of their nutritional status, autoimmune manifestations, and recurrent infections [34].

Apart from classical immunosuppression, other treatments have been described. These include targeted therapies, such as monoclonal antibodies directed against specific molecules that directly cause disease: CTLA4-Ig, anti-IL-6 monoclonal antibodies (tocilizumab), rituximab, and IL-2. The CTLA-4-Ig is a fusion protein composed of the Fc region of IgG, fused to the extracellular domain of CTLA-4. It is used mainly in patients with CTLA-4 haploinsufficiency and acts by controlling auto-inflammation, with improvement seen specifically in patients with interstitial lung disease; the CTLA-4-Ig is also used to treat patients with LRBA deficiency, who benefit from the addition of hydroxychloroquine which inhibits lysosomal degradation. Moreover, tocilizumab inhibits IL-6-related Th17 cells differentiation, that is mostly observed in patients with STAT3 GOF disease, while increasing the number of circulating Tregs [8].

Rituximab is an anti-CD20 monoclonal antibody used for $CD20^+$ B lymphocytes depletion in patients with autoimmune conditions. However, in IEI, it is mostly used to treat patients with evident autoimmune cytopenia and granulomatous lymphocytic interstitial lung disease (GLILD). Also, it has been used in patients with *FOXP3*, *CTLA4*, *CD25*, *LRBA,* and *STAT3* GOF mutations. Nevertheless, rituximab is associated with complications related to B cell depletion, such as increased risk of infections, which might exacerbate the severity of certain patients' conditions, especially in those presenting with increased susceptibility to infection.

Other targeted therapies include IL-2 cytokine administration, which enhances and maintains the function of $FOXP3^+$ Tregs by increasing CD25 expression. However, even when administered in low doses, it can worsen patients' conditions, such as thrombocytopenia, in addition to its toxic side effects [8]. Some studies are focused on enhancing native IL-2 with concurrent administration of engineered

autologous Tregs in order to avoid effector T cells activation caused by IL-2 cytokine infusion [8].

Nevertheless, patients who received either immunosuppression or HSCT showed similar outcomes, but those who underwent HSCT benefited more, mainly with longer disease-free survival [34]. A recent survey on HSCT outcome in patients with primary immune regulatory disorders indicated that transplantation was the most definitive treatment, especially in patients with regulatory T cells defects including IPEX and IPEX-like clinical phenotypes, with good overall 5-year survival rate regardless of the donor type, match, or conditioning regimen received prior to HSCT. Strikingly, HSCT was more successful in patients with inactive or quiescent disease, mainly after immunosuppressive therapy [145].

Lastly, gene therapy represents a solid therapeutic option, especially for patients with monogenic diseases affecting Tregs function, since the mutation effects can be readily accessible in immune cells. Many gene therapy approaches have been studied and tested in patients. The most studied approaches include ex vivo HSCs gene correction, where the patient's own stem cells are manipulated to correct the genetic mutation through a lentiviral vector and are then transferred back to the patient at a specific genome site in order to prevent oncogenic gene activation. Also, conventional $CD4^+$ T cells from IPEX patients can be reprogrammed to express wild-type FOXP3, and hence to suppress the disease. Other approaches include patient's HSCs gene editing through clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 ribonucleoproteins: this technique is based on a chemically modified guide RNA and homology-directed DNA repair, and it has been used in T cells from a patient with a nonsense mutation in *CD25* gene, with acceptable CD25 expression and function [8]. Despite all the efforts and scientific advances made so far, more studies are required for this technique to be fully implemented in clinical practice, and HSCT remains the most favorable curative treatment for patients presenting with IPEX and IPEX-like clinical phenotype.

References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40(1):24–64
2. Duclaux-Loras R, Charbit-Henrion F, Neven B, Nowak J, Collardeau-Frachon S, Malcus C et al (2018) Clinical heterogeneity of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: a French multicenter retrospective study. Clin Transl Gastroenterol 9(10)
3. Hwang JL, Park SY, Ye H, Sanyoura M, Pastore AN, Carmody D et al (2018) FOXP3 mutations causing early-onset insulin-requiring diabetes but without other features of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Pediatr Diabetes 19(3):388–392
4. Torgerson TR, Gambineri E, Ochs HD (2013) Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance. In: Ochs HD, Smith CIE, Puck JM (eds) Primary immunodeficiency diseases a molecular and genetic approach, 3rd edn. Oxford University Press, New York, pp 395–413

5. Bacchetta R, Barzaghi F, Roncarolo MG (2016) From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. Ann N Y Acad Sci 1417(1):5–22
6. Gambineri E, Mannurita SC, Hagin D, Vignoli M, Anover-Sombke S, DeBoer S et al (2018) Clinical, immunological, and molecular heterogeneity of 173 patients with the phenotype of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Front Immunol 9(NOV):2–5
7. Liu R, Li S, Yang W, Wang LIPEX (2013) Syndrome, FOXP3 and Cancer. J Syndr 1(1):1–15
8. Cepika AM, Sato Y, Liu JMH, Uyeda MJ, Bacchetta R, Roncarolo MG (2018) Tregopathies: monogenic diseases resulting in regulatory T-cell deficiency. J Allergy Clin Immunol 142(6):1679–1695
9. Li B, Samanta A, Song X, Iacono KT, Brennan P, Chatila TA et al (2007) FOXP3 is a homo-oligomer and a component of a supramolecular regulatory complex disabled in the human XLAAD/IPEX autoimmune disease. Int Immunol 19(7):825–835
10. Rudra D, Deroos P, Chaudhry A, Niec RE, Arvey A, Samstein RM et al (2012) Transcription factor Foxp3 and its protein partners form a complex regulatory network. Nat Immunol 13(10):1010–1019
11. Toubiana J, Okada S, Hiller J, Oleastro M, Gomez ML, Becerra JCA et al (2016) Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. Blood 127(25):3154–3164
12. Lopez-Herrera G, Tampella G, Pan-Hammarström Q, Herholz P, Trujillo-Vargas CM, Phadwal K et al (2012) Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet 90(6):986–1001
13. Powell BR, Buist NRM, Stenzel P (1982) An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. J Pediatr 100(5):731–737
14. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C et al (2000) JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. J Clin Invest 106(12):75–81
15. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L et al (2001) The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome [IPEX] is caused by mutations of FOXP3. Nat Genet 27(1):20–21
16. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N et al (2001) X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 27(1):18–20
17. Russell WL, Russell LB, Gower JS (1959) Exceptional inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex-chromosome constitution is female. Proc Natl Acad Sci 45(4):554–560
18. Godfrey VL, Wilkinson JE (1991) Russell LB. X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. Am J Pathol 138(6):1379–1387
19. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA et al (2001) Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 27(1):68–73
20. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133(5):775–787
21. Lam EWF, Brosens JJ, Gomes AR, Koo CY (2013) Forkhead box proteins: tuning forks for transcriptional harmony. Nat Rev Cancer 13(7):482–495
22. Lopes JE, Torgerson TR, Schubert LA, Anover SD, Ocheltree EL, Ochs HD et al (2006) Analysis of FOXP3 reveals multiple domains required for its function as a transcriptional repressor. J Immunol 177(5):3133–3142
23. Allan SE, Passerini L, Bacchetta R, Crellin N, Dai M, Orban PC et al (2005) The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. J Clin Invest 115(11):3276–3284
24. Smith EL, Finney HM, Nesbitt AM, Ramsdell F, Robinson MK (2006) Splice variants of human FOXP3 are functional inhibitors of human CD4 + T-cell activation. Immunology 119(2):203–211

25. Joly AL, Seitz C, Liu S, Kuznetsov NV, Gertow K, Westerberg LS et al (2018) Alternative splicing of FoxP3 controls regulatory T cell effector functions and is associated with human atherosclerotic plaque stability. Circ Res 122(10):1385–1394
26. Magg T, Mannert J, Ellwart JW, Schmid I, Albert MH (2012) Subcellular localization of FOXP3 in human regulatory and nonregulatory T cells. Eur J Immunol 42(6):1627–1638
27. Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4(4):330–336
28. Marson A, Kretschmer K, Frampton GM, Jacobsen ES, Polansky JK, MacIsaac KD et al (2007) Foxp3 occupancy and regulation of key target genes during T-cell stimulation. Nature 445(7130):931–935
29. Zheng Y, Josefowicz SZ, Kas A, Chu T-T, Gavin MA, Rudensky AY (2007) Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. Nature 445(7130):936–940
30. Wu Y, Borde M, Heissmeyer V, Feuerer M, Lapan AD, Stroud JC et al (2006) FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell 126(2):375–387
31. Nagai Y, Lam L, Greene MI, Zhang H (2019) FOXP3 and its cofactors as targets of immunotherapies. Engineering 5(1):115–121
32. Deng G, Song X, Fujimoto S, Piccirillo CA, Nagai Y, Greene MI (2019) Foxp3 post-translational modifications and treg suppressive activity. Front Immunol 10(OCT):2486
33. Chen C, Rowell EA, Thomas RM, Hancock WW, Wells AD (2006) Transcriptional regulation by Foxp3 is associated with direct promoter occupancy and modulation of histone acetylation. J Biol Chem 281(48):36828–36834
34. Barzaghi F, Amaya Hernandez LC, Neven B, Ricci S, Kucuk ZY, Bleesing JJ et al (2018) Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: an international multicenter retrospective study. J Allergy Clin Immunol 141(3):1036–1049.e5
35. Zama D, Cocchi I, Masetti R, Specchia F, Alvisi P, Gambineri E et al (2014) Late-onset of immunodysregulation, polyendocrinopathy, enteropathy, x-linked syndrome [IPEX] with intractable diarrhea. Ital J Pediatr 40(1):1–7
36. Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299(5609):1057–1061
37. Minami Y, Kono T, Miyazaki T, Taniguchi T (1993) The IL-2 receptor complex: its structure, function, and target genes. Annu Rev Immunol 11:245–268
38. Lin JX, Leonard WJ (2000) The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. Oncogene 19(21):2566–2576
39. O'Gorman WE, Dooms H, Thorne SH, Kuswanto WF, Simonds EF, Krutzik PO et al (2009) The initial phase of an immune response functions to activate regulatory T cells. J Immunol 183(1):332–339
40. Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D et al (2006) IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. Blood 108(5):1571–1579
41. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 155([3]):1151–1164
42. Refaeli Y, Van Parijs L, London CA, Tschopp J, Abbas AK (1998) Biochemical mechanisms of IL-2–regulated Fas-mediated T cell apoptosis. Immunity 8(5):615–623
43. Sharfe N, Dadi HK, Shahar M, Roifman CM (1997) Human immune disorder arising from mutation of the α chain of the interleukin-2 receptor. Proc Natl Acad Sci 94(7):3168–3171
44. Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW (2007) CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. J Allergy Clin Immunol 119(2):482–487
45. Goudy K, Aydin D, Barzaghi F, Gambineri E, Vignoli M, Mannurita SC et al (2013) Human IL2RA null mutation mediates immunodeficiency with lymphoproliferation and autoimmunity. Clin Immunol 146(3):248–261

46. Bezrodnik L, Caldirola MS, Seminario AG, Moreira I, Gaillard MI (2014) Follicular bronchiolitis as phenotype associated with CD25 deficiency. Clin Exp Immunol 175(2):227–234
47. Vignoli M, Ciullini Mannurita S, Fioravanti A, Tumino M, Grassi A, Guariso G et al (2019) CD25 deficiency: a new conformational mutation prevents the receptor expression on cell surface. Clin Immunol 201(January):15–19
48. Lorenzini T, Dotta L, Giacomelli M, Vairo D, Badolato R (2017) STAT mutations as program switchers: turning primary immunodeficiencies into autoimmune diseases. J Leukoc Biol 101(1):29–38
49. Kanai T, Jenks J, Nadeau KC (2012) The STAT5b pathway defect and autoimmunity. Front Immunol 3:234
50. Nadeau K, Hwa V, Rosenfeld RG (2011) STAT5b deficiency: an unsuspected cause of growth failure, immunodeficiency, and severe pulmonary disease. J Pediatr 158(5):701–708
51. Ambrosio R, Fimiani G, Monfregola J, Sanzari E, De Felice N, Salerno MC et al (2002) The structure of human STAT5A and B genes reveals two regions of nearly identical sequence and an alternative tissue specific STAT5B promoter. Gene 285(1–2):311–318
52. Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J et al (2003) Growth hormone insensitivity associated with a STAT5b mutation. N Engl J Med 349(12):1139–1147
53. Hwa V (2016) STAT5B deficiency: impacts on human growth and immunity. Growth Horm IGF Res Off J Growth Horm Res Soc Int IGF Res Soc 28:16–20
54. Hwa V, Little B, Adiyaman P, Kofoed EM, Pratt KL, Ocal G et al (2005) Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. J Clin Endocrinol Metab 90(7):4260–4266
55. Krummel MF, Allison JP (1996) CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med 183(6):2533–2540
56. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z et al (2008) CTLA-4 control over Foxp3+ regulatory T cell function. Science 322(5899):271–275
57. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N et al (2000) Immunologic self-tolerance maintained by CD25+ CD4+ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 192(2):303–310
58. Krummel MF, Allison JP (2011) Pillars article: CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. The journal of experimental medicine. 1995. 182: 459-465. J Immunol 187(7):3459–3465
59. Carreno BM, Collins M (2002) The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. Annu Rev Immunol 20:29–53
60. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA (1991) CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med 174(3):561–569
61. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R (1994) Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity 1(9):793–801
62. Schwartz J-CD, Zhang X, Fedorov AA, Nathenson SG, Almo SC (2001) Structural basis for co-stimulation by the human CTLA-4/B7-2 complex. Nature 410(6828):604–608
63. Stamper CC, Zhang Y, Tobin JF, Erbe DV, Ikemizu S, Davis SJ et al (2001) Crystal structure of the B7-1/CTLA-4 complex that inhibits human immune responses. Nature 410(6828):608–611
64. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM et al (2011) Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science 332(6029):600–603
65. Sansom DM (2015) Moving CTLA-4 from the trash to recycling. Science 349(6246):377–378
66. Walker LSK, Sansom DM (2015) Confusing signals: recent progress in CTLA-4 biology. Trends Immunol 36(2):63–70
67. Masteller EL, Chuang E, Mullen AC, Reiner SL, Thompson CB (2000) Structural analysis of CTLA-4 function in vivo. J Immunol 164(10):5319–5327

68. Dariavach P, Mattéi MG, Golstein P, Lefranc MP (1988) Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. Eur J Immunol 18(12):1901–1905
69. Ling V, Wu PW, Finnerty HF, Sharpe AH, Gray GS, Collins M (1999) Complete sequence determination of the mouse and human CTLA4 gene loci: cross-species DNA sequence similarity beyond exon borders. Genomics 60(3):341–355
70. Teft WA, Kirchhof MG, Madrenas J (2006) A molecular perspective of CTLA-4 function. Annu Rev Immunol 24:65–97
71. Gibson HM, Hedgcock CJ, Aufiero BM, Wilson AJ, Hafner MS, Tsokos GC et al (2007) Induction of the CTLA-4 gene in human lymphocytes is dependent on NFAT binding the proximal promoter. J Immunol 179(6):3831–3840
72. Mitsuiki N, Schwab C, Grimbacher B (2019) What did we learn from CTLA-4 insufficiency on the human immune system? Immunol Rev 287(1):33–49
73. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP et al (1995) Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 270(5238):985–988
74. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH (1995) Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3(5):541–547
75. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT et al (2014) Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 345(6204):1623–1627
76. Schwab C, Gabrysch A, Olbrich P, Patiño V, Warnatz K, Wolff D et al (2018) Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4-insufficient subjects. J Allergy Clin Immunol 142(6):1932–1946
77. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A et al (2014) Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 20(12):1410–1416
78. Hou TZ, Olbrich P, Soto JML, Sanchez B, Moreno PS, Borte S et al (2018) Study of an extended family with CTLA-4 deficiency suggests a CD28/CTLA-4 independent mechanism responsible for differences in disease manifestations and severity. Clin Immunol 188:94–102
79. Ueda H, Howson JMM, Esposito L, Heward J, Snook CG et al (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 423(6939):506–511
80. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z et al (2016) Spectrum of phenotypes associated with mutations in LRBA. J Clin Immunol 36(1):33–45
81. Wang J-W, Gamsby JJ, Highfill SL, Mora LB, Bloom GC, Yeatman TJ et al (2004) Deregulated expression of LRBA facilitates cancer cell growth. Oncogene 23(23):4089–4097
82. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C et al (2015) Autoimmune disease. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science 349(6246):436–440
83. Wang JW, Howson J, Haller E, Kerr WG (2001) Identification of a novel lipopolysaccharide-inducible gene with key features of both A kinase anchor proteins and chs1/beige proteins. J Immunol 166(7):4586–4595
84. Habibi S, Zaki-Dizaji M, Rafiemanesh H, Lo B, Jamee M, Gámez-Díaz L et al (2019) Clinical, immunologic, and molecular spectrum of patients with LPS-responsive beige-like anchor protein deficiency: a systematic review. J Allergy Clin Immunol Pract 7(7):2379–2386.e5
85. Gámez-Díaz L, August D, Stepensky P, Revel-Vilk S, Seidel MG, Noriko M et al (2016) The extended phenotype of LPS-responsive beige-like anchor protein [LRBA] deficiency. J Allergy Clin Immunol 137(1):223–230
86. Burnett DL, Parish IA, Masle-Farquhar E, Brink R, Goodnow CC (2017) Murine LRBA deficiency causes CTLA-4 deficiency in Tregs without progression to immune dysregulation. Immunol Cell Biol 95(9):775–788

87. Gámez-Díaz L, Neumann J, Jäger F, Proietti M, Felber F, Soulas-Sprauel P et al (2017) Immunological phenotype of the murine Lrba knockout. Immunol Cell Biol 95(9):789–802
88. Uzel G, Sampaio EP, Lawrence MG, Hsu AP, Hackett M, Dorsey MJ et al (2013) Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. J Allergy Clin Immunol 131(6):1611–1623.e3
89. van de Veerdonk FL, Plantinga TS, Hoischen A, Smeekens SP, Joosten LAB, Gilissen C et al (2011) STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N Engl J Med 365(1):54–61
90. Liu L, Okada S, Kong X-F, Kreins AY, Cypowyj S, Abhyankar A et al (2011) Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. J Exp Med 208(8):1635–1648
91. Eren Akarcan S, Ulusoy Severcan E, Edeer Karaca N, Isik E, Aksu G, Migaud M et al (2017) Gain-of-function mutations in STAT1: a recently defined cause for chronic mucocutaneous candidiasis disease mimicking combined immunodeficiencies. Case Rep Immunol 2017:2846928
92. Depner M, Fuchs S, Raabe J, Frede N, Glocker C, Doffinger R et al (2016) The extended clinical phenotype of 26 patients with chronic mucocutaneous candidiasis due to gain-of-function mutations in STAT1. J Clin Immunol 36(1):73–84
93. Tabellini G, Vairo D, Scomodon O, Tamassia N, Ferraro RM, Patrizi O et al (2017) Impaired natural killer cell functions in patients with signal transducer and activator of transcription 1 [STAT1] gain-of-function mutations. J Allergy Clin Immunol 140(2):553–564.e4
94. Bernasconi AR, Yancoski J, Villa M, Oleastro MM, Galicchio M, Rossi JG (2018) Increased STAT1 amounts correlate with the phospho-STAT1 level in STAT1 gain-of-function defects. J Clin Immunol 38:745–747
95. Zimmerman O, Olbrich P, Freeman AF, Rosen LB, Uzel G, Zerbe CS et al (2019) STAT1 gain-of-function mutations cause high total STAT1 levels with normal dephosphorylation. Front Immunol 10:1433
96. Delgoffe GM (2013) Vignali DAA. STAT heterodimers in immunity: a mixed message or a unique signal? JAK-STAT 2(1):e23060–e23060
97. Milner JD, Vogel TP, Forbes L, Ma CA, Stray-Pedersen A, Niemela JE et al (2015) Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. Blood 125(4):591–599
98. Todaro F, Tamassia N, Pinelli M, Moratto D, Dotta L, Grassi A et al (2019) Multisystem autoimmune disease caused by increased STAT3 phosphorylation and dysregulated gene expression. Haematologica 104:e322–e325
99. Haapaniemi EM, Kaustio M, Rajala HLM, van Adrichem AJ, Kainulainen L, Glumoff V et al (2015) Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. Blood 125(4):639–648
100. Sediva H, Dusatkova P, Kanderova V, Obermannova B, Kayserova J, Sramkova L et al (2017) Short stature in a boy with multiple early-onset autoimmune conditions due to a STAT3 activating mutation: could intracellular growth hormone signalling be compromised? Horm Res Paediatr 88:160–166
101. Mauracher AA, Eekels JJM, Woytschak J, van Drogen A, Bosch A, Prader S et al (2020) Erythropoiesis defect observed in STAT3 GOF patients with severe anemia. J Allergy Clin Immunol 145(4):1297–1301
102. Fabre A, Marchal S, Barlogis V, Mari B, Barbry P, Rohrlich P-S et al (2019) Clinical aspects of STAT3 gain-of-function germline mutations: a systematic review. J Allergy Clin Immunol Pract 7(6):1958–1969.e9
103. Zhang Z, Gothe F, Pennamen P, James JR, McDonald D, Mata CP et al (2019) Human interleukin-2 receptor β mutations associated with defects in immunity and peripheral tolerance. J Exp Med 216(6):1311–1327

104. Fernandez IZ, Baxter RM, Garcia-Perez JE, Vendrame E, Ranganath T, Kong DS et al (2019) A novel human IL2RB mutation results in T and NK cell-driven immune dysregulation. J Exp Med 216(6):1255–1267
105. Alroqi FJ, Charbonnier L-M, Keles S, Ghandour F, Mouawad P, Sabouneh R et al (2017) DOCK8 deficiency presenting as an IPEX-like disorder. J Clin Immunol 37(8):811–819
106. Kearney CJ, Randall KL, Oliaro J (2017) DOCK8 regulates signal transduction events to control immunity. Cell Mol Immunol 14(5):406–411
107. Keles S, Charbonnier LM, Kabaleeswaran V, Reisli I, Genel F, Gulez N et al (2016) Dedicator of cytokinesis 8 regulates signal transducer and activator of transcription 3 activation and promotes TH17 cell differentiation. J Allergy Clin Immunol 138(5):1384–1394.e2
108. Tangye SG, Pillay B, Randall KL, Avery DT, Phan TG, Gray P et al (2017) Dedicator of cytokinesis 8-deficient CD4+ T cells are biased to a TH2 effector fate at the expense of TH1 and TH17 cells. J Allergy Clin Immunol 139(3):933–949
109. Jabara HH, McDonald DR, Janssen E, Massaad MJ, Ramesh N, Borzutzky A et al (2012) DOCK8 functions as an adaptor that links TLR-MyD88 signaling to B cell activation. Nat Immunol 13(6):612–620
110. Sun X, Wang J, Qin T, Zhang Y, Huang L, Niu L et al (2018) Dock8 regulates BCR signaling and activation of memory B cells via WASP and CD19. Blood Adv 2(4):401–413
111. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ et al (2009) Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med 361(21):2046–2055
112. Engelhardt KR, McGhee S, Winkler S, Sassi A, Woellner C, Lopez-Herrera G et al (2009) Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J Allergy Clin Immunol 124(6):1289–302.e4
113. Su HC, Jing H, Angelus P, Freeman AF (2019) Insights into immunity from clinical and basic science studies of DOCK8 immunodeficiency syndrome. Immunol Rev 287(1):9–19
114. Tangye SG, Bucciol G, Casas-Martin J, Pillay B, Ma CS, Moens L et al (2019) Human inborn errors of the actin cytoskeleton affecting immunity: way beyond WAS and WIP. Immunol Cell Biol 97(4):389–402
115. Nunes-Santos CJ, Uzel G, Rosenzweig SD (2019) PI3K pathway defects leading to immunodeficiency and immune dysregulation. J Allergy Clin Immunol 143(5):1676–1687
116. Okkenhaug K (2013) Signaling by the phosphoinositide 3-kinase family in immune cells. Annu Rev Immunol 31:675–704
117. Preite S, Gomez-Rodriguez J, Cannons JL, Schwartzberg PL (2019) T and B-cell signaling in activated PI3K delta syndrome: from immunodeficiency to autoimmunity. Immunol Rev 291(1):154–173
118. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR et al (2013) Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. Science 342(6160):866–871
119. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U et al (2014) Dominant-activating germline mutations in the gene encoding the PI[3]K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. Nat Immunol 15(1):88–97
120. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L et al (2014) Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. J Clin Immunol 34([3]):272–276
121. Kracker S, Curtis J, Ibrahim MAA, Sediva A, Salisbury J, Campr V et al (2014) Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase δ syndrome. J Allergy Clin Immunol 134:233–236
122. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N et al (2017) Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. J Allergy Clin Immunol 13(2):597–606.e4
123. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J et al (2014) Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. J Exp Med 211(13):2537–2547

124. Deau M-C, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P et al (2014) A human immunodeficiency caused by mutations in the PIK3R1 gene. J Clin Invest 124(9):3923–3928
125. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Schölvinck L, Kalina T et al (2017) Genetic defects in PI3Kδ affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. Clin Immunol 176:77–86
126. Jardine S, Dhingani N, Muise AM (2019) TTC7A: Steward of intestinal health. Cell Mol Gastroenterol Hepatol 7(3):555–570
127. Chen R, Giliani S, Lanzi G, Mias GI, Lonardi S, Dobbs K et al (2013) Whole-exome sequencing identifies tetratricopeptide repeat domain 7A (TTC7A) mutations for combined immunodeficiency with intestinal atresias. J Allergy Clin Immunol 132(3):656–664.e17
128. Avitzur Y, Guo C, Mastropaolo LA, Bahrami E, Chen H, Zhao Z et al (2014) Mutations in tetratricopeptide repeat domain 7A result in a severe form of very early onset inflammatory bowel disease. Gastroenterology 146(4):1028–1039
129. Nakatsu F, Baskin JM, Chung J, Tanner LB, Shui G, Lee SY et al (2012) PtdIns4P synthesis by PI4KIIIα at the plasma membrane and its impact on plasma membrane identity. J Cell Biol 199(6):1003–1016
130. Bigorgne AE, Farin HF, Lemoine R, Mahlaoui N, Lambert N, Gil M et al (2014) TTC7A mutations disrupt intestinal epithelial apicobasal polarity. J Clin Invest 124(1):328–337
131. Samuels ME, Majewski J, Alirezaie N, Fernandez I, Casals F, Patey N et al (2013) Exome sequencing identifies mutations in the gene TTC7A in French-Canadian cases with hereditary multiple intestinal atresia. J Med Genet 50(5):324–329
132. Lemoine R, Pachlopnik-Schmid J, Farin HF, Bigorgne A, Debré M, Sepulveda F et al (2014) Immune deficiency-related enteropathy-lymphocytopenia-alopecia syndrome results from tetratricopeptide repeat domain 7A deficiency. J Allergy Clin Immunol 134(6):1354–1364.e6
133. Lien R, Lin Y-F, Lai M-W, Weng H-Y, Wu R-C, Jaing T-H et al (2017) Novel mutations of the tetratricopeptide repeat domain 7A gene and phenotype/genotype comparison. Front Immunol 8:1066
134. Notarangelo LD (2014) Multiple intestinal atresia with combined immune deficiency. Curr Opin Pediatr 26(6):690–696
135. Leveau C, Gajardo T, El-Daher M-T, Cagnard N, Fischer A, de Saint Basile G et al (2020) Ttc7a regulates hematopoietic stem cell functions while controlling the stress-induced response. Haematologica 105(1):59–70
136. Morton DJ, Kuiper EG, Jones SK, Leung SW, Corbett AH, Fasken MB (2018) The RNA exosome and RNA exosome-linked disease. RNA 24(2):127–142
137. Hartley JL, Zachos NC, Dawood B, Donowitz M, Forman J, Pollitt RJ et al (2010) Mutations in TTC37 cause trichohepatoenteric syndrome (phenotypic diarrhea of infancy). Gastroenterology 138(7):2388–2398, 2398.e1-2
138. Fabre A, Charroux B, Martinez-Vinson C, Roquelaure B, Odul E, Sayar E et al (2012) SKIV2L mutations cause syndromic diarrhea, or trichohepatoenteric syndrome. Am J Hum Genet 90(4):689–692
139. Vély F, Barlogis V, Marinier E, Coste M-E, Dubern B, Dugelay E et al (2018) Combined immunodeficiency in patients with trichohepatoenteric syndrome. Front Immunol 9:1036
140. Rider NL, Boisson B, Jyonouchi S, Hanson EP, Rosenzweig SD, Cassanova J-L et al (2015) Novel TTC37 mutations in a patient with immunodeficiency without diarrhea: extending the phenotype of trichohepatoenteric syndrome. Front Pediatr 3:2
141. Ohnuma K, Kishita Y, Nyuzuki H, Kohda M, Ohtsu Y, Takeo S et al (2020) Ski3/TTC37 deficiency associated with trichohepatoenteric syndrome causes mitochondrial dysfunction in Drosophila. FEBS Lett 594(13):2168–2181
142. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W et al (2018) The 2017 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol 38(1):129–143
143. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T et al (2020) Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. J Clin Immunol 40(1):66–81

144. Lin Y, Xu A, Zeng C, Cheng J, Li N, Niu H et al (2018) Somatic and germline FOXP3 mosaicism in the mother of a boy with IPEX syndrome. Eur J Immunol 48:885–887
145. Chan AY, Leiding JW, Liu X, Logan BR, Burroughs LM, Allenspach EJ et al (2020) Hematopoietic cell transplantation in patients with primary immune regulatory disorders [PIRD]: a Primary Immune Deficiency Treatment Consortium (PIDTC) survey. Front Immunol 11:239

Autoinflammatory Disorders

11

Mark Kacar and Sinisa Savic

Abstract

Autoinflammatory disorders represent an evolving group of conditions which were first mentioned as a separate disease category in 1999, after the genetic basis for tumor necrosis factor (TNF) receptor-associated periodic fever syndrome was identified. Since then, the boundaries of what defines an autoinflammatory condition continue to expand and include diseases which to a varying degree have features of immunodeficiency and classical autoimmunity. There has been considerable progress in mapping the genetic basis of these disorders and understanding the relevant biological pathways responsible for the inflammatory pathology. A targeted therapeutic approach using modern biologicals has in many cases led to significant improvements in disease management and outcomes. According to the latest classification from the International Union of Immunological Societies, there are almost 40 diseases being classed as autoinflammatory. It is beyond the scope of this chapter to describe each condition in detail. Instead, it will shed light on the more common, prototypic, and well-understood conditions which help elucidate autoinflammatory disease pathways in general.

Keywords

Autoinflammation · Pyrin · NLRP3 · TRAPS · Inflammasomopathies · Relopathies Interferonopathies

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11.1 Introduction

The autoinflammatory disorders (AID) comprise a heterogenous family of conditions characterized by varying degrees of pathological innate immune activity. The term initially covered only a handful of well-defined, monogenic conditions such as familial Mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic fever syndrome (TRAPS). Though barely out of its teens, this family of disorders has now grown to include nearly 40 monogenic conditions. In addition, the pathological processes associated with AID have been found to play a role in a wide range of more common conditions ranging from Alzheimer's disease to rheumatoid arthritis and others. Even the initial division between innate and adaptive has become less clear with time with conditions such as the interferonopathies, considered to be AIDs by many experts in the field, manifesting hallmarks of disordered innate and adaptive immunity simultaneously.

Typically, all AIDs are characterized by increased production and release of inflammatory mediators, although the exact intracellular pathway and the factors involved in triggering it vary between conditions. The excessive release of proinflammatory cytokines, whether interleukin-1 (IL-1) or tumor necrosis factor (TNF), interleukin-18 (IL-18), or interferon γ (IFN-γ), culminates in systemic and/or localized inflammation and can arise due to aberrations at practically every step of the transduction of inflammatory signals. Mutations in cell membrane receptors (as in TRAPS), intracellular machinery (as in FMF), transcription factors (as in the relopathies), and modifying enzymes involved in a wider array of cellular functions (as in the haploinsufficiency of A20) have all lead to an autoinflammatory phenotype.

Treatment options for the first of the AID were limited, nonspecific, and potentially toxic. However, with the advent of targeted biologic therapies, the outlook for patients suffering from AID has improved beyond recognition.

There have been many attempts to classify AID. For example, a recent article by Gul et al. attempted to divide the autoinflammatory disorders into "auto-"and "hyperinflammatory" based on whether initiation of the inflammatory cascade is spontaneous or triggered (by pathogens, physical, chemical, and other noxa) [1]. A more typical classification method relies on the mechanisms involved in the generation, propagation, and perpetuation of the inflammatory cascade. The following chapter will be based on the latter system of classification as it tends to group conditions more closely both by symptoms and therapeutic options available for management. The names used to describe autoinflammatory disorders will reflect the recommendations of a recent consensus paper on AID nomenclature, where available [2]. It is beyond the scope of this chapter to describe all AID in great detail. The focus will be given to relatively common disorders or conditions where the pathogenesis is better understood. Where relevant, the reader will be directed to additional literature (marked with *** and the appropriate citation number).

11.1.1 Inflammasomopathies

Inflammasomopathies are a group of disorders characterized by abnormal functioning of inflammasomes (Table 11.1), the intracellular complexes involved in caspase-driven production or processing of inflammatory cytokines. Regardless of whether the inflammasome functions as a pathogen pattern receptor (e.g., NAIP for T3SS) [3], a decoy for virulence factors (NLRP1b for *B. anthracis*) [4], or a receptor of cellular homeostasis (NALP3 for K+ ions) [5], the end results of inflammasome activation are dependent on the activation of caspases and further cleavage and activation of pro-inflammatory cytokines.

11.1.1.1 Pyrin-Associated Autoinflammatory Diseases (PAAD)

The discovery in 1997 of the *MEFV* (MEditerranean FeVer) gene encoding the protein pyrin is one of the definitive events in the history of autoinflammatory disease research [6, 7]. Pyrin is predominantly expressed in the cells of the innate immune system (neutrophils, monocytes, and dendritic cells) and also in synovial, pleural, dermal, and peritoneal fibroblasts, which in part explains some of the clinical manifestations of PAAD. The pyrin inflammasome acts as a sensor of RhoA GTPase impairment, a critical step in the recognition of pathogens such as *Yersinia pestis* and *C. difficile* [8]. The sensing of RhoA GTPase impairment caused by the effects of microbial toxins results in activation of the pyrin inflammasome and ultimately caspase-1-driven cleavage of pro-IL-1β to the active form, IL-1β (Fig. 11.1). Though the *MEFV* gene has been known about for over 20 years, pyrin's function has only recently been elucidated, and novel conditions stemming from its malfunction are still being described.

Familial Mediterranean Fever (FMF)

The majority of mutations associated with FMF are typically found in exon 10 of *MEFV* coding for the B30.2 domain of pyrin. Though the mode of inheritance is generally autosomal recessive, the mutations are almost all gain-of-function missense substitutions, leading to a lowered threshold of pyrin inflammasome activation. Recent reports of somatic mutations add further weight to this theory [10]. Though prevalence of FMF is highest in countries abutting the Mediterranean basin, it is not limited to this region and cases have been reported worldwide.

The disease presents by the age of 10 or 20 years in 65% and 90% of individuals, respectively. It is characterized by recurrent episodes of fever, serositis, arthritis, and/or rash lasting between 12 and 72 hours, interspersed by disease-free intervals lasting from weeks to years. Various triggers for attacks have been reported, including cold exposure, surgery, vigorous exercise, and menstruation.

Table 11.1 Inflammasomopathies

General pathway		Disease	Gene	Affected protein/ function	Mode of inheritance	Age of onset	Key clinical features	Fever pattern and duration	Treatment
Inflamma-somopa-thies	Pyrinopa-thies	Familial Mediterranean fever	*MEFV*	Pyrin	AR/AD	Infancy to adulthood	Peritonitis, arthralgia, arthritis, erysipeloid rash, and amyloidosis	Periodic, 24–72 h	Colchicine/anti-IL-1
		PAAND	*MEFV*	Pyrin	AD	Childhood	Neutrophilic dermatosis (pyoderma gangrenosum), arthralgia, and myalgia	Periodic, several weeks	Anti-IL-1/anti-TNF
		MKD	*MVK*	Mevalonate kinase	AR	Childhood	Lymphadenopathy, abdominal pain, joint pain, diarrhea, skin rashes, and headache, rarely amyloidosis	Random, 3–7 d	NSAIDs/corticosteroids/ anti-IL-1/anti-TNF/ anti-IL-6/HSCT
		PAPA*** [9]	*PSTPIP1*	PSTPIP-1/CD2 binding protein-1	AD	Childhood	Destructive pyogenic arthritis, skin ulcers, pyoderma gangrenosum, and cystic acne	/	Corticosteroids/anti-IL-1, anti-TNF
	NLRP3-AID	*Mild* (FCAS1)	*NLRP3*	NLRP3/NALP3	AD[a]	Infancy to adulthood	Cold-induced neutrophilic urticaria, conjunctivitis, general malaise, headache, arthralgia, and myalgia	Triggered, 30 min–72 h	Anti-IL-1
		Moderate (MWS)	*NLRP3*	NLRP3/NALP3	AD[a]	Infancy to adulthood	As above +/− episcleritis, hearing loss, meningitis, and oligoarthritis	Continuous with flares or triggered, 24–48 h	Anti-IL-1
		Severe (NOMID/ CINCA)	*NLRP3*	NLRP3/NALP3	AD[a]	Birth	As above + progressive cognitive impairment	Continuous with exacerbations	Anti-IL-1
		NLRP12-AID (FCAS2) *** [51]	*NLRP12*	Monarch-1 protein	AD	Childhood	Cold urticaria, arthralgia, myalgia, lymphadenopathy, oral ulcers, and abdominal pain	Triggered by cold, 12 h–2 wks	IL-1 blockade/ corticosteroids
		NLRC4-AID	*NLRC4*	NLRC4	AD	Birth	Infantile enterocolitis, macrophage activation syndrome, and *NLRP3*-AID-like symptoms	Triggered, variable	Anti-IL-1/anti-IL18
		NLRP1-AID*** [52]	*NLRP1*	NLRP1	AD	Infancy	Conjunctival, corneal, and laryngeal dyskeratosis, arthritis, chronic infections, and autoimmunity	Random, 1–7 d	Acitretin and anti-IL-1

AR autosomal recessive, *AD* autosomal dominant, *HSCT* hematopoietic stem cell transplantation

[a]Sporadic cases reported

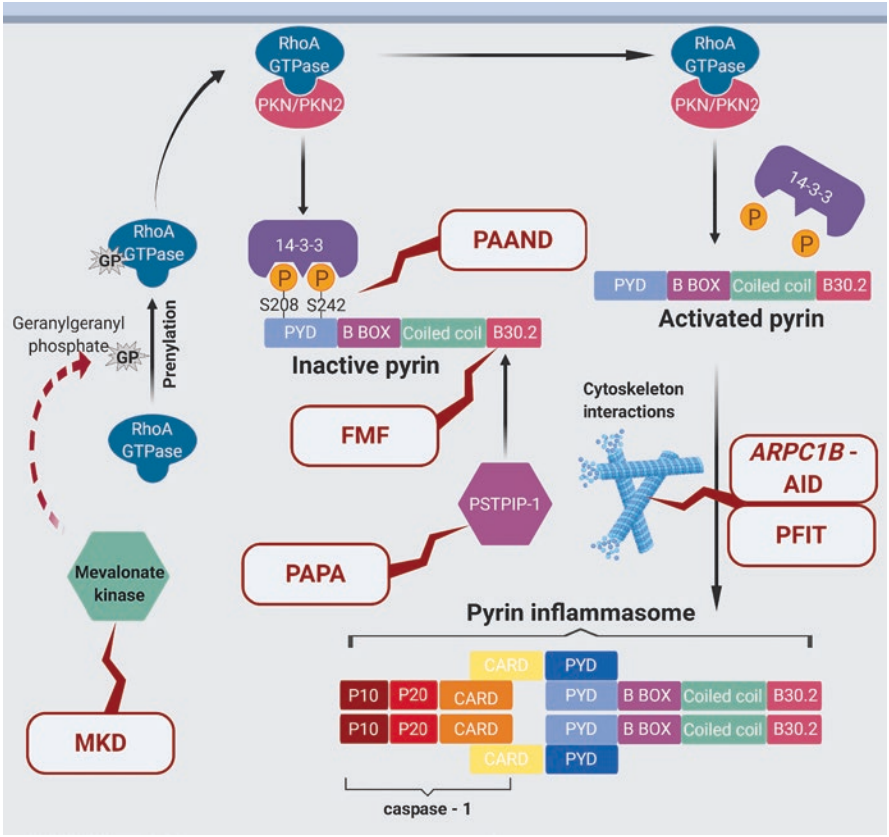

Fig. 11.1 The pyrin inflammasome and associated pathways. RhoA GTPase prenylation is crucial for enzyme membrane tethering and function. The 14-3-3 inhibitory protein binds to phosphorylated serine residues at positions 208 and 242; amino acid substitution results in constitutive inflammation. Mutations affecting the B30.2 domain result in a lowered threshold for inflammasome activation. Pyrin senses bacterial modification of RhoA GTPase, resulting in pyrin inflammasome assembly requiring cytoskeletal interactions. *MKD* mevalonate kinase deficiency, *GP* geranylgeranyl phosphate, *PKN* protein kinase, *PAAND* pyrin-associated autoinflammation with neutrophilic dermatosis, *FMF* familial Mediterranean fever, *PAPA* pyogenic arthritis, pyoderma gangrenosum, and acne*** [9]; PFIT, periodic fevers, immunodeficiency, and thrombocytopenia

Uncontrolled chronic inflammation can lead to end-organ damage due to amyloid deposition, most commonly affecting (but not limited to) the kidneys. A recent study from Turkey showed that patients with FMF have increased frequency of other inflammatory conditions, including ankylosing spondylitis, Henoch-Schönlein purpura, juvenile idiopathic arthritis, polyarteritis nodosa, multiple sclerosis, and Behçet's disease, when compared to the general population [11]. The diagnosis of FMF is a clinical one, using defined criteria and supported by genetic testing [12].

The first-line treatment of FMF has been colchicine since 1972 [13]. This has dramatically improved the outlook for patients with FMF and led to significant

reduction of amyloidosis [14, 15]. Patients who cannot tolerate colchicine, or more rarely have colchicine-resistant (cr) FMF, tend to respond well to the IL-1 receptor antagonist analogue anakinra [16] or the IL-1β-specific antibody canakinumab [17].

Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND)

Another condition arising from mutations in *MEFV* but manifesting an entirely different phenotype is PAAND. The dominantly inherited mutations associated with PAAND (p.S242R, p.S208T, p.S208C, and E244K) disrupt pyrin phosphorylation at these sites and therefore prevent binding of the inhibitory protein 14-3-3 (Fig. 11.1). This leads to constitutive activation of the pyrin inflammasome, resulting in a more severe phenotype than FMF, characterized by childhood-onset recurrent episodes of fever, neutrophilic dermatosis, arthralgias, and myalgias [18–20]. Treatment of PAAND is complex and response to therapy more difficult to predict. Anakinra has been used successfully in two patients, resulting in symptomatic improvement and normalization of CRP. However, positive response to anti-IL-1 inhibition is not universal, and in some selected cases, anti-TNF was shown to be more effective [18, 19].

Mevalonate Kinase Deficiency (MKD)

MKD belongs to a subset of conditions resulting in pyrin inflammasome activation stemming from errors in pyrin-associated pathways, but not mutations in the *MEFV* gene itself. Mevalonate kinase is an enzyme involved in the synthesis of isoprenoids required in numerous intracellular processes including geranylgeranylation of proteins, allowing for their tethering in the plasma membrane, as well as modulating enzymatic activities throughout the cell. Consequently, in MKD, there is reduction in geranylgeranyl pyrophosphate level and isoprenylation of RhoA and Rab GTPases resulting in their impaired function and enhanced activation of the pyrin inflammasome [21] (Fig. 11.1).

Autosomal recessive loss of function can be partial or complete, resulting in a broad disease spectrum with health and MKD at one end and mevalonic aciduria (MA) and *in utero* death at the other [22].

MKD is characterized by fever, abdominal pain, vomiting, diarrhea, hepatosplenomegaly, lymphadenopathy, maculopapular rash, periorbital erythema, arthralgias, and mucosal ulcers. It is believed to be the result of reduced mevalonate kinase activity ranging from 1.8% to 28% of normal, most commonly due to mutations affecting protein stability. MA, on the other hand, arises when MK activity is below 0.5% of normal [23, 24]. In addition to the inflammatory manifestations associated with MKD, MA is associated with dysmorphic facial features, growth retardation, neurological, and ocular abnormalities [25].

Diagnosis of MKD is based on genetic tests showing biallelic mutations in the *MKD* gene. If variants of unknown significance are found, MK enzymatic activity in leukocytes can be measured [23]. Urinary mevalonic acid levels are massively elevated in MA but can be normal or only mildly elevated during acute attacks of MKD.

The goal of therapy is to prevent, abort, or alleviate acute attacks and to normalize levels of inflammatory markers to prevent the development of systemic amyloidosis. The latter complication is far rarer in MKD than FMF. First-line therapy for acute MKD attacks consists of nonsteroidal anti-inflammatory drugs (NSAIDs) [26]. However additional biological therapies are frequently required for better disease control. Anti-IL-1 biologics, such as anakinra and canakinumab, are both effective, with the latter now being licensed for this indication [27–30]. Anti-TNF and, in some cases, anti-IL-6 biologics are also effective in selected cases [31–33]. More severe phenotypes might benefit from haematopoietic stem cell transplantation (HSCT) which corrects the inflammatory as well as the neurological phenotype [34, 35].

11.1.1.2 NLRP3-Associated Autoinflammatory Disease (NLRP3-AID)

NACHT, LRR, and PYD domain-containing protein 3 (NALP3/NLRP3), coded for by the *NLRP3* gene on chromosome 1q44, functions as a sensor for cellular damage and pathogens [36]. Detection of damage- or pathogen-associated molecular patterns (DAMP or PAMP, respectively) results in the assembly of the multimeric inflammasome complex which culminates in the caspase-1-driven production and processing of the pro-inflammatory cytokine IL-1β.

Gain-of-function *NLRP3* gene mutations inherited in an autosomal dominant manner (but also arising de novo and, more interestingly, as the result of somatic mutations) lead to constitutive activation of the NLRP3 inflammasome resulting in the NLRP3-AID spectrum [37–40]. Disease phenotypes range from mild (previously known as familial cold autoinflammatory syndrome [FCAS]), moderate (previously known as Muckle-Wells syndrome [MWS]) to severe NLRP3-AID (also known as chronic infantile neurological, cutaneous, and articular syndrome/neonatal-onset multisystem inflammatory disease [CINCA/NOMID]) [41, 42].

The mild form of NLRP3-AID is characterized by episodes of cold-induced symptoms including a non-pruritic urticaria-like rash, headache, conjunctivitis, and arthritis.

Moderate NLRP3-AID demonstrates signs of constitutive inflammasome activation with fevers, cutaneous rash, conjunctivitis, uveitis, sensorineural deafness, and potentially fatal amyloidosis. Age of onset is variable but tends to be later than in severe NLRP3-AID.

The latter is characterized by neonatal-onset disease manifesting as near-continuous inflammation characterized by fever, rash, sterile lymphocytic meningitis, sensorineural deafness, psychomotor retardation, and arthropathy.

Recently implemented diagnostic criteria for NLRP3-AID have shown to be effective across the entire spectrum, with an overall specificity of 94% and sensitivity of 81%. To meet the criteria of NLRP3-AID, a patient must demonstrate elevated inflammatory markers (CRP, serum amyloid A) as well as two or more of the following: urticaria-like rash, musculoskeletal symptoms (myalgia, arthralgia,

arthritis), skeletal abnormalities (epiphyseal overgrowth, frontal bossing), cold-induced episodes, sensorineural hearing loss, and/or chronic aseptic meningitis [43].

All three forms of NLRP3-AID respond readily to IL-1 blockade, and prompt treatment can ameliorate the inflammatory phenotype, prevent neurological sequelae, and revert hearing loss [17, 44, 45]. The development of skeletal abnormalities, however, appears to be independent of IL-1 activity and does not respond to anti-IL-1 therapy [42].

11.1.1.3 NLRC4-Related Autoinflammatory Disease (NLRC4-AID)

Nucleotide binding/leucine-rich repeat protein containing CARD (caspase-activation and recruitment domain) 4, NLRC4, is a component of arguably the best understood among all the inflammasomes, the eponymous NLRC4 inflammasome.

The NLRC4 inflammasome functions as a PAMP receptor, recognizing, among others, components of the *Salmonella* type III secretion system (T3SS) and flagellin [3]. It consists of a sensor (NLR family apoptosis inhibitory protein [NAIP]), nucleator (NLRC4), adaptor (apoptosis-associated speck-like protein containing a CARD [ASC]), and effector (caspase-1 [CASP1]) whose activation results in release of IL1-β, IL-18, and gasdermin D [46].

NLRC4-AID in humans has only been reported since 2014 when two teams concurrently reported on the presence of heterozygous, gain-of-function mutations in *NLRC4* in cases of very early-onset inflammatory bowel disease (VEO-IBD) and recurrent macrophage activation syndrome (MAS) [47, 48]. The reported cases responded variably to IL-1 and TNF-α blockade, whereas treatment with a novel IL-18 binding protein (IL-18BP) has shown great promise.

In addition to the MAS/VEO-IBD phenotype, multiple recent reports have described family members suffering from *NLRP3*-AID-like conditions responsive to anakinra, who were ultimately found to have germline mutations in *NLRC4* instead [49]. Another patient presenting with symptoms of severe *NLRP3*-AID was found to harbor a somatic mutation in *NLRC4* [50].

11.1.2 Relopathies

"Relopathy" is a novel umbrella term for conditions affecting the nuclear factor κ-light-chain enhancer of activated B cells (NF-κB) pathway. These conditions arise from mutations affecting a wide array of different proteins involved in ubiquitination and deubiquitination, as well as transcription factors (Fig. 11.2) (Table 11.2). The NF-κB pathway is downstream of the TNF receptor 1 (TNFR1), IL-1 receptor (IL1R), as well as pattern recognition receptors (PRR) (canonical pathway) and CD40 (noncanonical pathway). It is also activated in response to genomic stress – atypical pathway. As required for such a crucial pathway, it is tightly regulated through a system of ubiquitinases and deubiquitinases which dictate the rate of proteasomal degradation of NF-κB pathway components and allow for the phosphorylation and subsequent activation of intracellular kinases and, ultimately,

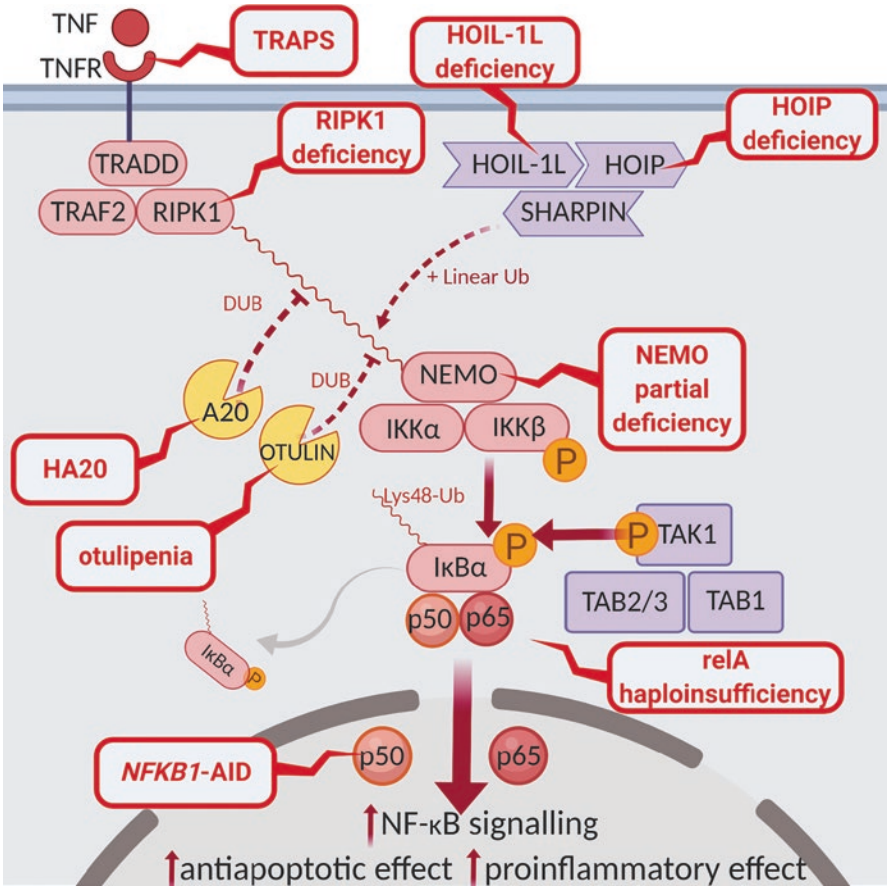

Fig. 11.2 The NF-κB pathway and associated autoinflammatory conditions. The deubiquitinases A20 and OTULIN remove Met-linked linear ubiquitin chains from NEMO, as well as RIPK1, ASC, and TNFR1 (not depicted). Absence of A20 and OTULIN results in impaired degradation of the ubiquitinated enzymes and constitutive activation of the NF-κB pathway. NEMO (IKK-γ) is a negative modulator of NF-κB and its partial (but not complete) deficiency leads to impaired inhibition of IKK-α and IKK-β. Impaired linear ubiquitination (as in LUBAC deficiency) of NEMO leads to decreased IkBα degradation which leads to impaired B-cell responses and immunodeficiency. Monocyte lineage cells in LUBAC deficiency are hyperresponsive to IL-1β (not shown)

NF-κB subunit translocation into the nucleus. Considering the number of important cellular processes regulated by this pathway, including inflammatory responses and cell survival, it is unsurprising that immunological disorders associated with dysregulation of NF-κB signaling have overlapping phenotypes. These range from predominant immunodeficiency to typical autoinflammation.

This section details relopathies with a predominantly autoinflammatory phenotype, whereas those with overlapping features of primary immunodeficiency are described later in this chapter.

Table 11.2 Relopathies

	Disease	Gene	Affected protein/ function	Mode of inheritance	Age of onset	Key clinical features	Immunodeficiency	Treatment
Relopathies	Haploinsufficiency of A20 (HA20)	*TNFAIP3*	NF-κB regulatory protein	AD[a] (some cases unclear)	Infancy to adulthood	Urogenital ulcers, uveitis, gastrointestinal symptoms, arthralgia/arthritis, fever, and neuroinflammation	/	Colchicine, systemic corticosteroids, anti-IL-1, anti-IL-6, anti-TNF, and baricitinib
	ORAS/ otulipenia	*OTULIN*	OTULIN; deubiquitinase	AR	Childhood	Neutrophilic dermatosis, panniculitis, fever, lipodystrophy, diarrhea, and swollen joints	/	Anti-TNF, corticosteroids, and anti-IL-1
	RELA (p65) haploinsufficiency	*RELA*	relA (p65) NF-κB subunit	AD	Childhood	IBD, mucocutaneous ulceration, vomiting, fevers, and leukocytosis	/	Anti-TNF
	NEMO partial deficiency	*IKBKG*	NF-κB essential modulator	XL	Birth	IBD, lymphocytic panniculitis/dermatitis, uveitis, ectodermal dysplasia, osteopetrosis, and lymphoedema	Hypogammaglobulinemia, impaired T-cell proliferation, and low B- and NK-cell counts	IgG replacement, antibiotics, HSCT, and anti-TNF
	Biallelic RIPK1 mutations	*RIPK1*	Receptor-interacting serine/ threonine kinase 1	AR	Childhood	Early-onset IBD, rash, and progressive polyarthritis	Lymphopenia and viral, bacterial, and fungal infections	HSCT
	HOIL-1/HOIP deficiency	*HOIL1 and HOIP*	HOIP, HOIL-1, and SHARPIN; components of LUBAC	AR	Infancy	Autoinflammatory attacks, amylopectinosis, and lymphangiectasia	Low B cells, hypogammaglobulinemia, recurrent pyogenic infections, and abnormal viral response	IgG replacement, antibiotics, anti-TNF, anti-IL-1, and corticosteroids
	NFKB1-AID	*NFKB1*	p50/p105 NF-κB subunit	AD	Variable	Febrile attacks, aphthous mucositis, small-vessel vasculitis, and hyperinflammatory response to surgery	Reduced class-switched memory B cells, hypogammaglobulinemia, and impaired specific antibody response	N/A

AD autosomal dominant, *AR* autosomal recessive, *XL* X-linked recessive, *IBD* inflammatory bowel disease, *HSCT* hematopoietic stem cell transplantation

[a]Sporadic cases reported

11.1.2.1 Haploinsufficiency of A20 (HA20)

Tumor necrosis α-induced protein 3 (*TNFAIP3*), also known as A20, is a deubiquitinase involved in the human NF-κB pathway and functions as a negative regulator of the NLRP3 inflammasome. Heterozygous missense or frameshift mutations in TNFAIP3 leading to reduced or absent A20 levels result in increased constitutive and triggered NF-κB activity and subsequent phosphorylation of p65, interferon regulatory factor 3 (IRF3), STAT1, and STAT 3, among others. This leads to the increased transcription of pro-inflammatory genes and increased production of pro-inflammatory cytokines such as IL-1β, TNF, IL-6, IFN γ, IL-17, and IL-18 [53].

Commonly, HA20 presents as an early-onset autoinflammatory disorder not unlike Behçet's disease, characterized by urogenital ulceration, ocular inflammation, arthralgia/arthritis, and systemic inflammation, with at least two reported cases of neuroinflammation. Interestingly, these cases are often in the presence of autoantibodies (but patients only rarely manifest overt autoimmune disease). Other reports have described cases presenting with conditions resembling Still's disease or rheumatoid arthritis [54, 55]. Additionally, one case was diagnosed as autoimmune lymphoproliferative syndrome (ALPS) with hepatosplenomegaly, lymphadenopathy, fever, rash, and hypogammaglobulinemia but was later found to have a heterozygous mutation in *TNFAIP3* [56].

Given the paucity of cases reported in literature, there is no universal treatment protocol for HA20. A trial of colchicine should be attempted for the Behçet-like patients, but many among them will require anti-cytokine (anti-IL-1, anti-IL-6, or anti-TNF) therapy [57]. Alternative treatments to consider include DMARDs, with JAK inhibition showing promise in a single case of HA20-associated severe neuroinflammation [58].

11.1.2.2 Otulipenia

The deubiquitinase OTULIN is responsible for the enzymatic removal of Met1-linked linear ubiquitin chains from numerous intracellular targets including RIPK1, ASC, NEMO (IKK-γ), and TNFR1 (Fig. 11.2) [59]. The attachment of linear ubiquitin chains to these proteins prevents their proteasomal degradation, and OTULIN is a necessary negative modulator of their activity. In the absence of OTULIN due to loss-of-function mutations, there is accumulation of polyubiquitinated NF-KB, ASC, and TNFR1 with markedly increased production of IL-1β, IL-6, and IFN-γ upon stimulation with LPS observed across all cell lines.

Disease stemming from homozygous loss of function due to missense or frameshift mutations has been reported in a handful of cases and is characterized by early-onset fevers, neutrophilic dermatosis/panniculitis, and lipodystrophy, as well as failure to thrive [60]. The profoundly hyperinflammatory state can be fatal. The condition is steroid-responsive, with a variable response to IL-1 blockade with anakinra and a reportedly good response to the anti-TNF drug infliximab.

11.1.2.3 RelA Haploinsufficiency

NF-κB is a hetero- or homodimer formed from subunits RelA (p65), RelB, c-Rel, NF-κB1, and NF-κB2, most commonly in the form of a RelA/NF-κB1 heterodimer

[61]. Haploinsufficiency of RelA arising from a premature stop codon in the *RELA* gene results in reduced NF-κB activity. This is associated with increased TNF-driven stromal cell (but not lymphocyte) apoptosis and, paradoxically for an autoinflammatory condition, reduced TNF-stimulated secretion of IL-6.

The mutation was first described in a family affected by variable mucocutaneous involvement with an obvious autosomal dominant pattern of inheritance [62]. The index case presented aged 3 years with an IBD-like phenotype characterized by fevers, leukocytosis, vomiting, and acute ileitis on biopsy. The patient was unsuccessfully treated with mesalamine, azathioprine, colchicine, and anakinra, finally achieving complete and sustained remission with infliximab and methotrexate. The affected family members manifested with a far milder condition characterized by diarrhea, oral and/or genital ulcers not requiring anti-TNF therapy.

Similar to cases of HA20, a single case of ALPS-like disease was reported in a patient RelA haploinsufficiency. The affected patient presented with idiopathic thrombocytopenic purpura, anemia, neutropenia, recurrent aseptic meningitis, and splenomegaly responsive to mycophenolate mofetil, IVIg, and rituximab [63].

11.1.3 Interferonopathies

Although stemming from very different underlying genetic defects, type I interferonopathies present with similar phenotypes (Table 11.3). This reflects the predominant effects of aberrant type I interferon signaling *via* the JAK/STAT pathway, culminating in nuclear entry of STAT dimers and transcription of IFN genes (Fig. 11.3). There is debate as to whether monogenic interferonopathies constitute true autoinflammatory disease. Proponents of their inclusion among SAID point toward the auto- or hyperinflammatory nature of the conditions, and opponents exclude them based on the common coexistence of autoantibodies and due to similar interferon signatures being present in a number of "classical" autoimmune conditions such as systemic lupus erythematosus and subsets of rheumatoid arthritis. Regardless of this uncertainty, the conditions warrant a brief overview due to their stereotypical presentation and the importance of their early diagnosis.

11.1.3.1 Aicardi-Goutières Syndrome (AGS)

AGS is a disorder characterized by early-onset neurological disease mimicking an *in utero* viral infection. The subtypes of the illness (AGS types 1–7) are each associated with a specific gene (*TREX1, RNASEH2B, RNASEH2C, RNASEH2A, SAMHD1, ADAR1, IFIH1,* respectively). The genes are involved with the processing of endogenous nucleic acids (TREX1, RNASEH2 subunits) or detection and response to exogenous nucleic acids (*SAMHD1, ADAR1, IFIH1*), giving credence to the theory that AGS stems from aberrant signaling of the interferon pathways responsible for responses to endogenous and exogenous nucleic acids [64, 65].

AGS is associated with a wide spectrum of clinical disease demonstrating phenotypical variations between different affected genes as well as among patients harboring mutations in the same gene. Neurological involvement is present in one-fifth

Table 11.3 Interferonopathies

	Disease	Gene	Affected protein/ function	Mode of inheritance	Age of onset	Key clinical features	Treatment
Interferonopathies	CANDLE/PRAAS	*PSMA3* *PSMB4* *PSMB8* *PSMB9* *POMP*	Proteasome	AR	Infancy	Skin eruptions, progressive lipodystrophy, hepatosplenomegaly, myositis, and violaceous perioral/ periorbital edema	Glucocorticoids and JAK inhibition
	Aicardi-Goutières syndrome	*TREX1*	Exonuclease	AR/AD	Infancy	Encephalopathy, thrombocytopenia, hepatosplenomegaly, and chilblains-like rash with tissue loss	Symptomatic treatment, JAK inhibition, and reverse transcriptase inhibitors
		RNASEH2A *RNASEH2B* *RNASEH2C*	Subunits of the RNAse H2 endo-nuclease complex				
		SAMHD1 *ADAR1* *IFIH1*	Control of dNTP pool				
	SAVI	*TMEM173*	Stimulator of Interferon Genes (STING)	AD and SM	Infancy	Cutaneous vasculopathy leading to ulcers and necrosis, Raynaud's phenomenon, livedo reticularis, ILD, and polyarthritis	JAK inhibition
	DNASE2 deficiency *** [75]	*DNASE2*	Deoxyribonuclease	AR	Infancy	Neonatal anemia, deforming arthropathy, glomerulonephritis, liver fibrosis, and anti-dsDNA antibodies	JAK inhibition

AR autosomal recessive, *AD* autosomal dominant, *SM* somatic mosaicism

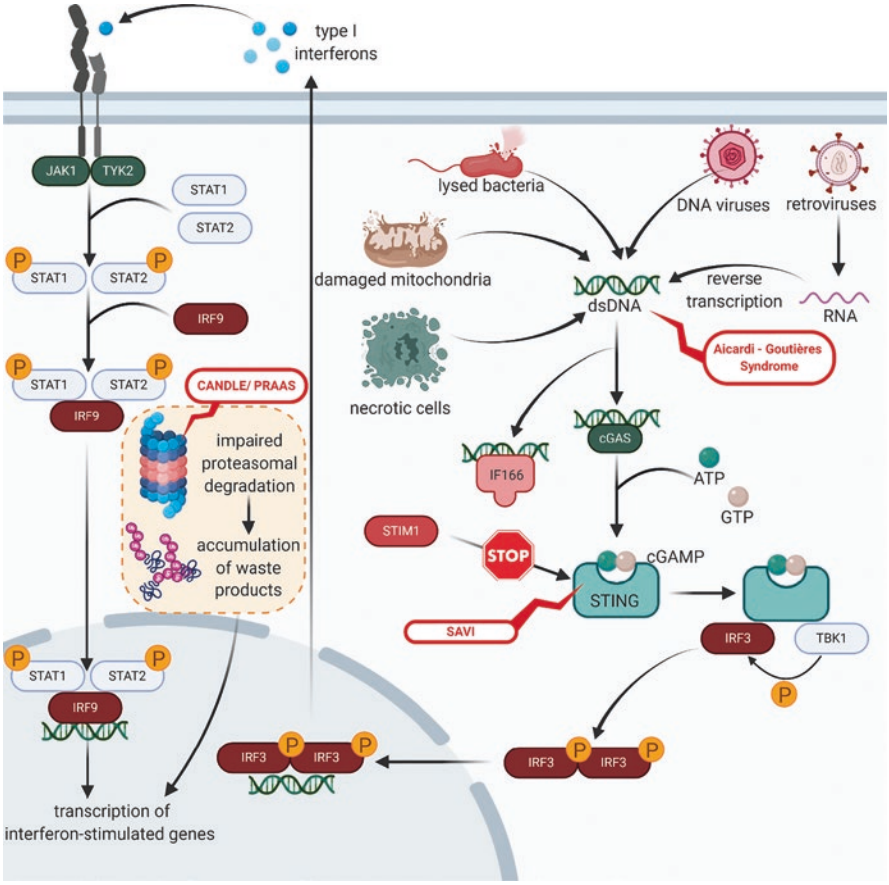

Fig. 11.3 Type I interferon-associated pathways. Detection of type I interferons by the interferon α/β receptor triggers the JAK/STAT pathway, resulting in nuclear entry of interferon regulatory factor (IRF) 9 associated with STAT1/2. Double-stranded (ds) DNA from numerous sources leads to cyclic guanosine monophosphate adenosine monophosphate (cGAMP)-triggered activation of STING, culminating in phosphorylation of IRF3 and production of type I interferons, resulting in a positive feedback loop. Impaired proteasomal degradation (as in PRAAS) leads to the accumulation of waste products, increased intracellular stress, and transcription of interferon-stimulated genes

of cases with spasticity, dystonia, seizures, microcephaly, and basal ganglia calcification being the most common presentations. Seemingly spontaneous fevers, as well as chilblains, are also associated with AGS, as is a lupus-like syndrome. Low-titer autoantibodies are commonly found in the sera of AGS patients [66–68].

11.1.3.2 STING-Associated Vasculopathy with Onset in Infancy (SAVI)

SAVI stems from gain-of-function mutations affecting exon 5 of the *TMEM173* gene coding for STING (Stimulator of INterferon Genes), a protein involved in coupling sensing of exogenous DNA to the type I interferon response. Unlike the

other interferonopathies described, SAVI is not associated with neurological manifestations but is instead characterized by an aggressive vasculitic process resulting in significant tissue loss, as well as progressive interstitial lung disease. These tissue-specific manifestations likely reflect the expression profile of STING, which can predominantly be found in alveolar macrophages and vascular endothelial cells. In addition, SAVI manifests with recurrent low-grade fevers and polyarthritis with antinuclear or RA-associated autoantibodies. The condition has a high mortality secondary to cardiopulmonary failure or infections [69–71].

11.1.3.3 Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated Temperature/ Proteasome-Associated Autoinflammatory Syndromes (CANDLE/PRAAS)

CANDLE/PRAAS can arise from mutations affecting various proteasomal subunits (*PSMB8*, *PSMB4, PSMB9, PSMA3*) or associated proteins (*POMP)* resulting in impaired enzymatic activity or protein stability. The ensuing deficiency in proteasomal degradation leads to intracellular accumulation of polyubiquitinated proteins and increased cellular stress which in turn stimulates IFN signaling. CANDLE/PRAAS is a neonatal- or infancy-onset condition associated with a typical annular acral rash with raised edges; myositis; progressive lipodystrophy affecting the face, upper extremities, and trunk; periodic fevers; and violaceous periorbital and perioral edema. It is characteristic of the condition that flares occur spontaneously or are triggered by innocuous stimuli such as viral infections, ambient cold air, and physical or psychological stress. Flares typically result in inflammation capable of affecting virtually any organ system, with reports of carditis, nephritis, epididymitis, and conjunctivitis [72, 73].

11.1.3.4 Treatment of Interferonopathies

Regardless of the syndrome involved, interferonopathies represent a therapeutic challenge since they are refractory to NSAIDs, colchicine, and DMARDs and only partially responsive to corticosteroids. A 2018 study investigating the use of baricitinib, an oral JAK 1/2 inhibitor, showed impressive steroid-sparing effects with both clinical and laboratory improvement [74].

11.1.4 Other Autoinflammatory Disorders

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

TRAPS is an autosomal dominant condition arising from heterozygous mutations in the *TNFRSF1A* gene. All pathogenic variants thus far described affect the extracellular domain of the receptor, with mutations affecting cysteine-rich domains (e.g., C88Y, T50M) resulting in the most severe phenotype (Table 11.4). Multiple molecular mechanisms likely contribute to the pathogenesis of TRAPS, reflecting the varied and opposing effects of the TNF signaling pathway. Proposed mechanisms include reduced TNFR1 cleavage; ligand-independent activation of mutant TNFR1;

Table 11.4 Other AID

	Disease	Gene	Affected protein/ function	Mode of inheritance	Age of onset	Key clinical features	Treatment
Others	TRAPS – high penetrance	*TNFRSF1A (T50M, C88Y)*	Tumor necrosis factor receptor	AD	Childhood	Skin rash, myalgia, serositis, periorbital edema, and fevers (7–14 d)	Corticosteroids/anti-IL-1/ anti-IL-6/etanercept
	TRAPS – low penetrance	*TNFRSF1A (R92Q, P46L)*	Tumor necrosis factor receptor	AD	Variable	Fevers, aphthous stomatitis, arthritis, pharyngitis, cervical adenitis, RA, and BD	Anti-TNF
	DIRA	*IL-1RN*	IL-1 receptor antagonist	AR	Infancy (fetal distress)	Pustular rash, osteolytic bone lesions, osteopenia, and hepatosplenomegaly	Anti-IL-1
	DITRA	*IL-36RN*	IL-36 receptor antagonist	AR	Variable	Pustular psoriasis (cutaneous pustulosis, fevers), SIRS, and asthenia	Anti-IL-1, anti-TNF, anti-IL-12/23, and anti-IL-17
	NOD2 – AID	*NOD2*	NOD-like receptor protein	AD and SM	Childhood	Polyarthritis (synovitis leading to contractures), panuveitis, dermatitis (micropapular rash, erythema nodosum-like nodules, erythematous plaques), and visceral involvement	Methotrexate, anti-TNF, anti-IL-1, anti-IL-6, and corticosteroids
	Monogenic systemic Still's disease *** [102]	*LACC1*	FAMIN; fatty acid metabolism regulator	AR	Childhood	Daily spiking fevers, erythematous rash, polyarthritis, serositis, and lymphadenopathy	Anti-IL-1, anti-IL-6, and corticosteroids

AR autosomal recessive, *AD* autosomal dominant, *SM* somatic mosaicism Monogenic Autoinflammatory Disorders with PID Overlap

enhanced activation of NF-κB and mitogen-activated protein kinase pathways; increased generation of mitochondrial oxygen species and subsequent activation of NLRP3 inflammasome; and activation of endonuclease inositol-requiring enzyme 1 due to TNFR1 misfolding and retention within the endoplasmic reticulum, leading to selective degradation of anti-inflammatory microRNAs and hyperresponsiveness to lipopolysaccharide [76].

Most patients with TRAPS are symptomatic from childhood. Clinical presentation includes high fevers typically lasting 7–14 days, myalgias, centrifugally spreading maculopapular rash often overlying areas of myalgia, serositis, and periorbital edema. Most patients report a relapsing-remitting course, with approximately one-eighth presenting with continuous inflammation with or without added flares. The low-penetrance mutations (e.g., R92Q, P46L) result in a milder and less well-defined phenotype and account for almost a third of the TRAPS cohort in the European database [77]. In some cases, the clinical picture resembles the PFAPA syndrome (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis) [78]. In addition, R92Q has been found at high frequency in other inflammatory disorders and is now believed to contribute to the pathogenesis of more complex disorders including early rheumatoid arthritis [79] and Behçet's disease [80].

One of the hallmarks of TRAPS is rapid and marked response to corticosteroids at doses equivalent to prednisolone 0.5–1 mg/kg/day, which induce and maintain remission but are associated with deleterious long-term effects. Depending on disease severity, corticosteroids and NSAIDs can suffice in patients with infrequent flares and a milder disease phenotype. The mainstay of treatment for more severe phenotypes is IL-1 blockade with anakinra or canakinumab, both of which achieve rapid remission [17, 81]. Colchicine is ineffective, while anti-TNF therapy can have mixed outcomes. For example, etanercept, a fusion protein composed of two TNFR2 and an Fc portion of IgG1, is effective for symptomatic control but does not always lead to biochemical remission and can be associated with secondary loss of effect [82]. Infliximab, a chimeric anti-TNF monoclonal antibody, can cause paradoxical worsening of TRAPS [83] and should be avoided. In rare instances, anti-IL-6 treatment has also been effective [84]. The management of R92Q- and P46L-associated disease is different to classical TRAPS since anti-TNF therapies might be a preferred treatment choice in these patients [78].

Based on the molecular mechanisms identified thus far, therapeutic approaches based on direct inhibition of NLRP3 or the IRE1 axis might be considered for the future management of this complex condition.

11.1.4.2 Deficiency of the IL-1 Receptor Antagonist (DIRA)

DIRA is an autosomal recessive condition presenting within weeks of birth, as well as being associated with preterm labor and fetal distress. The condition stems from mutations affecting the *IL1RN* gene on chromosome 2, with a marked founder effect in the Puerto Rican, Newfoundland Canadian, and Dutch population [85–87]. The mutation results in absent or truncated IL-1 receptor antagonist (IL1RA), a suppressor of IL-1α and IL-1β signaling, leading to unopposed pro-inflammatory signaling, as well as deranged osteoclast activity which is normally tightly regulated by IL-1α.

The condition is characterized by neonatal-onset cutaneous pustulosis, bone abnormalities (osteopenia, lytic lesions, sclerosis, flaring of ribs and clavicles), and systemic inflammation. It can be fatal if untreated. Despite high inflammatory activity, as demonstrated by elevated inflammatory markers and thrombocytosis, fever is not a common feature in DIRA. In addition to typical symptoms, there have been reports of arterial and venous thrombosis, as well as interstitial lung disease and hepatosplenomegaly [85–87].

DIRA responds dramatically to the glycosylated IL-1 receptor antagonist, anakinra, given subcutaneously at doses of 1 mg/kg/day but uptitrated in a treat-to-effect manner. The vast majority of patients with DIRA achieve complete remission with anakinra, whereas partial responders require additional corticosteroid cover.

11.1.4.3 Deficiency of Interleukin-36 Receptor Antagonist (DITRA)

The interleukin-36 receptor antagonist (IL-36Ra) belongs to the interleukin-1 family of cytokines and suppresses IL-36α, IL-β, and IL-γ signaling, especially in the skin and gut. Homozygous or compound heterozygous mutations in the *IL36RN* gene result in an inheritable form of generalized pustular psoriasis. The mutated IL-36Ra is less stable and has impaired binding to IL-1 receptor-resembling protein 2. This results in unopposed IL36-α, IL36-β, and IL36-γ signaling, leading to increased interleukin-8 production in keratinocytes. Unexpectedly, markedly increased IL-8 production secondary to stimulation with IL-1β and poly[I:C] has also been described [88].

Generalized pustular psoriasis is an autoinflammatory condition often (but not always) associated with psoriasis vulgaris. It manifests with episodes of high fever (40–42 °C), generalized erythematous rash, and pustulosis. Multiorgan involvement and fatality stemming from a septic shock-like condition have also been described. DITRA was first reported secondary to a P26L mutation found in nine consanguineous Tunisian families but has since been found to stem from other mutations as well [89, 90].

Treatment of DITRA has lacked consensus. Multiple anti-cytokine therapies have been attempted including inhibition of TNF-α, IL-1, IL-17, or IL-12/23, all with variable effect.

11.1.4.4 NOD2-Associated Granulomatous Disease

The nucleotide-binding oligomerization domain-containing protein 2 (NOD2) consists of two caspase activation and recruitment domains (CARD), a central NOD/NACHT domain and an LRR domain. The CARD domains are crucial modulators of the NF-κB pathway via the receptor-interacting protein kinase 2 (RIPK2), whereas the NOD domain is responsible for self-oligomerization of NOD2 and amplification of the inflammatory cascade. Mutations affecting the LRR domain have long been associated with Crohn's disease; however, mutations affecting the central NOD domain result in an autoinflammatory disorder known as Blau syndrome (BS). BS is typically associated with mutations affecting the evolutionarily preserved arginine residue at position 334 and is inherited in an autosomal dominant manner, with a milder phenotype being reported as arising from a somatic mutation affecting the same position [91].

The typical triad associated with BS consists of early-onset inflammatory polyarthritis, progressive panuveitis, and dermatitis. The arthritis tends to be associated with synovitis or tenosynovitis and contractures resulting in camptodactyly; however, joint destruction is exceedingly rare. Joint pain and stiffness tend to be well tolerated despite prominent swelling [92]. Eye involvement associated with BS tends to manifest insidiously within 2 years of birth, progressing to affect both the anterior (iridocyclitis) and posterior (retinal vasculitis, vitritis, and choroiditis) chamber of the eye, with 42% of patients reporting moderate to severe visual impairment [93]. Cutaneous manifestations of BS include a fine, pink- to tan-colored micropapular rash affecting the trunk, neck, and proximal extremities which can resemble ichthyosis vulgaris during its desquamatory phase. Less frequently, the condition manifests as mildly tender subcutaneous nodules affecting the shins, reminiscent of erythema nodosum.

Involvement of other organs has been reported in nearly half of patients, with kidney disease (granulomatous or interstitial nephritis, chronic renal insufficiency), lymphadenitis (peripheral and mediastinal), pericarditis, neuropathy of the facial nerve, and small-vessel vasculitis reported in recent years [94–97].

Treatment of BS remains problematic well into the age of biologic therapy. Joint and visceral involvement tend to respond well to methotrexate and anti-TNF therapy including adalimumab and infliximab [98]. The effect of anti-TNF in the management of ocular disease is less predictable, and there is emerging evidence for the efficacy of interleukin-1 blockade with anakinra or canakinumab [95, 99], as well as of IL-6 blockade with tocilizumab [100, 101].

11.2 Monogenic Autoinflammatory Disorders with PID Overlap

11.2.1 Relopathies

11.2.1.1 Receptor Interacting Protein Serine/Threonine Kinase 1 (RIPK1) Deficiency

An autosomal recessive condition resulting in the loss of RIPK1 demonstrates the delicate interplay between autoinflammation and immunodeficiency. RIPK1 is a kinase downstream of TNFR1, TLR3, and TLR4 and is responsible for the phosphorylation of MAP kinases (MAPKs) and NF-κB (Fig. 11.2). Absence of RIPK1 therefore results in reduced MAPK and NF-κB activity, with reduced production of IL-6 and IL10, or IL-12 and TNF upon stimulation by LPS or co-stimulation by LPS and IFN-γ, respectively. Upon stimulation with the potent T-cell mitogen phytohemagglutinin (PHA), there is disproportionate IL-1β production with concurrent reduction in IL-17 and IFN-γ [103, 104].

RIPK1 deficiency results in early-onset inflammatory bowel disease (IBD) and immunodeficiency characterized by lymphopenia and a propensity for bacterial, viral, and fungal infections. A proportion of cases also presented with progressive polyarthritis and/or rash. IL-1β is well known to be involved in the pathogenesis of IBD and arthritis, and impaired IL-10 signaling is associated with early-onset

idiopathic IBD. The imbalance of these two cytokines is therefore likely to be responsible for the inflammatory component of RIPK1 deficiency, whereas impaired signaling via the TNF receptor and the pattern receptors TLR3 and TLR4 is likely responsible for the impaired immune response.

Based on the cytokine abnormalities described, the condition appears amenable to IL-1 blockade. However, this has not been attempted in the limited cases described in literature to date. Of the handful of reported cases, one underwent hematopoietic stem cell transplantation which resulted in resolution of IBD and polyarthritis, as well as a marked reduction in infection frequency. In the remaining cases, there was a variable response to corticosteroids. Azathioprine was found to be ineffective in one patient who later succumbed to overwhelming sepsis.

A purely autoinflammatory disorder related to the heterozygous mutations in RIPK1 was described in unrelated families [105, 106]. Here, the mutant RIPK1 is resistant to cleavage by caspase 8, which would typically terminate signaling via this pathway. The resulting phenotype is that of early-onset disease characterized by high fevers, lymphadenopathy, splenomegaly, oral ulceration, and responsiveness to IL-6 blockade.

11.2.1.2 Nuclear Factor-κB Essential Modulator (NEMO) Partial Deficiency

NF-κB essential modulator (NEMO), also known as an inhibitor of the NF-KB kinase subunit γ (IKK-γ), is the modulatory subunit of the IKK complex (consisting of NEMO, IKKα, and IKKβ) responsible for the phosphorylation-triggered degradation of IκBα [107]. Degradation of IκBα results in the nuclear entry of NF-κB subunits and increased transcription of pro-inflammatory genes. NEMO represents one of the final steps in a wide variety of pathways as it lies downstream of the Toll-like receptor 4 (TLR4), IL-1 receptor (IL-1R), TNF-α receptor (TNF-αR), receptor activator of NF-κB (RANK), vascular endothelial growth factor 3 (VEGF3), and ectodysplasin-A receptor.

The *IKBKG* gene encoding NEMO is located on the X chromosome, and complete loss-of-function mutations result in incontinentia pigmenti in females and in utero death in males. Hypomorphic mutations affecting *IKBKG*, however, result in an X-linked recessive syndrome characterized by variable degrees of immunodeficiency, ectodermal dysplasia, osteopetrosis, lymphoedema and inflammation in the form of IBD, lymphocytic panniculitis or dermatitis, and uveitis [108–110].

The numerous disrupted pathways result in diminished cellular responses to lipopolysaccharide (LPS, *via* TLR4), IL-1 and IL-18 (*via* IL1R), TNF-α (*via* TNF-αR) which manifest with a highly variable degree of immunodeficiency associated with B-cell impairment, hypogammaglobulinemia with or without elevated IgM levels, normal T-cell numbers (with or without impaired proliferation), and a variable degree of NK-cell deficiency. The only immunological abnormality consistently associated with the condition seems to be impaired production of glycan-associated antibodies, explaining the extreme susceptibility of affected individuals to pneumococcus.

Ectodermal dysplasia, osteopetrosis, and lymphoedema are most likely due to impaired signaling downstream of ectodysplasin-A receptor, RANK, and VEGF3, respectively [111]. The underlying defect(s) responsible for autoinflammation and autoimmunity in the form of IBD, dermatitis, hemophagocytic lymphohistiocytosis, autoimmune hemolytic anemia, idiopathic thrombocytopenia, and Hashimoto thyroiditis remain to be elucidated.

Treatment of the condition relies on immunoglobulin replacement therapy, therapeutic and prophylactic antibiotic use, as well as hematopoietic stem cell transplantation in more severe cases [112]. HSCT appears to improve the immunodeficiency; however, the IBD seems to be mediated by cells and mechanisms outside the hematopoietic niche and was reported to respond to anti-TNF therapy [113, 114].

11.2.1.3 Linear Ubiquitin Chain Assembly Complex (LUBAC) Deficiency

LUBAC consists of regulatory subunits HOIL-1 (hemoxidized iron-regulatory protein 2 ubiquitin ligase 1) and SHARPIN (SHANK-associated RH domain-interacting protein), and the catalytic subunit HOIP (HOIL1-interacting protein) (Fig. 11.2). It is involved in the ubiquitin proteasome system responsible for the proteasomal degradation of various NF-κB pathway proteins including IKKβ and IκBα.

Since 2012, research led by Boisson et al. has shed light on conditions arising from hypomorphic mutations involving the *HOIL1* or *HOIP* genes [115]. Both conditions are characterized by immunodeficiency complicated by recurrent pyogenic infections, episodic autoinflammation (characterized by fevers, lymphadenopathy, hepatosplenomegaly), and varying degrees of amylopectinosis. The reported HOIP-deficient patient also suffered from lymphangiectasia. Homozygous mutations in either gene affect not only the mutated protein but also prevent the assembly and functioning of the LUBAC multimer.

HOIL-1 and HOIP deficiencies both result in impaired degradation of IκBα and linear ubiquitination of NEMO, leading to decreased fibroblast responsiveness to TNF and IL-1β and impaired CD40-mediated B-cell proliferation and specific antibody responses. Paradoxically, patients' monocyte lineage cells respond excessively when stimulated with IL-1β (but not with TNF) and are thought to be responsible for the autoinflammatory symptoms.

Treatment of the HOIP-deficient patient has thus far been symptom-based, with intravenous immunoglobulin replacement for the hypogammaglobulinemia, antibiotics for recurrent infections, and diet for fat malabsorption stemming from lymphangiectasia. The HOIL-1-deficient patient was treated with TNF-blocking agents which caused a transient reduction of inflammatory symptoms.

11.2.1.4 NF-κB1 Associated Autoinflammation

The NF-κB family of transcription factors consists of five proteins—NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA(p65), RelB, and c-Rel—all of which contain the Rel Homology Domain (RHD) and form homo- and heterodimers among themselves.

The *NFKB1* gene codes for p50/p105, which, after proteasomal degradation to p50, forms homo- or heterodimers with RelA (p65). The dimers then enter the

nucleus and function as transcription factors affecting more than 500 inflammation- and immunity-related genes.

Haploinsufficiency of p50 is a known cause of autosomal dominant common variable immunodeficiency (CVID) with autoimmune manifestations [116]. However, novel mutations affecting different regions of the *NFKB1* gene have been associated with a markedly (auto)inflammatory condition in addition to a variable degree of antibody deficiency.

Of the three novel heterozygous mutations described, the two affecting the RHD—p.H67R and p.R157X—are both associated with autoinflammation. The p.H67R mutation is associated with a Behçet's disease-like phenotype characterized by aphthous mucositis, fever, and arthritis in addition to hypogammaglobulinemia, impaired specific antibody responses, and a reduced proportion of class-switched memory B cells. Elegant experimental work conducted by Kaustio et al. revealed reduced NF-κB activation and impaired nuclear entry of the transcription factors, as well as reduced secretion of IL-1β [117].

The p.R157X mutation leads to an unstable protein end product which results in the depletion of p50/105. This leads to reduced NF-κB activity but, paradoxically, increased production of IL-1β following LPS-stimulated NLRP3 activation. The mutation is characterized by hyperinflammatory responses to surgery manifesting as postoperative necrotizing cellulitis with abscess formation, fever, and neutrophilia. The immunodeficiency associated with p.R157X is less pronounced, with variable degrees of antibody deficiency but consistently reduced proportions of class-switched memory B cells. Carriers of this mutation also demonstrate markedly variable penetrance, with two of the four documented carriers remaining asymptomatic.

Based on the molecular mechanisms underlying the conditions, targeted treatment with IL-1 or TNF blockade might be effective in addition to immunoglobulin replacement therapy. However, the limited literature available fails to report on the treatment approaches chosen in the handful of cases described.

11.2.1.5 Deficiency of Adenosine Deaminase 2 (DADA2)

DADA2 is an autosomal recessive condition with neurological, inflammatory, hematologic, immunologic, and dermatologic manifestations. The condition can occur sporadically or in endemic populations, with a 10% mutation carriage rate reported in the Georgian Jewish population [118]. ADA2 is an enzyme expressed by myeloid cells and excreted by cells belonging to the monocyte-macrophage-DC (dendritic cell) lineage. It is responsible for the removal of adenosine nucleotides, functions as a vascular endothelial growth factor, and promotes macrophage differentiation toward the M2 phenotype [119].

Loss-of-function mutations affecting the *CECR1* gene (coding for ADA2) can result in decreased dimerization, reduced stability, obscured binding sites, or loss of enzymatic activity. The mutations lead to a wide and variable spectrum of disease stemming from increased vascular endothelial fragility, skewing of

macrophages toward the pro-inflammatory M1 lineage, or reduced B-cell numbers [120].

DADA2 often presents early in life, but cases presenting in the fifth decade of life have also been reported. The phenotype is highly variable, with some cases demonstrating recurrent (but widely interspersed) hyperinflammatory flares with long asymptomatic periods in between. Mutations affecting *CECR1* were found to be present in almost a third of patients presenting with early-onset polyarteritis nodosa (PAN) [121].

The neurological manifestations of DADA2 include early-onset ischemic lacunar infarcts as well as hemorrhagic strokes in areas of previous lacunar infarcts, in the subarachnoid space, and in the parenchyma, both spontaneous and in the context of prophylactic anticoagulant use. As these strokes can be subclinical, it is recommended that cerebral MRI/MRA be performed in all patients diagnosed with DADA2.

Anemia, either secondary to chronic inflammation or stemming from pure red cell aplasia, can be a presenting symptom, as can be frank pancytopenia. Bone marrow examination tends to be rather nonspecific, with hyper- or hypocellularity being described in addition to inflammation-associated large granular lymphocytes.

Patients with DADA2 often present with livedo racemosa affecting the extremities but occasionally spreading to involve the trunk, neck, and face. The cutaneous pattern of livedo racemosa tends to be broader and interrupted compared to the finer and complete weblike pattern of livedo reticularis. In addition, DADA2 can present with subcutaneous nodules, erythema nodosum and urticaria, and hallmarks of insufficient perfusion such as Raynaud's phenomenon, skin atrophy, and ulceration.

The gastrointestinal tract can be affected as part of the polyarteritis nodosa-like vasculitis, with intestinal perforation secondary to ischemia an important cause of morbidity and mortality in DADA2, as well as with hepatic abnormalities such as hepatosplenomegaly, portal hypertension, and nodular regenerative hyperplasia on biopsy.

Immunologic manifestations of DADA2 are far less severe than those associated with deficiency of ADA1, namely, SCID, but a degree of immunodeficiency mainly affecting B cells is nonetheless common. Clinically, this can manifest as isolated IgM deficiency, impaired specific antibody response, or panhypogammaglobulinemia leading to a diagnosis of common variable immunodeficiency disorder (CVID) [122].

Definitive treatment of the hematological and immunological complications of DADA2 consists of hematopoietic stem cell transplantation (HSCT) which abrogates the phenotype [123]. Neurovascular complications tend to respond well to TNF-α blockade. Implementation of thromboprophylaxis is a complex decision, however, it might predispose to hemorrhagic stroke in the context of increased vascular fragility associated with DADA2 [124].

11.2.2 PLC-γ2-Associated Antibody Deficiency and Immune Dysregulation/Autoinflammatory PLC-γ2-Associated Antibody Deficiency and Immune Dysregulation (PLAID/APLAID)

Phospholipase Cγ2 (PL-Cγ2), an enzyme predominantly expressed by lymphoid and myeloid lineage cells, is involved in the processing of phosphatidylinositol bisphosphate (PIP2) into diacylglycerol (DAG) and inositol trisphosphate (IP3); this mediates the release of calcium from the ER into the cytoplasm, leading to cellular activation. Mutations affecting the *PLCG2* gene encoding PL-Cγ2 result in two related, but phenotypically distinct, entities [125].

The first such condition, PLC-γ2-associated antibody deficiency and immune dysregulation (PLAID), is an autosomal dominant disorder characterized by cold urticaria (triggered by evaporative cooling), recurrent infections, autoimmunity, and atopy (Table 11.5). The exon-skipping mutations associated with PLAID result in a constitutively activated PL-Cγ2 which paradoxically leads to B-cell, NK-cell, and mast cell anergy at physiological temperatures, but spontaneous activity on cold exposure. This probably accounts for the cold urticaria and granulomatous dermatitis involving cold-exposed areas. Patients with PLAID suffer from recurrent bacterial and viral respiratory tract infections, as well as frequent VZV reactivations and problematic fungal onychomycosis stemming from NK- and B-cell anergy. Laboratory findings associated with PLAID include panhypogammaglobulinemia, low percentage of class-switched memory B cells, and reduced numbers of CD57+ cells. The autoimmune complications most commonly associated with PLAID include thyroid disease and vitiligo. Nearly all PLAID patients are atopic. Treatment of PLAID is based on symptom management and lifestyle alterations (e.g., cold avoidance) [126].

The second autosomal dominant condition stemming from *PLCG2* mutations is associated with the substitution of evolutionarily highly conserved residues at positions 707 or 848. This results in supraphysiological calcium release upon PLCG activation by receptor kinases. The ensuing clinical presentation is characterized by early-onset epidermolysis bullosa, cutaneous pustulosis, ocular involvement with corneal ulceration, inflammatory bowel disease, and recurrent bacterial infections. Additional cases have described association with CNS vasculitis [127] and cutis laxa [128]. Despite the similar absence of class-switched memory B cells to PLAID, APLAID patients have thus far demonstrated a lesser degree of immunodeficiency, presenting with recurrent bacterial chest infections but no autoimmunity. Patients with APLAID fail to improve with NSAIDs and show partial or absent improvement with IL-1 or TNF blockade. They often rely on long-term corticosteroid therapy for symptom control [127–130].

Table 11.5 Other AIDs associated with immunodeficiency

Disease		Gene	Affected protein/ function	Mode of inheritance	Age of onset	Key clinical features	Treatment
Actin cytoskeleton dysregulation	Autoinflammatory periodic fevers, immunodeficiency, and thrombocytopenia (PFIT)	*WDR1*	WD repeat domain 1; degradation of intermediate filaments and polymerization of F-actin	AR	Infancy	Periodic fevers (3–7 d), thrombocytopenia, recurrent oral inflammation, and recurrent infections	Colchicine, corticosteroids, anti-IL-1, and HSCT
	ARPC1B deficiency	*ARPC1B*	Actin-related protein 2/3 complex subunit 1B; branching of F-actin	AR	Infancy	Recurrent infections, cutaneous vasculitis, thrombocytopenia, GI bleeding, atopy, arthritis, and lymphadenopathy	Antibiotics, corticosteroids, mycophenolate, sirolimus, and HSCT
PLCG2 AID	PLAID	*PLCG2*	Phospholipase Cγ2	AD	Childhood	Cold urticaria, granulomatous acral dermatitis, autoimmunity, atopy, and panhypogammaglobulinemia resulting in recurrent infections	Symptomatic (e.g., IVIg, cold avoidance)
	APLAID			AD	Childhood	Epidermolysis bullosa, corneal ulceration, IBD, and recurrent bacterial infections	Corticosteroids and anti-IL-1
	DADA2	*CECR1*	Adenosine deaminase 2	AR	Childhood	Livedo racemosa, polyarteritis nodosa (bowel ischemia, lacunar strokes, skin ulcers), pancytopenia, portal hypertension, arthralgia, and variable degree of antibody deficiency	Anti-TNF and bone marrow transplantation
	Congenital sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD)*** [136]	*TRNT1*	TRNA nucleotidyl transferase 1	AR	Birth	Severe congenital (microcytic) anemia, B-cell lymphopenia, panhypogammaglobulinemia, periodic fever, cardiomyopathy, sensorineural deafness, seizures, and developmental delay	Symptomatic (RBC transfusions, IVIg), anti-IL-1, and HSCT

AR autosomal recessive, *AD* autosomal dominant, *HSCT* hematopoietic stem cell transplantation, *IVIg* intravenous immunoglobulin, *RBC* red blood cell

11.2.3 Cytoskeletal Disorders

Immune disorders stemming from abnormalities of the cell's underlying cytoskeletal structure have been known about for nearly a century, with Wiskott-Aldrich syndrome (WAS) the best-known example. The cytoskeleton plays a crucial role in T-cell activation, cell-cell interactions, and chemotaxis. Immunodeficiency arising from disruption of cytoskeletal structures can therefore be explained by impairment of the above processes. The autoinflammatory component of cytoskeletal disorders, on the other hand, seems to arise from intracellular accumulation of cytoskeletal components, resulting in increased cellular stress.

11.2.3.1 Periodic Fever, Immunodeficiency, and Thrombocytopenia (PFIT)

The PFIT syndrome stems from homozygous mutations affecting the *WDR1* gene [131]. The gene product, WDR1, is comprised of 14 WD40 motifs arranged into two 7-motif propellers responsible for degradation of intermediate filaments as well as polymerization of F-actin [132]. Cells of affected patients demonstrate increased F-actin accumulation and increased production of IL-18 with normal levels of IL-1β and IL-18BP. The clinical phenotype, thus far only described in two pediatric cases of consanguineous Pakistani descent, is characterized by periodic fevers, sterile inflammation, immunodeficiency, and thrombocytopenia [131]. The inflammatory episodes can be spontaneous or triggered by innocuous viral infections, whereas the immunodeficiency manifests with severe infections including S. aureus septic arthritis and S. pneumoniae cellulitis (Table 11.5). Corticosteroids and colchicine have both been used in PFIT, eliciting a partial response, along with various DMARDs, all of which were ineffective. Surprisingly, one of the patients demonstrated partial improvement after treatment with anakinra, giving further substance to theories that it can suppress IL-18-mediated inflammation [133]. Despite the partial response to anakinra, the patient died due to unchecked multiorgan inflammation, whereas her younger sister underwent HSCT and remains symptom- and therapy-free.

11.2.3.2 *ARPC1B*-Associated Autoinflammatory Disease

The protein Arp 2/3 can associate with numerous proteins (including Wiskott-Aldrich syndrome protein) and is responsible for branching of F-actin. Homozygous mutations affecting the *ARPC1B* gene coding for the synonymous Arp 2/3 subunit result in a syndrome characterized by recurrent infections, (micro)thrombocytopenia, spontaneous bleeding, vasculitis, and atopy [134, 135].

The affected Arp 2/3 protein exists in two isotypes (ARPC1A and APC1B), with ARPC1B predominantly expressed in hematopoietic cells. The precise mechanisms responsible for the disease phenotype remain to be elucidated. However, impaired formation of proplatelets from megakaryocytes has been described in vitro and likely contributes to the platelet abnormalities [135]. Propensity for bacterial infections is believed to be secondary to impaired macrophage and neutrophil chemotaxis, whereas NK-cell dysfunction likely underlies the characteristic propensity for

viral infections. Atopy is believed to arise from T_{reg} dysfunction and subsequent exaggerated T_H2 response.

There is marked heterogeneity among the reported ARPC1B patients, with recurrent infections, cutaneous leukocytoclastic vasculitis, gastrointestinal hemorrhage, atopy, and inflammatory bowel disease reported in some, but not all, of the patients. Most of the patients reported thus far had normal platelet volumes and increased IgE, IgA, and eosinophil levels. Platelet counts were low or normal. The majority of patients are treated with prophylactic antibiotics, and the autoinflammatory complications respond to corticosteroids, sirolimus, and mofetil mycophenolate. HSCT remains the only definitive curative option, with five patients reportedly disease-free posttransplant [134, 135].

11.3 Conclusions

In this chapter, we have described a number of monogenic AID with varying degrees of autoinflammatory features. This includes classic conditions such as the original hereditary fever group (FMF, MVK, TRAPS, and NLRP3-AID) and conditions which have varying degrees of overlapping, autoimmune, and/or immunodeficiency features, such as NFkB1 haploinsufficiency. There are a number of biological processes involved in the pathogenesis of these disorders. Some of these are shared by a group of AID, for example, the relopathies, while in some cases, multiple coexisting, non-mutually exclusive molecular mechanisms are all involved in the pathogenesis of a single AID, such as in TRAPS.

The field of autoinflammatory disorders is rapidly evolving, and by the time this textbook is published, several novel conditions will have been discovered. Due to space limitations, we have not included polygenic autoinflammatory diseases, such as Still's disease or Schnitzler's syndrome. An additional chapter would be required to detail these complex but fascinating disorders.

Recognition and diagnosis of AID in routine clinical practice remain challenging. The wider use of genetic testing, although largely helpful, has its own challenges. Interpretation of genetic variants of unknown significance and relating these findings to the patient's clinical symptoms and overall diagnosis can be difficult. Although helpful diagnostic criteria have been developed for some AID, this is not the case for the vast majority. A high degree of clinical suspicion and specialist experience is therefore needed when dealing with patients with suspected AID. Exclusion of other more common disorders, such as infections and malignancy, which have overlapping clinical features with AID, is paramount.

Improved understanding of the molecular disturbances that underpin the pathogenesis of AIDs has helped to shape the treatment options for this patient group. In some cases, this has been life-transforming, for example, the use of IL-1-blocking agents in NLRP3-AID and TRAPS. In some other diseases such as HA20, there is no single best treatment option, but instead the choice of therapy must be individually tailored. Nevertheless, with multiple targeted biological treatments now available and wider use of novel therapies such as JAKi, it is likely that the majority of AID patients can achieve if not a cure, then at least reasonable disease control.

References

1. Gül A (2018) Dynamics of inflammatory response in autoinflammatory disorders: autonomous and hyperinflammatory states. Front Immunol 9:2422
2. Ben-Chetrit E, Gattorno M, Gul A, Kastner DL, Lachmann HJ, Touitou I et al (2018) Consensus proposal for taxonomy and definition of the autoinflammatory diseases (AIDS): a Delphi study. Ann Rheum Dis 77(11):1558–1565
3. Yang J, Zhao Y, Shi J, Shao F (2013) Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. Proc Natl Acad Sci 110(35):14408–14413
4. Chavarría-Smith J, Vance RE (2013) Direct proteolytic cleavage of NLRP1B is necessary and sufficient for inflammasome activation by anthrax lethal factor. PLoS Pathog 9(6):e1003452
5. Pétrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J (2007) Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. Cell Death Differ 14(9):1583–1589
6. Bernot A, Clepet C, Dasilva C, Devaud C, Petit JL, Caloustian C et al (1997) A candidate gene for familial Mediterranean fever. Nat Genet 17(1):25–31
7. Aksentijevich I, Centola M, Deng Z, Sood R, Balow JE, Wood G et al (1997) Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial mediterranean fever The International FMF Consortium* Introduction Group 4. Cell 90(4):797–807
8. Jamilloux Y, Magnotti F, Belot A, Henry T (2018) The pyrin inflammasome: From sensing RhoA GTPases-inhibiting toxins to triggering autoinflammatory syndromes. Pathog Dis 76(3):1–9
9. Wise CA (2002) Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. Hum Mol Genet 11(8):961–969
10. Shinar Y, Tohami T, Livneh A, Schiby G, Hirshberg A, Nagar M et al (2015) Acquired familial Mediterranean fever associated with a somatic MEFV mutation in a patient with JAK2 associated post-polycythemia myelofibrosis. Orphanet J Rare Dis 10(1):1–6
11. Balcı-Peynircioğlu B, Kaya-Akça Ü, Arıcı ZS, Avcı E, Akkaya-ulum ZY, Karadağ Ö et al (2020) Comorbidities in familial Mediterranean fever: analysis of 2000 genetically confirmed patients. Rheumatology 59(6):1372–1380
12. Livneh A, Langevitz P, Zemer D, Zaks N, Kees S, Lidar T et al (1997) Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 40(10):1879–1885
13. Goldfinger SE (1972) Colchicine for familial mediterranean fever. N Engl J Med 287(25):1302–1302. https://doi.org/10.1056/NEJM197212212872514
14. Zemer D, Revach M, Pras M, Modan B, Schor S, Sohar E et al (1974) A controlled trial of colchicine in preventing attacks of familial Mediterranean fever. N Engl J Med 291(18):932–934. https://doi.org/10.1056/NEJM197410312911803
15. Goldstein RC, Schwabe AD (1974) Prophylactic colchicine therapy in familial Mediterranean fever. A controlled, double-blind study. Ann Intern Med 81(6):792–794
16. Dinarello CA, van der Meer JWM (2013) Treating inflammation by blocking interleukin-1 in humans. Semin Immunol 25(6):469–484. https://doi.org/10.1016/j.smim.2013.10.008
17. De Benedetti F, Gattorno M, Anton J, Ben-Chetrit E, Frenkel J, Hoffman HM et al (2018) Canakinumab for the treatment of autoinflammatory recurrent fever syndromes. N Engl J Med 378(20):1908–1919. https://doi.org/10.1056/NEJMoa1706314
18. Moghaddas F, Llamas R, De Nardo D, Martinez-Banaclocha H, Martinez-Garcia JJ, Mesa-Del-Castillo P et al (2017) A novel pyrin-associated autoinflammation with neutrophilic dermatosis mutation further defines 14-3-3 binding of pyrin and distinction to familial Mediterranean fever. Ann Rheum Dis 76(12):2085–2094
19. Unless R, Act P, Rose W, If T, Rose W, Masters SL et al (2016) Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. Sci Transl Med 8(332):1–10. Available from: http://eprints.whiterose.ac.uk/98758/

20. Hong Y, ASI S, Nanthapisal S, Sebire N, Jolles S, Omoyinmi E et al (2019) Autoinflammation due to homozygous S208 MEFV mutation. Ann Rheum Dis 78(4):571–573. Available from: https://ard.bmj.com/content/early/2018/10/24/annrheumdis-2018-214102
21. Park YH, Wood G, Kastner DL, Chae JJ (2016) Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. Nat Immunol 17(8):914–921
22. Simon A, Kremer HPH, Wevers RA, Scheffer H, De Jong JG, Van Der Meer JWM et al (2004) Mevalonate kinase deficiency: evidence for a phenotypic continuum. Neurology 62(6):994–997. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15037710
23. Cuisset L, Drenth JPH, Simon A, Vincent MF, Van Der Velde Visser S, Van Der Meer JWM et al (2001) Molecular analysis of MVK mutations and enzymatic activity in hyper-IgD and periodic fever syndrome. Eur J Hum Genet 9(4):260–266
24. Houten SM, Kuis W, Duran M, De Koning TJ, Van Royen-Kerkhof A, Romeijn GJ et al (1999) Mutations in MVK, encoding mevalonate kinase, cause hyperimmunoglobulinaemia D and periodic fever syndrome. Nat Genet 22(2):175–177
25. Hoffmann GF, Charpentier C, Mayatepek E, Mancini J, Leichsenring M, Gibson KM et al (1993) Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. Pediatrics 91(5):915–921
26. Ter Haar NM, Oswald M, Jeyaratnam J, Anton J, Barron KS, Brogan PA et al (2015) Recommendations for the management of autoinflammatory diseases. Ann Rheum Dis 74(9):1636–1644
27. Bodar EJ, van der Hilst JCH, Drenth JPH, van der Meer JWM, Simon A (2005) Effect of etanercept and anakinra on inflammatory attacks in the hyper-IgD syndrome: Introducing a vaccination provocation model. Neth J Med 63(7):260–264
28. Tsitsami E, Papadopoulou C, Speletas MA (2013) Case of Hyperimmunoglobulinemia D Syndrome Successfully Treated with Canakinumab. Case Rep Rheumatol 2013:1–4
29. Curtis CD, Fox CC (2015) Treatment of adult hyper-IgD syndrome with canakinumab. J Allergy Clin Immunol Pract 3(5):817–818
30. Arostegui JI, Anton J, Calvo I, Robles A, Iglesias E, Lopez-Montesinos B et al (2017 Aug) Open-Label, Phase II Study to assess the efficacy and safety of Canakinumab treatment in active hyperimmunoglobulinemia D with periodic fever syndrome. Arthritis Rheumatol 69(8):1679–1688
31. Demirkaya E, Caglar MK, Waterham HR, Topaloglu R, Ozen S (2007) A patient with hyper-IgD syndrome responding to anti-TNF treatment. Clin Rheumatol 26(10):1757–1759
32. Topaloğlu R, Ayaz NA, Waterham HR, Yüce A, Gumruk F, Sanal Ö, Hyperimmunoglobulinemia D (2008) periodic fever syndrome; treatment with etanercept and follow-up. Clin Rheumatol 27(10):1317–1320
33. Shendi HM, Devlin LA, Edgar JD (2014) Interleukin 6 blockade for hyperimmunoglobulin d and periodic fever syndrome. J Clin Rheumatol 20(2):103–105
34. Arkwright PD, Abinun M, Cant AJ (2007) Mevalonic aciduria cured by bone marrow transplantation. N Engl J Med 357(13):1350–1350. https://doi.org/10.1056/NEJMc072018
35. Neven B, Valayannopoulos V, Quartier P, Blanche S, Prieur A-M, Debré M et al (2007) Allogeneic bone marrow transplantation in mevalonic aciduria. N Engl J Med 356(26):2700–2703. https://doi.org/10.1056/NEJMoa070715
36. Pelegrín P (2011) Inflammasome activation by danger signals. In: The inflammasomes. Springer, Basel
37. Saito M, Nishikomori R, Kambe N, Fujisawa A, Tanizaki H, Takeichi K et al (2008) Disease-associated CIAS1 mutations induce monocyte death, revealing low-level mosaicism in mutation-negative cryopyrin-associated periodic syndrome patients. Blood 111(4):2132–2141. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18063752
38. Zhou Q, Aksentijevich I, Wood GM, Walts AD, Hoffmann P, Remmers EF et al (2015) Cryopyrin-associated periodic syndrome caused by a myeloid-restricted somatic NLRP3 mutation. Arthritis Rheumatol 67(9):2482–2486

39. Rowczenio DM, Gomes SM, Aróstegui JI, Mensa-Vilaro A, Omoyinmi E, Trojer H et al (2017) Late-onset cryopyrin-associated periodic syndromes caused by somatic NLRP3 mosaicism-UK single center experience. Front Immunol 8:1410
40. Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD (2001) Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle–Wells syndrome. Nat Genet 29(3):301–305. Available from: http://www.nature.com/articles/ng756z
41. Aksentijevich I, Putnam CD, Remmers EF, Mueller JL, Le J, Kolodner RD et al (2007) The clinical continuum of cryopyrinopathies: Novel CIAS1 mutations in North American patients and a new cryopyrin model. Arthritis Rheum 56(4):1273–1285
42. Goldbach-Mansky R (2011) Current status of understanding the pathogenesis and management of patients with NOMID/CINCA. Curr Rheumatol Rep 13(2):123–131
43. Kuemmerle-Deschner JB, Ozen S, Tyrrell PN, Kone-Paut I, Goldbach-Mansky R, Lachmann H et al (2017) Diagnostic criteria for cryopyrin-associated periodic syndrome (CAPS). Ann Rheum Dis 76(6):942–947. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27707729
44. Neven B, Marvillet I, Terrada C, Ferster A, Boddaert N, Couloignier V et al (2010) Long-term efficacy of the interleukin-1 receptor antagonist anakinra in ten patients with neonatal-onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, articular syndrome. Arthritis Rheum 62(1):258–267
45. Kuemmerle-Deschner JB, Wittkowski H, Tyrrell PN, Koetter I, Lohse P, Ummenhofer K et al (2013) Treatment of Muckle-Wells syndrome: analysis of two IL-1-blocking regimens. Arthritis Res Ther 15(3):1–8
46. Canna SW, Girard C, Malle L, de Jesus A, Romberg N, Kelsen J et al (2017) Life-threatening NLRC4-associated hyperinflammation successfully treated with IL-18 inhibition. J Allergy Clin Immunol 139(5):1698–1701. Available from: https://www.sciencedirect.com/science/article/pii/S0091674916313525
47. Canna SW, de Jesus AA, Gouni S, Brooks SR, Marrero B, Liu Y et al (2014) An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nat Genet 46(10):1140–1146. Available from: http://www.nature.com/articles/ng.3089
48. Romberg N, Al Moussawi K, Nelson-Williams C, Stiegler AL, Loring E, Choi M et al (2014) Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. Nat Genet 46(10):1135–1139. Available from: http://www.nature.com/articles/ng.3066
49. Volker-Touw CML, de Koning HD, Giltay JC, de Kovel CGF, van Kempen TS, Oberndorff KMEJ et al (2017) Erythematous nodes, urticarial rash and arthralgias in a large pedigree with NLRC4-related autoinflammatory disease, expansion of the phenotype. Br J Dermatol 176(1):244–248
50. Kawasaki Y, Oda H, Ito J, Niwa A, Tanaka T, Hijikata A et al (2017) Identification of a high-frequency somatic NLRC4 mutation as a cause of autoinflammation by pluripotent cell–based phenotype dissection. Arthritis Rheumatol 69(2):447–459
51. Jéru I, Duquesnoy P, Fernandes-Alnemri T, Cochet E, Yu JW, Lackmy-Port-Lis M et al (2008) Mutations in NALP12 cause hereditary periodic fever syndromes. Proc Natl Acad Sci U S A 105(5):1614–1619
52. Grandemange S, Sanchez E, Louis-Plence P, Tran Mau-Them F, Bessis D, Coubes C et al (2017) A new autoinflammatory and autoimmune syndrome associated with NLRP1 mutations: NAIAD (NLRP1- associated autoinflammation with arthritis and dyskeratosis). Ann Rheum Dis 76(7):1191–1198
53. Zhou Q, Wang H, Schwartz DM, Stoffels M, Park YH, Zhang Y et al (2016) Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. Nat Genet 48(1):67–73. Available from: http://www.nature.com/articles/ng.3459
54. Matmati M, Jacques P, Maelfait J, Verheugen E, Kool M, Sze M et al (2011) A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. Nat Genet 43(9):908–912. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21841782

55. Lawless D, Pathak S, Scambler TE, Ouboussad L, Anwar R (2018) Savic S. A case of adult-Onset Still's disease caused by a novel splicing mutation in TNFAIP3 successfully treated with tocilizumab. Front Immunol 9:1–8
56. Takagi M, Ogata S, Ueno H, Yoshida K, Yeh T, Hoshino A et al (2017) Haploinsufficiency of TNFAIP3 (A20) by germline mutation is involved in autoimmune lymphoproliferative syndrome. J Allergy Clin Immunol 139(6):1914–1922. https://doi.org/10.1016/j.jaci.2016.09.038
57. Aeschlimann FA, Batu ED, Canna SW, Go E, Gül A, Hoffmann P et al (2018) A20 haploinsufficiency (HA20): Clinical phenotypes and disease course of patients with a newly recognised NF-kB-mediated autoinflammatory disease. Ann Rheum Dis 77(5):728–735. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29317407
58. Mulhern CM, Hong Y, Omoyinmi E, Jacques TS, D'Arco F, Hemingway C et al (2019) Janus kinase 1/2 inhibition for the treatment of autoinflammation associated with heterozygous TNFAIP3 mutation. J Allergy Clin Immunol 144(3):863–866. e5
59. Damgaard RB, Walker JA, Marco-Casanova P, Morgan NV, Titheradge HL, Elliott PR et al (2016) The Deubiquitinase OTULIN is an essential negative regulator of inflammation and autoimmunity. Cell 166(5):1215–1230.e20. https://doi.org/10.1016/j.cell.2016.07.019
60. Zhou Q, Yu X, Demirkaya E, Deuitch N, Stone D, Tsai WL et al (2016) Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. Proc Natl Acad Sci 113(36):10127–10132. https://doi.org/10.1073/pnas.1612594113
61. Hayden MS, Ghosh S (2012) NF-κB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev 26(3):203–234
62. Badran YR, Dedeoglu F, Leyva Castillo JM, Bainter W, Ohsumi TK, Bousvaros A et al (2017) Human RELA haploinsufficiency results in autosomal-dominant chronic mucocutaneous ulceration. J Exp Med 214(7):1937–1947. https://doi.org/10.1084/jem.20160724
63. Comrie WA, Faruqi AJ, Price SR, Zhang Y, Koneti Rao V, Su HC et al (2018) RELA haploinsufficiency in CD4 lymphoproliferative disease with autoimmune cytopenias. J Allergy Clin Immunol 141(4):1507–1510. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0091674917330117
64. Crow YJ (2011) Type I interferonopathies: a novel set of inborn errors of immunity. Ann N Y Acad Sci 1238(1):91–98
65. Lee-Kirsch MA, Wolf C, Kretschmer S, Roers A (2015) Type I interferonopathies—an expanding disease spectrum of immunodysregulation. Semin Immunopathol 37(4):349–357
66. Crow YJ, Rehwinkel J (2009) Aicardi-Goutieres syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity. Hum Mol Genet 18(R2):130–136
67. Rice GI, del Toro Duany Y, Jenkinson EM, Forte GMA, Anderson BH, Ariaudo G et al (2014) Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. Nat Genet 46(5):503–509. Available from: http://www.nature.com/articles/ng.2933
68. Crow YJ, Chase DS, Lowenstein Schmidt J, Szynkiewicz M, Forte GMA, Gornall HL et al (2015) Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, and IFIH1. Am J Med Genet Part A 167(2):296–312. https://doi.org/10.1002/ajmg.a.36887
69. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Sanchez GAM et al (2014) Activated STING in a vascular and pulmonary syndrome. N Engl J Med 371(6):507–518. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25029335
70. Jeremiah N, Neven B, Gentili M, Callebaut I, Maschalidi S, Stolzenberg M et al (2014) Inherited STING-activating mutation underlies a familial inflammatory. J Clin Invest 124(12):5516–5520
71. Omoyinmi E, Melo Gomes S, Nanthapisal S, Woo P, Standing A, Eleftheriou D et al (2015) Stimulator of interferon genes-associated vasculitis of infancy. Arthritis Rheumatol 67(3):808
72. Brehm A, Liu Y, Sheikh A, Marrero B, Omoyinmi E, Zhou Q et al (2016) Additive loss-of-function proteasome subunit mutations in CANDLE/PRAAS patients promote type I IFN production. J Clin Invest 126(2):795–795

73. Agarwal AK, Xing C, Demartino GN, Mizrachi D, Hernandez MD, Sousa AB et al (2010) PSMB8 encoding the β5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. Am J Hum Genet 87(6):866–872
74. Montealegre Sanchez GA, Reinhardt A, Ramsey S, Wittkowski H, Hashkes PJ, Berkun Y et al (2018) JAK1/2 inhibition with baricitinib in the treatment of autoinflammatory interferonopathies. J Clin Invest 128(7):3041–3052. https://doi.org/10.1172/JCI98814
75. Rodero MP, Tesser A, Bartok E, Rice GI, Della Mina E, Depp M et al (2017) Type i interferon-mediated autoinflammation due to DNase II deficiency. Nat Commun 8(1):1–15
76. Hashkes PJ, Laxer RM (2019) Textbook of autoinflammation. Springer International Publishing, Cham, Switzerland, Chapter 18, pages 329–345
77. https://www.printo.it/eurofever/autoinflammatory_diseases.asp. Eurofever Project [Internet]. [cited 2020 Feb 18]. Available from: https://www.printo.it/eurofever/
78. Pelagatti MA, Meini A, Caorsi R, Cattalini M, Federici S, Zulian F et al (2011) Long-term clinical Profile of children with the low-penetrance R92Q mutation of the tNFRSF1A gene. Arthritis Rheum 63(4):1141–1150
79. Jawaheer D, Seldin MF, Amos CI, Chen WV, Shigeta R, Monteiro J et al (2001) A Genomewide screen in multiplex rheumatoid arthritis families suggests genetic overlap with other autoimmune diseases. Am J Hum Genet 68(4):927–936
80. Direskeneli H, Saruhan-Direskeneli G, Amoura Z, Dode C, Wechsler B, Piette JC (2005) R92Q TNFRSF1A mutation and Behçet's disease: Comment on the article by Amoura et al. [5] (multiple letters). Arthritis Rheum 52(8):2583–2584
81. Gattorno M, Pelagatti MA, Meini A, Obici L, Barcellona R, Federici S et al (2008) Persistent efficacy of anakinra in patients with tumor necrosis factor receptor-associated periodic syndrome. Arthritis Rheum 58(5):1516–1520
82. Bulua AC, Mogul DB, Aksentijevich I, Singh H, He DY, Muenz LR et al (2012) Efficacy of etanercept in the tumor necrosis factor receptor-associated periodic syndrome: a prospective, open-label, dose-escalation study. Arthritis Rheum 64(3):908–913
83. Nedjai B, Hitman GA, Quillinan N, Coughlan RJ, Church L, McDermott MF et al (2009) Proinflammatory action of the antiinflammatory drug infliximab in tumor necrosis factor receptor-associated periodic syndrome. Arthritis Rheum 60(2):619–625
84. Vaitla PM, Radford PM, Tighe PJ, Powell RJ, McDermott EM, Todd I et al (2011) Role of interleukin-6 in a patient with tumor necrosis factor receptor-associated periodic syndrome. Arthritis Rheum 63(4):1151–1155
85. Reddy S, Jia S, Geoffrey R, Lorier R, Suchi M, Broeckel U et al (2009) An autoinflammatory disease due to homozygous deletion of the IL1RN locus. N Engl J Med 360(23):2438–2444
86. Aksentijevich I, Masters SL, Ferguson PJ, Dancey P, Frenkel J, Van Royen-Kerkhoff A et al (2009) An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. N Engl J Med 360(23):2426–2437
87. Thacker PG, Binkovitz LA, Thomas KB (2012) Deficiency of interleukin-1-receptor antagonist syndrome: a rare auto-inflammatory condition that mimics multiple classic radiographic findings. Pediatr Radiol 42(4):495–498
88. Gabay C, Towne JE (2015) Regulation and function of interleukin-36 cytokines in homeostasis and pathological conditions. J Leukoc Biol 97(4):645–652
89. Marrakchi S, Guigue P, Renshaw BR, Puel A, Pei XY, Fraitag S et al (2011) Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. N Engl J Med 365(7):620–628
90. Onoufriadis A, Simpson MA, Pink AE, Di Meglio P, Smith CH, Pullabhatla V et al (2011) Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. Am J Hum Genet [Internet] 89(3):432–437. https://doi.org/10.1016/j.ajhg.2011.07.022
91. De Inocencio J, Mensa-Vilaro A, Tejada-Palacios P, Enriquez-Merayo E, González-Roca E, Magri G et al (2015) Somatic NOD2 mosaicism in Blau syndrome. J Allergy Clin Immunol 136(2):484–487. e2

92. Rosé CD, Doyle TM, McIlvain-Simpson G, Coffman JE, Rosenbaum JT, Davey MP et al (2005) Blau syndrome mutation of CARD15/NOD2 in sporadic early onset granulomatous arthritis. J Rheumatol 32(2):373–375
93. Sarens IL, Casteels I, Anton J, Bader-Meunier B, Brissaud P, Chédeville G et al (2018) Blau syndrome–associated uveitis: preliminary results from an international prospective interventional case series. Am J Ophthalmol 187:158–166
94. Saini SK, Rose CD (1996) Liver involvement in familial granulomatous arthritis (Blau syndrome). J Rheumatol [Internet] 23(2):396–399. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8882056
95. Aróstegui JI, Arnal C, Merino R, Modesto C, Carballo MA, Moreno P et al (2007) NOD2 gene-associated pediatric granulomatous arthritis: Clinical diversity, novel and recurrent mutations, and evidence of clinical improvement with interleukin-1 blockade in a Spanish cohort. Arthritis Rheum 56(11):3805–3813
96. Becker ML, Martin TM, Doyle TM, Rosé CD (2007) Interstitial pneumonitis in Blau syndrome with documented mutation in CARD15. Arthritis Rheum 56(4):1292–1294
97. See Ting S, Ziegler J, Fischer E (1998) Familial granulomatous arthritis (Blau syndrome) with granulomatous renal lesions. J Pediatr 133(3):450–452
98. Milman N, Andersen CB, Hansen A, Van Overeem Hansen T, Nielsen FC, Fledelius H et al (2006) Favourable effect of TNF-α inhibitor (infliximab) on Blau syndrome in monozygotic twins with a de novo CARD15 mutation. APMIS 114(12):912–919
99. Simonini G, Xu Z, Caputo R, De Libero C, Pagnini I, Pascual V et al (2013) Clinical and transcriptional response to the long-acting interleukin-1 blocker canakinumab in Blau syndrome-related uveitis. Arthritis Rheum 65(2):513–518
100. Lu L, Shen M, Jiang D, Li Y, Zheng X, Li Y et al (2018) Blau syndrome with good reponses to Tocilizumab: a case report and focused literature review. Semin Arthritis Rheum 47(5):727–731
101. Rosé CD, Pans S, Casteels I, Anton J, Bader-Meunier B, Brissaud P et al (2015) Blau syndrome: cross-sectional data from a multicentre study of clinical, radiological and functional outcomes. Rheumatol (United Kingdom) 54(6):1008–1016
102. Wakil SM, Monies DM, Abouelhoda M, Al-Tassan N, Al-Dusery H, Naim EA et al (2015) Association of a mutation in LACC1 with a monogenic form of systemic juvenile idiopathic arthritis. Arthritis Rheumatol 67(1):288–295
103. Li Y, Führer M, Bahrami E, Socha P, Klaudel-Dreszler M, Bouzidi A et al (2019) Human RIPK1 deficiency causes combined immunodeficiency and inflammatory bowel diseases. Proc Natl Acad Sci 116(3):970–975. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30591564
104. Cuchet-Lourenço D, Eletto D, Wu C, Plagnol V, Papapietro O, Curtis J et al (2018) Biallelic RIPK1 mutations in humans cause severe immunodeficiency, arthritis, and intestinal inflammation. Science 361(6404):810–813. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30026316
105. Tao P, Sun J, Wu Z, Wang S, Wang J, Li W et al (2020) A dominant autoinflammatory disease caused by non-cleavable variants of RIPK1. Nature [Internet] 577(7788):109–114. https://doi.org/10.1038/s41586-019-1830-y
106. Lalaoui N, Boyden SE, Oda H, Wood GM, Stone DL, Chau D et al (2020) Mutations that prevent caspase cleavage of RIPK1 cause autoinflammatory disease. Nature [Internet] 577(7788):103–108. https://doi.org/10.1038/s41586-019-1828-5
107. Israël A (2010) The IKK complex, a central regulator of NF-kappaB activation. Cold Spring Harb Perspect Biol 2(3):a000158
108. Takada H, Nomura A, Ishimura M, Ichiyama M, Ohga S, Hara T (2010) NEMO mutation as a cause of familial occurrence of Behçet's disease in female patients. Clin Genet 78(6):575–579
109. Döffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A et al (2001) X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-κB signaling. Nat Genet 27(3):277–285

110. Hanson EP, Monaco-Shawver L, Solt LA, Madge LA, Banerjee PP, May MJ et al (2008) Hypomorphic NEMO mutation database and reconstitution system identifies phenotypic and immunologic diversity. J Allergy Clin Immunol 122(6):1169–1177
111. Orange JS, Geha RS (2003) Finding NEMO: genetic disorders of NF-κB activation. J Clin Invest 112(7):983–985
112. Miot C, Imai K, Imai C, Mancini AJ, Kucuk ZY, Kawai T et al (2017) Hematopoietic stem cell transplantation in 29 patients hemizygous for hypomorphic IKBKG/NEMO mutations. Blood 130(12):1456–1467
113. Mizukami T, Obara M, Nishikomori R, Kawai T, Tahara Y, Sameshima N et al (2012) Successful treatment with infliximab for inflammatory colitis in a patient with X-linked anhidrotic ectodermal dysplasia with immunodeficiency. J Clin Immunol 32(1):39–49
114. Klemann C, Pannicke U, Morris-Rosendahl DJ, Vlantis K, Rizzi M, Uhlig H et al (2016) Transplantation from a symptomatic carrier sister restores host defenses but does not prevent colitis in NEMO deficiency. Clin Immunol 164:52–56
115. Boisson B, Laplantine E, Prando C, Giliani S, Israelsson E, Xu Z et al (2012) Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. Nat Immunol 13(12):1178–1186
116. Tuijnenburg P, Lango Allen H, Burns SO, Greene D, Jansen MH, Staples E et al (2018) Loss-of-function nuclear factor κB subunit 1 (NFKB1) variants are the most common monogenic cause of common variable immunodeficiency in Europeans. J Allergy Clin Immunol 142(4):1285–1296
117. Kaustio M, Haapaniemi E, Göös H, Hautala T, Park G, Syrjänen J et al (2017) Damaging heterozygous mutations in NFKB1 lead to diverse immunologic phenotypes. J Allergy Clin Immunol 140(3):782–796
118. Elkan PN, Pierce SB, Segel R, Walsh T, Barash J, Padeh S et al (2014) Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy. N Engl J Med 370(10):921–931
119. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Zavialov AV, Lauvau G (2010) Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol 88(2):279–290
120. Zhou Q, Yang D, Ombrello AK, Zavialov AV, Toro C, Zavialov AV et al (2014) Early-onset stroke and vasculopathy associated with mutations in ADA2. N Engl J Med [Internet] 370(10):911–920. https://doi.org/10.1056/NEJMoa1307361
121. Caorsi R, Penco F, Grossi A, Insalaco A, Omenetti A, Alessio M et al (2017) ADA2 deficiency (DADA2) as an unrecognised cause of early onset polyarteritis nodosa and stroke: a multicentre national study. Ann Rheum Dis 76(10):1648–1656
122. Schepp J, Proietti M, Frede N, Buchta M, Hübscher K, Rojas Restrepo J et al (2017) Screening of 181 patients with antibody deficiency for deficiency of adenosine deaminase 2 sheds new light on the disease in adulthood. Arthritis Rheumatol 69(8):1689–1700
123. Hashem H, Kumar AR, Müller I, Babor F, Bredius R, Dalal J et al (2017) Hematopoietic stem cell transplantation rescues the hematological, immunological, and vascular phenotype in DADA2. Blood 130(24):2682–2688
124. Ombrello AK, Qin J, Hoffmann PM, Kumar P, Stone D, Jones A, Romeo T, Barham B, Pinto-Patarroyo G, Toro C, Soldatos A (2019) Treatment strategies for deficiency of adenosine deaminase 2. N Engl J Med 380(16):1582–1584
125. Gresset A, Hicks SN, Harden TK, Sondek J (2010) Mechanism of phosphorylation-induced activation of phospholipase C-γ isozymes. J Biol Chem 285(46):35836–35847
126. Ombrello MJ, Remmers EF, Sun G, Freeman AF, Datta S, Torabi-Parizi P et al (2012) Cold urticaria, immunodeficiency, and autoimmunity related to PLCG2 deletions. N Engl J Med 366(4):330–338
127. Morán-Villaseñor E, Saez-de-Ocariz M, Torrelo A, Arostegui JI, Yamazaki-Nakashimada MA, Alcántara-Ortigoza MA et al (2019) Expanding the clinical features of autoinflammation and phospholipase Cγ2-associated antibody deficiency and immune dysregulation by description of a novel patient. J Eur Acad Dermatol Venereol 33(12):2334–2339

128. Neves JF, Doffinger R, Barcena-Morales G, Martins C, Papapietro O, Plagnol V et al (2018) Novel PLCG2 Mutation in a patient with APLAID and Cutis Laxa. Front Immunol 9:2863
129. Zhou Q, Lee GS, Brady J, Datta S, Katan M, Sheikh A et al (2012) A hypermorphic missense mutation in PLCG2, encoding phospholipase Cγ2, causes a dominantly inherited autoinflammatory disease with immunodeficiency. Am J Hum Genet [Internet] 91(4):713–720. https://doi.org/10.1016/j.ajhg.2012.08.006
130. Novice T, Kariminia A, Del Bel KL, Lu H, Sharma M, Lim CJ et al (2019) A germline mutation in the C2 domain of PLCγ2 associated with gain-of-function expands the phenotype for PLCG2-related diseases. J Clin Immunol 40(2):267–276
131. Standing ASI, Malinova D, Hong Y, Record J, Moulding D, Blundell MP et al (2017) Autoinflammatory periodic fever, immunodeficiency, and thrombocytopenia (PFIT) caused by mutation in actin-regulatory gene *WDR1*. J Exp Med [Internet] 214(1):59–71. https://doi.org/10.1084/jem.20161228
132. Kile BT, Panopoulos AD, Stirzaker RA, Hacking DF, Tahtamouni LH, Willson TA et al (2007) Mutations in the cofilin partner Aip1/Wdr1 cause autoinflammatory disease and macrothrombocytopenia. Blood 110(7):2371–2380
133. Brydges SD, Broderick L, McGeough MD, Pena CA, Mueller JL, Hoffman HM (2013) Divergence of IL-1, IL-18, and cell death in NLRP3 inflammasomopathies. J Clin Invest 123(11):4695–4705
134. Volpi S, Cicalese MP, Tuijnenburg P, Tool ATJ, Cuadrado E, Abu-Halaweh M et al (2019) A combined immunodeficiency with severe infections, inflammation, and allergy caused by ARPC1B deficiency. J Allergy Clin Immunol i:2296–2299
135. Kahr WHA, Pluthero FG, Elkadri A, Warner N, Drobac M, Chen CH et al (2017) Loss of the Arp2/3 complex component ARPC1B causes platelet abnormalities and predisposes to inflammatory disease. Nat Commun 8:1–14
136. Wiseman DH, May A, Jolles S, Connor P, Powell C, Heeney MM et al (2013) A novel syndrome of congenital sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD). Blood 122(1):112–123

Lung in Cellular Immunodeficiencies

12

Emanuele Vivarelli and Lorenzo Cosmi

Abstract

The lung is a very complex organ, devoted to the oxygen supply to the whole body. The essential role of the respiratory system forces lung microenvironment to a continuous contact with air and with the external world. Lung epithelium and lung connective tissue are protected by an articulated and pervasive immune system, which fights the external microbial and toxic threats while preserving respiratory function and blood oxygenation. The balance between microbial clearance and preservation of tissue function is of pivotal importance because not only microbial growth but also immunopathology in this organ can be a serious threat to the whole organism survival. Primary immunodeficiencies (PID) can be associated with a wide range of systemic and lung complications (see Table 12.1); lung complications (see Table 12.2) in PID can be broadly divided in infections and chronic complications. In this chapter, we review the main clinical and epidemiological features associated with lung involvement in PID.

Keywords

Lung · Immunodeficiency · Pneumonia · Granuloma · Interstitial lung disease GLILD · Bronchiectasis · Chronic obstructive lung disease · Lymphoma

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Table 12.1 Clinical features in selected PIDs

PID	Infective complications	Autoimmunity/other features	Neoplastic diseases
SCID/CID	Pyogenic pneumonia Interstitial pneumonia (viral or PJ) Failure to thrive, bronchiectasis Chronic diarrhea Disseminated CMV, HZV infections Disseminated molluscum contagiosum Disseminated NTM/BCG infections Pyogenic meningitis Mucocutaneous candidiasis Invasive fungal infections (Aspergillus, Cryptococcus, dimorphic fungi)	ILD, PAP Generalized erythroderma Eczema Autoimmune cytopenias Granulomatous disease Arthritis, GPA Type I DM	Non-Hodgkin lymphoma Hodgkin lymphoma AML, ALL
HIGM	PJ/adenovirus pneumonia Pyogenic pneumonia Failure to thrive, bronchiectasis Disseminated CMV infections Disseminated NTM/BCG infections Cryptosporidium cholangitis Invasive fungal infections (Aspergillus, Cryptococcus, dimorphic fungi) Visceral leishmaniasis	Autoimmune neutropenia Immune thrombocytopenia Hemolytic anemia Hepatosplenomegaly Lymphadenopathy Sclerosing cholangitis Autoimmune hepatitis IBD, uveitis, arthritis Aphthous ulcers Osteopenia	Bile duct carcinoma Biliary tree tumors Hepatocellular carcinoma Neuroendocrine tumors Lymphoma
CVID	Pyogenic pneumonia, bronchiectasis Upper respiratory tract infections Giardia, *C. jejuni*, Salmonella spp., Norovirus infections Bacterial meningitis Septic arthritis	ILD, arthritis Autoimmune cytopenias Hepatosplenomegaly Lymphadenopathy Celiac-like disease, IBD Granulomatous disease Liver nodular hyperplasia	NHL, HL Gastric cancer
AD-HIES	Necrotizing pneumonia, empyema Pneumatocele, bronchiectasis Chronic airways infections (Aspergillus, *P. aeruginosa*, NTM) Mucocutaneous candidiasis	Eczema Vascular aneurysms High-arched palate Teeth abnormalities Scoliosis, osteopenia	NHL, HL
AR-HIES	Same as AD-HIES HZV, HPV, CMV, JC infections Molluscum contagiosum	Eczema, asthma Food allergy Vasculitis	Squamous cell carcinomas NHL, HL

Table 12.1 (continued)

PID	Infective complications	Autoimmunity/other features	Neoplastic diseases
GATA2 deficiency	NTM infections HPV, CMV, EBV, HSV infections *C. difficile* colitis Mucocutaneous candidiasis Invasive fungal infections (Aspergillus, Histoplasma)	Panniculitis, arthritis Erythema nodosum Lymphedema, PAP Sensorineural hearing loss Thrombosis	MDS, AML Squamous cell carcinomas Breast cancer

AD-HIES/AR-HIES autosomal dominant/autosomal recessive hyper-IgE syndrome, *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *BCG* bacillus Calmette-Guerin, *CID* combined immunodeficiency, *CMV* cytomegalovirus, *CVID* common variable immunodeficiency, *DM* diabetes mellitus, *EBV* Epstein-Barr virus, *GPA* granulomatosis with polyangiitis, *HIGM* hyper-IgM syndrome, *HL* Hodgkin lymphoma, *HPV* human papillomavirus, *HSV* herpes simplex virus, *JC* John-Cunningham, *IBD* inflammatory bowel disease, *ILD* interstitial lung disease, *MDS* myelodysplastic syndrome. *NHL* non-Hodgkin lymphoma, *NTM* nontuberculous mycobacteria, *PAP* pulmonary alveolar proteinosis, *PID* primary immunodeficiency, *PJ* Pneumocystis jiroveci, *RSV* respiratory syncytial virus, *SCID* severe combined immunodeficiency, *VZV* varicella-zoster virus

12.1 Lung Infections

The lung is an enormous organ with a very huge surface in contact with external world. For this reason, immune system has to patrol this large boundary to avoid microbial invasion. It is therefore not surprising that respiratory infections are so common in PID and contribute significantly to morbidity and mortality. Recurrent pneumonias, the most common form of recurrent lung infections, are considered one of the ten warning signs for PID by the Jeffrey Modell Foundation; in particular, at least two pneumonias per year in children and one pneumonia per year for more than 1 year in adults are the clinical signs that should raise suspicion for PID. The spectrum of lung infections is wide, ranging from forms clinically indistinguishable from community-acquired pneumonia to lung abscess and necrotizing pneumonia. The microorganisms responsible for lung infections are very different, according to the underlying immune defect.

12.1.1 Bacterial Infections

Recurrent bacterial infections of the lower respiratory tract due to encapsulated bacteria (*Streptococcus pneumoniae*, Haemophilus influenzae, Moraxella catarrhalis) is a prominent feature of primary humoral and complement immunodeficiencies, which are not the issue of this chapter; however, recurrent pneumonias by encapsulated bacteria are commonly found in other PID involving the cellular immunity that are characterized by a partial or total humoral defect such as common variable immunodeficiency (CVID) or immune dysregulation syndromes (CTLA-4

Table 12.2 Lung involvement in selected PIDs

PID	Lung infections	Lung autoimmunity	Bronchiectasis	Lung cancers
SCID/CID	*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *M. tuberculosis*, NTM, BCG, adenovirus, VZV, CMV, RSV, PJ, Cryptococcus spp., dimorphic fungi	GLILD, OP, PAP	Yes	DLBCL
HIGM	*S. pneumoniae*, *P. aeruginosa*, Serratia spp., *M. tuberculosis*, NTM, CMV, PJ, Cryptococcus spp., Aspergillus spp.	Absent	Yes	Absent
ICL	*M. tuberculosis*, NTM, BCG, Nocardia spp., VZV, CMV, PJ, Cryptococcus spp., Aspergillus spp.	OP, sarcoidosis-like disease	Rare	DLBCL
CVID	*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*	GLILD, OP, LIP	Yes	MZL, LYG, DLBCL
CTLA4 deficiency	*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, CMV	GLILD, OP	Yes	DLBCL
LRBA deficiency	*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, CMV	GLILD, OP	Yes	DLBCL
STAT1 GOF	*S. pneumoniae*, *H. influenzae*, *P. aeruginosa*, *S. aureus*, CMV, PJ, Cryptococcus spp., dimorphic fungi, *M. tuberculosis*	Absent	Yes	Absent
STAT3 GOF	*S. pneumoniae*, *H. influenzae*, *P. aeruginosa*, *S. aureus*	LIP, OP, DIP, GLILD, UIP	Yes	Absent
APDS	*S. pneumoniae*, *H. influenzae*, *P. aeruginosa*, *M. catarrhalis*, CMV, RSV, adenovirus, rhinovirus	Nodular lymphoid hyperplasia	Yes	DLBCL, MZL
AD-HIES/ AR-HIES	*S. aureus*, *S. pneumoniae*, *H. influenzae*, *P. aeruginosa*, Aspergillus spp., NTM, PJ	Absent	Yes	Absent
MSMD	Salmonella spp., Klebsiella spp., *M. tuberculosis*, NTM, BCG, dimorphic fungi	Absent	No	Occasional
GATA 2 deficiency	NTM, CMV, Aspergillus spp., Histoplasma spp.	OP, PAP	No	Absent

AD-HIES/AR-HIES autosomal dominant/autosomal recessive hyper-IgE syndrome, *APDS* activated PI3K-delta syndrome, *BCG* bacillus Calmette-Guerin, *CID* combined immunodeficiency, *CMV* cytomegalovirus, *CTLA4* cytotoxic T-lymphocyte antigen 4, *CVID* common variable immunodeficiency, *DIP* desquamative interstitial pneumonia, *DLBCL* diffuse large B-cell lymphoma, *GLILD* granulomatous and lymphocytic interstitial lung disease, *HIGM* hyper-IgM syndrome, *ICL* idiopathic CD4+ lymphopenia, *LIP* lymphocytic interstitial pneumonia, *LRBA* LPS-responsive beige-like anchor protein, *LYG* lymphomatoid granulomatosis, *MSMD* Mendelian susceptibility to mycobacterial disease, *MZL* marginal zone lymphoma, *NTM* nontuberculous mycobacteria, *OP* organizing pneumonia, *PAP* pulmonary alveolar proteinosis, *PID* primary immunodeficiency, *PJ* Pneumocystis jiroveci, *RSV* respiratory syncytial virus, *SCID* severe combined immunodeficiency, *STAT 1/3* signal transducer and activator of transcription 1/3, *UIP* usual interstitial pneumonia, *VZV* varicella-zoster virus

deficiency, LRBA deficiency, APDS; see below). In PID patients, these common bacteria cause pneumonia that is clinically indistinguishable from community-acquired pneumonia in immunocompetent hosts even if the clinical picture can be sometimes blunted. In PID patients, however, pneumonias caused by encapsulated bacteria show a more severe and aggressive course, are usually recurrent, heal more slowly than in immunocompetent host, and are more prone to complications such as lung abscesses; prolonged antibiotic course, intravenous route of administration, and hospitalization are frequently needed to cure these infections [1].

Among extracellular bacteria, *Staphylococcus aureus* is known to cause many community and health-care-associated infections, with a special tropism for skin, soft tissues, and lung. About 20–30% of the population is colonized by *S. aureus* at the mucosal surfaces, and even more people harbor this pathogen intermittently [2]. Immune response against *S. aureus* is dependent, as for many bacteria, on both innate and adaptive immune response. Innate immune response against *S. aureus* is critically dependent on neutrophils; epithelial surfaces can sense *S. aureus* lipoproteins via Toll-like receptor 2 (TLR2) and a variety of pattern recognition receptors which activate the inflammasome complex. The inflammasome complex can recruit neutrophils in an IL-1-dependent manner, as well as blood-derived monocytes and dendritic cells, which can present *S. aureus* antigens to lymphocytes in the secondary lymphoid organs [3]. Regarding adaptive immune response against *S. aureus*, it has been shown that humoral immune response is useful but is dispensable in the protection of the host because patients with primary antibody deficiencies don't show increased susceptibility to *S. aureus* infections [4]. Cellular immune response has a pivotal role in clearing *S. aureus* infections, especially Th17 response. Th17 lymphocytes are a subset of CD4+ lymphocytes, which are very important for the control of extracellular pathogens infections [5]. Th17 lymphocytes develop from activated CD4+ lymphocytes in response to proinflammatory cytokines such as IL-1, IL-6, IL-21, and IL-23, which are abundant in the inflammatory milieu; this subset depends on the transcriptional program induced by RORC transcription factor [6]. Th17 cells exploit at least two mechanisms to enhance clearance of extracellular pathogens. Th17 cells produce IL-17, which induces the production of chemokines such as granulocyte colony-stimulating factor (G-CSF) and C-X-C motif chemokine ligand 1 (CXCL1). These chemokines recruit neutrophils at the sites of inflammation. On top of this, IL-17 cells induce the expression of antimicrobial proteins such as Lipocalin-237, β-defensin, S100A7 (psoriasin), S100A8/9 (calprotectin), and cathelicidin (LL37) by the epithelium, resulting in pathogen control. In the human host, recurrent infections with *Staphylococcus aureus* are a hallmark of some particular PID, called hyper-IgE syndromes (HIES). HIES are a group of PID which are caused by mutations in STAT3, DOCK8, PMG3, and TYK2 genes; STAT3 mutations show an autosomal dominant pattern of inheritance (AD-HIES), while the other mutations are characterized by an autosomal recessive pattern of inheritance (AR-HIES). Signal Transducer and Activator of Transcription 3 (STAT3) is a transcription factor that signals downstream of the main Th17-inducing cytokines (IL-6, IL-21, IL-23); loss-of-function (LOF) mutations in this gene abolish Th17 subset, which is typically lacking in AD-HIES [7]. This immunological

feature is responsible for the high susceptibility to *S. aureus* lung infections. AD-HIES patients usually suffer from recurrent necrotizing pneumonias; the causative agents are not only *S. aureus* but also *S. pneumoniae* and H. influenzae. Common presenting symptoms are fever, cough, dyspnea, and hypoxemia. However, systemic signs of infection can be mild, with some patients presenting with low-grade fever, normal white blood cell count, and normal inflammatory markers. Airways inflammation is severe with production of highly tenacious mucus, and the infection has a highly destructive course, with necrosis, liquefaction, and cavitation of lung tissue, that may lead to the formation of pneumatoceles and bronchiectasis, that can be colonized by pathogens such as Aspergillus spp. and Pseudomonas spp. Other complications are bronchopleural fistulae, parapneumonic effusions, empyema, and hemoptysis [8]. *S. aureus* infections are frequent in phagocytic disorders and innate immunity disorders involving NF-kB pathway, too. In these conditions, necrotizing pneumonias not only by *S. aureus* but also by bacteria such as *Klebsiella pneumoniae*, Serratia spp., and *Burkholderia cepacia* complex are common. In chronic granulomatous disease (CGD) and syndromes characterized by congenital neutropenia, due to the pivotal role of phagocytes in the control of these bacteria [1], lung abscesses and necrotizing pneumonias frequently occur. *Pseudomonas aeruginosa* infections are another hallmark of phagocyte defect and syndromes characterized by congenital neutropenia but are also common in patients affected by lung bronchiectasis.

Intracellular bacteria that are typically associated with cellular immunity defect and particularly with phagocyte defects are Nocardia spp. Nocardia spp. are aerobic, filamentous, gram-positive bacteria, with variable acid-fast staining, widely distributed in soil, air, and water. Nocardia spp. reach the lungs by inhalation; in PID patients, the infection can easily disseminate in any organ, especially in central nervous system (brain abscess). The clinical picture is protean, including pneumonia, lung abscess, lung masses, or chronic cavitary disease; chest imaging shows lung infiltrates/nodules that can cavitate. Treatment regimens include trimethoprim-sulfamethoxazole and carbapenems, but mortality can be high, depending on the extension of the disease, brain involvement, and the immune status of the patient [9].

12.1.2 Mycobacterial Infections

The genus Mycobacterium includes at least three groups of human pathogenic bacteria, characterized by acid-fast staining due to the particular composition and thickness of the cell wall. M. tuberculosis complex encompasses a few species including M. tuberculosis (also known as Koch's bacillus, KB) responsible for human tuberculosis; *M. leprae* causes leprosy, while nontuberculous mycobacteria (NTM) has been associated with many clinical illnesses in the human host, depending on the immune status and the specific mycobacterial species. Considering PID patients, the main lung pathogens are KB and NTM.

KB is an extremely successful human pathogen, infecting approximately one-third of the world population; about 8.6 million new cases and 1.3 million deaths

worldwide have been estimated in 2012 [10]. *M. tuberculosis* is an exclusively human pathogen with no animal reservoirs and is mainly transmitted by aerosolized droplets generated by coughing of people with active tuberculosis. The outcomes of KB infection are dramatically dependent on the balance between the pathogen and the human immune response. In normal host, KB reaches the lower airways and lung alveoli, where it meets the alveolar macrophages. Alveolar macrophages, together with dendritic cells, engulf KB and produce a bunch of important cytokines such as IL-1, IL-12, IL-18, and TNF-α, stimulating inflammation and neutrophils recruitment in the lung; neutrophils, via phagocytosis of KB and their destruction by respiratory burst and neutrophil extracellular traps, have a very important role in the early host defense against KB. Alveolar macrophages are activated by the local production of interferon γ (IFNγ) in response to IL-12, becoming more able to destroy engulfed KB [11]. However, KB possesses many strategies to elude intracellular destruction and survive inside host macrophages, so adaptive immune response is necessary to control KB replication. The adaptive immune response is mainly a cellular response and is directed against a wide array of KB antigens, more than 80 [12]. Alveolar macrophages and lung dendritic cells, infected by KB, are the pivotal antigen-presenting cells known to activate adaptive host immune response. CD4+ lymphocytes, activated in the secondary lymphoid organs, migrate to the infected lung, and there they establish a Th1 immune response, producing IFNy and promoting the activation of macrophages and the formation of granuloma, the hallmark of KB immune response. In the granuloma, KB is imprisoned and cannot disseminate or cause lung disease; however, granuloma are highly dynamic structures, where immune surveillance is constantly needed to ensure KB infection control. Clinically, the first contact of KB with human host is usually asymptomatic or barely symptomatic, with fever, night sweats, constitutional symptoms, cough, or other immune complex-mediated manifestations, such as erythema nodosum [13]. KB infection becomes latent after primary infection but is prone to reactivate, with the classical radiological appearance of upper lobe opacity. In immunocompromised host, KB infection can show a more severe course, with severe endobronchial and hematogenous dissemination; the clinical picture often shows severe respiratory compromise (dyspnea, cough, hemoptysis, chest pain, pleural effusion, respiratory failure) and severe systemic involvement, with fever and constitutional symptoms. Dissemination with extrapulmonary organs involvement, such as kidney and central nervous system involvement, is not unusual. Moreover, long-term complications of KB infection, such as bronchiectasis, fibrothorax, and multiple lung cavitary lesions, can develop; these complications are responsible of severe respiratory long-term morbidity.

NTM are a wide group of ubiquitous free-living mycobacteria, with more than 150 different species described; they can be found in soil and water and can be divided, depending on the growth velocity in cultures, into rapid-growing (less than 7 days) and slow-growing (7 days or more). The most common pathogens are *Mycobacterium avium* complex, Mycobacterium abscessus complex, and Mycobacterium kansasii. NTM are not usually pathogenic for immunocompetent patients but represent a substantial threat to immunocompromised host; they mostly

cause lung infections but can also cause skin, bone, and eye infections [14]. NTM lung disease is difficult to diagnose and requires a high index of suspicion. The clinical picture is not specific and closely mimics tuberculosis; the patients usually complain of cough, increased sputum, dyspnea, low-grade fever, and weight loss. Chest imaging is very important to raise the diagnostic suspicion: NTM disease shows two main radiological patterns, fibrocavitary and nodular bronchiectatic [15]. The fibrocavitary pattern resembles tuberculosis and typically affects elderly men with underlying lung disease. This form is characterized by thin-walled cavities with areas of increased opacity, usually located in the upper lobes; pleural thickening and volume loss by fibrosis with traction bronchiectasis are frequent. The nodular bronchiectatic form shows bilateral, multilobar bronchiectasis, especially in the middle and lower lung fields, with small nodules on chest imaging. This pattern of NTM lung disease occurs predominantly in elderly nonsmoking women without underlying lung disease and appears more commonly in those with a thin body habitus [15]. The gold standard for NTM disease diagnosis is culture, which allows for genotypic identification. Species identification is of paramount importance to guide therapy [15]; however, it is important to remember that NTM are found commonly in the environment, especially in water, so attention must be paid to the possible contamination of the specimen used to isolate NTM in culture. Furthermore, it must be noted that asymptomatic NTM colonization can be found in patients with chronic lung disease, such as bronchiectasis. The clinicians need, therefore, to integrate clinical, microbiological, immunological, and radiological evidence to make a proper diagnosis of NTM disease and to distinguish it from tubercular mycobacteriosis [16]. To this end, sometimes, lung biopsy is needed, with histopathological examination and biopsy specimen culture.

Due to the complex interaction between host immunity and mycobacteria, there are many different PID that are associated with susceptibility to KB and NTM infections. Severe combined immunodeficiencies (SCID) are a group of PID characterized by very low levels of autologous T lymphocytes, with or without an associated lack of B cells (B− or B+, respectively). These patients are susceptible to a very wide range of pathogens, due to the lack of T cell immunity, and mycobacterial infections are part of the disease spectrum. X-linked Hyper-IgM Syndrome (HIGM) is a disease characterized by the lack of CD40 ligand (CD40L), a molecule expressed on cell membrane in activated lymphocytes and antigen-presenting cells, with a pivotal role in immunoglobulin class switching and lymphocyte and phagocyte activation. CD40L signals through the NEMO-NF-kB pathway to enhance the production of IL-12; the loss of this signal is probably the reason for the susceptibility to KB and NTM of these patients [10]. The importance of IL12 and IFNγ signaling in mycobacterial defense is underlined by a group of monogenic disorders, called Mendelian susceptibility to mycobacterial disease, which affects many genes in this pathway, such as IL-12 receptor, IFNγ receptor, STAT-1, and NEMO [17]. Though this PID are not T cell defects, they provide important insight into the immune response against mycobacteria and into the pivotal role of innate immunity, together with adaptive immunity, in the control of mycobacterial diseases. Another defect with increased susceptibility to mycobacterial disease, especially NTM infections,

is GATA 2 haploinsufficiency, which is a protean disorder with features of immunodeficiency, pulmonary alveolar proteinosis, congenital lymphedema, and high risk of myelodysplastic syndrome/leukemia. The susceptibility to mycobacteria in these patients seems to be due, however, to defective function of dendritic cells, but a monocyte/macrophage lineage defect is also present [10].

Another mycobacterial infection, which is typical of PID, is the infection due to bacillus Calmette-Guerin (BCG). BCG is an attenuated strain originated from *M. bovis* at the beginning of the twentieth century; it has been used since 1921 as a vaccine against tuberculosis. BCG administration has been acknowledged useful to prevent severe form of tuberculosis in young children (up to 5 years of age) but is not protective for pulmonary tuberculosis, especially in adults. Due to its overall safety, it is recommended by the World Health Organization in endemic and especially in low-income countries [18]. BCG, however, can cause infections in patients with PID because it is a live vaccine, and so patients with severely impaired cellular immunity can have troubles in controlling BCG replication. BCG disease can cause only mild local lymphadenopathy (a disease called BCGitis) or cause severe disseminated disease (a disease called BCGosis). BCGosis can show important lung involvement with nonspecific clinical symptoms; cough, low-grade fever, and malaise are the typical clinical picture, with lung nodules or infiltrates at chest imaging [18]. Lymphadenopathy involving multiple regions, liver and spleen lesions, and osteomyelitis can also be present in BCGosis; the clinical picture is usually severe and mandates aggressive therapy with at least four antimycobacterial drugs [18]. BCG infection has been described in SCID, DiGeorge syndrome, immune dysregulation syndromes (STAT1 gain-of-function mutations, activated phosphatidylinositide 3-kinase d syndrome [APDS]-1 and 2), CGD, and Mendelian susceptibility to mycobacterial disease.

12.1.3 Viral Infections

Viral infections are typical infections in cellular PID because immune response against viruses is a pivotal function of cellular response. Therefore, quantitative and qualitative defects in cellular response are linked to susceptibility to viruses, as well as some PID characterized by defective innate immunity.

12.1.3.1 Adenovirus

Human adenoviruses (HAdV) are a double-stranded DNA, non-enveloped group of viruses, known to cause a wide range of clinical pictures in immunocompetent and immunocompromised hosts [19]. HAdV are divided in seven species (termed A to G) with over 50 types, discovered by both a serotyping and a genomic approach [19]. The typical routes of transmission are inhalation of aerosolized droplets or direct conjunctival inoculation, but the oral-fecal route or transmission via contaminated surfaces or devices is also possible due to the high resistance to disinfectants of HAdV, which are able to retain infective properties for several weeks in the moisture-free environment. The infection is usually common in children and in

adults attending crowded and closed settings, such as the military recruits. Viral entry in cells is mediated by coxsackie-adenovirus receptor (CAR) but also by CD46; tissue tropism is wide, including conjunctival epithelium, lung, and gastrointestinal epithelial surfaces. After primary infection, HAdV can establish latent infections in the majority of human hosts, persisting in lymphoid cells, lung epithelial cells, central nervous system, and the entire gastrointestinal tract [19, 20]; these latent infections are prone to reactivation with a significant risk of disseminated disease in immunocompromised hosts. Human innate response is the first line of defense, slowing down viral replication for a while; subsequently, adaptive response, mediated by CD4+ and CD8+ lymphocytes, ensues, allowing viral clearance. The adaptive response produces effector T cell, mainly cytotoxic, against a core set of immunodominant epitopes, belonging to viral capsid and common to almost all HAdV types; this adaptive response is protective in human adults.

Considering PID patients, HAdV respiratory tract infections and disseminated disease are not uncommon in SCID, and the mortality can be very high, up to 55% [21]. HAdV pneumonia, such as other viral pneumonias, has no specific clinical picture; it cannot be distinguished by pneumonia caused by other pathogens, such as bacteria. Fever, cough, shortness of breath, respiratory failure, and crackles on chest auscultation can be present; radiologically, ground-glass opacities and even consolidations are found. Therefore, the clinical suspicion, associated with the knowledge that viral pneumonia is quite common even in immunocompetent individuals, mandates microbiological assessment in all patients. HAdV infections in immunocompromised hosts can easily disseminate, involving gastrointestinal tract (colitis), liver (hepatitis), central nervous system (encephalitis), and the eye (retinitis). Admission to intensive care unit (ICU) is not uncommon for these patients, with unfavorable outcomes. The treatment is supportive; in a few case reports, therapy with cidofovir has been tried.

12.1.3.2 Herpesviridae

Herpesviridae is a wide family of double-stranded DNA viruses, with a worldwide distribution. Herpesviridae can be divided in three groups: alphaherpesvirinae (HSV 1–2, VZV), betaherpesvirinae (CMV, HHV6–7), and gammaherpesvirinae (EBV, HHV-8). They are very complex viruses responsible for several infections but also for many immunomodulatory and tumorigenic effects due to their ability to integrate their DNA into host DNA.

Herpes simplex viruses (HSV) 1 and 2 cause one of the most prevalent infection in the world; their seroprevalence, in the US population, is, respectively, 58% and 17% [22]. The transmission is by contact with infected skin or secretions and can occur during subclinical viral shedding, when viruses replicates in host infected cells without symptoms. HSV usually causes painful and ulcerating vesicles in the labial (HSV-1) or anogenital (HSV-2) region; in the immunocompromised host, HSV can affect the oropharyngeal region and, by contiguous spread or aspiration, can reach the lower respiratory tract causing tracheobronchitis and pneumonia. Clinically, lower respiratory tract involvement manifests as fever, cough, dyspnea, bronchospasm, and chest pain; the radiological picture is not specific, showing

bilateral symmetric ground-glass opacities or consolidations. HSV infections can easily disseminate affecting extrapulmonary organs such as the liver and the central nervous system; HSV viremia has a high mortality in ICU (27%) [23]. HSV pneumonia is a rare cause of pneumonia in immunocompromised host and is difficult to diagnose due to the lack of specificity of the clinical and radiological features and of the detection of HSV in the airways; it is not uncommon to detect HSV in bronchoalveolar lavage fluid due to asymptomatic viral shedding in immunocompromised host, even if high HSV viral load seems to be associated with worse outcome.

Herpes zoster virus (HZV) is usually associated with chicken pox (primary infection), a febrile illness with diffuse vesicular exanthem, and with shingles, a dermatomal vesicular skin rash, arising as a result of the reactivation of HZV, persisting in sensory ganglia after primary infection. HZV pneumonia is a complication of chicken pox, with an estimated incidence of 2.3 in 400 cases [23]. It usually occurs within 7 days from the onset of the rash, but respiratory symptoms may precede rash. Symptoms are nonspecific, including cough, dyspnea, and respiratory failure; typical chest radiological signs of VZV pneumonia include diffuse interstitial nodular opacities that are often more severe than expected for the degree of clinical symptoms. Lung involvement can be a complication of HZV reactivation, but it's much rarer, with low mortality rates (<2%). HZV infection can be observed in PID with adaptive immune cellular response impairment, such as in SCID and idiopathic CD4+ lymphopenia, where disseminated disease is not so rare.

Approximately 50–90% of the world population are cytomegalovirus (CMV) seropositive, depending on age and location [24]. CMV is found in blood, secretions, and organs of infected individuals, so transmission is quite easy. Primary CMV infection in immunocompetent host is usually mild, characterized by fever, cervical or diffuse lymphadenopathy, myalgias, and pharyngitis. However, especially in immunocompromised host, CMV primary infection or CMV reactivation from a latent infection can involve other organs, such as the eye, the central nervous system, the liver, and the lung. In the lung, CMV can infect many cell types, including alveolar macrophages, dendritic cells, alveolar epithelium, and fibroblast-like stromal cells; infection can occur via direct spread from the upper airways or via bloodstream. CMV interferes with host immune response in many ways by inhibiting adaptive response (MHC downregulation, inhibition of T cell receptor costimulators, production of viral peptides that mimic inhibitory cytokines or act as "traps" of cytokines involved in lymphocyte migration and activation) and innate immune response (downregulation of NK cell response); the host immune system is unable to clear the infection but, however, can limit viral replication by cellular immunity. CMV pneumonia presents as a lung infection with a wide spectrum of severity; clinical picture includes fever, cough, dyspnea, and hypoxemia disproportionate to radiological findings. Chest imaging is not specific for the diagnosis, showing ground-glass opacities, consolidations, and lung nodules, so bronchoalveolar lavage with PCR analysis is needed to demonstrate CMV infection. CMV pneumonia has been observed in a range of different PID. The impairment of cellular response observed in SCID and HIGD is a strong risk factor for CMV infection; quite often, CMV infection can lead to death in SCID patients. CMV infections have been

observed in other PID, including immune dysregulation disorders (APDS syndrome, CTLA4 haploinsufficiency, ICOS deficiency), in DOC8 deficiency (autosomal recessive hyper-IgE syndrome), and in GATA2 deficiency.

12.1.3.3 Respiratory Syncytial Virus (RSV)

RSV is an enveloped single-stranded RNA virus belonging to the Paramyxoviridae family, genus Pneumovirus. Two main subtypes, called A and B, are known, identified by important differences in the viral proteins. RSV is the causative agent of bronchiolitis, a lower respiratory tract infection typical of newborn and children aged less than 2, especially preterm infants; the infection is a typical airborne disease, transmitted via infected respiratory secretions but also via contaminated fomites. Clinically, the disease is characterized by dyspnea, wheezing, tachypnea, and poor feeding, usually preceded by upper respiratory tract infection symptoms; chest imaging shows lung air trapping. RSV bronchiolitis often leads to hospitalization of the affected children, with higher mortality in PID patients. RSV immune response involves both the humoral branch and the cellular branch of immune system. The antibodies prevent viral dissemination in the respiratory tract, but the clearance of viruses is performed by the cellular immune response. Accordingly, children with T and B cell impairment are at higher risk of RSV infections; furthermore, PID children exhibit higher risk of ICU admission and longer hospital stay than immunocompetent children [25]. Survivors of RSV infection are at high risk of developing asthma in their lifetime, and reinfections are associated with wheezing and asthmatic exacerbations in older children [25]. In many children at risk of severe RSV disease, a preventive strategy has been developed, namely, the IgG1 monoclonal antibody palivizumab, directed toward RSV fusion protein; palivizumab prevents viral entry in cells and limits hospitalizations. The use of palivizumab is advised in SCID patients during RSV season to prevent severe RSV disease. RSV infection treatment is mainly supportive, but in severe cases, intravenous immunoglobulins and ribavirin have been tried, with some evidence of clinical efficacy [26].

12.1.4 Fungal Infections

Fungi are a wide and heterogeneous group of eukaryotic organisms; only a few species are pathogenic for the human host. The efficacy in clearing fungal infections is a hallmark of a healthy immune system; lung and invasive fungal infections are therefore rare in normal host, but they are common in immunocompromised hosts and in many PID. Immunity against fungi in lung relies on both innate immune response and adaptive immune response. Lung epithelial cells (LEC), as well as dendritic cells, activated by pattern recognition receptors in response to inhaled fungi (dectin receptors and Toll-like receptors), act as antigen-presenting cells and activate local innate immunity; neutrophils are particularly important in clearing extracellular fungi and are recruited by the IL-17 production by innate lymphoid cells, attracted in the lungs by NF-kB-driven chemokines production. Adaptive immunity is, however, crucial in eradicating fungal infections. Intracellular fungi,

such as Pneumocystis jiroveci, are preferentially cleared by a strong IFNy-dependent Th1 response, mediated by CD4+ lymphocytes. Extracellular fungi immune response, such as response against Candida spp. and Aspergillus spp., is heavily dependent on Th17 response, which can elicit neutrophil-rich inflammation in lung tissue [27]. The clinical presentation is wide and isn't pathognomonic, ranging from asymptomatic lesions found serendipitously on chest imaging to acute respiratory failure. The nonspecific clinical picture mandates a thorough and aggressive diagnostic approach, which can include high-resolution chest computed tomography (HRCT), bronchoscopy with bronchoalveolar lavage fluid culture, and transbronchial and/or surgical biopsy. Here, we will review the main type of fungal lung infections in PID.

12.1.4.1 Pneumocystis jiroveci

Pneumocystis jiroveci is a ubiquitous fungus which was known to cause pneumonia (PCP) epidemics in malnourished children during the Second World War. An important reservoir of *P. jiroveci* is children; most children are infected early in life and transmit the infection via the airborne route. PCP can therefore arise as a primary infection or as a reactivation of a previous infection.

PCP was a rare disease till the eighties when HIV epidemic started. The strongest risk factor for PCP is, in fact, depression of T cells response, particularly CD4+ function impairment, and PCP is actually considered an AIDS-defining illness in HIV-positive individuals; now, many other patients are considered at risk of PCP due to iatrogenic immunosuppression in malignancy, transplantation, and systemic autoimmune diseases. However, many PID are known to be associated with deadly PCP. The most common PID associated with PCP are SCID, HIGM, Wiskott-Aldrich syndrome (WAS), and idiopathic CD4+ lymphopenia; PCP is quite rare in PID with disturbed IL-17 axis, such as HIES. From a clinical point of view, PCP onset can be insidious; children often present with dyspnea, low-grade fever, and dry cough, and clinical examination may not show adventitious lung sounds. Hypoxemia and respiratory failure can, however, develop rapidly, owing to a severe interstitial and parenchymal involvement as suggested by the typical CT scan appearance (diffuse ground-glass opacities). Patients usually are admitted to the hospital, and intensive care unit with mechanical ventilation is often needed, with a high mortality rate. In non-HIV-infected patients, even if there aren't specific data for PID patients, mortality rate seems to be higher than in HIV-infected patients, ranging from 30 to 40%, but reaching 80.9 and 86.8%, respectively, for patients requiring invasive mechanical ventilation and for those with an acute respiratory distress syndrome [28]. For these reasons, therapies such as hematopoietic stem cell transplantation (HSCT) and antibiotic prophylaxis need to be used in all the patients at risk without delay once they are diagnosed.

12.1.4.2 Aspergillus spp.

Aspergillus spp. is a mold responsible for the majority of human invasive mold disease. About 200,000 cases of invasive aspergillosis are estimated worldwide and are often observed in immunocompromised hosts, such as patients with

hematological malignancies and patients treated with HSCT [29]. Aspergillus can be found in soil, decaying vegetation, food, air, and the water supply; its spores, also called conidia, reach the respiratory tract by inhalation and lodge in the lower respiratory tract due to the small dimensions (2–3 mm) [30]. Conidia can germinate, and the ensuing filamentous hyphae can spread in lung tissue and disseminate in remote organs hematogenously. The immune response against Aspergillus spp. relies heavily on neutrophil activation. Conidia lodging in the lower airways are mainly removed and destroyed by resident monocytes/macrophages; conidia which germinate efficiently activate innate immune response by engaging pattern recognition receptors such as dectin-1 and dectin-2. IL-1, IL-17, TNF-α, and IL-22 are rapidly produced via inflammasome- and non-inflammasome-mediated pathways [30]. LEC are then activated and produce chemokines which recruit neutrophils in the lung. Neutrophil can kill conidia and hyphae in a NAPDH oxidase-dependent or -independent way; they can also inhibit hyphal growth by iron sequestration in the extracellular space by secreting lactoferrin. It is not surprising, therefore, that Aspergillus spp. infections are common in patients with quantitative or qualitative neutrophil defects, such as patients with syndromes associated with congenital neutropenia, leukocyte adhesion deficiency, and CGD; the latter disease, in particular, is associated with an array of defects in NADPH oxidase, which impair oxidative burst, essential for conidia and hyphae killing. Lymphocytes are less important in the immune response against Aspergillus spp., and in fact, PID characterized by T cell and/or B cell defects are not associated with invasive aspergillosis. HIES patients can develop lung and disseminated aspergillosis; however, in these patients, the predisposing factor is the presence of lung cavities as a result of previous necrotizing bacterial pneumonias, which can be colonized by Aspergillus spp. [29, 31]. The clinical picture of pulmonary aspergillosis is not specific. Usually, patients experience fever unresponsive to wide-spectrum antibiotics, but many patients can be afebrile; cough, chest pain, and hemoptysis, because of lung infarction due to mold-induced vascular obstruction., can be seen. However, as the disease progresses, worsening hypoxemia develops leading to respiratory failure, and dissemination to remote site can be observed; seizure. or other focal signs can show, for example, cerebral involvement [29].

12.1.4.3 *Cryptococcus neoformans*

Cryptococcus neoformans is a ubiquitous free-living yeast with polysaccharide capsule, which is usually found in soil, but it's an accidental human pathogen. *C. neoformans* is responsible for more than one million cases of cryptococcosis and about 650,000 deaths annually; cryptococcosis can be considered, therefore, the most prevalent fatal fungal disease worldwide [29]. Desiccated yeast cells or spores are usually inhaled and reach the lungs, where primary infection occurs; primary infection is usually asymptomatic, and yeasts are buried into granuloma where they are innocuous. Cryptococcosis is often related to the reactivation of fungi in conditions of host immunodepression or, much more rarely, to an aggressive primary infection. Immune response against Cryptococcus is mainly dependent on T cells [32]. At the beginning of the infection, dendritic cells and alveolar

macrophages present cryptococcal antigens to CD4+ lymphocytes, eliciting a strong Th1 response. Th1 response is necessary to clear fungi and limit infection, as Cryptococcus can survive intracellularly in phagocytes and even exploit them to penetrate in central nervous system, the most dangerous target organ in cryptococcosis. Indeed, the strongest risk factor for Cryptococcus infection is low CD4+ cell count, as can be seen in HIV-infected patients, idiopathic CD4+ lymphocytopenia, and combined immunodeficiencies [33]. Cryptococcal pneumonia has a wide spectrum of clinical pictures. Some patients may be asymptomatic, showing only well-defined single or multiple noncalcified nodules and pulmonary infiltrates on chest imaging. Usually, patients present with fever and cough, and respiratory failure can ensue rapidly. However, isolated cryptococcal pneumonia is rare, and cryptococcosis often shows signs of disseminated disease, such as CNS involvement (headache, meningeal irritation, cranial nerve palsies, altered mental status) and skin involvement.

12.1.4.4 Endemic Fungi

The last group of pathogenic fungi (dimorphic fungi) which can cause lung infections in PID patients are endemic fungi, a wide group of fungi characterized by specific geographical distribution [34]. Coccidioides spp. and *Histoplasma capsulatum* are endemic in the American continent, dwelling, respectively, in desert areas and in tropical areas with high humidity. Paraccidioides spp. are endemic in South America and is found in the tropical and very humid regions, especially in acidic soil where coffee and sugarcanes are cultivated. Talaromyces marneffei is highly endemic in Thailand, Vietnam, southern China, and other subtropical areas in Southeast Asia. Blastomyces dermatitidis grows in wet soils, and the most significant endemic epicenter is in eastern US between the Ohio and Mississippi River valleys. These fungi grow as molds in the environment while undergoing morphological switch to the yeast form, or spherules in Coccidioides, at body temperatures of mammalian hosts. They usually reach the lung by inhalation where they have to face innate immune system, especially alveolar macrophages and neutrophils, and adaptive immune response, mainly of Th1 type. In normal host, alveolar macrophages engulf and eliminate inhaled yeasts, and the infection is usually asymptomatic; in immunocompromised host, however, the yeasts proliferate inside macrophages, and systemic dissemination may occur via reticuloendothelial system. Endemic fungi may be responsible for infections in PID patients, especially in patients with IFNγ pathway defects such as IL12Rβ1 deficiency, IFNγR1, and STAT1 gain-of-function (GOF) mutations, highlighting the importance of IL12/IFNγ pathway in the immune response against this group of fungi. Moreover, dimorphic fungi infections have been described in adult patients producing anti-IFNγ antibodies. In patients with HIGM, dimorphic fungi infections have been described; CD40L deficiency, in these patients, impairs IL-12 production by dendritic cells and the production of IFNγ by T cells. Dimorphic fungi infections are not typical in CGD, suggesting that killing of intracellular fungi can be achieved by phagocytes in a NAPDH oxidase-independent way.

12.2 Chronic Lung Complications

12.2.1 Bronchiectasis and Chronic Obstructive Lung Disease

Chronic obstructive pulmonary disease (COPD) in PID is a serious complication with high morbidity and mortality, mainly due to bronchiectasis. PID are probably an underrecognized cause of COPD, with a prevalence ranging from 2 to 4% in general COPD cohorts, according to the few studies published [35]. A recent systematic review in CVID patients shows a prevalence of bronchiectasis of 34% [36]. Although bronchiectasis has been extensively studied in CVID and in other predominantly humoral PID (X-linked agammaglobulinemia), it can be found in all PID; in immune dysregulation syndromes, such as APDS, CTLA-4 haploinsufficiency, and LRBA deficiency; and in phagocytic defects such as CGD (Tables 12.1 and 12.2).

Bronchiectasis is a disease characterized by abnormal and irreversible enlargement of the airways in the lung. Recurrent infections produce an inflammatory response in the bronchi, leading to mucus accumulation and impairment of mucociliary clearance. As a consequence, bacteria can easily proliferate and colonize bronchi, which gradually lose their normal shape and function and dilate. These abnormally dilated bronchi further enhance bacterial colonization, establishing a vicious circle in which abnormal bronchi gradually become more and more dilated. Bronchiectasis can be a localized complication of infections such as pneumonia or tuberculosis or can be widespread as it is usually observed in PID. In PID, subclinical infections are even more important than symptomatic ones, when we consider the causes of lung function decline, because they can promote continuous low-grade bronchial inflammation. Subclinical infections are well documented in PID patients. The presence of H. influenzae, Adenovirus spp., and Rhinovirus spp. in the bronchoalveolar lavage fluid of asymptomatic CVID patients has been reported [37]. PID patients also clear infections at a slower rate than immunocompetent persons; rhinovirus shedding in patients with PID lasted on average 40.9 days (95% CI, 26.4–55.4 days) compared to 11.4 (8.2–14.7) days and 10.1 (7.4–12.9) days in immunocompetent children and adults, respectively [38]. The rate of lung function decline is fast, and a substantial proportion of patients has chronic lung disease at diagnosis, considering the diagnostic delay, which continues to be huge. The average decline of forced expiratory volume in 1 s (FEV1) in CVID patients is 45 mL/year, a value that is larger than the value observed not only in healthy people (19.6 and 17.6 mL/year for males and females, respectively) but also in continuous smoker (38.2 and 23.9 mL/year in males and females, respectively) [39, 40]. The clinical picture of lung bronchiectasis is characterized by chronic cough with large volume of sputum with a mean (SD) daily volume of 38 ± 34 mL at diagnosis. The sputum is often discolored, with a green or yellow purulence, depending on airway inflammation and bacterial infection/colonization [41]. Patients with bronchiectasis often experience recurrent bronchial infections and pneumonias due to altered local defense with frequent bacterial colonization. Other common symptoms include

dyspnea, hemoptysis, chest pain, and wheeze; chronic fatigue is common. In children, symptoms are similar, with prolonged "wet" cough unresponsive to long courses of antibiotic therapy and recurrent respiratory infections. Particular attention must be paid to children presenting with asthma who don't improve with specific treatment, especially children with cough variant asthma. The most frequent clinical sign in adults, but also in children, is crackles, present in 69.9–73% at the time of diagnosis, most commonly in the lower lung zones [42]. These respiratory sounds are usually expiratory and can improve or even disappear after cough. Wheezes, cyanosis, and clubbing are less common signs. Physical examination, however, can be completely normal. Lung function tests can demonstrate lung obstruction with reduction of FEV1 and reduction of the ratio between FEV1 and forced vital capacity (FVC); lung hyperinflation, as documented by increased lung static volumes such as total lung capacity and residual volume, can be present. A reduction of the diffusing capacity of the lung for carbon monoxide (DLCO), which is regarded as expression of parenchymal injury, can also be demonstrated. These findings, however, are not specific, and in some patients, lung function tests show normal lung function. The gold standard for bronchiectasis diagnosis is the chest computed tomography (CT) because chest X-ray is not sufficiently sensitive and specific for detecting the disease. The radiological hallmark of bronchiectasis is bronchial dilatation; if the internal diameter of the bronchial lumen is greater than that of the adjacent artery (bronchioarterial ratio > 1), a diagnosis of bronchiectasis can be made. Other radiological findings often found in patients with bronchiectasis include a lack of tapering and airway visibility within 1 cm of the costal pleural surface or touching mediastinal pleura, bronchial wall thickening, interlobular septal thickening, emphysema, and a mosaic attenuation pattern due to small airway disease. There are many controversial issues in the treatment of bronchiectasis in patient not affected by cystic fibrosis. First-line treatment encompasses respiratory physiotherapy techniques and pulmonary rehabilitation. Patients are also encouraged to undergo vaccination against influenza and pneumococcal disease. Many patients are also treated with inhaled therapy such as corticosteroids, long-acting antimuscarinic drugs, and long-acting beta-adrenergic drugs, but the evidence of efficacy and effectiveness for these drugs is not strong. Other drugs that are widely used to enhance mucus clearance are mucolytics and nebulized isotonic or hypertonic saline. Long-term inhaled or oral antibiotics are commonly used for patients with frequent exacerbations (more than three exacerbations for year). Inhaled colistin is preferred, according to the British Thoracic Society Guidelines [43], in patients with *Pseudomonas aeruginosa* colonization. Long-term oral antibiotic therapy can be used in all patients with non-cystic fibrosis bronchiectasis and frequent exacerbations. The antibiotics used are usually macrolides such as azithromycin or erythromycin. Macrolide antibiotics show an excellent lung distribution and have anti-inflammatory properties, which partly explain their capacity to reduce exacerbation together with their ability to control bacterial growth. A recent Italian placebo controlled study [44], performed in patients with predominantly antibody deficiency, showed an hazard risk of 0.5 for exacerbations and hospitalizations in patients receiving azithromycin versus patients receiving placebo.

12.2.2 Interstitial Lung Disease

Interstitial lung disease (ILD) is a group of respiratory illnesses characterized by inflammation and fibrosis involving lung interstitium, alveolar spaces, lymphatics, and intrathoracic lymph nodes. ILD is a frequent complication in PID patients due to immune dysregulation and autoimmunity that are seen in many PID.

The most common form of ILD in PID is granulomatous-lymphocytic ILD (GLILD), observed in CVID and in CVID-like syndromes such as hypomorphic mutations of recombination-activating gene 1 (RAG1), haploinsufficiency of cytotoxic T lymphocyte antigen-4 (CTLA4), and deficiency of lipopolysaccharide responsive beige-like anchor protein (LRBA). GLILD is defined, according to a Consensus Statement of the British Lung Society [45], as "a distinct clinico-radiopathological ILD occurring in patients with CVID, associated with a lymphocytic infiltrate and/or granuloma in the lung, and in whom other conditions have been considered and where possible excluded." GLILD is strongly associated with immune dysregulation, and this is the reason why GLILD is not observed in congenital agammaglobulinemia and in HIGM. GLILD is part of a wide spectrum of noninfective lung complications that have been described in CVID. Two independent studies have demonstrated that patients with noninfective lung complications have a 11-fold higher risk of death than patients with only infective complications [46, 47]. This complication is quite relevant because approximately 30% of CVID patients have ILD [48]. GLILD is usually considered as an organ-specific involvement of a systemic granulomatous disease which is associated with CVID. In patients with GLILD, granulomatous involvement of the spleen, liver, and lymph nodes may be also the first manifestation of the disease [49]. Granulomatous involvement of the skin and the gastrointestinal tract is also possible as well as autoimmune manifestations, such as autoimmune cytopenias. The most common symptoms are dyspnea and cough, with progressive lung function worsening. Many patients are also asymptomatic, and so chest imaging is mandatory in CVID patients to screen them for subclinical ILD. No serological biomarkers are available though elevated beta-2 microglobulin and IgM have been associated with GLILD. Lung function tests can show a reduction of DLCO even before that a restrictive syndrome develops (a concomitant obstructive syndrome can be seen due to bronchiectasis). However, many patients have normal lung function tests, and restrictive syndrome is a late pattern, which appears when lung injury is advanced and only partially treatable. Chest imaging is of paramount importance in the detection of GLILD in PID patients. The typical CT pattern in GLILD is quite different from the usual findings in CVID patients, consisting of micronodules with ground-glass density, centrilobular distribution, and mainly located in mid-lower lobes (see Fig. 12.1). The most important radiological differential diagnosis to be made is with sarcoidosis, where the nodules are mainly present in the mid-upper lobes with a perilymphatic distribution [50]. Diffuse intrathoracic lymphadenopathy is common, and bilateral smooth interlobular septal thickening at lower lobes are other common alterations. Fibrotic alterations such as reticular opacities can be present, while cystic alterations are unusual [50]. Flexible bronchoscopy is usually performed in

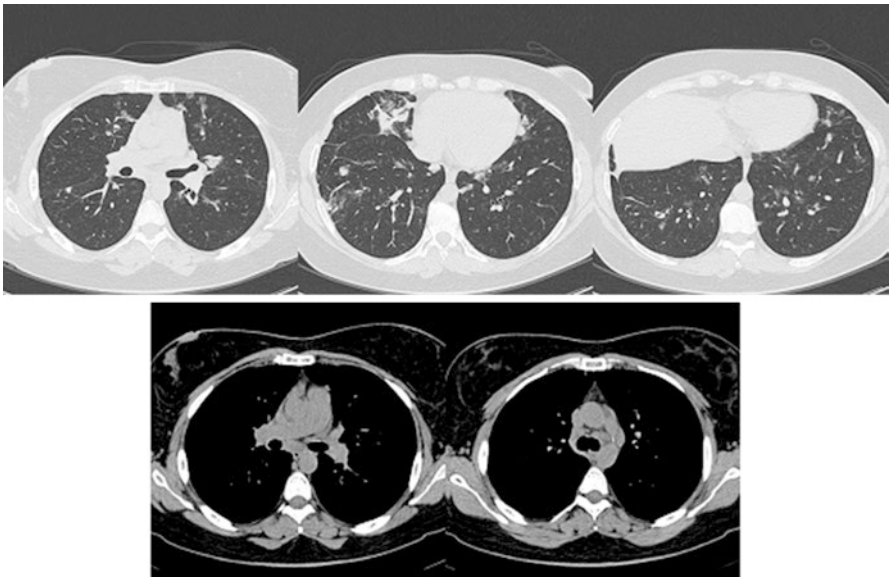

Fig. 12.1 Chest CT of a female patient affected by CVID showing granulomatous involvement of the lung (GLILD). Nodular and ground-glass opacities are seen in lung parenchyma, while lymphadenopathies are present in the mediastinum (personal archive). Courtesy of Dr Vivarelli

patients to rule out infections, by the culture of bronchoalveolar lavage fluid, in which usually lymphocytosis is present, like in many ILD such as sarcoidosis, lymphocytic interstitial pneumonia (LIP), and hypersensitivity pneumonia [51].

The diagnosis, due to the absence of pathognomonic clinical and radiological signs, relies on surgical lung biopsy, which can be performed using a mini-invasive approach, the video-assisted thoracic surgery (VATS).

GLILD encompasses a wide spectrum of disorders with a variable histopathologic appearance. The pathological hallmarks of GLILD are lymphoid proliferation and the presence of noncaseating granuloma. Lymphoid proliferation in the lung is expressed by several histopathological aspects, such as follicular bronchiolitis and LIP. Follicular bronchiolitis is a disease characterized by hyperplasia of lymphoid tissue in the small airways, where lymphoid follicles with germinal centers are predominantly found in a peribronchiolar distribution. In CVID patients, significant interstitial lymphocytic infiltration and fibrosis are usually associated with peribronchial lymphoid infiltrates, while isolated peribronchial or interstitial distribution is quite rare [52]. Lymphocytes infiltrating peribronchial and interstitial lung tissue are mainly CD4+, but also CD8+ and even B lymphocytes are described [52]. The third histopathological feature is noncaseating granuloma, resembling sarcoid granuloma, which is not very frequent and is lacking in some case series [53]. Lung granulomata don't show complete correlation with extrapulmonary granulomata as they can lack in lung biopsies in the presence of extrapulmonary granulomata. The etiology of granulomata is not known though EBV and HHV-8 have been

hypothesized as possible causes [54]. Nodular lymphoid hyperplasia and reactive lymphoid infiltrates have also been reported [55].There is no consensus across medical literature regarding the treatment of GLILD. There is a wide consensus that immunosuppressor drugs are required to control aberrant activation of immune cells in the lungs. First-line therapy is usually corticosteroids even if they are not sufficient to achieve control in a proportion of patients; second-line immunosuppressive drugs used in these patients are azathioprine, rituximab, and mycophenolate [45].

Organizing pneumonia (OP) is a rare ILD occurring in patients with CVID [56]; OP has been described in patients with CVID and in combined immunodeficiency and can coexist with GLILD. OP pathogenesis is related to an abnormal response to bronchial epithelium injury; the agents which cause lung injury can be inhalational agents, drugs, or infections, but many cases are idiopathic. The injured epithelium reacts by production of granulation tissue; the granulation tissue is made up by loose collagen-embedded fibroblasts and myofibroblasts and lymphocytic infiltration is commonly seen in lung interstitium. The granulation tissue invades the alveoli, the alveolar ducts, and the terminal bronchioles, filling these spaces and forming intraluminal polyps. The symptoms are not specific; patients complain of cough, dyspnea, malaise, and fever, and OP is, therefore, a very close mimic of bacterial pneumonia but usually shows a subacute clinical course. The bronchoalveolar lavage fluid cellular analysis is not specific and can demonstrate lymphocytosis. Chest CT has a pivotal role in the diagnosis; the classical radiological pattern consists of focal subpleural and/or peribronchovascular consolidation areas, often bilateral and asymmetrical, with a predominant lower lobes location. Air bronchogram can be noted in the consolidations; ground-glass opacity or areas of traction bronchiectasis (reversible under steroid treatment) can be commonly seen. These features are usually migratory, with some abnormalities disappearing spontaneously and new areas of consolidation appearing simultaneously in different sites. OP usually displays a very good response to steroid therapy; however, relapses are frequent.

12.2.3 Lung Neoplasms

Immune system has the heavy burden of immune surveillance against cancer; PID have been associated, in fact, with a higher risk of cancer as confirmed by several studies [57, 58]. One of the largest cohort of PID patients, the United States Immunodeficiency Network Registry, demonstrated 1.42-fold excess relative risk of cancer in subjects with PID compared with the age-adjusted population [59]. The higher risk of cancer in PID patients was mainly due to lymphomas (especially B cell lymphomas), with a 10-fold higher risk in men and an 8-fold higher risk in women in comparison with age-adjusted population. Most common solid tumor malignancies such as prostate, breast, colorectal, and lung cancer are not increased in PID patients, with the only exception of gastric cancer in CVID patients [59].

There are many potential mechanisms that can explain the heightened susceptibility to cancer of PID patients. Many PID are associated with stem cell or lymphocytes development defects; the wide range of molecular defects, which cause protein

and vesicles mistrafficking, endoplasmic reticulum stress, destabilization of the mitochondrial membrane potential, disturbed energy metabolism, abnormal glycosylation, and deregulated actin polymerization, represent not only the molecular basis of the immune defects but also of enhanced tumorigenesis. Another leading cause of enhanced tumorigenesis in PID is DNA repair defects, which lead to genetic instability, as is commonly seen in ataxia telangiectasia, Nijmegen breakage syndrome, and Bloom syndrome; SCID patients with RAG1 mutations have high risk of hematological malignancies and have demonstrated distinct DNA breaks and differentiation blocks in T cell development, which can be responsible for enhanced tumorigenesis [60]. There are, however, many other mechanisms which interact with the disturbed immune system and can enhance tumorigenesis in PID patients. Chronic inflammation, repeated infections by oncogenic pathogens such as HPV and EBV, and defective immune surveillance are also pivotal in the process that leads to malignancy [60]. The lung malignancies commonly encountered in population, such as squamous cell carcinoma, adenocarcinoma, and small cell lung cancer, don't show raised incidence in PID patients. Primary lung malignancies in PID patients belong to the family of lymphoproliferative disorders. Primary pulmonary lymphomas (PPL) are defined as clonal lymphoid proliferations affecting one or both lungs (parenchyma and/or bronchi) in a patient without extrapulmonary involvement at the time of diagnosis or the subsequent 3 months. Pulmonary lymphoproliferative neoplasms are rare tumors, accounting for less than 1% of all lung tumors, less than 1% of all non-Hodgkin lymphomas (NHL), and 3–4% of all extranodal NHL; metastatic involvement of the lungs, by either solid tumor malignancies or extrapulmonary lymphoproliferative disorders, is much more common [61]. PPL arise from bronchus-associated lymphoid tissue (BALT) that is a part of mucosa-associated lymphoid system (MALT), an organized cluster of B and T cells that perform immune surveillance at the boundaries of the body, i.e., the mucosal surfaces; this tissue develops in young children as a result of antigenic stimulation and usually regresses in adults. BALT can reappear in patients undergoing chronic antigenic stimulation, such as in patients with chronic or recurrent infections. There are many clinical types of PPL, but the most frequent are marginal zone lymphoma of MALT type (MZL), diffuse large B cell lymphoma (DLBCL), and lymphomatoid granulomatosis (LYG).

MZL is the most common among PPL, accounting for at least 70% of the cases [62]. Patients with MZL may be completely asymptomatic (up to 50% of the patients) or complain of nonspecific symptoms, such as fever, cough, dyspnea, weight loss, and hemoptysis. Chest CT usually shows mono or bilateral nodules measuring less than 5 cm, with solid, ground-glass, or mixed CT attenuation and peribronchovascular distribution; mosaic attenuation pattern is possible if the tumor infiltrates small airways, while hilar lymphadenopathy and pleural effusions are rare. Fluorodeoxyglucose positron emission tomography (FDG-PET) can yield false negative results due to the indolent growth of the lymphoma. The diagnosis, however, can be made only by tissue biopsy, performed via bronchoscopy or CT-guided percutaneous biopsy. MZL has a good clinical prognosis and an indolent clinical course. The 5- and 10-year survival ranges from 84 to 88%. Asymptomatic

patients with limited disease can be managed with a "watch and wait" approach, while patients with advanced disease are usually treated with a combination of chemotherapy and anti-CD20 therapy (rituximab). Radiotherapy and surgical resection can be considered in selected cases. MZL carries the risk of histologic transformation to DLBCL, like all indolent lymphomas; however, the risk seems to be low [61].

DLBCL is the second most common PPL type, accounting for 10–20% of all PPL. It has an aggressive clinical course, and so systemic spread of the disease is frequent at the time of the diagnosis. Unlike MZL, patients are usually symptomatic, complaining of respiratory symptoms (cough, dyspnea, rarely hemoptysis) and systemic "B" symptoms (fever, weight loss, night sweats). DLBCL presents, on chest CT, as a single or multiple well-defined rounded solid masses; the lesions are often located peripherally in the lower lobes and can show necrosis with subsequent cavitation in about 50% of the cases [61]. Radiological findings can overlap with MZL, but pleural effusions are more frequently observed. Bronchoscopy is usually abnormal, showing stenosis and infiltration of the bronchi, and allows diagnostic biopsy in most patients; FDG-PET normally demonstrates metabolic activity in the lesions. DLBCL therapy is usually chemotherapy because the tumor is widespread at the diagnosis in most patients. Chemotherapy consists of the same multi-agent regimens used in high-grade nodal lymphomas, including cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). Recent studies show a good clinical response in primary pulmonary DLBCL. A complete response was achieved in most patients treated with CHOP (94%), and the 10-year progression-free survival was 90% [63].

LYG is an angiocentric and angiodestructive disease that is driven by clonal proliferation of atypical large B cells that are EBV-infected lymphocytes. Atypical large B cells are associated with an abundant infiltrate of histiocytes, plasma cells, and reactive T cells. This cellular infiltrate accumulates around vessels, which are progressively invaded, with lumen occlusion and vessel destruction. Lung parenchyma is the most common primary site of involvement (80–90%), but synchronous extrapulmonary involvement is frequent, mainly skin (50%) and central nervous system (30%) involvement and, more rarely, kidney and liver involvement [61]. Patients are almost always symptomatic at the time of the diagnosis, with cough and dyspnea. Systemic symptoms such as fever, weight loss, and night sweats can occur, as well as hemoptysis. The most common chest CT features are multiple nodules ranging from 5 to 10 mm with a basal and peribronchovascular distribution. The nodules can coalesce in larger masses which undergo necrosis with subsequent cavitation; this imaging features closely mimic the presentation of vasculitides such as granulomatosis with polyangiitis and eosinophilic granulomatosis with polyangiitis. LYG has a poor prognosis, with a median survival of approximately 4 years; the prognosis is associated with the proportion of EBV-infected large B cells (the higher the proportion, the worse the prognosis). Aggressive LYG has been treated with chemotherapy, with different drug regimens, but no evidence-based recommendations are available because of the rarity of the disease. DLBCL arising from LYG is possible, and sometimes they are histologically indistinguishable.

The main reason which explains the susceptibility of PID to primary lymphoproliferative neoplasms is chronic or recurrent infections by transforming viruses such as Epstein-Barr virus (EBV). EBV belongs to Herpesviridae family and is a double-stranded DNA virus which is transmitted mainly by saliva of infected patients, where the virus is shed together with mucosal cells asymptomatically during subclinical phases of viral replication. EBV can replicate in mucosal cells and in lymphocytes during the lytic cycle, with subsequent host cell destruction and release of new virions. The lytic cycle can also be replaced by viral DNA integration in host cell DNA and expression of a selected subset of regulatory proteins and RNAs which induce lymphocytes proliferation and immortalize B cells; it has been shown, for example, that the expression of viral proteins LMP1 and LMP2A, which EBV uses to promote the transformation of EBV-infected cells in long-lived memory cells, mimics CD40 and B cell receptor signaling, respectively [64]. The immune response to EBV is different, depending on the phase of EBV infection; the acute infection is controlled by a rapid activation of innate immunity (NK cells) and a fast expansion of CD8+ T cells, which control viral replication by destroying viral reservoirs, while the latent infection, which is responsible for the lymphoproliferative complications, is controlled by T cell response and T-B cell interactions. As a consequence, PID characterized by defects in innate immunity have not been associated with chronic EBV infections, suggesting that innate immunity response is dispensable in the protection against EBV latency. Of note, PID with chronic EBV infection susceptibility can be divided in PID with broad nonselective viral susceptibility and in PID with selective EBV infection susceptibility. Considering the first group of diseases, SCID patients and less severe combined immunodeficiency (e.g., hypomorphic RAG1/RAG2 mutations) patients show impairment in B and/or T cell development and susceptibility to a broad range of viral infection. Other defects such as CTPS1 and RASGRP1 mutations affect selectively T cell proliferation in response to viral antigens, without affecting cytokine production or other effector functions. Other PID in the first group, such as APDS and CORO1A and STK4 deficiencies, are associated with increased apoptosis, decreased survival, and elevated T cell senescence; in APDS, both CD8 T cells and NK cells exhibit an aberrant exhausted, senescent phenotype associated with elevated restimulation- or activation-induced cell death (RICD or AICD) and poor ability to kill EBV-infected B cells [65]. Regarding PID with a selective susceptibility to chronic EBV infection and subsequent lymphoproliferative disorders, these diseases are characterized by a prominent defect in T cell antigen-driven proliferation and activation, such as can be seen in ITK deficiency (ITK is a kinase downstream to TCR signaling), CD27 and CD70 deficiency (costimulatory molecules promoting EBV-specific T cell expansion), and CD137 deficiency (another costimulatory molecule enhancing T cell antigen-driven proliferation); the cytotoxicity, however, is not strongly impaired in this PID as can be seen in genetic hemophagocytic lymphohistiocytosis syndromes. MAGT1 haploinsufficiency impairs magnesium influx in T cells, disrupting normal ITK function, and impairs N-glycosylation of some intracellular protein such as NKG2D, enhancing its destruction by ubiquitin-proteasome pathway. NKG2D is a receptor expressed on cell membrane by NK cells and CD8+ lymphocytes, which

binds to MHC class I-homologous proteins, which are upregulated in infected or cancer cells; NKG2D triggers NK- and CD8+ -mediated cytotoxicity promoting infected or cancer cells destruction.

As can be seen from this overview, PID can be considered tumor-promoting disorders due to a wide variety of reasons, ranging from intrinsic defects in host cells to chronic oncogenic infections. This awareness is of paramount importance in the management of PID patients and will gain more and more importance in the future; in fact, targeted therapies and gene therapies will be increasingly used in these patients, and the improved survival will enhance the need for oncological surveillance and management protocols in these patients.

References

1. Nonas S (2015) Pulmonary manifestations of primary immunodeficiency disorders. Immunol Allergy Clin N Am 35:753–766
2. Verhoeven PO, Gagnaire J, Botelho-Nevers E, Grattard F, Carricajo A, Lucht F et al (2014) Detection and clinical relevance of *Staphylococcus aureus* nasal carriage: an update. Expert Rev Anti-Infect Ther 12:75–89
3. Bekeredjian-Ding I, Stein C, Uebele J (2017) The innate immune response against *Staphylococcus aureus*. Curr Top Microbiol Immunol:385–418
4. Karauzum H, Datta SK (2017) Adaptive immunity against Staphylococcus aureus. Curr Top Microbiol Immunol. https://doi.org/10.1007/82_2016_1
5. Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F (2014) Th17 and non-classic Th1 cells in chronic inflammatory disorders: two sides of the same coin. Int Arch Allergy Immunol 164:171–177
6. Patel DD, Kuchroo VK (2015) Th17 Cell pathway in human immunity: lessons from genetics and therapeutic interventions. Immunity 43:1040–1051
7. Vogel TP, Milner JD, Cooper MA (2015) The Ying and Yang of STAT3 in human disease. J Clin Immunol 35:615–623
8. Nicolaou EV, Bartlett AH (2017) Necrotizing pneumonia. Pediatr Ann 46:e65–e68
9. Shariff M, Gunasekaran J (2016) Pulmonary nocardiosis: review of cases and an update. Can Respir J 2016:1–4
10. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, Camcioglu Y, Parvaneh N, El Azbaoui S et al (2015) Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. Immunol Rev 264:103–120
11. Matucci A, Maggi E, Vultaggio A (2014) Cellular and humoral immune responses during tuberculosis infection: useful knowledge in the era of biological agents. J Rheumatol 41:17–23
12. Lindestam Arlehamn CS, Gerasimova A, Mele F, Henderson R, Swann J, Greenbaum JA et al (2013) Memory T cells in latent *Mycobacterium tuberculosis* infection are directed against three antigenic Islands and largely contained in a CXCR3+CCR6+ Th1 subset. PLoS Pathog 9:e1003130
13. Lyon SM, Rossman MD (2017) Pulmonary tuberculosis. Microbiol Spectr 5:726
14. Mortaz E, Moloudizargari M, Varahram M, Movassaghi M, Garssen J, Dizagie MK et al (2018) What immunological defects predispose to non-tuberculosis mycobacterial infections. Iran J Allergy Asthma Immunol 17:100–109
15. Ryu YJ, Koh WJ, Daley CL (2016) Diagnosis and treatment of nontuberculous mycobacterial lung disease: Clinicians' perspectives. Tuberc Respir Dis (Seoul) 79:74–84
16. Cosmi L, Maggi L, Santarlasci V, Liotta F, Frosali F, Angeli R et al (2007) Detection by flow cytometry of ESAT-6- and PPD-specific circulating CD4+ T lymphocytes as a diagnostic tool for tuberculosis. Int Arch Allergy Immunol 143:1–9

17. Bustamante J (2020) Mendelian susceptibility to mycobacterial disease: recent discoveries. Hum Genet. https://doi.org/10.1007/s00439-020-02120-y
18. Hassanzad M, Valinejadi A, Darougar S, Hashemitari SK, Velayati AA (2019) Disseminated bacille calmette-guérin infection at a glance: a mini review of the literature. Adv Respir Med 87:239–242
19. Lion T (2014) Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev 27:441–462
20. Garnett CT, Erdman D, Xu W, Prevalence GLR (2002) Quantitation of species C adenovirus DNA in human mucosal lymphocytes. J Virol 76:10608–10616
21. Echavarría M (2008) Adenoviruses in immunocompromised hosts. Clin Microbiol Rev 21:704–715
22. Xu F, Sternberg MR, Kottiri BJ, McQuillan GM, Lee FK, Nahmias AJ et al (2006) Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. J Am Med Assoc 296:964–973
23. Reid GE, Lynch JP, Weigt S, Sayah D, Belperio JA, Grim SA et al (2016) Herpesvirus respiratory infections in immunocompromised patients: epidemiology, management, and outcomes. Semin Respir Crit Care Med 37:603–630
24. Restrepo-Gualteros SM, Gutierrez MJ, Villamil-Osorio M, Arroyo MA, Nino G (2019) Challenges and clinical implications of the diagnosis of cytomegalovirus lung infection in children. Curr Infect Dis Rep 21:24
25. Lanari M, Vandini S, Capretti MG, Lazzarotto T, Faldella G (2014) Respiratory syncytial virus infections in infants affected by primary immunodeficiency. J Immunol Res 2014:1–6
26. Barr R, Green CA, Sande CJ, Drysdale SB (2019) Respiratory syncytial virus: diagnosis, prevention and management. Ther Adv Infect Dis 6:204993611986579
27. McDermott AJ, Klein BS (2018) Helper T-cell responses and pulmonary fungal infections. Immunology 155:155–163
28. Roux A, Gonzalez F, Roux M, Mehrad M, Menotti J, Zahar JR et al (2014) Update on pulmonary Pneumocystis jirovecii infection in non-HIV patients. Med Mal Infect 44:185–198
29. Schmiedel Y, Zimmerli S (2016) Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and Pneumocystis pneumonia. Swiss Med Wkly. https://doi.org/10.4414/smw.2016.14281
30. Tischler BY, Hohl TM (2019) Menacing mold: recent advances in aspergillus pathogenesis and host defense. J Mol Biol 431:4229–4246
31. Danion F, Rouzaud C, Duréault A, Poirée S, Bougnoux M-E, Alanio A et al (2019) Why are so many cases of invasive aspergillosis missed? Med Mycol 57:S94–S103
32. Li Z, Lu G, Meng G (2019) Pathogenic Fungal Infection in the Lung. Front Immunol 10. https://doi.org/10.3389/fimmu.2019.01524
33. Lionakis MS (2019) Primary immunodeficiencies and invasive fungal infection. Curr Opin Infect Dis 32:531–537
34. Lee PP, Lau Y-L (2017) Cellular and molecular defects underlying invasive fungal infections—revelations from endemic mycoses. Front Immunol 8. https://doi.org/10.3389/fimmu.2017.00735
35. Berger M, Geng B, Cameron DW, Murphy LM, Schulman ES (2017) Primary immune deficiency diseases as unrecognized causes of chronic respiratory disease. Respir Med 132:181–188
36. Ramzi N, Jamee M, Bakhtiyari M, Rafiemanesh H, Zainaldain H, Tavakol M et al (2020) Bronchiectasis in common variable immunodeficiency: A systematic review and meta-analysis. Pediatr Pulmonol 55:292–299
37. Kainulainen L, Nikoskelainen J, Vuorinen T, Tevola K, Liippo K, Ruuskanen O (1999) Viruses and bacteria in bronchial samples from patients with primary hypogammaglobulinemia. Am J Respir Crit Care Med 159:1199–1204
38. Peltola V, Waris M, Kainulainen L, Kero J, Ruuskanen O (2013) Virus shedding after human rhinovirus infection in children, adults and patients with hypogammaglobulinaemia. Clin Microbiol Infect 19:E322–E327

39. Kohansal R, Martinez-Camblor P, Agustí A, Sonia Buist A, Mannino DM, Soriano JB (2009) The natural history of chronic airflow obstruction revisited: an analysis of the Framingham Offspring cohort. Am J Respir Crit Care Med 180:3–10
40. Chen Y, Stirling RG, Paul E, Hore-Lacy F, Thompson BR, Douglass JA (2011) Longitudinal decline in lung function in patients with primary immunoglobulin deficiencies. J Allergy Clin Immunol 127:1414–1417
41. Quint JK, Smith MP (2019) Paediatric and adult bronchiectasis: diagnosis, disease burden and prognosis. Respirology 24:413–422
42. King PT, Holdsworth SR, Freezer NJ, Villanueva E, Holmes PW (2006) Characterisation of the onset and presenting clinical features of adult bronchiectasis. Respir Med 100. https://doi.org/10.1016/j.rmed.2006.03.012
43. T Hill A, L Sullivan A, D Chalmers J, De Soyza A, Stuart Elborn J, Andres Floto R et al (2019) British Thoracic Society Guideline for bronchiectasis in adults. Thorax 74:1–69
44. Milito C, Pulvirenti F, Cinetto F, Lougaris V, Soresina A, Pecoraro A et al (2019) Double-blind, placebo-controlled, randomized trial on low-dose azithromycin prophylaxis in patients with primary antibody deficiencies. J Allergy Clin Immunol 144:584–593. e7
45. Hurst JR, Verma N, Lowe D, Baxendale HE, Jolles S, Kelleher P et al (2017) British Lung Foundation/United Kingdom primary immunodeficiency network consensus statement on the definition, diagnosis, and management of granulomatous-lymphocytic interstitial lung disease in common variable immunodeficiency disorders. J Allergy Clin Immunol Pract 5:938–945
46. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B et al (2008) Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood 112:277–286
47. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C (2012) Morbidity and mortality in common variable immune deficiency over 4 decades. Blood 119:1650–1657
48. Baumann U, Routes JM, Soler-Palacín P, Jolles S (2018) The lung in primary immunodeficiencies: new concepts in infection and inflammation. Front Immunol 9. https://doi.org/10.3389/fimmu.2018.01837
49. Yazdani R, Abolhassani H, Asgardoon M, Shaghaghi M, Modaresi M, Azizi G et al (2017) Infectious and noninfectious pulmonary complications in patients with primary immunodeficiency disorders. J Investig Allergol Clin Immunol 27:213–224
50. Torigian DA, LaRosa DF, Levinson AI, Litzky LA, Miller WT (2008) Granulomatous-lymphocytic interstitial lung disease associated with common variable immunodeficiency: CT findings. J Thorac Imaging 23:162–169
51. Meyer KC, Raghu G (2011) Bronchoalveolar lavage for the evaluation of interstitial lung disease: is it clinically useful? Eur Respir J 38:761–769
52. Rao N, MacKinnon AC, Routes JM (2015) Granulomatous and lymphocytic interstitial lung disease: A spectrum of pulmonary histopathologic lesions in common variable immunodeficiency—histologic and immunohistochemical analyses of 16 cases. Hum Pathol 46:1306–1314
53. Patel S, Anzilotti C, Lucas M, Moore N, Chapel H (2019) Interstitial lung disease in patients with common variable immunodeficiency disorders: several different pathologies? Clin Exp Immunol 198:212–223
54. Aghamohammadi A, Abolhassani H, Rezaei N, Kalantari N, Tamizifar B, Cheraghi T et al (2010) Cutaneous granulomas in common variable immunodeficiency: case report and review of literature. Acta Dermatovenerol Croat
55. Cinetto F, Scarpa R, Rattazzi M, Agostini C (2018) The broad spectrum of lung diseases in primary antibody deficiencies. Eur Respir Rev 27:180019
56. Boujaoude Z, Arya R, Rafferty W, Dammert P (2013) Organising pneumonia in common variable immunodeficiency. Case Rep 2013:bcr2013008905
57. Kersey JH, Spector BD, Good RA (1974) Cancer in children with primary immunodeficiency diseases. J Pediatr 84:263–264
58. Gathmann B, Mahlaoui N, Gérard L, Oksenhendler E, Warnatz K, Schulze I et al (2014) Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J Allergy Clin Immunol 134:116–126. e11

59. Mayor PC, Eng KH, Singel KL, Abrams SI, Odunsi K, Moysich KB et al (2018) Cancer in primary immunodeficiency diseases: cancer incidence in the United States Immune Deficiency Network Registry. J Allergy Clin Immunol 141:1028–1035
60. Hauck F, Voss R, Urban C, Seidel MG (2018) Intrinsic and extrinsic causes of malignancies in patients with primary immunodeficiency disorders. J Allergy Clin Immunol 141:59–68. e4
61. Tang V, Vijhani P, Cherian S, Ambelil M, Estrada-Y-Martin R (2018) Primary pulmonary lymphoproliferative neoplasms. Lung India 35:220–230
62. William J, Variakojis D, Yeldandi A, Raparia K (2013) Lymphoproliferative neoplasms of the lung: a review. Arch Pathol Lab Med 137:382–391
63. Neri N, Nambo MJ, Avilés A (2011) Diffuse large B-cell lymphoma primary of lung. Hematology 16:110–112
64. Latour S, Fischer A (2019) Signaling pathways involved in the T-cell-mediated immunity against Epstein-Barr virus: Lessons from genetic diseases. Immunol Rev 291:174–189
65. Edwards ESJ, Bier J, Cole TS, Wong M, Hsu P, Berglund LJ et al (2019) Activating PIK3CD mutations impair human cytotoxic lymphocyte differentiation and function and EBV immunity. J Allergy Clin Immunol 143:276–291. e6

Gut Involvement in Cellular Immunodeficiencies

13

Arianna Troilo, Natalie Frede, Chiara Della Bella, and Mario Milco D'Elios

Abstract

Primary cellular immunodeficiencies may often initially present with manifestations affecting the gastrointestinal tract. Not only infections but also inflammatory, autoimmune, and in some cases malignant manifestations may suggest the presence of a defect in the immune system.

Gastrointestinal manifestations are present in 5–50% of patients with primary immunodeficiency, which is not surprising since the intestine is the largest lymphoid organ.

Keywords

Autoimmunity · Infections · Cancer · Gastric cancer · Gastric lymphoma *Helicobacter pylori* · Intestinal cancer · Liver · Pancreas · Autoimmune anemia Spleen

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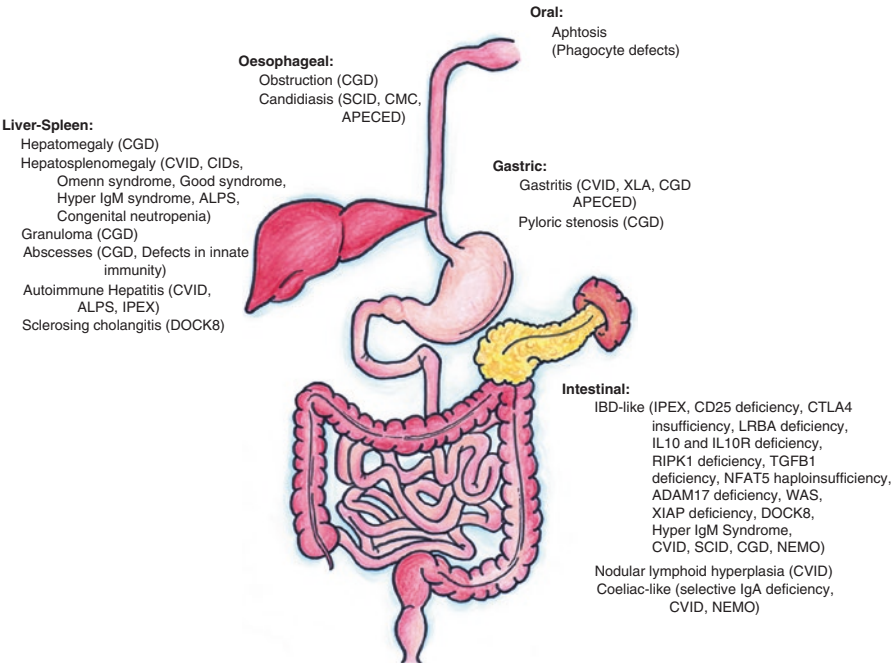

Fig. 13.1 Overview of primary immunodeficiencies affecting different parts of the gastrointestinal tract
Primary cellular immunodeficiencies can affect any site of the gastrointestinal tract. Not only infections but also inflammatory, autoimmune, and malignant manifestations may occur in PID patients

The type of manifestation depends not only on the nature of the immune defect but also on the affected part of the gastrointestinal tract (Fig. 13.1), in addition to the pathogenic stimulus, from pathogens to allergens.

Patients with primary cellular immunodeficiencies can have inflammatory, autoimmune, and malignant manifestations in any part of the gastrointestinal tract.

The symptomatology is often confused with the clinical symptoms of common gastrointestinal diseases, from the most common gastroenteritis to autoimmune or inflammatory diseases such as celiac disease or inflammatory bowel disease. Atypical presentation regarding symptomatology, age of onset of symptoms, and, above all, a characteristic histology as well as non-response to common treatments can lead to suspicion that a defect in the immune system is at the basis of the symptoms.

In this case, a simple protein electrophoresis screening and immunoglobulin and total protein assay may already help to distinguish primary hypogammaglobulinemia from protein loss. The study of vaccine responses and a lymphocyte profile may coarsely point between a humoral immune defect and a cellular or combined defect. Humoral immune defects and gastrointestinal manifestations of these have been extensively discussed in the previous volume of this trilogy. In this chapter we

will focus on the gastrointestinal manifestations of primary cellular immunodeficiencies. An overview of the main gastrointestinal symptoms and pathohistological findings can be found in Table 13.1.

Table 13.1 Overview of main gastrointestinal symptoms and pathohistological findings in cellular primary immunodeficiencies

	Cellular PID	Gastrointestinal involvement	Histology
B and T cell defects	SCID	Infectious diarrhea	Hypocellular lamina propria Villous atrophy GVHD-like
	DOCK8 deficiency	Infectious diarrhea Eosinophilic esophagitis Enteropathy	
	NEMO syndrome	Infectious and inflammatory colitis	Acute inflammation, edema Superficial cryptitis Mucosal ulceration
	Omenn syndrome	Diarrhea, malabsorption Enterocolitis	
Phagocyte defects	CGD	Infectious diarrhea, granuloma IBD-like enterocolitis Oral aphthosis, anal fistulas, vomiting, Anorexia abdominal pain Liver abscesses	Granulomas, pigmented macrophages, eosinophils
	LAD-1	Omphalitis Perirectal abscesses, periodontitis Diarrhea Hepatosplenomegaly IBD-like colitis	
	G6PC3	IBD-like colitis Chronic diarrhea with steatorrhea	
Regulatory T cell defects	IPEX	Severe enteropathy Watery/bloody diarrhea	Villous atrophy, mucosal erosion and ulcerations Lymphocytic infiltrates in lamina propria and submucosa
	CD25 deficiency	Enteropathy with chronic diarrhea	IPEX-like villous atrophy
	CTLA4 insufficiency	Atrophic gastritis Diarrhea Crohn's disease	Ulcerative lesions as well as deep T cell infiltrates and inflammatory changes in the submucosa
	LRBA deficiency		

(continued)

Table 13.1 (continued)

	Cellular PID	Gastrointestinal involvement	Histology
Immune dysregulation with colitis	IL10 and IL10R deficiency	Enterocolitis, perianal disease, and fistula	Ulcerations, pseudopolyps, granulation, inflammatory infiltrate,s and abscesses
	RIPK1 deficiency	Diarrhea IBD-like colitis	Chronic inflammation with erosions Apoptotic bodies within the cryptic bases Depletion of lamina propria plasma cells
	NFAT5 haploinsufficiency	IBD-like autoimmune enteropathy	Intraepithelial lymphocytosis Apoptotic enterocytes Absence of goblet cells
	TGFB1 deficiency	Severe very early-onset inflammatory bowel disease	Epithelial inflammatory infiltrates, mucosal ulceration, crypt abscesses
	ADAM17 deficiency	Early-onset chronic/bloody diarrhea	
Lymphoprolif.	XIAP deficiency	Early-onset IBD Splenomegaly	
Defined syndromes	Wiskott-Aldrich syndrome	Bloody diarrhea Malabsorption	Cobblestone appearance, inflammatory pseudopolyps, cryptitis, and crypt abscesses
	DiGeorge syndrome	Autoimmune enteropathy and celiac disease, abdominal pain, vomiting, gastroesophageal reflux, chronic constipation, abnormal permeability, laryngotracheoesophageal and gastrointestinal structural defects	
	Chronic mucocutaneous candidiasis	Candida esophagitis	
	APECED syndrome	Autoimmune gastritis and pancreatic insufficiency	

13.1 B and T Cell Defects

13.1.1 Severe Combined Immune Deficiency

Combined T and B immunodeficiencies affect B cell and T cell functions; therefore GI disorders are frequent and occur in almost 90% of patients and can be life-threatening if untreated [1, 2]. Diarrhea and malabsorption can occur due to bacterial infections but more often viral infections, such as rotavirus, cytomegalovirus, Epstein-Barr virus, adenovirus, or opportunistic infections such as *P. aeruginosa* [3]. Rotavirus vaccination was demonstrated to be able to cause chronic infection in infants with SCID [4]. Frequent gastrointestinal manifestations include candidiasis (oral, esophageal, perianal, and more rarely intestinal). Oral and esophageal candidiasis can contribute to the failure to thrive by reducing oral intake in affected children [5]. *E. coli, Giardia, Cryptosporidium, Salmonella,* and *Shigella* infections were also described [6]. GI manifestations can also be present due to GVHD after bone marrow transplantation or blood transfusions.

Gastrointestinal biopsies show a hypocellular lamina propria without lymphocytes or plasma cells. Cytomegalovirus and adenovirus were identified in gastrointestinal biopsies of SCID patients. Villous atrophy may be present following infections. A GVHD-like process affecting the colon and the small intestine was described not only in patients who had received bone marrow transplantations or blood transfusions but also in pre-bone marrow transplantation biopsies [7, 8]. Infections as well as GVHD can also affect the liver (adenovirus and CMV hepatitis have been described, as well as rotavirus [9, 10]). Acute GVHD of the pancreas and viral pancreatitis have also been observed [11].

13.1.2 DOCK8 Deficiency

Gastrointestinal viral infections with Epstein-Barr virus, rotavirus, herpes simplex virus, as well as hepatitis A, B, and C viruses were described in DOCK8-deficient patients [12–15]. Hepatobiliary infection with *Cryptosporidium* has also been described and can be particularly damaging and difficult to treat [16]. Parasitic infections include *Entamoeba histolytica* and *Giardia lamblia* infections [13, 17, 18]. Sclerosing cholangitis secondary to chronic infection has also been reported in DOCK8-deficient patients [17]. Eosinophilic esophagitis has also been observed [19].

Intestinal complications can lead to failure to thrive and growth stunting [13]. Malabsorption resulting in failure to thrive may be caused not only by infections but also by allergic or autoimmune enteropathy. Chronic diarrhea caused by enteropathy can be a prominent feature to the degree that the condition may present as IPEX-like disease [20].

13.1.3 NFKB Essential Modulator Deficiency (NEMO) Syndrome

X-linked recessive primary immune deficiency due to a genetic mutation affecting the inhibitor of nuclear factor kappa-B kinase subunit gamma (IKBKG) gene, which codes for the NEMO protein, is characterized by abnormal development of the immune system, affecting lymphocytes, dendritic cells, and phagocytes. Patients present with susceptibility to bacterial and fungal infections. Gut infections were described in more than 20% of the patients, including severe adenoviral gastroenteritis [21].

A study by Hanson et al. describing NEMO phenotypes showed 21% of patients to have inflammatory colitis, almost half of them suffered from intractable diarrhea and failure to thrive [22]. IBD in NEMO patients was shown to affect both, the small and the large intestine. In the histology acute inflammation, edema, superficial cryptitis, and mucosal ulceration were found as well as abundance of neutrophils within the lamina propria [23].

13.1.4 Omenn Syndrome

Omenn syndrome is an autosomal recessive form of severe combined immunodeficiency (SCID) characterized by erythroderma, desquamation, alopecia, chronic diarrhea, failure to thrive, lymphadenopathy, eosinophilia, hepatosplenomegaly, and elevated serum IgE levels. Genetic defects in *RAG1*, *RAG2,* or *DCLRE1C*/ARTEMIS can be the molecular cause of the disease.

Patients can present with gastrointestinal symptoms such as intractable diarrhea, malabsorption, infections with enteric pathogens, and failure to thrive. IBD-like enterocolitis was described in *RAG1*/*RAG2* defects as well as in patients with ARTEMIS deficiency [24, 25].

13.2 Congenital Defects of Phagocyte Number or Function

The focus of this section will be on oxidative burst defects, where a characteristic intestinal involvement has been described. However, symptoms at the level of the gastrointestinal tract can also occur in patients with other defects of function and number of phagocytes. In particular, in a study of 14 patients with glucose-6-phosphatase catalytic subunit 3 (G6PC3) deficiency, three patients developed Crohn's disease, and five had chronic diarrhea with steatorrhea [26].

13.2.1 Chronic Granulomatous Disease (CGD)

Gastrointestinal (GI) involvement is a common and recurring problem in CGD, especially in those with X-linked inheritance [27]. Chronic granulomatous disease (CGD) is caused by defects in the subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex in phagocytes, which plays an

essential role in the production of superoxide anion radicals crucial for the killing of catalase-positive organisms. Phagocytes from patients affected by CGD are therefore unable to effectively kill intracellular and some extracellular microorganisms [28]. Therefore, patients with CGD start to suffer from recurrent life-threatening bacterial and fungal infections soon after birth, which can affect the skin as well as the gastrointestinal and respiratory tract, as well as other organs where high numbers of phagocytes are present (e.g., the liver) [29].

The most frequent pathogens are *Staphylococcus aureus, Burkholderia cepacia, Serratia marcescens, Aspergillus species, Chromobacterium violaceum, and Nocardia species* [30]. Although the average survival age of CGD patients has increased over time, infections remain a major cause of death. In a study by Yu et al., serum levels of GI-specific pathogen antibodies in patients with CGD were measured, detecting high levels of these antibodies. However, these did not correlate with the presence or absence of intestinal inflammation and therefore did not explain the etiopathogenesis of intestinal manifestations [31].

Around 70% of CGD patients present with gastrointestinal symptoms like non-infectious diarrhea, oral aphthosis, anal fistulas, vomiting, anorexia, and abdominal pain. Gastrointestinal involvement in CGD can mimic other inflammatory bowel diseases [32, 33]. Specifically, gastrointestinal involvement can present as Crohn-like disease, protein-losing enteropathy, or noncaseating granulomatous colitis. The entire GI tract can be affected by granulomata, and symptoms can include dysphagia, dysmotility, or obstruction [34].

A study of 87 CGD patients with gastrointestinal symptoms who underwent endoscopy showed that 95% of patients with gastrointestinal symptoms presented a pathological histological analysis, with frequent presence of microgranulomas, pigmented macrophages and eosinophils in both acute and chronic inflammation and independently of the presence of neutrophils. The most affected segment of the GI tract was the colon. Some biopsies of duodenum and ileum showed shortening of the villi. It was almost never possible to correlate inflammatory infiltrates with a trigger infection [35]. In a retrospective study of 20 CGD patients with inflammatory gastrointestinal manifestations, 55% complained of nonspecific diarrhea, with severe endoscopic appearance not correlating to gravity of the symptoms. The colon was extensively involved in 44% of the patients, and characteristic features (epithelioid granulomas, pigmented macrophages, and increased eosinophils) were present in 78% of patients [36].

Treatment of gastrointestinal involvement frequently includes antibiotics or other anti-infective agents, steroids, and other immunomodulatory or immunosuppressive therapies, where benefits must be weighed against the risk of infectious complications, as well as at times surgery. Hematopoietic stem cell transplantation constitutes an option for severely affected patients.

Hepatic involvement is also frequent in CGD. Liver abscesses are a common complication, frequently detected pathogens are *S. aureus* and *Pseudomonas aeruginosa.* Abscesses with *S. aureus* were reported in around 27% of CGD patients [37]. Drug-induced hepatotoxicity is also frequent. Nodular regenerative

hyperplasia and hepatosplenomegaly have also been described [38]. Vascular injuries to the microvasculature of the liver from repeated systemic and hepatic infections may lead to noncirrhotic portal hypertension, which is associated with high mortality [39].

13.2.2 Leukocyte Adhesion Deficiency Type 1

Leukocyte adhesion deficiency type 1 (LAD-1) is an autosomal recessive primary immunodeficiency, caused by mutations in the integrin β2 gene (ITGB2), which encodes the common β subunit of the β2 integrin family, also termed CD18. The impairment of integrin function results in impaired leukocyte adhesion to the vascular wall, inhibiting polymorphonuclear (PMN) migration to tissue sites and pus formation, which is accompanied by neutrophilia [40]. Patients classically present with omphalitis during the newborn period, nonpurulent skin and soft tissue infections, perirectal abscesses, periodontitis, and defective wound healing [41, 42]. The most frequent pathogens are *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella* sp. Also fungal infections may occur [43].

Other gastrointestinal manifestations include diarrhea and hepatosplenomegaly. Furthermore, chronic ileocolitis resembling idiopathic inflammatory bowel disease (IBD) with inflammation and ulceration of the colon and the terminal ileum was described [44, 45]. Cord blood transplantation may lead to remission of IBD-like disease [46].

13.3 Diseases of Immune Dysregulation

The gastrointestinal system is often considered the largest lymphoid organ of the body, and its lumen is densely populated by vast numbers of microorganisms, which regularly come into contact with resident immune cells. Furthermore, food antigens offer a continuous challenge. Thus, a tight regulation is necessary in order to maintain homeostasis; barrier functions and maintenance of tolerance are of utmost importance. In immune dysregulation syndromes, these mechanisms fail, and therefore many of these syndromes are associated with enteropathy and gastrointestinal inflammation. Here, we present a number of characteristic examples, though this list is by no means exhaustive and patients with almost any immunodysregulatory disease may develop gastrointestinal manifestations.

13.3.1 Regulatory T Cell Defects

13.3.1.1 IPEX Syndrome

The immune dysregulation polyendocrinopathy X-linked (IPEX) syndrome is caused by mutations in the transcription factor FOXP3, which leads to dysfunction

of regulatory T cells and consequently immune dysregulation and susceptibility to autoimmunity [47]. Hallmarks of IPEX syndrome are a severe enteropathy, which is often intractable, and early-onset polyendocrinopathy, frequently including type 1 diabetes. Enteropathy presents as refractory secretory, i.e., watery but sometimes also bloody diarrhea in the first months of life, and often does not respond to enteric rest [48]. Therefore, patients frequently suffer from failure to thrive and malnutrition and require total parenteral nutrition.

Due to loss of oral tolerance, patients may present with severe food allergy and IgE levels can be increased [49]. Anti-enterocyte, anti-harmonin, or anti-villin autoantibodies are frequently detectable in patients with enteropathy [50, 51]. Enteropathy may lead to protein loss including hypogammaglobulinemia. Severe enteropathy may furthermore facilitate translocation of bacteria from the gut, which can lead to infectious complications.

Histopathological findings include partial or total villous atrophy with mucosal erosion and ulcerations up to destruction of the small bowel mucosa. Lymphocytic infiltrates in the lamina propria and submucosa constitute a typical finding and may also occur in other organs [50, 52]. Involvement of the colon may be present.

Treatment consists of total parenteral nutrition and immunosuppressors, such as cyclosporin A, tacrolimus, sirolimus, and corticosteroids, although immunosuppression may lead to opportunistic infections [53, 54]. Hematopoietic stem cell transplantation constitutes a curative approach and was demonstrated to lead to gut immune reconstitution [55, 56].

13.3.1.2 CD25 Deficiency

CD25 deficiency constitutes a rare regulatory T cell defect, which leads to an IPEX-like syndrome [57]. Patients may present with severe early-onset autoimmune enteropathy with chronic diarrhea very similar to IPEX, which may also be accompanied by protein loss. Even histopathological findings resemble IPEX with villous atrophy being a prominent finding [58]. However, patients show a greater susceptibility to infections (viral, bacterial, and fungal, often opportunistic) than IPEX patients, which may complicate immunosuppressive treatment [57, 59]. Hematopoietic stem cell transplantation is curative [58].

13.3.1.3 CTLA4 Insufficiency

CTLA4 insufficiency is probably the most common regulatory T cell defect and is also commonly associated with gastrointestinal disease. Schwab et al. reported 59% of patients to have gastrointestinal involvement, however varying in severity [60]. Diarrhea is a common occurrence, with a number of patients being diagnosed with concomitant Crohn's disease [61]. Also atrophic gastritis was reported in a number of cases. Histopathological findings include ulcerative lesions as well as deep T cell infiltrates and inflammatory changes in the submucosa.

Treatment with CTLA4 fusion proteins abatacept and belatacept may lead to improvement of clinical symptoms including enteropathy.

13.3.1.4 LRBA Deficiency

A detailed account of LRBA deficiency may be found in the second book of this series, Humoral Primary Immunodeficiencies [62].

13.3.2 Immune Dysregulation with Colitis

13.3.2.1 IL10 and IL10R Deficiency

IL10 constitutes an important immunoregulatory cytokine produced by regulatory T cells, monocytes, macrophages, and B cells, but also epithelial cells. Loss of function mutations in the cytokine IL10 itself or the IL10 receptor chains IL10RA or IL10RB lead to very early-onset inflammatory bowel disease usually manifesting within the first year of life [63, 64]. Patients present with a severe and difficult to treat enterocolitis, which is frequently accompanied by perianal disease and fistula formation and leads to failure to thrive. Extraintestinal manifestations may include folliculitis, recurrent respiratory disease, and arthritis [65]. Immunological workup normally shows only subtle abnormalities, such as a decreased CD4/CD8 T cell ratio and immunoglobulin levels, which may be elevated or decreased. Histopathological findings may include ulcerations, pseudopolyps, granulation, inflammatory infiltrates, and abscesses.

Disease often is refractory to conventional immunosuppressive treatment, necessitating surgical interventions. Hematopoietic stem cell transplantation constitutes a curative approach [65, 66].

13.3.2.2 RIPK1 Deficiency

The receptor-interacting serine/threonine-protein kinase 1 (RIPK1) plays an important role in controlling inflammation and cell death responses. RIPK1 deficiency leads to a primary immunodeficiency with immune dysregulation and intestinal inflammation. Depending on the genetic mutation, patients were described to either present with a combined immunodeficiency phenotype including diarrhea or with a predominantly early-onset IBD phenotype [67]. Other extraintestinal manifestations include a progressive polyarthritis [68]. The patients were shown to have lymphopenia with impaired B and T cell differentiation and an increased susceptibility to recurrent viral, bacterial, and fungal infections. There are only few data available, but histologic features of RIPK1 deficiency include chronic inflammation with erosions, increased apoptotic bodies within the cryptic bases, and depletion of lamina propria plasma cells [67]. Hematopoietic stem cell transplantation can be an effective treatment addressing immunodeficiency, enteropathy, and arthritis [68].

13.3.2.3 NFAT5 Haploinsufficiency

Boland et al. described a patient with a heterozygous mutation in NFAT5, who developed an IBD-like autoimmune enteropathy starting at the age of 7 years. Histopathological findings included intraepithelial lymphocytosis, abundant apoptotic enterocytes, as well as an absence of goblet cells, while anti-goblet cell

antibodies were positive. Extraintestinal manifestations included eczema and recurrent respiratory tract infections [69].

13.3.2.4 TGFB1 Deficiency

TGF-beta is an immunomodulatory cytokine, which plays an important role in tissue homeostasis and wound healing. In 2018, Kotlarz et al. described three patients from two families with TGFB1 deficiency presenting with severe very early-onset inflammatory bowel disease and encephalopathy including epilepsy, brain atrophy, and global developmental delay [70]. Two out of three patients also had recurrent infections. Histological findings included inflammatory infiltrates in the epithelium with mucosal ulceration and crypt abscesses. Two of the three patients experienced rapid neurological deterioration and ultimately passed away due to infectious complications aged 25 and 39 months [70].

13.3.2.5 ADAM17 Deficiency

ADAM17, also called TNF-α-converting enzyme (TACE), constitutes a metalloproteinase or sheddase, which is essential for TNF-α shedding from the cell surface. *ADAM17* deletion was described to lead to inflammatory skin and bowel disease with early-onset chronic diarrhea and failure to thrive [71]. The diarrhea was bloody with malabsorptive characteristics and accompanied by an inflammatory skin condition with psoriasiform erythroderma and generalized pustular rashes. Patient's PBMCs exhibited impaired TNF-α production. So far, only two patients from one kindred have been described.

13.3.3 Immune Dysregulation with Lymphoproliferative Defects

13.3.3.1 XIAP Deficiency

XIAP deficiency or XLP2 is a clinically heterogeneous immune dysregulation syndrome, which may lead to EBV-triggered lymphoproliferative disease, splenomegaly, hemophagocytic lymphohistiocytosis (HLH), and a Crohn-like enteropathy. Depending on the cohort, 0–29% of patients were reported to suffer from colitis/enteropathy [72–74]. Also atypical presentations of (very) early-onset inflammatory bowel disease have been described as a common clinical manifestation of XIAP deficiency [75, 76]. In a XIAP-deficient cohort described by Aguilar et al., IBD was the main or most severe clinical finding in half of the patients [72]. Also female carriers with skewed X-inactivation toward the mutated allele may develop an IBD phenotype [72, 77]. Patients initially presenting with IBD may go on to develop HLH in the further course of disease. IBD in XIAP deficiency is frequently severe, may be accompanied by abscess formation and perineal fistulae, and is often difficult to treat. The distribution often mimics Crohn's disease. Histopathological findings include crypt abscesses, infiltrates, and epithelioid granulomas. Disease is often refractory to immunosuppressive medication and requires aggressive treatment and surgery. Hematopoietic stem cell transplantation may lead to resolution of IBD and may also be considered in order to avoid HLH, especially in young patients [76, 78].

13.4 Defined Syndromes with Immunodeficiency and Gastrointestinal Involvement

13.4.1 Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome (WAS) is a rare X-linked immune deficiency that is characterized by eczema, thrombocytopenia, and recurrent sinopulmonary infections. Disease occurs due to disruption of the integrity of the cytoskeleton [79]. Wiskott-Aldrich syndrome is associated with a higher risk of autoimmunity, including cytopenias, vasculitis, arthritis, as well as inflammatory bowel disease presenting often with bloody diarrhea and malabsorption [80]. Inflammatory bowel disease in WAS may present as either ulcerative colitis or Crohn's disease and is found in 10% of the patients [81, 82]. Endoscopic and histopathological findings include cobblestone appearance, inflammatory pseudopolyps, cryptitis, and crypt abscesses, although only limited data on histopathology are available due to the high risk of bleeding complications [82, 83]. Laboratory findings may include eosinophilia, elevated IgE levels, as well as specific IgE to food allergens [84, 85]. Patients with mutations in *WAS* may also present with a (very) early-onset IBD phenotype without the typical triad of thrombocytopenia, eczema, and susceptibility to infections [86].

Treatment options include immunosuppressive treatment, such as steroids or cyclosporin, as well as for severe cases gene therapy and hematopoietic stem cell transplantation [80, 87, 88].

13.4.2 DiGeorge Syndrome

DiGeorge syndrome is characterized by thymic aplasia with impaired T lymphocyte maturation. B cells are usually not affected [89]. In patients with DiGeorge syndrome, autoimmune diseases occur at an increased frequency, including autoimmune enteropathy and celiac disease [90]. A monocentric study evaluating 26 patients with DiGeorge syndrome found GI involvement in the 58% of patients. Patients presented with abdominal pain, vomiting, gastroesophageal reflux, chronic constipation, as well as abnormal intestinal permeability [91]. Patients with complete DiGeorge syndrome may present shortly after birth with recurrent severe infections, chronic diarrhea, and failure to thrive. This form of the disease is fatal unless a thymus or bone marrow transplant is performed [92].

22q11 deletion syndromes may be accompanied by laryngotracheoesophageal and gastrointestinal structural defects, which may include cleft lip/palate defects, velopharyngeal incompetence with dysphagia and feeding difficulties, esophageal atresia, mal- or nonrotation, and Hirschsprung disease [93–96].

13.4.3 Chronic Mucocutaneous Candidiasis and APECED Syndrome

These syndromes are characterized by impaired T cell proliferation and cytokine production in response to *Candida albicans* antigens. Candida esophagitis can result in reduced oral intake and failure to thrive.

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome occurs due to mutations in AIRE gene. This subgroup of patients can present also autoimmune gastritis and pancreatic insufficiency, as leading to weight loss, as gastrointestinal manifestations [97].

13.5 Conclusions

Patients with primary immunodeficiencies frequently present with GI symptoms, which are often associated with a high morbidity and sometimes also with an increased mortality. GI manifestations of PID can mimic common gastrointestinal diseases and may not always be obvious/may be difficult to diagnose, especially in patients where GI disease precedes other manifestations of PID. Unusually severe or chronic infections resistant to usual treatments, as well as the isolation of uncommon pathogens, are suggestive of a possible defect in the immune system.

In particular, IBD-like disease is a common manifestation of cellular immunodeficiencies, and an early-onset disease should suggest an underlying immune defect, especially in countries with frequent consanguineous marriages.

Flow cytometric analysis and phagocytic respiratory burst tests can easily screen for the most common cellular immunodeficiencies associated with this clinical presentation, whereas TREC analysis permits to screen for SCID. However, in recent years, genetic analysis has become more widely available and should be considered in any patient with very early-onset IBD, as the identification of an underlying genetic defect may help anticipate other disease manifestations, which may arise in the future and facilitate treatment decisions especially in severely affected patients.

References

1. Lai Ping So A, Mayer L (1997) Gastrointestinal manifestations of primary immunodeficiency disorders. Semin Gastrointest Dis 8(1):22–32
2. Rosen FS (1986) Defects in cell-mediated immunity. Clin Immunol Immunopathol 41(1):1–7
3. Jarvis WR, Middleton PJ, Gelfand EW (1983) Significance of viral infections in severe combined immunodeficiency disease. Pediatr Infect Dis 2(3):187–192
4. Bakare N, Menschik D, Tiernan R, Hua W, Martin D (2010) Severe combined immunodeficiency (SCID) and rotavirus vaccination: reports to the Vaccine Adverse Events Reporting System (VAERS). Vaccine 28(40):6609–6612

5. Patil S, Rao RS, Majumdar B, Anil S (2015) Clinical appearance of oral candida infection and therapeutic strategies. Front Microbiol 6:1391
6. Stephan JL, Vlekova V, Le Deist F, Blanche S, Donadieu J, De Saint-Basile G et al (1993) Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. J Pediatr 123(4):564–572
7. Snover DC, Filipovich AH, Ramsay NK, Weisdorf SA, Kersey JH (1985) Graft-versus-host-disease-like histopathological findings in pre-bone-marrow transplantation biopsies of patients with severe T cell deficiency. Transplantation 39(1):95–97
8. Lee EY, Clouse RE, Aliperti G, DeSchryver-Kecskemeti K (1991) Small intestinal lesion resembling graft-vs-host disease. A case report in immunodeficiency and review of the literature. Arch Pathol Lab Med 115(5):529–532
9. Washington K, Gossage DL, Gottfried MR (1993) Pathology of the liver in severe combined immunodeficiency and DiGeorge syndrome. Pediatr Pathol 13(4):485–504
10. Gilger MA, Matson DO, Conner ME, Rosenblatt HM, Finegold MJ, Estes MK (1992) Extraintestinal rotavirus infections in children with immunodeficiency. J Pediatr 120(6):912–917
11. Washington K, Gossage DL, Gottfried MR (1994) Pathology of the pancreas in severe combined immunodeficiency and DiGeorge syndrome: acute graft-versus-host disease and unusual viral infections. Hum Pathol 25(9):908–914
12. Sanal O, Jing H, Ozgur T, Ayvaz D, Strauss-Albee DM, Ersoy-Evans S et al (2012) Additional diverse findings expand the clinical presentation of DOCK8 deficiency. J Clin Immunol 32(4):698–708
13. Engelhardt KR, Gertz ME, Keles S, Schäffer AA, Sigmund EC, Glocker C et al (2015) The extended clinical phenotype of 64 patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol 136(2):402–412
14. Alsum Z, Hawwari A, Alsmadi O, Al-Hissi S, Borrero E, Abu-Staiteh A et al (2013) Clinical, immunological and molecular characterization of DOCK8 and DOCK8-like deficient patients: single center experience of twenty-five patients. J Clin Immunol 33(1):55–67
15. Betts K, Abusleme L, Freeman AF, Sarmadi M, Fahle G, Pittaluga S et al (2015) A 17-year old patient with DOCK8 deficiency, severe oral HSV-1 and aggressive periodontitis—a case of virally induced periodontitis? J Clin Virol 63:46–50
16. Al-Herz W, Ragupathy R, Massaad MJ, Al-Attiyah R, Nanda A, Engelhardt KR et al (2012) Clinical, immunologic and genetic profiles of DOCK8-deficient patients in Kuwait. Clin Immunol 143(3):266–272
17. Shah T, Cale C, Hadzic N, Jones A (2014) Dedicator of cytokinesis 8 deficiency: a predisposition to sclerosing cholangitis. Clin Immunol 155(1):71–73
18. Al-Herz W, Chu JI, van der Spek J, Raghupathy R, Massaad MJ, Keles S et al (2016) Hematopoietic stem cell transplantation outcomes for 11 patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol 138(3):852–859. e3
19. Biggs CM, Keles S, Chatila TA (2017) DOCK8 deficiency: insights into pathophysiology, clinical features and management. Clin Immunol 181:75–82
20. Alroqi FJ, Charbonnier LM, Keles S, Ghandour F, Mouawad P, Sabouneh R et al (2017) DOCK8 deficiency presenting as an IPEX-like disorder. J Clin Immunol 37(8):811–819
21. Picard C, Casanova JL, Puel A (2011) Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. Clin Microbiol Rev 24(3):490–497
22. Hanson EP, Monaco-Shawver L, Solt LA, Madge LA, Banerjee PP, May MJ et al (2008) Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. J Allergy Clin Immunol 122(6):1169–1177. e16
23. Cheng LE, Kanwar B, Tcheurekdjian H, Grenert JP, Muskat M, Heyman MB et al (2009) Persistent systemic inflammation and atypical enterocolitis in patients with NEMO syndrome. Clin Immunol 132(1):124–131
24. Hönig M, Schwarz K (2006) Omenn syndrome: a lack of tolerance on the background of deficient lymphocyte development and maturation. Curr Opin Rheumatol 18(4):383–388

25. Puzenat E, Rohrlich P, Thierry P, Girardin P, Taghian M, Ouachee M et al (2007) Omenn syndrome: a rare case of neonatal erythroderma. Eur J Dermatol 17(2):137–139
26. Desplantes C, Fremond ML, Beaupain B, Harousseau JL, Buzyn A, Pellier I et al (2014) Clinical spectrum and long-term follow-up of 14 cases with G6PC3 mutations from the French Severe Congenital Neutropenia Registry. Orphanet J Rare Dis 9:183
27. Marciano BE, Rosenzweig SD, Kleiner DE, Anderson VL, Darnell DN, Anaya-O'Brien S et al (2004) Gastrointestinal involvement in chronic granulomatous disease. Pediatrics 114(2):462–468
28. Arnold DE, Heimall JR (2017) A review of chronic granulomatous disease. Adv Ther 34(12):2543–2557
29. Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S et al (2015) Common severe infections in chronic granulomatous disease. Clin Infect Dis 60(8):1176–1183
30. McCabe RP (2002) Gastrointestinal Manifestations of Non-AIDS Immunodeficiency. Curr Treat Opt Gastroenterol 5(1):17–25
31. Yu JE, De Ravin SS, Uzel G, Landers C, Targan S, Malech HL et al (2011) High levels of Crohn's disease-associated anti-microbial antibodies are present and independent of colitis in chronic granulomatous disease. Clin Immunol 138(1):14–22
32. Rieber N, Hector A, Kuijpers T, Roos D, Hartl D (2012) Current concepts of hyperinflammation in chronic granulomatous disease. Clin Dev Immunol 2012:252460
33. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L et al (2009) Chronic granulomatous disease: the European experience. PLoS One 4(4):e5234
34. Magnani A, Brosselin P, Beauté J, de Vergnes N, Mouy R, Debré M et al (2014) Inflammatory manifestations in a single-center cohort of patients with chronic granulomatous disease. J Allergy Clin Immunol 134(3):655–662. e8
35. Alimchandani M, Lai JP, Aung PP, Khangura S, Kamal N, Gallin JI et al (2013) Gastrointestinal histopathology in chronic granulomatous disease: a study of 87 patients. Am J Surg Pathol 37(9):1365–1372
36. Angelino G, De Angelis P, Faraci S, Rea F, Romeo EF, Torroni F et al (2017) Inflammatory bowel disease in chronic granulomatous disease: an emerging problem over a twenty years' experience. Pediatr Allergy Immunol 28(8):801–809
37. Winkelstein JA, Marino MC, Johnston RB, Boyle J, Curnutte J, Gallin JI et al (2000) Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore) 79(3):155–169
38. Hussain N, Feld JJ, Kleiner DE, Hoofnagle JH, Garcia-Eulate R, Ahlawat S et al (2007) Hepatic abnormalities in patients with chronic granulomatous disease. Hepatology 45(3):675–683
39. Feld JJ, Hussain N, Wright EC, Kleiner DE, Hoofnagle JH, Ahlawat S et al (2008) Hepatic involvement and portal hypertension predict mortality in chronic granulomatous disease. Gastroenterology 134(7):1917–1926
40. Etzioni A (2009) Genetic etiologies of leukocyte adhesion defects. Curr Opin Immunol 21(5):481–486
41. Levy-Mendelovich S, Rechavi E, Abuzaitoun O, Vernitsky H, Simon AJ, Lev A et al (2016) Highlighting the problematic reliance on CD18 for diagnosing leukocyte adhesion deficiency type 1. Immunol Res 64(2):476–482
42. Roberts MW, Atkinson JC (1990) Oral manifestations associated with leukocyte adhesion deficiency: a five-year case study. Pediatr Dent 12(2):107–111
43. Wolach B, Gavrieli R, Wolach O, Stauber T, Abuzaitoun O, Kuperman A et al (2019) Leucocyte adhesion deficiency—a multicentre national experience. Eur J Clin Investig 49(2):e13047
44. D'Agata ID, Paradis K, Chad Z, Bonny Y, Seidman E (1996) Leucocyte adhesion deficiency presenting as a chronic ileocolitis. Gut 39(4):605–608
45. Uzel G, Kleiner DE, Kuhns DB, Holland SM (2001) Dysfunctional LAD-1 neutrophils and colitis. Gastroenterology 121(4):958–964
46. Jain S, Gozdziak P, Morgan A, Burt RK (2013) Remission of Crohn's disease after cord blood transplantation for leukocyte adhesion deficiency type 1. Bone Marrow Transplant 48(7):1006–1007

47. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C et al (2000) JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest 106(12):R75–R81
48. Wildin RS, Smyk-Pearson S, Filipovich AH (2002) Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet 39(8):537–545
49. Torgerson TR, Linane A, Moes N, Anover S, Mateo V, Rieux-Laucat F et al (2007) Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene. Gastroenterology 132(5):1705–1717
50. Patey-Mariaud de Serre N, Canioni D, Ganousse S, Rieux-Laucat F, Goulet O, Ruemmele F et al (2009) Digestive histopathological presentation of IPEX syndrome. Mod Pathol 22(1):95–102
51. Barzaghi F, Amaya Hernandez LC, Neven B, Ricci S, Kucuk ZY, Bleesing JJ et al (2018) Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: an international multicenter retrospective study. J Allergy Clin Immunol 141(3):1036–1049. e5
52. Bennett CL, Ochs HD (2001) IPEX is a unique X-linked syndrome characterized by immune dysfunction, polyendocrinopathy, enteropathy, and a variety of autoimmune phenomena. Curr Opin Pediatr 13(6):533–538
53. Yong PL, Russo P, Sullivan KE (2008) Use of sirolimus in IPEX and IPEX-like children. J Clin Immunol 28(5):581–587
54. Kobayashi I, Kawamura N, Okano M (2001) A long-term survivor with the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. N Engl J Med 345(13):999–1000
55. Gambineri E, Ciullini Mannurita S, Robertson H, Vignoli M, Haugk B, Lionetti P et al (2015) Gut immune reconstitution in immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome after hematopoietic stem cell transplantation. J Allergy Clin Immunol 135(1):260–262
56. Rao A, Kamani N, Filipovich A, Lee SM, Davies SM, Dalal J et al (2007) Successful bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. Blood 109(1):383–385
57. Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW (2007) CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. J Allergy Clin Immunol 119(2):482–487
58. Vignoli M, Ciullini Mannurita S, Fioravanti A, Tumino M, Grassi A, Guariso G et al (2019) CD25 deficiency: a new conformational mutation prevents the receptor expression on cell surface. Clin Immunol 201:15–19
59. Sharfe N, Dadi HK, Shahar M, Roifman CM (1997) Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. Proc Natl Acad Sci U S A 94(7):3168–3171
60. Schwab C, Gabrysch A, Olbrich P, Patiño V, Warnatz K, Wolff D et al (2018) Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4-insufficient subjects. J Allergy Clin Immunol 142(6):1932–1946
61. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A et al (2014) Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 20(12):1410–1416
62. D'Elios MM, Rizzi M (2019) Humoral primary immunodeficiencies. Springer, Cham
63. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F et al (2009) Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med 361(21):2033–2045
64. Glocker EO, Frede N, Perro M, Sebire N, Elawad M, Shah N et al (2010) Infant colitis—it's in the genes. Lancet 376(9748):1272
65. Engelhardt KR, Shah N, Faizura-Yeop I, Kocacik Uygun DF, Frede N, Muise AM et al (2013) Clinical outcome in IL-10- and IL-10 receptor-deficient patients with or without hematopoietic stem cell transplantation. J Allergy Clin Immunol 131(3):825–830

66. Kotlarz D, Beier R, Murugan D, Diestelhorst J, Jensen O, Boztug K et al (2012) Loss of interleukin-10 signaling and infantile inflammatory bowel disease: implications for diagnosis and therapy. Gastroenterology 143(2):347–355
67. Li Y, Führer M, Bahrami E, Socha P, Klaudel-Dreszler M, Bouzidi A et al (2019) Human RIPK1 deficiency causes combined immunodeficiency and inflammatory bowel diseases. Proc Natl Acad Sci U S A 116(3):970–975
68. Cuchet-Lourenço D, Eletto D, Wu C, Plagnol V, Papapietro O, Curtis J et al (2018) Biallelic. Science 361(6404):810–813
69. Boland BS, Widjaja CE, Banno A, Zhang B, Kim SH, Stoven S et al (2015) Immunodeficiency and autoimmune enterocolopathy linked to NFAT5 haploinsufficiency. J Immunol 194(6):2551–2560
70. Kotlarz D, Marquardt B, Barøy T, Lee WS, Konnikova L, Hollizeck S et al (2018) Human TGF-β1 deficiency causes severe inflammatory bowel disease and encephalopathy. Nat Genet 50(3):344–348
71. Blaydon DC, Biancheri P, Di WL, Plagnol V, Cabral RM, Brooke MA et al (2011) Inflammatory skin and bowel disease linked to ADAM17 deletion. N Engl J Med 365(16):1502–1508
72. Aguilar C, Lenoir C, Lambert N, Bègue B, Brousse N, Canioni D et al (2014) Characterization of Crohn disease in X-linked inhibitor of apoptosis-deficient male patients and female symptomatic carriers. J Allergy Clin Immunol 134(5):1131–1141. e9
73. Marsh RA, Madden L, Kitchen BJ, Mody R, McClimon B, Jordan MB et al (2010) XIAP deficiency: a unique primary immunodeficiency best classified as X-linked familial hemophagocytic lymphohistiocytosis and not as X-linked lymphoproliferative disease. Blood 116(7):1079–1082
74. Yang X, Kanegane H, Nishida N, Imamura T, Hamamoto K, Miyashita R et al (2012) Clinical and genetic characteristics of XIAP deficiency in Japan. J Clin Immunol 32(3):411–420
75. Worthey EA, Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B et al (2011) Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med 13(3):255–262
76. Girardelli M, Arrigo S, Barabino A, Loganes C, Morreale G, Crovella S et al (2015) The diagnostic challenge of very early-onset enterocolitis in an infant with XIAP deficiency. BMC Pediatr 15:208
77. Dziadzio M, Ammann S, Canning C, Boyle F, Hassan A, Cale C et al (2015) Symptomatic males and female carriers in a large Caucasian kindred with XIAP deficiency. J Clin Immunol 35(5):439–444
78. Tsuma Y, Imamura T, Ichise E, Sakamoto K, Ouchi K, Osone S et al (2015) Successful treatment of idiopathic colitis related to XIAP deficiency with allo-HSCT using reduced-intensity conditioning. Pediatr Transplant 19(1):E25–E28
79. Candotti F (2018) Clinical Manifestations and Pathophysiological Mechanisms of the Wiskott-Aldrich Syndrome. J Clin Immunol 38(1):13–27
80. Dupuis-Girod S, Medioni J, Haddad E, Quartier P, Cavazzana-Calvo M, Le Deist F et al (2003) Autoimmunity in Wiskott-Aldrich syndrome: risk factors, clinical features, and outcome in a single-center cohort of 55 patients. Pediatrics 111(5 Pt 1):e622–e627
81. Folwaczny C, Ruelfs C, Walther J, König A, Emmerich B (2002) Ulcerative colitis in a patient with Wiskott-Aldrich syndrome. Endoscopy 34(10):840–841
82. Hsieh KH, Chang MH, Lee CY, Wang CY (1988) Wiskott-Aldrich syndrome and inflammatory bowel disease. Ann Allergy 60(5):429–431
83. Esmaeilzadeh H, Bordbar MR, Dastsooz H, Silawi M, Fard MAF, Adib A et al (2018) A novel splice site mutation in WAS gene in patient with Wiskott-Aldrich syndrome and chronic colitis: a case report. BMC Med Genet 19(1):123
84. Root AW, Speicher CE (1963) The triad of thrombocytopenia, eczema, and recurrent infections (Wiskott-Aldrich syndrome) associated with milk antibodies, giant-cell pneumonia, and cytomegalic inclusion disease. Pediatrics 31:444–454
85. Orange JS, Stone KD, Turvey SE, Krzewski K (2004) The Wiskott-Aldrich syndrome. Cell Mol Life Sci 61(18):2361–2385

86. Ohya T, Yanagimachi M, Iwasawa K, Umetsu S, Sogo T, Inui A et al (2017) Childhood-onset inflammatory bowel diseases associated with mutation of Wiskott-Aldrich syndrome protein gene. World J Gastroenterol 23(48):8544–8552
87. Buchbinder D, Nugent DJ, Fillipovich AH (2014) Wiskott-Aldrich syndrome: diagnosis, current management, and emerging treatments. Appl Clin Genet 7:55–66
88. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA (1994) A multiinstitutional survey of the Wiskott-Aldrich syndrome. J Pediatr 125(6 Pt 1):876–885
89. McDonald-McGinn DM (2018) 22q11.2 deletion syndrome: a tiny piece leading to a big picture. Am J Med Genet A 176(10):2055–2057
90. Digilio MC, Giannotti A, Castro M, Colistro F, Ferretti F, Marino B et al (2003) Screening for celiac disease in patients with deletion 22q11.2 (DiGeorge/velo-cardio-facial syndrome). Am J Med Genet A 121A(3):286–288
91. Giardino G, Cirillo E, Maio F, Gallo V, Esposito T, Naddei R et al (2014) Gastrointestinal involvement in patients affected with 22q11.2 deletion syndrome. Scand J Gastroenterol 49(3):274–279
92. Markert ML, Sarzotti M, Ozaki DA, Sempowski GD, Rhein ME, Hale LP et al (2003) Thymus transplantation in complete DiGeorge syndrome: immunologic and safety evaluations in 12 patients. Blood 102(3):1121–1130
93. Digilio MC, Marino B, Bagolan P, Giannotti A, Dallapiccola B (1999) Microdeletion 22q11 and oesophageal atresia. J Med Genet 36(2):137–139
94. Kilic SS, Gurpinar A, Yakut T, Egeli U, Dogruyol H (2003) Esophageal atresia and tracheoesophageal fistula in a patient with Digeorge syndrome. J Pediatr Surg 38(8):E21–E23
95. Campbell IM, Sheppard SE, Crowley TB, McGinn DE, Bailey A, McGinn MJ et al (2018) What is new with 22q? An update from the 22q and You Center at the Children's Hospital of Philadelphia. Am J Med Genet A 176(10):2058–2069
96. Jackson O, Crowley TB, Sharkus R, Smith R, Jeong S, Solot C et al (2019) Palatal evaluation and treatment in 22q11.2 deletion syndrome. Am J Med Genet A 179(7):1184–1195
97. Humbert L, Cornu M, Proust-Lemoine E, Bayry J, Wemeau JL, Vantyghem MC et al (2018) Chronic mucocutaneous candidiasis in autoimmune polyendocrine syndrome type 1. Front Immunol 9:2570

Malignancies in Cellular Immunodeficiencies

14

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Abstract

Primary immunodeficiency diseases (PID) are a heterogeneous group of over 450 different genetically defined inborn errors of immunity, the hallmark of which is a predisposition to severe, unusual or recurrent infections. In addition, there are frequently accompanying features of immune dysregulation and an increased risk of hematopoietic and other malignancies. Although several forms of PID are associated with an increased risk of malignancy, this chapter will focus specifically on malignancies in patients with cellular immunodeficiencies. This group of disorders, their underlying immunopathology, potential pathophysiological mechanisms contributing to evolution of malignant disease, and details of the associated malignancies which have been reported will be discussed.

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Malignancy is a major cause of morbidity and mortality in patients with PID. Hence, clinician awareness of malignancy risks, regular screening and early investigation of symptoms are imperative. In addition, malignancy may be the first sign of an underlying immunodeficiency, particularly in the context of an atypical or early presentation together with other supportive clinical features. A history of previous malignancy in a patient with a cellular immunodeficiency, or risk of future malignant disease development should also prompt consideration of definitive therapies such as allogeneic hematopoietic stem cell transplantation or gene therapy.

Keywords
Primary immunodeficiency diseases (PID) · Combined immunodeficiencies · Immune dysregulation · Malignancy · Lymphoma · Lymphoproliferation

14.1 Introduction

Primary immunodeficiency diseases (PID) are a heterogeneous group of over 450 genetically defined inborn errors of immunity [1]. These conditions have been classified by the International Union of Immunological Sciences (IUIS) into ten groups according to the predominant underlying immunological defect: immunodeficiencies affecting cellular and humoral immunity, combined immunodeficiencies with associated or syndromic features, predominantly antibody deficiencies, diseases of immune dysregulation, congenital defects of phagocyte number or function, defects of intrinsic and innate immunity, autoinflammatory disorders, complement deficiencies, bone marrow failure syndromes and phenocopies of inborn errors of immunity [1]. Clinical and immunological phenotypes are broad; however the hallmark of PID is severe, recurrent or unusual infection. Other common associations include features of immune dysregulation, autoimmunity and a predisposition to malignancy. It is well recognized that the incidence of malignant disease is considerably higher in individuals with PID as compared with otherwise healthy populations, with an earlier age of presentation and worse clinical outcomes in this patient group [2, 3]. Both hematological and non-hematological malignancy constitute a major cause of morbidity and mortality in patients with PID and represent the second most common cause of death in this patient group, after infection [2].

In this review, we will focus on the malignancies observed in patients with cellular immunodeficiencies. The disorders which will be discussed fall within the IUIS subclassification of immunodeficiencies affecting cellular and humoral immunity (including severe combined immunodeficiency (SCID) and other forms of combined immunodeficiency generally less profound than SCID), combined immunodeficiencies with associated or syndromic features, and diseases of immune dysregulation. Cellular immunodeficiencies with reported associated malignancies will be reviewed, including disease pathophysiology, possible mechanisms of increased malignancy risk, and details of the specific hematopoietic and non-hematopoietic malignancies observed in each patient group. A comprehensive overview of all

cellular immunodeficiencies with reported malignant disease associations is presented in Table 14.1, and selected examples will be discussed in more detail.

Management of patients with cellular immunodeficiencies is highly variable and depends upon the underlying diagnosis and patient-specific factors. Therapy is therefore individualized for each patient. However, in general, a significant part of management includes preventing infectious complications through antimicrobial prophylaxis, early treatment of infection and implementing immunoglobulin replacement therapy. However, these interventions frequently do little to reduce the inherent risk of developing an associated malignancy. Definitive therapy with allogeneic hematopoetic stem cell transplantation (alloHSCT) or corrective gene therapy (GT) reduces the risk of malignancy in individuals with PID by restoring normal immunological function [4, 5]. As such, these definitive therapies should be considered in patients with cellular immunodeficiencies with a past history of malignancy and those at high risk of developing malignant disease in the future.

It is worth noting that in some cases, malignancy may be the presenting feature of PID, and there should be a low threshold to investigate for an underlying immunodeficiency, where indicated [6, 7]. In particular, specific investigations to identify an underlying PID should be considered if there are any of the following features: a positive family history of PID; malignancy presenting with atypical features (including an unexpectedly early age of presentation); a preceding history of recurrent, severe or unusual infections; prior autoimmune cytopenias; persistent leukocytosis, lymphoproliferation or hepatosplenomegaly [7]; and evidence of chronic viral infections (particularly EBV or CMV). Accurate diagnosis of an underlying PID in a patient presenting with malignant disease has the potential to alter their treatment course. For example, allogeneic hematopoietic stem cell transplantation (alloHSCT) may be recommended in first complete remission, which may not otherwise be indicated in the absence of a PID diagnosis.

14.2 Proposed Mechanisms of Cancer Development in Cellular Immunodeficiencies

A recent review by Hauck et al. examined the etiology of malignancy in patients with PID and categorized these according to intrinsic (primary) or extrinsic (secondary) mechanisms [8]. *Intrinsic* causes were identified as (a) defects in stem cell, myeloid and lymphoid development, differentiation, and apoptosis; (b) defects in lymphocyte (co-)signalling, the cytoskeleton, cytotoxicity, and metabolism; and (c) defects of chromosome stability, DNA repair, and telomeric maintenance. *Extrinsic* causes included (a) transforming infections (viral infections), (b) chronic tissue inflammation, and (c) impaired specific tumor immunosurveillance [8]. Impaired tumor immunosurveillance was previously thought to be the major mechanism of malignancy in PID [9, 10]; however more recently it has been suggested that this may be a more peripheral etiological factor [6, 8].

A decreased ability to eliminate altered cells has also been postulated as a causal mechanism. In the majority of cancer patients, complex, dynamic interactions exist

Table 14.1 Cellular immunodeficiencies and reported malignant disease associations

IUIS classification	Disease	Gene	Inheritance	OMIM	Reported malignancies/pre-malignant states	References
Immunodeficiencies affecting cellular and humoral immunity						
T-B+ SCID						
	γc (common gamma chain deficiency)	*IL2RG*	XL	308380	Lymphoma, leukemia, pre-malignant LPD, renal and pulmonary leiomyomata (post-allo-HSCT)	[3, 8, 70]
	JAK3 deficiency	*JAK3*	AR	600173	Lymphoma, leukemia, pre-malignant LPD	[3, 8]
	IL7Rα deficiency	*IL7R*	AR	146661	Lymphoma, leukemia, pre-malignant LPD	[3, 8]
	Coronin-1A deficiency	*CORO1A*	AR	605000	Lymphoma (B cell, EBV-related), leukemia, pre-malignant LPD	[8, 55]
T-B-SCID						
	RAG deficiency	*RAG1*	AR	179615	Lymphoma, leukemia, pre-malignant LPD, carcinoma, sarcoma	[8, 70]
	RAG deficiency	*RAG2*	AR	179616	Lymphoma, leukemia, pre-malignant LPD, carcinoma, sarcoma	[8, 70]
	DCLRE1C (Artemis) deficiency	*DCLRE1C*	AR	605988	Lymphoma (including EBV-associated lymphoma), leukemia, pre-malignant LPD	[3, 8, 71]
	DNA PKcs deficiency	*PRKDC*	AR	615966	Lymphoma, leukemia, carcinoma, sarcoma	[8]
	Cernunnos/XLF deficiency	*NHEJ1*	AR	611290	Lymphoma, leukemia, carcinoma, sarcoma	[8]
	DNA ligase IV deficiency	*LIG4*	AR	601837	Lymphoma, leukemia, carcinoma, sarcoma	[3, 8, 72]

IUIS classification	Disease	Gene	Inheritance	OMIM	Reported malignancies/pre-malignant states	References
	ADA deficiency	*ADA*	AR	608958	Lymphoma (particularly Burkitt lymphoma in un-transplanted patients on PEG-ADA replacement)	[3, 8, 27, 28]
Combined immunodeficiency, generally less profound than SCID						
	X-linked hyper IgM syndrome	*CD40L*	XL	308230	Carcinoma of the liver, pancreas, biliary tract, neuroectodermal endocrine tumors	[3, 22, 23]
	Zap 70 deficiency	*ZAP70*	AR	269840	Lymphoma (B-NHL, EBV-associated), leukemia, pre-malignant LPD	[8, 73]
	IKAROS deficiency	*IKZF1*	AD DN	603023	B-ALL	[74]
	DOCK8 deficiency	*DOCK8*	AR	243700	Burkitt lymphoma, cutaneous T cell lymphoma/leukemia, pre-malignant LPD, SCC	[8, 16]
	RHOH deficiency	*RHOH*	AR	602037	Lymphoma (B cell, Burkitt), leukemia, pre-malignant LPD	[8, 75]
	STK4 deficiency	*STK4*	AR	614868	Lymphoma (B cell including HL, T-cell, EBV-associated), leukemia, pre-malignant LPD (EBV-associated)	[8, 76, 77]
	ITK deficiency	*ITK*	AR	186973	Lymphoma, leukemia, pre-malignant LPD	[8, 55, 78]
	MALT1 deficiency	*MALT1*	AR	615468	Lymphoma, leukemia, pre-malignant LPD	[8, 79]
	CARD11 deficiency	*CARD11*	AR LOF	615206	Lymphoma	[8, 80]
	CARD11 deficiency	*CARD11*	AD GOF	606445	Lymphoma (B cell, HL), leukemia (CLL), LPD (BENTA)	[8, 80]
	OX40 deficiency	*TNFRSF4*	AR	615593	Lymphoma (Kaposi), leukemia, pre-malignant LPD	[8, 81]

(continued)

Table 14.1 (continued)

IUIS classification	Disease	Gene	Inheritance	OMIM	Reported malignancies/pre-malignant states	References
Combined immunodeficiencies with associated or syndromic features						
Immunodeficiency with congenital thrombocytopenia						
	Wiskott-Aldrich syndrome	*WAS*	XL	300392	Lymphoma (HL, NHL), DLBCL, laryngeal NHL, leukemia, cerebellar astrocytoma, Kaposi sarcoma, smooth muscle tumors, pre-malignant LPD	[3, 8, 25, 42, 44, 82]
	WIP deficiency	*WIPF1*	AR	602357	Lymphoma, leukemia, pre-malignant LPD	[8]
DNA repair defects (other than those listed above)						
	Ataxia-telangiectasia	*ATM*	AR	607585	Lymphoma (B and T cell), lymphoid leukemias, carcinoma, sarcoma, epithelial tumors, brain tumors Carriers: breast cancer; modest risk of other malignancies including colorectal and gastric cancers	[3, 8, 25, 34–37]
	Nijmegen breakage syndrome (NBS)	*NBS1*	AR	602667	Lymphoma: T, B, NHL, DLBCL, T-LBL, leukemia, brain tumors, sarcoma, carcinoma	[3, 8, 38, 39]
	Bloom syndrome	*BLM*	AR	604610	Lymphoma (NHL, HL), leukemia, sarcoma, carcinoma, solid tumors (breast, skin, gastrointestinal)	[8, 25, 83]
	Immunodeficiency with centromeric instability and facial anomalies (ICF types 1-4)	*DNMT3B* *ZBTB24* *CDCA7* *HELLS*	AR	602900 614064 609937 603946	Lymphoma (HL), leukemia, carcinoma, sarcoma (including angiosarcoma), MDS	[8, 84–86]

IUIS classification	Disease	Gene	Inheritance	OMIM	Reported malignancies/pre-malignant states	References
	PMS2 deficiency	*PMS2*	AR	600259	Constitutional mismatch repair syndrome, lymphoma, leukemia, carcinoma (including colorectal, endometrial), sarcoma, brain tumors	[8, 87, 88]
	RNF168 deficiency	*RNF168*	AR	612688	MDS	[8]
	MCM4 deficiency	*MCM4*	AR	602638	B cell lymphoma	[1, 89]
	POLE1 deficiency	*POLE1*	AR	174762	Lymphoma (T cell, B cell), colorectal cancer, MDS	[8, 90]
	POLE2 deficiency	*POLE2*	AR	602670	LPD	[91]
	Ligase I deficiency	*LIG1*	AR	126391	Lymphoma	[70]
Thymic defects with additional congenital anomalies						
	22q11.2 deletion/ DiGeorge/ velocardiofacial syndrome	*22q11.2 del*	AD	602054	Lymphoma (predominantly B cell lymphoma), hepatoblastoma, renal cell carcinoma, neuroblastoma	[3, 8, 92–94]
	DiGeorge/velocardiofacial syndrome	*Unknown*	Sporadic	–		
	TBX1 deficiency	*TBX1*	AD	602054		
Immuno-osseous dysplasias						
	Cartilage-hair hypoplasia	*RMRP*	AR	157660	Lymphoma (typically NHL), skin cancer (SCC, BCC), carcinoma (neuroendocrine, thyroid, vocal cord), plasmacytoma, myelodysplasia	[95, 96]
Hyper IgE syndromes						
	AD-hyper IgE syndrome (STAT3 deficiency)	*STAT3*	AD LOF	147060	Lymphoma (B cell (NHL, HL, EBV-associated, T cell), pulmonary adenocarcinoma	[97–99]

(continued)

Table 14.1 (continued)

IUIS classification	Disease	Gene	Inheritance	OMIM	Reported malignancies/pre-malignant states	References
	CARD11 deficiency (heterozygous)	*CARD11*	AD LOF	617638	Lymphoma (EBV-related, T-cell, LGL, mycosis fungoides), leukemia	[80, 100]
Calcium channel defects						
	ORAI-1 deficiency	*ORAI1*	AR	610277	Lymphoma, pre-malignant LPD	[8, 101]
	STIM1 deficiency	*STIM1*	AR	605921	Lymphoma, pre-malignant LPD	[8, 101]
Other defects						
	PNP deficiency	*PNP*	AR	164050	Lymphoma, leukemia, pre-malignant LPD, CNS EBV-associated lymphoma	[8, 102, 103]
Diseases of immune dysregulation						
Familial hemophagocytic lymphohistiocytosis (FHL) syndromes						
	Perforin deficiency (FHL2)	*PRF1*	AR	170280	Lymphoma, leukemia, pre-malignant LPD	[3, 8, 70]
	UNC13D/Munc13-4 deficiency (FHL3)	*UNC13D*	AR	608897	Lymphoma, leukemia, pre-malignant LPD	[8, 70]
	Syntaxin 11 deficiency (FHL4)	*STX11*	AR	605014	Lymphoma, leukemia, pre-malignant LPD	[8, 70]
	STXBP2/Munc 18-2 deficiency (FHL5)	*STXBP2*	AR/AD	601717	Lymphoma, leukemia, pre-malignant LPD	[8, 70]
	FAAP24 deficiency	*FAAP24*	AR	610884	EBV-driven LPD	[70, 104]
FHL syndromes with hypopigmentation						
	Chediak-Higashi syndrome	*LYST*	AR	606897	Leukemia	[3, 70]
	Hermansky-Pudlak type II	*AP3B1*	AR	603401	Lymphoma (HL), SCC, retinoblastoma	[105–107]
	Griscelli syndrome, type II	*RAB27A*	AR	603868	Leukemia, myelodysplastic syndrome	[3, 70]
Regulatory T cell defects						
	IPEX	*FOXP3*	XL	300292	Lymphoma	[70]

IUIS classification	Disease	Gene	Inheritance	OMIM	Reported malignancies/pre-malignant states	References
	CTLA4 haploinsufficiency (ALPS-V)	*CTLA4*	AD	123890	Lymphoma, leukemia, pre-malignant LPD	[8, 58, 59]
	LRBA deficiency	*LRBA*	AR	606453	Lymphoproliferation, lymphoma	[70, 108]
	STAT3 GOF mutation	*STAT3*	AD GOF	102582	Lymphoma (HL), leukemia (LGL), pre-malignant LPD (malignancy risk persists in non-hematopoietic tissues post-alloHSCT)	[8, 109]
Autoimmunity with or without lymphoproliferation						
	APECED (APS-1), autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy	*AIRE*	AR/AD	240300	Oral and esophageal squamous cell carcinoma	[14, 15]
Immune dysregulation with colitis						
	IL-10R deficiency	*IL10RA*	AR	146933	Lymphoma (B cell)	[110]
	IL-10R deficiency	*IL10RB*	AR	123889	Lymphoma (B cell)	[110]
Autoimmune lymphoproliferative syndrome (ALPS, Canale-Smith syndrome)						
	ALPS-FAS	*TNFRSF6*	AD/AR/somatic (het germline mutation plus somatic mutation or loss of second allele)	134637	Lymphoma (B cell (HL, NHL), T cell), leukemia, pre-malignant LPD	[3, 8, 111]
	ALPS-FASLG	*TNFSF6*	AR	134638	Lymphoma, leukemia, pre-malignant LPD	[8]

(continued)

Table 14.1 (continued)

IUIS classification	Disease	Gene	Inheritance	OMIM	Reported malignancies/pre-malignant states	References
Susceptibility to EBV and lymphoproliferative conditions						
	SAP deficiency (XLP1)	*SH2D1A*	XR	308240	Lymphoma (predominantly B cell lymphoma), gut lymphoma (NHL), pre-malignant LPD	[3, 8, 25, 55]
	CD27 deficiency	*CD27*	AR	615122	Lymphoma (B-NHL, EBV-associated, T cell), pre-malignant LPD, oral and anal cancers	[8, 54, 55, 112, 54]
	CD70 deficiency	*CD70 (TNFSF7)*	AR	602840	Lymphoma (B cell (predominantly HL), EBV-associated), pre-malignant LPD	[8, 54, 113]
	CTPS1 deficiency	*CTPS1*	AR	615897	Lymphoma (B cell, EBV-associated), pre-malignant LPD	[8, 114]
	CD137 deficiency (41BB)	*TNFRSF9*	AR	602250	Lymphoma (B cell, EBV -associated), lymphoproliferation	[115]
	RASGRP1 deficiency	*RASGRP1*	AR	603962	Lymphoma (B cell, EBV-associated), pre-malignant LPD	[55, 116]
	RLTPR deficiency	*CARMIL2*	AR	610859	Lymphoma, pre-malignant LPD, EBV and lymphoproliferative conditions, other malignancy, EBV-associated smooth muscle tumors	[8, 117]
	X-linked magnesium, EBV and neoplasia (XMEN) syndrome	*MAGT1*	XL	300853	Lymphoma (particularly EBV-associated), leukemia, pre-malignant LPD	[8, 52]

AD autosomal dominant, *AR* autosomal recessive, *XL* X-linked, *XR* X-linked recessive, *ITK* IL2-inducible T cell kinase, *LPD* lymphoproliferative disease, *HL* Hodgkin's disease (lymphoma), *NHL* non-Hodgkin's lymphoma, *DLBCL* diffuse large B cell lymphoma, *LBL* lymphoblastic leukemia, *SCC* squamous cell carcinoma, *BCC* basal cell carcinoma, *BENTA* B cell expansion with NF-kB and T cell anergy, *LGL* large granular lymphocytic leukemia

between malignant cells, the tumor microenvironment and the host immune response. For some patients the tumor can be eliminated, usually requiring both chemotherapy and radiotherapy in addition to anti-tumor immunity. However, in others, there exists a functional equilibrium between tumor elimination and tumor escape. Tumor-associated antigens and neo-antigens expressed by malignant cells stimulate anti-tumor immune responses, which subject the tumor to immune editing by various mechanisms [11]. In patients with cellular immunodeficiencies such endogenous anti-tumor immune responses are impaired and often unable to control tumor growth. In addition, intrinsic genetic heterogeneity of the tumor also impacts on disease progression and susceptibility to tumor-directed immunity. Defects in genes which act as tumor suppressors also contribute to oncogenesis in patients with cellular immunodeficiencies. For example, the loss of DOCK8 expression observed in cancers such as squamous cell carcinoma (SCC) and neuroblastoma suggests that this protein plays a key role in tumor suppression [12], and thus potentially contributes to oncogenesis in patients with DOCK8 deficiency.

14.2.1 The Role of Chronic Infection in Oncogenesis in Cellular Immunodeficiencies

Patients with PID are susceptible to a range of infectious diseases, and in many cellular immunodeficiencies, chronic infection itself can also be an etiological factor in the development of malignancy. This is the case for some viral, protozoal, and fungal pathogens, a few examples of which are discussed below.

In addition to EBV, which will be reviewed in detail separately, other oncogenic herpesviruses have been identified as contributing to tumorigenesis in patients with PID. Patients with Wiskott-Aldrich syndrome and deficiencies in OX-40, STIM1, and MAGT1 (XMEN syndrome) have a predisposition to human herpes virus 8 (HHV8)-associated disease, including Kaposi sarcoma (reviewed in [13]).

Patients with PID predisposing to chronic mucocutaneous candidiasis, such as APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) syndrome, have an increased risk of oral and esophageal SCC. While the pathogenesis is multifactorial, the role of chronic candidal infection, including direct carcinogenic effects and chronic mucosal inflammation, plays a central role in SCC development in these patients [14, 15].

Other patients at significantly increased risk of human papillomavirus (HPV) infections include those with DOCK8 deficiency (affecting 40% of patients in one cohort) [12, 16] and SCID due to mutations in *IL-2Rγ* and JAK3 deficiency who develop severe, recalcitrant cutaneous HPV infection despite successful alloHSCT. The development of HPV-associated warts appears to be independent of chimerism or immune reconstitution [17]. In one reported case series, 50% of patients with these forms of SCID developed severe warts, with a median time of onset of 8 years following alloHSCT [17]. It has been hypothesized that a lack of correction in non-hematopoietic cells such as keratinocytes, which express both common γ-chain-dependent cytokine receptors and JAK3 [18], may result in

aberrant local immune responses post-transplant. One study observed no clear difference in NK cell number or function in the patients who developed warts post-transplant compared with those who did not [17], although lower numbers of NK cells were noted in affected patients in a second, smaller case series [19]. Although no cases of malignancy have been reported in this patient group to date, this remains a possibility and requires long-term follow-up [5], as malignant transformation to squamous cell carcinoma has been described in canine models of IL-2Rγ deficiency with HPV infection [20].

Chronic cryptosporidial infection has been implicated as a risk factor for cancer development in the general population [21], and patients with CD40 ligand deficiency (X-linked hyper IgM syndrome) have a predisposition to infection with this protozoan parasite. Chronic *Cryptosporidium* infection drives a chronic inflammatory response in the hepatobiliary system, frequently resulting in sclerosing cholangitis and cirrhosis, along with an increased predisposition to adenocarcinoma of the liver, biliary tree and pancreas, and neuroectodermal endocrine tumors [22, 23].

It is likely that tumorigenesis in patients with cellular immunodeficiencies is multifactorial, occurring as a result of a combination of intrinsic and extrinsic factors, which are variable depending on the underlying genetic and immunological abnormalities [8]. Figure 14.1 summarizes postulated etiological factors in the development of cancer in cellular immunodeficiencies.

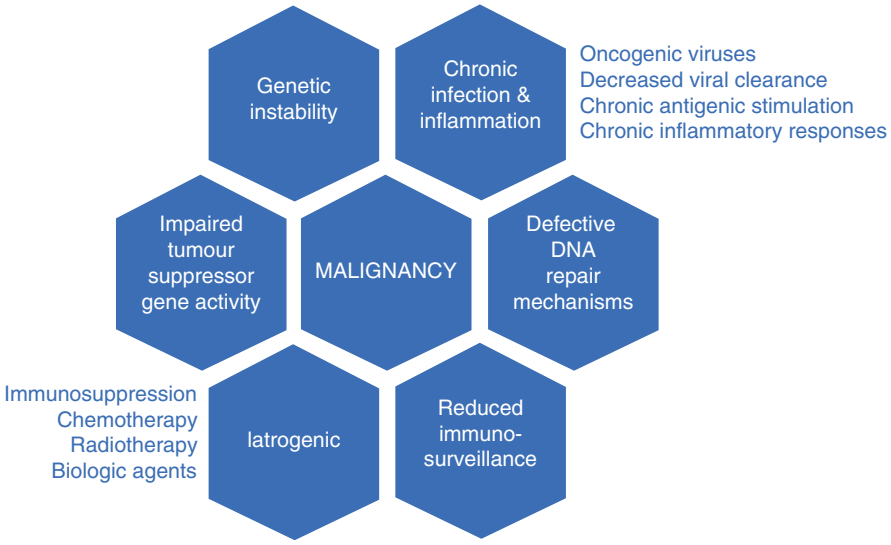

Fig. 14.1 Potential pathophysiological mechanisms contributing to malignancy in patients with cellular immunodeficiencies. Cancer development in patients with primary immunodeficiency diseases is multifactorial, including intrinsic mechanisms (genetic instability, defective DNA repair mechanisms, impaired tumor suppressor gene activity) and extrinsic mechanisms (chronic infection and tissue inflammation, impaired specific tumor immunosurveillance). Iatrogenic effects from immunosuppressive therapies, chemo- and radiotherapy and biologic agents also contribute. Frequently, more than one mechanism contributes to the evolution of malignant disease. Adapted with permission from Refs. [2, 3, 6, 8]

14.3 Specific Cellular Immunodeficiencies Associated with Malignant Disease

A summary of cellular immunodeficiencies known to predispose to malignant disease, their genetic bases and reported cancer associations is presented in Table 14.1, and selected examples are reviewed in greater detail below.

14.3.1 Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) is one of the most severe forms of PID, with the majority of patients presenting in early infancy with severe, life-threatening infections with opportunistic and other pathogens, failure to thrive, autoimmune manifestations, and, in some cases, associated dysmorphic features. Patients with SCID have low or absent naïve T cells, with variably reduced B and NK cells, depending upon the underlying genetic defect [1]. SCID is associated with high levels of morbidity and mortality; thus early identification and urgent institution of supportive and definitive therapy, with either alloHSCT or GT, is indicated. Previous data has suggested that outcomes are significantly improved if alloHSCT is performed prior to 3.5 months of age [24], before the onset of severe infections, their sequelae and other complications.

The majority of infants with SCID undergo alloHSCT or GT early in life, thereby correcting their inborn error of immunity and mitigating their intrinsic risk of malignancy. However, those who have not undergone definitive therapy remain at significant risk of developing hematological and other cancers [25]. One example is ADA deficiency SCID managed with enzyme replacement therapy. Adenosine deaminase (ADA) is a ubiquitously expressed enzyme, which catalyzes the conversion of adenosine to inosine, and 2′-deoxyadenosine to 2′-deoxyinosine. An absence of this enzyme, due to recessively inherited mutations in the *ADA* gene, results in the cellular accumulation of toxic by-products, including adenosine, 2′-deoxyadenosine and deoxyribunucleotides [26]. Although ubiquitously expressed, tissues are differentially affected. Lymphocytes and thymic tissue are particularly sensitive to these by-products, accumulation of which blocks lymphocyte differentiation, causes rapid apoptosis and induces thymic involution, resulting in pan-lymphopenia, and classically a T-/B-/NK- SCID phenotype, although delayed-onset ADA deficiency has also been described (reviewed in [26]).

As for other forms of SCID, early, definitive therapy with alloHSCT or GT is indicated. However, due to the nature of the condition and availability of PEG-ADA replacement therapy, this can be administered to bridge the gap until corrective therapy is instituted. Due to either a lack of a suitable alloHSCT donor, or inability to access GT, some patients have remained on ADA replacement therapy for several years. Although this therapy is highly effective in the detoxification of cells and restoration of lymphocyte number and function, there are some challenges associated with its use, including reduced efficacy over time. Patients with an uncorrected defect remain at a higher risk of malignancy, as evident from a recent review

reporting nine cases of lymphoma in ADA-deficient patients, five of which were EBV-related and two of which involved extra-nodal sites (the brain and lung, respectively) [27]. In this series, three of the patients had undergone attempted correction prior to the onset of lymphoma: two patients underwent haplo-HSCT with poor engraftment and incomplete correction, and one underwent GT with poor detoxification, requiring re-commencement of enzyme replacement therapy [27, 28].

The pathophysiological mechanisms resulting in lymphoma development in this patient group likely relate to the intrinsic cellular defect, decreased tumor surveillance and effect of oncogenic viruses [27]. However, the lack of ADA might be expected to be a protective factor against lymphomagenesis as apoptosis of abnormal lymphocytes is enhanced [27]. Interestingly, this has been exploited by the use of ADA inhibitors to treat cutaneous T cell lymphoma [29]. It has been postulated that the mutation may revert in lymphoma cells, as this phenomenon has been observed in some patients with ADA deficiency [27].

14.3.2 Double-Stranded DNA Breakage Repair Syndromes/ Chromosomal Instability Syndromes

Double-stranded DNA breakage repair is essential for normal immunological function and a variety of other cellular processes. Defective DNA repair mechanisms result in chromosomal instability and breakage, with sequelae including a predisposition to malignancy and a variety of other clinical features [30]. Several molecules are key to effective DNA repair, including the ATM and NBS1 proteins, as depicted in Fig. 14.2. Mutations in the genes encoding these proteins give rise to the clinical syndromes of ataxia-telangiectasia and Nijmegen breakage syndrome, respectively. These immunodeficiencies and the associated predisposition to malignancy are discussed below.

14.3.3 Ataxia-Telangiectasia

Ataxia-telangiectasia (AT) occurs as a result of homozygous or compound heterozygous mutations in the *ATM* gene, the product of which (ATM) has a key role in several cellular processes, including cell cycle checkpoint control, mitogenic signal transduction, intracellular protein transport, double-stranded DNA-break repair and phosphorylation of tumor suppressor genes [31, 32]. The clinical features of AT include early-onset, progressive cerebellar degeneration with ataxia, oculocutaneous telangiectasia, recurrent sinopulmonary infections owing to defects in cellular and humoral immunity and radiosensitivity [33]. Patients with AT are at an increased risk of malignant disease, with one third of patients developing cancer, particularly lymphoid leukemia and lymphoma [3] in childhood and young adulthood, with onset of other malignancies such as carcinomas and brain tumors later in life [34, 35]. Generally, observed malignancies tend to have an earlier onset and a worse prognosis than in the general population.

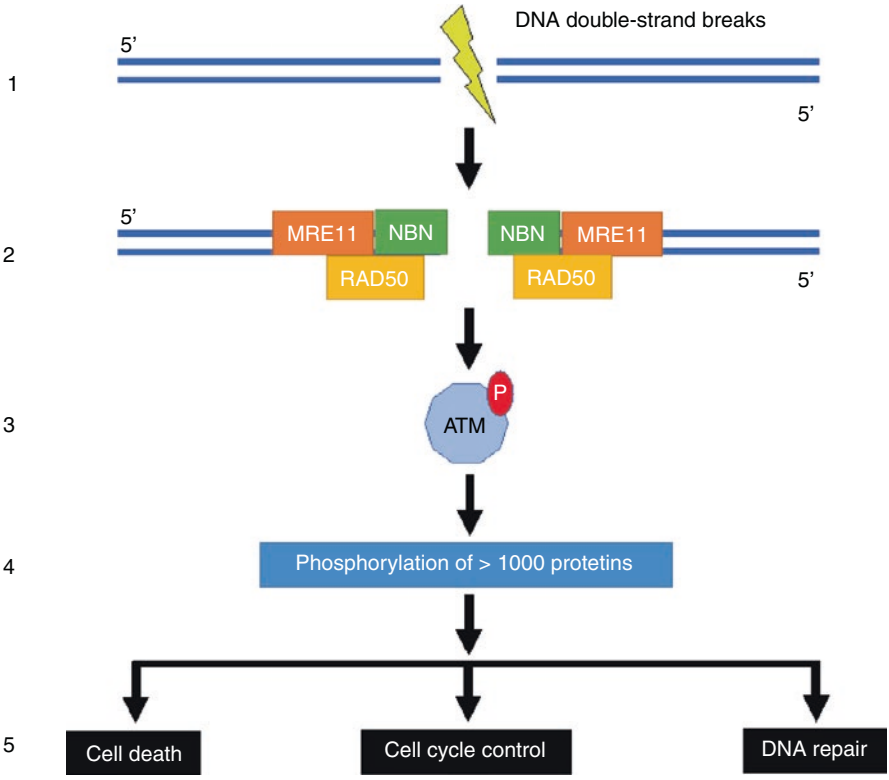

Fig. 14.2 The role of ATM, NBS1 and other proteins in the repair of double-stranded DNA breaks. DNA double-strand break (DSB) repair mechanisms are critical for cellular processes in a variety of tissues. (1) DSB are identified by Nibrin (NBN), which forms the MRN complex (along with MRE11 and RAD50). (2) The MRN complex activates ATM (3), which phosphorylates over 1000 downstream proteins, regulating DNA damage response pathways (5) including DNA repair (by homology-directed repair and non-homologous end joining processes), cell cycle control, and cell death. Defective ATM and NBN proteins, due to mutations in the *ATM* and *NBS1* genes, give rise to the clinical syndromes of ataxia-telangiectasia and Nijmegen breakage syndrome, respectively. In these disorders, DSB repair processes are ineffective, giving rise to the predisposition to malignancy observed in these conditions. Reproduced with permission from reference [30]

ATM deficiency promotes tumor formation through several different mechanisms, and typically the type of malignancy observed depends on the specific underlying mutation and presence or absence of residual ATM kinase activity [36]. An absence of ATM results in an inability to halt the cell cycle required to repair double-stranded DNA breaks, which are essential for successful V(D)J recombination events in the T cell receptor and immunoglobulin genes, thus contributing to the development of lymphoid malignancies [36]. ATM phosphorylates p53 protein, a product of the tumor suppressor gene TP53 which promotes cell cycle arrest and apoptosis of DNA-damaged cells [32]. In the presence of *ATM* gene mutations, p53 function is impaired.

Carriers of *ATM* mutations also have a higher risk of malignancy. In particular, a higher frequency of breast cancer is observed in heterozygous carriers compared to the general population, with one UK study of 1160 relatives of AT patients from 132 families reporting a relative risk (RR) of 2.23 overall [37], similar to rates reported in other cohorts. This was significantly higher, reaching an almost fivefold increased risk (RR = 4.94) in younger individuals aged under 50 years [37]. *ATM* gene products phosphorylate the breast cancer susceptibility gene 1 (BRCA1) tumor suppressor protein, which is one factor potentially explaining this observation. A more modest increase in risk was noted for other cancers, including colorectal and gastric cancers [37].

14.3.4 Nijmegen Breakage Syndrome

Nijmegen breakage syndrome (NBS) is an autosomal recessive condition, arising due to mutations in the *NBS* (also *NBS1*, *Nibrin*, *p95*) gene [1]. Nibrin, the gene product of *NBS*, lies within the same pathway as ATM (Fig. 14.2), with immunological findings similar to those of ataxia-telangiectasia, including cellular and humoral defects, radiosensitivity, and chromosomal instability [30]. Nibrin, along with proteins RAD50 and MRE11, forms the MRN complex, which recognizes double-stranded DNA breaks and enables downstream activation of ATM [30]. Affected patients have dysmorphic features including microcephaly, short stature and "bird-like" facies, along with a markedly increased risk of malignancy [38]. Forty percent of patients will develop a malignancy before the age of 20 years [39]. The vast majority of these are hematological malignancies (lymphoma, particularly non-Hodgkin's lymphoma, NHL) and leukemia. Rhabdomyosarcoma and medulloblastoma have also been reported [30, 39].

14.3.5 Combined Immunodeficiencies

Combined immunodeficiencies (CID) affect both cellular and humoral immunity, and are subclassified into those CID that are generally less profound than SCID, and those with associated or syndromic features (including immunodeficiency with congenital thrombocytopenia, some DNA repair defects, thymic defects with additional congenital anomalies, immuno-osseous dysplasias, hyper IgE syndromes, defects of vitamin B12 and folate metabolism, anhidrotic ectodermodysplasia with immunodeficiency, calcium channel defects, and other defects) [1]. Immunodeficiency diseases falling into these categories in which malignancy has been described are summarized in Table 14.1, and two conditions, Wiskott-Aldrich syndrome and DOCK8 deficiency, are discussed in further detail below.

14.3.6 Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome (WAS) occurs as a result of X-linked mutations in the *WAS* gene, giving rise to reduced or abnormal WAS protein (WASP) expression [40]. WASP is expressed in all hematopoietic lineages and has an essential role regulating the actin cytoskeletal complex. The clinical phenotype of WAS is broad, with features including thrombocytopenia with small volume platelets, bleeding diathesis, severe eczema, autoimmunity, severe infections and hypogammaglobulinemia, with a variable spectrum of severity [41].

The prevalence of malignancy in patients with severe forms of WAS has been reported to be 13–22%, with an average age of onset of 9.5 years [41–43]. The presence of autoimmunity as a feature of the disease has been noted to be a risk factor for cancer development [42]. Malignancy is also observed in patients with milder forms of WAS. In a review of 173 patients with mild WAS, the overall prevalence of malignancy was 5%, lower than that observed classical WAS, with a higher median age of onset (34 years) [44]. Of the cancers observed, lymphoid malignancies predominate. B cell NHL is the most common form, this is often extra-nodal in nature and frequently EBV-associated [42]. A variety of other hematological malignancies have been described in patients with WAS, including lymphoblastic leukemia, EBV-associated lymphoproliferative disease, myelodysplasia and myeloproliferative disorders [41, 44]. Reported non-lymphoreticular malignancies include glioma, neuroma, Kaposi sarcoma, seminoma, testicular carcinoma, and pancreatic cancer [41, 44]. In addition, patients with WAS are prone to developing benign lymphadenopathy with histological features consistent with follicular reactive hyperplasia [41].

Defective immune surveillance due to inherent abnormalities in T, NK, and dendritic cells is proposed to be the major pathophysiological mechanism accounting for the increased risk of malignancy in WAS, along with a reduced ability to eliminate abnormal cells [41]. In particular, WAS patient cells exhibit a reduced ability to lyse B cell lymphoma cells due to impaired cytotoxic T and NK cell function, which likely contributes to lymphomagenesis in the context of WAS [45]. In addition, WASP and its associated protein WIP (WASP-interacting protein) have been shown to act as tumor suppressors in T cell lymphomas, alluding to the importance of these proteins in the maintenance of genomic stability [46].

All features of WAS are potentially curable by alloHSCT [41], including a reduction in malignancy risk. This is evidenced by three case series reporting the outcomes of alloHSCT in 334 patients with WAS, where only one patient developed lymphoma [47] and one patient developed squamous cell carcinoma [48]. No other incidences of malignancy were reported, with the exception of a few cases of EBV-related PTLD, arising in the early post-transplant period of lymphopenia [47, 49].

14.3.7 DOCK8 Deficiency

Recessively inherited mutations in the *DOCK8* gene give rise to the clinical syndrome of autosomal recessive hyper IgE syndrome, manifested by severe atopic disease including allergies and eczema, severe viral skin infections, recurrent sinopulmonary infections, and a predisposition to malignancy [16]. In one case series of 136 patients, 23 (17%) developed malignancies, eight of whom died as a result [16]. The median age of onset of malignancy was 12 years, and the most common types of cancer were hematological (11 patients) and epithelial (9 patients), with the 5 remaining patients developing other forms of malignancy [16].

The etiology of this increased risk of malignancy is likely partly explained by the effects of uncontrolled viral infection, with EBV infection giving rise to lymphoma, and HPV infection to squamous cell carcinoma [12]. As with other immunodeficiencies, reduction in tumor surveillance capabilities is another potential mechanism resulting in cancer development [50].

DOCK8 deficiency is associated with a poor long-term prognosis without intervention, and as such, alloHSCT is recommended for most affected patients [50]. Allogeneic HSCT has been performed in over 100 patients with this condition to date, with good outcomes achieved overall, and in those with prior malignancy, alloHSCT has been effective in preventing recurrence [50, 51], as demonstrated in one series of 136 patients with DOCK8 deficiency, 36 of whom underwent alloHSCT [16]. Twelve of these patients had a history of malignant disease pre-transplant and remained in remission, and one patient died from progressive lymphoma post-alloHSCT. Only one case of post-alloHSCT malignancy has been observed (thyroid cancer following total body irradiation) [51].

14.3.8 Immune Dysregulatory Disorders

Disorders of immune dysregulation include familial hemophagocytic lymphohistiocytosis (FHL syndromes), FHL syndromes with hypopigmentation, regulatory T cell defects, autoimmunity with or without lymphoproliferation, immune dysregulation with colitis, autoimmune lymphoproliferative syndrome (ALPS) and susceptibility to EBV and lymphoproliferative conditions [1]. Immune dysregulatory disorders associated with malignant disease are summarized in Table 14.1, and the role of EBV infection in many of these conditions is discussed below in further detail, along with a discussion of malignancy in X-linked lymphoproliferative syndromes (XLP) and CTLA4-haploinsufficiency.

14.3.9 EBV-Associated Malignancies in Cellular Immunodeficiency

The gamma-herpes virus Epstein-Barr virus (EBV, also known as human herpes virus 4 (HHV-4)) is a B-tropic, oncogenic virus [13]. It is well recognized that an

inability to control EBV and resultant EBV-related pathology (chronic active EBV (CAEBV), lymphoproliferation, fulminant mononucleosis, HLH, and EBV-associated malignancy) are a feature of many forms of primary immunodeficiency [52, 53]. Abnormal T and NK cell function, both hypo- and hyper-active TCR signalling, and defective cytotoxic T cell activity in these conditions result in an inability to adequately control EBV infection and contribute to tumorigenesis [13]. EBV is most commonly associated with various histological subsets of B cell lymphomas and epithelial carcinomas [13].

A series of monogenic immunodeficiencies have been described that predispose to EBV infection and EBV-related pathology, including lymphoma. In particular, patients with mutations in *SH2D1A* (XLP type 1), *XIAP* (XLP type 2), *ITK*, *RASGRP1*, *CD70*, *CD27*, *MAGT1*, *PRKCD*, *CARMIL2, CTPS1, CORO1A, STK4, and TNFRSF9* [1, 53–55] have been identified to be at high risk of EBV-driven lymphoproliferative disease and other complications [53, 56]. The role of these molecules in EBV immunity and the consequences of abnormalities in associated pathways are given in Fig. 14.3. These disorders and details of their associated malignancies are summarized in Table 14.1.

14.3.10 CTLA-4 Haploinsufficiency

Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is a negative regulator of the T cell immunological response, and heterozygous mutations in the *CTLA4* gene (resulting in *CTLA4* haploinsufficiency) give rise to an immune dysregulatory syndrome, with a broad range of clinical features including recurrent infections, autoimmunity, lymphoproliferation, and lymphocytic tissue infiltrates [57, 58]. *CTLA4* haploinsufficient patients are also at a higher risk of malignancy, with a prevalence of 12.9% (17 patients in a cohort of 131 symptomatic carriers) [59]. Lymphoma (Hodgkin, Burkitt, and DLBCL) was the predominant malignancy, followed by gastric cancer, both of which were associated with EBV infection in the majority of cases [59]. Multiple myeloma and metastatic melanoma were reported in two other patients. Several patients in this cohort who developed malignancy were receiving corticosteroid therapy, and it has been postulated that additional immunosuppression may also be a factor in the development of malignancy in this patient cohort, along with the intrinsic T cell defect and effects of uncontrolled oncogenic viral infection, chronic inflammation and defective immune surveillance [59].

14.3.11 Activated Phosphoinositide 3-Kinase Delta Syndrome (APDS)

Activated phosphoinositide 3-kinase delta syndrome (APDS) type 1 arises as a result in autosomal dominant, gain-of-function mutations in the *PIK3CD* gene, which although classified by the IUIS as a "predominantly antibody deficiency syndrome" [1], gives rise to an immunodeficiency syndrome manifested by recurrent

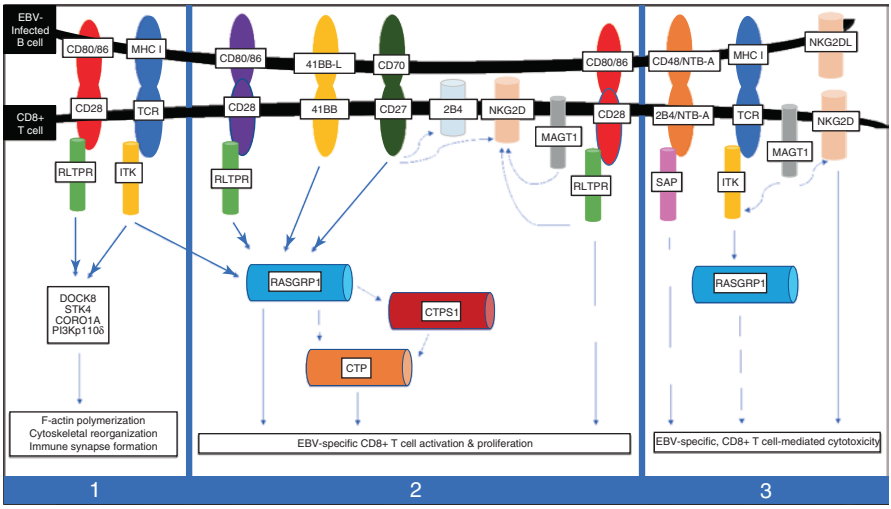

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Fig. 14.3 Major signalling pathways required for CD8+ T cell-mediated EBV immunity. Effective CD8+ T cell-mediated immunity against EBV requires formation of an effective immunological synapse, adequate CD8+ T cell priming, and optimal function of EBV-specific CD8+ T cells. Each essential process and major signalling pathways are detailed in the panels below: (1) *Formation of an effective immunological synapse between the CD8+ T cell and EBV-infected B cell.* Interactions between MHC I and the T cell receptor (TCR), and CD80/86 and CD28 and subsequent signalling through these receptors, including RLTPR-dependent activation of CD28, induce cytoskeletal arrangements and F-actin polymerization requiring DOCK8, STK4, CORO1A and PI3K p110δ. Mutations in genes encoding these proteins and ITK and RLTPR result in loss of naïve CD8+ T cells, increased apoptosis and accelerated immunosenescence. (2) *CD8+ T cell priming.* This requires engagement of MHC I/TCR, CD28/CD80-86, CD27/CD70 and 4-IBB/4-IBBL and intact signalling via ITK and RASGRP1, resulting in DNA synthesis via induction of CTPS1 and EBV-specific CD8+ T cell proliferation, and maintenance of activating receptors (2B4 and NKG2D) via MAGT1 signalling. Mutations in genes encoding MAGT1, CD70, CD27, ITK, RLTPR, RASGRP1 and CTPS1 inhibit CD8+ T cell priming, expansion and reduced expression of activating receptors. (*3) Optimal EBV-specific CD8+ T cell function.* After initial expansion of these cells, SAP mediates engagement of receptors 2B4/NTB-A, and the interaction between NKG2D and its ligands promotes cytotoxic responses of the EBV-specific CD8+ T cells. Mutations in genes encoding SAP, ITK, MAGT1 and RASGRP1 result in reduced CD8+ T cell cytotoxicity against infected B cells. These mechanisms contribute to EBV-related pathology, including malignancy, in patients with cellular immunodeficiencies due to mutations in the aforementioned genes. Adapted from Ref. [56]

respiratory tract infections, bronchiectasis, lymphoproliferation, and a predisposition to lymphoma [60]. The gene product of *PIK3CD,* the p110delta subunit of phosphoinositide 3-kinase delta, has a key role in intracellular signalling and also in the immune response to herpesviruses such as EBV, as depicted in Fig. 14.3 [61, 62]. Patients with APDS1 are predisposed to non-malignant lymphoproliferation affecting the lymph nodes, liver, spleen, and the intestinal and respiratory mucosa [62]. Reported malignancies include B cell lymphoma (DLBCL, HL, marginal zone

lymphoma (MZL), lymphoplasmacytic lymphoma (LPL)), which may be EBV-associated, and one case of a primary cutaneous anaplastic large cell lymphoma [62].

A second form of APDS has also been described, referred to as APDS2, caused by autosomal dominant mutations in the *PIK3R1* gene which encode the regulatory subunit of class IA phosphoinositide 3-kinases (p85α, p55α, and p50α) [63, 64]. Similar to patients with APDS1, APDS2 is associated with severe infections, lymphoproliferative disease and a predisposition to lymphoma [63]. In one cohort, 28% of patients developed a malignancy, predominantly lymphoma (HL, DLBCL, MZL, CLL), with a cumulative risk of lymphoma development of 78% by age 40 years [63].

14.3.12 GATA2 Deficiency

GATA2 is a zinc finger transcription factor essential for hematopoiesis, and mutations in the *GATA2* gene give rise to a clinical syndrome previously termed "MonoMac": predisposition to infection (disseminated non-tuberculous mycobacterial infection, viral (particularly HPV and EBV) and fungal infections), severe monocytopenia, and B and NK cell lymphopenia [65]. This disorder is classified by the IUIS as a phagocytic defect [1]. Other clinical features include lymphedema and pulmonary alveolar proteinosis and a predisposition to myelodysplasia, leukemia, and lymphoma [66]. In addition to myeloid leukemias, patients with GATA2 deficiency are also prone to developing HPV-related carcinomas, particularly affecting the vulva and cervix, and other malignancies including EBV-associated leiomyosarcoma [65].

14.4 Secondary Malignancies Following alloHSCT for Cellular Immunodeficiencies

Despite alloHSCT effectively correcting the underlying immunological defect in cellular immunodeficiencies and reducing the overall risk of malignancy, late effects of the transplantation procedure itself also need to be considered, particularly the risk of secondary malignancy or relapse of prior malignancy. This malignancy risk can be attributed to a number of factors, including the underlying genetic abnormality and its distribution in other non-hematopoietic cells, the use of alkylating agents as conditioning chemotherapy, a history of previous lymphoma (including remission status at the time of transplant), and persistent viral infections [8]. The degree of donor chimerism and immune reconstitution may also be important [5]. Excluding EBV-associated PTLD, the overall risk of developing malignancy post-alloHSCT for all forms of PID has been found to be small, with secondary malignancy occurring in 0.6% of patients (21/3340 patients across three case series, reviewed in [5]), although this rate may be higher in adults transplanted for PID. This suggests that the rate of second malignancy is not increased in PID patients compared to non-PID transplant recipients.

A review of 87 patients who underwent alloHSCT for DNA double-strand breakage repair disorders (Nijmegen breakage syndrome, DNA ligase IV deficiency, Cernunnos-XLF deficiency and ataxia-telangiectasia) did not identify any cases of secondary malignancy [67]. Appropriate conditioning regimens should be employed for patients with radiosensitive (Artemis) SCID and DNA breakage repair disorders, including the avoidance of alkylating agents and radiotherapy, which may increase the risk of secondary malignancies and other complications due to the underlying genetic defect affecting other cell types [67]. Given the small, but potential risk of secondary malignancy in patients following alloHSCT for PID, long-term surveillance and follow-up is recommended [5, 67].

14.5 Considerations in Clinical Management

Clinicians managing patients with cellular immunodeficiencies should be aware of the increased risk of malignancy in this patient group and arrange regular review and screening based on clinical history, physical examination and investigations as indicated, with early evaluation if there is suspicion of malignant disease [6]. Careful evaluation should be made to differentiate malignancy from benign lymphoproliferation or other bone marrow abnormalities, which can also be a feature of many forms of PID [41, 68]. This may require repeated biopsies to exclude clonal expansion of lymphocytes. Surveillance and screening approaches need to be individualized, as they will vary depending on the underlying disorder and individual patient factors. Routine recommendations regarding a healthy lifestyle to reduce other factors contributing to general cancer risks, including avoiding smoking and sun exposure, should also be made. Other individualized advice may also be provided in specific circumstances, for example, reduction of exposure to unnecessary ionizing radiation in DNA repair and radiosensitivity syndromes.

Hematologists and oncologists should also consider whether their patients presenting with malignancy may have an underlying PID and consider investigation and referral accordingly for immunological assessment [6, 7]. Clinical features suggesting an underlying immunodeficiency include a positive family history, a preceding history of recurrent, severe or unusual infections and features of immune dysregulation or autoimmunity (particularly cytopenias). Atypical features relating to the malignancy, in terms of type or earlier than expected age of presentation, should also prompt investigation for an underlying PID.

The management of malignant disease in patients with cellular immunodeficiencies necessitates a personalized approach to therapy and requires close communication between immunologists and hematologists/oncologists [6, 69]. Due to their intrinsic immunodeficiency, patients may have a higher than usual risk of complications such as infection as a result of further immunosuppression with chemotherapeutic agents. Consideration must be given to appropriate antimicrobial prophylaxis and early investigation and treatment for any infectious complications. In addition, chemotherapeutic regimens may need to be modified for patients with

radiosensitivity syndromes and DNA breakage disorders [67], avoiding radiomimetic and some chemotherapeutic agents, including cyclophosphamide, methotrexate, vincristine, and etoposide [3]. Additional patient factors including pre-existing organ damage and potential drug toxicities should also be considered [6].

As discussed earlier in this chapter, there is a clear role for definitive therapy with alloHSCT or GT to correct the underlying immunological defect in several forms of cellular immunodeficiency, which reduces the risk of primary or recurrent malignancy [4, 5]. A small risk of secondary malignancy following alloHSCT remains, which is variable depending on the underlying disorder and other factors, and hence long-term follow-up of these patients is essential [5, 67].

14.6 Conclusion

In summary, cellular immunodeficiencies are a broad group of primary immunodeficiency diseases, which predispose affected patients to a variety of complications due to infection, immune dysregulation and malignant disease. The pathophysiological mechanisms conferring this cancer susceptibility are multifactorial and include both intrinsic, disease-specific factors and extrinsic influences, including the effects of chronic infection and persistent inflammation. Patients with cellular immunodeficiencies are at a significant risk of developing hematological and other malignancies, with associated high levels of morbidity and mortality. Clinicians managing patients with cellular immunodeficiencies should have a low threshold for screening for and investigating suspected malignancy and consider corrective therapies with alloHSCT or GT to lower the inherent risks of cancer development. In addition, malignant disease may be the initial presenting problem for some patients with an underlying primary immunodeficiency, and this diagnosis should be considered in those with atypical presentations and a positive family history, preceding history of severe, recurrent or unusual infections or features of immune dysregulation.

References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40(1):24–64
2. Shapiro RS (2011) Malignancies in the setting of primary immunodeficiency: implications for hematologists/oncologists. Am J Hematol 86(1):48–55
3. Salavoura K, Kolialexi A, Tsangaris G, Mavrou A (2008) Development of cancer in patients with primary immunodeficiencies. Anticancer Res 28(2b):1263–1269
4. Gennery AR, Albert MH, Slatter MA, Lankester A (2019) Hematopoietic stem cell transplantation for primary immunodeficiencies. Front Pediatr 7:445
5. Gennery AR, Lankester A (2019) Long term outcome and immune function after hematopoietic stem cell transplantation for primary immunodeficiency. Front Pediatr 7:381
6. Kindler O, Quehenberger F, Benesch M, Seidel MG (2018) The Iceberg Map of germline mutations in childhood cancer: focus on primary immunodeficiencies. Curr Opin Pediatr 30(6):855–863

7. Sanchez-Ramon S, Bermudez A, Gonzalez-Granado LI, Rodriguez-Gallego C, Sastre A, Soler-Palacin P (2019) Primary and secondary immunodeficiency diseases in oncohaematology: warning signs, diagnosis and management. Front Immunol 10:586
8. Hauck F, Voss R, Urban C, Seidel MG (2018) Intrinsic and extrinsic causes of malignancies in patients with primary immunodeficiency disorders. J Allergy Clin Immunol 141(1):59–68. e4
9. Burnet FM (1970) The concept of immunological surveillance. Prog Exp Tumor Res 13:1–27
10. Burnet M (1957) Cancer; a biological approach. I. The processes of control. Br Med J 1(5022):779–786
11. Schreiber RD, Old LJ, Smyth MJ (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 331(6024):1565–1570
12. Su HC, Jing H, Angelus P, Freeman AF (2019) Insights into immunity from clinical and basic science studies of DOCK8 immunodeficiency syndrome. Immunol Rev 287(1):9–19
13. Damania B, Münz C (2019) Immunodeficiencies that predispose to pathologies by human oncogenic γ-herpesviruses. FEMS Microbiol Rev 43(2):181–192
14. Borchers J, Pukkala E, Mäkitie O, Laakso S (2020) Patients with APECED have increased early mortality due to endocrine causes, malignancies and infections. J Clin Endocrinol Metab 105(6):e2207–e2e13
15. Rautemaa R, Hietanen J, Niissalo S, Pirinen S, Perheentupa J (2007) Oral and oesophageal squamous cell carcinoma—a complication or component of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS-I). Oral Oncol 43(6):607–613
16. Aydin SE, Kilic SS, Aytekin C, Kumar A, Porras O, Kainulainen L et al (2015) DOCK8 deficiency: clinical and immunological phenotype and treatment options—a review of 136 patients. J Clin Immunol 35(2):189–198
17. Laffort C, Le Deist F, Favre M, Caillat-Zucman S, Radford-Weiss I, Debre M et al (2004) Severe cutaneous papillomavirus disease after haemopoietic stem-cell transplantation in patients with severe combined immune deficiency caused by common gammac cytokine receptor subunit or JAK-3 deficiency. Lancet (London, England) 363(9426):2051–2054
18. Nishio H, Matsui K, Tsuji H, Tamura A, Suzuki K (2001) Immunolocalisation of the janus kinases (JAK)—signal transducers and activators of transcription (STAT) pathway in human epidermis. J Anat 198(Pt 5):581–589
19. Kamili QUA, Seeborg FO, Saxena K, Nicholas SK, Banerjee PP, Angelo LS et al (2014) Severe cutaneous human papillomavirus infection associated with natural killer cell deficiency following stem cell transplantation for severe combined immunodeficiency. J Allergy Clin Immunol 134(6):1451–1453. e1
20. Goldschmidt MH, Kennedy JS, Kennedy DR, Yuan H, Holt DE, Casal ML et al (2006) Severe papillomavirus infection progressing to metastatic squamous cell carcinoma in bone marrow-transplanted X-linked SCID dogs. J Virol 80(13):6621–6628
21. Kalantari N, Gorgani-Firouzjaee T, Ghaffari S, Bayani M, Ghaffari T, Chehrazi M (2020) Association between Cryptosporidium infection and cancer: a systematic review and meta-analysis. Parasitol Int 74:101979
22. Yazdani R, Fekrvand S, Shahkarami S, Azizi G, Moazzami B, Abolhassani H et al (2019) The hyper IgM syndromes: epidemiology, pathogenesis, clinical manifestations, diagnosis and management. Clin Immunol 198:19–30
23. Hayward AR, Levy J, Facchetti F, Notarangelo L, Ochs HD, Etzioni A et al (1997) Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. J Immunol 158(2):977–983
24. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC et al (2014) Transplantation outcomes for severe combined immunodeficiency, 2000–2009. N Engl J Med 371(5):434–446
25. Mueller BU, Pizzo PA (1995) Cancer in children with primary or secondary immunodeficiencies. J Pediatr 126(1):1–10
26. Sauer A, Brigida I, Carriglio N, Aiuti A (2012) Autoimmune dysregulation and purine metabolism in adenosine deaminase deficiency. Front Immunol 3:265

27. Migliavacca M, Assanelli A, Ponzoni M, Pajno R, Barzaghi F, Giglio F et al (2018) First occurrence of plasmablastic lymphoma in adenosine deaminase-deficient severe combined immunodeficiency disease patient and review of the literature. Front Immunol 9:113
28. Hirschhorn R, Nicknam MN, Eng F, Yang DR, Borkowsky W (1992) Novel deletion and a new missense mutation (Glu 217 Lys) at the catalytic site in two adenosine deaminase alleles of a patient with neonatal onset adenosine deaminase- severe combined immunodeficiency. J Immunol 149(9):3107–3112
29. Argnani L, Broccoli A, Zinzani PL (2017) Cutaneous T-cell lymphomas: focusing on novel agents in relapsed and refractory disease. Cancer Treatment Rev 61:61–69
30. Taylor AMR, Rothblum-Oviatt C, Ellis NA, Hickson ID, Meyer S, Crawford TO et al (2019) Chromosome instability syndromes. Nat Rev Dis Primers 5(1):64
31. Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L et al (1995) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268(5218):1749–1753
32. Rotman G, Shiloh Y (1998) ATM from gene to function. Hum Mol Genet 7(10):1555–1563
33. Crawford TO (1998) Ataxia telangiectasia. Semin Pediatr Neurol 5(4):287–294
34. Taylor AM, Metcalfe JA, Thick J, Mak YF (1996) Leukemia and lymphoma in ataxia telangiectasia. Blood 87(2):423–438
35. Suarez F, Mahlaoui N, Canioni D, Andriamanga C, Dubois d'Enghien C, Brousse N et al (2015) Incidence, presentation, and prognosis of malignancies in ataxia-telangiectasia: a report from the French national registry of primary immune deficiencies. J Clin Oncol 33(2):202–208
36. Reiman A, Srinivasan V, Barone G, Last JI, Wootton LL, Davies EG et al (2011) Lymphoid tumours and breast cancer in ataxia telangiectasia; substantial protective effect of residual ATM kinase activity against childhood tumours. Br J Cancer 105(4):586–591
37. Thompson D, Duedal S, Kirner J, McGuffog L, Last J, Reiman A et al (2005) Cancer risks and mortality in heterozygous ATM mutation carriers. J Natil Cancer Inst 97(11):813–822
38. van der Burgt I, Chrzanowska KH, Smeets D, Weemaes C (1996) Nijmegen breakage syndrome. J Med Genet 33(2):153–156
39. Pastorczak A, Szczepanski T, Mlynarski W (2016) International Berlin-Frankfurt-Munster ALLhgvwg. Clinical course and therapeutic implications for lymphoid malignancies in Nijmegen breakage syndrome. Eur J Med Genet 59(3):126–132
40. Derry JM, Ochs HD, Francke U (1994) Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. Cell 79(5):922
41. Candotti F (2018) Clinical manifestations and pathophysiological mechanisms of the Wiskott-Aldrich syndrome. J Clin Immunol 38(1):13–27
42. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA (1994) A multiinstitutional survey of the Wiskott-Aldrich syndrome. J Pediatr 125(6 Pt 1):876–885
43. Imai K, Morio T, Zhu Y, Jin Y, Itoh S, Kajiwara M et al (2004) Clinical course of patients with WASP gene mutations. Blood 103(2):456–464
44. Albert MH, Bittner T, Stachel D, Nonoyama S, Notarangelo L, Burns S et al (2008) Clinical phenotype and long term outcome in a large cohort of X-linked thrombocytopenia (XLT)/Mild Wiskott-Aldrich-syndrome patients. Blood 112(11):90
45. De Meester J, Calvez R, Valitutti S, Dupre L (2010) The Wiskott-Aldrich syndrome protein regulates CTL cytotoxicity and is required for efficient killing of B cell lymphoma targets. J Leukoc Biol 88(5):1031–1040
46. Menotti M, Ambrogio C, Cheong TC, Pighi C, Mota I, Cassel SH et al (2019) Wiskott-Aldrich syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. Nat Med 25(1):130–140
47. Moratto D, Giliani S, Bonfim C, Mazzolari E, Fischer A, Ochs HD et al (2011) Long-term outcome and lineage-specific chimerism in 194 patients with Wiskott-Aldrich syndrome treated by hematopoietic cell transplantation in the period 1980–2009: an international collaborative study. Blood 118(6):1675–1684

48. Elfeky RA, Furtado-Silva JM, Chiesa R, Rao K, Amrolia P, Lucchini G et al (2018) One hundred percent survival after transplantation of 34 patients with Wiskott-Aldrich syndrome over 20 years. J Allergy Clin Immunol 142(5):1654–1656. e7
49. Iguchi A, Cho Y, Yabe H, Kato S, Kato K, Hara J et al (2019) Long-term outcome and chimerism in patients with Wiskott-Aldrich syndrome treated by hematopoietic cell transplantation: a retrospective nationwide survey. Int J Hematol 110(3):364–369
50. Albert MH, Freeman AF (2019) Wiskott-Aldrich Syndrome (WAS) and dedicator of cytokinesis 8- (DOCK8) deficiency. Front Pediatr 7:451
51. Aydin SE, Freeman AF, Al-Herz W, Al-Mousa HA, Arnaout RK, Aydin RC et al (2019) Hematopoietic stem cell transplantation as treatment for patients with DOCK8 deficiency. J Allergy Clin Immunol Pract 7(3):848–855
52. Li FY, Chaigne-Delalande B, Su H, Uzel G, Matthews H, Lenardo MJ (2014) XMEN disease: a new primary immunodeficiency affecting Mg^{2+} regulation of immunity against Epstein-Barr virus. Blood 123(14):2148–2152
53. Tangye SG, Palendira U, Edwards ES (2017) Human immunity against EBV-lessons from the clinic. J Exp Med 214(2):269–283
54. Ghosh S, Köstel Bal S, Edwards ESJ, Pillay B, Jimenez-Heredia R, Rao G et al (2020) Extended clinical and immunological phenotype and transplant outcome in CD27 and CD70 deficiency. Blood 136(23):2638–2655. https://doi.org/10.1182/blood.2020006738. PMID: 32603431
55. Latour S, Winter S (2018) Inherited immunodeficiencies with high predisposition to Epstein-Barr virus-driven lymphoproliferative diseases. Front Immunol 9:1103
56. Tangye SG (2020) Genetic susceptibility to EBV infection: insights from inborn errors of immunity. Hum Genet 139(6-7):885–901
57. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A et al (2014) Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 20(12):1410–1416
58. Schwab C, Gabrysch A, Olbrich P, Patino V, Warnatz K, Wolff D et al (2018) Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4-insufficient subjects. J Allergy Clin Immunol 142(6):1932–1946
59. Egg D, Schwab C, Gabrysch A, Arkwright PD, Cheesman E, Giulino-Roth L et al (2018) Increased risk for malignancies in 131 affected CTLA4 mutation carriers. Front Immunol 9:2012
60. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U et al (2014) Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. Nat Immunol 15(1):88–97
61. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR et al (2013) Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. Science 342(6160):866–871
62. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N et al (2017) Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: A large patient cohort study. J Allergy Clin Immunol 139(2):597–606. e4
63. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L et al (2016) Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: A cohort study. J Allergy Clin Immunol 138(1):210–218. e9
64. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P et al (2014) A human immunodeficiency caused by mutations in the PIK3R1 gene. J Clin Invest 124(9):3923–3928
65. Vinh DC, Patel SY, Uzel G, Anderson VL, Freeman AF, Olivier KN et al (2010) Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood 115(8):1519–1529
66. Hsu AP, McReynolds LJ, Holland SM (2015) GATA2 deficiency. Curr Opin Allergy Clin Immunol 15(1):104–109
67. Slack J, Albert MH, Balashov D, Belohradsky BH, Bertaina A, Bleesing J et al (2018) Outcome of hematopoietic cell transplantation for DNA double-strand break repair disorders. J Allergy Clin Immunol 141(1):322–328. e10

68. Kiykim A, Eker N, Surekli O, Nain E, Kasap N, Akturk H et al (2020) Malignancy and lymphoid proliferation in primary immune deficiencies; hard to define hard to treat. Pediatr Blood Cancer 67(2):e28091
69. Sánchez-Ramón S, Bermúdez A, González-Granado LI, Rodríguez-Gallego C, Sastre A, Soler-Palacín P et al (2019) Primary and Secondary immunodeficiency diseases in oncohaematology: warning signs, diagnosis, and management. Front Immunol 10:586
70. Duan L, Grunebaum E (2018) Hematological malignancies associated with primary immunodeficiency disorders. Clin Immunol 194:46–59
71. Moshous D, Pannetier C, Chasseval Rd R, Deist Fl F, Cavazzana-Calvo M, Romana S et al (2003) Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis. J Clin Invest 111(3):381–387
72. Jiang J, Tang W, An Y, Tang M, Wu J, Qin T et al (2016) Molecular and immunological characterization of DNA ligase IV deficiency. Clin Immunol 163:75–83
73. Hoshino A, Takashima T, Yoshida K, Morimoto A, Kawahara Y, Yeh TW et al (2018) Dysregulation of Epstein-Barr Virus infection in hypomorphic ZAP70 mutation. J Infect Dis 218(5):825–834
74. Kuehn HS, Boisson B, Cunningham-Rundles C, Reichenbach J, Stray-Pedersen A, Gelfand EW et al (2016) Loss of B cells in patients with heterozygous mutations in IKAROS. N Engl J Med 374(11):1032–1043
75. Crequer A, Troeger A, Patin E, Ma CS, Picard C, Pedergnana V et al (2012) Human RHOH deficiency causes T cell defects and susceptibility to EV-HPV infections. J Clin Invest 122(9):3239–3247
76. Schipp C, Schlütermann D, Hönscheid A, Nabhani S, Höll J, Oommen PT et al (2018) EBV negative lymphoma and autoimmune lymphoproliferative syndrome like phenotype extend the clinical spectrum of primary immunodeficiency caused by STK4 Deficiency. Front Immunol 9:2400
77. Abdollahpour H, Appaswamy G, Kotlarz D, Diestelhorst J, Beier R, Schäffer AA et al (2012) The phenotype of human STK4 deficiency. Blood 119(15):3450–3457
78. Linka RM, Risse SL, Bienemann K, Werner M, Linka Y, Krux F et al (2012) Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. Leukemia 26(5):963–971
79. Juilland M, Thome M (2018) Holding All the CARDs: how MALT1 controls CARMA/CARD-dependent signaling. Front Immunol 9:1927
80. Lu HY, Bauman BM, Arjunaraja S, Dorjbal B, Milner JD, Snow AL et al (2018) The CBM-opathies-A rapidly expanding spectrum of human inborn errors of immunity caused by mutations in the CARD11-BCL10-MALT1 complex. Front Immunol 9:2078
81. Byun M, Ma CS, Akçay A, Pedergnana V, Palendira U, Myoung J et al (2013) Inherited human OX40 deficiency underlying classic Kaposi sarcoma of childhood. J Exp Med 210(9):1743–1759
82. Filipovich AH, Heinitz KJ, Robison LL, Frizzera G (1987) The immunodeficiency cancer registry. a research resource. Am J Pediatr Hematol Oncol 9(2):183–184
83. German J, Passarge E (1989) Bloom's syndrome. XII. Report from the Registry for 1987. Clin Genet 35(1):57–69
84. Polityko A, Khurs O, Rumyantseva N, Naumchik I, Kosyakova N, Tönnies H et al (2010) Two siblings with immunodeficiency, facial abnormalities and chromosomal instability without mutation in DNMT3B gene but liability towards malignancy; a new chromatin disorder delineation? Mol Cytogenet 3:5
85. van den Brand M, Flucke UE, Bult P, Weemaes CM, van Deuren M (2011) Angiosarcoma in a patient with immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome. Am J Med Genet A 155A(3):622–625
86. Hagleitner MM, Lankester A, Maraschio P, Hultén M, Fryns JP, Schuetz C et al (2008) Clinical spectrum of immunodeficiency, centromeric instability and facial dysmorphism (ICF syndrome). J Med Genet 45(2):93–99

87. van der Klift HM, Mensenkamp AR, Drost M, Bik EC, Vos YJ, Gille HJ et al (2016) Comprehensive mutation analysis of PMS2 in a large cohort of probands suspected of lynch syndrome or constitutional mismatch repair deficiency syndrome. Hum Mutat 37(11):1162–1179
88. Tabori U, Hansford JR, Achatz MI, Kratz CP, Plon SE, Frebourg T et al (2017) Clinical management and tumor surveillance recommendations of inherited mismatch repair deficiency in childhood. Clin Cancer Res 23(11):e32–ee7
89. Orange JS (2013) Natural killer cell deficiency. J Allergy Clin Immunol 132(3):515–525
90. Logan CV, Murray JE, Parry DA, Robertson A, Bellelli R, Tarnauskaitė Ž et al (2018) DNA polymerase Epsilon deficiency causes IMAGe syndrome with variable immunodeficiency. Am J Hum Genet 103(6):1038–1044
91. Frugoni F, Dobbs K, Felgentreff K, Aldhekri H, Al Saud BK, Arnaout R et al (2016) A novel mutation in the POLE2 gene causing combined immunodeficiency. J Allergy Clin Immunol 137(2):635–638. e1
92. Sato T, Tatsuzawa O, Koike Y, Wada Y, Nagata M, Kobayashi S et al (1999) B-cell lymphoma associated with DiGeorge syndrome. Eur J Pediatr 158(7):609
93. Kim KY, Hur JA, Kim KH, Cha YJ, Lee MJ, Kim DS (2015) DiGeorge syndrome who developed lymphoproliferative mediastinal mass. Korean J Pediatr 58(3):108–111
94. Ramos JT, López-Laso E, Ruiz-Contreras J, Giancaspro E, Madero S (1999) B cell non-Hodgkin's lymphoma in a girl with the DiGeorge anomaly. Arch Dis Child 81(5):444–445
95. Eisner JM, Russell M (2006) Cartilage hair hypoplasia and multiple basal cell carcinomas. J Am Acad Dermatol 54(2 Suppl):S8–S10
96. Vakkilainen S, Taskinen M, Klemetti P, Pukkala E, Mäkitie O (2019) A 30-year prospective follow-up study reveals risk factors for early death in cartilage-hair hypoplasia. Front Immunol 10:1581
97. Chandesris MO, Melki I, Natividad A, Puel A, Fieschi C, Yun L et al (2012) Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. Medicine (Baltimore) 91(4):e1–e19
98. Jiao H, Tóth B, Erdos M, Fransson I, Rákóczi E, Balogh I et al (2008) Novel and recurrent STAT3 mutations in hyper-IgE syndrome patients from different ethnic groups. Mol Immunol 46(1):202–206
99. Oztop I, Demirkan B, Tarhan O, Kayahan H, Yilmaz U, Kargi A et al (2004) The development of pulmonary adenocarcinoma in a patient with Job's syndrome, a rare immunodeficiency condition. Tumori 90(1):132–135
100. Dorjbal B, Stinson JR, Ma CA, Weinreich MA, Miraghazadeh B, Hartberger JM et al (2019) Hypomorphic caspase activation and recruitment domain 11 (CARD11) mutations associated with diverse immunologic phenotypes with or without atopic disease. J Allergy Clin Immunol 143(4):1482–1495
101. Feske S, Picard C, Fischer A (2010) Immunodeficiency due to mutations in ORAI1 and STIM1. Clin Immunol 135(2):169–182
102. Al-Saud B, Al Alawi Z, Hussain FB, Hershfield M, Alkuraya FS, Al-Mayouf SMA (2020) Case with purine nucleoside phosphorylase deficiency suffering from late-onset systemic lupus erythematosus and lymphoma. J Clin Immunol 40(6):833–839
103. Watson AR, Evans DI, Marsden HB, Miller V, Rogers PA (1981) Purine nucleoside phosphorylase deficiency associated with a fatal lymphoproliferative disorder. Arch Dis Child 56(7):563–565
104. Daschkey S, Bienemann K, Schuster V, Kreth HW, Linka RM, Hönscheid A et al (2016) Fatal lymphoproliferative disease in two siblings lacking functional FAAP24. J Clin Immunol 36(7):684–692
105. Burges M, King B, McGee R, Brennan R, Wilson M (2019) Unilateral retinoblastoma in a patient with Hermansky-Pudlak syndrome. Ophthalmic Genet 40(1):83–85
106. Iwata Y, Kobayashi T, Arima M, Numata S, Yagami A, Okamura K et al (2017) Case of Japanese Hermansky-Pudlak syndrome patient with deeply invasive squamous cell carcinoma and multiple lesions of actinic keratosis on the face and neck. J Dermatol 44(2):219–220

107. Lorenzi L, Tabellini G, Vermi W, Moratto D, Porta F, Notarangelo LD et al (2013) Occurrence of nodular lymphocyte-predominant hodgkin lymphoma in hermansky-pudlak type 2 syndrome is associated to natural killer and natural killer T cell defects. PLoS One 8(11):e80131
108. Gámez-Díaz L, August D, Stepensky P, Revel-Vilk S, Seidel MG, Noriko M et al (2016) The extended phenotype of LPS-responsive beige-like anchor protein (LRBA) deficiency. J Allergy Clin Immunol 137(1):223–230
109. Fabre A, Marchal S, Barlogis V, Mari B, Barbry P, Rohrlich PS et al (2019) Clinical aspects of STAT3 gain-of-function germline mutations: a systematic review. J Allergy Clin Immunol Pract 7(6):1958–1969. e9
110. Zheng C, Huang Y, Hu W, Shi J, Ye Z, Qian X et al (2019) Phenotypic characterization of very early-onset inflammatory bowel disease with interleukin-10 signaling deficiency: based on a large cohort study. Inflamm Bowel Dis 25(4):756–766
111. Straus SE, Jaffe ES, Puck JM, Dale JK, Elkon KB, Rösen-Wolff A et al (2001) The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. Blood 98(1):194–200
112. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S et al (2013) Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. Haematologica 98(3):473–478
113. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M et al (2017) Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. J Exp Med 214(1):91–106
114. Martin E, Palmic N, Sanquer S, Lenoir C, Hauck F, Mongellaz C et al (2014) CTP synthase 1 deficiency in humans reveals its central role in lymphocyte proliferation. Nature 510(7504):288–292
115. Alosaimi MF, Hoenig M, Jaber F, Platt CD, Jones J, Wallace J et al (2019) Immunodeficiency and EBV-induced lymphoproliferation caused by 4-1BB deficiency. J Allergy Clin Immunol 144(2):574–583. e5
116. Salzer E, Cagdas D, Hons M, Mace EM, Garncarz W, Petronczki ÖY et al (2016) RASGRP1 deficiency causes immunodeficiency with impaired cytoskeletal dynamics. Nat Immunol 17(12):1352–1360
117. Schober T, Magg T, Laschinger M, Rohlfs M, Linhares ND, Puchalka J et al (2017) A human immunodeficiency syndrome caused by mutations in CARMIL2. Nat Commun 8:14209

Autoimmunity in Cellular Immunodeficiencies

15

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Abstract

The dual function of the immune system is to defend the host against pathogens and not to be reactive against self-antigens. The failure of these two missions is represented by primary immunodeficiencies (PID) and autoimmunity (AI), respectively. As such, PID and AI could be simplistically thought as mutually exclusive.

However, over 25% of patients with PID present one or more autoimmune disease with organ- or non-organ-specific manifestations, with an increased risk up to 120 times for autoimmune cytopenias. Defective lymphocyte maturation and activation, impaired central and peripheral tolerance, immunodysregulation and defective apoptosis are responsible of this paradoxical association.

In this chapter we briefly review autoimmune manifestations associated with PID categorized as (i) SCID/CID, (ii) LOCID, (iii) APECED, (iv) IPEX, (v) hypogammaglobulinemias, (vi) immunodysregulation disorders, and (vii) ALPS, describing each prototypical molecular defect and delving into the mechanisms that contribute to the development of AI.

Keywords

Immunodeficiency · Immunodysregulation · Tolerance · Autoimmunity Autoimmune diseases · Cytopenias · Hemolytic anemia

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15.1 Introduction

There is no dark side in the moon really—(Eclipse, Pink Floyd 1973)

Although the Jeffrey Modell Foundation has listed ten warning signs to identify patients likely to have primary IDs [1], it would be wise to add autoimmune manifestations as the eleventh. Selective IgA deficiency (sIgAD), the most common ID in Western countries, was already known to associate with several autoimmune disorders, such as cytopenias and other organ-specific AI [2]. Further, almost 50 years ago the rarer hyper-IgM deficiency was found as able to develop autoantibodies to blood elements [3]. ID and AI have been only a curious and unexplained combination for years and considered as opposite functional poles of the immune system since recently. Now, a series of cellular and humoral IDs were found to associate with AI, and it is clear that autoimmune diseases may not merely complicate the course but can be the initial feature of an ID. Along with this, a mounting number of reviews addressing the complex dysfunction of the immune system in IDs with AI has been recently published [4–6].

This *dark side of the moon*, i.e., the apparent paradox of the immune system activated against self when failing against external offenders, is going to be revealed and *as a matter of fact, it's not all dark*. **Hypotheses and facts** concur **to explain this association**.

First, monogenic IDs identified mutations for single immune regulators explaining the break of immunological tolerance. Gene mutations in SCID/CID (Omenn or DiGeorge syndrome *in primis*), APECED and IPEX/IPEX-like diseases undermine, respectively, central and peripheral tolerance. LRBA/CTLA4 mutations, some STAT GOF/LOF, X-linked Bruton syndrome and other hypogammaglobulinemias affect the fine mechanisms of immune regulation or B cell activation. Gene defects in ALPS and ALPS-related diseases alter apoptosis. Ataxia-telangiectasia and Wiskott-Aldrich syndrome characterized by interference in T cell activation are complicated by AI. All these pictures represent human in vivo experiments, frequently with more overt clinical manifestations than knockout animal models themselves.

Secondly, pathogens per se may contribute to the selection of the response. Pressure delivered by repeated infections may up- and dysregulate the production of proinflammatory cytokines redirecting T helper differentiation towards pathogenic (instead of protective) functional phenotypes. At the same time, the wide spectrum of colonizing pathogens may expose a mass of antigens, few of them cross-reacting with self-inciting residual responses against wrong targets (molecular mimicry) and others possibly resulting in polyclonal T cell activation (superantigens).

Finally, cell damage may produce DAMPs because of unresolved infections and unrelated (heterologous) CD8+, and CD4+ T cells might be activated following an antigen-specific, although inefficient, response (bystander activation).

Several similarities link AI to IDs and polygenic rheumatic diseases (SLE and RA) indirectly confirm shared pathomechanisms.

First, GWAS in Caucasian and Asian cohorts identified shared polymorphisms in MHC-unrelated genes in RA and some IDs as a proof of their regulatory role in tolerance [7]. Gene variants of complement proteins or GOF variants of the IFN-α signaling pathways or LOF mutations of other non-MHC genes are demonstrated in SLE analogously to the so-called interferonopathies. These findings could explain familial predisposition to AI resembling familial clustering of IDs [6].

Second, the frequent co-occurrence of autoimmune diseases within the same individual (polyautoimmunity) suggests a complex immune dysregulation as observed in some IDs where organ- and non-organ-specific AI may co-exist. Thus, the concept of the kaleidoscope could be proposed in IDs not dissimilarly from AI [8].

The **prevalence of AI in IDs** has been rarely addressed, and an intrinsic difficulty resides into the large variety of distribution and prevalence of IDs within different geographic areas and size of the national or international registries. The European Society for Immunodeficiencies (ESID) has recently renewed its registry, a large database collecting patients from 31 different countries, including some from Middle East, with more than 28.000 patients inserted. From the French national registry (CEREDIH), Fischer et al. [9] based the most relevant study retrospectively considering clinical pictures of dysregulation in 2183 screened ID patients aged 6 months to 92 years. One out four (26.2%) exhibited one or more AI and inflammatory manifestation, the general risk ranging 3–14 times compared to general population. Autoimmune cytopenias were the most prevalent (120-fold higher risk) with AHA stratospherically above all (830-fold). Even if gastrointestinal tract and skin were the most affected organs after the blood elements, rheumatologic disorders (i.e., RA) and large vessel vasculitides were overrepresented (at least tenfold). CVID and T cell defects were at highest risk for AI, and the overall survival time was significantly shorter when AI complicated IDs at any age (p 0.004). Prospective studies are still lacking.

If AI should be added to the ten warning signs of ID, is there any **special sign** which should **alert** in clinical practice? There is no specific and shared recommendation on this; nonetheless some suggestions might be proposed.

First, classes of immunoglobulins should be always determined as the prevalence of AI in sIgAD is globally about 36% whereas the prevalence of this immune defect is highly variable worldwide (overall prevalence 1:382 in Europe) [10].

Secondly, as cytopenias are the most frequent complication of IDs, all the patients, not only children, should be ideally screened for IDs in case of AHA, ITP or both. In the French cohort, it was estimated that given 360 pediatric cases of AHA, 15% of them (50 patients) will be ID [9]. As the risk to develop AI against blood elements persists through the ages, adult patients should not be deprived of the opportunity to be screened. Sex does not represent an adjunctive value as males are equally affected as females. Inflammatory arthritis of any kind and joint pain represent an alarm when occurring in the childhood as the risk is increased 40-fold in IDs and undoubtedly when associated with systemic autoinflammation [9]. See Table 15.1.

Finally, as AI manifestations may occur throughout the patient's lifetime and ID may be clinically highly variable (from severe to nuanced) also in monogenic

Table 15.1 Frequency of the most common autoimmune manifestations in a selected group of immunodeficiencies and typical monogenic defects

Clinical presentation	SCID/CID/LOCID							APECED	IPEX	Hypogammaglobulinemias						Immunodysregulation			ALPS	
	SCID	CID-G/AI	DGS	DGS	DGS	WAS	APDS			AG	sIgAD	CSR		CVID						RALD
Prototypical gene defect(s)	IL2RG, ADA, RAG1, RAG2	RAG1, RAG2	22q11del	ZAP70, ITK, LCK	CARD11, MALT1	WASP, WIP, ARPC1B	PIK3CD, PIK3R1, MTOR	AIRE	FOXP3, CD25, STAT5B	BTK	n.d.	CD40L	AICDA	TACI, BAFFR, ICOS, NFKB	ADA2	CTLA4, LRBA, DEF6	STAT1 GOF	STAT3 GOF	Fas, FASL, CASP8	NRAS, KRAS
Clinical autoimmunity																				
Cytopenias	+++	+++	++	++	++	+	+++	-	++	+++	++	++	++	+++	+++	+++	+++	+++	+++	+++
Thyroiditis	++	+	++	-	+	-	+	+	+	++	++	+	-	+	+	+	++	+	+	-
Endocrinopathies	-	-	-	-	+	-	-	+++	+++	-	-	-	-	+	-	+	++	-	-	-
Enteropathy	+	+	-	-	-	+	+	+	+++	+	-	-	+	+	+	+	++	-	-	-
Arthritis	-	-	+	+	-	+	+	-	+	-	-	+	-	+	+	+	-	++	-	++
Alopecia/Vitiligo	-	+	-	-	-	-	-	+	+	-	-	-	-	+	-	+	++	-	-	-
Lung disease	-	+	-	-	+	-	+	+	-	-	-	-	-	+	+	++	-	+	-	-
Vasculitis	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+++	-	-	-	-	-
Glomerulonephritis	-	+	-	-	-	+	+	-	-	-	-	+	-	-	+	+	-	-	+	+
Systemic Lupus Erythematosus	-	-	-	-	-	-	-	-	-	-	+	+	-	+	++	++	-	-	-	++
CNS infiltration	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	++	-	-	-	-
Hepatitis	-	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	-	-

SCID severe combined immunodeficiency, *CID-G/AI* combined immunodeficiency-granulomatous autoimmunity, *DGS* DiGeorge syndrome, *WAS* Wiskott-Aldrich syndrome, *APDS* activated p110delta syndrome, *APECED* autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, *IPEX* immunodysregulation polyendocrinopathy enteropathy X-linked, *AG* agammaglobulinemia, *sIgAD* selective IgA deficiency, *CSR* class-switch recombination defects, *CVID* common variable immunodeficiency, *ALPS* autoimmune lymphoproliferative syndrome, *RALD* RAS-associated lymphoproliferative syndrome

diseases, age does not represent a criterion to exclude adult patients from the screen any time.

In this chapter we provide an overview of mono- and polygenic IDs of the cellular compartment of the immune system presenting AI disorders. Other immune deficiencies such as interferonopathies, complement deficiencies and opsonization defects, or autoinflammatory disorders equally associated with manifestations of AI are beyond the purpose of this review.

15.2 Autoimmunity in Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) is characterized by several gene mutations affecting lymphocyte maturation (IL2RG, JAK3, IL7R, etc.), VDJ recombination (RAG1, RAG2, Artemis, etc.), or purine metabolism (ADA, PNP). Clinically, they manifest in early childhood with failure to thrive and susceptibility to opportunistic infections. T lymphocytes are usually absent with variable B and/or NK cells depending on the underlying molecular defect.

15.2.1 RAG Deficiencies and AI

Recombinase genes (RAG1 and RAG2) are critical for VDJ recombination during the development of both T and B lymphocyte. Thus, biallelic RAG1 or RAG2 mutations are associated with a classical T-B-NK+ SCID phenotype. Other mutations may maintain residual enzymatic activity with a heterogeneous spectrum of manifestations, ranging from Omenn syndrome, leaky/atypical SCID, delayed-onset combined ID with granulomas and/or autoimmunity (CID-G/AI), to milder phenotypes mimicking CVID or sIgAD [11].

Omenn syndrome is paradigmatic as a SCID variant associated with hypomorphic variants in which T cells are normally present but oligoclonally expanded, skewed towards a Th2 phenotype, activated and autoreactive [12]. Clinically, eosinophilia, elevated IgE and widespread organ lymphocytic infiltration causing lymphadenopathies, hepatosplenomegaly, erythroderma, and enteropathy are present.

Whereas AI is rare in infants with typical SCID, autoimmune cytopenias and vasculitis are often observed in Omenn syndrome and leaky SCID. Interestingly, CID-G/AI is associated with multi-organ granulomatous lesions, mainly involving skin, lung and liver, in addition to organ-specific AI, including nephritis, alopecia, vitiligo, psoriasis, myasthenia gravis and Guillain-Barré syndrome [11].

The reasons why SCID patients may or may not develop AI are different and reside on the degree of oligoclonality and amount of residual recombinase activity. The lower the levels, the more restricted TCR and BCR repertoires and the early-onset severe phenotypes. Vice versa, the higher the levels, the broader repertoire and better immune response, but more autoreactive clones and AI. The reduction of the TCR repertoire is associated with a skewed V, D, and J gene segment usage and a bias towards the use of proximal TRAV/TRAJ combination [13]. Loop length and

diversity of CDR3 (complementarity determining region 3) are reduced, while hydrophobic amino acids are enhanced [14]. In particular, the presence of hydrophobic residues at position 6 and 7 of CDR3β is associated with the development of autoreactive T lymphocytes [15].

Immunodysregulation may be also favored by environmental factors, i.e., chronic infections and altered microbiome interactions. The vaccine-strain rubella virus is contained inside the granulomas of patients with various T cell defects, including RAG deficiency [16]. In a mouse model of Omenn syndrome, microbiota would be a critical driver of AI, as responsible of the gut barrier impairment and mucosal expansion of self-reactive T cell clones, whereby manipulation of the microbiome with antibiotics reverted the inflammation [17].

Third, disruption of central and peripheral tolerance plays a major role in the immunodysregulation in RAG mutations. A correct thymic development requires the crosstalk between CD40+ RANK+ medullary thymic epithelial cells (mTEC) and CD40L+ RANKL+ CD4+ thymocytes. This interaction induces the expression of the autoimmune regulator (AIRE) by mTECs, which in turn increases the presentation of tissue-restricted antigens. Patients and animal models with defective recombinase activity exhibit a reduced-size thymus with a disrupted intra-thymic architecture, a lower AIRE and FoxP3 expression, and enrichment of self-reactive T cells. In atypical SCID and CID-G/AI, a disproportionate loss of Treg cells and increased self-reactive conventional T cells are indeed seen [18].

In addition, B cell tolerance is impaired, and high numbers of autoantibodies are found in RAG-deficient patients, including anti-cytokine (anti-IFN-α and anti-IFN-ω) antibodies [19]. Physiologically, RAG re-expression is critical for the B cell receptor editing in the bone marrow, and rearrangements in kappa and lambda light-chain loci reduce the frequency of self-reactive B lymphocytes. Finally, B cell-activating factor BAFF plays a cornerstone role in the survival of peripheral B cells as in physiological conditions anergic and self-reactive B cells have lower levels of BAFF receptor (BAFFR) than naïve B cells and are thus unable to survive [20]. In RAG-deficient patients, B cell lymphopenia induces high BAFF levels sustaining the survival of self-reactive B cells [21].

15.2.2 Thymic Defects and AI

Thymic development defects, similarly to RAG mutations, induce tolerance breakdown and raise of ID and AI. **DiGeorge syndrome (DGS)** is due to chromosome 22q11.2 deletions, with the consequence of parathyroid, thymus, cardiac and facial malformations. This ID varies from a severe SCID-like phenotype to almost normal. The prevalence of AI ranges between 10% and 33% [22, 23] with autoimmune cytopenias as the most commonly reported and possibly preceding infectious manifestations. Autoimmune polyendocrinopathies have been also observed, such as atrophic gastritis, T1D and thyroid diseases [24]. Thymus of DGS has a perturbed distribution of thymocytes, altered thymic output, and lower frequency of mature CD4+ and CD8+ T cells, in addition to reduced proportions and function of Tregs both into the

thymus and peripheral blood [25]. Interestingly enough, the immunophenotypic abnormalities of the peripheral blood predict DGS patients at risk of hematologic AI and correlate with survival [26].

15.3 AI in Late-Onset Combined Immunodeficiencies (LOCID)

Late-onset combined immunodeficiencies (LOCID) include a group of patients with severe infections for severe defects of cell-mediated immunity (e.g., opportunistic infections) and/or $CD4^+$ lymphocyte counts $<200 \times 10^6$ cells/L [27]. According to several investigations, LOCID patients tend to have a higher frequency of consanguinity and higher prevalence of lymphoproliferative disorders with hepatosplenomegaly and lymphadenopathy. Moreover, they usually present with granulomatous disease, gluten-resistant enteropathy and some features of AI. These patients might be misdiagnosed as CVID, because of frequent hypogammaglobulinemia [27]. Genetic studies, thanks to next-generation sequencing (NGS), are progressively defining new (and old) monogenic causes of LOCID [28]. Clear examples of LOCID are represented by three groups of diseases: (1) defective TCR signaling, (2) defective actin cytoskeleton, and (3) defective T cell activation/immunosenescence.

15.3.1 Defective TCR Signaling

TCR/CD3 complex engagement triggers a cascade of events resulting in T lymphocyte selection, activation, migration and effector functions, paralleled by Treg cell development and activation. The interaction between TCR and MHC-peptide complex activates Src family kinase LCK which phosphorylates CD3 ITAMs (immunoreceptor tyrosine-based activation motifs). ZAP70 (Zeta-associated protein of 70 kDa) is activated by LCK, binds to the phosphorylated ITAMs and phosphorylates downstream the adaptor molecules SLP-76 and LAT (Linker of Activated T cells). ITK (interleukin-2-inducible tyrosine kinase) upon interaction with phosphorylated SLP-76 and LAT undergoes autophosphorylation. When IDs involve proximal TCR signaling, severe abnormalities in T lymphocyte development and function with susceptibility to infections and immune dysregulation are described [29].

ZAP70 deficiency typically lacks circulating CD8+ T cells, whereas CD4+ T lymphocytes are present but do not proliferate. Hypomorphic variants with partial defects in ZAP70 signaling exhibit a peculiar immunodysregulated phenotype, similar to Omenn syndrome, with wheezing, erythroderma, lymphadenopathy, eosinophilia and high serum IgE [30]. Interestingly enough, an overwhelming AI was documented in a family with compound heterozygous ZAP70 mutations, one hypomorphic allele and one with gain of function, with bullous pemphigoid, colitis, hemophilia because of factor VIII autoantibody and nephrotic syndrome [31].

Defects in LCK present with recurrent respiratory infections, nodular skin lesions, arthritis, vasculitis, and ITP [32]. **ITK deficiency** clinically manifests

infections, especially from herpes virus, and autoimmune cytopenias, lymphadenopathy, hepatosplenomegaly and EBV-associated lymphoproliferative disease, usually localized to the lung [33].

After the proximal TCR engagement (ZAP70, SLP-76, LAT, ITK, LAT), phospholipase C-γ1 (PLC-γ1) is activated, allowing hydrolysis of phosphatidylinositol (4,5) diphosphate (PIP_2) to inositol (1,4,5) trisphosphate (IP_3) and diacylglycerol (DAG), release of Ca^{2+} from endoplasmic reticulum (ER) stores, Ca^{2+} influx, activation of Erk, reorganization of the actin cytoskeleton and activation of transcriptional program.

Ca^{2+} release from ER stores is called SOCE (store-operated Ca^{2+} entry). It is activated by CRAC (Ca^{2+} release-activated Ca^{2+}) channels which are composed by forming ion-conducting pore on the plasma membrane ORAI1 and STIM1 and 2 (stromal interaction molecule) located in the ER. **LOF mutations of the CRAC complex proteins,** ORAI1 and STIM1, are characterized by a SCID-like disease with AI, muscular hypotonia, and ectodermal dysplasia, with defects in sweat gland function and dental enamel formation [34].

Caspase recruitment domain (CARD) proteins, B cell CLL/lymphoma 10 (BCL10) and MALT1 paracaspase (MALT1) form adaptor complexes (CBM) critical in downstream signaling of numerous membrane receptors, including innate immunity pattern-recognition receptors (PRR) and GPCRs in non-hematopoietic cells. TCR and BCR signaling is mediated by the CBM complex expressing CARD11, which finely regulates cell activation, proliferation, metabolism in addition to survival pathways such as NF-kB, JNK and mTOR signaling. Numerous phenotypes associated with **defects in the CBM complex** have been recently described [35]. **Mutations of CARD11** manifest with different phenotypes. Biallelic LOF associates with a SCID/CID-like phenotype [36]. Monoallelic germline or somatic GOF variants cause a polyclonal B cell expansion with NF-kB and T cell anergy (**BENTA**) [37]. Heterozygous dominant-negative mutations are responsible for CARD11-associated atopy with dominant interference of NF-kB signaling (**CADINS**). CADINS is characterized by variable degrees of ID, including viral and bacterial infections in skin and airways, respectively, low to normal Ig and prominent atopic features, namely, asthma and atopic dermatitis, followed by food allergy, eosinophilic esophagitis and rhino-conjunctivitis. AI is found in 20% of the patients, most commonly alopecia, ITP, neutropenia and bullous pemphigoid [38].

15.3.2 Defective Actin Cytoskeleton

Defects in the assembly and dynamics of the actin cytoskeleton are an expanding group of IDs. Polymerization of actin plays an active role in multiple cellular processes, such as proliferation, endo- and exocytosis, intracellular trafficking and migration. Some of those defects are associated with significant inflammation and AI [39].

Wiskott-Aldrich syndrome protein (WASP) and WASP-interacting protein (WIP) are nucleation-promoting factors that promote F-actin branching. Defects in these genes are associated with Wiskott-Aldrich syndrome (WAS), which is the prototype of actin assembly defects. WAS is characterized by a triad: ID, microthrombocytopenia and eczema. Classical WASP defects are X-linked, while WIP defects are associated with autosomal recessive phenocopy. Hematological malignancies and AI are common complications of WAS and are often associated with the most severe forms. Reported autoimmune diseases include AHA, neutropenia, small and large vessels vasculitis, IBD and glomerulonephritis. A broad spectrum of autoantibodies has been observed [40]. Numerous mechanisms have been implicated in the WAS-associated AI: Tregs dysfunction, loss of tolerance and expansion of autoreactive B cells, defective Fas-mediated apoptosis and phagocytosis of apoptotic cells [41].

Arp2/3 complex (actin-related protein 2/3 complex) is required for assembly and branching of actin filaments. ARPC1B acts as a bridge between the actin filaments and the Arp2/3 complex. Biallelic **mutations in ARPC1B** have been reported in patients with a WAS-like ID, in which AI is prominent. Patients typically presents with lung and skin infections, bleeding, failure to thrive, early-onset eczema and atopic features. Cutaneous small vessel vasculitis, IBD and arthritis are between the most common presentations [42].

DOCK8 (dedicator of cytokinesis 8) is a guanine nucleotide exchange factor that activates Rho GTPases such as CDC42. DOCK8 activation is regulated by MST1 (mammalian sterile 20-like 1), also known as STK4 (serine/threonine kinase 4). **DOCK8 deficiency** causes autosomal recessive hyper-IgE syndrome, associated with a profound combined ID. Patients suffer from severe bacterial, viral (severe warts, CMV, EBV, HPV, HSV, VZV) and fungal (mucocutaneous candidiasis) infections, food allergies and malignancies. They have decreased and dysfunctional Tregs and produce autoantibodies [43]. Described autoimmune manifestations include AHA, colitis, sclerosing colitis and vasculitis. However, overt AI is uncommon, and it has been speculated that the profound T effector function deficiency might protect DOCK8-deficient patients from AI [44].

Disassembly of actin filaments is also crucial in cytoskeletal dynamics. Depolymerization is mediated by the recruitment of coronin, cofilin, and Aip1 (actin-interacting protein 1), encoded by *WDR1* (WD repeat-containing protein 1). **WDR1 deficiency** is embryonic lethal; however hypomorphic mutations have been recently discovered being the cause of the **lazy leukocyte syndrome** described in the 1970s. Affected patients suffer from severe pyogenic infections, defective wound healing, chronic stomatitis leading to oral stenosis, neutropenia, thrombocytopenia and autoinflammation with periodic fever [45].

15.3.3 Defective T Cell Activation/Immunosenescence

mTOR (serine/threonine kinase mechanistic/mammalian target of rapamycin) is a sensor detecting clues coming from the microenvironment and controlling cell

growth, proliferation and terminal cell differentiation. mTOR is part of a complex pathway including phosphoinoside3-kinase (PI3K), AKT and S6 kinase [46]. Hyperactivation of this pathway switches cells towards an anabolic state and impairs T cell function. Accordingly, naïve $CD4^+$ repertoire is extremely limited and counterbalanced by an excess of terminally differentiated senescent T $CD8^+$ cells. mTOR hyperactivation might be the consequence of heterozygous GOF mutations of two PI3Kdelta subunits, p110delta catalytic and p85alfa regulatory subunit. These mutations are responsible for the **activated PI3Kdelta syndrome (APDS) 1 and 2**, respectively. APDS1 was formerly named **PASLI** (p110delta activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency) [47].

The clinical picture is usually characterized by the presence of both ID and immunodysregulation. Classically, all the patients present recurrent respiratory tract infections, commonly pneumonia and bronchiectasis, these latter higher in the APDS1 cohort. Bacterial pathogens are more frequently capsulated (*Streptococcus pneumoniae*, *Haemophilus influenzae*). Half of the patients show persistent/recurrent severe herpes virus infections, with EBV viremia detected in 50% of them. More than three quarter of the patients show features of non-infective immune complications, the most common being non-neoplastic lymphoproliferation, clinically evidenced by diffuse chronic lymphadenopathy, splenomegaly and/or hepatomegaly. Evolution to lymphoma and other malignancies are more common in APDS2 patients (25%). Over than 1/3 of the patients present with some autoimmune features. AI is common in APDS1 with organ- or non-organ-specific manifestations: cytopenias (e.g., Coombs-positive AHA), GN, thyroiditis, insufficiency of exocrine pancreas, seronegative arthritis and sclerosing cholangitis. Recurrent pericarditis and enteropathy are also possible. In APDS2 AI manifestations are similar although less frequent (AHA, ITP and arthritis) in addition to organ-specific diseases (T1D, autoimmune hepatitis). Growth retardation is a prerogative of APDS2. Although most of the patients have been misdiagnosed as CVID, Ig deficiency is not a typical feature. Pneumococcal vaccine response is reduced in 90% of the patients, but IgG and IgA are low in less than half of the patients, whereas IgM levels are high and APDS1 and 2 need to be differentially diagnosed from hyper-IgM syndromes [48, 49].

Despite the differences between APDS1 and 2 cohorts, there is no genotype-phenotype correlation. Similarly, no different treatments between the two cohorts have been suggested [50]. Antimicrobial prophylaxis, immunoglobulin replacement and HSCT are used. Autoimmune manifestations have been treated with steroids and rituximab, with good clinical response, although followed by sustained B cell lymphopenia. Sirolimus has been used in non-lymphomatous lymphoproliferation but with less benefits on AI and cytopenias. Leniolisib, a potent oral p110delta subunit inhibitor, seems promising to control lymphoproliferation with no significant adverse reactions so far [50].

15.4 AI in Primary Defects of Central Tolerance

The paradigmatic link between IDs and AI is represented by the **autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED)** also **called autoimmune polyendocrine syndrome (APS)-1**. APS-1 is a complex disorder with predominant organ-specific manifestations as parathyroids, thyroid, adrenal glands and gonad involvement. As a ID, 80% of patients are also affected by chronic mucocutaneus candidiasis [51].

Characteristically, the production of organ- and non-organ-specific autoantibodies in APECED is strictly linked to the failure of central tolerance [52], that is, physical elimination or functional anergy of harmful self-reactive lymphocytes before their maturation [53]. The expression of self-antigens within the thymus is tightly regulated by a transcriptional regulating and chromatin remodeling complex called AIRE. *AIRE* mutations have a knockout effect on the development of regulatory T (Treg) cells which account for the clinical features of the disease [53]. Indeed, the different clinical phenotypes described so far might be explained by the large amount of mutations described, the most common being R257* in exon 6, the so-called Finnish mutation [54].

Chronic mucocutaneous candidiasis (CMC) is usually the first manifestation of the disease, appearing during the first 5 years of age and caused by the presence of anti-interleukin (IL)-17 and anti-IL-22 autoantibodies [55]. Chronic hypoparathyroidism and adrenal insufficiency that constitute the classical triad of APS-1 might appear up to the fourth decade of life [51]. Chronic hypoparathyroidism is usually the first endocrine organ hit by AI with a vague clinical presentation (muscle cramps and mild paresthesia), as hypocalcemia seems to be slowly progressive. Fever may be the precipitating factor, leading to seizure and, eventually, to diagnosis [56]. Relevant autoantigens have not been discovered yet. Vice versa, autoantibodies against a recognized target (anti-12-hydroxilase) can be detected many years before the onset of adrenal insufficiency, being fatigue, weight loss and increased pigmentation the most common (and non-specific) symptoms [57]. Hypergonadotropic hypogonadism, T1D, thyroid diseases and pituitary defects are other endocrine features [57]. Gastrointestinal AI (i.e., autoimmune gastritis and hepatitis) is also common. Malabsorption and steatorrhea may be explained by pancreatic failure or activity of anti- intestinal endocrine cell autoantibodies [58]. Cutaneous vasculitis, Sjögren's syndrome, and celiac disease have also been described. Autoimmune encephalitis may occur but is not common [59]. Hormone replacement therapy is necessary for the endocrine failure, and immunosuppressive therapy, including rituximab, is used to control clinical symptoms and the autoimmune manifestations [60]. Finally, ectodermal abnormalities are also common and appear early during the disease course. Although autoimmune mechanisms might explain the clinical picture, no specific autoantibodies have been reported so far. Dental enamel hypoplasia, pitted nail dystrophy, alopecia, and vitiligo are the most common manifestations, and an urticarial rash associated with fever might appear at first [61].

15.5 AI in Primary Defects of Peripheral Tolerance

Peripheral tolerance is the general mechanism of inactivation of mature lymphocytes that escaped the central tolerance but recognizing self antigens. It may occur by re-exposure to the antigen (anergy), death by apoptosis or active suppression by Tregs [62]. $CD4^+$ regulatory T cells express high levels of CD25 (IL-2 receptor alpha chain), CD127 (IL-7 receptor) and the transcription factor FOXP3 (forkhead box P3) [63]. FOXP3 is, indeed, critical for the development and function of Treg cells [63].

15.5.1 IPEX

Hemizygous mutations of *FOXP3* gene located on the X-chromosome are responsible for the **immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)** syndrome associated with a deficiency of Treg cells, and the subsequent failure of peripheral tolerance [62].

Even though the typical presentation of the disease is characterized by severe enteropathy, T1D and severe eczema during the first months of life, diagnosis can be difficult as it may manifest later in childhood with isolated features and failure to thrive [64]. No correlation genotype-phenotype has been described so far and the cause of this heterogeneity remains obscure [63].

Enteropathy occurs usually early in life (within 7 months of age), occurs in >90% of the patients and causes watery diarrhea and malabsorption that eventually brings to failure to thrive. In one half of these patients, histology demonstrates small bowel villous atrophy, probably due to anti-harmonin and anti-villin autoantibodies [64]. Skin manifestations are common with eczema and/or exfoliative dermatitis [63]. Severe food allergy typically associates with elevated IgE levels with overall normal immunoglobulins [64]. It is probably caused by inappropriate skewing towards a type-2 immunity due to defective Treg cells and reduced T cell receptor repertoire [65].

T1D usually follows enteritis and skin disease and is marked by anti-glutamic acid decarboxylase (GAD) or anti-islet cell antibodies. Besides this classic triad, other common manifestations of AI include thyroid dysfunction, cytopenias (anemia, neutropenia, thrombocytopenia), renal involvement (GN and interstitial nephritis), autoimmune hepatitis and interstitial lung disease. The response to common immunosuppressant such as corticosteroids or cyclosporine A is usually transient and weak. Thus, early hematopoietic stem cell transplantation (HSCT) is the only curative approach with a long-term survival (over 30 years) in 50% [64].

15.5.2 IPEX-Like Syndromes

IL-2 is pivotal for the survival and proliferation of all T lymphocytes [66]. Treg cells are characterized by the isolate presence of the low-affinity IL-2 receptor

CD25 (IL-2 receptor alpha chain) able to equally bind IL-2, differently from the other T cell populations expressing the high-affinity receptor for IL-2, constituted by beta (CD122) and gamma (CD132) chains in addition to CD25. In this way, Treg cells compete and deprive other T cells from IL-2, with the consequence of reduced differentiation and proliferation and, ultimately, suppression of the immune response [63]. The IL-2 high-affinity receptor signals through the STAT5b transduction pathway, allowing FOXP3 transcription. However, transcription factor STAT5b is shared by other signaling pathways and is pivotal for growth hormone (GH) [67]. For this reason, mutations of CD25 and STAT5b show a clinical picture similar but not identical to IPEX (so-called IPEX-like syndromes), where AI and ID are present at the same time and, in case of STAT5b, together with GH insensitivity and dwarfism [63]. At variance with IPEX, however, patients with IPEX-like syndromes less likely suffer from T1D and food allergies which generally develop later in life [64].

CD25 deficiency is an IPEX-like syndrome characterized by severe enteropathy, eczema, AI (such as T1D), lymphoproliferation, systemic lymphadenopathy and susceptibility to chronic infections, especially towards herpes family viruses [68].

STAT5b deficiency is a rare autosomal recessive disorder which combines GH insensitivity syndrome (impaired postnatal growth in the presence of normal-to-high GH levels) and features of an IPEX-like syndrome with infections. Dominant-negative STAT5b mutations have been recently described with predominant growth defects and none-to-mild immune dysregulation. This latter is evidenced by eczema, celiac disease and autoimmune thyroiditis [69].

Ten-year survival is significantly higher in IPEX-like than IPEX patients (81.5% vs 65%), but 30-year survival is poor in both groups, and in both cohorts, HSCT improves the survival rate, supporting its early implementation [64].

15.6 AI in Immunoglobulin Defects

Activation, proliferation and survival of B cells, correct somatic hypermutation of immunoglobulin (Ig) genes and antibody class switch recombination (CSR) are critical points for a successful (and regulated) humoral response. Thus, any perturbation of these checkpoints alters the quantity of circulating immunoglobulins and, only apparently, paradoxical AI development. The main clinical syndromes discussed in this chapter are agammaglobulinemia, selective IgA deficiency, class-switch recombination defects and common variable immunodeficiency (CVID).

Bruton agammaglobulinemia is a rare X-linked ID with agammaglobulinemia (XLA) characterized by the absence of mature B cells and Ig-secreting plasma cells, severe antibody deficiency and recurrent infections. More than 500 different mutations (nonsense, splice defects, deletions, insertions) of the *BTK* gene located on the long arm of the X-chromosome have been described. Mutated BTK (Bruton tyrosine kinase) impairs B cell development from pro-B to pre-B lymphocytes as being involved downstream into signal transduction after a successful immunoglobulin

heavy-chain rearrangement [70]. Severe recurrent infections are the mainstay of this ID. This may account for reactive manifestations resembling clear AI (arthritis secondary to *Mycoplasma* infections, GNs, IBDs due to *Campylobacter* or *Enteroviruses*), but XLA is characterized per se by AI although much less frequently than CVID or other Ig defects. Fewer than one third of the patients may develop painful joints, but frank formal arthritis is only occasionally observed. Thrombocytopenia, anemia and leukopenia affect less than 10% of patients, and neutropenia seems to be due to infective agents rather than autoantibodies [71].

Selective IgA deficiency (SIgAD) is the most common ID in both adults and children with a highly variable prevalence also in populations of similar descent [72]. Similarly, SIgAD is highly heterogeneous in its immunological defects: a) defects of B cells (altered class switch and Ig rearrangement, increased B cell apoptosis, reduced survival of IgA+ plasma cells or reduced terminal differentiation of IgA plasmablasts), b) unbalanced production of cytokines crucial for class switch or maturation of B lymphocytes (TGF-beta, IL-10 or IL-21) or c) defective T cell help [73]. Patients with sIgAD may carry several chromosomal abnormalities and cytogenetic defects (monosomy, trisomy, translocation or deletions) [74], but low IgA levels can be found in a series of inborn errors of immunity. Beyond possible infectious susceptibility, allergic diseases, gastrointestinal disorders and malignancies, sIgAD characteristically associate with AI with 5–30% prevalence [73, 75]. Individuals with symptomatic or asymptomatic SIgAD indeed show an increased ability to produce autoantibodies not necessarily associated with overt AI and antithyroid autoantibodies (anti-thyroglobulin or -microsomal antigens), Coomb's positivity, anti-nuclear and anti-cardiolipin antibodies, can be detected. When autoimmunity clinically develops, cytopenias (ITP, AHA), thyroid diseases (Graves' disease, Hashimoto's thyroiditis), T1D, celiac disease, RA or SLE can occur. Almost one fifth of SIgAD affected adult patients develops at least one of the autoimmune diseases [76], whereas in children celiac disease is the most prevalent (about 15%) followed by thyroid disease and then vitiligo, psoriasis, T1D, and alopecia as seen in one recent cohort undergoing a long follow-up [77].

Class-switch recombination (CSR) is an intrachromosomal deletional recombination event leading to the production of all the isotypes of immunoglobulins, but IgM, in mature B cells. Thus, CSR-ID are characterized by normal/elevated circulating IgM in the absence/low levels of IgG, IgA and IgE. They are thus also known as **hyper-IgM syndromes (HIGM)**. CSR-ID/HIGM are rare, far less common than classic CVID (at most 1/1,000,000), and are classified in five different disorders (HIGM1–5 and X-linked hyper-IgM syndrome [XHIM]) because different genes are involved, namely, CD40L and CD40, AICDA, UNG, and IKBKG [78].

CD40 is codified on 20q12-q13.2 and plays an essential role for T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation [75, 79]. Only in 2001 absence of CD40 expression was related to the rare autosomal recessive HIGM3.

CD40L encoding gene (*CD40LG*) is located in the X chromosome and its defects are responsible for the most frequent XHIM. It codifies for the activated T cell

receptor CD40L (CD154) which engages the B-cell costimulatory receptor CD40 to induce the production of antibodies of correct affinity. In mice CD40L hyperexpression associates with accelerated lymphocyte apoptosis, high titers of autoantibodies and SLE-like [80]. In humans, *CD40LG* microduplications associate with AI (cytopenias, arthralgias, thyroid disease) [81].

In general, every perturbation of CD40-CD40L interaction correlates with AI and XHIGM and HIGM3 patients are similarly affected by autoimmune manifestations. Autoimmune cytopenias (neutropenia, AHA, ITP), hypothyroidism, IBD, and kidney disease are described in addition to infections. HIGM2 is an autosomal recessive (mostly) or autosomal dominant (rarely) inherited defect of *AICDA*. The gene encodes the DNA editing enzymatic protein **AID** (activation-induced cytidine deaminase) involved in somatic hypermutation and Ig gene conversion [82]. Cytopenias, hepatitis, IBD, and arthritis are the most common manifestations occurring in about one fifth of the patients, but also SLE and uveitis have been described and autoreactive antibodies detected into the circulation [83, 84]. The recognition of the genetic defects of HIGM4 still lacks, and the syndrome has a mild course with involvement of blood series, uvea and joints [85]. So far, no manifestations of AI have been recognized, although expected, in HIGM5 deficiency related to **UNG** (uracil N-glycosylase) [86].

Other diseases including IDs exhibit increased levels of IgM both occasionally (RAG-2 deficiencies) and regularly (APDS, PMS2 gene mutations, A-T or NBS, CVID). Both PMS2 (postmeiotic segregation increased 2) gene mutations and NBS (Nijmegen breakage syndrome) are constitutional mismatch repair deficiencies primarily characterized by malignancies in the absence (PMS2) or presence (NBS) of ID. AI occurs only in the latter, mainly described in individuals of Slavic descent, with single descriptions of AHA and juvenile idiopathic arthritis (JIA)-like polyarthritis [87]. Revisions of AI in selected IDs with hyper IgM are available [78, 88].

CVID is the most common symptomatic hypogammaglobulinemia in adults. It is characterized by reduced immunoglobulins, impaired production of specific antibodies, autoimmunity and lymphoproliferation. However, CVID is an extremely heterogeneous syndrome. In fact, it is extensively used as a broad umbrella diagnosis. Monogenic forms account for a variable percentage of the patients, depending on selection criteria and sequencing technique. Genes that have been implicated in monogenic CVID include ICOS, TACI, BAFF-R, TWEAK, CD19, CD81, CD21, CD20, CD27, IL-21, IL21R, LRBA, CTLA4, PRKCD, PLCG2, NF-KB1, NF-KB2, PIK3CD, PIK3R1, VAV1, RAC2, BLK, IKZF1 and IRF2BP2 [89]. Moreover, the discovery of the molecular defects underlying some CVID patients greatly helped understating the pathogenesis of the apparently paradoxical autoimmune and lymphoproliferative complications. Some of the most complex clinical pictures characterized by overwhelming autoimmune phenomena have been re-classified as "immunodysregulation disorders" [90] and are discussed separately in this manuscript, despite the clinical presentation often mimics CVID.

B cell-activating factor (BAFF) regulates antibody response through the binding of its three receptors: (i) **BAFF-R (BAFF-receptor),** promoting survival and maturation of peripheral B cells; (ii) BCMA (calcium modulator and B cell maturation antigen), promoting the survival of long-lived bone marrow plasma cells; and (iii) **TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor),** promoting differentiation and survival of plasma cells, inhibition of B cell expansion and induction of CSR towards IgG and IgA [91, 92]. In mice, deletions in the BAFF-encoding gene or BAFFR deficiency interrupt maturation of transitional B cells causing ID. In humans the homozygous deletion within the exon 2 of the *TNFRSF13C* gene produces an altered transmembrane region of BAFFR with late-onset IgG and IgM hypogammaglobulinemia, severe lymphopenia and possibly infections [93]. Altered extracellular or intracellular chain of BAFFR due to missense mutations is more common and may be found in CVID, but its contribution remains elusive [94]. Several genetic defects (biallelic and monoallelic loss-of-function variants) in *TNFRSF13B* TACI-encoding gene have been found in almost 10% of the patients with CVID, mostly heterozygous C104R and A181E point mutations, with associated variable clinical phenotypes [95]. Variants have also been detected in sIgAD, IgG subclass deficiency, asymptomatic relatives of CVID and 1–2% general population; thus the combination with other defect(s) is likely involved into overt ID [89, 96]. More interestingly, BAFF and its receptors are highly involved in AI. $TACI^{-/-}$ or BAFF transgenic mice develop a SLE-like disease. In humans BAFF/APRIL concentrations are increased in RA and SLE and correlate with disease activity and autoantibody production. High levels of BAFF maintain the pool of transitional B cells in which high numbers of autoreactive lymphocytes are included [91, 92, 97]. In CVID, levels of BAFF are indeed increased [98]. In TACI as well as in BAFFR variants, cytopenias, rheumatic diseases (sacroiliitis) and IBD may develop [89].

ICOS (inducible T cell costimulator) is a T cell-associated receptor belonging to the CD28/CTLA-4 family. ICOS-ICOS-L interaction is crucial for antibody production, effector T cell responses and tolerance [99]. In ICOS mutations (missense mutations, homozygous deletions, compound heterozygous mutations), clinical AI with cytopenias, rheumatic disease, and early onset of Crohn's-like colitis are found together with altered B cell numbers, hypogammaglobulinemia, impaired CTLA-4 expression, abnormal cytokine production and T_H1/T_H2 unbalance, repeated respiratory and/or viral infections [100, 101] without any clear genotype-phenotype correlation.

NF-κB (nuclear factor "kappa-light-chain-enhancer" of activated B cells) represent a family of transcription factors (NF-κB1, NF-κB2, RelA, RelB, c-Rel) crucial for cell activation [102]. Deficiency of NF-κB1 (heterozygous LOF variants) and NF-κB2 (germline dominant-negative heterozygous mutations) is responsible for CVID with early onset (NF-κB2) or ranging from infants to aged individuals (NF-κB1). ID varies from mild hypogammaglobulinemias to severe forms with only NF-κB1 mutations associated with organ-specific AI (cytopenias, alopecia areata or universalis, vitiligo, and Hashimoto thyroiditis) [103].

15.7 Diseases of Immunodysregulation

15.7.1 Checkpoints Defects

Lymphocyte deactivation after antigen recognition requires negative signals which are provided by a series of surface receptors and/or regulatory cells. CTLA-4, ICOS, OX40, and PD1 exert downregulation activities and control lymphocyte proliferation and differentiation. Thus, mutations of genes coding for these checkpoint molecules are followed by lymphoproliferation and abnormal antigen recognition including self in the context of different degrees of ID.

Cytotoxic T lymphocyte antigen 4 (CTLA-4) (CD152) is a homodimer coded by four exons acting as negative regulator of immunity [104]. *CTLA4* gene is located on 2q33.3 where *CD28* and *ICOS* are also placed [105]. CTLA-4 is constitutively expressed on Tregs [106] where it represents a sine-qua-non molecule for the suppressive function being *CTLA4,* a target gene of FOXP3 [106, 107]. Functionally, it binds CD80 and CD86 with greater (500–2500-fold) affinity and avidity than its homolog CD28, thus competing with this latter and terminating cell activation. Deprivation of IL-2 is one of the mechanisms of cell deactivation by CTLA-4 [108]. Actually, homozygous *CTLA4*$^{-/-}$ mice succumb for fatal AI and lymphoproliferation [109]. In humans, several observations link the *CTLA4* gene with thyroid autoimmunity (hypothyroidism, Graves'), dysregulated IgE production, insulin-dependent diabetes and malignancies [110, 111]. Conversely, the fusion protein with agonist properties abatacept is a useful therapeutic tool in autoimmune diseases including RA [112].

Heterozygous germline mutations in CTLA4 have been described for the first time in 2014 [113, 114]. Since then, more than 50 heterozygous *CTLA4* germline mutations have been identified (missense, insertions or deletions, nonsense) all placed in exons 1–2-3 [115]. Asymptomatic or mildly symptomatic carriers of *CTLA4* mutation have been found in the same families of the affected individuals.

The largest known cohort of *CTLA4* mutation carriers including 133 subjects and 28 novel mutations has clarified the complex spectrum of the clinical manifestations in heterozygous CTLA-4 insufficiency [116]. Three out four of the patients had CVID and respiratory tract infections or sepsis. AI is eventually represented by cytopenias (30%) frequently bi- or tri-lineage (ITP, AHA, pure red cell aplasia or autoimmune neutropenia). Autoimmune endocrinopathies (thyroid diseases and T1D, Addison disease), psoriatic arthritis, alopecia, autoimmune encephalitis, atrophic gastritis, celiac disease and primary biliary cholangitis may also occur. Immune dysregulation finally manifests as non-malignant or malignant lymphoproliferation and solid organ neoplasms. This kaleidoscopic picture is completed by other clinical manifestations as atopic dermatitis (about 60%), neurological features (about 30%), mental and/or growth retardation. Phenotypically, lymphopenia with reduced CD19+ CD27+ IgD- and relative increase of CD21^{low} are found [116]. A complete review on CTLA-4 in relation to immunodeficiency has been recently provided [115].

In 2012 mutations in chromosomal region 4q31 encoding **LRBA (lipopolysaccharide-responsive beige-like anchor protein)**, already suspected as an ID candidate gene [117], were described [118]. Homozygous individuals exhibited a deleterious ID with hypogammaglobulinemia and repeated severe infections. AI was almost invariably the onset of the disease (ITP followed by AHA) plus autoimmune enteropathy resembling Crohn's disease, atrophic gastritis with autoantibodies against intrinsic factor, hypothyroidism, myasthenia gravis, and arthritis. Disturbed B cell development and reduced plasmablast formation were initially described, and, in 2015, marked depletion of Tregs with defective levels of canonical markers (FOXP3, CD25, Helios and CTLA-4) was added [119]. LBRA is codified into the BEACH (BEige and Chediak-Higashi syndrome) family of cytoplasmic proteins that regulate intracellular vesicle trafficking and exocytosis [120]. CTLA4 and LRBA co-localize within recycling endosomes and the TGN, but the tail of CTLA-4 needs to interact with the pleckstrin homology (PH)-like and the BEACH domains of LRBA to be maintained intracellularly stored [121]. In LRBA-deficient individuals CTLA4 mRNA levels are thus normal and LRBA controls the expression of CTLA-4 at post-translational level [121].

Recessive-inherited mutations in LRBA deficiency vary (complete absence of the protein, truncated protein with altered expression of PH, BEACH or WD40 domains, truncated protein devoid of the BEACH and WD40 domains, normal expression of a not-functional protein) [122]. The clinical course is close to CTLA-4 deficiency: hypogammaglobulinemia, repeated infections, interstitial lung disease, neurologic disease (cerebral or cerebellar atrophy, myasthenia gravis, space-occupying lesions usually of granulomatous origin), skin diseases resembling atopic dermatitis, and chronic diarrhea. About AI, besides ITP and AIHA, autoimmune thyroid disease and autoimmune enteropathy are the most common, but, also, vitiligo, celiac or sprue-like disease with villous atrophy, T1D and juvenile idiopathic arthritis may occur [122].

One more mutated protein associated with decreased ability to recycle CTLA-4 is **DEF6 (Differentially Expressed in FDCP6 homolog)** also named as IRF4 binding protein (IBP) or SWAP-70-like adaptor of T cells (SLAT) [123, 124]. It activates downstream of TCR and promotes Ca2+ signaling, NFAT1 activation and T cell adhesion [125]. In AI it was invoked in early-onset large vessel vasculitis and other systemic autoimmune manifestations [126]. Very recently, three patients from two families from Iraqi and Pakistan were described as carrying homozygous or compound heterozygous mutations with reduced or complete loss of expression of DEF6 protein. In addition to increased susceptibility to viral and bacterial infections, mixed autoimmune/autoinflammatory pictures were present: IBD and atrophic gastritis due to lymphocytic infiltration with severe diarrhea, AHA, ITP, high levels of anti-neutrophil cytoplasmic antibodies (ANCA) and anti-phospholipid autoantibodies, successful treatment with abatacept. Cycling CTLA-4 was reduced, CTLA-4 did not effectively reach surfaces and CD80 capture and transendocytosis were impaired. A defective interaction between DEF6 and the small GTPase RAB11 necessary for correct recycling endosomes is responsible for this novel ID [125].

The interaction between **tumor necrosis factor receptor OX40 (CD134)** and its cognate ligand OX40L (CD134L, CD252) has been long supposed as a possible therapeutic target in AI. OX40 is encoded by the *TNFRSF4* gene placed on chromosome 1. Lack of OX40 is characterized by reduced numbers of Tregs and reduced IFN-ɣ production [127]. However, only one healthy female carrying an autosomal recessive OX40 deficiency has been described [128]. More recently, a single case with a homozygous nonsense mutation of the *RC3H1* gene, affecting OX40, ICOS, and TNF expression, has been described, but the clinical phenotype was hyperinflammation and presentation as relapsing HLH instead of ID and AI [129].

15.7.2 Defects of JAK/STAT Signaling

Maturation, survival, and differentiation of B cells and Ig class switch are also dependent by a correct signaling after the engagement of the cell surface receptors for cytokines, chemokines, growth factors or hormones. The activation of different combinations of JAKs (Janus kinase signal transducer) is responsible for the recruitment of various STATs (activator of transcription signaling pathway), depending on the type (I or II) of the cytokine receptor. This chain of events is involved in host defense but also in AI as redirecting T cell differentiation, and JAK inhibitors are successful therapeutic arms for increasing numbers of autoimmune diseases [130, 131].

STAT1 is principally activated upon binding of type I (α and β) and II (ɣ) interferons in addition to IL-2, IL-10, IL-21 and IL-27. While LOF mutations on *STAT1* gene are included into the group of MSMD (Mendelian Susceptibility to Mycobacterial Disease) as deficient in the IL-12/IFN-ɣ axis, GOF mutations associate with CMC (chronic mucocutaneous candidiasis) [132, 133]. Heterozygous GOF STAT1 mutations are characterized by ID (hypogammaglobulinemia, CMC, disseminated fungal infections, viral and *Staphylococcus aureus* infections) and AI in more than 30% of the patients, seldom as the prevalent feature. AI is typically represented by hypothyroidism, T1D, autoimmune cytopenias, and SLE, but other manifestations can occur such as IBD, autoimmune hepatitis, arthritis, multiple sclerosis, as well as cerebral vascular aneurysms, even if infrequently [133]. A single case of fatal Takayasu arteritis has been described [134].

STAT3 is activated downstream after type I, II and III interferons, IL-6, IL-10, IL-17, IL-21, IL-22 and IL-23 signaling. Autosomal dominant-negative STAT3 LOF variants are the cause of hyper-IgE syndrome, also known as Job's syndrome. Those patients display defective T_H17 lymphocyte polarization and IL-17 production. They present clinically with a wide array of infectious diseases (candidiasis, *S. aureus*) and typical phenotypical features (facies, pneumatoceles, connective tissue malformations). They often present with symptoms suggestive of AI diseases such as arthritis and Raynaud's, skin, and mucosal ulcerations, but seldomly meet diagnostic criteria. Between 4.8% and 14.5% have a possible AI connective tissue disease. Organ-specific diseases like T1D or thyroiditis are rare [135].

STAT3 GOF germline mutations (more than 28 described, autosomal dominant trait inheritance) enhance transcriptional activity of STAT3, STAT3 DNA-binding activity, or delay STAT3 dephosphorylation. At the same time, STAT1 and STAT5 phosphorylation decreases and STAT5 is crucial for growth hormone signaling. STAT3 hyperactivation favors T_H17 differentiation and suppresses Treg function. In fact, *STAT3* polymorphisms associate with increased predisposition to psoriasis and multiple sclerosis [136]. Patients with STAT3 heterozygous GOF mutations exhibit multiple different clinical features as short stature, inconstant recurrent infections proper of ID (disseminated non-tuberculous mycobacteria, viruses and fungi) with low IgG levels and reduced class-switched memory B lymphocytes, non-malignant lymphoproliferation (lymphadenopathy, splenomegaly) and AI [137]. Autoimmune disorders range from cytopenias (about 50% of the patients), to T1D with infancy onset (25%), enteropathies (50%), primary hypothyroidism (25%), and interstitial lung disease (about one third) [138].

15.7.3 Miscellaneous Disorders

Adenosine deaminase 2 (ADA2) is an extracellular enzyme, secreted by monocytes and dendritic cells involved into the catabolism of purines [139]. It also regulates the balance between M1 and M2 macrophages. M1 macrophages induce TNF-α production and enhance inflammation, characterized by vascular injury and upregulation of neutrophil-expressed proinflammatory genes. Thus, anti-TNF-α drugs have shown efficacy in the treatment of DADA2 vasculitis and prevention of strokes [139]. **Homozygous mutations in ADA2** (**DADA2**) exhibit different clinical phenotypes, with hematological, immunological and vascular involvement. Although it is generally accepted that the lower the activity of ADA2, the worse is the clinical phenotype, no clear genotype-phenotype correlation exists. Clinically, livedo reticularis and erythema nodosum are classical signs. Cutaneous vasculitis can be severe, with skin ulcers, and systemic vasculitis mimicking panarteritis nodosa (PAN), with juvenile onset. Both central and peripheral nervous systems (ischemic and hemorrhagic strokes) can occur. Strokes can recur in approximately one half of the patients [139]. Peripheral neuropathy is common. Arthritis is a frequent finding. The clinical picture also includes the hematological involvement: pure red cell aplasia (sometimes mimicking Diamond-Blackfan anemia) and bone marrow failure signs (including neutropenia and pancytopenia). Finally, hepatosplenomegaly, diffuse lymphadenopathy and hematological malignancies are observed. Homozygous ADA2 mutations have been recently found in patients with hypogammaglobulinemia and recurrent infections (**CVID-like DADA2**) [140].

Ikaros is a zinc-finger transcription factor encoded by *IKZF1* gene, strongly expressed in lymphoid and hematopoietic progenitors. Ikaros homodimers bind to DNA at the pericentromeric heterochromatin regions, acting mainly as a gene repressor [141]. In humans Ikaros regulates B cell development, commitment and activation, Ig gene recombination together with EBF1 and PAX5 [142] but also the differentiation of dendritic cells. In **IKZF1 haploinsufficiency** plasmacytoid

dendritic cells are absent, but conventional dendritic cells are normal or expanded [143]. In Ikaros deficiency very low B cell counts, raised CD8+ T lymphocytes counts, and reduction of two or more Ig classes are observed. Autoimmune manifestations include arthritis, SLE, and ITP [144]. Combined ID with myeloid abnormalities (neutropenia, eosinopenia and reduced myeloid dendritic cells) and increased susceptibility to hematological malignancies, especially B cell and T cell acute lymphoblastic leukemia, are seen [141].

GATA2 is a member of a family of zinc finger transcription factors critical for generation and function of stem cells, progenitor cells and the following cell lineages [145]. **Heterozygous mutations into *GATA2* gene** (deletions, mutations in exons or intronic regulatory regions) associate with a spectrum of human diseases, which follows an autosomal dominant pattern of inheritance, due to haploinsufficiency: hematological abnormalities, recurrent infections, alveolar proteinosis and pulmonary dysfunction, lymphedema, thrombotic events, congenital deafness and hematological malignancies. AI manifests with panniculitis (up to 50% of the patients), erythema nodosum, Sweet syndrome, arthritis and SLE-like, hypothyroidism, autoimmune hepatitis or primary biliary cholangitis [146, 147].

15.8 Autoimmune Lymphoproliferative Syndrome (ALPS)

Peripheral tolerance is crucial for a healthy immune system, because the deletion of every autoreactive T cell is impossible in central lymphoid organs. Peripheral tolerance thus prevents dangerous expansion and activation of autoreactive T cells in secondary lymphoid organs and peripheral tissues. Among the several effector mechanisms responsible for peripheral tolerance, the Fas-Fas ligand (FasL) system plays an important role in eliminating autoreactive lymphocytes. Fas (CD95) is a member of the TNF superfamily expressed in T, B and antigen-presenting cells, including dendritic cells. It is a homotrimer with three extracellular cysteine-rich domains, one transmembrane domain and a functional intracellular domain or Death Domain (DD). Its ligand (Fas-L) equally belongs to the TNF superfamily and is a homotrimer. Fas-DD allows the interaction between Fas and a group of cytoplasmic DD-containing proteins, named FADD (Fas-associated DD). FADD also contains a Death Effector Domain (DED), recruiting other cytoplasmic DED-containing proteins such as procaspase-8 and procaspase-10. This cascade promotes the assembly of the Death Inducing Signalling Complex (DISC). Procaspase-8 and procaspase-10 are recruited to the DISC, converted into their active forms, thus activating other procaspase with their proteolytic action. This process culminates in cellular apoptosis. The DISC is negatively regulated by the enzymatically inactive caspase homolog FLIP (Flice-Inhibitory Protein), which inhibits procaspase activation [148].

At rest, Fas and FasL are not expressed on the cell surface, as appearing within 24 hours after cell activation. Initially, due to a relative abundance of FLIP, T cells are resistant to apoptosis, becoming more susceptible one week after the activation.

Fas-FasL system is crucial for the downregulation of the normal immune response, as preventing persistent immune activation and tissue damage. Fas is also involved in AICD (activation-induced cell death), preventing AI. Dendritic cells continuously patrol peripheral tissues, sampling and presenting antigens to naïve T cells. Repeated TCR stimulation by autoantigens in the absence of costimulatory signals triggers the expression of Fas and FasL in activated T cells with deletion of activated T cells and dendritic cells. In this way autoreactive cells are removed in the peripheral tissues.

Mutations of Fas-FasL system demonstrate the importance of these molecules. **Autoimmune lymphoproliferative syndrome (ALPS)** depends on several different gene mutations of *Fas*, *FasL* and *CASP10*. De novo or inherited *Fas* mutations arise in hematopoietic progenitors. Mutations are mostly autosomal dominant and less frequently recessive and not fully penetrant [149].

Mutated lymphocytes display a survival advantage which explains the clinical hallmark of ALPS, i.e., lymphoproliferation. Lymphocytes infiltrate secondary lymphoid organs (SLO), such as spleen and lymph nodes, explaining the usual clinical presentation of ALPS with splenomegaly and lymphadenopathy. Lymphoproliferation is mediated by an abnormal CD4-CD8-TCRαβ+ subset, namely, double negative cells (DNC). Spleen infiltration is responsible for marginal zone-like B cell deficiency and poor pneumococcal polysaccharide response, a critical risk factor for pneumococcal sepsis. Loss of Fas signaling impairs deletion of autoreactive lymphocytes, resulting in the clinical picture of autoimmune cytopenias which commonly occur in ALPS. AHA, ITP, and autoimmune neutropenia can be severe, and due to autoantibodies. Other autoimmune diseases, such as glomerulonephritis and uveitis, are much rarer and probably coincidental. It is supposed that ALPS accelerates organ-specific AI that would have happened later in the life. The survival advantage of lymphocytes in ALPS is also a risk factor for malignant lymphoproliferation, in particular Hodgkin lymphoma (50-fold) and non-Hodgkin lymphoma (14-fold) [150].

ALPS clinical phenotype is not limited to Fas, FasL or caspase-10 mutations. The same phenotype is shared by other primary IDs, collectively called **ALPS-like syndromes**. Here, many other mechanisms are involved, but all with disturbed apoptosis. Defects in Fas signaling involving FADD and caspase-8 have been found, but the clinical picture is consistent with combined IDs, given the role of these proteins in lymphocyte proliferation [149].

Other IDs share disturbed apoptosis. STAT3 can downregulate the expression of Fas while enhancing many anti-apoptotic proteins of the BCL-2 family. Thus, **STAT3 GOF mutation,** previously discussed in this manuscript, may exhibit an ALPS-like phenotypes [151].

Somatic **mutations of KRAS and NRAS** are responsible for constitutive activation of the RAS pathway followed by BIM expression defect. This is the molecular pathogenesis of a block of activated cell autonomous death, i.e., cell death after growth factor deprivation. Lymphocytes of these patients are resistant to IL-2 starvation in vitro. From a clinical point of view, lymphoproliferation and autoimmune

cytopenias are the hallmarks of this syndrome, often associated with monocytosis without any DNT expansion [148].

Finally, immune checkpoint defects, such as **CTLA-4 haploinsufficiency** and **LRBA deficiency**, can mimic ALPS. The removal of the inhibitory effect of CTLA-4 on lymphocytes is a risk factor for lymphoproliferation and infiltration of SLO and peripheral tissues. Consistently, these patients often display AI manifestations as described above [148].

15.9 Therapeutic Perspectives in IDs with Autoimmune Complications

The growing knowledge of the molecular defects in immune dysregulation syndromes has paved the way to targeted therapy. See Fig. 15.1.

APDS due to GOF mutations in PIK3CD or PIK3R1 genes is characterized by PI3K hyperactivity with many and complex effects on the immune system, including AI and lymphoproliferation. A downstream effector of PI3K is mTOR (mammalian target of rapamycin), a conserved serine/threonine kinase that participates in two complexes, mTORC1 and 2. APDS, where high mTOR activity is present, is indeed included in the so-called immune TOR-opathies [46]. For this reason, the use of **sirolimus**, an antibiotic produced by *Streptomyces hygroscopicus* and inhibiting mTOR, is reasonable. Sirolimus has been used in APDS as effective in reversing hepatosplenomegaly and lymphadenopathy, even if less efficient in cytopenias and gastrointestinal disease [50]. Selective p110δ inhibitors are currently under evaluation as possible more targeted and safe options. The most promising molecule, leniolisib, was nicely able to revert lymphoproliferation (hepatosplenomegaly, lymphadenopathy) and fatigue and normalize immunological parameters (transitional B cells, senescent T cells, naive B cells) [50].

Sirolimus was also clinically efficient in ALPS, as reducing lymphocytes' survival and removing the stop to apoptosis. In some studies, sirolimus also reduced lymphoproliferation and DNC numbers and improved autoimmune cytopenias [152]. It decreases effector T cell proliferation with little impairment of Treg function. IPEX syndrome takes advantage of this activity of sirolimus [152] exhibiting fewer side effects than **calcineurin inhibitors**, usually representing the first-line therapy.

Other IDs might be susceptible of targeted therapy, i.e., CTLA-4 haploinsufficiency and LRBA deficiency. **Abatacept**, a fusion protein joining the extracellular domain of CTLA-4 to the Fc region of IgG1, has been successfully used in RA to dampen joint inflammation, but it is also able to inhibit lymphocyte activation. The absence of the natural CTLA-4 protein in these disorders can be replaced by abatacept. Although used into a small number of patients, a significant improvement in lymphadenopathy, colitis, autoimmune cytopenias and lymphocytic interstitial lung disease was observed [153]. Notably, abatacept seems to restore immunological balance, as increasing naive T cells and partially restoring vaccine response [153] without any long-term complications.

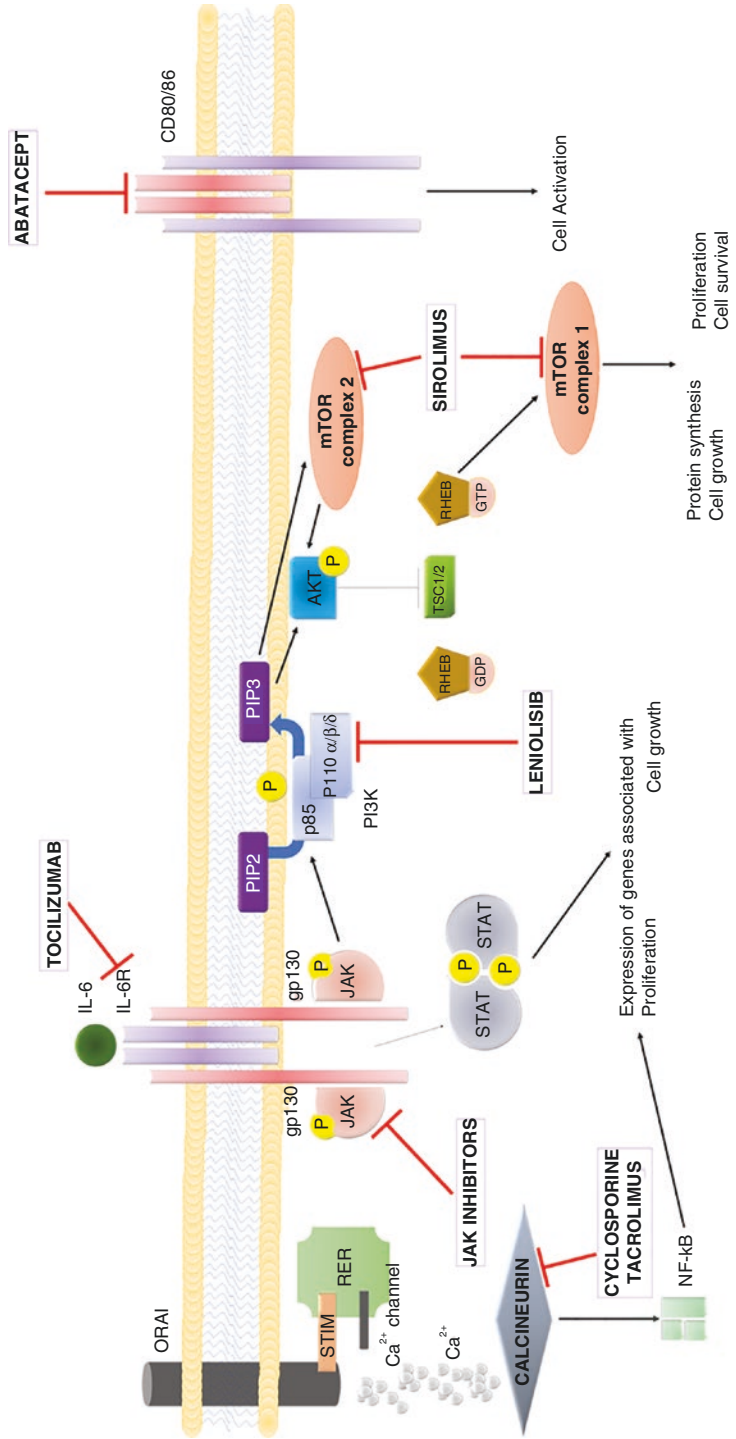

Fig. 15.1 Molecular pathways and possible targeted immunomodulatory drugs for the treatment of autoimmune manifestations associated with monogenic primary immunodeficiencies

GOF STAT1 mutations increase STAT1 expression or phosphorylation or impair STAT1 dephosphorylation. STAT1 is placed downstream several cytokine receptors which transmit their signals by JAK1, JAK2 and JAK3. Therefore, inhibition of JAKs is an appealing approach to reverse immune dysregulation in these patients. Selective inhibitors of JAK (JAKi) have been recently developed to cure RA and psoriatic arthritis. Five **JAK inhibitors** are now on the market: ruxolitinib (JAK1/JAK2 inhibitor), tofacitinib (JAK1/JAK3 inhibitor), baricitinib (JAK1/JAK2 inhibitor), filgotinib (JAK1 selective inhibitor), and decernotinib (JAK3 selective inhibitor). JAKi have been used in limited case series with good clinical efficacy in cytopenias, interstitial lung diseases and enteropathy. JAKi also improved the control of infections and ameliorated immunological parameters such as NK cell cytotoxicity and proportion of T_H1, T_H17, and Tfh cells [133, 153].

STAT3 GOF mutations enhance transcriptional activity of STAT3 or delay STAT3 dephosphorylation. Here, two targeted approaches have been tried. Given the important role of STAT3 in IL-6 signaling, the anti-IL-6 receptor monoclonal antibody **tocilizumab** has been administered to few patients with only a partial effect. JAKi alone or together with anti-IL-6 strategies have been tried and the combination of the two seems to be particularly effective [153].

Besides targeted therapies, **hematopoietic stem cell transplantation (HSCT)** offers the fascinating prospective of a complete cure of IDs, as providing a new immune system. This therapy is now safe, reaching high survival percentage (90%).

When approaching HSCT, the type of ID must be first considered. HSCT is suitable for defects stemming from hematopoietic compartment but not for thymic stromal or other extra-hematopoietic defects. Secondly, timing of HSCT needs to be considered. Best results are obtained in patients undergoing HSCT early, before anatomical injuries or severe infections develop. In young adults, HSCT must be wisely chosen, weighting accurately benefits versus risks. HSCT is lifesaving and urgent when SCID is diagnosed. This therapeutic option is otherwise challenging in immune dysregulation syndromes, although likely to be useful as avoiding long-term disease and treatment-related injuries. However few data have been indeed published. As targeted therapy is available, the opportunity of HSCT should be individualized and HSCT still remains a controversial option in humoral IDs, such as CVID [154].

New strategies have been developed to improve the access to HSCT. **Matched-related donor** (MRD), which is the gold standard for HSCT, may be difficult, because of the high frequency of consanguinity in patients with IDs. **Matched unrelated donor (MUD)** may be the answer, but patients often belong to ethnic minorities, underrepresented in donor registries. **Haplo-identical transplant** with selective ex vivo depletion of αβ T lymphocytes (as responsible for graft-versus-host disease, GVHD) and B lymphocytes (possibly harboring EBV), as well as **HSCT with genetically modified αβ T lymphocytes** with an added caspase suicide gene, allowed remarkable survival in ID cohorts. In the latter, the activator of the caspase suicide gene **rimiducid** is administered if GVHD arises, to selectively remove donor's T lymphocytes [154]. Further, less toxic conditioning regimens and

T lymphocytes specifically directed against viral epitopes from donor banks have been used to control post-HSCT infections with remarkably improved outcomes.

Gene therapy is the last and more promising therapy for IDs as aimed to directly correct the responsible gene defect(s) by modification(s) of host genome. Retroviruses have been extensively used as vectors for DNA modification, as able to integrate into host DNA. Gamma-retroviruses, simple retroviruses without additional regulatory genes, have been used in the past. However, they were not so efficient and raised safety concerns, because of significant rate of insertional mutagenesis with possible development of leukemia. Thus, lentiviruses are now used as devoid of insertional mutagenesis and more efficient in transducing non-dividing cells. Gene therapy has been used in ADA-SCID, X-linked SCID, WAS and CGD, with good results in children. Also, adult patients poorly responding to HSCT seem to benefit, but fewer data are available. Gene therapy for ADA-SCID is now available on the market [154].

Two more advanced approaches to gene therapy are **autologous gene-corrected T cell therapy** and **gene editing**. In the former gene therapy is restricted to T cells only or to a specific T cell subset only. The latter aims to correct the gene defect. Specific endonucleases introduce breaks into the host DNA and exploit the normal DNA repairing systems to restore gene function. These approaches are currently under evaluation on preclinical animal models and clinical studies are eagerly awaited [154].

References

1. Modell V, Orange JS, Quinn J, Modell F (2018) Global report on primary immunodeficiencies: 2018 update from the Jeffrey Modell Centers Network on disease classification, regional trends, treatment modalities, and physician reported outcomes. Immunol Res 66:367–380. https://doi.org/10.1007/s12026-018-8996-5
2. Liblau RS, Bach J-F (1992) Selective IgA deficiency and autoimmunity. Int Arch Allergy Immunol 99:16–27. https://doi.org/10.1159/000236330
3. Rosen FS, Kevy SV, Merler E, Janeway CA, Gitlin D (1961) Dysgammaglobulinæmia and recurrent bacterial infection. Lancet 277:700. https://doi.org/10.1016/s0140-6736(61)91725-1
4. Amaya-Uribe L, Rojas M, Azizi G, Anaya J-M, Gershwin ME (2019) Primary immunodeficiency and autoimmunity: a comprehensive review. J Autoimmun 99:52–72. https://doi.org/10.1016/j.jaut.2019.01.011
5. Walter JE, Ayala IA, Milojevic D (2019) Autoimmunity as a continuum in primary immunodeficiency. Curr Opin Pediatr 31:851–862. https://doi.org/10.1097/mop.0000000000000833
6. Schmidt RE, Grimbacher B, Witte T (2017) Autoimmunity and primary immunodeficiency: two sides of the same coin? Nat Rev Rheumatol 14:7–18. https://doi.org/10.1038/nrrheum.2017.198
7. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K et al (2014) Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 506:376–381. https://doi.org/10.1038/nature12873
8. Shoenfeld Y (1993) The kaleidoscope of autoimmunity. Autoimmunity 15:245–252. https://doi.org/10.3109/08916939309019934
9. Fischer A, Provot J, Jais J-P, Alcais A, Mahlaoui N, Adoue D et al (2017) Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. J Allergy Clin Immunol 140:1388–1393.e8. https://doi.org/10.1016/j.jaci.2016.12.978

10. Singh K, Chang C, Gershwin ME (2014) IgA deficiency and autoimmunity. Autoimmun Rev 13:163–177. https://doi.org/10.1016/j.autrev.2013.10.005
11. Delmonte OM, Schuetz C, Notarangelo LD (2018) RAG deficiency: two genes, many diseases. J Clin Immunol 38:646–655. https://doi.org/10.1007/s10875-018-0537-4
12. Milner JD, Fasth A, Etzioni A (2008) Autoimmunity in Severe Combined Immunodeficiency (SCID): lessons from patients and experimental models. J Clin Immunol 28:29–33. https://doi.org/10.1007/s10875-007-9159-y
13. Lee YN, Frugoni F, Dobbs K, Tirosh I, Du L, Ververs FA et al (2016) Characterization of T and B cell repertoire diversity in patients with RAG deficiency. Sci Immunol 1:eaah6109. https://doi.org/10.1126/sciimmunol.aah6109
14. Daley SR, Koay H-F, Dobbs K, Bosticardo M, Wirasinha RC, Pala F et al (2019) Cysteine and hydrophobic residues in CDR3 serve as distinct T-cell self-reactivity indices. J Allergy Clin Immunol 144:333–336. https://doi.org/10.1016/j.jaci.2019.03.022
15. Stadinski BD, Shekhar K, Gómez-Touriño I, Jung J, Sasaki K, Sewell AK et al (2016) Hydrophobic CDR3 residues promote the development of self-reactive T cells. Nat Immunol 17:946–955. https://doi.org/10.1038/ni.3491
16. Buchbinder D, Hauck F, Albert MH, Rack A, Bakhtiar S, Shcherbina A et al (2019) Rubella virus-associated cutaneous granulomatous disease: a unique complication in immune-deficient patients, not limited to DNA repair disorders. J Clin Immunol 39:81–89. https://doi.org/10.1007/s10875-018-0581-0.
17. Rigoni R, Fontana E, Guglielmetti S, Fosso B, D'Erchia AM, Maina V et al (2016) Intestinal microbiota sustains inflammation and autoimmunity induced by hypomorphic RAG defects. J Exp Med 213:355–375. https://doi.org/10.1084/jem.20151116
18. Rowe JH, Stadinski BD, Henderson LA, Ott de Bruin L, Delmonte O, Lee YN et al (2017) Abnormalities of T-cell receptor repertoire in CD4 + regulatory and conventional T cells in patients with RAG mutations: Implications for autoimmunity. J Allergy Clin Immunol 140:1739–1743.e7. https://doi.org/10.1016/j.jaci.2017.08.001
19. Walter JE, Rosen LB, Csomos K, Rosenberg JM, Mathew D, Keszei M et al (2015) Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency. J Clin Invest 125:4135–4148. https://doi.org/10.1172/JCI80477
20. Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu H-B et al (2004) Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. Immunity 20:441–453. https://doi.org/10.1016/S1074-7613(04)00079-2
21. Walter JE, Rucci F, Patrizi L, Recher M, Regenass S, Paganini T et al (2010) Expansion of immunoglobulin-secreting cells and defects in B cell tolerance in Rag-dependent immunodeficiency. J Exp Med 207:1541–1554. https://doi.org/10.1084/jem.20091927
22. Lima K, Abrahamsen TG, Wolff AB, Husebye E, Alimohammadi M, Kämpe O et al (2011) Hypoparathyroidism and autoimmunity in the 22q11.2 deletion syndrome. Eur J Endocrinol 165:345–352. https://doi.org/10.1530/EJE-10-1206
23. Gennery AR (2002) Antibody deficiency and autoimmunity in 22q11.2 deletion syndrome. Arch Dis Child 86:422–425. https://doi.org/10.1136/adc.86.6.422
24. McLean-Tooke A, Spickett GP, Gennery AR (2007) Immunodeficiency and autoimmunity in 22q11.2 deletion syndrome. Scand J Immunol 66:1–7. https://doi.org/10.1111/j.1365-3083.2007.01949.x
25. Marcovecchio GE, Bortolomai I, Ferrua F, Fontana E, Imberti L, Conforti E et al (2019) Thymic epithelium abnormalities in DiGeorge and down syndrome patients contribute to dysregulation in T cell development. Front Immunol 10. https://doi.org/10.3389/fimmu.2019.00447
26. Montin D, Marolda A, Licciardi F, Robasto F, Di Cesare S, Ricotti E et al (2019) Immunophenotype anomalies predict the development of autoimmune cytopenia in 22q11.2 deletion syndrome. J Allergy Clin Immunol Pract 7:2369–2376. https://doi.org/10.1016/j.jaip.2019.03.014
27. Malphettes M, Gérard L, Carmagnat M, Mouillot G, Vince N, Boutboul D et al (2009) Late-onset combined immune deficiency: a subset of common variable immunodeficiency with

severe T cell defect. Clin Infect Dis Off Publ Infect Dis Soc Am 49:1329–1338. https://doi.org/10.1086/606059
28. Ameratunga R, Lehnert K, Woon S-T, Gillis D, Bryant VL, Slade CA et al (2018) Review: diagnosing common variable immunodeficiency disorder in the era of genome sequencing. Clin Rev Allergy Immunol 54:261–268. https://doi.org/10.1007/s12016-017-8645-0
29. Notarangelo LD (2014) Immunodeficiency and immune dysregulation associated with proximal defects of T cell receptor signaling. Curr Opin Immunol 31:97–101. https://doi.org/10.1016/j.coi.2014.10.003
30. Au-Yeung BB, Shah NH, Shen L, Weiss A (2018) ZAP-70 in signaling, biology, and disease. Annu Rev Immunol 36:127–156. https://doi.org/10.1146/annurev-immunol-042617-053335
31. Chan AY, Punwani D, Kadlecek TA, Cowan MJ, Olson JL, Mathes EF et al (2016) A novel human autoimmune syndrome caused by combined hypomorphic and activating mutations in ZAP-70. J Exp Med 213:155–165. https://doi.org/10.1084/jem.20150888
32. Hauck F, Randriamampita C, Martin E, Gerart S, Lambert N, Lim A et al (2012) Primary T-cell immunodeficiency with immunodysregulation caused by autosomal recessive LCK deficiency. J Allergy Clin Immunol 130:1144–1152.e11. https://doi.org/10.1016/j.jaci.2012.07.029
33. Stepensky P, Weintraub M, Yanir A, Revel-Vilk S, Krux F, Huck K et al (2011) IL-2-inducible T-cell kinase deficiency: clinical presentation and therapeutic approach. Haematologica 96:472–476. https://doi.org/10.3324/haematol.2010.033910
34. Lacruz RS, Feske S (2015) Diseases caused by mutations in ORAI1 and STIM1. Ann N Y Acad Sci 1356:45–79. https://doi.org/10.1111/nyas.12938
35. Lu HY, Biggs CM, Blanchard-Rohner G, Fung S-Y, Sharma M, Turvey SE (2019) Germline CBM-opathies: from immunodeficiency to atopy. J Allergy Clin Immunol 143:1661–1673. https://doi.org/10.1016/j.jaci.2019.03.009
36. Greil J, Rausch T, Giese T, Bandapalli OR, Daniel V, Bekeredjian-Ding I et al (2013) Whole-exome sequencing links caspase recruitment domain 11 (CARD11) inactivation to severe combined immunodeficiency. J Allergy Clin Immunol 131:1376–1383.e3. https://doi.org/10.1016/j.jaci.2013.02.012
37. Brohl AS, Stinson JR, Su HC, Badgett T, Jennings CD, Sukumar G et al (2015) Germline CARD11 mutation in a patient with severe congenital B cell lymphocytosis. J Clin Immunol 35:32–46. https://doi.org/10.1007/s10875-014-0106-4
38. Dorjbal B, Stinson JR, Ma CA, Weinreich MA, Miraghazadeh B, Hartberger JM et al (2019) Hypomorphic caspase activation and recruitment domain 11 (CARD11) mutations associated with diverse immunologic phenotypes with or without atopic disease. J Allergy Clin Immunol 143:1482–1495. https://doi.org/10.1016/j.jaci.2018.08.013
39. Tangye SG, Bucciol G, Casas-Martin J, Pillay B, Ma CS, Moens L et al (2019) Human inborn errors of the actin cytoskeleton affecting immunity: way beyond WAS and WIP. Immunol Cell Biol 97:389–402. https://doi.org/10.1111/imcb.12243
40. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA (1994) A multiinstitutional survey of the Wiskott-Aldrich syndrome. J Pediatr 125:876–885. https://doi.org/10.1016/S0022-3476(05)82002-5
41. Maillard MH, Cotta-de-Almeida V, Takeshima F, Nguyen DD, Michetti P, Nagler C et al (2007) The Wiskott-Aldrich syndrome protein is required for the function of CD4+CD25+Foxp3+ regulatory T cells. J Exp Med 204:381–391. https://doi.org/10.1084/jem.20061338
42. Volpi S, Cicalese MP, Tuijnenburg P, Tool ATJ, Cuadrado E, Abu-Halaweh M et al (2019) A combined immunodeficiency with severe infections, inflammation, and allergy caused by ARPC1B deficiency. J Allergy Clin Immunol 143:2296–2299. https://doi.org/10.1016/j.jaci.2019.02.003
43. Biggs CM, Keles S, Chatila TA (2017) DOCK8 deficiency: insights into pathophysiology, clinical features and management. Clin Immunol 181:75–82. https://doi.org/10.1016/j.clim.2017.06.003
44. Janssen E, Kumari S, Tohme M, Ullas S, Barrera V, Tas JMJ et al (2017) DOCK8 enforces immunological tolerance by promoting IL-2 signaling and immune synapse formation in Tregs. JCI Insight 2:e94298. https://doi.org/10.1172/jci.insight.94298

45. Kuhns DB, Fink DL, Choi U, Sweeney C, Lau K, Priel DL et al (2016) Cytoskeletal abnormalities and neutrophil dysfunction in WDR1 deficiency. Blood 128:2135–2143. https://doi.org/10.1182/blood-2016-03-706028
46. Jung S, Gámez-Díaz L, Proietti M, Grimbacher B (2018) "Immune TOR-opathies," a novel disease entity in clinical immunology. Front Immunol 9:966. https://doi.org/10.3389/fimmu.2018.00966
47. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K (2016) PI3Kδ and primary immunodeficiencies. Nat Rev Immunol 16:702–714. https://doi.org/10.1038/nri.2016.93
48. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N et al (2017) Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. J Allergy Clin Immunol 139:597–606.e4. https://doi.org/10.1016/j.jaci.2016.06.021
49. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L et al (2016) Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: a cohort study. J Allergy Clin Immunol 138:210–218.e9. https://doi.org/10.1016/j.jaci.2016.03.022
50. Coulter TI, Cant AJ (2018) The treatment of activated PI3Kδ syndrome. Front Immunol 9:2043–2043. https://doi.org/10.3389/fimmu.2018.02043
51. Aaltonen J, Björses P, Perheentupa J, Horelli-Kuitunen N, Palotie A, Peltonen L et al (1997) An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet 17:399–403. https://doi.org/10.1038/ng1297-399
52. Hinterberger M, Aichinger M, Prazeres da Costa O, Voehringer D, Hoffmann R, Klein L (2010) Autonomous role of medullary thymic epithelial cells in central CD4+ T cell tolerance. Nat Immunol 11:512–519. https://doi.org/10.1038/ni.1874
53. Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D (2005) The cellular mechanism of aire control of T cell tolerance. Immunity 23:227–239. https://doi.org/10.1016/j.immuni.2005.07.005
54. Stolarski B, Pronicka E, Korniszewski L, Pollak A, Kostrzewa G, Rowińska E et al (2006) Molecular background of polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome in a Polish population: novel AIRE mutations and an estimate of disease prevalence. Clin Genet 70:348–354. https://doi.org/10.1111/j.1399-0004.2006.00690.x
55. Sarkadi AK, Taskó S, Csorba G, Tóth B, Erdős M, Maródi L (2014) Autoantibodies to IL-17A may be correlated with the severity of mucocutaneous candidiasis in APECED patients. J Clin Immunol 34:181–193. https://doi.org/10.1007/s10875-014-9987-5
56. Alimohammadi M, Björklund P, Hallgren Å, Pöntynen N, Szinnai G, Shikama N et al (2008) Autoimmune polyendocrine syndrome Type 1 and NALP5, a parathyroid autoantigen. N Engl J Med 358:1018–1028. https://doi.org/10.1056/nejmoa0706487
57. Ferre EMN, Rose SR, Rosenzweig SD, Burbelo PD, Romito KR, Niemela JE et al (2016) Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. JCI Insight 1. https://doi.org/10.1172/jci.insight.88782
58. Gianani R, Eisenbarth GS (2003) Autoimmunity to gastrointestinal endocrine cells in autoimmune polyendocrine syndrome Type I. J Clin Endocrinol Metab 88:1442–1444. https://doi.org/10.1210/jc.2003-030247
59. Mazza C, Buzi F, Ortolani F, Vitali A, Notarangelo LD, Weber G et al (2011) Clinical heterogeneity and diagnostic delay of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome. Clin Immunol 139:6–11. https://doi.org/10.1016/j.clim.2010.12.021
60. Popler J, Alimohammadi M, Kämpe O, Dalin F, Dishop MK, Barker JM et al (2012) Autoimmune polyendocrine syndrome type 1: utility of KCNRG autoantibodies as a marker of active pulmonary disease and successful treatment with rituximab. Pediatr Pulmonol 47:84–87. https://doi.org/10.1002/ppul.21520
61. Perheentupa J (2006) Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Clin Endocrinol Metab 91:2843–2850. https://doi.org/10.1210/jc.2005-2611
62. Georgiev P, Charbonnier L-M, Chatila TA (2019) Regulatory T cells: the many faces of Foxp3. J Clin Immunol 39:623–640. https://doi.org/10.1007/s10875-019-00684-7

63. Cepika A-M, Sato Y, Liu JM-H, Uyeda MJ, Bacchetta R, Roncarolo MG (2018) Tregopathies: monogenic diseases resulting in regulatory T-cell deficiency. J Allergy Clin Immunol 142:1679–1695. https://doi.org/10.1016/j.jaci.2018.10.026
64. Gambineri E, Ciullini Mannurita S, Hagin D, Vignoli M, Anover-Sombke S, DeBoer S et al (2018) Clinical, immunological, and molecular heterogeneity of 173 patients with the phenotype of immune dysregulation, polyendocrinopathy, enteropathy, X-Linked (IPEX) syndrome. Front Immunol 9:2411. https://doi.org/10.3389/fimmu.2018.02411
65. Santoni de Sio FR, Passerini L, Restelli S, Valente MM, Pramov A, Maccari ME et al (2018) Role of human forkhead box P3 in early thymic maturation and peripheral T-cell homeostasis. J Allergy Clin Immunol 142:1909–1921.e9. https://doi.org/10.1016/j.jaci.2018.03.015
66. Mitra S, Leonard WJ (2018) Biology of IL-2 and its therapeutic modulation: mechanisms and strategies. J Leukoc Biol 103:643–655. https://doi.org/10.1002/JLB.2RI0717-278R
67. Kanai T, Jenks J, Nadeau KC (2012) The STAT5b pathway defect and autoimmunity. Front Immunol 3. https://doi.org/10.3389/fimmu.2012.00234
68. Vignoli M, Ciullini Mannurita S, Fioravanti A, Tumino M, Grassi A, Guariso G et al (2019) CD25 deficiency: a new conformational mutation prevents the receptor expression on cell surface. Clin Immunol 201:15–19. https://doi.org/10.1016/j.clim.2019.02.003
69. Klammt J, Neumann D, Gevers EF, Andrew SF, Schwartz ID, Rockstroh D et al (2018) Dominant-negative STAT5B mutations cause growth hormone insensitivity with short stature and mild immune dysregulation. Nat Commun 9:2105. https://doi.org/10.1038/s41467-018-04521-0
70. Vetrie D (1993) The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. Nature 364:362–362. https://doi.org/10.1038/364362a0
71. Hernandez-Trujillo VP, Scalchunes C, Cunningham-Rundles C, Ochs HD, Bonilla FA, Paris K et al (2014) Autoimmunity and inflammation in X-linked agammaglobulinemia. J Clin Immunol 34:627–632. https://doi.org/10.1007/s10875-014-0056-x
72. Yel L (2010) Selective IgA deficiency. J Clin Immunol 30:10–16. https://doi.org/10.1007/s10875-009-9357-x
73. Yazdani R, Azizi G, Abolhassani H, Aghamohammadi A (2017) Selective IgA deficiency: epidemiology, pathogenesis, clinical phenotype, diagnosis, prognosis and management. Scand J Immunol 85:3–12. https://doi.org/10.1111/sji.12499
74. Abolhassani H, Aghamohammadi A, Hammarström L (2016) Monogenic mutations associated with IgA deficiency. Expert Rev Clin Immunol 12:1321–1335. https://doi.org/10.1080/1744666x.2016.1198696
75. Castigli E, Geha RS (2008) TACI, isotype switching, CVID, and IgAD. Natl Inst Allergy Infect Dis NIH:343–348. https://doi.org/10.1007/978-1-59745-569-5_38
76. Nechvatalova J, Pikulova Z, Stikarovska D, Pesak S, Vlkova M, Litzman J (2012) B-lymphocyte subpopulations in patients with selective IgA deficiency. J Clin Immunol 32:441–448. https://doi.org/10.1007/s10875-012-9655-6
77. Lougaris V, Sorlini A, Monfredini C, Ingrasciotta G, Caravaggio A, Lorenzini T et al (2019) Clinical and laboratory features of 184 Italian pediatric patients affected with selective IgA deficiency (SIgAD): a Longitudinal Single-Center Study. J Clin Immunol 39:470–475. https://doi.org/10.1007/s10875-019-00647-y
78. Davies EG, Thrasher AJ (2010) Update on the hyper immunoglobulin M syndromes. Br J Haematol 149:167–180. https://doi.org/10.1111/j.1365-2141.2010.08077.x
79. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ (2009) Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev 229:152–172. https://doi.org/10.1111/j.1600-065x.2009.00782.x
80. Kishi Y, Aiba Y, Higuchi T, Furukawa K, Tokuhisa T, Takemori T et al (2010) Augmented antibody response with premature germinal center regression in CD40L transgenic mice. J Immunol 185:211–219. https://doi.org/10.4049/jimmunol.0901694
81. Le Coz C, Trofa M, Syrett CM, Martin A, Jyonouchi H, Jyonouchi S et al (2018) CD40LG duplication-associated autoimmune disease is silenced by nonrandom X-chromosome inactivation. J Allergy Clin Immunol 141:2308–2311.e7. https://doi.org/10.1016/j.jaci.2018.02.010

82. Shinkura R, Ito S, Begum NA, Nagaoka H, Muramatsu M, Kinoshita K et al (2004) Separate domains of AID are required for somatic hypermutation and class-switch recombination. Nat Immunol 5:707–712. https://doi.org/10.1038/ni1086
83. Quartier P, Bustamante J, Sanal O, Plebani A, Debré M, Deville A et al (2004) Clinical, immunologic and genetic analysis of 29 patients with autosomal recessive hyper-IgM syndrome due to activation-induced cytidine deaminase deficiency. Clin Immunol 110:22–29. https://doi.org/10.1016/j.clim.2003.10.007
84. Durandy A, Cantaert T, Kracker S, Meffre E (2013) Potential roles of activation-induced cytidine deaminase in promotion or prevention of autoimmunity in humans. Autoimmunity 46:148–156. https://doi.org/10.3109/08916934.2012.750299
85. Imai K, Catalan N, Plebani A, Maródi L, Sanal Ö, Kumaki S et al (2003) Hyper-IgM syndrome type 4 with a B lymphocyte–intrinsic selective deficiency in Ig class-switch recombination. J Clin Invest 112:136–142. https://doi.org/10.1172/jci18161
86. Yousif AS, Stanlie A, Begum NA, Honjo T (2014) Opinion: uracil DNA glycosylase (UNG) plays distinct and non-canonical roles in somatic hypermutation and class switch recombination. Int Immunol 26:575–578. https://doi.org/10.1093/intimm/dxu071
87. Chrzanowska KH, Gregorek H, Dembowska-Bagińska B, Kalina MA, Digweed M (2012) Nijmegen breakage syndrome (NBS). Orphanet J Rare Dis 7:13. https://doi.org/10.1186/1750-1172-7-13
88. Giardino G, Gallo V, Prencipe R, Gaudino G, Romano R, De Cataldis M et al (2016) Unbalanced immune system: immunodeficiencies and autoimmunity. Front Pediatr 4. https://doi.org/10.3389/fped.2016.00107
89. Bogaert DJA, Dullaers M, Lambrecht BN, Vermaelen KY, De Baere E, Haerynck F (2016) Genes associated with common variable immunodeficiency: one diagnosis to rule them all? J Med Genet 53:575–590. https://doi.org/10.1136/jmedgenet-2015-103690
90. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40:24–64. https://doi.org/10.1007/s10875-019-00737-x
91. Zhang Y, Li J, Zhang Y-M, Zhang X-M, Tao J (2015) Effect of TACI signaling on humoral immunity and autoimmune diseases. J Immunol Res 2015:1–12. https://doi.org/10.1155/2015/247426
92. Smulski CR, Kury P, Seidel LM, Staiger HS, Edinger AK, Willen L et al (2017) BAFF- and TACI-Dependent processing of BAFFR by ADAM proteases regulates the survival of B cells. Cell Rep 18:2189–2202. https://doi.org/10.1016/j.celrep.2017.02.005
93. Warnatz K, Salzer U, Rizzi M, Fischer B, Gutenberger S, Bohm J et al (2009) B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. Proc Natl Acad Sci 106:13945–13950. https://doi.org/10.1073/pnas.0903543106
94. Smulski CR, Eibel H (2018) BAFF and BAFF-receptor in B cell selection and survival. Front Immunol 9. https://doi.org/10.3389/fimmu.2018.02285
95. Salzer U, Chapel HM, Webster ADB, Pan-Hammarström Q, Schmitt-Graeff A, Schlesier M et al (2005) Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat Genet 37:820–828. https://doi.org/10.1038/ng1600
96. Castigli E, Wilson SA, Garibyan L, Rachid R, Bonilla F, Schneider L et al (2005) TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat Genet 37:829–834. https://doi.org/10.1038/ng1601
97. Vincent FB, Morand EF, Schneider P, Mackay F (2014) The BAFF/APRIL system in SLE pathogenesis. Nat Rev Rheumatol 10:365–373. https://doi.org/10.1038/nrrheum.2014.33
98. Knight AK, Radigan L, Marron T, Langs A, Zhang L, Cunningham-Rundles C (2007) High serum levels of BAFF, APRIL, and TACI in common variable immunodeficiency. Clin Immunol 124:182–189. https://doi.org/10.1016/j.clim.2007.04.012
99. Sharpe AH, Freeman GJ (2002) The B7–CD28 superfamily. Nat Rev Immunol 2:116–126. https://doi.org/10.1038/nri727

100. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Dräger R et al (2003) Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat Immunol 4:261–268. https://doi.org/10.1038/ni902
101. Abolhassani H, El-Sherbiny YM, Arumugakani G, Carter C, Richards S, Lawless D et al (2019) Expanding clinical phenotype and novel insights into the pathogenesis of ICOS deficiency. J Clin Immunol. https://doi.org/10.1007/s10875-019-00735-z
102. BEINKE S, LEY SC (2004) Functions of NF-κB1 and NF-κB2 in immune cell biology. Biochem J 382:393–409. https://doi.org/10.1042/bj20040544
103. Tuijnenburg P, Lango Allen H, Burns SO, Greene D, Jansen MH, Staples E et al (2018) Loss-of-function nuclear factor κB subunit 1 (NFKB1) variants are the most common monogenic cause of common variable immunodeficiency in Europeans. J Allergy Clin Immunol 142:1285–1296. https://doi.org/10.1016/j.jaci.2018.01.039
104. Schwartz J-CD, Zhang X, Fedorov AA, Nathenson SG, Almo SC (2001) Structural basis for co-stimulation by the human CTLA-4/B7-2 complex. Nature 410:604–608. https://doi.org/10.1038/35069112
105. Ling V, Wu PW, Finnerty HF, Agostino MJ, Graham JR, Chen S et al (2001) Assembly and annotation of human chromosome 2q33 sequence containing the CD28, CTLA4, and ICOS gene cluster: analysis by computational, comparative, and microarray approaches. Genomics 78:155–168. https://doi.org/10.1006/geno.2001.6655
106. Jago CB, Yates J, Câmara NOS, Lechler RI, Lombardi G (2004) Differential expression of CTLA-4 among T cell subsets. Clin Exp Immunol 136:463–471. https://doi.org/10.1111/j.1365-2249.2004.02478.x
107. Hori S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061. https://doi.org/10.1126/science.1079490
108. Walker LSK, Sansom DM (2011) The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. Nat Rev Immunol 11:852–863. https://doi.org/10.1038/nri3108
109. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP et al (1995) Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 270:985–988. https://doi.org/10.1126/science.270.5238.985
110. Heward JM, Allahabadia A, Armitage M, Hattersley A, Dodson PM, Macleod K et al (1999) The development of graves' disease and the CTLA-4 gene on chromosome 2q331. J Clin Endocrinol Metab 84:2398–2401. https://doi.org/10.1210/jcem.84.7.5820
111. Jury EC, Flores-Borja F, Kalsi HS, Lazarus M, Isenberg DA, Mauri C et al (2010) Abnormal CTLA-4 function in T cells from patients with systemic lupus erythematosus. Eur J Immunol 40:569–578. https://doi.org/10.1002/eji.200939781
112. Moreland L, Bate G, Kirkpatrick P (2006) Abatacept. Nat Rev Drug Discov 5:185–186. https://doi.org/10.1038/nrd1989
113. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A et al (2014) Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 20:1410–1416. https://doi.org/10.1038/nm.3746
114. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT et al (2014) Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 345:1623–1627. https://doi.org/10.1126/science.1255904
115. Mitsuiki N, Schwab C, Grimbacher B (2018) What did we learn from CTLA-4 insufficiency on the human immune system? Immunol Rev 287:33–49. https://doi.org/10.1111/imr.12721
116. Schwab C, Gabrysch A, Olbrich P, Patiño V, Warnatz K, Wolff D et al (2018) Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4-insufficient subjects. J Allergy Clin Immunol 142:1932–1946. https://doi.org/10.1016/j.jaci.2018.02.055
117. Alangari A, Alsultan A, Adly N, Massaad MJ, Kiani IS, Aljebreen A et al (2012) LPS-responsive beige-like anchor (LRBA) gene mutation in a family with inflammatory bowel disease and combined immunodeficiency. J Allergy Clin Immunol 130:481–488.e2. https://doi.org/10.1016/j.jaci.2012.05.043

118. Lopez-Herrera G, Tampella G, Pan-Hammarström Q, Herholz P, Trujillo-Vargas CM, Phadwal K et al (2012) Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet 90:986–1001. https://doi.org/10.1016/j.ajhg.2012.04.015
119. Charbonnier L-M, Janssen E, Chou J, Ohsumi TK, Keles S, Hsu JT et al (2015) Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. J Allergy Clin Immunol 135:217–227. https://doi.org/10.1016/j.jaci.2014.10.019
120. Cullinane AR, Schäffer AA, Huizing M (2013) The BEACH is hot: a LYST of emerging roles for BEACH-domain containing proteins in human disease. Traffic Cph Den 14:749–766. https://doi.org/10.1111/tra.12069
121. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C et al (2015) Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science 349:436–440. https://doi.org/10.1126/science.aaa1663
122. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z et al (2015) Spectrum of phenotypes associated with mutations in LRBA. J Clin Immunol 36:33–45. https://doi.org/10.1007/s10875-015-0224-7
123. Gupta S, Lee A, Hu C, Fanzo J, Goldberg I, Cattoretti G et al (2003) Molecular cloning of IBP, a SWAP-70 homologous GEF, which is highly expressed in the immune system. Hum Immunol 64:389–401. https://doi.org/10.1016/s0198-8859(03)00024-7
124. Tanaka Y, Bi K, Kitamura R, Hong S, Altman Y, Matsumoto A et al (2003) SWAP-70-like adapter of T cells, an adapter protein that regulates early TCR-initiated signaling in Th2 lineage cells. Immunity 18:403–414. https://doi.org/10.1016/s1074-7613(03)00054-2
125. Serwas NK, Hoeger B, Ardy RC, Stulz SV, Sui Z, Memaran N et al (2019) Human DEF6 deficiency underlies an immunodeficiency syndrome with systemic autoimmunity and aberrant CTLA-4 homeostasis. Nat Commun 10:3106. https://doi.org/10.1038/s41467-019-10812-x
126. Canonigo-Balancio AJ, Fos C, Prod'homme T, Bécart S, Altman A (2009) SLAT/Def6 plays a critical role in the development of Th17 cell-mediated experimental autoimmune encephalomyelitis. J Immunol Baltim Md 1950 183:7259–7267. https://doi.org/10.4049/jimmunol.0902573
127. Webb GJ, Hirschfield GM, Lane PJL (2015) OX40, OX40L and autoimmunity: a comprehensive review. Clin Rev Allergy Immunol 50:312–332. https://doi.org/10.1007/s12016-015-8498-3
128. Byun M, Ma CS, Akçay A, Pedergnana V, Palendira U, Myoung J et al (2013) Inherited human OX40 deficiency underlying classic Kaposi sarcoma of childhood. J Exp Med 210:1743–1759. https://doi.org/10.1084/jem.20130592
129. Tavernier SJ, Athanasopoulos V, Verloo P, Behrens G, Staal J, Bogaert DJ et al (2019) A human immune dysregulation syndrome characterized by severe hyperinflammation with a homozygous nonsense Roquin-1 mutation. Nat Commun 10:4779–4779. https://doi.org/10.1038/s41467-019-12704-6
130. O'Shea JJ, Plenge R (2012) JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. Immunity 36:542–550. https://doi.org/10.1016/j.immuni.2012.03.014
131. Villarino AV, Kanno Y, O'Shea JJ (2017) Mechanisms and consequences of Jak–STAT signaling in the immune system. Nat Immunol 18:374–384. https://doi.org/10.1038/ni.3691
132. Lorenzini T, Dotta L, Giacomelli M, Vairo D, Badolato R (2016) STAT mutations as program switchers: turning primary immunodeficiencies into autoimmune diseases. J Leukoc Biol 101:29–38. https://doi.org/10.1189/jlb.5ri0516-237rr
133. Notarangelo LD, Fleisher TA (2017) Targeted strategies directed at the molecular defect: toward precision medicine for select primary immunodeficiency disorders. J Allergy Clin Immunol 139:715–723. https://doi.org/10.1016/j.jaci.2017.01.004

134. Maeshima K, Ishii K, Shibata H (2018) An adult fatal case with a STAT1 Gain-of-function mutation associated with multiple autoimmune diseases. J Rheumatol 46:325–327. https://doi.org/10.3899/jrheum.180210
135. Heimall JR, Collins KR, Milner JD, Holland SM, Freeman AF, Siegel R (2010) Autoimmune phenotypes in patients with autosomal dominant hyper-IgE/Job's syndrome. J Allergy Clin Immunol 125:AB9. https://doi.org/10.1016/j.jaci.2009.12.066
136. Hillmer EJ, Zhang H, Li HS, Watowich SS (2016) STAT3 signaling in immunity. Cytokine Growth Factor Rev 31:1–15. https://doi.org/10.1016/j.cytogfr.2016.05.001
137. Vogel TP, Milner JD, Cooper MA (2015) The Ying and Yang of STAT3 in human disease. J Clin Immunol 35:615–623. https://doi.org/10.1007/s10875-015-0187-8
138. Fabre A, Marchal S, Barlogis V, Mari B, Barbry P, Rohrlich P-S et al (2019) Clinical aspects of STAT3 gain-of-function germline mutations: a systematic review. J Allergy Clin Immunol Pract 7:1958–1969.e9. https://doi.org/10.1016/j.jaip.2019.02.018
139. Sahin S, Adrovic A, Kasapcopur O (2020) A monogenic autoinflammatory disease with fatal vasculitis. Curr Opin Rheumatol 32:3–14. https://doi.org/10.1097/bor.0000000000000669
140. Lee PY, Kellner ES, Huang Y, Furutani E, Huang Z, Bainter W et al (2020) Genotype and functional correlates of disease phenotype in deficiency of adenosine deaminase 2 (DADA2). J Allergy Clin Immunol. https://doi.org/10.1016/j.jaci.2019.12.908
141. Boutboul D, Kuehn HS, Van de Wyngaert Z, Niemela JE, Callebaut I, Stoddard J et al (2018) Dominant-negative IKZF1 mutations cause a T, B, and myeloid cell combined immunodeficiency. J Clin Invest 128:3071–3087. https://doi.org/10.1172/JCI98164
142. Eskandarian Z, Fliegauf M, Bulashevska A, Proietti M, Hague R, Smulski CR et al (2019) Assessing the functional relevance of variants in the IKAROS family Zinc Finger Protein 1 (IKZF1) in a Cohort of patients with primary immunodeficiency. Front Immunol 10:568–568. https://doi.org/10.3389/fimmu.2019.00568
143. Bigley V, Cytlak U, Collin M (2019) Human dendritic cell immunodeficiencies. Semin Cell Dev Biol 86:50–61. https://doi.org/10.1016/j.semcdb.2018.02.020
144. Hoshino A, Okada S, Yoshida K, Nishida N, Okuno Y, Ueno H et al (2017) Abnormal hematopoiesis and autoimmunity in human subjects with germline IKZF1 mutations. J Allergy Clin Immunol 140:223–231. https://doi.org/10.1016/j.jaci.2016.09.029
145. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR et al (2014) GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood 123:809–821. https://doi.org/10.1182/blood-2013-07-515528
146. McReynolds LJ, Calvo KR, Holland SM (2018) Germline GATA2 mutation and bone marrow failure. Hematol Oncol Clin North Am 32:713–728. https://doi.org/10.1016/j.hoc.2018.04.004
147. Collin M, Dickinson R, Bigley V (2015) Haematopoietic and immune defects associated with GATA2 mutation. Br J Haematol 169:173–187. https://doi.org/10.1111/bjh.13317
148. Rieux-Laucat F (2017) What's up in the ALPS. Curr Opin Immunol 49:79–86. https://doi.org/10.1016/j.coi.2017.10.001
149. Rieux-Laucat F, Magérus-Chatinet A, Neven B (2018) The autoimmune lymphoproliferative syndrome with defective FAS or FAS-ligand functions. J Clin Immunol 38:558–568. https://doi.org/10.1007/s10875-018-0523-x
150. Straus SE, Jaffe ES, Puck JM et al (2001) The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. Blood 98(1):194–200. Blood 2017;130:232–232. https://doi.org/10.1182/blood-2017-05-787341
151. Nabhani S, Schipp C, Miskin H, Levin C, Postovsky S, Dujovny T et al (2017) STAT3 gain-of-function mutations associated with autoimmune lymphoproliferative syndrome like disease deregulate lymphocyte apoptosis and can be targeted by BH3 mimetic compounds. Clin Immunol 181:32–42. https://doi.org/10.1016/j.clim.2017.05.021

152. Vignesh P, Rawat A, Singh S (2016) An update on the use of immunomodulators in primary immunodeficiencies. Clin Rev Allergy Immunol 52:287–303. https://doi.org/10.1007/s12016-016-8591-2
153. Delmonte OM, Castagnoli R, Calzoni E, Notarangelo LD (2019) Inborn errors of immunity with immune dysregulation: from bench to bedside. Front Pediatr 7:353–353. https://doi.org/10.3389/fped.2019.00353
154. Castagnoli R, Delmonte OM, Calzoni E, Notarangelo LD (2019) Hematopoietic stem cell transplantation in primary immunodeficiency diseases: current status and future perspectives. Front Pediatr 7:295–295. https://doi.org/10.3389/fped.2019.00295

Differential Diagnostic in Cellular Immunodeficiencies

16

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Abstract

More than 400 monogenic Inborn Errors of Immunity (IEI) are known, and the number is increasing rapidly. Their diagnosis might be considered as definitive by the identification of the underlying molecular defect and of a known monogenic pathological variant in a patient with a given clinical and immunological phenotype. Phenotype of IEI patients includes susceptibility to bacterial, fungal, and viral infection diseases and also auto-inflammatory and autoimmune disorders and increased incidence of malignancies. Despite the advances of genetic diagnostic technologies, many patients with clinical and immunological phenotypes compatible with a possible IEI diagnosis still lack a definitive genetic diagnosis or are misdiagnosed. Early diagnosis on a primary immunodeficiency condition might help to prevent long-term organ damages. This can result in diagnostic delay and worsen prognosis. Therefore, a differential clinical, immunological, and genetic diagnostic workup is required for the majority of IEI patients, knowing that phenotype varies also within the same clinical entity and it might change with time.

Keywords

T cell immune defects · B cell immune defects · Innate immunity defects · Secondary hypogammaglobulinemias · Flow cytometry · Inborn errors of immunity

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Our knowledge of the spectrum of primary immune deficiencies, now called Inborn Errors of Immunity (IEI), has expanded over the last decade. Today, more than 400 monogenic IEI are known, and the number is increasing rapidly [1]. The IEI diagnosis might be considered as definitive by the identification of the underlying molecular defect and of a known monogenic pathological variant in a patient with a given clinical and immunological phenotype [2]. IEI conditions are caused by monogenic germline mutations that result in loss of expression, loss of function (LOF; amorphic/hypomorphic), or gain of function (GOF; hypermorphic) of the encoded protein. Heterozygous lesions may underlie autosomal dominant traits by GOF, haploinsufficiency, or negative dominance. Biallelic lesions typically cause autosomal recessive traits by LOF of the encoded protein (rarely GOF), while X-linked recessive traits arise from LOF of genes on the X chromosome, either in the hemizygous state in males or in the homozygous state in females. Rare X-linked dominant traits can also arise from LOF or GOF variants [3].

However, despite the advances of genetic diagnostic technologies, many patients with clinical and immunological phenotypes compatible with a possible IEI diagnosis still lack a definitive genetic diagnosis or are misdiagnosed. Thus, differential diagnostics are required for the majority of IEI. Differential diagnosis might be based on clinical phenotypes, and the International Union of Immunological Societies (IUIS) has recently published a phenotype-driven diagnostic consensus paper to help in the diagnostic process and to reduce the misdiagnosis [4]. Severe, recurrent, or unusual infections are the major clinical features and the main clinical presentation in the majority of patients with IEI. However, IEI predispose to the development of not only infectious diseases but also of inflammatory, autoimmune, allergic, and neoplastic conditions [5, 6]. Moreover, the clinical spectrum varies also within the same clinical entity, and it might change at the time of the clinical onset or during follow-up. Thus, the clinical presentation should be considered in the differential diagnosis but not as the only criterion, because many clinical conditions increase the susceptibility to recurrent infections (Table 16.1). Pathogen identification is helpful to drive the suspicious of a given IEI condition (Table 16.2).

Table 16.1 Main clinical conditions associated with recurrent infections

Anatomical defects
Asplenia
Asthma
a1-anti-trypsin deficiency
Chronic obstructive bronchial pathologies
Ciliary dyskinesia
Cystic fibrosis
Gastroesophageal reflux
Iron deficiency
Malnutrition
Other genetic disorders
Secondary immune deficiencies (SID)

Table 16.2 Main microorganisms responsible for recurrent infection in primary cellular immune deficiencies

Cellular defect	Pathogen
B cell defects	*Haemophilus influenzae*
	Streptococcus pneumoniae
	Moraxella catarrhalis
	Neisseria meningitidis
	Mycoplasma pneumoniae
	Klebsiella
	Ureaplasma urealyticum
	Campylobacter
	Helicobacter pylori
	Giardia lamblia
	Enteroviruses
T cell defects and combined T and B cell defects	Viral infections (CMV, EBV, VZV, HSV, adenovirus, HHV8, HPV, molluscum contagiosum, RSV)
	Fungal infections (*Candida, Aspergillus, Cryptococcus, Histoplasma, Pneumocystis jiroveci/carinii, Microsporidium, Cryptosporidium*)
	Parasitic infections (*Toxoplasma*)
	Bacterial infections (*Mycobacterium* spp., *Salmonella*, *Staphylococcus aureus, E. coli*, *Klebsiella*)
Defects of innate cells	*Pseudomonas*
	Staphylococcus aureus
	Salmonella
	E. coli
	Klebsiella
	Serratia marcescens
	Enterobacteria
	Mycobacteria
	Aspergillus
	Candida
	Nocardia
	Herpes viruses

16.1 Differential Diagnosis of Defects of the Adaptive Cellular Immunity

16.1.1 T Cell Immune Deficiencies

Severe combined immunodeficiency diseases (SCID) are a group of IEI usually associated with severe or life-threatening infections and complete or partial lack of mature T cells in the periphery. They are often difficult to recognize due to the heterogeneity of the genetic basis and to the variability of clinical phenotypes, including infections, immune dysregulation, and lymphoproliferative diseases. Moreover,

Table 16.3 Differential diagnosis of lymphopenias

Inherited causes	Acquired causes
Severe combined immunodeficiency Activated phosphoinositide 3-kinase delta syndrome (APDS) DiGeorge syndrome Wiskott-Aldrich syndrome Ataxia-telangiectasia WHIM syndrome Trisomy 21 CHARGE syndrome RAC2 deficiency Noonan syndrome Nijmegen syndrome	Infectious diseases Sepsis Idiopathic CD4 lymphopenia Autoimmune disorders Lymphoproliferative disorders and aplastic anemia Cancer (advanced or metastatic stage) Radiation Steroid therapy and chemotherapy Protein-losing conditions: – Severe burns – Amyloidosis – Disease – Inflammatory bowel disease – Zinc deficiency

hypomorphic mutations in SCID-causing genes may allow residual development of autologous T cells and reduced T cell function [7].

Most SCID patients exhibit a T cell lymphopenia defined by CD3+ T cells <300/μL that may be accompanied by an absence or decrease in B and natural killer (NK) cells [3]. A profound lymphopenia requires a differential diagnosis with other known causes of lymphopenia (Table 16.3). The term lymphopenia or "lymphocytopenia" refers to a count of less than 1000 absolute lymphocyte counts (ALC) per microliter of blood in adults or less than 3000 lymphocytes per microliter of blood in children.

SCID should be differentiated from many other T cell immunodeficiencies including those presenting with atypical phenotypes and associated with the presence of T cells, called combined immunodeficiencies (CID). CID include a spectrum of disorders severely affecting T cell function and leading to cellular and humoral immune dysfunction. The distinction between CID and SCID might be difficult since CID patients might also have T cell lymphopenia and opportunistic, recurrent viral or bacterial infections. On the other hand, atypical SCID might not have T cell lymphopenia as in late-onset ADA deficiency and in ZAP70 deficiency with reduced CD8 T cell levels but normal CD4 T cell. Some CID patients may have hypergammaglobulinemia, whereas other patients may present with hypogammaglobulinemia and functional antibody deficiency [8, 9].

SCID and CID differential diagnosis might be a difficult process in particular with patients presenting (a) hypomorphic RAG mutations with delayed-onset disease and granulomatous or autoimmune manifestations [10–13], (b) dysgammaglobulinemia with hyper-IgM phenotype [14], and (c) idiopathic CD4 lymphopenia [15]. SCID should be also differentiated from Omenn syndrome characterized by early postnatal onset of erythroderma, diarrhea, life-threatening infections, failure to thrive, low T cell proliferation, low frequency of naïve T cells, increased IgE level or absolute eosinophil count, and peripheral expansion of self-reactive oligoclonal

T cell clonotypes. Eczema is also frequent and may occasionally be misdiagnosed with the Wiskott-Aldrich syndrome (WAS) [8].

A differential diagnosis of SCID and CID should also be taken into consideration in patients with secondary immunodeficiencies such as HIV disease and in patients with other well-defined IEI such as ataxia-telangiectasia, hyper-IgE syndrome, dyskeratosis congenita, and bone marrow failure disorders and/or in patients who do not fulfill diagnostic criteria. Moreover, diagnosis of SCID and CID should be differentiated with conditions characterized by thymic dysfunction, due to abnormalities of the stromal component of the thymus [9]. In particular, few patients with DiGeorge or 22q11.2 Del and 10p deletion syndromes show an immunological phenotype overlapping a T(−) B(+) NK(+) SCID patients. However, features uniquely associated such as facial defects, cardiovascular malformations, and hypocalcemia help to identify this condition, except in pauci-symptomatic patients [9].

Nowadays, the inclusion of SCID and other IEI to newborn screening programs (NBS) is becoming standard in many countries around the world, supporting early diagnosis, improving management, and increasing survival of patients. To date, population-based NBS represents the best strategy for the early identification of affected newborns prior to the onset of infections and other complications. However, if thymic output is not severely affected, NBS may fail to detect some CID, such as ZAP70, ORAI1, or MHC class II deficiencies, and SCID due to hypomorphic mutations [16, 17].

16.1.2 Maternal Engraftment

A frequent finding in SCID is the presence of circulating maternal T cells due to the inability of the child's immune system to reject the maternal cells transfused at the time of delivery that might be found at high frequency (up to 1000/μL) and can persist for months. Cytogenetics, restriction fragment length polymorphism (RFLP) analysis, short tandem repeat analysis (STR), and human leukocyte antigen (HLA) typing are useful in the recognition of maternal T cells [9].

16.1.3 B Cell Immune Deficiencies

Most B cell immune deficiencies present with hypogammaglobulinemia. A condition of hypogammaglobulinemia, defined as an impaired production of antibodies caused by defects in B cell number and maturation, by impaired survival and activation of B cells, and by defects in the interaction of B cells with T cells, is an immunological feature common to many primary immunodeficiencies. Hypogammaglobulinemias are characterized by low levels of any isotype (IgG, IgA, IgM, IgE, and IgG subclasses). When serum hypogammaglobulinemia is evident, clinicians must consider a diagnosis of a primary antibody defect in patients of all ages. Besides a quantitative assessment, a functional defect of B cells should be also demonstrated by the failure to mount an antibody response after infections

Table 16.4 List of differential diagnosis of B cell defects

Primary immunodeficiencies, including CID
Chromosomal abnormalities
18q syndrome
Trisomy 8
Trisomy 21
Monosomy 22
Secondary hypogammaglobulinemias including:
Protein-losing syndromes (enteropathy, lymphangiectasia, nephropathy, severe burns)
Chronic lymphocytic leukemia
Multiple myeloma
B cell malignancies
Bone marrow transplantation HSCT
Congenital AIDS and other viral infections (EBV, CMV)
Drugs

or immunization [18–20]. When B cells are present in a symptomatic hypogammaglobulinemic patients, exclusion of other known causes of B cell defects should be addressed (Table 16.4).

Some patients with a low IgG level and impaired vaccine responses may not fulfill criteria for CVID because IgA or IgM level is not low. This is a form of hypogammaglobulinemia with antibody deficiency that should not be called CVID (it may be called "unspecified IgG deficiency" or "unspecified hypogammaglobulinemia"). Alternatively, IgG and IgA levels may be low, but vaccine responses may appear normal by standard criteria. In all these cases, patients should be assessed repeatedly over time because immunoglobulin levels and antibody function may wane to the point that the above criteria are met and a diagnosis of CVID may be conferred [19]. The age of a patient with a serum hypogammaglobulinemia should be taken into account since a physiological condition of B cell defect is observed in infants below 4 years of age. Moreover, serum immunoglobulin levels vary with age, so age-specific cutoffs should be used when performing laboratory testing. In addition, it should be considered that most of the patients with transient hypogammaglobulinemia of infancy will normalize IgG values, mostly within 2 to 4 years of age [18]. A laboratory workup including complete blood count with differentials, immune cell phenotype assessment by flow cytometry, measurement of serum immunoglobulins, and human immunodeficiency virus (HIV) antibody test can identify children who need further testing and referral to a subspecialist for a suspected primary cellular immunodeficiency [21].

Primary immunodeficiencies with autosomal recessive inheritance should also be considered in the differential diagnosis of patients with early-onset hypogammaglobulinemia particularly in consanguineous families [19].

The differential diagnosis for B cell defects can vary depending on age of onset. It includes XLA, transient hypogammaglobulinemia of infancy, CVID, autosomal recessive agammaglobulinemias, or defects in related genes in the BCR signaling pathway, monogenic B cell defects, X-linked lymphoproliferative syndrome, and combined T and B cell immunodeficiency with hypogammaglobulinemia [22].

As a general rule, lack of B lymphocytes excludes transient hypogammaglobulinemia of infancy, CVIDs, class-switch recombination defects, X-linked lymphoproliferative disease, TACI deficiency, LRBA deficiency, BAFF receptor deficiency, TWEAK defects, NFKB2 deficiency, WHIM syndrome, ICOS deficiency, selective IgA deficiency, and isolated IgG subclass deficiency. The lack of B cells supports the diagnosis of XLA and of other forms of agammaglobulinemia caused by rare mutations in genes encoding the μ heavy chain, Igα, Igβ, λ5, B cell linker (BLNK), leucine-rich repeat-containing 8 (LRRC8), the p85α subunit of phosphoinositide 3-kinase defects (PI3K), the CD19 deficiency, the CD20 deficiency, and the CD21 deficiency [23].

Absence of T cells directs the workup toward defects associated with severe combined immunodeficiency [18].

A comprehensive search for mutations in known primary immunodeficiency genes may be warranted in patients with B cell defects [20].

16.1.3.1 CVID and CID

A highly heterogeneous pattern of T cell abnormalities has been observed in CVID patients. In fact, patients with B cell defects might be misdiagnosed as CVIDs and might better receive a diagnosis of CID or unclassified antibody deficiencies. ESID registry data show that a diagnosis of CVID is still being used too often in children and that some CVID patients were later classified as combined immunodeficiency or as agammaglobulinemia instead of CVID based on the identification of a genetic cause [20]. This has important consequences because the identification of other diagnoses such as CID might imply a completely different therapeutic approach, including stem cell transplantation or targeted treatment. This difficulty in diagnostic differential will disappear with increased application of genetic tests including the use of NGS panels, exome, or genome diagnostics. It is important to recognize that in some patients where the genetic test identifies the correct IEI, the phenotype might differ from the expected genotype-associated, clinical picture [20].

16.1.3.2 Chromosomal Abnormalities

Several chromosomal abnormalities (chromosome 18q-syndrome, monosomy 22, trisomy 8, and trisomy 21) are currently identified as causes of B cell defects, could manifest with recurrent infections, and could mimic CVID. The incidence of 18q deletion syndrome is reported as 1:10,000 live births. The characteristic features of the syndrome are short stature, hearing loss, hypotonia, mental retardation, and endocrine disorders, accompanied by autoimmunity. Moreover, also IgA deficiency has been reported in 18th chromosomal abnormalities. The immunological defect was limited to an inability to synthesize IgA molecule chains in a normal amounts [24]. Trisomy 8, together with other chromosomal abnormalities, is currently identified as causes of B cell defect; could manifest with recurrent infections, diarrhea, malnutrition, and pernicious anemia; and mimic CVID. Trisomy 21 is associated with changes characteristic of primary immunodeficiencies such as susceptibility to infection, autoimmunity, and a high malignancy risk. The abnormalities of the immune system associated with Down syndrome include mild to moderate T and B

cell lymphopenia, with marked decrease of naive lymphocytes, impaired mitogen-induced T cell proliferation, reduced specific antibody responses to immunizations, and defects of neutrophil chemotaxis associated with infections predominantly of the respiratory tract [18].

16.1.3.3 Secondary Cellular Immune Defects

When patients with defects of cellular immunity are encountered, a vigorous search should be undertaken for secondary treatable causes. Secondary immune deficiencies (SID) might develop in the course of different infectious diseases and/or in consequence of immune suppressive or immune modulatory treatment. Deficiencies may involve innate immunity and adaptive T and B cell immunity. SID patients are thus a heterogeneous population which include patients with HIV and CMV infection, hematological malignancies, and malignant solid tumors, hematopoietic stem cell transplant (HSCT) recipients, solid organ transplant (SOT) recipients, patients on immune suppressive or immune modulatory treatment for systemic autoimmune and inflammatory diseases, and other minor conditions [25].

Immunocompetent patients with cancer can develop immune suppression secondary to the cancer, from chemotherapy or from post-transplant immunosuppressive therapy. This is particularly evident for B cell chronic lymphocytic leukemia and for lymphoproliferative disorders or plasma cell dyscrasias, all conditions that have to be carefully ruled out at initial evaluation of a patient with a suspicious cellular immune deficiency [26]. Patients suffering from B cell malignancies are prone to acquired B cell defects with specific antibody failure and suffer from severe, recurrent, or opportunistic infections, all hallmarks of antibody failure [27].

Classical conditions of SID due to malignant B cell lymphoproliferation or conditioning regimes with alkylating agents in transplant recipients are being outnumbered by SID conditions caused by an ever-increasing number of therapeutic monoclonal antibodies (MAbs), fusion proteins, and engineered T cells with potential to directly or indirectly target the B cell lineage and plasma cells [28]. With the advent of many new potent therapeutic options and their combination with classical drugs, the spectrum of cellular immune deficiency states caused by innovative treatment strategies has greatly broadened [29] (Table 16.5).

Table 16.5 Drug-induced immune cellular defects

Classical drugs	New drugs
Glucocorticoids	Anti-CD20 therapy (rituximab, ocrelizumab, ibritumomab tiuxetan, obinutuzumab, ofatumumab)
Alkylating agents	Anti-TACI (atacicept)
Mycophenolate	Anti-BAFF therapy (belimumab, tabalumab, blisibimod)
Cyclophosphamide	Anti-CD25 (daclizumab and basiliximab)
Carbamazepine	Anti-CD52 (alemtuzumab)
Phenytoin	Anti-IL6/IL6R (tocilizumab)
D-Penicillamine	Anti-CD3/CD19 (blinatumomab)
	Anti-CD22 (epratuzumab, inotuzumab, moxetumomab pasudotox)

16.2 Differential Diagnosis in Primary Cellular Defects of the Innate Immunity

IEI of innate immunity typically underlie increased susceptibility to infectious diseases caused by narrow range of microbes (Table 16.1), but might also give rise to auto-inflammation. The genetic theory of infectious diseases, classically focused on deciphering rare variants underlying sporadic cases of severe infection, has culminated in unraveling the Mendelian predisposition of TB cases. It should be note here that also impaired adaptive cellular immunity causes defective innate immune responses as shown by the role of NF-kB pathway in the innate defense to *S. pneumoniae, S. aureus and, Candida albicans*. Patients with defects in genes coding for interleukin-1 receptor-associated kinase (IRAK)-4 and for myeloid differentiation primary receptor gene 88 (MyD88) suffer from infections presenting with weak and delayed fever and increase of inflammatory markers caused by a limited number of pathogens due to their impaired recognition of Toll-like receptors (TLR), but have normal T cell and B cell responses. Mutations within innate-immune signaling pathways including IRAK4 and MyD88 have been described in association with a hyper-IgE-like syndrome. Misdiagnosis of IEI as allergy is well described and may be complicated by co-occurrence of allergy as a manifestation. Comparison of sensitization patterns shows marked differences between patients with DOCK8, STAT3, or atopic dermatitis (AD). Allergic symptoms and skin prick test results correlate well with specific IgE results in DOCK8 and AD patients and can be useful in directing dietary exclusion [30].

In contrast, STAT3-HIES patients show remarkably similar specific IgE values and skin prick testing to healthy individuals despite extremely high total IgE levels. The lifetime frequency and severity of food allergies for STAT3-HIES remain higher than reported by healthy controls but is greatly diminished relative to atopic controls with similar IgE levels. One explanation for this apparent paradox is the profound impairment of mast cell degranulation associated with STAT3 loss of function. Physiochemical analysis of the skin barrier in STAT3-HIES and AD individuals suggests an additional explanation: dermal integrity remains remarkably preserved in STAT3-HIES in contrast to atopic dermatitis patients. Recently reported novel gene defects in patients with a phenotype of staphylococcal infections, allergy, and elevated IgE have shed light on the clinical expression of impaired IL-6 signaling. Indeed, the consequences of excessive IL-6 signaling in humans have been well recognized in patients suffering from various inflammatory conditions. Patients with defects in gene coding for IL-6R show atopic dermatitis, elevated IgE and recurrent cold *S. aureus* skin abscesses as well as lung infections, mild hypogammaglobulinemia, and reduced inflammatory responses (serum CRP and fever) despite systemic infection [31].

Immune dysregulation is a feature of many IEI, including those affecting neutrophil function. Phagocytes play a crucial role in bacterial and fungal killing via phagocytosis and generation of reactive oxygen species. Both quantitative and functional defects have been described. Neutrophils and other phagocytes are essential effectors of innate immunity and rapidly respond to the presence of invading

bacteria, fungi, and parasites. Inflammatory signals activate adhesion, chemotaxis, phagocytosis, and release of oxidants, proteases, and other molecules aimed at microbial killing. These same processes are important for appropriate responses to wounds or sterile inflammation. Hence, IEI with functional phagocyte defects can display recurrent severe bacterial and fungal infections and aberrant inflammation that is not always related to infection [32].

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome associated with autosomal recessive mutations in genes encoding perforin and a group of proteins required for secretion of perforin-containing cytotoxic granules [33]. However, HLH is also a frequent manifestation of other genetic disorders such as X-linked lymphoproliferative syndromes and can manifest in the context of severe infections including viral infections or sepsis/systemic inflammatory response syndrome, autoimmune and autoinflammatory diseases, or malignancies such as lymphomas, all considered as "secondary HLH," "acquired HLH," or "macrophage activation syndrome." Affected patients usually present with clinical and laboratory manifestations that cannot be readily distinguished from those observed in patients with defects in cytotoxicity. In addition, HLH syndrome has been described in patients with SCID, CID, and CGD. All patients have high levels of sCD25 and impaired NK cell cytotoxicity. A differential diagnosis between primary HLH and secondary HLH due to primary T cellular immune deficiencies is the relatively low sCD25 levels and high ferritin levels in most patients with T cell deficiencies, reflecting the lack of T cells in SCID and the low T cell counts and/or impaired T cell function in CID patients. Some data suggest that a ferritin:sCD25 ratio of ≥ 3 should be viewed as suggestive of SCID/CID in an infant with the HLH syndrome.

GATA2 deficiency shows a clinical and immunological heterogeneity found even between individuals with disease, even among those having the same genetic lesion. The majority have infections, mainly herpes viral infections. Immunological defects are isolated NK defects, isolated monocytopenia, B cell deficiency, and dendritic cell deficiency. Decreased NK cell numbers in the periphery correlate to an increase number of clinical complications, while asymptomatic carriers of GATA2 mutations are often CMV seronegative and have higher frequencies of NK cells in peripheral blood.

16.3 The Role of Flow Cytometry in the Differential Diagnosis of Cellular Immune Deficiencies

The application of flow cytometry is an essential analysis to the diagnosis of cellular defects in IEI patients. Multiparametric flow cytometry (MFC) represents a rapid, highly reproducible, and sensitive diagnostic technology for IEI which is characterized by a wide range of T cell perturbations and a broad clinical and genetic heterogeneity [21]. T cell development, T and B cell subsets, cytokine signaling, and intracellular signaling pathways can be phenotypically assessed by the combined use of antibodies to cell surface marker expression. It is widely known that by flow

cytometry it became possible to define the stages and phenotypes of T and B cell development in human bone marrow and how mutations affect this process. Flow cytometry has been used to identify other populations such as monocytes, dendritic cell subsets, innate lymphoid cells, and ILC precursors [21]. As an example, the diagnosis of HLA class II deficiency relies on the lack of detection of HLA-DR, e.g., -DQ, and -DP molecules at the surface of all cells that normally express HLA class II molecules (B cells, monocytes, dendritic cells) and on the surface of γ-interferon (IFN- γ)-activated cells (T cells, fibroblasts, etc.). The vast majority of causative IEI gene mutations abolishs protein expression and flow cytometry identifies the absence of the encoded protein. However, in other IEI variants the protein expression is not affected. The development of intracellular staining protocols to detect expression of SAP, XIAP, or DOCK8 expedites the rapid diagnosis of the X-linked lymphoproliferative diseases or an autosomal recessive form of hyper-IgE syndrome (HIES) [21].

T cell subset frequencies might be used to investigate to estimate the relative disease severity and could possibly support the differential diagnosis [34]. The immunological heterogeneity of IEI makes the differential diagnosis more difficult, and patients might not have flow cytometry parameters different from healthy donors. Flow cytometry in CVID might help to differentiate from CID due to the presence of lymphopenia with reduced CD4+ with a trend to an accelerated T cell exhaustion leading to an inefficient immune response and the risk to develop immune-dysregulation phenomena. For patients with thymic defects, flow cytometry is extremely heterogeneous with patients presenting a more severe clinical phenotype similar to those found in CID. Flow cytometry is useful also to identify lymphoid infiltration of various organs, most predominantly in the lungs, liver, gut, and bone marrow [21].

16.4 The Role of Quantification of T Cell Receptor Excision Circles and Ig Kappa Deleting Recombination Excision Circles in the Differential Diagnosis of Cellular Immune Deficiencies

The association of flow cytometry analysis with the quantification of T cell receptor excision circles (TRECs) and with multiplex TRG rearrangement quantification helps in the differential diagnosis of IEI. Newly formed T cells carry T cell receptor excision circles (TRECs), whereas in memory T cell populations, these are extremely diluted. The use of Ig kappa deleting recombination excision circles (KRECs) of intron RSS-Kde rearrangements to examine B cell replication has been also validated. It has to be underlined that although newborn screening for T cell and B cell lymphopenia is highly effective, at least 80% of newborns with a positive test (reduced TRECs and/or KRECs) turn out to be “false positives” and do not have SCID or XLA. Thus, for the definitive diagnosis and for the differential diagnosis, all abnormal results require drawing of a second blood sample for flow cytometry analysis [35].

References

1. Bousfiha A, Jeddane L, Picard C, Ailal F, Gaspar B, Al-Herz W et al (2018) The 2017 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol 38:129–114. https://doi.org/10.1007/s10875-017-0465-8
2. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T et al (2018) International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol 38:96–128. https://doi.org/10.1007/s10875-017-0464-9
3. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40(1):24–64
4. Notarangelo LD, Fischer A, Geha RS, Casanova J-L, Chapel H, Conley ME et al (2009) Primary immunodeficiencies: 2009 update. International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies. J Allergy Clin Immunol 124:1161–1178. https://doi.org/10.1016/j.jaci.2009.10.013
5. Quinti I, Agostini C, Tabolli S, Brunetti G, Cinetto F, Pecoraro A et al (2012) Malignancies are the major cause of death in patients with adult onset common variable immunodeficiency. Blood 120:1953–1954. https://doi.org/10.1182/blood-2012-05-431064
6. Marrella V, Poliani PL, Notarangelo DL, Grassi F, Rag Defects VA (2014) Thymic stroma: lessons from animal models. Front Immunol 5:259. https://doi.org/10.3389/fimmu.2014.00259
7. Notarangelo LD (2013) Partial defects of T cell development associated with poor T cell function. J Allergy Clin Immunol 131:1297–1305. https://doi.org/10.1016/j.jaci.2013.01.020
8. Fischer A (2001) Primary T-lymphocyte immunodeficiencies. Clin Rev Allergy Immunol 20(1):3–26
9. Cirillo E, Cancrini C, Azzari C, Martino S, Martire B, Pession A et al (2019) Clinical, immunological, and molecular features of typical and atypical severe combined immunodeficiency: report of the Italian Primary Immunodeficiency Network. Front Immunol 10:1908. Published online 2019 Aug 13. https://doi.org/10.3389/fimmu.2019.01908
10. Cassani B, Poliani PL, Moratto D, Sobacchi C, Marrella V, Imperatori L et al (2010) Defect of regulatory T cells in patients with Omenn syndrome. J Allergy Clin Immunol 125:209–216. https://doi.org/10.1016/j.jaci.2009.10.023
11. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B et al (2008) An immunodeficiency disease with RAG mutations and granulomas. N Engl J Med 358:2030–2038. https://doi.org/10.1056/NEJMoa073966
12. De Ravin SS, Cowen EW, Zarember KA, Whiting-Theobald NL, Kuhns DB, Sandler NG et al (2010) Hypomorphic Rag mutations can cause destructive midline granulomatous disease. Blood 116:1263–1271. https://doi.org/10.1182/blood-2010-02-267583
13. Avila EM, Uzel G, Hsu A, Milner JD, Turner ML, Pittaluga S et al (2010) Highly variable clinical phenotypes of hypomorphic RAG1 mutations. Pediatrics 126:e1248–e1252. https://doi.org/10.1542/peds.2009-3171
14. Chou J, Hanna-Wakim R, Tirosh I, Kane J, Fraulino D, Lee YN et al (2012) A novel homozygous mutation in recombination activating gene 2 in 2 relatives with different clinical phenotypes: Omenn syndrome and hyper-IgM syndrome. J Allergy Clin Immunol 130:1414–1416. https://doi.org/10.1016/j.jaci.2012.06.012
15. Kuijpers TW, Ijspeert H, van Leeuwen EM, Jansen MH, Hazenberg MD, Weijer KC et al (2011) Idiopathic CD4+ T lymphopenia without autoimmunity or granulomatous disease in the slipstream of RAG mutations. Blood 117:5892–5896. https://doi.org/10.1182/blood-2011-01-329052
16. Cifaldi C, Brigida I, Barzaghi F, Zoccolillo M, Ferradini V, Petricone D et al (2019) Targeted NGS platforms for genetic screening and gene discovery in primary immunodeficiencies. Front Immunol 10:316. Published online 2019 Apr 11. https://doi.org/10.3389/fimmu.2019.00316

17. Borte S, von Döbeln U, Hammarström L (2013) Guidelines for newborn screening of primary immunodeficiency diseases. Curr Opin Hematol 20(1):48–54. https://doi.org/10.1097/MOH.0b013e32835a9130
18. Quinti I, Milito C, Carello R, Pulvirenti F (2019) Differential diagnosis in hypogammaglobulinemia. In: D'Elios M, Rizzi M (eds) Humoral Primary Immunodeficiencies. Rare Diseases of the Immune System. Springer, Cham. https://doi.org/10.1007/978-3-319-91785-6_19
19. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT et al (2016) International Consensus Document (ICON): common variable immunodeficiency disorders. J Allergy Clin Immunol Pract 4:38–59. https://doi.org/10.1016/j.jaip.2015.07.025
20. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, on behalf of the ESID Registry Working Party et al (2019) The European Society for Immunodeficiencies (ESID) registry working definitions for the clinical diagnosis of inborn errors of immunity. J Allergy Clin Immunol Pract 7(6):1763–1770. https://doi.org/10.1016/j.jaip.2019.02.004
21. Ma CS, Tangye SG (2019) Flow cytometric-based analysis of defects in lymphocyte differentiation and function due to inborn errors of immunity. Front Immunol 10:2108. https://doi.org/10.3389/fimmu.2019.02108
22. Fried AJ, Francisco A (2009) Bonilla pathogenesis, diagnosis, and management of primary antibody deficiencies and infections. Clin Microbiol Rev 22(3):396–414. https://doi.org/10.1128/CMR.00001-09
23. Al-Herz W, Bousfiha A, Casanova JL, Chapel H, Conley M, Cunningham-Rundles C et al (2011) Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. Front Immunol 2:54. https://doi.org/10.3389/fimmu.2011.00054
24. Finley SC, Cooper MD, Finley WH, Uchida IA, Noto TA, Roddam RF (1969) Immunological profile in a chromosome 18 deletion syndrome with IgA deficiency. J Med Genet 6:388–393. https://doi.org/10.1136/jmg.6.4.388
25. Mouthon L, Fermand JP, Gottenberg JE (2013) Management of secondary immune deficiencies: what is the role of immunoglobulins? Curr Opin Allergy Clin Immunol 13(Suppl. 2):S56–S67. https://doi.org/10.1097/01.all.0000433132.16436.b5
26. Rossignol J, Terriou L, Robu D, Willekens C, Hivert B, Pascal L et al (2015) Radioimmunotherapy ((90) Y-Ibritumomab Tiuxetan) for posttransplant lymphoproliferative disorders after prior exposure to rituximab. Am J Transplant 15(7):1976–1981. https://doi.org/10.1111/ajt.13244
27. Morrison VA (2010) Infectious complications of chronic lymphocytic leukaemia: pathogenesis, spectrum of infection, preventive approaches. Best Pract Res Clin Haematol 23(1):145–153. https://doi.org/10.1016/j.beha.2009.12.004
28. Visentin A, Compagno N, Cinetto F, Imbergamo S, Zambello R, Piazza F et al (2015) Clinical profile associated with infections in patients with chronic lymphocytic leukemia. Protective role of immunoglobulin replacement therapy. Haematologica. https://doi.org/10.3324/haematol.2015.126763
29. Dhalla F, Lucas MM, Schuh AA, Bhole MM, Jain RR, Patel SY et al (2014) Antibody deficiency secondary to chronic lymphocytic leukemia: Should patients be treated with prophylactic replacement immunoglobulin? J Clin Immunol 34(3):277–282. https://doi.org/10.1007/s10875-014-9995-5
30. Renner ED, Hartl D, Rylaarsdam S et al (2009) Comèl-Netherton syndrome—defined as primary immunodeficiency. J Allergy Clin Immunol 124:536–543
31. Moens L, Meyts I (2020) Inborn errors of innate immunity. Curr Opin Immunol 62:79–90
32. Mace EM, Orange JS (2019) Emerging insights into human health and NK cell biology from the study of NK cell deficiencies. Immunol Rev 287:202–225. https://doi.org/10.1111/imr.12725
33. Bode SF, Ammann S, Al-Herz W, Bataneant M, Dvorak CC, Gehring S, Inborn Errors Working Party of the EBMT et al (2015) The syndrome of hemophagocytic lymphohistiocytosis in primary immunodeficiencies: implications for differential diagnosis and pathogenesis. Haematologica 100:978–988. https://doi.org/10.3324/haematol.2014.121608

34. Attardi E, Di Cesare S, Amodio D, Giancotta C, Cotugno N, Cifaldi C et al (2019) Phenotypical T cell differentiation analysis: a diagnostic and predictive tool in the study of primary immunodeficiencies. Front Immunol 10:2735. https://doi.org/10.3389/fimmu.2019.02735
35. Korsunskiy I, Blyuss O, Gordukova M, Davydova N, Zaikin A, Zinovieva N et al (2020) Expanding TREC and KREC Utility in primary immunodeficiency diseases diagnosis. Front Immunol 11:320. https://doi.org/10.3389/fimmu.2020.00320

Management of Cellular Immunodeficiencies

17

Carlo Agostini and Alessandro Dell'Edera

Abstract

In this chapter, we will highlight the general strategies and tools in the clinical management of primary cellular immunodeficiency with predominant T cell and/or B cell dysfunction. Hematopoietic stem cell transplantation (HSCT) represents a curative treatment in certain patients with cellular immunodeficiency; enzyme replacement therapy and gene therapy may be further options in very specific settings. Apart from disease-specific treatments, antibiotic therapy and prophylaxis and replacement of immunoglobulin G are therefore the mainstay of treatment, also after HSCT. The monitoring of long-term consequences of infections on airways architecture and function is pivotal in cellular immunodeficiency. Moreover, the management of immune-mediated complications, encompassing a range of clinical issues as interstitial lung diseases, systemic granulomatosis, immune dysregulation, autoimmune cytopenia, and enteropathy, requires a multidisciplinary approach. Finally, a proper management of T and B cellular immune deficiencies allows an early detection of lymphoproliferative complications and cancer.

Keywords

Management · Cellular immunodeficiencies · Immunoglobulin replacement therapy · Antibiotic prophylaxis · Noninfectious complications

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17.1 Introduction

Primary cellular immunodeficiencies are a group of rare genetic disorders characterized mainly by deficiencies of T lymphocyte counts and/or function and/or B lymphocyte defect. The clinical spectrum of primary immunodeficiencies (PID) is extremely broad, and the management mostly depends on the underlying immunological defect and its functional consequences that may have infectious and noninfectious implications. Together with infections, T and B cell immunodeficiencies, indeed, predispose to the development of autoimmunity, allergy, chronic lung disease (including bronchiectasis, asthma, COPD, interstitial lung diseases), gastrointestinal disease with or without malabsorption, systemic or localized granulomatous disease, liver disease, splenomegaly, lymphadenopathy, and neoplastic conditions [1] (see Fig. 17.1). The management of these noninfectious conditions needs to be integrated with the prophylaxis and management of infections, requiring a multidisciplinary approach; pediatric and adult immunologists should thus favor the interplay between different healthcare professionals as pulmonologists, cardiologists, geneticists, gastroenterologists, and neurologists. Finally, primary immunodeficiencies are lifelong conditions: a psychological support could also be necessary, especially for younger patients. This chapter will recapitulate the milestones of the clinical management of primary cellular immunodeficiency associated with predominantly T cell and/or B cell dysfunction, which have been partly discussed in previous chapters.

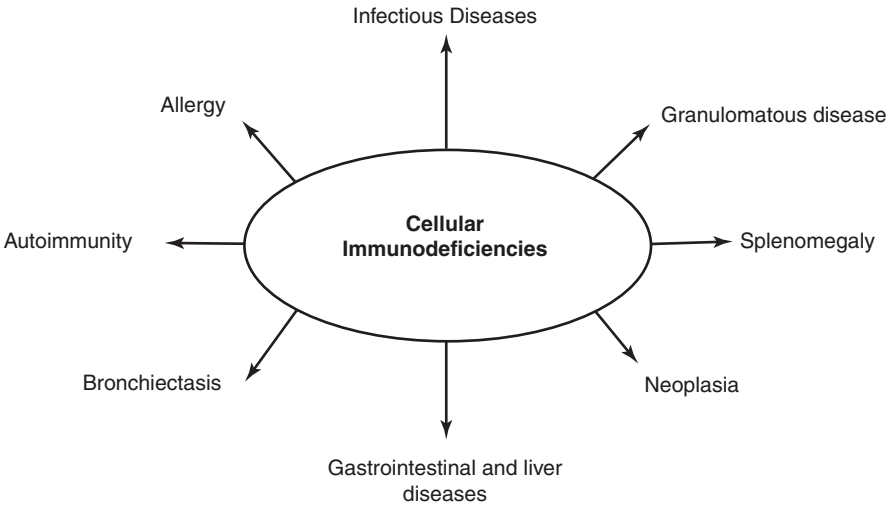

Fig. 17.1 Complications of cellular immunodeficiencies

17.2 Management of T Cell Immune Deficiencies

Patients are classified as severe combined immune deficiency (SCID) if their mature peripheric T lymphocytes are absent or extremely low (CD3+ T cells <500 cell/mL) [2]. Patients affected by SCID usually present during the first year of life recurrent invasive bacterial, viral (particularly cytomegalovirus, parainfluenza, and rotavirus), and opportunistic infections such as *Pneumocystis jiroveci* pneumonia (PJP), *Candida,* and *Aspergillus* species. The clinical manifestations are often characterized by respiratory and gastrointestinal infections, although meningitis, arthritis, urinary tract infection, and systemic infections can also occur [3]. Epstein-Barr virus (EBV) infection has been reported and it can rarely determine immune dysregulation phenomena together with the development of B cell lymphoma or hemophagocytic lymphohistiocytosis [4]. Patients with SCID should be placed in protective isolation and receive symptomatic treatments, parenteral nutritional support due to chronic diarrhea, intensive treatment of ongoing infections, and/or prophylaxis for infections. Although B cells are present in many types of SCID (common gamma chain deficiency [IL2RG], JAK3 deficiency), antibody production results profoundly impaired due to the absence of adequate co-stimulation by CD4+ T cells [5]. Moreover, patients with SCID often present with recurrent sinopulmonary infections caused by encapsulated organisms (*Streptococcus pneumoniae* and *Haemophilus influenzae*), as well parainfluenza, respiratory syncytial virus, adenovirus, and CMV. Thus, patients with SCID must be treated with antibiotic prophylaxis for PJP and immunoglobulin replacement therapy (IgRT) (see Table 17.1). Both of these therapies have been shown to reduce the risk of infection before and after treatment with hematopoietic stem cell transplantation (HSCT). Enzyme replacement therapy with intramuscular injections of ADA (adenosine deaminase) coupled with polyethylene glycol has been successfully used to improve immune system function in patients with ADA-SCID.

17.2.1 Management of Immune Dysregulation

A diagnosis of primary immunodeficiency does not represent an absolute contraindication to immune suppressive therapy, in the presence of immune-mediated complications. Corticosteroids are indeed widely used in treating autoimmune disease and immune dysregulation, especially as first-line therapy, in T cell dysfunction. Ciclosporin is frequently used as “steroid-sparing” agents. Rapamycin inhibits T cell proliferation while selectively increasing the number of Treg cells, then it can be used in the treatment of patients with autoimmune lymphoproliferative syndrome (ALPS) [6]. A wide variety of biologicals are already being used to block cytokines

Table 17.1 Differences in clinical manifestations and management of cellular PID

Cellular PID	Clinical infections and noninfectious complications	Pathogens	Management
SCID, CID *(T and B cell defects)*	Systemic, invasive, or opportunistic infections are common. Diarrhea, eczema and infections. Unusual infection or unusually severe course of infection Failure to thrive Autoimmune or chronic inflammatory disorders Chronic lung diseases Lymphoproliferative disorders Cancer	Viruses (CMV, EBV, VZV, HSV, adenovirus, HHV8, HPV, molluscum contagiosum, RSV) Fungi (*Candida, Aspergillus, Cryptococcus, Histoplasma*, PCJ) Protozoa (*Toxoplasma*, *Microsporidium*, *Cryptosporidium*) Intracellular bacteria (*Mycobacterium* spp., *Salmonella*, *Listeria*) Extracellular bacteria	*PJP prophylaxis*: TMP-SMX dosed as 4–6 mg/kg/day of TMP component divided twice daily 3 days per week (after 30 days of life) *Fungal prophylaxis*: fluconazole 6 mg/kg daily or oral amphotericin B Treatment of acute infections Avoid environmental exposure and live vaccines IgRT Enzyme replacement therapy (ADA-SCID) HSCT Gene therapy
CVID, Bruton agammaglobulinemia, TACI deficiency, class-switch recombination defects (CD40L deficiency) *(B cells defects)*	Recurrent bacterial sinopulmonary infections (otitis media, sinusitis, and pneumonia). Diarrhea due to *Giardia lamblia*. Sometimes meningitidis. Systemic, invasive, or opportunistic infections are uncommon Enteropathy Autoimmunity Interstitial lung disease Chronic lung disease Lymphoproliferative disorders Cancer	Encapsulated bacteria (*Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis*)	IgRT *Antibiotic prophylaxis*: azithromycin 5 mg/kg 3 times weekly (maximum 250 mg three times weekly) Treatment of acute infections Appropriate vaccinations Diet, nutritional support, diagnosis and management of inflammatory bowel diseases Immune suppressive treatment Early detection of pulmonary complications, asthma/COPD treatment, pulmonary rehabilitation when required Surveillance for early detection (and treatment) of neoplastic complications

Abbreviations: *SCID* severe combined immune deficiency, *CID* combined immune deficiency, *ADA* adenosine deaminase, *CVID* common variable immunodeficiency, *IgRT* immunoglobulin replacement therapy, *HSCT* hematopoietic stem cell transplantation, *TMP-SMX* trimethoprim-sulfamethoxazole, *COPD* chronic obstructive pulmonary disease, *CMV* cytomegalovirus, *EBV* Epstein-Barr virus, *VZV* varicella-zoster virus, *HSV* herpes simplex virus, *HHV8* human herpesvirus 8, *HPV*, human papillomavirus, *RSV* respiratory syncytial virus, *PCJ Pneumocystis jiroveci*

(such as TNF-alpha, IL-1, IL-6, IL-12/23, and IL-17) in the treatment of autoimmune diseases, and their potential in patients with T cell deficiencies has to be carefully considered. Finally, in patients with thrombocytopenia, as in Wiskott-Aldrich syndrome (WAS), the use of recombinant thrombopoietin may be an option for supportive treatment [7].

17.2.2 Management of Complications of HSCT

HSCT is a high-risk treatment but can potentially provide a definitive correction for most PID. A major concern when a patient undergoes a HSCT is infections. Common infectious diseases occur after HSCT. Post-HSCT infectious complications are usually classified according to the time after HSCT: pre-engraftment, immediate post-engraftment, and late post-engraftment period. The infectious diseases that occur before the engraftment are similar to those that develop during the neutropenic phase after chemotherapy. The majority of infections in this period are due to bacteria, with Gram-positive organisms predominating over Gram negatives, *Clostridium difficile*, fungal infections, and herpes simplex virus (HSV) reactivation (see Table 17.2) [8]. In neutropenic patients during the pre-engraftment phase, infections can progress rapidly and it can be difficult to distinguish between

Table 17.2 Common pathogens, risk factors, and antimicrobial prophylaxis according to the various time periods after HSCT

	Pre-engraftment period (1st 2–4 weeks)	Immediate post-engraftment period (2nd and 3rd month)	Late post-engraftment period (after 2nd and 3rd month)
Pathogens	Gram-negative bacteria (especially enteric bacteria)	Gram-negative bacteria (especially enteric bacteria)	Encapsulated bacteria (*S. pneumoniae, H. influenzae*)
	Gram-positive cocci (mainly viridans group streptococci)	Gram-positive cocci	*Nocardia*
	Clostridium difficile		Tuberculosis, NTM
	Candida, Aspergillus	*Aspergillus* and PCJ	*Aspergillus* and PCJ
	HSV	EBV, CMV, HHV-6, JC virus	EBV, CMV, VZV
Risk factors	Mucositis, central venous catheter, neutropenia, organ dysfunction due to conditioning regimen	Acute GVHD	Chronic GVHD
		Immune-modulating viruses	Hyposplenism, decrease in opsonization
Antimicrobial prophylaxis	Consider fluoroquinolone and fluconazole during neutropenia	PCJ prophylaxis	PCJ prophylaxis
		Antiviral prophylaxis	Antiviral prophylaxis

Abbreviations: *CMV* cytomegalovirus, *EBV* Epstein-Barr virus, *VZV* varicella-zoster virus, *HSV* herpes simplex virus, *HHV-6* human herpesvirus 6, HHV8; *PCJ Pneumocystis jiroveci*, *JC* John Cunningham virus (human polyomavirus 2), *NTM* nontuberculous mycobacteria

bacterial infection and noninfectious fever. For this reason, empirical antimicrobial therapy is strongly recommended in all patients with febrile neutropenic episodes: the broad-spectrum β-lactam antibiotic is the primary choice. After HSCT, PCJ prophylaxis with trimethoprim/sulfamethoxazole (single-strength 80/400 mg daily or double-strength 160/800 mg three times weekly) is recommended from engraftment to at least ≥6 months and as long as receiving immunosuppressive therapy is ongoing [9]. CMV can reactivated after HSCT and the spectrum of CMV infection is extensive extremely broad: from asymptomatic DNAemia to esophagitis, gastritis, colitis, hepatitis, pneumonia, retinitis, and encephalitis. In addition, CMV reactivation can determine a state of graft failure or immunosuppression that may permit the development of concurrent bacterial and/or fungal infections. CMV pneumonia or encephalitis can be fatal despite aggressive anti-CMV therapy [10]. Management of CMV is categorized into prevention, preemptive treatment, and definitive treatment. Preemptive therapy is anti-CMV treatment even in the absence of clinical symptoms in cases with CMV infection (reinfection or reactivation). Most transplantation centers introduce preemptive therapy rather than routine prevention because of cost-benefit ratio and adverse drug reactions. In 2017, the U.S. Food and Drug Administration has approved letermovir for the prevention of CMV infection in adult CMV-seropositive patients undergoing allogeneic HSCT, after the evidence that letermovir prophylaxis resulted in a significantly lower risk of clinically significant CMV infection than placebo [11]. Currently available anti-CMV agents are ganciclovir, valganciclovir, foscarnet, and cidofovir. Moreover, it has been noted that graft versus host disease (GVHD) or the use of monoclonal antibodies can increase the incidence of CMV infections [12, 13]. Finally, acyclovir prophylaxis is recommended to reduce the incidence of HSV and varicella-zoster virus (VZV) infections not only before engraftment but also in the long term until the immunosuppressant is stopped: some studies showed that acyclovir shall be maintained for at least 1 year after allogeneic HSCT and for 6 to 12 months after autologous HSCT [14].

17.3 Management of B Cell Immune Deficiencies

B cell defects are a heterogeneous group of disorders with variable reduction in B cell numbers and function, sharing the reduction in or absence of serum Ig and/or specific antimicrobial antibodies [15]. Most infections are caused by encapsulated bacteria, particularly *Streptococcus pneumoniae* and *Haemophilus influenzae* (Table 17.1). Both recurrent or chronic bronchitis and pneumonia can result in chronic lung disease such as bronchiectasis and interstitial lung disease due to the chronic inflammation and fibrotic process in the pulmonary interstitium. Patients affected by B cell defects frequently suffer from recurrent otitis media and chronic sinusitis since childhood. Infants typically present first bacterial infections once maternal IgG have disappeared from circulation, which occurs between 3 and 18 months of age. Also common are gastrointestinal infections with *Giardia lamblia* and *Cryptosporidium* spp. (the latter mainly in patients with certain class-switch recombination defects) or CNS infection with bacterial meningitidis [16].

Despite an appropriate IgG replacement therapy, as described for combined immunodeficiencies, low IgM and particularly low IgA serum levels may be risk factors for *S. pneumoniae* and *H. influenzae* lung colonization [17]. In contrast to T cell defects, most B cell defects are associated with an otherwise normal response to viral infection. Numerical or functional B cell defects can be divided into three major categories: (i) defects in early B cell development (agammaglobulinemia), (ii) class-switch recombination defects (e.g., hyper-IgM syndromes), and (iii) common variable immunodeficiency (CVID). In the presence of B cell defects, as in CVID and X-linked agammaglobulinemia, the management may include IgRT, antibiotics for treatment, and prophylaxis of infections, but also respiratory rehabilitation programs and proper therapy for noninfectious complications [18]. Despite the reduction of bacterial infections due to IgRT [19], patients with cellular immunodeficiencies remain more susceptible to complications because of an associated T cell defect that requires particular attention.

17.3.1 Management of Pulmonary Complications

Among B cell immunodeficiencies, CVID is the most associated with pulmonary complications, such as bronchiectasis and noninfectious interstitial lung diseases (ILDs) [20]. Bronchiectasis presents as atypical bronchial and bronchiolar dilatations, resulting from repeated episodes of infection and inflammation with the destruction of the airways and lung parenchyma, consequent decline in lung function [21]. Also, it has also been demonstrated that, once the remodeling process is ongoing, airway inflammation gets worse even in the absence of bacterial infection, facilitated by the neutrophil accumulation and the augmentation of pro-inflammatory milieu. The management of bronchiectasis, apart from antibiotic prophylaxis, may require physiotherapy programs. Some studies have evidenced how the high incidence of chronic lung diseases in B cell immunodeficiencies is a direct consequence of diagnostic delay, severity of the infectious respiratory phenotype, and difficulty to find appropriate treatment strategies [22]. Thus, screening for chronic lung disease by high-resolution chest tomography (HRCT) scan is a cornerstone of disease management. Chronic airway inflammation due to recurrent infections may also lead to airway hyper-reactivity state with reversible or fixed obstruction [23]. Thus, annual testing with spirometry and CO transfer measurement is recommended (see Table 17.3). Another great challenge is currently represented by the management of ILDs [24]. ILD has not been only described in CVID but also in patients with CID such as cytotoxic T lymphocyte antigen 4 (CTLA-4) haploinsufficiency, lipopolysaccharide (LPS)-responsive and beige-like anchor protein (LRBA) deficiency, and signal transducer and activator of transcription 3 (STAT-3) gain of function (GOF). Interestingly, it is not found in hyperimmunoglobulin (IgM) syndromes or in XLA [25]. Different ILD patterns have been described in lung biopsies, including granulomas and all forms of pulmonary lymphoid hyperplasia. The term "granulomatous-lymphocytic interstitial lung disease" (GLILD) has been adopted to encompass all these features of ILD in CVID patients [26]. Its pathogenesis and appropriate management are currently under investigation. GLILD is usually described as part of a

Table 17.3 Monitoring and management of complications in B cell immune deficiencies

At diagnosis	During follow-up	
	Annually	Every 5 years
According to HRCT and PFTs (before and after BD) with DLCO measurement: • Tailored doses of IgG • Route of administration • Need for antibiotic prophylaxis • Pulmonary rehabilitation	PTFs and DLCO measurement • **Obstructive pattern** → inhaled steroid or steroid/LABA: In case of bronchiectasis → azithromycin prophylaxis + pulmonary rehabilitation + toilet bronchoscopy • **Restrictive pattern and/or DLCO reduction** with/without dry cough or dyspnea on exercise (**ILD suspicion**) → 6MWT, CPET, HRCT scan, bronchoscopy (microbiology, BALF analysis) → consider transbronchial biopsy (or cryobiopsy) to rule out a lymphoproliferative disorder Abdominal ultrasound EGDS	HRCT scan

Abbreviations: *HRCT* high-resolution chest tomography, *PFTs* pulmonary function tests, *DLCO* diffusing capacity for carbon monoxide, *6MWT* 6-minute walking test, *CPET* cardiopulmonary exercise testing, *BALF* bronchoalveolar lavage fluid, *LABA* long-acting beta agents, *EGDS* esophagogastroduodenoscopy

multisystem granulomatous/inflammatory disease, potentially involving the lymph nodes, spleen, liver, gastrointestinal tract, and other organs. The presence of splenomegaly, immune cytopenia, low serum IgA levels, higher IgM levels, and percentage expansion of CD21low B have been suggested as clinical predictors of GLILD [27]. A reduction in DLCO/gas transfer at routine lung function tests may be the earlier sign of an underlying ILD, deserving radiologic investigation. In case of radiological suspicion of ILD, a bronchoscopy in order to obtain a bronchoalveolar lavage fluid (BALF) analysis (microbiology and lymphocytic subpopulation study) should be performed, as well as a lung or nodal biopsy in order to confirm GLILD diagnosis and rule out hematological malignancies, when appropriate. Specific therapeutic guidelines for GLILD are currently lacking, but there are promising results with immunosuppressive agents on T cell and B cell (methotrexate, azathioprine [28], mycophenolate [29], sirolimus [30], anti-CD20 and anti-TNF agents [31]), thus suggesting how both lymphocyte subpopulations may play an active role in disease progression.

17.3.2 Management of Other Immunological Complications

Noninfectious complications of B cell immune deficiencies also include autoimmune cytopenias, enteropathy, and neoplastic diseases. Treatment of autoimmune cytopenias (mainly autoimmune thrombocytopenia and autoimmune hemolytic anemia) is mainly based on corticosteroids and anti-CD20 treatment. Currently, there is no evidence of advantages in splenectomy for the treatment of cytopenias in CVID [32]. Moreover, the indication for any immunosuppressive therapy in PID

should be considered very carefully for the risk of developing opportunistic infections like PCJ pneumonia. At least 20% of CVID patients have chronic gastrointestinal symptoms (including bloating, discomfort, malabsorption, and diarrhea) [33]. The histological evidence of mucosal inflammation in CVID enteropathy is similar to villous atrophy in celiac disease, although recent works underlined the different immunologic hallmarkers of T cell subpopulations [34]. About 5% of CVID with enteropathy present severe symptoms and may also benefit from treatment with low-dose immunomodulators, rituximab, and anti-TNF agents [35]. Finally, another relevant complication and cause of morbidity and mortality during the management of B cell immune deficiencies is cancer. Lymphoma and gastric carcinoma are the most represented neoplastic diseases [36]. The finding of EBV-driven lymphoproliferative diseases in patients with hypogammaglobulinemia and history of recurrent bacterial infections should raise the suspicion of a CD27-CD70 axis deficiency [37]

17.4 Immunoglobulin Replacement Therapy and Antibiotic Prophylaxis

SCID is a medical emergency as patients are extremely susceptible to developing additional severe and debilitating infections. When pulmonary infections are severe and recurrent, patients may develop irreversible lung damage such as bronchiectasis [38]. Many patients cannot produce appropriate antibodies; therefore, immunoglobulin G (IgG) replacement therapy should be initiated as soon as the immune deficit is diagnosed [39]. Both intravenous IgG (IVIG) and subcutaneous IgG (SCIG) have been regarded therapeutically equivalent (same efficacy for prevention of bacterial infections) [17]. Moreover, SCIG have showed some advantages in less systemic adverse events, improved quality of life, and stable IgG levels and disadvantages in having more local infusion site reactions and requirement of frequent infusions (weekly vs. monthly) [40] (see Table 17.4). The serum

Table 17.4 Differences in intravenous vs. subcutaneous IgRT

IVIG	SCIG
Intravenous administration, 100% and immediate bioavailability	Subcutaneous administration, gradual absorption, venous access is not required
Monthly infusions	Frequent infusions (generally weekly, up to every 3–4 weeks with fSCIG)
Higher peaks, less stable serum IgG levels	Stable and possibly higher serum IgG trough levels
No difference in quality of life	Improved quality of life [41]
More systemic adverse events	Less systemic adverse events, but more local infusion site reactions
Hospital-based, may be home-based according to the single country	Home-based after initial training

Abbreviations: *fSCIG* facilitated subcutaneous immunoglobulin

IgG trough level, defined as concentration preceding the next dose of IgG infusion, has been regarded as an important guide to therapy. An IVIG dose of 400–600 mg/kg every 4 weeks or a dose that maintains serum IgG trough levels above 500 mg/dL is desirable. Measurement of serum IgG trough level is necessary every 3 months until a steady state is achieved and then every 6 months if the patient is stable. For persons who have a high catabolism of infused IgG (e.g., during a period of active infection), measuring serum IgG levels and adjusting to higher dosages or shorter intervals may be required. Recent recommendations have noticed that individualized treatment plans and route of administration based on patient's preferences increase the therapeutic compliance to IgRT [42]. Adjunct therapy as azithromycin prophylaxis has been suggested for its antimicrobial, immunomodulatory, and anti-inflammatory properties. A recent study showed that long-term oral azithromycin prophylaxis (250 mg once daily for 3 consecutive days per week) in patients affected by XLA and CVID presenting chronic infection-related pulmonary diseases significantly may reduce the number of acute respiratory exacerbations per patient-year [43].

17.5 Neonatal Screening and Vaccination in the Management of T and B Cell Immune Deficiencies

Analysis of thymopoiesis can also help in the management of T cell immune deficiencies. T cell receptor excision circles (TREC), which are fragments of genomic DNA formed during V(D)J recombination in thymocytes, have been shown to reflect thymus activity. TREC levels in patients with cellular immunodeficiencies are typically below 400/mg DNA. TREC levels can be quantified by PCR, even from Guthrie cards, and it has become the mainstay of neonatal screening programs of SCID/CID. Thus, an early diagnosis can help in the management of cellular immunodeficiency. Moreover, detection of kappa-deleting recombination circles (KRECs), co-products during B cell formation, by PCR can estimate the average number of B cell divisions and may be a possible target for neonatal screening of B cell immunodeficiencies. KREC concentration in CVID patients has been found to be lower than in controls (58). This may enable an earlier diagnosis and management, especially starting sooner the IgRT, providing better clinical outcome and prevention of chronic immunologic complications. The role of vaccination in cellular immunodeficiencies will be discussed in the next chapter. In addition, vaccination is also used as diagnostic tool to assess specific antibody response to protein and/or polysaccharide antigens [44]. In particular, the specific IgG measured in pre- and post-vaccination in response to 23-valent pneumococcal polysaccharide vaccine is the most used test to evaluate a T-independent antibody response and the residual function of B lymphocytes. A recent 6-year longitudinal study showed that anti-23 pneumococcal serotype IgA level is a parameter capable of detecting CVID patients at risk of developing greater frequency of respiratory infections, chronic lung damage, and noninfectious complications over time [45].

References

1. Bazregari S, Azizi G, Tavakol M, Asgardoon MH, Kiaee F, Tavakolinia N et al (2017) Evaluation of infectious and non-infectious complications in patients with primary immunodeficiency. Cent-Eur J Immunol 42(4):336–341
2. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME et al (2015) Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol 35(8):696–726
3. Fischer A, Notarangelo LD, Neven B, Cavazzana M, Puck JM (2015) Severe combined immunodeficiencies and related disorders. Nat Rev Dis Primer 1:15061
4. Latour S, Fischer A (2019) Signaling pathways involved in the T-cell-mediated immunity against Epstein-Barr virus: Lessons from genetic diseases. Immunol Rev 291(1):174–189
5. Gernez Y, Baker MG, Maglione PJ (2018) Humoral immunodeficiencies: conferred risk of infections and benefits of immunoglobulin replacement therapy. Transfusion (Paris) 58(Suppl 3):3056–3064
6. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells - PubMed [Internet]. [cited 2020 Oct 19]. Available from: https://pubmed.ncbi.nlm.nih.gov/15746082/
7. Imbach P, Crowther M (2011) Thrombopoietin-receptor agonists for primary immune thrombocytopenia. N Engl J Med 365(8):734–741
8. Gudiol C, Garcia-Vidal C, Arnan M, Sánchez-Ortega I, Patiño B, Duarte R et al (2014) Etiology, clinical features and outcomes of pre-engraftment and post-engraftment bloodstream infection in hematopoietic SCT recipients. Bone Marrow Transplant 49(6):824–830
9. Cho S-Y, Lee H-J, Lee D-G (2018) Infectious complications after hematopoietic stem cell transplantation: current status and future perspectives in Korea. Korean J Intern Med 33(2):256–276
10. Boeckh M, Geballe AP (2011) Cytomegalovirus: pathogen, paradigm, and puzzle. J Clin Invest 121(5):1673–1680
11. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF et al (2017) Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med 377(25):2433–2444
12. Cantoni N, Hirsch HH, Khanna N, Gerull S, Buser A, Bucher C et al (2010) Evidence for a bidirectional relationship between cytomegalovirus replication and acute graft-versus-host disease. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant 16(9):1309–1314
13. Marchesi F, Pimpinelli F, Ensoli F, Mengarelli A (2018) Cytomegalovirus infection in hematologic malignancy settings other than the allogeneic transplant. Hematol Oncol 36(2):381–391
14. Kim DH, Kumar D, Messner HA, Minden M, Gupta V, Kuruvilla J et al (2008) Clinical efficacy of prophylactic strategy of long-term low-dose acyclovir for Varicella-Zoster virus infection after allogeneic peripheral blood stem cell transplantation. Clin Transpl 22(6):770–779
15. Modern management of primary B-cell immunodeficiencies - PubMed [Internet]. [cited 2020 Oct 30]. Available from: https://pubmed.ncbi.nlm.nih.gov/22122788/
16. Common Variable Immune Deficiency: Dissection of the Variable [Internet]. [cited 2020 Oct 30]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6435035/
17. Chapel HM, Spickett GP, Ericson D, Engl W, Eibl MM, Bjorkander J (2000) The comparison of the efficacy and safety of intravenous versus subcutaneous immunoglobulin replacement therapy. J Clin Immunol 20(2):94–100
18. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT et al (2016) International consensus document (ICON): common variable immunodeficiency disorders. J Allergy Clin Immunol Pract 4(1):38–59
19. Busse PJ, Razvi S, Cunningham-Rundles C (2002) Efficacy of intravenous immunoglobulin in the prevention of pneumonia in patients with common variable immunodeficiency. J Allergy Clin Immunol 109(6):1001–1004

20. The Lung in Primary Immunodeficiencies: New Concepts in Infection and Inflammation [Internet]. [cited 2020 Oct 30]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6096054/
21. Cole PJ (1986) Inflammation: a two-edged sword--the model of bronchiectasis. Eur J Respir Dis Suppl 147:6–15
22. Mooney D, Edgar D, Einarsson G, Downey D, Elborn S, Tunney M (2017) Chronic lung disease in common variable immune deficiency (CVID): a pathophysiological role for microbial and non-B cell immune factors. Crit Rev Microbiol 43(4):508–519
23. Primary immune deficiency diseases as unrecognized causes of chronic respiratory disease - PubMed [Internet]. [cited 2020 Oct 31]. Available from: https://pubmed.ncbi.nlm.nih.gov/29229095/
24. Prasse A, Kayser G, Warnatz K (2013) Common variable immunodeficiency-associated granulomatous and interstitial lung disease. Curr Opin Pulm Med 19(5):503–509
25. Schussler E, Beasley MB, Maglione PJ (2016) Lung disease in primary antibody deficiencies. J Allergy Clin Immunol Pract 4(6):1039–1052
26. Hurst JR, Verma N, Lowe D, Baxendale HE, Jolles S, Kelleher P et al (2017) British Lung Foundation/United Kingdom Primary immunodeficiency network consensus statement on the definition, diagnosis, and management of granulomatous-lymphocytic interstitial lung disease in common variable immunodeficiency disorders. J Allergy Clin Immunol Pract 5(4):938–945
27. Hartono S, Motosue MS, Khan S, Rodriguez V, Iyer VN, Divekar R et al (2017) Predictors of granulomatous lymphocytic interstitial lung disease in common variable immunodeficiency. Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol 118(5):614–620
28. Use of combination chemotherapy for treatment of granulomatous and lymphocytic interstitial lung disease (GLILD) in patients with common variable immunodeficiency (CVID)—PubMed [Internet]. [cited 2020 Oct 31]. Available from: https://pubmed.ncbi.nlm.nih.gov/22930256/
29. Bucciol G, Petrone A, Putti MC. Efficacy of mycophenolate on lung disease and autoimmunity in children with immunodeficiency. Pediatr Pulmonol 2017;52(10):E73–E76. [cited 2020 Oct 31]
30. Deyà-Martínez A, Esteve-Solé A, Vélez-Tirado N, Celis V, Costa J, Cols M et al (2018) Sirolimus as an alternative treatment in patients with granulomatous-lymphocytic lung disease and humoral immunodeficiency with impaired regulatory T cells. Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol 29(4):425–432
31. Ng J, Wright K, Alvarez M, Hunninghake GM, Wesemann DR (2019) Rituximab monotherapy for common variable immune deficiency-associated granulomatous-lymphocytic interstitial lung disease. Chest 155(5):e117–e121
32. Notarangelo LD (2009) Primary immunodeficiencies (PIDs) presenting with cytopenias. Hematol Am Soc Hematol Educ Program:139–143
33. Gastrointestinal Disease in Patients with Common Variable Immunodeficiency: A Retrospective Observational Study | Journal of the Canadian Association of Gastroenterology | Oxford Academic [Internet]. [cited 2020 Oct 31]. Available from: https://academic.oup.com/jcag/article/3/4/162/5355634
34. Malesza IJ, Malesza M, Krela-Kaźmierczak I, Zielińska A, Souto EB, Dobrowolska A et al (2020) Primary humoral immune deficiencies: overlooked mimickers of chronic immune-mediated gastrointestinal diseases in adults. Int J Mol Sci [Internet] 21(15) [cited 2020 Oct 31]
35. Chua I, Standish R, Lear S, Harbord M, Eren E, Raeiszadeh M et al (2007) Anti-tumour necrosis factor-α therapy for severe enteropathy in patients with common variable immunodeficiency (CVID). Clin Exp Immunol 150(2):306–311
36. Quinti I, Agostini C, Tabolli S, Brunetti G, Cinetto F, Pecoraro A et al (2012) Malignancies are the major cause of death in patients with adult onset common variable immunodeficiency. Blood 120(9):1953–1954
37. Latour S, Winter S (2018) Inherited immunodeficiencies with high predisposition to Epstein-Barr virus-driven lymphoproliferative diseases. Front Immunol 9:1103
38. Truong T (2013) The overlap of bronchiectasis and immunodeficiency with asthma. Immunol Allergy Clin N Am 33(1):61–78

39. Pachlopnik Schmid J, Güngör T, Seger R (2014) Modern management of primary T-cell immunodeficiencies. Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol. 25(4):300–313
40. Comparative study of subcutaneous versus intravenous IgG replacement therapy in pediatric patients with primary immunodeficiency diseases: a multicenter study in Argentina | SpringerLink [Internet]. [cited 2020 Oct 16]. Available from: https://link.springer.com/article/10.1007%2Fs10875-013-9916-z
41. Pulvirenti F, Cinetto F, Pecoraro A, Carrabba M, Crescenzi L, Neri R et al (2019) Health-related quality of life in patients with CVID Under different schedules of immunoglobulin administration: prospective multicenter study. J Clin Immunol 39(2):159–170
42. Chapel H, Gardulf A (2013) Subcutaneous immunoglobulin replacement therapy: the European experience. Curr Opin Allergy Clin Immunol 13(6):623–629
43. Milito C, Pulvirenti F, Cinetto F, Lougaris V, Soresina A, Pecoraro A et al (2019) Double-blind, placebo-controlled, randomized trial on low-dose azithromycin prophylaxis in patients with primary antibody deficiencies. J Allergy Clin Immunol 144(2):584–593. e7
44. Cavaliere FM, Graziani S, Duca ED, Bilotta C, Sgrulletti M, Quinti I et al IgM, IgA and IgG response to conjugate polysaccharides in children with recurrent respiratory infections. Scand J Immunol:e12955
45. Pulvirenti F, Milito C, Cavaliere FM, Mezzaroma I, Cinetto F, Quinti I (2020) IGA Antibody induced by immunization with pneumococcal polysaccharides is a prognostic tool in common variable immune deficiencies. Front Immunol [Internet] 24. [cited 2020 Nov 1]:11

Vaccines in Cellular Immunodeficiencies

18

Chiara Azzari and Clementina Canessa

Abstract

Infectious complications are a major cause of morbidity and mortality in patients with primary immune deficiencies. Prevention of infectious diseases by vaccines is among the most effective healthcare measures mainly for these subjects. As in humoral immune deficiencies, there are some specific aspects that need to be deepened. Firstly, response to vaccine is often seriously compromised in patients affected by cellular immunodeficiencies so that some vaccines result inefficacious or even dangerous in particular conditions. However, subjects affected by a cellular immunodeficiency vary in their degree of immunosuppression and susceptibility to infection depending on the kind of defect and, therefore, represent a heterogeneous population with regard to immunization. Secondly, the susceptibility to specific pathogens has to be considered. Therefore, it becomes very important for clinicians to distinguish which vaccines are useful and not deleterious for patients, depending on the type of cellular defect. The aim of this chapter is to issue recommendations based on published scientific literature and practical experience about how and when vaccines can be used in primary cellular immune deficiencies, in order to facilitate physician decisions and to ensure the best immune protection with the lowest risk to the health of the patient.

Keywords

Vaccine · Cellular · Primary immunodeficiency · Severe · Combined · Syndromic Innate immunity

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18.1 Introduction

In cellular immunodeficiencies, the increased susceptibility to infectious diseases results in extremely high morbidity and mortality and has a major role in determining the prognosis. Due to the generally severe course of infections and to the poor response to conventional antimicrobial treatment, prevention is crucial in this population.

Therefore, vaccination as well as immunoglobulin replacement therapy and antimicrobial prophylaxis represent the most important tools of individual prevention. As in humoral immunodeficiencies, some issues have to be considered carefully, first of all the safety of vaccines. In fact, when the cellular defect is profound, the risk of proliferation and dissemination of live viral or bacterial agents is high.

Secondly, if the cellular defect is severe, most of the vaccines have an extremely low immunogenicity, so that specific protection against pathogen can't be obtained.

Moreover, in most of the cases, these patients are under immunoglobulin substitutive therapy; therefore, besides their primary inability to mount an adequate immune response, the interference generated by infused antibodies has to be taken in account. In view of all this, in patients affected by severe cellular immune defects, the greatest possible immunization of all contacts becomes fundamental in order to take the best advantage of herd immunity effect. Finally, special awareness should be raised about specific pathogen susceptibility in the different types of cellular immunodeficiencies (PIDs) so that certain vaccines could be specifically recommended. The aim of this chapter is to report vaccine schedule recommendations in patients with primary cellular immune disorders, based on the currently available evidence and on the standardized experience and practice. Specific recommendations will be made only regarding well-defined and studied conditions. Viable and nonviable vaccines will be analyzed separately in each disease category; in particular cellular deficiencies will be classified in a simplified manner as follows: severe combined immunodeficiencies, combined immunodeficiencies, and combined immunodeficiencies with associated or syndromic feature.

Moreover, vaccine recommendations in defects of innate immunity in intrinsic immunity with susceptibility to specific organisms, in congenital defects of phagocytes, and in complement deficiencies will be reviewed. Vaccines in particular conditions, as contacts of patients with cellular immunodeficiency and patients undergoing stem cell transplant, will be discussed, as well.

In recent years, thanks to the rapid evolution of diagnostic techniques, more and more genetic defects have been discovered. The chapter will offer general principles that could be applicable also into newly discovered clinical entities instead of focusing on specific defects that are still poorly characterized. Specific recommendations will be made only regarding well-defined and studied conditions. Viable and nonviable vaccines will be analyzed separately in each disease category. It's important to notice that providing specific recommendations for each type of cellular immunodeficiency is difficult for many reasons: the rarity of the single conditions, the

exclusion of immunocompromised individuals from pre-licensure vaccine tests, the lack of high-quality data, and the extreme heterogeneity of clinical expression even among patients with the same molecular defect. Therefore, authors think that a tailor-made approach based on general principles and on specific evaluation of the patient's immune function and a precise assessment of risk-benefits to ensure the greatest protection and to prevent risks of adverse events has to be considered the most appropriate.

18.2 General Principles

The International Union of Immunological Societies (IUIS) Expert Committee for Primary Immunodeficiencies has cataloged and classified all known cellular deficiencies into two main groups, both included in the group named "immunodeficiencies affecting cellular and humoral immunity": severe combined immunodeficiencies (SCID), defined by CD3 T cell lymphopenia (defined by CD3+ T cells <300/μL), and combined immunodeficiencies (CID) generally less profound than severe combined immunodeficiency. Besides these, there is the group of CID with associated or syndromic features [1]. Viable and nonviable vaccines have different general indications and contraindications in the above-cited categories. Inactivated vaccines can be considered safe in all patients. Nevertheless, in patients with profound impairment of cellular immune system, their benefit is unlikely and their use is not fully justified especially when receiving immunoglobulin replacement therapy. On the other hand, live vaccines have always to be avoided in SCID, whether they should be used with greater caution in patients with other cellular defects. In the same classification, just updated, combined immunodeficiencies with associated or syndromic features, congenital defects of phagocyte number or function, defects in intrinsic and innate immunity, and complement deficiencies are cited and constitute different groups. In turn, each group includes multiples genetic defects; in the following discussion, only principal clinical entities will be considered.

18.3 Vaccination in Severe Combined Immunodeficiencies

No live viral or bacterial vaccines should be given to SCID patients before immune reconstitution who should otherwise receive passive immunization with immunoglobulins [2]. Immunodeficient patients who have received hematopoietic stem cell transplantation (HCT) but who continue to have incomplete immune reconstitution or are undergoing immunosuppression should not be given live viral or bacterial vaccines [3]. In particular, no live viral (oral poliovirus, measles, mumps, rubella, varicella, yellow fever, herpes zoster, smallpox, rotavirus, or live attenuated influenza virus) or live bacterial (BCG or *S. typhi* Ty21a) vaccines should be administered in SCID patients. In fact, in these subjects the impaired generation of a diverse repertoire of mature T lymphocytes leads to a severe T lymphopenia with a lack of a T- and B-dependent-specific

antibody response. Even if B cell counts may be normal or increased in some patients with SCID, their maturation is incomplete and fail to produce specific antibodies. For these reasons, there is a very high risk of vaccine-induced infection. Disseminated vaccine-acquired varicella and vaccine-acquired rubella have been reported in a 13-month-old female with an atypical SCID due to IL7R mutation [4]. All patients receive immunoglobulin substitutive therapy and have exogenous protective antibody titers. Oral polio vaccine (OPV) should not be administered to SCID patients. In most countries, inactivated poliovirus vaccine (IPV) has replaced OPV vaccine. Recently in the United States a case of vaccine-associated paralytic poliomyelitis has been described in a child with SCID and a history of OPV vaccination in India [5]. Other cases of oral vaccine-derived poliovirus infections have been reported in subjects with SCID [6, 7]. Live-attenuated *M. bovis* bacille Calmette-Guérin (BCG) is routinely administered in most countries within the first month of life. However, only a small number of SCID patients receive diagnosis before the age of 1 month, as the median age at diagnosis is 138.5 days [8]. BCG complications, including disseminated BCG infections, have been observed in patients with all of the underlying genetic types of severe combined immunodeficiency (SCID). However, it is not known which type of SCID is more susceptible to BCG complications, which prohibits BCG administration to any patient with SCID [9–11]. Moreover, in countries where newborn screening for SCID is available, BCG vaccination should be shortly postponed. Oral rotavirus vaccine is also a live vaccine that is recommended at 2 months of life in the United States and in several European countries. In the United States from February 3, 2006, to January 15, 2010, nine cases of SCID and rotavirus vaccination in infants between 3 and 9 months of age have been reported. All but one presented with diarrhea among other symptoms. Stool rotavirus testing was positive in all the children, and the virus was identified as the vaccine strain in six cases. Prolonged viral shedding was documented in five patients. Fortunately, no death occurred [12]. Thus, as for other live vaccines, SCID is a contraindication for rotavirus vaccination [13]. However, a delay in rotavirus vaccination cannot be considered because rotavirus vaccination must be administered early in life to prevent the first yet most severe infection in children. The only strategy to avoid rotavirus vaccination in SCID patients is early diagnosis through newborn screening. Killed or inactivated vaccines are safe because they cannot replicate. Nevertheless, their effectiveness is very limited. Bacterial conjugate polysaccharide vaccines, including pneumococcal, meningococcal, and *H. influenzae* vaccines, and influenza-inactivated vaccine are recommended even in patients with complete T cell defect, but immune response to those vaccines is likely to be poor. However, whereas patients are passively protected by donors' immunoglobulins against diphtheria, poliomyelitis, tetanus, hepatitis B, and pertussis, they are not by capsulated bacteria since most of the population is not immunized. Thus, it is worth vaccinating them against these pathogens [14] (Table 18.1).

Table 18.1 Vaccination in primary cellular immunodeficiencies and in defects of innate immunity

	TDP	IPV	Hib	HBV	Influenza	Pneumococcus in S. pneumoniae	Meningococcus in N. meningitidis	MMR Varicella	Rotavirus	BCG *S. typhi*
SCID	No[a]	No[a]	Yes[b]	No[a]	Yes[b]	Yes[b]	Yes[b]	No	No	No
CID	Yes[b]	Yes[b]	Yes	Yes[b]	Yes	Yes	Yes	No[c]	No[c]	No
MSMD	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Invasive bacterial infections	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
CMCD	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
TLR deficiency	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
IL-12/IFN-gamma pathway deficiency	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Complement deficiency	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No data available
Congenital defects of phagocytes (CGD, LAD, MPO neutropenia)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[d]	Yes[d]	No

[a]Not recommended: these vaccines are safe but probably ineffective
[b]May be administered, the response to these vaccines is likely to be poor
[c]Generally contraindicated but they could be considered according to patient's immune system function
[d]Not recommended in LAD and CHs

18.4 Vaccination in Combined Immunodeficiencies

In this group of patients, inactivated viral and bacterial vaccines are always safe and are generally indicated; however, the immunological response could be poor. Vaccines against *S. pneumoniae, H. influenzae,* and *N. meningitidis* are recommended because they are T cell-independent antigens.

Influenza-inactivated vaccine is recommended, as well. In patients with impairment of T-cell functions, live bacterial vaccines are always contraindicated. Live viral vaccines are generally contraindicated, but live measles, mumps, rubella, and varicella vaccines could be considered in patients according to the immune system function: if CD4 + T lymphocytes are greater than or equal to 500 cells/μL, CD8+ T lymphocytes are greater than or equal to 200 cells/μL and proliferative response to mitogens is normal, and they can be administered safely.

The Centers for Disease Control and Prevention (CDC) recommends higher values of CD4+ T cells if the child is less than 6 years old: at least 1000 CD4+ T cells/μL if between 1 and 6 years, at least 1500 CD4 T cells/μL under the year if age [2, 15, 16]. Patients affected by mild DiGeorge syndrome can have a CID immunological phenotype. Before administering a live viral vaccine, in order to reduce the risks, it is worth evaluating the lymphocyte count and mitogen responsiveness [17, 18]: those with ≥500 CD4 cells/ L, ≥200 CD8 cells/ L and a normal mitogen response or, more simply according to Hofstetter et al. [18], those with CD4 cells/L ≥ 25% can receive measles-mumps-rubella (MMR) and varicella vaccines. Focusing on mild DiGeorge syndrome, Perez et al. retrospectively analyzed adverse events following MMR and varicella vaccine administration in these patients [19] and found that only 9% experienced adverse events, none of which was severe. A comparison of the patients who tolerated the vaccine and those who reported adverse events showed that there was no statistically significant difference in current age, age at the time of vaccination, or T cell subset counts. Similar immunogenicity and safety findings have been reported by Al-Sukaiti et al. [18, 20] (Table 18.1).

18.5 Vaccination in Combined Immunodeficiencies with Associated or Syndromic Features

18.5.1 Hyper-IgE Syndrome

In hyper-IgE syndrome (HIES) patients, all killed/inactivated/recombinant vaccines are recommended, and in particular anti-*H. influenza*e, anti-pneumococcal, and all the anti-meningococcal are indicated, since they don't evoke a T-mediated immune response. Anti-influenza-inactivated vaccine is recommended as well, as it can't be dangerous. Nevertheless, these vaccines could not be protective totally, depending on variable immune defect.

A variable capacity to produce protective antibody response has been demonstrated in these patients [21]. In particular, the administration of two doses of

conjugate vaccines (13-valent pneumococcal and tetravalent anti-meningococcal MenACWY vaccines) at 12-month interval may be useful.

Live attenuated viral vaccines can be used without risks in AD-HIES patients. Conversely, live vaccines should not be administered in HIES patients with DOCK8 or PGM3 mutations with T immune defects, as evaluated by CD4+ T cell counts ≥500 cells/μL, CD8+ T cells ≥200 cells/μL, and normal T cell response to mitogen. Conversely, live attenuated bacterial vaccines (BCG and *Salmonella typhi* vaccines) are always contraindicated because of the common association with a functional defect of antibacterial response [14].

18.5.2 Wiskott-Aldrich Syndrome

Killed, inactivated, and recombinant vaccines are all recommended, using the conjugated forms preferably in Wiskott-Aldrich syndrome (WAS). In particular patients aged 2–5 years should receive one dose of 13-valent pneumococcal conjugate vaccine (PCV13) if they have received three doses of PCV (either 7-valent PCV [PCV7] or PCV13) before age 24 months and two doses of PCV13 (8 weeks apart) if they have received an incomplete schedule of ≤2 doses of PCV7 (PCV7 or PCV13) before age 24 months [17]. Live attenuated viral and bacterial vaccines are contraindicated, as a defective number or function of T lymphocytes is common in these patients [22].

18.5.3 Ataxia-Telangiectasia

Patients affected by ataxia-telangiectasia can receive all killed/inactivated/recombinant vaccines and conjugated vaccines against *S. pneumoniae, H. influenzae*, and *N. meningitidis*, are recommended. Although there is reduced specific antibody production against polysaccharides, conjugated vaccines are effective. Inactivated influenza vaccine is strongly suggested, as well. Regarding live attenuated vaccines, they can be administered only after a careful evaluation of number and function of T lymphocytes, in particular only if CD4+ T lymphocytes are ≥500 cells/μL, CD8+ T lymphocytes are ≥200 cells/μL, and T lymphocyte mitogen response is normal [23, 24].

18.5.4 DiGeorge Syndrome

When the form is complete, patients share the same recommendations of patients affected by SCID regarding vaccines: all live attenuated viral and bacterial vaccines are contraindicated because of the potential risk of vaccine-related diseases. Conversely, vaccines against capsulated germs and inactivated influenza vaccine are recommended [14, 15].

Regarding "partial" syndrome, as mentioned before, the same recommendations observed in CID are to be followed. Vaccines against *S. pneumoniae, H. influenzae*, and *N. meningitidis* and influenza are recommended. Live attenuated viral vaccines can be administered if CD4+ T cells are ≥500 cells/μL, CD8+ T cells are ≥200 cells/μL, and T cell response to mitogen is normal. If these criteria are not satisfied, delaying the vaccination with immunological monitoring is advised [14, 15].

18.5.5 IPEX Syndrome (Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked)

In IPEX patients none of killed/inactivated/recombinant vaccines can provoke damage, so that all can be administered [25]. However, studies on response to those vaccines are not available. Regarding live attenuated vaccines, some cautions are needed. It's well known that immunoglobulins, number, and distribution of lymphocyte subpopulations are normal. T repertoire is polyclonal and naïve, and memory cells are comparable to those of control subjects of the same age. T lymphocyte mitogen response is normal or increased. Life vaccines could then be used, theoretically. Nevertheless, every single case has to be carefully evaluated and a complete immunologic evaluation is needed before any vaccine. In fact, sometimes cytokine production can be altered. Moreover, many patients are treated with immunosuppressive agents because of autoimmunity and that prohibits live vaccines [14].

18.5.6 APECED Syndrome (Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy)

All killed, inactivated, and recombinant vaccines are safe in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome.

Since lymphocytic defect is not always specific for *C. albicans*, live attenuated vaccines could be contraindicated; their use has to be carefully evaluated and immunologic tests have to be performed: lymphocyte subpopulations and T lymphocyte mitogen response. Nevertheless, live attenuated vaccines are at present contraindicated for the lack of studies [14] (Table 18.2).

18.6 Vaccines in Defects of Innate Immunity

Inborn errors of innate immunity encompass a wide group of congenital immunodeficiencies mainly characterized by recurrent bacterial invasive infections and viral and fungal infections often in the absence of a significant inflammatory response. Recently, the International Union of Immunological Societies (IUIS) Expert Committee on Primary Immunodeficiency (PIDs) classified these disorders in three main groups: intrinsic defects of innate immunity including Mendelian susceptibility to mycobacterial disease (MSMD), herpes simplex encephalitis, Toll-Like

Table 18.2 Vaccination in syndromic immunodeficiencies

	TDP	IPV	Hib	HBV	Influenza	Pneumococcus in S. pneumoniae	Meningococcus in N. meningitidis	MMR varicella	Rotavirus	BCG *S. typhi*
Complete DiGeorge syndrome	No[a]	No[a]	Yes[b]	No[a]	Yes[b]	Yes[b]	Yes[b]	No	No	No
Partial DiGeorge syndrome	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[c]	Yes[c]	No
Ataxia-telangiectasia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[c]	No data available	No
Wiskott-Aldrich syndrome	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Hyper-IgE syndrome	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[c,d]	Yes[c,d]	No
IPEX syndrome	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No data available	No data available	No data available
APECED syndrome	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No data available	No data available	No data available

[a]Not recommended: these vaccines are safe but probably ineffective
[b]May be administered, the response to these vaccines is likely to be poor
[c]Can be administered only if T CD4+ lymphocytes ≥500 cells/μL, T CD8+ lymphocytes ≥200 cells/μL and T lymphocytes mitogen response is normal. Center for Disease Control and Prevention recommends higher CD4+ levels if children are under 6 years: al least 1000 CD4+ cells/μL between 1 and 6 years, at least 1500 cells/μL under 1 year of life (*Red Book, 31st Edition 2018, Report of the Committee on Infectious Diseases*)
[d]Can be administered only in AD-HIES, generally

receptor (TLR) signaling pathway deficiencies, and disorders with predisposition to invasive fungal infections; congenital defect of the phagocyte number, function, or both; and complement pathway deficiencies [1] (Table 18.1).

18.6.1 Vaccination in Defects in Intrinsic Immunity with Susceptibility to Specific Organisms

In patients with defects in innate immunity that predispose to invasive bacterial infections, all the killed/inactivated/recombinant vaccines are safe and indicated. In particular, inherited mutations in IRAK4, NEMO, and MYD88 make patient susceptible to invasive bacterial infections, mostly caused by *S. pneumoniae*, *S. aureus*, and *P. aeruginosa* [1]. Thus, patients have to be vaccinated with both 13-valent pneumococcal conjugate vaccine (PCV13) and 23-valent pneumococcal polysaccharide vaccine (PPSV23), *Haemophilus* type b conjugate vaccine, and *Neisseria meningitidis* conjugate (MenACWY) and subcapsular protein (MenB) vaccine [14]. Moreover, patients require repeated vaccination. However, a fatal pneumococcal meningitis despite PCV13 vaccination 6 weeks before and a satisfactory IgG response to vaccine antigens in a 7-year-old girl with IRAK-4 deficiency has been reported [26]. Patients that present Mendelian susceptibility to mycobacterial disease (MSMD), due to mutations in genes involved in the IL-12/IFN-γ axis, can't receive live bacterial vaccines. In fact, after BCG vaccine, patients with MSMD can develop localized (such as lymphadenitis or osteomyelitis) or disseminated (fever, weight loss, anemia, and hepatosplenomegaly) complications. In 1996, a case of fatal disseminated BCG infection led to the identification of the IFN-γ receptor deficiency [27].

In 1998, inherited IL-12 deficiency was identified in a patient with a history of BCG lymphadenitis and *Salmonella enteritidis*-disseminated infection [28]. Disseminated BCG osteomyelitis was found in a patient with heterozygous mutation in STAT1 [29].

In patients affected by chronic mucocutaneous candidiasis disease (CMCD), killed/inactivated/recombinant vaccines can generally be administered, while live attenuated pathogens are contraindicated. In fact, in a cohort of 274 patients with STAT1 GOF mutations, Toubiana et al. reported localized and disseminated disease caused by BCG vaccine and severe disease due to live viral vaccine (smallpox and measles) [30].

18.6.2 Vaccines in Phagocytic Defects

Even in the absence of controlled trials, vaccines with inactivated germs are useful, safe, and well tolerated in patients with phagocytic defects [17]. Nevertheless, immunologic response can be compromised. In fact, it has been reported that patients affected by chronic granulomatous disease (CGD) display significantly lower antibody titers against measles and not fully characterized abnormalities of

the B cell compartment, hence a suspect defect in long-term maintenance of the memory response [31]. Regarding the administration of vaccines with live viruses, an increased risk of bacterial complication of viral infections (such as staphylococcus infections on varicella lesions) should be considered in patients with neutropenia, so that these vaccines are recommended in those patients. However, in a few cases affected by leukocyte adhesion deficiency (LAD) or Chediak-Higashi syndrome (CHs), which is characterized by a failure in releasing the cytolytic granules, severe side effects have been reported as a consequence of the impairment of cell-mediated and cytolytic activity [32]. Thus, live viral vaccines are contraindicated in these categories of patients [14]. As for attenuated live bacterial vaccines, an increased risk to develop a disseminated form of mycobacteriosis following BCG vaccination has been reported even a long time after the vaccination. Multiple BCG reactivations have been described, as well [32]. Several studies show that 62–75% of the CGD patients with mycobacterial complications had BCG-related disease [33–35]. Therefore, BCG vaccination is contraindicated in patients with a diagnosis of CGD. Despite the lack of data, live *Salmonella typhi* vaccines should be avoided, due to the high occurrence of such infections in CGD patients, while inactivated *Salmonella typhi* vaccines can be used [14].

18.6.3 Vaccines in Complement Deficiency

All types of vaccines are generally safe in patients with complement deficiency (CD). Post-vaccine immunocomplex-mediated glomerulonephritis has been described in a patient with C2 deficiency who had received the first dose of the combined vaccine with purified antigens; but, the presence of specific antigens was not detected in glomerular immunocomplexes [36]. Concerning the effectiveness of vaccination, all vaccines, including viral vaccines, can be considered sufficiently immunogenic. In particular, conjugate vaccines (pneumococcal, anti-*Haemophilus,* and anti-meningococcus) are strongly recommended in patients suffering from both early component and late component deficiency. Few studies are available on immunogenicity of these vaccines: the serum bactericidal and opsono-phagocytosis activity of patients with CD, who had received an anti-meningococcal tetravalent polysaccharidic vaccine (MPSV), was similar or only slightly lower than those of healthy subjects. However, a significantly increased risk of meningococcal disease persisted in the years following the vaccine administration, especially in the cohort of children who had developed a lower antibody titer [37, 38]. A subsequent study, performed on 22 C2-deficient patients, who had received a tetravalent polysaccharide vaccine, reported a normal antibody response against the serogroups C, Y, and W, but lower against the serogroup A [39]. For these reasons, additional immunization against these pathogens is indicated: a booster dose of tetravalent conjugate meningococcal vaccine every 3 years for 2-month- to 6-year-old patients or every 5 years for patients older than 6 years of age [17]. Furthermore, pneumococcal conjugate vaccine (PCV13) should be followed by pneumococcal polysaccharide 23 vaccine (PPV23) at least 6 months later, to retain protection levels of antibodies [40, 41].

18.7 Vaccinations for Household Members and Caregivers of Patients with Cellular Immunodeficiency

It is mandatory that all close contacts of a patient with cellular defect are immunized against all vaccine preventable diseases, whenever this is possible. In fact, when the patient is forbidden to receive any vaccine, the only way to protect him is through protection of the related people. It is then fundamental to verify that all the contacts are vaccinated: if not already protected, they can receive all killed, inactivated, and recombinant vaccines. In particular, in older household members, it is suggested to administer a booster of anti-pertussis vaccine, since protection obtained through a previous infection is likely to decrease over time. The booster should be repeated every 10 years. In the case of bacterial infection, such as pertussis or meningitis, isolating the patient, observing all the hygienic measures, and, in case of meningitis due to *Neisseria meningitidis or Haemophilus influenzae*, administering antibiotic prophylaxis to the subject are advised. Regarding live attenuated vaccines, OPV should be avoided in household contacts of patients with cellular immunodeficiency because of documented risk of transmission and possible vaccine-related complications [15]. Only inactivated vaccine IPV should be administered.

Measles, mumps, rubella, varicella, and rotavirus can be administered to family members or other close contacts susceptible to infection, since the risk of developing the disease is extremely rare.

Yellow fever and *Salmonella typhi* (Ty21a) can be administered to contacts, as well. Particularly, adults with primary immune deficiency should avoid changing the diaper to children vaccinated with rotavirus in the 4 weeks following the immunization. It is also recommended to verify the immune status against varicella in adult contacts, since they could be not vaccinated and not protected by natural immunization. If a household member had a rash after varicella vaccine, the risk of transmission of infection to an immunosuppressed patient would be very low. The only risky case would be if blisters appeared in correspondence of the inoculation site: in that case it would be better to isolate the patient and to treat him with prophylactic specific immunoglobulins (a single dose within 96 h after exposure) and to treat the contact with antiviral therapy. In the rare case of measles in a household member, patient must receive specific immunoglobulins within 6 days from exposure [2, 17]. As reported by the ACIP, live attenuated influenza vaccine should not be administered in people who care for patients affected by cellular immunodeficiency, in particular SCID, patients who received hematopoietic stem cell transplantation (HSCT) within 2 months, and patients receiving treatment for graft versus host disease (GVHD). Otherwise, the contact with immunosuppressed patients within 7 days after vaccination should be avoided due to the risk of virus transmission [42]. On the contrary, inactivated influenza annual vaccine is recommended in household contacts [2, 17].

18.8 Vaccination in Patients with Cellular Immunodeficiency Undergoing HSCT or Gene Therapy

The loss of vaccine immunity that occurs after SCT is affected by many factors: the strength of pretransplant immunity of the patient and the donor's immune status, the age of the patient at the time of transplantation, the combination of pretransplant chemotherapy regimens and/or radiation therapy, the occurrence of GVHD, and the immunosuppressive therapy following transplantation.

The risk of losing the vaccine immunity is similar after allogeneic and autologous SCT so that recommendations about vaccines are the same. In both cases, vaccination schedule has to be started from the beginning after transplant, considering the patient naïve for any antigens.

In literature, data regarding the effectiveness of vaccines in patients undergoing allogeneic hematopoietic stem cell transplantation for a primary immunodeficiency are limited. It is known that the count of B cells takes 3–12 months to return to normal values. Furthermore, newly generated B cells often show defective Ag-specific response during the first year after transplantation, due to a limited capacity of naïve B cells to undergo isotype switching and somatic mutations [43]. The majority of circulating T cells in the first year after transplantation are T memory/effector, derived from the graft and able to respond to antigens encountered by the donor before transplantation. The naïve T cells capable of responding to new antigens are generated only 6–12 months after transplant, and this occurs earlier in young children than in older ones [44].

Inactivated/recombinant vaccines are safe and are not associated with an increased risk of side effects compared to healthy patients. In general, these vaccines have to be scheduled starting from 1 year after transplant. However, they should be considered in every single case and should be given 6 months after stopping any immunosuppressive therapy.

Three doses of DTP-Polio-Hib-HBsAg, separately or in combination according to age (hexavalent can be used up to the 7th year), two doses of conjugate pneumococcal vaccine, two doses of MenB, and two doses of conjugate MenACWY vaccine should be given. Inactivated influenza vaccine should be given annually [17]. In particular, this vaccine is recommended for all SCT patients at least 4–6 months after SCT [17]. Live vaccines should not be used within 24 months from SCT or in patients with GVHD or immunosuppressive therapy ongoing [45].

Specifically, two doses of MMR and varicella vaccines should be given 24 months after HSCT, but a preliminary immunologic evaluation including lymphocyte subset count and T cell proliferation test is always highly recommended [14].

Moreover, the last Ig infusion must have been drawn up at least 11 months before, no GVHD has to be present, and immunosuppressive therapy must have been stopped at least 3 months before [46].

Donor who has not received recommended vaccinations should be vaccinated for his/her own health; in fact, vaccination of donor aimed to the recipient's benefit is not recommended. Vaccination of donor with live vaccines should be avoided within 4 weeks of donation [17].

Regarding vaccination in patients who had undergone gene therapy, there is no literature available, yet. Nevertheless, we could assume that the same rules of the transplant apply: vaccines will be safe and fully effective when immune reconstitution will be complete.

References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, Franco JL, Holland SM, Klein C, Morio T, Ochs HD, Oksenhendler E, Picard C, Puck J, Torgerson TR, Casanova JL, Sullivan KE (2020) Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40(1):24–64. https://doi.org/10.1007/s10875-019-00737-x
2. Shearer WT, Fleisher TA, Buckley RH, Ballas Z, Ballow M, Blaese RM et al (2014) Recommendations for live viral and bacterial vaccines in immunodeficient patients and their close contacts. J Allergy Clin Immunol 133:961–966
3. American Academy of Pediatrics (2012) Immunization in special clinical circumstances. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS (eds) Red Book: 2012 Report of the committee on infectious diseases. American Academy of Pediatrics, Elk Grove Village (IL), pp 74–90
4. Bayer DK, Martinez CA, Sorte HS, Forbes LR, Demmler-Harrison GJ, Hanson IC, Pearson NM, Noroski LM, Zaki SR, Bellini WJ, Leduc MS, Yang Y, Eng CM, Patel A, Rodningen OK, Muzny DM, Gibbs RA, Campbell IM, Shaw CA, Baker MW, Zhang V, Lupski JR, Orange JS, Seeborg FO, Stray-Pedersen A (2014) Vaccine-associated varicella and rubella infections in severe combined immunodeficiency with isolated CD4 lymphocytopenia and mutations in IL7R detected by tandem whole exome sequencing and chromosomal microarray. Clin Exp Immunol 178:459–469
5. Trimble R, Atkins J, Quigg TC, Burns CC, Wallace GS, Thomas M, Mangla AT, Infante AJ (2014) Vaccine-associated paralytic poliomyelitis and BCG-osis in an immigrant child with severe combined immunodeficiency syndrome—Texas, 2013. MMWR Morb Mortal Wkly Rep 63:721–724
6. Shaghaghi M, Parvaneh N, Ostad-Rahimi P, Fathi SM, Shahmahmoodi S, Abolhassani H, Aghamohammadi A (2014) Combined immunodeficiency presenting with vaccine- associated paralytic poliomyelitis: a case report and narrative review of literature. Immunol Invest 2013 43(3):292–298
7. Canessa C, Romano F, Lippi F, Bianchi L, Kashef S, Rezaei N, Moriondo M, Nieddu F, Martini M, Azzari C (2013) Bcgitis and vaccine-derived poliovirus infection in a patient with a novel deletion in RAG1 binding site. Int J Immunopathol Pharmacol 26:511–515
8. Pai S-Y, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, Kapoor N, Hanson IC, Filipovich AH, Jyonouchi S, Sullivan KE, Small TN, Burroughs L, Skoda-Smith S, Haight AE, Grizzle A, Pulsipher MA, Chan KW, Fuleihan RL, Haddad E, Loechelt B, Aquino VM, Gillio A, Davis J, Knutsen A, Smith AR, Moore TB, Schroeder ML, Goldman FD, Connelly JA, Porteus MH, Xiang Q, Shearer WT, Fleisher TA, Kohn DB, Puck JM, Notarangelo LD, Cowan MJ, O'Reilly RJ et al (2014) Transplantation outcomes for severe combined immunodeficiency, 2000–2009. N Engl J Med 371:434–446

9. Norouzi S, Aghamohammadi A, Mamishi S, Rosenzweig SD, Rezaei N (2012) Bacillus Calmette-Guérin (BCG) complications associated with primary immunodeficiency diseases. J Infect 64:543–554
10. Stephan JL, Vlekova V, Le Deist F, Blanche S, Donadieu J, De Saint-Basile G, Durandy A, Griscelli C, Fischer A (1993) Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. J Pediatr 123:564–572
11. Yeganeh M, Heidarzade M, Pourpak Z, Parvaneh N, Rezaei N, Gharagozlou M, Movahedi M, Shabestari MS, Mamishi S, Aghamohammadi A, Moin M (2008) Severe combined immunodeficiency: a cohort of 40 patients. Pediatr Allergy Immunol 19:303–306
12. Bakare N, Menschik D, Tiernan R, Hua W, Martin D (2010) Severe combined immunodeficiency (SCID) and rotavirus vaccination: Reports to the Vaccine Adverse Events Reporting System (VAERS). Vaccine 28:6609–6612
13. Centers for Disease Control and Prevention (CDC) (2010) Addition of severe combined immunodeficiency as a contraindication for administration of rotavirus vaccine. MMWR Morb Mortal Wkly Rep 59:687–688
14. Martire B, Azzari C, Badolato R, Canessa C, Cirillo E, Gallo V, Graziani S, Lorenzini T, Milito C, Panza R, Moschese V, with Italian Network for Primary Immunodeficiencies (IPINET) (2018 Jun 7) Vaccine 36(24):3541–3554
15. Red Book 2018–2021 (2018). 31st Edition. Report of Pediatrics Committee on Infectious Diseases American Academy of Pediatrics
16. Centers for Disease Control and Prevention (1994) Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. Official authorized addenda: human immunodeficiency virus infection codes and official guidelines for coding and reporting ICD-9-CM. MMWR Recomm Rep 43(RR-12):1–19
17. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, Bousvaros A, Dhanireddy S, Sung L, Keyserling H, Kang I (2014) 2013 IDSA clinical practice guideline for vaccination in the immunocompromised host. Clin Infect Dis 58:e44–e100
18. Hofstetter AM, Jakob K, Klein NP, Dekker CL, Edwards KM, Halsey NA, Baxter R, Williams SE, Graham PL 3rd, LaRussa P (2014) Live vaccine use and safety in DiGeorge syndrome. Pediatrics 133:e946–e954
19. Perez EE, Bokszczanin A, McDonald-McGinn D, Zackai EH, Sullivan KE (2003) Safety of live viral vaccines in patients with chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). Pediatrics 112:e325
20. Al-Sukaiti N, Reid B, Lavi S, Al-Zaharani D, Atkinson A, Roifman CM et al (2010) Safety and efficacy of measles, mumps, and rubella vaccine in patients with DiGeorge syndrome. J Allergy Clin Immunol 126:868–869
21. Leung DYM, Ambrosino DM, Arbeit RD, Newton JL, Geha RS (1988) Impaired antibody responses in the hyperimmunoglobulin E syndrome. J Allergy Clin Immunol 81:1082–1087
22. Albert MH, Notarangelo LD, Ochs HD (2011) Clinical spectrum, pathophysiology and treatment of the Wiskott–Aldrich syndrome. Curr Opin Hematol 18:42–48
23. Giovannetti A, Mazzetta F, Caprini E, Aiuti A, Marziali M, Pierdominici M, Cossarizza A, Chessa L, Scala E, Quinti I, Russo G, Fiorilli M (2002) Skewed T-cell receptor repertoire, decreased thymic output, and predominance of terminally differentiated T cells in ataxia telangiectasia. Blood 100(12):4082–4089
24. Staples ER, McDermott EM, Reiman A, Byrd PJ, Ritchie S, Taylor AMR, Davies EG (2008) Immunodeficiency in ataxia telangiectasia is correlated strongly with the presence of two null mutations in the ataxia telangiectasia mutated gene. Clin Exp Immunol 153:214–220
25. Cesaro S, Giacchino M, Fioredda F, Barone A, Battisti L, Bezzio S et al (2014) Guidelines on vaccinations in paediatric haematology and oncology patients. Biomed Res Int 2014:707691
26. Issekutz A, Top K, McKelvie B, Issekutz T, Letenyi D, McCusker C (2012) Fatal pneumococcal sepsis/meningitis in seven-year-old girl with IRAK-4 deficiency despite antibiotic prophylaxis and repeated pneumococcal vaccination. J Clin Immunol 32:S93

27. Jouanguy E, Altare F, Lamhamedi S, Revy P, Emile JF, Newport M, Levin M, Blanche S, Seboun E, Fischer A, Casanova JL (1996) Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guérin infection. N Engl J Med 335:1956–1961
28. Altare F, Lammas D, Revy P, Jouanguy E, Döffinger R, Lamhamedi S, Drysdale P, Scheel-Toellner D, Girdlestone J, Darbyshire P, Wadhwa M, Dockrell H, Salmon M, Fischer A, Durandy A, Casanova JL, Kumararatne DS (1998) Inherited interleukin 12 deficiency in a child with bacille Calmette- Guerin and Salmonella enteritidis disseminated infection. J Clin Invest 102:2035–2040
29. Boudjemaa S, Dainese L, Heritier S, Masserot C, Hachemane S, Casanova JL, Coulomb A, Bustamante J (2017) Disseminated bacillus Calmette-Guerin osteomyelitis in twin sisters related to STAT1 gene deficiency. Pediatr Dev Pathol 20:255–261
30. Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC, Ouachée-Chardin M, Fouyssac F, Girisha KM, Etzioni A, Van Montfrans J, Camcioglu Y, Kerns LA, Belohradsky B, Blanche S, Bousfiha A, Rodriguez-Gallego C, Meyts I, Kisand K, Reichenbach J, Renner ED, Rosenzweig S, Grimbacher B, van de Veerdonk FL, Traidl-Hoffmann C, Picard C, Marodi L, Morio T, Kobayashi M, Lilic D, Milner JD, Holland S, Casanova JL, Puel A, International STAT1 Gain-of-Function Study Group (2016) Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. Blood 127(25):3154–3164
31. Cotugno N, Finocchi A, Cagigi A, Di Matteo G, Chiriaco M, Di Cesare S, Rossi P, Aiuti A, Palma P, Douagi I (2015) Defective B-cell proliferation and maintenance of long-term memory in patients with chronic granulomatous disease. J Allergy Clin Immunol 135:753–761. e2
32. Oyachi N, Obana K, Suzuki T, Kimura S, Chino K, Oyama T, Takano K (2013) Costal BCG osteomyelitis developing 1 year after BCG vaccination. Pediatr Int 55:641–643
33. Lee PP, Lee P (2015) Disseminated bacillus Calmette-Guérin and Susceptibility to Mycobacterial Infections—Implications on Bacillus Calmette-Guérin Vaccinations *
34. Bustamante J, Aksu G, Vogt G, de Beaucoudrey L, Genel F, Chapgier A, Filipe-Santos O, Feinberg J, Emile JF, Kutukculer N, Casanova JL (2007) BCG-osis and tuberculosis in a child with chronic granulomatous disease. J Allergy Clin Immunol 120:32–38
35. Winkelstein JA, Marino MC, Johnston RB, Boyle J, Curnutte J, Gallin JI, Malech HL, Holland SM, Ochs H, Quie P, Buckley RH, Foster CB, Chanock SJ, Dickler H (2000) Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore) 79:155–169
36. Kersnik Levart T (2013) Post-vaccine glomerulonephritis in an infant with hereditary C2 complement deficiency: case study. Croat Med J 54:569–573
37. Schlesinger M, Kayhty H, Levy R, Bibi C, Meydan N, Phagocytic Killing LJ (2000) Antibody response during the first year after tetravalent meningococcal vaccine in complement-deficient and in normal individuals. J Clin Immunol 20:46–53
38. Fijen CA, Kuijper EJ, Drogari-Apiranthitou M, Van Leeuwen Y, Daha MR, Dankert J (1998) Protection against meningococcal serogroup ACYW disease in complement-deficient individuals vaccinated with the tetravalent meningococcal capsular polysaccharide vaccine. Clin Exp Immunol 114:362–369
39. Brodszki N, Skattum L, Bai X, Findlow H, Borrow R, Jönsson G (2015) Immune responses following meningococcal serogroups A, C, Y and W polysaccharide vaccination in C2-deficient persons: Evidence for increased levels of serum bactericidal antibodies. Vaccine 33:1839–1845
40. Eibl MM, Wolf HM (2015) Vaccination in patients with primary immune deficiency, secondary immune deficiency and autoimmunity with immune regulatory abnormalities. Immunotherapy 7:1273–1292
41. Poolman J, Borrow R (2011) Hyporesponsiveness and its clinical implications after vaccination with polysaccharide or glycoconjugate vaccines. Expert Rev Vaccines 10:307–322
42. Grohskopf LA, Sokolow LZ, Olsen SJ, Bresee JS, Broder KR, Karron RA (2015) Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices, United States, 2015–16 Influenza Season. MMWR Morb Mortal Wkly Rep 64:818–825

43. Olkinuora H, Käyhty H, Davidkin I, Roivainen M, Ölander R-M, Kantele JM, Siitonen S, Vettenranta K (2012) Immunity after (re)vaccination of paediatric patients following haematopoietic stem cell transplantation. Acta Paediatr 101:e373–e377
44. Kennedy LB, Li Z, Savani BN, Ljungman P (2017) Measuring immune response to commonly used vaccinations in adult recipients of allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 23:1614–1621
45. Hilgendorf I, Freund M, Jilg W, Einsele H, Gea-Banacloche J, Greinix H, Halter J, Lawitschka A, Wolff D, Meisel R (2011) Vaccination of allogeneic haematopoietic stem cell transplant recipients: Report from the International Consensus Conference on Clinical Practice in chronic GVHD. Vaccine 29:2825–2833
46. Ljungman P, Cordonnier C, Einsele H, Englund J, Machado CM, Storek J, Small T (2009) Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant 44:521–526. https://doi.org/10.1038/bmt.2009.263

Gene Therapy in Cellular Immunodeficiencies

19

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Abstract

The treatment of cellular primary immunodeficiencies has benefitted from significant advances in the field of allogeneic stem cell transplantation (alloHSCT). However, while this therapy is curative for many PIDs, the procedure requires a suitably matched donor and carries significant risks of morbidity and mortality from complications such as graft-versus-host disease (GVHD). Autologous gene therapy (GT) approaches using stem cells isolated from patients and modified *ex vivo* using viral vectors or gene editing techniques have the potential to offer curative therapy for PID without the immunological complications of

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alloHSCT. GT for PID has been developed over the last 30 years, and while several setbacks have been encountered along the way, there is now a licensed GT product for ADA-SCID, and promising results from phase I/II clinical trials have demonstrated that GT may offer clinical efficacy comparable to alloHSCT in several other PIDs. Developments in the field are broadening the application of GT, and we expect that this therapeutic modality may become standard of care for the management of several PIDs in the near future. This chapter explores the development of GT over the last 30 years and outlines its role in the management of cellular primary immunodeficiencies.

Keywords

Gene therapy · Therapeutics · Primary immunodeficiency · Gene editing Clinical trials

19.1 Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) has for many years been the only curative treatment option for primary immunodeficiencies (PIDs) [1]. The procedure has been performed for PIDs for over 50 years, and advances in this field have led to survival rates of over 95% in pediatric severe combined immune deficiency (SCID) cohorts [2]. The development of HLA-haploidentical stem cell transplantation using posttransplant cyclophosphamide (PTCy) or graft manipulation with α/β T cell and B cell depletion has reduced problems with donor availability, and survival outcomes are now comparable to HLA-matched HSCT [3–5]. However, alloHSCT still carries a risk of graft-versus-host disease (GVHD) and graft failure, which can result in significant morbidity and mortality. AlloHSCT carries higher risks in older cohorts with non-SCID PIDs, thus limiting the application of the procedure for some patients [6, 7].

Autologous gene therapy (GT) completely removes the risk of GVHD and the requirement for a suitably matched donor [8, 9]. Autologous procedures are less toxic than allogeneic approaches, as the lack of immunogenicity permits engraftment of HSCs with reduced intensity conditioning and removes the need for immune suppression as GVHD prophylaxis. Current autologous GT approaches using lentiviral vectors have demonstrated clinical benefits at least equivalent to alloHSCT, without the associated immunological complications [10–12]. This exciting emerging field has already shown impressive, long-term results for a number of conditions with the potential to offer curative therapies for many more PIDs and supplant alloHSCT as the standard of care for these rare but devastating disorders.

Ex vivo GT involves harvesting hematopoietic stem cells (HSCs) from a patient, either by direct aspiration from the bone marrow or from mobilized peripheral blood using GCSF and plerixafor to allow collection of large numbers of CD34+ HSCs (Fig. 19.1). Harvested HSCs are cultured ex vivo in conditions that favor

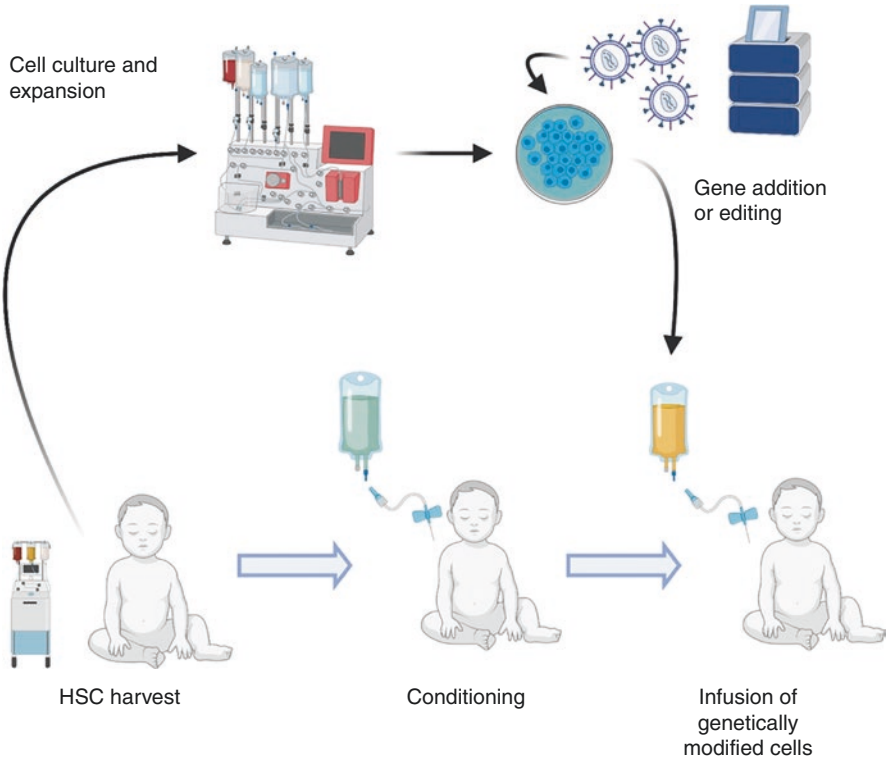

Fig. 19.1 Overview of autologous gene therapy (GT) procedure. Created with permission from BioRender.com

expansion of cells with long-term repopulating potential [9, 13, 14]. Gene transfer or gene editing is performed under sterile conditions, and necessary quality checks are performed to ensure that the genetically modified product is safe to be reinfused to the patient, termed GMP (good manufacturing practice) compliant. Prior to reinfusion, the patient receives conditioning with chemo-immunotherapy to deplete HSCs and permit engraftment of the genetically modified stem cells. Varying degrees of conditioning can be used to optimize engraftment but minimize side effects in different PIDs, for example, myeloreductive conditioning in SCID compared to more intense conditioning in GT trials for chronic granulomatous disease (CGD) [12, 15].

The genetically modified cells are reinfused to the patient and engraft in the bone marrow. After engraftment, the genetically modified HSCs have the potential to self-renew for the lifetime of the recipient and, as they differentiate, give rise to gene-corrected progeny across all immuno-hematopoietic lineages [9, 16].

Retroviruses and lentiviruses were identified as candidate vectors for the transduction of HSCs due to their ability to efficiently integrate their DNA into host cells.

HSCs are an ideal cellular target because they are long-lived, can be accessed with relative ease for *ex vivo* manipulation, and are multipotent, so genetic correction will be passed on to all progeny across different cell lineages [16, 17]. Prior to a detailed discussion of GT in cellular primary immunodeficiencies, it is worth evaluating which genetic disorders are most suitable for a GT approach.

Most importantly, the nature of the genetic defect needs to be considered. PIDs are excellent candidates for GT due to the clear link in many subtypes between defined monogenic defects and a clinical phenotype of immune dysfunction and dysregulation. PIDs arising from monogenic germline mutations can result in reduced or absent protein expression (loss of function, LOF) or increased or overactive protein expression (gain of function, GOF). Mutations can be heterozygous (haploinsufficiency or dominant negative) or homozygous [18]. The mutational landscape and variability in both genetic penetrance and clinical phenotype are also important. It is easier to target and then validate the efficacy of GT for diseases caused by single mutations that have a clear clinical phenotype, as opposed to diseases with heterogeneous mutational landscapes and variable clinical phenotype. Another important factor relates to the normal expression and function of the encoded protein. For example, it is helpful to know whether supra-physiological levels of ubiquitously expressed proteins are potentially harmful. It is logical therefore that a disease caused by homozygous LOF mutations resulting in reduced or absent expression of a ubiquitously expressed protein would be the least complicated disorder to attempt a GT approach. Indeed, adenosine deaminase-deficient SCID (ADA-SCID) fits this description, and it is therefore not surprising that ADA-SCID was the first genetic disorder for which *ex vivo* GT was attempted and for which GT approaches are now at the most advanced stage of clinical development.

As GT has advanced, the application of the technology to more complex disorders has begun and preclinical proof-of-concept studies are leading to clinical trials. Where normal protein expression is tightly regulated and/or lineage restricted, correction of expression by GT should, where possible, replicate this. Gene editing will prove to be particularly useful in these disorders where correction in situ allows physiological gene regulation from all native regulatory elements. Finally, the effect of transgene expression on the HSCs and/or their progeny is important. Where the transgene confers a significant survival advantage, the corrected cells are more likely to proliferate in vivo.

This is a rapidly changing field and developments are broadening the application of GT to an increasing number of PIDs. Newer gene editing techniques have the potential to overcome some of the limitations of viral gene addition strategies [9]. This chapter will describe the history of GT and some of the early problems that were encountered with the technique. The early applications of GT will be described before outlining the application of GT to different PIDs. The limitations of gene addition will be considered, before explaining how gene editing may overcome some of these issues. Finally, gene editing techniques will be explained and their application to PID GT outlined.

19.2 History of Gene Therapy (GT)

The pathway to the successful application of *ex vivo* HSC GT to PIDs has not been straightforward. The concept of using viral vectors to insert a functional copy of the defective gene directly into the cellular chromosomal DNA was met with initial excitement. The first PID for which GT was attempted was ADA-SCID, a multisystem, life-threatening disorder resulting from the absence of an essential enzyme, adenosine deaminase. Although a multisystem disorder, it is the immunological defect which is fatal and only this feature which can be corrected by both HSCT and GT [19]. The first clinical trials in humans, published in 1995, used gammaretroviral (γRV) vectors to introduce the ADA gene initially into T lymphocytes and then into HSCs [15, 20–22]. Most patients received low-intensity conditioning chemotherapy prior to administration of gene-corrected HSCs. The results of these early trials showed great promise with evidence of metabolic correction and immune reconstitution. Importantly, the gene-corrected cells persisted in the patients treated, demonstrating that HSC GT could offer a cure for ADA-SCID.

Encouraged by the results in the ADA-SCID trials, researchers began working on GT approaches for X-linked SCID (X-SCID), which results from mutations in the common cytokine receptor gamma chain (IL-2 receptor gene (*IL2RG*)). Again, early results using γRV vectors were encouraging with successful immune reconstitution and persistence of gene-corrected cells. However, optimism turned to concern when five of the 20 patients treated developed leukemia [23, 24]. This was caused by integration of the vector close to known proto-oncogenes and subsequent leukemic transformation. Although this was clearly of great concern, these early trials demonstrated the potential that HSC GT had for treating patients with X-SCID with one comparative study demonstrating that gene-corrected autologous HSCs offered improved immune reconstitution compared to haploidentical alloHSCT [25]. Leukemogenesis was also observed in a clinical trial using γRV vector-based therapy for Wiskott-Aldrich syndrome (WAS). While nine of the ten patients treated demonstrated correction of WAS protein (WASP) expression, seven patients developed leukemia due to vector integration close to proto-oncogenes. In this trial additional chromosomal translocations were also observed [26, 27]. A further trial in a third PID, chronic granulomatous disease (CGD), using γRV vectors encountered a similar problem. Insertional activation of ectopic viral integration site 1 (*EVI1*) leads to oligoclonal hematopoiesis and myelodysplasia with monosomy 7 in patients treated [28]. Further investigation demonstrated that the transgene was being silenced by methylation of promoter elements in the long terminal repeats whereas the enhancer elements were unaffected, resulting in mutagenesis [29, 30]. Interestingly, insertional mutagenesis was not observed in any of the patients treated with γRV vectors for ADA-SCID, despite similar vector integration patterns, for reasons which remain unclear [15, 31].

Although it was shown that leukemogenesis requires the acquisition of several secondary genetic lesions in addition to the initial event resulting from vector

insertion, there was significant concern about the ongoing safety of GT using γRV platforms [24]. This prompted the development of alternative vectors with the aim of improving safety. Typically, γRV vectors use strong viral promoters in the long terminal repeat (LTR) sequences to drive transgene expression. Self-inactivating (SIN)-γRV-vectors were developed by mutating the LTR sequences and inserting a less powerful mammalian promoter [32, 33]. Furthermore, a new gene transfer platform was developed based on the human immunodeficiency virus (HIV), a lentivirus (LV) which has an integration pattern associated with a lower risk of oncogene activating insertions [34–36]. The LV gene transfer vectors utilize the basic platform of HIV enabling insertion of genetic material into chromatin. Additional significant alterations removing their pathogenic potential and utilizing other viral envelopes for packaging have been made, thus enabling a range of cell types to be targeted [37, 38]. LV vectors were not only shown to have a more neutral insertion profile compared to γRVs but were also more efficient at gene transfer [34]. Induction of cell cycling by cytokine stimulation is required for retrovirus integration [39]. This pre-stimulation step prolongs the *ex vivo* manipulation and has been shown to reduce the long-term repopulating potential of the manipulated HSCs and impair their engraftment [40, 41]. Lentiviruses in contrast are able to transduce proliferating and non-proliferating cells, reducing the amount of *ex vivo* manipulation required and preserving HSC function [42, 43].

Since the development of SIN-γRV and SIN-LV vectors, no insertional oncogenic events have been reported to date, and although the theoretical risks remain, these platforms appear to be safe. To date, 29 phase I/II HSC GT clinical trials have been completed, are in follow-up or are recruiting for the most common monogenic PIDs (SCID, WAS and CGD) [44]. Indeed, a SIN-γRV vector for ADA-SCID was the first HSC GT product to receive marketing approval in 2016 [45]. *Strimvelis*, a γRV vector containing the adenosine deaminase (ADA) coding DNA (cDNA), is now available in Europe for patients with ADA-SCID and is currently licensed for use in patients who lack a suitable sibling donor for alloHSCT [31, 46]. The results of these trials and the application of HSC GT to specific PIDs will be discussed later in this chapter.

As the field develops, the number of PIDs for which HSC GT can be applied expands. In parallel, there has been a change in the demographics of patients who may benefit from HSC GT procedures. GT trials in SCID have exclusively treated infants and very young children, as survival beyond this is not possible without definitive treatment. As HSC GT approaches were developed for non-SCID PIDs, the possibility of treating adolescents and adult patients has arisen. There are many patients with PIDs such as WAS and CGD who survive to adulthood without an alloHSCT procedure, due to earlier mild disease phenotype, better supportive care, lack of a suitable donor for alloHSCT or the presence of comorbidities which increase the risks of an allogeneic procedure [6, 7]. There were concerns that older patients may not retain their ability to recover a full T cell repertoire using an autologous GT approach. The first report of HSC GT in adults was published in 2017 in a 30-year-old patient with WAS for whom a HLA-matched donor was not available and a haploidentical alloHSCT was deemed too high risk due to pre-existing

disease-related comorbidities [47]. Fears regarding differing reconstitution patterns in older patients appeared to be unfounded, and this first patient underwent rapid engraftment and expansion of a polyclonal pool of gene-corrected T cells and had sustained gene marking in myeloid and B cell lineages [47]. Adult patients have since been recruited to HSC GT trials including the recent, international, multicenter, phase I trial of lentivirus-based gene therapy for X-linked CGD. This trial recruited nine patients, but six of these were aged over 18 years old and the oldest was 27. Following GT, all patients had successful engraftment of gene-modified HSCs, and seven of those treated remained free of new infections at last follow-up [12]. Indeed, with advances in alloHSCT and haploidentical approaches reducing problems with donor availability, it may be that older patients, who have a higher risk of GVHD post alloHSCT, will stand to benefit most from autologous GT procedures [48].

The pathway to successful GT for cellular PIDs has been far from straightforward. However, the early tragic adverse events have driven a huge number of scientific advances in vector development and gene delivery. Optimism has returned to the field, as several clinical trials of GT approaches have demonstrated promising results in an increasing number of cellular PIDs.

19.3 Current Status of Gene Therapies in PID

19.3.1 Severe Combined Immune Deficiencies, SCID

19.3.1.1 ADA-SCID

HSC GT approaches for the commonest genetic causes of SCID were some of the first to be developed, and consequently are at the most advanced stage of clinical development. As previously stated, ADA-SCID was the first application of HSC GT. The disease is an ideal candidate for an autologous approach due to the relatively small size of the *ADA* cDNA (1.5 kb), simplifying cloning into a viral vector. Ubiquitous expression of ADA makes it safer to use the strong viral promoters in the γRV LTRs to drive transcription of the gene [49]. Untreated, ADA-SCID is fatal in infancy, and prior to GT, treatment options were either chronic enzyme-replacement therapy or alloHSCT. Due to the inability of a patient with ADA-SCID to reject sibling HSCs, alloHSCT can be performed in the absence of conditioning. In later GT clinical trials for ADA-SCID, prompt and secure engraftment of gene-corrected HSCs following low-dose busulfan conditioning (≤25% myeloablative dose) was observed [50, 51]. The finding that improved multi-lineage engraftment could be achieved with the addition of reduced intensity conditioning (RIC) has resulted in the adoption of conditioning for most autologous GT applications, even for underlying diseases with profound T cell lymphopenia [45]. As previously mentioned, a γRV-vector GT product, *Strimvelis*, is licensed in Europe for ADA-SCID and is available for patients who lack a HLA-matched donor for alloHSCT [46]. This is a fresh cell product meaning that patients must travel to Milan (the only treatment center) for the procedure. This fresh cell product model was used in many

clinical trials, but the advent of cryopreserved products will improve logistical accessibility, although probably not economic accessibility.

A SIN-LV approach for ADA-SCID has also been developed, and efficacy has been further improved by codon optimization of the ADA-cDNA, expressed under the control of the elongation factor 1α short (EFS) promoter. Promising preclinical studies of this product have led to clinical trials utilizing preconditioning with a single dose of busulfan (4–5 mg/kg) or more recently targeted AUC, prior to infusion of gene-modified HSCs [22, 52]. To date, over 30 patients have been treated with lentiviral GT in the United States and the United Kingdom with 100% survival and no complications associated with vector insertion [44, 53]. Longer-term follow-up is required to assess extent and durability of immune reconstitution.

19.3.1.2 X-Linked Common Gamma Chain SCID

For X-SCID, initial trials with γRV vectors were complicated by insertional mutagenesis. Subsequently, a trial of a SIN-γRV vector-transduced HSCs in X-SCID delivered without pre-conditioning demonstrated similar efficacy in terms of immune reconstitution compared to the earlier γRV vector trials but with less clustering of insertion sites around proto-oncogenes [32]. AlloHSCT can be performed in X-SCID without any conditioning; however, as B cell development is preserved, this may result in mixed chimerism in non-T cell lineages and persistence of recipient HSCs. The experience of autologous HSC GT in X-SCID has been similar, and gene-corrected T cells have been shown to consistently develop in the absence of conditioning, as gene correction results in functional T cell survival and proliferation conferring a competitive advantage. However, as no gene correction is seen in the myeloid compartment and B cells, patients remained immunoglobulin dependent [32]. Humoral immunity can be restored by HSC GT using low-dose busulfan conditioning prior to infusion of gene-marked stem cells [54]. At the time of writing, there are several clinical trials for X-SCID using LV vectors and low-dose busulfan conditioning [44]. Preliminary results of a dual-center phase I/II trial recently reported were extremely encouraging. After a median follow-up of 16.4 months, the eight patients treated demonstrated normal T cell and NK cell numbers and sustained gene marking across all lineages. The patients demonstrated normal IgM levels and antibody responses [54]. While longer-term follow-up will be required to assess the durability of the immune reconstitution, lentiviral GT for X-SCID appears very promising.

19.3.1.3 RAG1 and Artemis SCID

Due to the severity of the clinical phenotype, SCID caused by mutations in recombination genes are obvious candidates for HSC GT. Mutations in the *Artemis* gene and in recombinase-activating gene 1 and 2, *RAG1* and *RAG2* result in V(D)J recombination defects that cause severe impairment of T cell and immunoglobulin receptor rearrangement. Clinically this results in profound immune dysregulation [55]. A varied spectrum of combined immunodeficiency results from *RAG1* defects with the clinical phenotype resulting from hypomorphic mutations being particularly variable [56].

In addition to the absence of T and B cells, *Artemis* mutations result in cellular radiosensitivity and a predisposition to malignancy [57]. *Artemis* SCID is difficult to treat with alloHSCT, as the conditioning therapy is poorly tolerated due to the underlying sensitivity to ionizing radiation and alkylating chemotherapy [58]. A HSC GT approach, using SIN-LV vectors with transgene expression driven by the phosphoglycerate kinase (PGK) promoter, has been shown in murine models to result in restoration of T and B cell repertoires [57]. More recently, a SIN-LV vector incorporating the *Artemis* cDNA under the influence of the endogenous *Artemis* promoter has been shown to restore T and B cell function in vivo following adoptive transfer of transduced murine stem cells [59]. A humanized SIN-LV vector based on this has been shown to correct the radiosensitivity of fibroblasts isolated from Artemis SCID patients. Restoration of T and B cell development after transduction of mobilized CD34+ cells isolated from an Artemis SCID patient has also been demonstrated [58]. This vector has now entered phase I clinical trials in the United States (NCT03538899).

HSC GT approaches for *RAG1* deficiency have previously used γRV vectors which restored T and B cell function but resulted in lymphoproliferation [60]. A SIN-LV-based approach incorporating codon-optimized *RAG1* cDNA has demonstrated adequate immune reconstitution, but only at high vector copy numbers in a backbone unsuitable for large-scale production [61]. Generation of low RAG1 expression levels in cells transduced with this vector with a lower copy number resulted in incomplete thymic reconstitution and the development of an Omenn-like syndrome with autoreactive T cells [62]. While the development of GT for *RAG1* deficiency has been challenging, at the time of writing a SIN-LV-based approach has recently been reported. In the development of this vector, different promoters were tested and an MND (myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer binding site substituted promoter)-driven vector was found to result in restoration of *RAG1* deficiency at lower vector copy numbers. Using this vector, B and T cell reconstitution was observed in mice with adequate *RAG1* expression [63]. This approach is expected to enter phase I clinical trials in the next 12 months.

19.3.2 Non-SCID PIDs

19.3.2.1 Chronic Granulomatous Disease, CGD

Mutations in genes coding for the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex are responsible for CGD, a multisystem PID characterized by hyperinflammation and severe, recurrent bacterial and fungal infections. The most common, X-linked variant of the disease is caused by mutations in *CYBB* which encodes the gp91phox subunit of NADPH oxidase. The three autosomal recessive forms of CGD are rarer and cumulatively account for a third of CGD cases [64]. AlloHSCT has been shown to be curative in CGD across all age groups [65, 66].

As previously mentioned, HSC GT was attempted for CGD some 15 years ago with γRV vectors, but initial results were disappointing. The first trials in 1995 and 1997 for AR-CGD and X-CGD, respectively, used murine Moloney retroviral vectors to transduce HSCs, which were then administered without conditioning [67, 68]. Contrary to GT in SCID, the genetically modified HSCs did not have a significant survival advantage over uncorrected HSCs, and thus very low levels of engraftment and persistence were observed [68]. Following the observation in ADA-SCID GT that low-dose busulfan conditioning aided engraftment, subsequent CGD trials have used pre-conditioning prior to infusion of gene-modified HSCs [50]. In 2005, a German group used a γRV vector derived from a murine spleen focus-forming virus which was expected to offer improved but not specific gene transfer to the myeloid lineage [69]. While improved gene marking in myeloid cells was observed initially, unfortunately this trial was complicated by potent enhancer element-driven clonal expansion following gene insertion in the *EVI1/MDS1* gene complex and the development of a monosomy 7-derived MDS [28, 70]. A later trial in 2006 employing the same γRV vector used in the original 1997 trial but with busulfan conditioning achieved improved, but still poor long-term correction of neutrophils (1.1% at 6 months, as measured by flow cytometric analysis of dihydrorhodamine (DHR) oxidation) [70].

In order to mitigate the risk of insertional mutagenesis while improving gene marking in myeloid cells, a SIN-LV vector has been designed for X-CGD which encodes the codon-optimized human *CYBB* cDNA driven by a novel chimeric myeloid-specific promoter [71, 72]. This vector entered multicenter clinical trials in the United States and Europe, and the initial results of nine patients have recently been reported. At 6 months, 16–46% of neutrophils were oxidase-positive in treated patients with stable vector copy numbers [12]. This trial included six patients aged >18 years at time of entry to the study, providing evidence that GT is safe and effective in older patients with CGD. A similar SIN-LV-based strategy for AR-CGD is also in development [64]. Many older patients with CGD have significant comorbidities as a result of many years of infections and antimicrobial therapy and/or refractory inflammation. AlloHSCT for adults with CGD is safe and effective, but the TRM is higher than for children, particularly if only unrelated donors are available [6, 73, 74]. These promising results offer hope of a curative therapy for older CGD patients who may otherwise have limited treatment options. Longer follow-up and expanded studies are needed to assess clinical efficacy of HSC GT against alloHSCT in lower-risk patients.

19.3.2.2 Wiskott-Aldrich Syndrome (WAS)

WAS is an X-linked PID characterized by recurrent infections, thrombocytopenia and eczema and frequently complicated by autoimmunity and lymphoid malignancy. It results from mutations in the WAS gene which encodes WASP. WASP regulates the polymerization of actin and is critical for immunological synapse formation, cell migration and cytotoxicity [75]. The first GT trial for WAS used a γRV

vector. WASP expression was driven by a strong viral promoter, but this resulted in insertional mutagenesis due to gene insertion close to proto-oncogenes, and the majority of patients treated developed acute leukemia or myelodysplasia [26, 27].

As previously noted, SIN-LV vectors have a more neutral insertion pattern compared to γRV vectors. A SIN-LV vector was developed that used a fragment of the endogenous *WAS* gene promoter [76]. This approach entered clinical trials in Europe and the United States in 2010 with a reduced intensity conditioning regimen consisting of busulfan and fludarabine. Over 20 patients have now been treated and results are encouraging. The GT procedure resulted in good immune reconstitution. Engraftment of gene-marked cells was maintained with several patients now having over 5 years of follow-up. WASP expression was increased across all lineages, and a significant clinical improvement was noted with seven of eight patients in one cohort ceasing their immunoglobulin replacement therapy [77]. Platelet count recovery was variable, but many patients have become independent of platelet transfusions [10, 77, 78]. As previously mentioned, this trial included the first demonstration that HSC GT could be safely performed in adults with PID when a 30-year-old patient was successfully treated. This adult patient had significant disease-related comorbidities and no HLA-matched donor available. Following GT, they were able to discontinue immunosuppression for autoimmune complications and stop immunoglobulin replacement therapy [47]. No genotoxicity has been observed with prolonged follow-up, and this approach appears to be a very promising and safe alternative to alloHSCT for WAS.

19.3.2.3 Leukocyte Adhesion Defect Type 1 (LAD-1)

LAD-1 results from defects in the *ITGB2* gene which encodes the CD18 integrin subunit expressed at the plasma membrane. Reduced membrane expression of CD18 results in impaired neutrophil migration and manifests clinically as severe, recurrent bacterial infections. Untreated, few patients survive past infancy and alloHSCT remains the only curative therapy. GT approaches with γRV vectors failed to result in sustained gene marking [79]. Successful preclinical work using lentiviral vectors in murine and canine models, however, has led on to human clinical trials in Europe and the United States (NCT03825783, NCT03812263) using busulfan conditioning [80, 81].

19.4 GT Approaches in Preclinical Development

19.4.1 RAG2 Deficiency

Correction of *RAG2* deficiency has been demonstrated using *RAG2* knockout mice and a γRV vector, although a selective advantage of transduced lymphoid cells was noted, raising concerns of insertional mutagenesis [82]. A SIN-LV vector containing codon-optimized *RAG2* driven by the ubiquitous chromatin opening element

(UCOE) has been shown to correct *RAG2* deficiency in a murine model without any potentially oncogenic events in 28 treated mice [83]. A clinical trial is being planned.

Hypomorphic mutations in recombination activating genes result in the distinct phenotype, Omenn syndrome (as seen when low-level *RAG1* activity was generated in the early *RAG1* SIN-LV preclinical study) [62]. More recently, a SIN-LV vector, encoding *RAG2*, corrected the immunodeficiency and autoimmunity in a murine model of Omenn syndrome ($RAG2^{R229Q/R229Q}$). This preclinical work demonstrates that HSC GT may be effective in an autoinflammatory environment where the risks of alloHSCT may be higher [84].

19.4.2 IPEX Syndrome

Regulatory T cells (T_{reg}) require the FoxP3 transcription factor for normal development, and mutations in FoxP3 result in the devastating PID, immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. IPEX syndrome is characterized by severe autoimmunity with enteropathy, type 1 diabetes and eczema [85]. AlloHSCT is the only curative therapy; however, overall survival from this procedure in IPEX syndrome is similar to patients who received chronic immunosuppression and was frequently complicated by immune-mediated complications [85]. It has been shown in murine adoptive transfer experiments that ex vivo correction using a LV vector encoding *FoxP3* and the EF1α promoter can generate functional T_{reg}-like cells from conventional $CD4^+$ T cells [86]. However, it is possible that a T cell-based strategy may not generate adequate numbers of T_{reg}-like cells that persist long term [87]. Modified HSCs with their self-renewing properties may circumvent the potential problems of a T cell transfer approach. A California-based group recently demonstrated lineage-specific FoxP3 expression and abrogation of autoimmunity following adoptive transfer of LV-transduced HSCs in a mouse model of IPEX syndrome. They found that ubiquitous FoxP3 expression was detrimental to HSC proliferation and differentiation, with marked defects in peripheral mature cells when a LV vector containing the FoxP3 cDNA used the MNDU3 promoter. An alternative LV vector was designed incorporating the endogenous FoxP3 promoter and including some of the FoxP3 regulatory regions. HSCs transduced with this alternative LV vector were able to confer lineage-specific gene expression in the progeny of the transduced HSCs [88]. GT approaches for IPEX syndrome have not entered clinical trials at the time of writing although this is expected in the near future. The development of this preclinical proof-of-concept GT approach for IPEX syndrome highlights how the choice of promoter can influence the success of the strategy.

19.4.2.1 Familial Hemophagocytic Lymphohistiocytosis (FHL)

In PIDs where the defect is confined to lymphoid cells, there is the potential for employing a strategy where gene-modified T cells alone are transferred as opposed

to HSCs. The rapidly advancing field of adoptive cellular therapies has demonstrated that genetically modified autologous T cells can persist and proliferate in vivo [89, 90]. A T cell approach is particularly attractive when the transgene is not expressed at the progenitor level such as the perforin 1 (*PRF1*) gene. Mutations in *PRF1* account for the majority of cases of familial hemophagocytic lymphohistiocytosis (FHL) [91]. In a murine model of FHL ($Prf^{-/-}$), $CD8^+$ T cells transduced with a γRV vector incorporating the perforin cDNA successfully engrafted and demonstrated restored cytotoxic function [91]. Clinical trials are needed in order to assess whether T cell GT could be an effective long-term treatment or whether this should be used as a bridging therapy to more definitive treatment such as alloHSCT or HSC GT. Indeed, the potential of HSC GT for perforin defects has also been demonstrated in a preclinical setting. LV vectors expressing the human perforin gene under the influence of a phosphoglycerate kinase (PGK) promoter resulted in perforin expression in mature T and natural killer (NK) cells following gene transfer to murine progenitor cells and adoptive transfer experiments [92]. Perforin GT has not entered clinical trials at the time of writing although trials are in the planning stages. Gene therapy approaches are also being developed for the other major cause of FHL, Munc 13-4 defects [92].

19.4.2.2 X-Linked Lymphoproliferative Disease 1 (XLP1)

X-linked lymphoproliferative disease 1 (XLP1) is another PID for which a T cell GT strategy may be effective. Mutations in the *SH2D1A* gene result in defects in the SLAM-associated protein (SAP), an intracellular adaptor protein, important for normal T cell and NK-mediated cytotoxicity [93]. AlloHSCT remains the only curative therapy for XLP1, and outcomes are influenced by the presence or absence of active disease at the time of transplantation [94]. As patients with active disease at transplantation have inferior outcomes, GT may be particularly beneficial for this group. Promising preclinical work has demonstrated that T cells transduced with an LV vector incorporating *SAP* cDNA were able to engraft in *SAP*-deficient mice. T cells from patients affected by XLP1 demonstrated improved cytotoxicity following transduction with this LV vector [95]. These results suggest that autologous T cell GT may present an alternative therapeutic option for patients with XLP1 and a clinical trial is being planned. In parallel, HSC gene editing approaches are also in preclinical development (Booth, personal communication).

19.5 Gene Editing

While SIN-γRV and SIN-LV HSC GT approaches are either "in clinic" or in the advanced stages of development for a number of disorders, many challenges to this approach remain. While there have been no instances of insertional mutagenesis with SIN vectors, a theoretical risk of genotoxicity from semi-random integration of the transgene after gene transfer remains [8, 9]. SIN-γRV and SIN-LV HSC GT approaches have been proven to be highly successful in some PIDs such as ADA-SCID where ubiquitous protein expression is needed and overexpression of the

protein does not have adverse effects [50]. However, even in diseases where LV-mediated gene addition has been successful such as WAS and variants of CGD, the extent of gene transfer and engraftment of the genetically modified HSCs can vary between patients and trials [10]. For other PIDs, gene addition strategies are unlikely to be successful.

Diseases resulting from defects in genes which require stringent control of expression such as those involved in cell activation or intracellular signalling may be less likely to benefit from traditional gene addition, and indeed such strategies may carry significant risks. PIDs which fit this description include X-linked agammaglobulinemia (XLA) caused by mutations in Bruton tyrosine kinase (*BTK*) and X-linked hyper-IgM syndrome, caused by mutations in CD40 ligand [96]. In recent years, gene editing technologies have made it possible to repair genetic defects, insert additional genetic material or remove deleterious sequences with relative ease. For the GT field this technology offers great potential, as for the first time, a gene can be repaired or altered in its native site retaining endogenous regulatory elements [96, 97].

Gene editing has been made possible by the development of designer DNA endonucleases that can introduce a double-stranded DNA break at a specific target sequence. Transcription activator-like effector nuclease (TALENs), zinc-finger nucleases and meganucleases can all perform a similar function; however in 2012 the development of the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats associated with Cas9 endonuclease) RNA-based system has spurred the development of gene editing strategies [98]. The CRISPR-Cas9 system enables the same high specificity of sequence targeting of the other endonuclease systems yet is much easier to use due to the guidance of the Cas9 endonuclease to its target site being governed by Watson-Crick base pairing [97, 99]. Left to its own devices, DNA preferentially repairs through error-prone non-homologous end joining (NHEJ). However, in 1994 it was demonstrated that the introduction of a "donor" DNA template could result in homology-directed repair (HDR) (Fig. 19.2) [100]. The "donor" DNA template can be single- or double-stranded DNA with homology arms which extend either side of double-strand DNA break. Adeno-associated virus serotype 6 (AAV6) vectors have been proven to be a highly efficient platform to introduce a "donor" template to T lymphocytes and HSCs, and high levels of HDR have been observed using a combination of CRISPR-Cas9 (or TALENS) and AAV6 vectors [101–103]. AAV6 vectors have been developed without an integrative capacity by removing *rep* and *cap* from the viral genome [104]. A gene cassette up to 4.8 kilobases long can be cloned into the AAV6 genome between the inverted terminal repeats (ITRs) at either end of the single-stranded DNA (ssDNA) genome. When cells are transduced with an AAV6 vector at the same time as a double-strand DNA break is made, the AAV6 vector enters the cell nucleus and provides the repair template that enables HDR to occur. The AAV6 DNA is lost when the cell divides as the episomal DNA is not replicated in the process of cell division [102, 105–107]. The genetic edit which resulted from the induced HDR however is passed on to the progeny of the cell resulting in permanent modification of the genome.

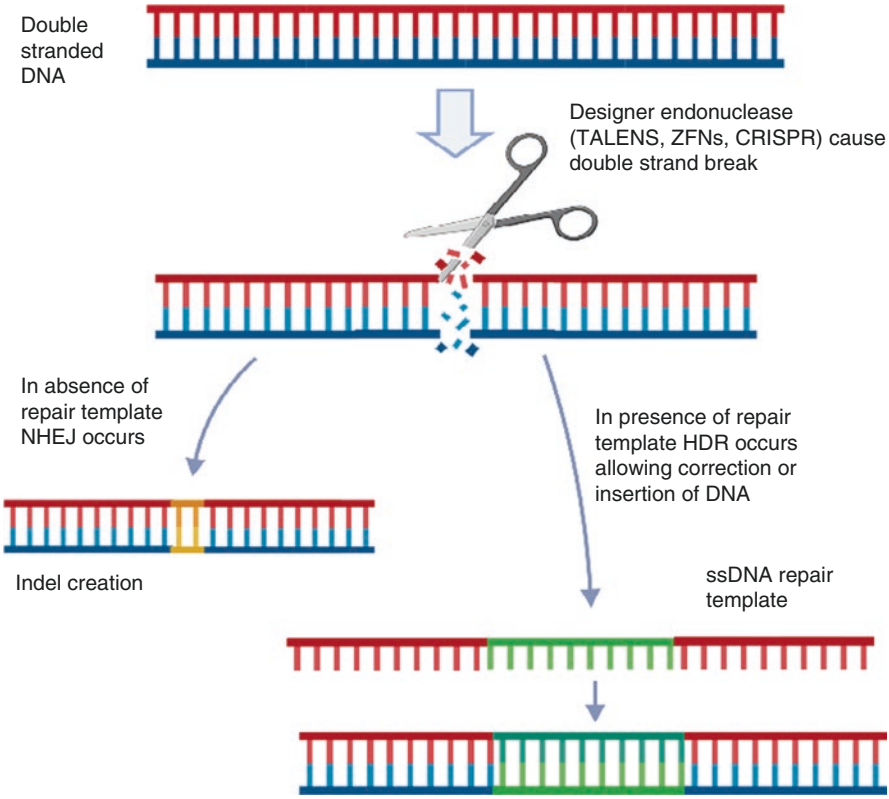

Fig. 19.2 Schematic representation of DNA repair mechanisms utilized in gene editing. Created with permission from BioRender.com

Gene editing techniques have entered clinical trials in humans. The first study modified the CCR5 co-receptor for HIV using ZFNs in CD4+ T cells [108]. At the time of writing, there are 17 clinical trials of gene editing-based therapeutic interventions worldwide listed on international clinical trial registries. These include gene editing of the CCR5 co-receptor in HSCs, gene editing of immune checkpoints such as PD1 in T cells to treat malignancies, and gene editing for the treatment of the hemoglobinopathies; sickle cell anemia; and β-thalassemia major. While there are no gene editing-based strategies for PID in clinical trials at the present time, several promising preclinical studies have been conducted. Proof of concept has been demonstrated for many PIDs including ADA-SCID, X-SCID, X-CGD, IPEX syndrome, WAS, and CD40 ligand deficiency [101, 103, 109–112].

There are several different gene editing approaches that can be utilized depending on the mutational landscape of the disease being treated. The first, *direct*

repair of the disease-causing mutation, is appropriate when a single mutation is responsible for the majority of clinical cases. An example of this in PID is the direct repair of the *CYBB* 676 locus in $CD34^+$ cells for the treatment of X-CGD. This proof-of-concept study confirmed repair in >20% of HSCs, which was sufficient to restore NADPH oxidase function in myeloid cells [111]. The second gene editing approach is to *insert a cDNA cassette into the endogenous locus of a particular gene*. Examples in PID are SCIDX1, hyper-IgM syndrome, X-linked agammaglobulinemia (XLA) and WAS [101, 103, 113, 114]. The preclinical studies in hyper-IgM syndrome and XLA are particularly noteworthy as these are two PIDs for which a gene addition strategy is unlikely to be successful due to concerns about regulation of the transgene. Expression of CD40L (in hyper-IgM syndrome) has been achieved with gene addition strategies, but unregulated expression resulted in lymphoproliferation [115]. Similarly, gene addition strategies have been successful in preclinical studies for XLA, but as BTK confers a significant selective advantage to the expressing cells, gene editing is likely to be a safer option as the endogenous regulatory machinery can remain intact [116, 117]. Similarly, heterozygous mutations in immune regulatory proteins such as CTLA4 or where GOF mutations result in PID such as mutations in APDS1, gene editing may be able to achieve physiological correction in a way that would not be possible using viral gene addition.

While gene editing offers many exciting therapeutic possibilities, safety concerns have yet to be fully addressed. Double-strand breaks are a potential source of genomic instability which may increase risk of oncogenic mutations and translocations [118]. Off-target gene edits are also a concern. Careful gRNA design and *in silico* simulations are a starting point for reducing this risk [97]. Improvements in editing specificity have been made to help mitigate off-target editing, for example, by reducing the exposure of DNA to the nuclease by delivering Cas9-gRNA as a ribonucleoprotein complex [119]. Several assays also exist to assess genome-wide off-target edits in preclinical validation experiments [120, 121]. While genome-wide screening is useful for detecting off-target effects of gene editing, the clinical relevance of any mutations is difficult to predict [122, 123]. While in vivo safety has been demonstrated using adoptive transfer experiments in murine models, they may not predict results in humans. Careful analysis of the gene editing therapeutics currently in phase I clinical trials will be required to ascertain any potential toxic effects in humans.

19.6 Future Developments, Challenges, and Conclusions

The exciting field of gene therapy has a bright future. A new class of autologous curative therapies will hopefully be available for patients with a variety of monogenic PIDs in the near future. Gene editing has the potential to offer autologous HSC-based therapies for a wider spectrum of PIDs and refine existing GT approaches. However, despite the significant progress made, several challenges

need to be overcome before HSC GT becomes the standard of care for the management of PID.

The advantages of GT over alloHSCT need to be assessed against contemporary transplant practice, which itself has benefitted from major advances. Advances in haploidentical transplantation have reduced issues with donor availability, while *ex vivo* graft manipulation, improved GVHD prophylaxis and better prophylaxis and treatment of infections have all reduced the morbidity and mortality associated with allogeneic transplant [124]. The benefits of GT need to be assessed against the increased costs compared to alloHSCT in order to make this a feasible option for healthcare providers and the patients that use them. While there are increasing numbers of patients with long-term follow-up post-GT, longer-term toxicities can only be assessed with time and as larger numbers of patients are treated. The genotoxic effects of gene editing in humans are currently unknown. Clinical trials of gene-edited products are underway; thus, initial safety analyses will hopefully reveal in the near future whether this novel group of therapeutics is truly feasible and safe in humans. Longer-term follow-up will be needed to assess the persistence of gene-edited cells and the durability of any beneficial effects.

Conditioning regimens for GT (and alloHSCT) currently use chemo-, radio-, and/or serotherapy. While reduced intensity regimens have reduced overall toxicity without compromising engraftment, adverse late effects as a result of conditioning therapy do occur. The ability to avoid these agents would be a significant therapeutic advance and likely broaden the appeal and application of GT procedures. Antibody-based conditioning agents that deplete HSCs are being developed. An approach using a hematopoietic cell-specific immunotoxin, saporin (SAP), conjugated to an antibody targeting CD45 can result in >90% engraftment of donor cells after a single dose in an in vivo model using immunocompetent mice [125]. Another approach targets c-kit (CD117), a dimeric transmembrane receptor tyrosine kinase expressed by HSCs. It has been shown that anti-mouse c-kit monoclonal antibodies can deplete HSCs and permit engraftment of exogenous HSCs [126–128]. CD45 is present on lymphocytes in addition to HSCs so using a combined CD45-c-kit-targeted approach would result in lymphodepletion in a similar way to alkylating agents which is undesirable. In order to circumvent this, c-kit (CD117) antibody drug conjugates have been developed, e.g., streptavidin-saporin-anti-CD117, and similarly to the CD45 targeting approaches, this agent leads to >99% depletion of host-HSCs without causing clinically significant side effects [127, 129]. Phase I trials of non-genotoxic conditioning agents are now in progress in the context of alloHSCT for SCID, and early results are promising with evidence of sustained engraftment of multipotent HSCs; however, it remains to be seen if these agents will permit stem cell engraftment in a non-SCID setting [130]. Should these new agents prove to be successful in the non-SCID *adult* setting, the ability to perform autologous GT procedures without alkylating agents or irradiation will increase the advantages and applicability of GT strategies while reducing the risks further.

Historically, GT procedures involved infusion of a fresh product meaning that patients had to travel to a site where the *ex vivo* manipulation, conditioning, infusion

and recovery had to take place. The ability to deliver a cryopreserved product manufactured centrally will improve the availability of GT and may reduce the cost of the therapy [131]. Limited capacity to manufacture cellular therapy and GT products is an issue as the technology is more widely adopted. However, as the results of clinical trials in gene and cell therapy have demonstrated the utility of these products for the treatment of a variety of disorders, interest and development of manufacturing capabilities has increased. Large-scale, serum-free, good manufacturing practice (GMP) compliant automated systems are now available for virus manufacture. These platforms reduce the use of animal-derived products and the handling required, thus lowering the risk of contamination [132, 133]. The development of culture media which maintains HSC potency as well as the availability of transduction enhancers which improve the efficiency of gene transfer will also help lower the cost of manufacture as less virus per product will be required [134–136]. Together these developments will help lower the cost and improve the availability of GT products.

In conclusion, while significant challenges remain, there is undoubtedly the potential that GT may become the standard of care for many PIDs in the not-to-distant future. Sustained international collaborative efforts between scientists, clinicians and industry will be required in order to make these treatments available to patients affected by these rare but devastating diseases. We look forward to the advances in the years to come and hope that even as you read this chapter, we are a few steps closer to making GT an effective therapy for patients affected by cellular PIDs.

References

1. Slatter MA, Gennery AR (2018) Hematopoietic cell transplantation in primary immunodeficiency—conventional and emerging indications. Expert Rev Clin Immunol 14(2):103–114
2. Gennery AR, Lankester A (2019) Inborn errors working party of the European Society for B, Marrow T. Long term outcome and immune function after hematopoietic stem cell transplantation for primary immunodeficiency. Front Pediatr 7:381
3. McCurdy SR, Kasamon YL, Kanakry CG, Bolanos-Meade J, Tsai HL, Showel MM et al (2017) Comparable composite endpoints after HLA-matched and HLA-haploidentical transplantation with post-transplantation cyclophosphamide. Haematologica 102(2):391–400
4. McCurdy SR, Luznik L (2019) How we perform haploidentical stem cell transplantation with posttransplant cyclophosphamide. Blood 134(21):1802–1810
5. Balashov D, Shcherbina A, Maschan M, Trakhtman P, Skvortsova Y, Shelikhova L et al (2015) Single-center experience of unrelated and haploidentical stem cell transplantation with TCR alphabeta and CD19 depletion in children with primary immunodeficiency syndromes. Biol Blood Marrow Transplant 21(11):1955–1962
6. Morris EC, Albert MH (2019) Allogeneic HSCT in adolescents and young adults with primary immunodeficiencies. Front Pediatr 7:437
7. Fox TA, Chakraverty R, Burns S, Carpenter B, Thomson K, Lowe D et al (2018) Successful outcome following allogeneic hematopoietic stem cell transplantation in adults with primary immunodeficiency. Blood 131(8):917–931
8. Booth C, Gaspar HB, Thrasher AJ (2016) Treating immunodeficiency through HSC gene therapy. Trends Mol Med 22(4):317–327

9. Naldini L (2019) Genetic engineering of hematopoiesis: current stage of clinical translation and future perspectives. EMBO Mol Med 11(3):e9958
10. Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C et al (2013) Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science 341(6148):1233151
11. De Ravin SS, Wu X, Moir S, Anaya-O'Brien S, Kwatemaa N, Littel P et al (2016) Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 8(335):335ra57
12. Kohn DB, Booth C, Kang EM, Pai SY, Shaw KL, Santilli G et al (2020) Lentiviral gene therapy for X-linked chronic granulomatous disease. Nat Med 26(2):200–206
13. Fares I, Chagraoui J, Lehnertz B, MacRae T, Mayotte N, Tomellini E et al (2017) EPCR expression marks UM171-expanded CD34(+) cord blood stem cells. Blood 129(25):3344–3351
14. Radtke S, Adair JE, Giese MA, Chan YY, Norgaard ZK, Enstrom M et al (2017) A distinct hematopoietic stem cell population for rapid multilineage engraftment in nonhuman primates. Sci Transl Med 9(414):eaan1145
15. Aiuti A, Slavin S, Aker M, Ficara F, Deola S, Mortellaro A et al (2002) Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science 296(5577):2410–2413
16. Morgan RA, Gray D, Lomova A, Kohn DB (2017) Hematopoietic stem cell gene therapy: progress and lessons learned. Cell Stem Cell 21(5):574–590
17. Kondo M, Wagers AJ, Manz MG, Prohaska SS, Scherer DC, Beilhack GF et al (2003) Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu Rev Immunol 21:759–806
18. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A et al (2020) Human inborn errors of immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 40(1):24–64
19. Whitmore KV, Gaspar HB (2016) Adenosine deaminase deficiency—more than just an immunodeficiency. Front Immunol 7:314
20. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M et al (1995) T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. Science 270(5235):475–480
21. Bordignon C, Notarangelo LD, Nobili N, Ferrari G, Casorati G, Panina P et al (1995) Gene therapy in peripheral blood lymphocytes and bone marrow for ADA- immunodeficient patients. Science 270(5235):470–475
22. Gaspar HB, Bjorkegren E, Parsley K, Gilmour KC, King D, Sinclair J et al (2006) Successful reconstitution of immunity in ADA-SCID by stem cell gene therapy following cessation of PEG-ADA and use of mild preconditioning. Mol Ther 14(4):505–513
23. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E et al (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 118(9):3132–3142
24. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H et al (2008) Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 118(9):3143–3150
25. Touzot F, Moshous D, Creidy R, Neven B, Frange P, Cros G et al (2015) Faster T-cell development following gene therapy compared with haploidentical HSCT in the treatment of SCID-X1. Blood 125(23):3563–3569
26. Braun CJ, Boztug K, Paruzynski A, Witzel M, Schwarzer A, Rothe M et al (2014) Gene therapy for Wiskott-Aldrich syndrome--long-term efficacy and genotoxicity. Sci Transl Med 6(227):227ra33
27. Braun CJ, Witzel M, Paruzynski A, Boztug K, von Kalle C, Schmidt M et al (2014) Gene therapy for Wiskott-Aldrich Syndrome-Long-term reconstitution and clinical benefits, but increased risk for leukemogenesis. Rare Dis 2(1):e947749

28. Stein S, Ott MG, Schultze-Strasser S, Jauch A, Burwinkel B, Kinner A et al (2010) Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med 16(2):198–204
29. Brendel C, Muller-Kuller U, Schultze-Strasser S, Stein S, Chen-Wichmann L, Krattenmacher A et al (2012) Physiological regulation of transgene expression by a lentiviral vector containing the A2UCOE linked to a myeloid promoter. Gene Ther 19(10):1018–1029
30. Brendel C, Hanseler W, Wohlgensinger V, Bianchi M, Tokmak S, Chen-Wichmann L et al (2013) Human miR223 promoter as a novel myelo-specific promoter for chronic granulomatous disease gene therapy. Hum Gene Ther Methods 24(3):151–159
31. Aiuti A, Brigida I, Ferrua F, Cappelli B, Chiesa R, Marktel S et al (2009) Hematopoietic stem cell gene therapy for adenosine deaminase deficient-SCID. Immunol Res 44(1–3):150–159
32. Hacein-Bey-Abina S, Pai SY, Gaspar HB, Armant M, Berry CC, Blanche S et al (2014) A modified gamma-retrovirus vector for X-linked severe combined immunodeficiency. N Engl J Med 371(15):1407–1417
33. Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ et al (2011) Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 3(97):97ra79
34. Modlich U, Navarro S, Zychlinski D, Maetzig T, Knoess S, Brugman MH et al (2009) Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. Mol Ther 17(11):1919–1928
35. Montini E, Cesana D, Schmidt M, Sanvito F, Bartholomae CC, Ranzani M et al (2009) The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. J Clin Invest 119(4):964–975
36. Biffi A, Bartolomae CC, Cesana D, Cartier N, Aubourg P, Ranzani M et al (2011) Lentiviral vector common integration sites in preclinical models and a clinical trial reflect a benign integration bias and not oncogenic selection. Blood 117(20):5332–5339
37. Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D et al (1998) A third-generation lentivirus vector with a conditional packaging system. J Virol 72(11):8463–8471
38. Zufferey R, Dull T, Mandel RJ, Bukovsky A, Quiroz D, Naldini L et al (1998) Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. J Virol 72(12):9873–9880
39. Roe T, Reynolds TC, Yu G, Brown PO (1993) Integration of murine leukemia virus DNA depends on mitosis. EMBO J 12(5):2099–2108
40. Barquinero J, Segovia JC, Ramirez M, Limon A, Guenechea G, Puig T et al (2000) Efficient transduction of human hematopoietic repopulating cells generating stable engraftment of transgene-expressing cells in NOD/SCID mice. Blood 95(10):3085–3093
41. Peters SO, Kittler EL, Ramshaw HS, Quesenberry PJ (1996) Ex vivo expansion of murine marrow cells with interleukin-3 (IL-3), IL-6, IL-11, and stem cell factor leads to impaired engraftment in irradiated hosts. Blood 87(1):30–37
42. Sutton RE, Reitsma MJ, Uchida N, Brown PO (1999) Transduction of human progenitor hematopoietic stem cells by human immunodeficiency virus type 1-based vectors is cell cycle dependent. J Virol 73(5):3649–3660
43. Millington M, Arndt A, Boyd M, Applegate T, Shen S (2009) Towards a clinically relevant lentiviral transduction protocol for primary human CD34 hematopoietic stem/progenitor cells. PLoS One 4(7):e6461
44. Pai SY (2019) Treatment of primary immunodeficiency with allogeneic transplant and gene therapy. Hematol Am Soc Hematol Educ Program 2019(1):457–465
45. Aiuti A, Roncarolo MG, Naldini L (2017) Gene therapy for ADA-SCID, the first marketing approval of an ex vivo gene therapy in Europe: paving the road for the next generation of advanced therapy medicinal products. EMBO Mol Med 9(6):737–740
46. Hoggatt J (2016) Gene therapy for "Bubble Boy" disease. Cell 166(2):263
47. Morris EC, Fox T, Chakraverty R, Tendeiro R, Snell K, Rivat C et al (2017) Gene therapy for Wiskott-Aldrich syndrome in a severely affected adult. Blood 130(11):1327–1335
48. Pai SY, Cowan MJ (2014) Stem cell transplantation for primary immunodeficiency diseases: the North American experience. Curr Opin Allergy Clin Immunol 14(6):521–526

49. Ferrua F, Aiuti A (2017) Twenty-five years of gene therapy for ADA-SCID: from bubble babies to an approved drug. Hum Gene Ther 28(11):972–981
50. Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L et al (2009) Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 360(5):447–458
51. Candotti F, Shaw KL, Muul L, Carbonaro D, Sokolic R, Choi C et al (2012) Gene therapy for adenosine deaminase-deficient severe combined immune deficiency: clinical comparison of retroviral vectors and treatment plans. Blood 120(18):3635–3646
52. Carbonaro DA, Jin X, Wang X, Yu XJ, Rozengurt N, Kaufman ML et al (2012) Gene therapy/bone marrow transplantation in ADA-deficient mice: roles of enzyme-replacement therapy and cytoreduction. Blood 120(18):3677–3687
53. Kuo CY, Kohn DB (2016) Gene therapy for the treatment of primary immune deficiencies. Curr Allergy Asthma Rep 16(5):39
54. Mamcarz E, Zhou S, Lockey T, Abdelsamed H, Cross SJ, Kang G et al (2019) Lentiviral gene therapy combined with low-dose busulfan in infants with SCID-X1. N Engl J Med 380(16):1525–1534
55. Lagresle-Peyrou C, Benjelloun F, Hue C, Andre-Schmutz I, Bonhomme D, Forveille M et al (2008) Restoration of human B-cell differentiation into NOD-SCID mice engrafted with gene-corrected CD34+ cells isolated from Artemis or RAG1-deficient patients. Mol Ther 16(2):396–403
56. Abolhassani H, Wang N, Aghamohammadi A, Rezaei N, Lee YN, Frugoni F et al (2014) A hypomorphic recombination-activating gene 1 (RAG1) mutation resulting in a phenotype resembling common variable immunodeficiency. J Allergy Clin Immunol 134(6):1375–1380
57. Benjelloun F, Garrigue A, Demerens-de Chappedelaine C, Soulas-Sprauel P, Malassis-Seris M, Stockholm D et al (2008) Stable and functional lymphoid reconstitution in artemis-deficient mice following lentiviral artemis gene transfer into hematopoietic stem cells. Mol Ther 16(8):1490–1499
58. Punwani D, Kawahara M, Yu J, Sanford U, Roy S, Patel K et al (2017) Lentivirus mediated correction of artemis-deficient severe combined immunodeficiency. Hum Gene Ther 28(1):112–124
59. Multhaup MM, Podetz-Pedersen KM, Karlen AD, Olson ER, Gunther R, Somia NV et al (2015) Role of transgene regulation in ex vivo lentiviral correction of artemis deficiency. Hum Gene Ther 26(4):232–243
60. Lagresle-Peyrou C, Yates F, Malassis-Seris M, Hue C, Morillon E, Garrigue A et al (2006) Long-term immune reconstitution in RAG-1-deficient mice treated by retroviral gene therapy: a balance between efficiency and toxicity. Blood 107(1):63–72
61. Pike-Overzet K, Rodijk M, Ng YY, Baert MR, Lagresle-Peyrou C, Schambach A et al (2011) Correction of murine Rag1 deficiency by self-inactivating lentiviral vector-mediated gene transfer. Leukemia 25(9):1471–1483
62. van Til NP, Sarwari R, Visser TP, Hauer J, Lagresle-Peyrou C, van der Velden G et al (2014) Recombination-activating gene 1 (Rag1)-deficient mice with severe combined immunodeficiency treated with lentiviral gene therapy demonstrate autoimmune Omenn-like syndrome. J Allergy Clin Immunol 133(4):1116–1123
63. Garcia-Perez L, van Eggermond M, van Roon L, Vloemans SA, Cordes M, Schambach A et al (2020) Successful preclinical development of gene therapy for recombinase-activating gene-1-deficient SCID. Mol Ther Methods Clin Dev 17:666–682
64. Thrasher AJ, Williams DA (2017) Evolving gene therapy in primary immunodeficiency. Mol Ther 25(5):1132–1141
65. Horwitz ME, Barrett AJ, Brown MR, Carter CS, Childs R, Gallin JI et al (2001) Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft. N Engl J Med 344(12):881–888
66. Gungor T, Teira P, Slatter M, Stussi G, Stepensky P, Moshous D et al (2014) Reduced-intensity conditioning and HLA-matched haemopoietic stem-cell transplantation in patients with chronic granulomatous disease: a prospective multicentre study. Lancet 383(9915):436–448

67. Malech HL, Maples PB, Whiting-Theobald N, Linton GF, Sekhsaria S, Vowells SJ et al (1997) Prolonged production of NADPH oxidase-corrected granulocytes after gene therapy of chronic granulomatous disease. Proc Natl Acad Sci U S A 94(22):12133–12138
68. Malech HL, Choi U, Brenner S (2004) Progress toward effective gene therapy for chronic granulomatous disease. Jpn J Infect Dis 57(5):S27–S28
69. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U et al (2006) Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 12(4):401–409
70. Kang EM, Choi U, Theobald N, Linton G, Long Priel DA, Kuhns D et al (2010) Retrovirus gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils. Blood 115(4):783–791
71. Santilli G, Almarza E, Brendel C, Choi U, Beilin C, Blundell MP et al (2011) Biochemical correction of X-CGD by a novel chimeric promoter regulating high levels of transgene expression in myeloid cells. Mol Ther 19(1):122–132
72. Brendel C, Rothe M, Santilli G, Charrier S, Stein S, Kunkel H et al (2018) Non-clinical efficacy and safety studies on G1XCGD, a lentiviral vector for ex vivo gene therapy of X-linked chronic granulomatous disease. Hum Gene Ther Clin Dev 29(2):69–79
73. Liese JG, Jendrossek V, Jansson A, Petropoulou T, Kloos S, Gahr M et al (1996) Chronic granulomatous disease in adults. Lancet 347(8996):220–223
74. Liese J, Kloos S, Jendrossek V, Petropoulou T, Wintergerst U, Notheis G et al (2000) Long-term follow-up and outcome of 39 patients with chronic granulomatous disease. J Pediatr 137(5):687–693
75. Ochs HD, Thrasher AJ (2006) The Wiskott-Aldrich syndrome. J Allergy Clin Immunol 117(4):725–738. quiz 39
76. Dupre L, Trifari S, Follenzi A, Marangoni F, Lain de Lera T, Bernad A et al (2004) Lentiviral vector-mediated gene transfer in T cells from Wiskott-Aldrich syndrome patients leads to functional correction. Mol Ther 10(5):903–915
77. Ferrua F, Cicalese MP, Galimberti S, Giannelli S, Dionisio F, Barzaghi F et al (2019) Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: interim results of a non-randomised, open-label, phase 1/2 clinical study. Lancet Haematol 6(5):e239–ee53
78. Hacein-Bey Abina S, Gaspar HB, Blondeau J, Caccavelli L, Charrier S, Buckland K et al (2015) Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. JAMA 313(15):1550–1563
79. Bauer TR Jr, Hickstein DD (2000) Gene therapy for leukocyte adhesion deficiency. Curr Opin Mol Ther 2(4):383–388
80. Leon-Rico D, Aldea M, Sanchez-Baltasar R, Mesa-Nunez C, Record J, Burns SO et al (2016) Lentiviral vector-mediated correction of a mouse model of leukocyte adhesion deficiency type I. Hum Gene Ther 27(9):668–678
81. Hunter MJ, Tuschong LM, Fowler CJ, Bauer TR Jr, Burkholder TH, Hickstein DD (2011) Gene therapy of canine leukocyte adhesion deficiency using lentiviral vectors with human CD11b and CD18 promoters driving canine CD18 expression. Mol Ther 19(1):113–121
82. Yates F, Malassis-Seris M, Stockholm D, Bouneaud C, Larousserie F, Noguiez-Hellin P et al (2002) Gene therapy of RAG-2−/− mice: sustained correction of the immunodeficiency. Blood 100(12):3942–3949
83. van Til NP, de Boer H, Mashamba N, Wabik A, Huston M, Visser TP et al (2012) Correction of murine Rag2 severe combined immunodeficiency by lentiviral gene therapy using a codon-optimized RAG2 therapeutic transgene. Mol Ther 20(10):1968–1980
84. Capo V, Castiello MC, Fontana E, Penna S, Bosticardo M, Draghici E et al (2018) Efficacy of lentivirus-mediated gene therapy in an Omenn syndrome recombination-activating gene 2 mouse model is not hindered by inflammation and immune dysregulation. J Allergy Clin Immunol 142(3):928–941. e8

85. Barzaghi F, Amaya Hernandez LC, Neven B, Ricci S, Kucuk ZY, Bleesing JJ et al (2018) Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: an international multicenter retrospective study. J Allergy Clin Immunol 141(3):1036–1049. e5
86. Passerini L, Rossi Mel E, Sartirana C, Fousteri G, Bondanza A, Naldini L et al (2013) CD4(+) T cells from IPEX patients convert into functional and stable regulatory T cells by FOXP3 gene transfer. Sci Transl Med 5(215):215ra174
87. Riley JL, June CH, Blazar BR (2009) Human T regulatory cell therapy: take a billion or so and call me in the morning. Immunity 30(5):656–665
88. Masiuk KE, Laborada J, Roncarolo MG, Hollis RP, Kohn DB (2019) Lentiviral gene therapy in HSCs restores lineage-specific Foxp3 expression and suppresses autoimmunity in a mouse model of IPEX syndrome. Cell Stem Cell 24(2):309–317. e7
89. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ et al (2014) Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 371(16):1507–1517
90. Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW et al (2015) Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med 7(303):303ra139
91. Ghosh S, Carmo M, Calero-Garcia M, Ricciardelli I, Bustamante Ogando JC, Blundell MP et al (2018) T-cell gene therapy for perforin deficiency corrects cytotoxicity defects and prevents hemophagocytic lymphohistiocytosis manifestations. J Allergy Clin Immunol 142(3):904–913. e3
92. Carmo M, Risma KA, Arumugam P, Tiwari S, Hontz AE, Montiel-Equihua CA et al (2015) Perforin gene transfer into hematopoietic stem cells improves immune dysregulation in murine models of perforin deficiency. Mol Ther 23(4):737–745
93. Parolini S, Bottino C, Falco M, Augugliaro R, Giliani S, Franceschini R et al (2000) X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. J Exp Med 192(3):337–346
94. Booth C, Gilmour KC, Veys P, Gennery AR, Slatter MA, Chapel H et al (2011) X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease. Blood 117(1):53–62
95. Panchal N, Houghton B, Diez B, Ghosh S, Ricciardelli I, Thrasher AJ et al (2018) Transfer of gene-corrected T cells corrects humoral and cytotoxic defects in patients with X-linked lymphoproliferative disease. J Allergy Clin Immunol 142(1):235–245. e6
96. Kohn DB, Kuo CY (2017) New frontiers in the therapy of primary immunodeficiency: from gene addition to gene editing. J Allergy Clin Immunol 139(3):726–732
97. Porteus MH (2019) A New Class of Medicines through DNA Editing. N Engl J Med 380(10):947–959
98. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096):816–821
99. Chakrabarti AM, Henser-Brownhill T, Monserrat J, Poetsch AR, Luscombe NM, Scaffidi P (2019) Target-specific precision of CRISPR-mediated genome editing. Mol Cell 73(4):699–713. e6
100. Rouet P, Smih F, Jasin M (1994) Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. Proc Natl Acad Sci U S A 91(13):6064–6068
101. Kuo CY, Long JD, Campo-Fernandez B, de Oliveira S, Cooper AR, Romero Z et al (2018) Site-specific gene editing of human hematopoietic stem cells for X-linked hyper-IgM syndrome. Cell Rep 23(9):2606–2616
102. Hirsch ML, Samulski RJ (2014) AAV-mediated gene editing via double-strand break repair. Methods Mol Biol 1114:291–307
103. Hubbard N, Hagin D, Sommer K, Song Y, Khan I, Clough C et al (2016) Targeted gene editing restores regulated CD40L function in X-linked hyper-IgM syndrome. Blood 127(21):2513–2522

104. Surosky RT, Urabe M, Godwin SG, McQuiston SA, Kurtzman GJ, Ozawa K et al (1997) Adeno-associated virus Rep proteins target DNA sequences to a unique locus in the human genome. J Virol 71(10):7951–7959
105. Daya S, Berns KI (2008) Gene therapy using adeno-associated virus vectors. Clin Microbiol Rev 21(4):583–593
106. Bak RO, Dever DP, Porteus MH (2018) CRISPR/Cas9 genome editing in human hematopoietic stem cells. Nat Protoc 13(2):358–376
107. Khan IF, Hirata RK, Russell DW (2011) AAV-mediated gene targeting methods for human cells. Nat Protoc 6(4):482–501
108. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G et al (2014) Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N Engl J Med 370(10):901–910
109. Joglekar AV, Hollis RP, Kuftinec G, Senadheera S, Chan R, Kohn DB (2013) Integrase-defective lentiviral vectors as a delivery platform for targeted modification of adenosine deaminase locus. Mol Ther 21(9):1705–1717
110. Lombardo A, Genovese P, Beausejour CM, Colleoni S, Lee YL, Kim KA et al (2007) Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Nat Biotechnol 25(11):1298–1306
111. De Ravin SS, Li L, Wu X, Choi U, Allen C, Koontz S et al (2017) CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease. Sci Transl Med 9:372
112. Goodwin M, Lee E, Lakshmanan U, Shipp S, Froessl L, Barzaghi F et al (2020) CRISPR-based gene editing enables FOXP3 gene repair in IPEX patient cells. Sci Adv 6(19):eaaz0571
113. Clough Cea ASGCT (2016) 19th Annual meeting: abstracts. Mol Ther 24(Suppl 1):S1–S304
114. Pavel-Dinu M, Wiebking V, Dejene BT, Srifa W, Mantri S, Nicolas CE et al (2019) Gene correction for SCID-X1 in long-term hematopoietic stem cells. Nat Commun 10(1):1634
115. Sacco MG, Ungari M, Cato EM, Villa A, Strina D, Notarangelo LD et al (2000) Lymphoid abnormalities in CD40 ligand transgenic mice suggest the need for tight regulation in gene therapy approaches to hyper immunoglobulin M (IgM) syndrome. Cancer Gene Ther 7(10):1299–1306
116. Sather BD, Ryu BY, Stirling BV, Garibov M, Kerns HM, Humblet-Baron S et al (2011) Development of B-lineage predominant lentiviral vectors for use in genetic therapies for B cell disorders. Mol Ther 19(3):515–525
117. Yu PW, Tabuchi RS, Kato RM, Astrakhan A, Humblet-Baron S, Kipp K et al (2004) Sustained correction of B-cell development and function in a murine model of X-linked agammaglobulinemia (XLA) using retroviral-mediated gene transfer. Blood 104(5):1281–1290
118. Brunet E, Jasin M (2018) Induction of chromosomal translocations with CRISPR-Cas9 and other nucleases: understanding the repair mechanisms that give rise to translocations. Adv Exp Med Biol 1044:15–25
119. Hendel A, Bak RO, Clark JT, Kennedy AB, Ryan DE, Roy S et al (2015) Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat Biotechnol 33(9):985–989
120. Wienert B, Wyman SK, Richardson CD, Yeh CD, Akcakaya P, Porritt MJ et al (2019) Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq. Science 364(6437):286–289
121. Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar VV, Thapar V et al (2015) GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nat Biotechnol 33(2):187–197
122. Morgens DW, Wainberg M, Boyle EA, Ursu O, Araya CL, Tsui CK et al (2017) Genome-scale measurement of off-target activity using Cas9 toxicity in high-throughput screens. Nat Commun 8:15178
123. Kuscu C, Arslan S, Singh R, Thorpe J, Adli M (2014) Genome-wide analysis reveals characteristics of off-target sites bound by the Cas9 endonuclease. Nat Biotechnol 32(7):677–683
124. Juric MK, Ghimire S, Ogonek J, Weissinger EM, Holler E, van Rood JJ et al (2016) Milestones of hematopoietic stem cell transplantation—from first human studies to current developments. Front Immunol 7:470

125. Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA et al (2016) Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. Nat Biotechnol 34(7):738–745
126. Czechowicz A, Kraft D, Weissman IL, Bhattacharya D (2007) Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. Science 318(5854):1296–1299
127. Czechowicz A, Palchaudhuri R, Scheck A, Hu Y, Hoggatt J, Saez B et al (2019) Selective hematopoietic stem cell ablation using CD117-antibody-drug-conjugates enables safe and effective transplantation with immunity preservation. Nat Commun 10(1):617
128. Chhabra A, Ring AM, Weiskopf K, Schnorr PJ, Gordon S, Le AC et al (2016) Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy. Sci Transl Med 8(351):351ra105
129. Kwon HS, Logan AC, Chhabra A, Pang WW, Czechowicz A, Tate K et al (2019) Anti-human CD117 antibody-mediated bone marrow niche clearance in nonhuman primates and humanized NSG mice. Blood 133(19):2104–2108
130. Agarwal R, Dvorak CC, Kwon HS, Long-Boyle JR, Prohaska SS, Brown JW, Le A, Guttman-Klein A, Weissman IL, Cowan MJ, Logan AC, Weinberg KI, Parkman R, Roncarolo M-G, Shizuru JA (2019) Non-genotoxic anti-CD117 antibody conditioning results in successful hematopoietic stem cell engraftment in patients with severe combined immunodeficiency. Blood 134(Suppl. 1):800
131. Morgenstern DA, Ahsan G, Brocklesby M, Ings S, Balsa C, Veys P et al (2016) Post-thaw viability of cryopreserved peripheral blood stem cells (PBSC) does not guarantee functional activity: important implications for quality assurance of stem cell transplant programmes. Br J Haematol 174(6):942–951
132. Valkama AJ, Leinonen HM, Lipponen EM, Turkki V, Malinen J, Heikura T et al (2018) Optimization of lentiviral vector production for scale-up in fixed-bed bioreactor. Gene Ther 25(1):39–46
133. Bauler M, Roberts JK, Wu CC, Fan B, Ferrara F, Yip BH et al (2020) Production of lentiviral vectors using suspension cells grown in serum-free media. Mol Ther Methods Clin Dev 17:58–68
134. Tajer P, Pike-Overzet K, Arias S, Havenga M, Staal FJT (2019) Ex vivo expansion of hematopoietic stem cells for therapeutic purposes: lessons from development and the Niche. Cell 8(2):169
135. Masiuk KE, Zhang R, Osborne K, Hollis RP, Campo-Fernandez B, Kohn DB (2019) PGE2 and Poloxamer Synperonic F108 enhance transduction of human HSPCs with a beta-globin lentiviral vector. Mol Ther Methods Clin Dev. 13:390–398
136. Petrillo C, Thorne LG, Unali G, Schiroli G, Giordano AMS, Piras F et al (2018) Cyclosporine H overcomes innate immune restrictions to improve lentiviral transduction and gene editing in human hematopoietic stem cells. Cell Stem Cell 23(6):820–832. e9

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