Genetic Disorders of Immune Regulation

5

Carsten Speckmann, Jan Rohr, and Stephan Ehl

Core Messages

- Susceptibility to infection and immune dysregulation are associated in many primary immunodeficiency diseases.
- Some primary immunodeficiencies cause immune dysregulation in the absence of susceptibility to infection.
- The common pathophysiological feature of familial hemophagocytic lymphohistiocytosis (FHL) is an impairment in cellular cytotoxicy. The clinical picture is one caused by an excessive activation of the immune system and not by an uncontrolled infection.
- Chediak-Higashi syndrome, Griscelli syndrome II, Hermansky Pudlak syndrome II and p14 deficiency are autosomal recessive diseases with oculocutaneous hypopigmentation and variable signs of immunodeficiency. The molecular basis are defects in the biogenesis, transport or delivery of secretory lysosomes, leading to a risk of hemophagocytic lymphohistiocytosis.
- X-Linked Lymphoproliferative syndrome (XLP) has three main disease manifestations: fulminant infectious mononucleosis, dysgammaglobulinemia and lymphoma. Disease onset is usually triggered by Epstein-Barr virus (EBV)-infection and patients may be asymptomatic prior to EBV-infection.
- Autoimmune lymphoproliferative syndrome (ALPS) is a benign Lymphoproliferative disease caused by defective apoptosis of peripheral lymphocytes. The clinical hallmarks are lymphoproliferation and autoimmune disease, in particular autoimmune cytopenia.
- Typical manifestations of APECED (autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy) are chronic or recurrent mucocutaneous candidiasis, hypoparathyroidism and adrenocortical failure. The pathogenetic basis of APECED is a disturbed development of immunological tolerance in the thymus due to mutations in the gene encoding AIRE (autoimmune regulator).
- Immunodysregulation, polyenocrinopathy, enteropathy, X-linked (IPEX) is caused by mutations in the gene "forkhead box P3" (*FOXP3*), which is central for the generation and function of regulatory CD4+T cells. IPEX usually manifests within the first year of life with severe diarrhea, failure to thrive, eczematous skin lesions, diabetes mellitus and/or other endocrinopathies.

5.1 Introduction

The primary role of the immune system is defense against infection. Antimicrobial immune responses are highly dynamic processes that involve rapid expansion and contraction of immune cell populations, targeted exertion of highly potent effector functions, and secretion of soluble mediators that have antimicrobial properties and influence cell functions and interactions. To maintain homeostasis, both innate and adaptive immune responses require tight regulation. Exaggerated inflammatory responses can be the consequence of uncontrolled activation of the immune system and failure to control immune responses against host antigens causes autoimmunity. There are many checkpoints that help to maintain homeostasis in the immune system involving a variety of cells and mediators. It is therefore not surprising that genetic deficiencies in many immunologically relevant molecules can lead to immune dysregulation in addition to absence of susceptibility to infection.

Failure to regulate immune responses may lead to various clinical manifestations including (benign) lymphoproliferation, febrile inflammatory responses and autoimmunity. In many cases, infections trigger these aberrant responses. In some circumstances, failure to appropriately control pathogens contributes to their maintenance, but in others, no exogenous stimulus can be identified. The molecular and cellular mechanisms responsible for immune dysregulation vary in different forms of primary immunodeficiencies. In many diseases, several mechanisms are involved. Immunodeficiencies associated with immune dysregulation include antibody deficiencies, T cell deficiencies, phagocytes defects and complement deficiencies. These diseases are discussed in the respective sections of this book. This chapter describes several immunodeficiency syndromes that predominantly manifest with immune dysregulation. This includes the familial hemophagocytic syndromes, the closely related immunodeficiencies with hypopigmentation, XLP (X-Linked Lymphoproliferative syndrome, ALPS (Autoimmune Lymphoproliferative syndrome), APECED (Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy) and IPEX (Immunodysregulation, polyenocrionpathy, enteropathy, X-linked).

5.2 Familial Hemophagocytic Lymphohistiocytosis *(Perforin Deficiency, MUNC13-4 Deficiency, Syntaxin 11 Deficiency)*

5.2.1 Definition

Familial hemophagocytic lymphohistiocytosis (FHL) is a group of genetically determined, life-threatening diseases caused by the uncontrolled proliferation of activated lymphocytes and histiocytes secreting high amounts of inflammatory cytokines [55]. The symptoms were first described in 1952 and include prolonged fever, hepatosplenomegaly, pancytopenia and neurological symptoms [51]. Currently, there are four known forms of FHL (FHL1-4), for three of which the causative genes have been identified: FHL-2 (OMIM#603553) is caused by mutations in the gene encoding perforin (*PRF1*, OMIM*170280) [169], FHL-3 (OMIM#608898) is due to mutations in the gene encoding MUNC 13-4 (*MUNC 13-4* or *UNC13D*, OMIM*608897) [52] and FHL-4 (OMIM#603552) is caused by mutations in the gene encoding syntaxin 11(*STX11*, OMIM*605014) [197]. All of these proteins are involved in cellular cytotoxicity mediated by NK cells and T cells [56]. FHL-1 has been linked to chromosome 9q21.3-22, however its genetic basis is still unknown [126]. In addition, there are further familial forms of the disease whose genetic basis remains to be elucidated.

5.2.2 Etiology

Contact-dependent cellular cytotoxicity by NK cells and CD8+ cytotoxic T cells (CTL) is one of the key effector mechanisms of the immune system against intracellular pathogens such as viruses and intracellular bacteria [93]. Cellular cytotoxicity is mediated by cytotoxic granules in the cytoplasm of NK cells and CTL containing perforin, granzymes and other components. After target cell recognition and formation of an appropriate contact area between effector and target cell (the immunological synapse), granules migrate to the site of cell contact, fuse with the plasma membrane and their contents are secreted into the intracellular space. Perforin and granzymes then cooperate to mediate rapid apoptosis of the target cell (Fig. 5.1) [171].

Perforin, MUNC13-4 and Syntaxin 11 are all expressed in NK cells and CTL. Perforin is a pore-forming protein that can insert into the lipid bilayer of target cell membranes causing cell death by osmotic lysis and allowing entry of apoptosis-inducing granzymes [22]. MUNC13-4 is involved in vesicle priming and MUNC13-4 deficiency results in defective exocytosis despite polarization of lytic granules and docking with the plasma membrane [122]. Syntaxin 11 is also expressed in APC and an impaired interaction between CTL and APC may contribute to FHL-4 [197]. However, the association of syntaxin 11 with other lysosomal proteins and the recent description of impaired CTL and NK cell degranulation in patients with syntaxin 11 deficiency suggests that it is also important for granule exocytosis [24].

In the context of its antimicrobial function, perforin-dependent cytotoxicity also plays an important role in the maintenance of T cell homeostasis [37]. During infections, pathogen-specific T cells undergo a massive expansion and activate their direct and indirect antimicrobial effector pathways including cytotoxicity and release of inflammatory cytokines such as interferon-gamma (IFN-γ). These pathways are also used by NK cells and lead to control of pathogen replication in infected tissues, but also to the elimination of antigen-presenting

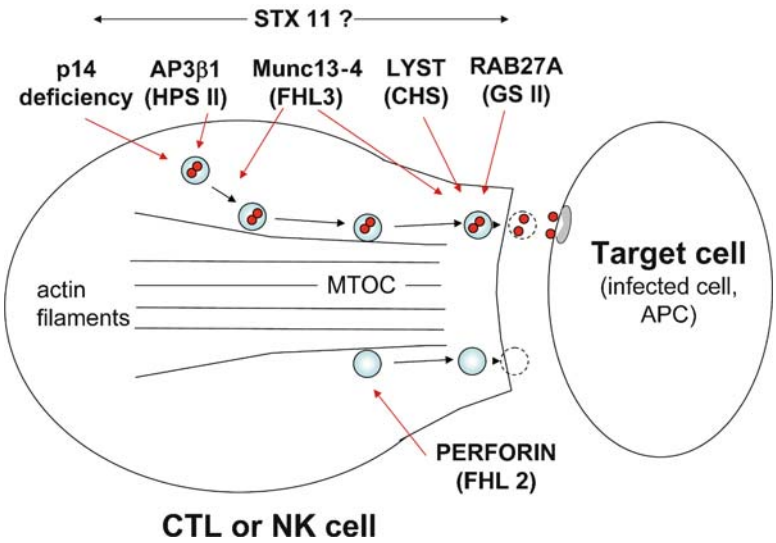

Fig. 5.1 Pathogenesis of cytotoxicity defects. Adapted from [38]

cells (APC). Both of these processes lead to a reduction in the level of antigenic stimulation of T cells. As a consequence, most of the effector T cells die leaving a pool of memory T cells that can mediate recall responses on further exposure to antigen. In the absence of perforin-dependent cytotoxicity, this "negative feed-back loop" is ineffective [38, 112]. Prolonged stimulation by APC and impaired pathogen control leads to uncontrolled expansion and persistence of the activated CTL.

Uncontrolled secretion of cytokines by activated CTL and NK cells leads to the hyperinflammatory state characteristic of hemophagocytic lymphohistiocytosis (HLH). Experiments with perforin-deficient mice have identified IFN-γ as a key cytokine involved [91]. IFN-γ is toxic to hematopoietic cells, which contributes to the cytopenia of HLH [15]. It is also a crucial activator of macrophages and tissue infiltration by macrophages with increased phagocytic activity are key features of HLH. This includes phagocytosis of blood cells in bone marrow and other infiltrated organs such as the liver, spleen or the brain, the demonstration of which is relevant for the diagnosis of the disease.

5.2.3 Clinical Manifestations

In about 85% of patients with FHL, the disease manifests within the first year, in 70% before 6 months of life [89]. A short period of absence of symptoms and normal development after birth is typical. Although the full picture of HLH is rather characteristic, the initial clinical presentation of the disease is highly variable. In most patients, high fever unresponsive to antibiotic therapy, often undulating, in combination with pallor, vomiting and weight loss, are the first signs of the disease. Hepatosplenomegaly is usually pronounced and progressive, lymphadenopathy can only be observed in about 30% of patients. Jaundice and edema, purpura and bleeding and nonspecific skin rashes may also be present. Neurological symptoms can manifest at the beginning of the disease, but more commonly develop later during disease progression. Typical symptoms and signs include irritability, bulging fontanelle, hyper- or hypotonia, seizures and apathy or coma [70]. These symptoms and signs of HLH may be progressive, leading to a lethal outcome if untreated, or may be remittant occurring in several bouts that ultimately lead to a lethal episode. Although a milder course of the disease with recurrent exacerbations and remissions has been observed in some patients with syntaxin 11 deficiency [151], clinical criteria do usually not allow to differentiate between the different genetic variants of the disease. Late-onset forms of FHL have been described, in particular in patients with missense mutations in perforin. These patients manifested late into adulthood and frequently showed atypical presentations including predominantly neurological disease [53] or aplastic anemia [167].

5.2.4 Diagnosis

Due to the nonspecific symptoms and signs, the diagnosis of FHL is difficult, in particular in patients with an incomplete, late-onset manifestation of the disease (Table 5.1). The two important challenges are to diagnose the hemophagocytic syndrome and to verify a genetically determined form of the disease. Typical

Table 5.1 Diagnostic criteria for hemophagocytic lymphohistiocytosis (www.histio.org)

Category	Criteria
Clinical criteria	Fever Splenomegaly
Laboratory criteria	Cytopenia ≥2 lineages
	Hypertriglyceridemia +/− hypofibrinogenemia
Histopathologic criteria	Hemophagocytosis in bone marrow, spleen or lymphnode
New criteria	Impaired NK cell function
	Ferritin >500 µg/L
	Soluble IL-2 receptor >2,400 u/ml

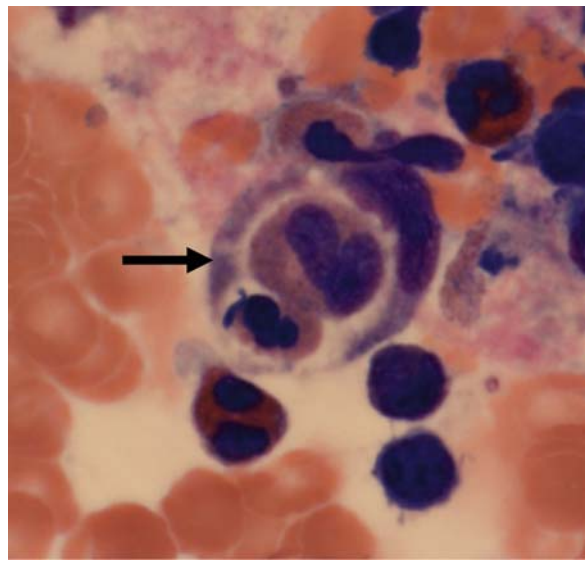

Fig. 5.2 Bone marrow aspirate smear from a hemophagocytic lymphohistiocytosis-patient showing a macrophage engulfing a granulocyte and a red cell precursor (hemophagocytosis)

laboratory findings of HLH include anemia, thrombocytopenia and, to a lesser extent, leukopenia. Clinical chemistry reveals signs of liver dysfunction including hypertriglyceridemia, hyperbilirubinemia, elevated transaminases, highly elevated ferritin (>500 ng/ml), hyponatremia and hypoproteinemia [89]. In addition, coagulation abnormalities are common, in particular hypofibrinogenemia. Analysis of the cerebrospinal fluid frequently shows mononuclear pleocytosis and increased protein, but may also be normal despite the presence of significant magnetic resonance imaging (MRI) changes such as hyperdense areas, atrophy or brain edema [70].

Immunological findings include markedly decreased cytotoxic activity by NK cells and increased levels of activated CD8+ T cells. High levels of several cytokines including TNF-α, IFN-γ, IL-1 and IL-6 can be demonstrated as well as high levels of soluble CD8 or soluble CD25 (sCD25, sIL-2R) reflecting the massive T cell, NK cell and macrophage activation [77]. The major histopathological finding is the infiltration of various organs by activated CTL and macrophages. Hemophagocytosis of erythrocytes and leukocytes is frequently observed, but may be absent (Fig. 5.2). Most organs can be infiltrated, but most frequently the spleen, liver, lymph nodes, bone marrow and central nervous system (CNS).

Diagnostic guidelines for the diagnosis of HLH have been established and may help in the differential diagnosis [76, 78]. Five of the following eight criteria must be fulfilled:

- Fever
- Splenomegaly
- Cytopenia >/= 2/3 lineages (Hb <9 mg/l, Platelets <100,000/ul, Neutrophils <1,000/ul)
- Hypertriglyceridemia and/or hypofibrinogenemia
- Hemophagozytosis in bone marrow, spleen, lymph nodes or CSF
- Reduced NK cell activity
- Ferritin > 500 ng/ml
- sCD25 > 2,400 U/ml

In parallel to the diagnostic evaluation for HLH, the question must be addressed, whether this is a genetic or (more frequently) a secondary form of the disease. A positive family history and parental consanguinity and an early age at manifestation may suggest a familial form. However, secondary forms due to infections, hematopoietic malignancies or autoimmune disease may also manifest in the first year of life [89]. Since infections also contribute to the manifestation of HLH in genetic cases, a careful microbiological work-up is required [43]. This includes blood and cerebrospinal fluid (CSF) cultures, diagnostic evaluation for viral infections [Epstein-Barr virus (EBV) in particular, but also cytomegalovirus (CMV), human immunodeficiency virus (HIV), adenovirus, enterovirus, parvovirus or human herpesvirus-6 (HHV-6)], fungal infections (aspergillus), bacterial or parasitic infections (congenital lues, miliar tuberculosis, leishmaniosis, malaria, and brucellosis). Visceral leishmaniosis is particularly difficult to diagnose and may require repeated very careful analysis of bone marrow smears in addition to serological tests. It is not a rare cause of HLH and should be actively sought for [61].

Demonstration of an infectious trigger of HLH may allow directed therapy, but does not discriminate

between primary and secondary forms of the disease. Although useful diagnostic algorithms have been proposed [2], the role of phenotypic functional immunological tests has not been prospectively evaluated. Absent intracellular staining of perforin in NK cells can support the diagnosis of FHL-2, but variants of the disease with remaining perforin expression have been reported [53]. Absent NK cytotoxicity and is a typical features of FHL, but can also be observed in secondary forms of the disease (Fig. 5.3a). Normalization of NK cell activity during remission is important evidence for a secondary form of the disease. Measurement of CTL mediated cytotoxicity can be more informative, since it is not compromised during active HLH. Recently, measurement of expression of the lysosomal marker protein CD107 on CTL or NK cells has been introduced as a parameter to quantify secretion of lytic granules (Fig. 5.3b) [14]. Reduced degranulation can be observed in patients with FHL-3, FHL-4 or yet undefined genetic disorders of degranulation [24, 110]. However, the test has not yet been used in a sufficient number of patients with secondary HLH to judge its usefulness for differentiating between primary and secondary forms of the disease. The CD107 assay is also useful in the diagnosis of patients with more complex lysosomal trafficking disorders leading to hypopigmentation and immunodeficiency [49]. Hair microscopy and evaluation of granule morphology in granulocytes may be helpful in differentiating the FHL variants from these diseases (see Sect. 5.3 for more details).

Genetic analysis can help to establish a definite diagnosis of perforin, MUNC13-4 or syntaxin 11 deficiencies. However, in a relevant proportion of cases, diagnosis of FHL still is a diagnosis of exclusion, depending on many anamnestic, clinical, laboratory, immunological and genetic criteria.

5.2.5 Management

Without treatment, FHL is usually lethal within the first year of life. Forms with very early onset of HLH tend to be more aggressive. There is no established prophylaxis to prevent HLH in patients with a genetic diagnosis of FHL prior to the manifestation of HLH. Current protocols for the treatment of HLH (HLH-2004) include chemotherapeutic (etoposide) or immunotherapeutic (anti-thymocyte globulin) regimes in association with cyclosporine A and dexamethasone [78, 131]. Appropriate antimicrobial treatment may help to control the infectious trigger, although this will only sometimes modify the course of the disease. This includes the use of rituximab for control of EBV infection. Intrathecal methotrexate (MTX) may help to treat the neurocerebral involvement and to limit further relapse. Unfortunately, these treatments are not always effective in controlling the primary disease and frequently fail to control relapses. At present, hematopoietic stem cell transplantation (HSCT) is the only curative treatment. The success of

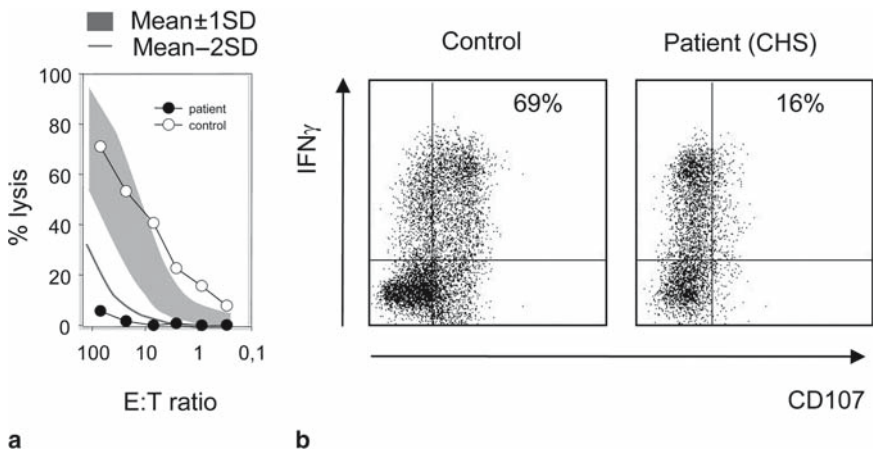

Fig. 5.3 (**a**) Results of an NK cell cytotoxicity assay showing severly impaired cytotoxic activity of patient cells in comparison to cells from a healthy control. (**b**) CD8+ T cell degranulation assay. Short-term PHA-blasts were stimulated with anti-CD3/anti-CD28 and stained for markers of T cell activation (IFN-γ) and degranulation (using the lysosomal marker protein CD107). The T cells from a patient with Chediak-Higashi syndrome fail to degranulate despite normal activation

HSCT depends on the extent of control of HLH prior to transplantation. Parital chimerism appears to be sufficient to prevent HLH reactivation in most cases. The estimated 3-year survival for patients with confirmed FHL in the HLH-94 study was about 50% [79] and similar numbers have been reported in a recent single-center study of 48 patients [131]. New targeted immunotherapeutic approaches are needed for the better control of the severe immune dysregulation prior to HSCT.

5.3 Immunodeficiency with Hypopigmentation *(Chediak-Higashi Syndrome, Griscelli Syndrome, Type II, Hermansky-Pudlak Syndrome, Type II, p14 Deficiency)*

5.3.1 Definition

The occurrence of immunodeficiency and immune dysregulation in patients with oculocutaneous hypopigmentation provides one of the fascinating examples, how basic cell biological processes impinge on several organ systems and cause complex human diseases that require precise differential diagnosis and interdisciplinary patient care. Chediak-Higashi (CHS, OMIM#214500), Griscelli syndrome type II (GSII, OMIM#607624), Hermansky Pudlak syndrome type II (HPSII, OMIM#608233) and p14 deficiency (OMIM#610798) are four autosomal-recessive diseases with hypopigmentation and variable signs of immunodeficiency. While CHS, GSII and HPSII all bear a common risk to develop HLH, this has not yet been reported in p14 deficiency. The genetic basis of these disorders is mutations in genes involved in the biogenesis, transport or delivery of secretory lysosomes (SL).

5.3.2 Etiology

Secretory lysosomes are cellular organelles which are involved in trafficking and exocytosis of intracellular proteins. They have important functions in several cell types, including melanocytes, neuronal cells, platelets, granulocytes, mast cells, NK cells and T cells [32, 170]. The protein machinery required for adequate biogenesis, transport and delivery of secretory lysosomes has a variable composition in each cell type. Therefore, the clinical phenotype of diseases due to genetic defects in proteins involved in lysosomal trafficking varies widely despite the similar cell biological basis. This has led to their classification as predominantly dermatologic, hematologic, hemostaseologic or immunologic disorders. While the seven genetically defined HPS variants predominantly present as bleeding disorders [186], the most important clinical problem of patients with CHS and GS type II is immunodeficiency [38] and immune dysregulation. Neurological defects characterize GS type I and CHS, while patients with GS type III only show oculocutaneous hypopigmentation [113]. This latter feature links all of these syndromes.

CHS [28, 80] is caused by mutations in the *Lysosomal Trafficking Regulator* (*LYST*, OMIM*606897) gene [7], encoding the CHS protein. This protein interacts with components of the t-SNARE (Soluble N-ethylmaleimide attachment protein receptor) complex, which plays a key role in vesicle docking and fusion [170, 171]. Several lysosomal proteins including MHC II, CTLA-4, granzymes and perforin are sorted abnormally in cells from CHS patients [171] and the *LYST* defect also leads to the formation of abnormally enlarged granules [44]. These granules can polarize to the immunological synapse, but cannot fuse with the membrane accounting for defective cytotoxic activity of NK cells and CTL in patients with CHS [32]. The giant granules [44] also form in other cells of the immune system and may cause the impaired chemotactic responses and cell-killing defects observed in CHS. CHS is also expressed in neurons and platelets and abnormal vesicle transport in these cells probably explains the neurological manifestations and the bleeding tendency associated with this disease [170].

GS type II [68] is caused by mutations in the gene (*RAB27A*, OMIM*603868) encoding the GTPase RAB27A [114]. This protein is required for granules to move from the microtubule organizing center (MTOC) to the cell membrane. The granules move along the microtubulus, but fail to detach from it [32, 170, 171]. An interaction between RAB27A and MUNC13-4 has been described [171]. The protein is expressed in NK cells and CTL explaining the cytotoxicity defect in patients with GSII. It is not expressed in neurons [170].

HPS type II is due to mutations in the gene (*AP3B1*, OMIM*603401) encoding the β-subunit of AP-3, an endosomal adaptor protein [40, 86, 92]. CTL from HPSII patients set up a MTOC upon activation, but granules completely fail to travel towards the immunological synapse [32, 170, 171]. AP-3 is also a key

regulator for sorting neutrophil specific elastase [12], which may explain the chronic neutropenia observed in HPSII patients [49]. The protein is also expressed in lysosome-related organelles of osteoclasts and pulmonary epithelial cells which explains bone abnormalities and progressive pulmonary fibrosis in this disease [64].

p14 is an adaptor molecule which seems to play a crucial role in controlling the configuration of the late endosomal compartment [182]. A hypomorphic mutation in the gene (*MAPBPIP*, OMIM*610389) encoding p14 has recently been described in four patients with hypopigmentation [21], short stature and immunodeficiency. p14 deficiency leads to decreased cytotoxic activity by CTL, disturbed B cell differentiation and chronic neutropenia, but the precise molecular mechanism remains to be defined.

5.3.3 Clinical Manifestations

The clinical manifestations of CHS are highly variable [38, 170]. Albinism ranges from mild pigmentary dilution of the skin and hair to severe hypopigmentation resulting in full oculocutaneous hypopigmentation with photophobia and nystagmus. The storage pool deficiency of platelets leads to a bleeding disorder with easy bruisability. Neurological manifestations include seizures, mental retardation, which may be progressive, cranial nerve palsies and progressive peripheral neuropathy. There is a significant susceptibility to pyogenic infections. About 85% of patients develop HLH, also termed "accelerated phase" in this context [38, 170].

Patients with GSII have a more prominent hypopigmentation phenotype than patients with CHS and usually show silvery gray hair and light skin [113, 171]. In some patients, neurological symptoms have been observed, but this is not a constant feature of the disease [96]. Platelet defects are not associated. The risk of developing HLH is higher in GS than in CHS [112, 114, 171]. Most patients die within the first 10 years of life from HLH, if not treated by HSCT.

Oculocutaneous hypopigmentation is also pronounced in patients with HPSII (Fig. 5.4) [40, 49, 86, 92, 161, 162, 171]. These patients also show neutropenia, a moderate bleeding tendency, hepatosplenomegaly, mild facial dysmorphia and dysplastic acetabulae, developmental delay, pulmonary fibrosis and susceptibility to bacterial infections. One of eight patients described so far developed HLH at the age of 3 years [49, 171]. The risk for developing HLH therefore still remains unclear.

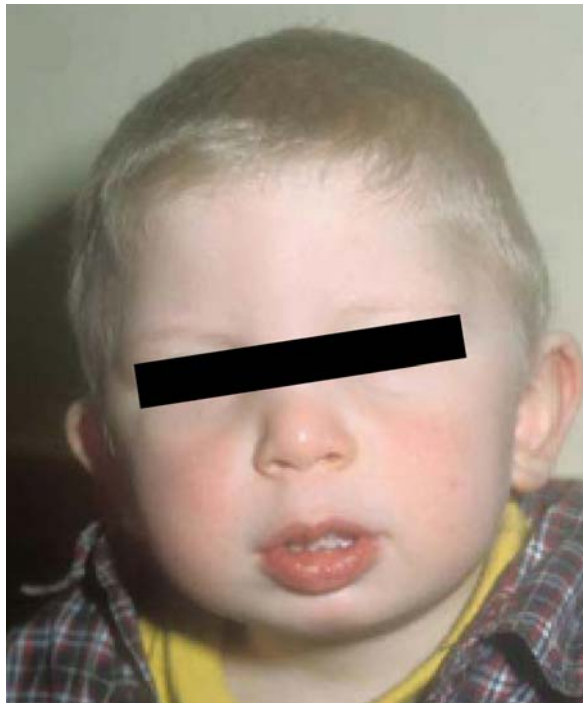

Fig. 5.4 Oculocutaneous hypopigmentation in a patient with Hermansky-Pudlack syndrome, type II

The four reported patients with p14 deficiency showed oculocutaneous hypopigmentation, neutropenia, short stature, coarse facial features and recurrent bronchopulmonary infections [21, 161, 162]. HLH has not yet been observed in p14 deficient patients.

5.3.4 Diagnosis

In any patient with immunodeficiency and hypopigmentation, a disorder of lysosomal trafficking should be suspected. Careful clinical evaluation, in particular with respect to dysmorphy, bleeding and neurodevelopmental issues may help to differentiate the different disorders (Table 5.2). Light microscopy of hair shafts is a simple, helpful extension of these clinical observations (Fig. 5.5). Hairs from CHS-patients present with evenly distributed regular melanin granules, larger than those seen in normal hairs. In contrast, hair from GSII-patient exhibit bigger and irregular melanin granules. In a single patient with HPSII, unevenly distributed small clumps of pigment were identified [21, 49, 161, 162]. The distribution of melanin in hair shafts of p14 deficient patients has so far not been reported.

Table 5.2 Differential diagnosis of oculocutaneous hypopigmentation and immunodeficiency

	Griscelli II	Chediak-Higashi	Hermansky-Pudlak II	p14 deficiency
Oculocutaneous hypopigmentation	+	+	+	+
Immunodeficiency	+	+	+	+
Developmental delay	–	+	–/+	–
Neutropenia	–	–	+	+
Bleeding disorder	–	+	+	–

The presence of giant azurophilic, PAS-positive granular inclusions in peripheral blood leukocytes (Fig. 5.6) is virtually diagnostic of CHS [44]. Chronic G-CSF responsive neutropenia is a characteristic feature of HPSII [49], differentiates it from other forms of HPS and is also prominent in p14 deficiency [92]. A prolonged bleeding time suggests CHS or HPSII, but is not observed in all patients with these disorders. Reduced or absent NK cell cytotoxicity, which can be restored by IL-2, has been reported in all syndromes except for p14 deficiency. Decreased CTL cytotoxicity is usually observed and CTL degranulation as assessed by CD107 expression has been shown to be reduced in all syndromes [49] except for p14 deficiency.

5.3.5 Management

General management of patients with oculocutaneous hypopigmentation and immunodeficiency includes control of infections and bleeding (in CHS and HPSII). Severe bleeding episodes during surgery responded well to tranexamic acid in individual cases [49]. The chronic neutropenia in HPSII and p14 deficiency can usually be well controlled using G-CSF [21, 49], but care should be taken to use this drug in HLH episodes. Patients with p14 defeciency were noted to have reduced memory B cells and specific antibodies upon vaccination were partially missing. IgG substitution might therefore be indicated in selected individuals [21].

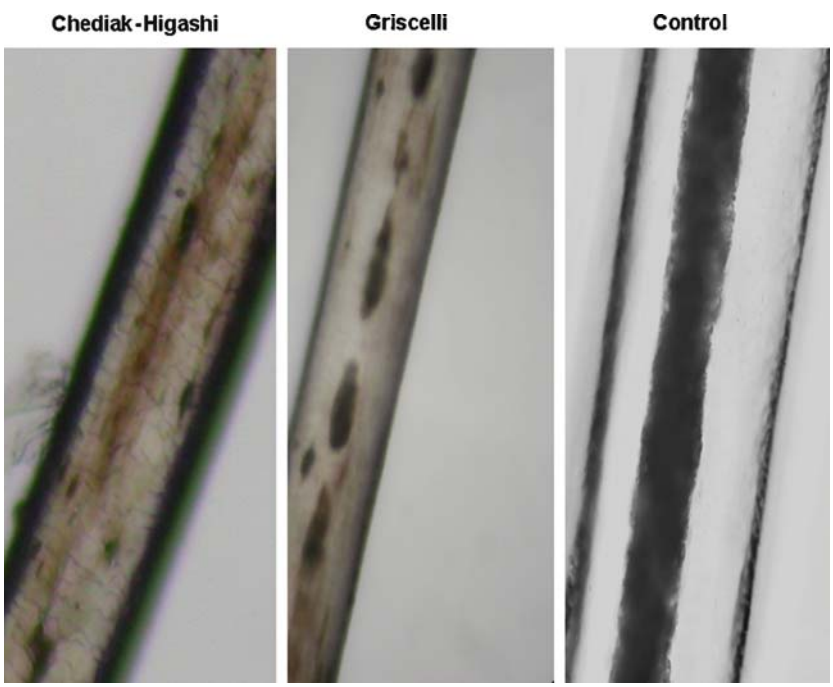

Fig. 5.5 Light-microscopic hair shaft analysis of Chediak-Higashi and Griscelli syndrome in comparison to a control. (Courtesy of N. Parvaneh; Tehran, Iran.)

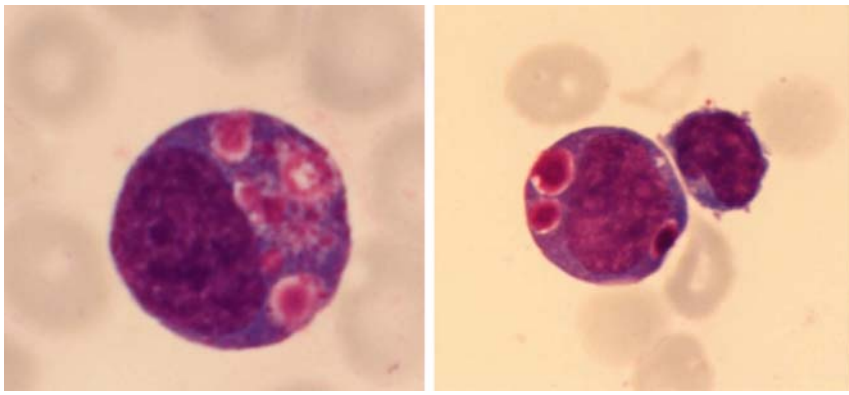

Fig. 5.6 Blood smear from a patient with Chediak-Higashi syndrome showing giant granules in the cytoplasm of leukocytes. (Courtesy of A. Karow; Freiburg, Germany.)

Patients who develop HLH require specific treatment protocols (see Sect. 2.5.2 for more details). If drug-free remissions can be achieved, relapses are frequently observed. Due to the high risk of developing lethal HLH, early evaluation for HSCT should be performed in all patients with genetically confirmed GSII and in most patients with CHS. HSCT is the only available causative therapy for these diseases [38, 170]. The role for preemptive HSCT in HPSII is uncertain. Long-term follow-up indicates that the development and progression of extrahematopoietic manifestations of these diseases including neurological symptoms is unfortunately not arrested by HSCT.

5.4 X-Linked Lymphoproliferative Syndrome (XLP) *(SAP Deficiency, XIAP Deficiency)*

5.4.1 Definition

X-linked lymphoproliferative disease (XLP), also called Purtilo syndrome, is a genetic disorder of immune regulation which has first been described more than 30 years ago [71, 140]. The disease manifestations are variable and mainly include fulminant infectious mononucleosis, dysgammaglobulinemia and lymphoma. In many cases, disease onset is triggered by EBV infection while prior to this event patients usually appear clinically healthy. Two variants of XLP have been described. XLP-1 (OMIM#308240) is caused by mutations in the "src homology 2-domain containing gene 1A" (*SH2D1A*, OMIM*300490) encoding a protein named "signaling lymphocytic activation molecule (SLAM)-associated protein" (SAP). XLP-2 (OMIM#300635) is caused by mutations in the gene encoding X-linked inhibitor-of-apotosis (*XIAP*, OMIM*300079). The genetic basis of further forms of the disease remains to be characterized.

5.4.2 Etiology

SAP is a small cytosolic adaptor protein that modulates intracellular signal transduction of receptors of the SLAM-family in T lymphocytes, NK cells and NKT cells (natural killer T cells) [160]. The interaction of SAP with SLAM-family receptors modulates the activation and interaction of T, B and NK cells. It influences cytokine production, cytotoxicity and antibody production [33, 75, 102, 139]. The role of SAP in this large variety of immune cell functions contributes to the heterogeneity of clinical presentations encountered in patients with XLP.

XLP-patients show defects in cytotoxicity in NK cells and CD8+ T cells [11, 132, 159, 178] that can be corrected by transfection of SAP [159]. The impaired cytotoxicity probably contributes to the increased susceptibility of XLP patients to HLH that usually develops in the context of an acute EBV infection. It is likely, that failure to eliminate proliferating EBV-infected B cells leads to prolonged antigen presentation with the subsequent hyperactivation of CTL and macrophages which is characteristic of HLH. This "fulminant infectious mononucleosis" is therefore not primarily due to a failure to control the pathogen (EBV viral loads can be moderate), but due to a failure to maintain immune homeostasis after a strong viral trigger infection. At present, it is unknown why this syndrome is triggered selectively by EBV, although its ability to induce excep-

tionally strong CTL responses certainly contributes. Therapeutic elimination of B lymphocytes may prevent the progression of EBV infection towards fulminant infectious mononucleosis [116]. The observed dysfunction of T lymphocytes and NK cells [11, 121, 132] may also impair the immunological surveillance and therefore facilitate the development of lymphomas in XLP-patients [50, 156].

Patients with XLP show defective immunoglobulin isotype switching and a reduced number of memory B cells [107, 108]. These humoral abnormalities are not due to an intrinsic defect in B cells, but probably arise from insufficient IL-10 secretion and upregulation of ICOS (Inducible costimulator) by CD4+ T cells [107]. They explain the hypogammaglobulinemia that frequently develops in XLP patients. SAP also seems to play a central role in the regulation of the synthesis of several cytokines, some of which lead to a shift of the Th1/Th2 balance towards the peferential production of Th1 cytokines [25, 35, 107, 121, 191]. SAP is required for recruitment of the tyrosine kinase Fyn to receptors of the SLAM-familiy [27, 101, 102]. The presence of Fyn is essential for NKT cell development [47, 60]. This may explain that XLP-patients show a lack of NKT cells [30, 123, 133]. Since NKT cells have immunoregulatory functions [99, 179], their absence may further contribute to the dysregulated immune response observed in XLP patients.

The pathophysiology leading to an XLP-phenotype in XIAP deficiency (XLP-2) is currently unknown. In agreement with the known functions of XIAP as an inhibitor of caspases [48], lymphocytes from XIAP-deficient patients display increased susceptibility to apoptotic stimuli [148]. Excessive lymphocyte apoptosis might impair the control of EBV infection in XIAP deficiency. Similar to XLP-1, XIAP deficiency also leads to a lack of NKT cells [148]. Interestingly, 2B4-mediated NK cytotoxicity has been shown to be normal in XIAP deficiency [148].

5.4.3 Clinical Manifestations

Although subtle immunological abnormalities can be observed in all XLP patients, the patients usually do not show clinical signs of immunodeficiency or immune dysregulation within the first years of life. XLP typically presents after EBV infection with one of three phenotypes: (1) fulminant infectious mononucleosis, (2) dysgammaglobulinemia, or (3) lymphoma (Table 5.3). More than one XLP-phenotype can present in a single patient simultaneously or consecutively. Although EBV is the most frequent trigger for the manifestation of XLP, dysgammaglobulinemia or lymphoma can also occur in the absence of prior EBV infection [23, 65, 125, 172, 174]. There is no good genotype–phenotype correlation even within members of the same family harboring identical mutations in *SH2D1A* [3, 117, 174, 175]. Female carriers of mutations in *SH2D1A* are usually clinically well, although abnormal patterns of antibody responses to EBV have been described [152].

Fulminant infectious mononucleosis (FIM) occurs in 50–60% of reported patients. It typically occurs between 2 and 3 years of life, but can also manifest at later timepoints [174]. Early clinical signs are similar to "ordinary" infectious mononucleosis including fever, malaise, pharyngitis, anorexia, lymphadenopathy and (hepato)-splenomegaly, but the course is more severe. The disease progresses towards HLH with severe hepatitis, bone marrow hypoplasia leading to thrombocytopenia and anemia, as well as central nervous system inflammation [118]. EBV-induced HLH in XLP patients is difficult to control and frequently has a lethal outcome.

However, EBV-infection does not lead to fulminant mononucleosis in all XLP-patients [174]. Patients who survive infectious mononucleosis often develop a combined immunodeficiency whose onset peaks between 6 and 9 years of age [157, 174]. It may in part be due to the extensive necrosis within lymphoid organs and bone marrow during EBV infection [156]. The resulting dysgammyglobulinemia leads to recurrent infections, which often affect the sinuses, middle ears or lungs and are typically caused by pyogenic encapsulated bacteria. Cutaneous, gastrointestinal or even systemic infections, such as sepsis, meningitis or septic arthritis have also been described. The clinical and immunological phenotype may be indistinguishable from common variable immunodeficiency (CVID) [85].

XLP patients carry a high risk of developing lymphomas [141]. In about 30% of XLP-patients lymphoma is the initial manifestation, usually around the age of 5 years [157, 174]. The vast majority of lymphomas occurring in XLP-patients are of B cell origin, approximately half of which are of the Burkitt-type [73]. The clinical picture includes lymphadenopathy, fever, weight loss, night sweats and reduced activity. Since lymphomas in XLP-patients are often localized extranodally and in particular in the ileocoecal region, abdominal complaints such as pain, nausea, vomiting, diarrhea and

Table 5.3 Clinical and laboratory findings in XLP patients

Clinical findings	Laboratory findings
Male gender	Activated CD8+ T cells increased
	Reduced memory B cells
	Lack of NKT cells
	Variable impairments in cytotoxicity
Fulminant infectious mononucleosis:	
Fever	Pancytopenia
Malaise	Hemophagocytosis
Pharyngitis	Elevated liver enzymes
Lymphadenopathy	Disturbed coagulation studies
(Hepato-) splenomegaly	Hyperferritinemia
Encephalopathy	High levels of soluble IL-2 receptor
Bleeding disorder	EBV-PCR positive
Lymphoma:	
Lymphadenopathy	Often of B cell origin
Fever	Often Burkitt-type
Night sweats	
Weight loss	
Malaise	
Intestinal symptoms	

decreased appetite are common [73]. Less common presentations of XLP include aplastic anemia, lymphoid vasculitis and a pulmonary lymphoid granulomatosis [46, 141].

5.4.4 Diagnosis

XLP should be suspected in any male patient with severe infectious mononucleosis progressing to HLH. Progressive cytopenia may not be present at the beginning, but usually develops after the first 2 weeks of illness. At this timepoint, bone marrow examination may reveal a massive infiltration by lymphocytes as well as hemophagocytosis and extensive necrosis [118]. Liver dysfunction can progress to liver failure culminating in hepatic encephalopathy and impaired coagulation [66]. Other diagnostic markers of EBV-induced HLH are described in the Sect. 5.2.4. EBV-DNA can usually be found by PCR in blood and various tissues. EBV viral load may not be extraordinarily elevated, since clinical disease is a reflection of a pathological immune reaction triggered by EBV and not of uncontrolled EBV-proliferation. Antibodies to EBV can be detected, but the pattern is variable. Antibodies towards Epstein-Barr nuclear antigen (EBNA) are frequently absent, also in patients who survive the acute EBV infection. In contrast, antibody levels against the EBV-viral capsid antigen (VCA) can be either low or elevated [72]. Most XLP patients are hypogammaglobulinemic and some display increased IgM- or IgA-levels [67, 134, 172]. Specific antibody responses may be impaired. A positive family history concerning males that have died from severe infections or unknown causes, patients with susceptibility to infections and (extranodal) lymphomas in patients with a CVID-like clinical picture should prompt diagnostic evaluation for XLP [117, 168].

Lymphocyte phenotyping shows a number of cellular immunological abnormalities, but most of them are poorly specific. They include a high percentage of activated CD8+ T cells leading to a decreased ratio of CD4/CD8 T cells, reduced numbers of CD27+B memory cells, a lack of switched memory B cells and NKT cells [30, 108, 109, 123, 133]. Cytotoxicity assays may reveal an impaired function of NK and T cells [11, 25,

45, 102, 132, 159, 178], but increased NK cytotoxicity during acute EBV infection has also been reported [85]. Analysis of SAP protein expression by western blot or flow cytometry has been successfully used to diagnose XLP [65]. The diagnosis is usually confirmed by detection of mutations in the *SH2D1A* gene. However, approximately 40% of the patients presenting with an XLP phenotype do not have mutations in *SH2D1A* [34, 194]. It remains to be established how many of these have mutations in the *XIAP* gene or in regulatory sequences affecting *SH2D1A*-expression.

5.4.5 Management

It has been suggested that all identified XLP-patients should receive immunoglobulin replacement therapy [63, 156, 173]. Although Ig replacement helps to ameliorate the susceptibility to bacterial infection in XLP-patients with dysgammaglobulinemia, it does not prevent primary EBV infection [109, 127, 157] or the development of lymphoma or other manifestations of XLP. In XLP-patients developing lymphoma, remissions can usually be achieved by standard chemotherapy protocols, but relapses are very common.

Treatment of fulminant mononucleosis relies on the principles of treatment for HLH [79, 87]. In particular, the administration of etoposide has been beneficial in several patients [88, 115, 128, 157]. A few patients with XLP, whose acute EBV infection has been treated with a combination of rituximab, steroids, Ig substitution and acyclovir, did not show progression towards fulminant infectious mononucleosis [116, 128]. A beneficial effect of rituximab is plausible considering the pathogenesis of the disease, but more experience is needed before this approach can be recommended as an alternative to a full HLH protocol.

XLP can currently only be cured by allogenic HSCT [3, 69, 81, 94, 100, 138, 157, 163, 184, 190, 196]. The reported experience suggests that transplantations before the age of 15 years may be more successful than in older patients [69]. Even though a benefit from the selection of EBV-positive donors has not yet been proven, theoretically the presence of donor-derived, fully functional EBV-specific T-lymphocytes in the graft might be helpful. Most patients have been transplanted using a myeloablative conditioning regime. However, in severely compromised patients with organ dysfunction due to fulminant infectious mononucleosis, reduced-intensity conditioning might be considered because of its lower level of toxicity. It has been suggested that adoptive immunotherapy with donor-derived EBV-specific cytotoxic T cells could control the increased risk of EBV-reactivation after reduced-intensity conditioning [154].

Prognosis for patients with XLP is still very poor with approximately 70% of patients dying within the first 10 years of life [157]. The fatality rate is highest in fulminant infectious mononucleosis reaching 96%, whereas it is 65% in lymphoma and about 50% in dysgammaglobulinemia [157].

5.5 Autoimmune Lymphoproliferative Syndrome (ALPS) *(ALPS Ia, Ib, IIa, IIb, III)*

5.5.1 Definition

Autoimmune lymphoproliferative syndrome (ALPS, OMIM#601859) is a genetically heterogenous syndrome that can be inherited in an autosomal-dominant and autosomal-recessive fashion or may arise due to somatic mutations in hematopoietic progenitors. The clinical manifestations include chronic nonmalignant lymphadenopathy and/or splenomegaly and autoimmune manifestations, mainly autoimmune anemia, thrombocytopenia and neutropenia as well as hematological malignancies. The molecular basis of most cases of ALPS is mutations in genes involved in CD95 mediated apoptosis (CD95, CD95L, caspase 8 and 10). However, a significant proportion of patients lack mutations in these genes and in some of these no defect in extrinsic apoptosis can be demonstrated, suggesting alternative pathways of disease pathogenesis.

5.5.2 Etiology

The CD95 death receptor pathway is crucial for lymphocyte apoptosis induction [4] and defects in the molecular machinery of this and probably other extrinsic and intrinsic pathways of lymphocyte apoptosis are the pathophysiologic basis of ALPS [57, 147]. CD95 is a member of the death receptor family, a familiy of transmembrane proteins containing a similar intracellular death domain (Fig. 5.7). Activation of CD95 by binding of its ligand CD95L requires formation of homotrimers of both molecules [136]. Their interaction mediates formation of the death inducing signaling complex (DISC), which is formed by interaction of the death domains of CD95 trimers with the adaptor protein

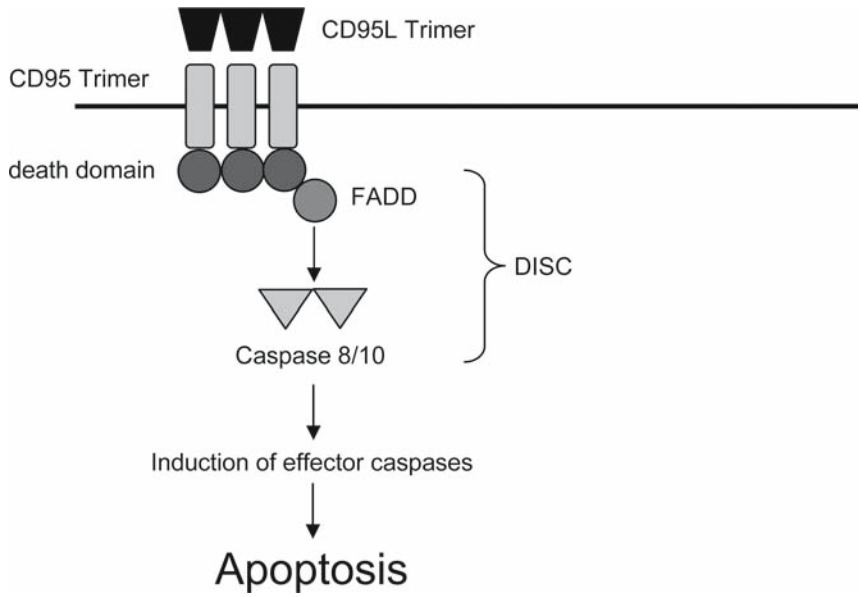

Fig. 5.7 Simplified overview of the CD95 mediated apoptosis pathway Both CD95 ligand and its receptor need to be trimerized to be activated. Upon interaction a so called "death-inducing signaling complex" (*DISC*) is initiated. This complex consists of the trimerized death domain, the adaptor molecule *FADD* (Fas-associated death domain) and activated caspase 8 (also called FLICE-1) and caspase 10 (FLICE-2). This complex activates effector caspases which then further mediate the induction of apoptosis

FADD (Fas-associated death domain) and subsequent recruitment and activation of the proteases caspase 8 and 10 [145]. These molecules cleave multiple downstream targets including effector caspases that induce the death of the cell. Apart from this receptor-mediated "extrinsic" pathway of lymphocyte apoptosis, an "intrinsic" pathway triggered by cytokine deprivation, DNA damage or treatment with cytotoxic drugs has also been described [129, 130]. That pathway is dependent on the induction of mitochondrial enzymes. Members of the bcl-2 familiy of proteins such as BIM are the key molecules involved and disturbed extrinsic apoptosis can also cause an ALPS phenotype [129].

CD95 is highly expressed on activated B and T cells and accumulation due to impaired death and of these cells leads to chronic enlargement of lymphoid tissues, in particular lymph nodes, liver and spleen [103]. Both B and T cells accumulate, but the most characteristic lymphocyte population in patients with ALPS are CD4-CD8- T cells that express a α/β T cell receptor (so called double negative or DNT cells) [19]. These cells probably represent previously activated mature T cells that have lost CD8 coreceptor expression. DNT cells are polyclonal and express markers such as CD11b, CD45RA, CD57, HLA-DR and perforin consistent with their linear differentiation from activated cytotoxic T cells [19]. It is speculated that DNT cells may show specificity for autoantigens. They may also contribute to the pathogenesis of ALPS by producing high amounts of IL-10 and other Th2 cytokines favoring the production of autoantibodies [59].

According to the different molecular causes, a classification for ALPS has been proposed (Table 5.4). It should be noted that there is no clear consensus on the terminology and due to ongoing progress in this field, the classification has to be regarded as provisional.

ALPS 0 is a consequence of homozygous null mutations in *CD95* (*TNFRSF6*, OMIM*134637). Affected patients present the most severe phenotype of the disease [39, 59]. ALPS Ia is caused by heterozygous mutations in *CD95*. In 75% of the affected patients, the mutation is found within the intracellular death domain [165]. Both the healthy and the mutant alleles are expressed, but mixed trimers of mutant and normal CD95 proteins do not transduce the death signal effectively [145, 146]. This explains the autosomal dominant inheritance of the disease. The penetrance of the disease is highly variable even within families. Both localization and type of mutation and environmental factors seem to influence the clinical phenotype. A single patient with a dominant *CD95L* (*TNFSF6,* OMIM*134638) mutation has been described [192]. Although he lacked DNT cells

Table 5.4 Current classification of ALPS

Type	Genetic basis
0[a]	Homozygous mutation in *CD95*
Ia	Mutation in *CD95*
Ib	Mutation in *CD95L*
Ic[a]	Homozygous mutation in *CD95L*
Im[a]	Somatic mutation in *CD95*
IIa	Mutation in caspase 10
IIb	Mutation in caspase 8
III	ALPS phenotype, no mutation in known genes
IV[a]	Intrinsic pathway apoptosis defects

[a]Suggested classification by the authors who described these variants; not generally accepted

and splenomegaly and rather presented with features of systemic lupus erythematodes, the disease was classified as ALPS Ib. A recent report described a patient with a homozygous mutation in *CD95L* associated with a more typical phenotype and suggested classification of this genotype as ALPS Ic [39]. A classical ALPS phenotype has also been observed in several patients with somatic rather than germline mutations in *CD95* [82]. These patients have been classified as ALPS Im.

Mutations in caspase 10 (*CASP10*, OMIM*601762) have been associated with an ALPS phenotype and were classified as ALPS IIa (OMIM#603909) [185, 195]. Mutations in caspase 8 (*CASP8*, OMIM*601763) cause ALPS IIb (OMIM#607271). The clinical phenotype of these patients is more severe and includes a severe immunodeficiency due to activation defects of T and B cells in addition to the defect in apoptosis [29]. There is a significant number of patients with autoimmunity, lymphoproliferation and elevated DNT cells were no mutation in Fas, FasL or Caspases 8 and 10 can be found [146]. In most published cohorts this ALPS III population represents up to 30% of the affected individuals [42, 165]. In about 50% of these patients, there is no defect in CD95 mediated apoptosis, indicating the relevance of other intrinsic and extrinsic pathways of apoptosis for the disease. As proof of concept, a patient with an activating *NRAS* (OMIM+164790) mutation leading to a defect in cytokine withdrawal-induced apoptosis has recently been described [129] with some features of ALPS and significant propensity to hematopoietic tumors. It was suggested to classify this disorder as ALPS IV.

5.5.3 Clinical Manifestations

The phenotype of ALPS is highly variable. Onset of disease ranges from birth to 15 years of age, but usually occurs within the first 2–5 years of life [146, 165]. The typical presentation includes features of lymphoproliferation (splenomegaly, chronic non-malignant lymphadenopathy), in many cases accompanied by autoimmune cytopenia of one or more lineages. Patients within the National Institutes of Health (NIH) cohort presented with lymphadenopathy in >90%, splenomegaly in 88% and hepatomegaly in 72%. Coombs positive anemia occurred in 51%, autoimmune thrombocytopenia in 47% and neutropenia in 23% of the patients [165]. Autoimmune cytopenia may also be the first manifestation of the disease in the absence of lymphoproliferation. Therefore, any patient with autoimmune bi- or tricytpenia (Evans syndrome) should be investigated for ALPS [180]. Besides hematological symptoms many other signs of autoimmunity like aczematous skin lesions, hepatitis, uveitis, thyreoditis or glomerulonephritis have been described. The risk to develop malignancy is estimated to be around 10% [146, 166] for ALPS Ia patients and is mainly due to B cell derived lymphomas. ALPS patients do usually not carry an increased susceptibility to infection, but lymphoproliferation leading to local problems and autoimmune neutropenia may predispose to bacterial infections in some patients

5.5.4 Diagnosis

A combination of clinical and laboratory criteria have been suggested for the diagnosis of ALPS (Table 5.5). These include chronic nonmalignant lymphadenopathy and/or splenomegaly persisting for at least 6 months, defective CD95-mediated lymphocyte apoptosis in vitro (Fig. 5.8a) and elevated circulating DNT cells (>1% or >20/μl) (Fig. 5.8b). DNT cells should be quantified repeatedly as levels may fluctuate. These required features may be supported by a positive family history, the demonstration of mutations in disease causing genes or typical histopathological findings. Follicular hyperplasia in lymph nodes and lymphoid hyperplasia of the white pulp are commonly found

Table 5.5 Current diagnostic criteria for ALPS

Required:

- Chronic nonmalignant lymphoproliferation
- >1% TCR α/β+ CD4-CD8- DN T cells in blood or lymph node
- Defective lymphocyte apoptosis in vitro

Supporting:

- Autoimmunity (i.e., cytopenias and skin)/autoantibodies
- Mutations in *CD95*, *CD95L*, caspase 8 or 10 genes

in ALPS [105], but are not exclusive to this disease. The mentioned criteria will help to establish the diagnosis in patients with ALPS 0, Ia, II and some of the patients with type III. However, mutations in *CD95* have been described in some patients with Evans syndrome without features of lymphoproliferation [180]. Moreover, patients with type Ib, Ic, Im, IV and many patients with type III do not show impaired apoptosis in vitro. Furthermore, DNA analysis from PBMC (peripheral blood mononuclear cell) of patients with type Im is normal, because the *CD95* mutation can only be clearly detected in sorted DNT cells. Thus, although the currently proposed diagnostic criteria help to identify the typical patient with ALPS Ia, they are of little help in identifying other variants of the disease or in excluding the diagnosis. Several other laboratory parameters may therefore be useful in supporting the diagnosis, in particular for type III. These include markers of T cell activation such as expression of HLA-DR, high levels of soluble CD25, elevated CD5+ B cells, hypergammaglobulinemia, elevated IL-10, characteristic abnormalities of lipid metabolism including low HDL cholesterol and low APO-A1 serum levels.

5.5.5 Management

The clinical management of patients with ALPS is mainly focused on the problems of lymphoproliferation and autoimmunity. Neither corticosteroids nor immunosuppressive drugs such as azathioprine, cyclosporine or mycophenolate reliably shrink the size of spleen or lymph nodes in ALPS patients [146].

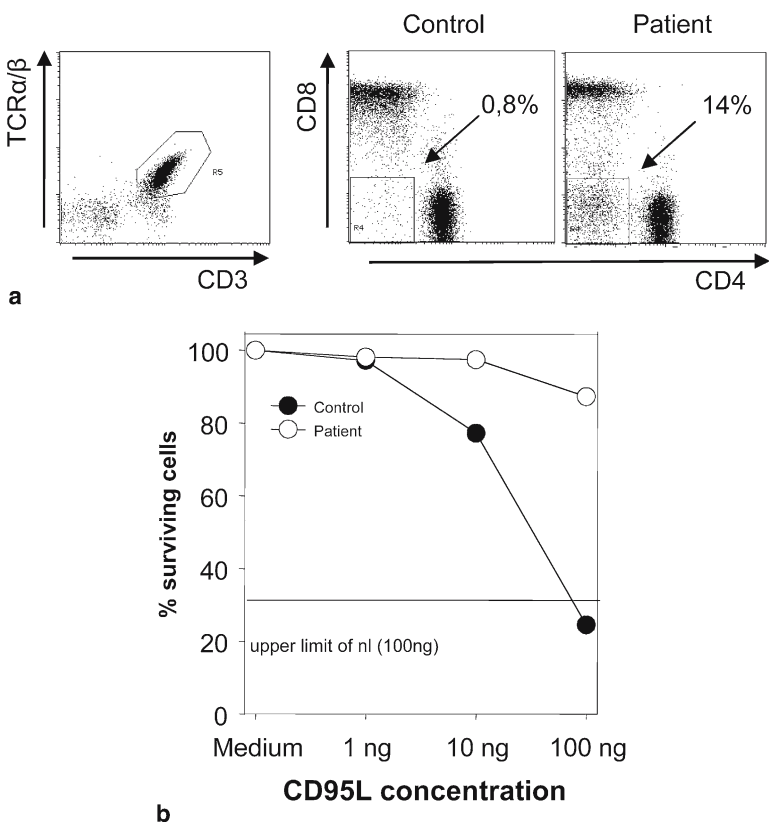

Fig. 5.8 Characteristic immunological findings in patients with ALPS. (**a**) Flow cytometric evaluation of peripheral blood lymphocytes. In ALPS patients, the number of CD3+TCRα/β+ CD4-/CD8- T cells (DNTs) is typically elevated. (**b**) Induction of apoptosis in T cell blasts after stimulation with CD95L. Patients with germline ALPS mutations typically show a reduced fraction of apoptotic cells. In contrast, cells from patients with somatic *CD95* mutations undergo apoptosis similar to healthy controls

Other agents like Fansidar [183], rapamycin [181] or arsenic trioxide [20] were either of limited benefit (Fansidar) or so far only shown to be effective in mice. Half of the patients reported from the NIH cohort have been splenectomized [165]. Indications for splenectomy included refractory hemolysis or thrombocytopenia, rupture or simply tremendous size which limits quality of life or planned pregnancy. Acute hemolytic crisis or significant thrombocytopenia requires immunosuppressive therapy. Intravenous methylprednisolone pulses (10–30 mg/kg) followed by oral prednisolone (1–2 mg/kg) may be of rapid benefit, with or without addition of Ig replacement therapy (1–2 g/kg). Mycophenolate mofetil (MMF) has been successfully used as a steroid sparing agent [144] in some cases of refractory cytopenia rituximab was used successfully [74]. A challenging problem is monitoring of ALPS patients for lymphoma development. In many cases, repeated lymph node biopsy is warranted since imaging studies, including MRI or PET-CT (positron emission tomography-computed tomography), are not helpful in differentiating between benign and malignant lymphoproliferation [143]. HSCT can cure the disease and has been performed in some patients with very severe refractory cytopenias [164]. Due to the severe phenotype it is the treatment of choice in patients with type 0 disease. The life expectancy of patients with ALPS is not significantly reduced; reported deaths were mainly due to sepsis after splenectomy, bleedings and lymphoma.

5.6 Autoimmune Polyendocrinopathy with Candidiasis and Ectodermal Dystrophy (APECED)

5.6.1 Definition

Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED, OMIM#240300), also called Autoimmune Polyendocrine syndrome Type I (APS-I), is an autosomal recessive disorder of immune regulation. The disease is characterized by multiorgan autoimmunity leading to chronic mucocutaneous candidiasis (CMC), hypoparathyroidism and adrenocortical failure as the most common phenotypic manifestations. The genetic basis of APECED are mutations in the gene encoding AIRE (autoimmune regulator) [120, 164], a protein involved in the development of central immunological tolerance.

5.6.2 Etiology

The autoimmune regulator gene (*AIRE*, OMIM*607358) encodes a 545-amino acid protein with multiple functional domains that are typical of a transcriptional regulator [120]. The AIRE protein is expressed at low levels in most lymphoid tissues, but most prominently in medullary thymus epithelial cells (mTEC) [120]. The subcellular localization is mostly nuclear [18]. AIRE is thought to have a role in the regulation of transcription [17] and the induction and maintenance of thymic self-tolerance. Experiments with AIRE-deficient mice have allowed the development of a model for the pathogenesis of APECED [1]. AIRE induces the ectopic expression of a battery of antigens that are otherwise restricted to peripheral tissues [1]. T cells that acquire specificity for these self-antigens during the rearrangement of the T cell receptor genes in the thymus encounter these antigens on thymic medullary epithelial cells and are subsequently negatively selected [1]. Lack of AIRE expression allows these self-reactive T cells to escape thymic deletion and these cells then cause the typical multiorgan autoimmunity in the periphery.

5.6.3 Clinical Manifestations

The clinical phenotype of APECED is very heterogenous (Table 5.6). In the majority of patients, the first symptom is recurrent mucocutaneous candidiasis, which is frequently difficult to treat [135]. It usually presents as oral or nail disease, but may also manifest with substernal pain on swallowing as isolated esophageal candidiasis. CMC is common in European patients, but may be much less common elsewhere. Only four of 24 Iranian Jewish patients developed CMC in the course of their disease [135]. Since APECED patients do not show an increased susceptibility to other infections including other fungal or systemic candida infections, this symptom does probably not reflect impairment of an immunological effector pathway. It is possible that it rather reflects an element of ectodermal dysplasia, which locally facilitates the establishment of candida infection.

Table 5.6 Frequency of Clinical Features of APECED[a]

Disease component	Frequency/prevalence
Endocrine components	*Percent (%)*
Hypoparathyroidism	79
Addison disease	72
Ovarian failure	60
Hypothyroidism	4
Type 1 diabetes	12
Pernicious anemia	13
Nonendocrine components	*Percent (%)*
Mucocutaneous candidiasis	100
Enamel hypoplasia	77
Vitiligo	13
Alopecia	72
Nail dystrophy	52
Malabsorption	18
Autoimmune hepatitis	12
Keratopathy	35
Rare disease components	*Number of cases*
Central diabetes insipidus	6
Growth hormone deficiency	8
Adrenocorticotropin deficiency	3
Gonadotropin deficiency	2
Hyperthyroidism	3
Autoimmune hemolytic anemia	3
IgA deficiency	>18
Asplenia	18
Choleithiasis	7
Periodic fever with rash	11
Sjögren syndrome	20
Oral squamous cell carcinoma	8

[a]Adapted from [135]

The first endocrine manifestation of APECED is usually hypoparathyroidism that often manifests before school-age [135]. It is more frequent in affected females than males. Adrenocortical failure is the third component of the classical clinical triad. Both cortisol and aldosterone deficiency should be actively diagnosed in the presence of symptoms including determination of cortisol levels, adrenocorticotropic hormone (ACTH) stimulation tests and determination of plasma renin activity. Further autoimmune endocrinopathies include diabetes mellitus, usually manifesting beyond 10 years of age, growth hormone deficiency, ovarian failure and – more rarely – male hypogonadism [135].

A variety of other autoimmune manifestations have been described in APECED patients. These include skin diseases such as alopecia, which may remain patchy, but can also become universal, vitiligo and urticarial rashes frequently associated with fever. Gastrointestinal complaints include autoimmune hepatitis, chronic diarrhea and pernicious anemia due to antiparietal cell or intrinsic factor antibodies. keratoconjunctivitis and tubulointerstitial nephritis have also been described. The most frequent manifestation of ectodermal dysplasia is enamel hypoplasia of permanent teeth. All of these disease manifestations should alert the physician to carefully look for other components of APECED.

5.6.4 Diagnosis

The diagnosis of APECED can usually be made on clinical grounds. The triad of candidiasis, hypoparathyroidism and adrenal failure is the most typical presentation of the disease. If a patient presents two of these major features, the clinical diagnosis is established. Due to the large spectrum and the high variability of clinical manifestations, a high index of suspicion is necessary. Among 91 patients with APECED in a Finnish cohort, 89% presented with one of the components of the classical triad CMC, hypoparathyroidism or adrenal failure [135]. It is therefore useful to consider the diagnosis in any patient with CMC beyond the first year of life and in any patient under 30 years of age with hypothyroidism or adrenocortical failure. Routine immunological tests are not helpful for confirming the diagnosis of APECED. One study has described reduced suppressive activity of regulatory T cells, but these results need to be confirmed [135].

The majority of APECED patients have a variety of autoantibodies [135]. The presence of organ-specific autoantibodies does not strictly correlate with autoimmune disease in that organ, but detection of autoantibodies frequently predates the onset of autoimmune disease. Antibodies directed against 21-hydroxilase and side-chain cleavage enzymes are detected in 75 and 61%, respectively, of APECED patients with Addison's disease. Patients with autoimmune hepatitis frequently develop antibodies against tryptophan hydroxilase and aromatic L-amino acid

decarboxylase. The latter antibodies as well as antibodies to GAD65 (glutamic acid decarboxylas) are frequently detected in patients with intestinal dysfunction. Cutaneous autoimmune manifestations such as alopecia and vitiligo are associated with antibodies against SOX10 or tyrosine hydroxilase. Several other tissue-specific autoantibodies have been described in individual patients. Recently, high titer neutralizing immunoglobulin G autoantibodies to most IFN-alpha subtypes and especially IFN-omega have been described in early samples of APECED patients and may have diagnostic value [135].

Definite confirmation of the diagnosis relies on genetic analysis. Common mutations have been described in several populations. The R257X mutation is found in 83% of Finnish APECED chromosomes and is found in many European and North American APECED patients [17]. It involves a CpG dinucleotide, a well-known mutational hot-spot, but a founder effect has not been excluded. A 1,094–1,106 deletion is common in North America [17], while many Italian APECED patients carry the R139X mutation [17] and the Y85C mutation is frequently found in Iranian Jews.

Although heterozygous family members are usually asymptomatic, a significant proportion of APECED patients carry mutations of the *AIRE* gene on one allele only. This may reflect a gene-dosage effect of the regulation of AIRE dependent thymic transcripts [17], but may also indicate other genetic loci that influence central tolerance.

5.6.5 Management

Treatment of patients with APECED requires immunological, endocrinological, gastrointestinal and psychosocial expertise. Most patients require continuous hormone replacement therapy, calcium and vitamin D supplements and systemic antibiotics for candidal infections. In the presence of chronic diarrhea, hypoparathyroidism may be difficult to treat because it impairs the ability to absorb calcium and vitamin D, and hypocalcemia will aggravate the diarrhea. Candidiasis needs to be treated carefully, since uncontrolled chronic candidiasis entails the risk of squamous cell carcinoma, which occurs in up to 10% of patients [135]. For this reason, patients should also be strongly advised to avoid smoking. Immunosuppressive therapy has a limited role in APECED; it may help to treat hepatitis, Keratoconjunctivitis and, in some cases, intestinal dysfunction. Since bone marrow-derived cells can not correct the genetic defect, stem cell transplantation including gene therapy of hematopoietic stem cells has no role in the treatment of APECED.

Once the diagnosis is established, regular follow-up is critical for these patients. This must include accessibility of an expert when a new component of the disease becomes manifest. Follow-up investigations must include ALT (alanine aminotransferase): determinations to monitor liver function, monitoring of calcemia and blood and urine glucose, as well as endocrine function tests for adrenal failure. Regular ophthalmological investigations are also important. Patients need to be instructed about the natural course of the disease and genetic issues have to be discussed.

The unpredictable course of the disease with a continuous risk of developing severe autoimmune diseases implies a strong psychological burden and an impaired quality of life for APECED patients. However, under close medical guidance for many patients the life expectancy is not significantly reduced. Causes of premature death include acute Addissonian crisis or fulminant hepatic failure [135].

5.7 Immunodysregulation, Polyendocrinopathy, Enteropathy, X-Linked (IPEX)

5.7.1 Definition

Immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX, OMIM#304790) is an X-linked recessively inherited disorder of immune regulation first mentioned in 1982 [137]. The disease usually manifests within the first year of life with severe diarrhea and failure to thrive, early-onset diabetes mellitus, thyroid disease, autoimmune cytopenia and variable skin lesions as the most common abnormalities. The genetic basis of IPEX is mutations in the gene "forkhead box P3" (*FOXP3*, OMIM*300292), encoding a protein involved in the thymic generation and peripheral function of regulatory CD4+ T cells.

5.7.2 Etiology

FOXP3 is a protein of 431 amino acids that has a number of structural features characteristic of a transcription factor. This includes motives required for nuclear import and binding to DNA. The gene is expressed predominantly in lymphoid tissues, particularly in CD4+

$CD25^{bright}$ regulatory T cells (Treg). Treg are anergic CD4+ T cells, which upon activation can suppress the proliferation and IL-2 production of naïve and memory CD4+ T cells through a contact-dependent, cytokine-independent mechanism. To be functionally active, FOXP3 forms a homodimer and acts as a transcriptional repressor of cytokine promoters such as NFκB and NFAT (nuclear factor of activated T-cells).

FOXP3 is a "critical differentiation switch" of Treg [187] and has been shown to be essential for the thymic development and function of these cells [58, 84, 95]. Mice deficient in FOXP3 ("scurfy" or FOXP3 knockout mice) lack functional Treg. More conflicting results have been obtained in humans and IPEX patients with normal numbers of Treg have been described [5, 36]. However, they are impaired in their ability to suppress immune activation – with the degree of dysfunction depending on the type of *FOXP3* mutation and the strength of T cell receptor mediated signals [5, 36]. Absence or dysfunction of Treg leads to loss of peripheral tolerance by allowing uncontrolled activation and cytokine production of self-reactive lymphocytes.

Apart from being a lineage-specific differentiation factor, FOXP3 also represses the production of multiple proinflammatory cytokines, such as IL-2, IL-4 and IFN-γ [13, 155, 193]. In mice as well as in humans, mutations in *FOXP3* have been reported to lead to TCR-hyperresponsiveness and a reduced requirement for costimulatory signals manifesting in spontaneous cell proliferation, defective apoptosis and autoimmunity [31, 142]. FOXP3 therefore appears to have important functions in Treg as well as in effector T cells [5, 13], both of which contribute to the clinical manifestations of IPEX syndrome such as autoimmune inflammatory disease.

In some patients displaying the clinical characteristics of IPEX, no mutations in *FOXP3* have been found. In these patients, mutations in regulatory elements of FOXP3 expression or yet unknown proteins interacting with FOXP3 could lead to the disease onset [9]. CD25 is a marker of regulatory T cells and, in mice, lack of CD25 leads to the spontaneous development of autoimmunity [83, 176, 177, 189]. It is therefore not surprising that one of the two published patients with CD25 deficiency showed clinical manifestations very similar to IPEX syndrome [26, 150] (see Sect. 2.15 for more details).

5.7.3 Clinical Manifestations

So far, only affected males have been reported, whereas female carriers of *FOXP3* mutations are asymptomatic. The most prominent feature is a severe watery or mucoid-bloody diarrhea typically starting in early infancy and leading to failure to thrive (Table 5.7). These gastrointestinal manifestations are associated with villous atrophy and lymphocytic infiltrates in the small bowel mucosa. Almost all of the patients also develop early-onset insulin-dependant diabetes mellitus in the first year of life. Anti-islet cell antibodies can frequently be detected. Thyroid disease including hypo- and hyperthyreoidism also represents a common manifestation of the disease and may be associated with elevated thyrotropin levels or antithyroid microsomal antibodies. The majority of patients also show signs of skin disease, which can manifest as erythroderma, exfoliative dermatitis, eczema, and psoriasis-like or ichtyosisform dematitis. Autoimmune hemolytic anemia, thrombocytopenia and neutropenia are also fairly common and usually associated with the presence of autoantibodies. Renal diseases, like glomerulonephritis or interstitial nephritis may occur in up to 50% of the patients [36, 62]. IPEX patients show an increased susceptibility to severe infections, including sepsis, meningitis, pneumonia and osteomyelitis. The most common pathogens include enterococcus, staphylococcus, cytomegalovirus and candida. However, it is a matter of debate, whether the increased susceptibility to severe infections is caused by the FOXP3 defect itself or secondary to impaired barrier function of skin and bowels, autoimmune neutropenia or the immunosuppressive treatment.

Hypofunctional mutations of *FOXP3* can lead to milder phenotypes and a later disease onset [9, 16]. Apart from the typical presentation as an infant with severe bowel disease, diabetes and eczema, any male patient presenting with features of several

Table 5.7 Clinical and laboratory findings in IPEX patients

Clinical findings	Laboratory findings
Male gender	Villous atrophy and lymphocytic infiltrates in small bowel mucosa
Severe diarrhea	Autoantibodies
Failure to thrive	Elevated liver enzymes
Diabetes mellitus	Elevated IgE
Other endocrinopathy	Eosinophilia
Variable skin lesions	Autoimmune cytopenia
Nephritis	
Increased susceptibility to infections	

autoimmune diseases, in particular of the gut and endocrine organs, should therefore raise the suspicion of IPEX.

5.7.4 Diagnosis

The various autoimmune features in IPEX often appear sequentially rather than simultaneously, rendering the clinical diagnosis difficult at the onset of the first characteristic symptoms. Suspicion must be high in patients with severe inflammatory bowel disease and failure to thrive or early-onset diabetes in the first year of life. Biopsies of the small bowels show marked villous atrophy and lymphocytic infiltration of the mucosa, submucosa and lamina propria. The histological picture can be very similar to celiac disease or inflammatory bowel diseases such as Crohn's disease and ulcerative colitis [187]. The presence of antigliadin and antiendomysium antibodies (Ab) does not rule out a diagnosis of IPEX [36], but the disease does usually not respond to a gluten-free diet. Elevations in aminotransferases as well as signs of cholestasis are common [188].

A variety of autoantibodies can be found in IPEX-patients. Anti-islet cell Ab occur most frequently, but anti-insulin Ab, anti-GAD Ab, anti-microsomal Ab, anti-thyroglobulin Ab, anti-smooth muscle Ab, anti-enterocyte Ab, anti-erythrocyte Ab, anti-platelet Ab or anti-neutrophil Ab can also be commonly detected. Most patients show a marked elevation of IgE levels, frequently also eosinophilia. Anemia, thrombocytopenia, neutropenia have been reported at different time points after disease onset. Routine immunological tests including specific antibody responses and lymphocyte proliferation tests are normal. Flow cytometry reveals a lack of regulatory T lymphocytes coexpressing CD4, CD25 and FOXP3. The diagnosis of IPEX can be confirmed by genetic analysis of the *FOXP3* gene.

5.7.5 Management

IPEX is a life-threatening disease and the vast majority of patients die during the first years of life [10, 188]. Early and aggressive therapy is therefore required. In the majority of cases, the diarrhea is unresponsive to dietary changes or even enteric rest and often parenteral nutrition is required to ensure thriving. The diabetes mellitus in IPEX patients can be very difficult to control as insulin requirement can vary substantially and rapidly [119].

Supportive measures such as transfusions of erythrocytes or thrombocytes may be necessary to treat symptoms arising from autoimmune hemolytic anemia or immune thrombocytopenia, respectively. Invasive infections have been reported with Staphylococci, Enterococci, CMV, Klebsiella or Candida species [54, 62, 90, 97, 104, 119, 134, 149]. IPEX-patients presenting with signs of infection should therefore be receive broad spectrum antimicrobial therapy until a causing agent has been identified. The skin lesions in IPEX have been treated with topical cyclosporine A, prednisone and dapsone [6, 124].

Different immunosuppressive agents such as cyclosporine A, tacrolimus, sirolimus, azathioprine, infliximab or steroids have been used either alone or in different combinations with varying success [8, 16, 36, 41, 98, 104, 153, 158, 188] Usually, only partial control of disease activity can be achieved and not maintained for a longer period of time. Other problems related to the immunosuppressive treatment were drug toxicity (especially renal toxicity) and the occurrence of infections due to the profound immunosuppression. Recently, a combination of sirolimus with either methotrexate or azathioprine has been described as an effective treatment with little toxicity in three cases of IPEX [16]. Exacerbations of disease activity can be related to immune stimulating events such as infections or vaccinations [16, 137, 187].

HSCT is the currently the only curative treatment for IPEX [8, 62, 111, 188]. In most patients, symptoms improve markedly during the conditioning therapy. However, the outcome of HSCT has been very poor as most patients died within the first years after HSCT [8, 62, 111, 188]. Since myeloablative conditioning approaches have been associated with a poor outcome, several groups have recently used a nonmyeloablative, reduced intensity conditioning regime consisting of Fludarabine and either melphalane and Campath [142] or Busulfan and Anti-Thymocyte Globulin [106]. The results were promising, but the numbers were small and long-term results have not been reported. In most patients, diarrhea resolved after HSCT, but the reversion of endocrinopathies has been variable [142] – probably due to previous nonreversible damage in endocrine organs. Establishment of mixed chimerism after HSCT is sufficient to achieve a clinical remission [8, 111, 188].

Gene therapy, even though it has so far not been tried in humans suffering from IPEX, seems to be

promising since even small numbers of cells normally expressing FOXP3 appear to be sufficient for a substantial amelioration of the clinical symptoms [8, 187].

References

1. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D (2002) Projection of an immunological self shadow within the thymus by the aire protein. Science 298:1395–1401
2. Arico M, Allen M, Brusa S, Clementi R, Pende D, Maccario R, Moretta L, Danesino C (2002) Haemophagocytic lymphohistiocytosis: proposal of a diagnostic algorithm based on perforin expression. Br J Haematol 119:180–188
3. Arkwright PD, Makin G, Will AM, Ayres M, Gokhale DA, Fergusson WD, Taylor GM (1998) X linked lymphoproliferative disease in a United Kingdom family. Arch Dis Child 79:52–55
4. Ashkenazi A, Dixit VM (1998) Death receptors: signaling and modulation. Science 281:1305–1308
5. Bacchetta R, Passerini L, Gambineri E, Dai M, Allan SE, Perroni L, Dagna-Bricarelli F, Sartirana C, Matthes-Martin S, Lawitschka A, Azzari C, Ziegler SF, Levings MK, Roncarolo MG (2006) Defective regulatory and effector T cell functions in patients with FOXP3 mutations. J Clin Invest 116:1713–1722
6. 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 137:373–378
7. Barbosa MD, Nguyen QA, Tchernev VT, Ashley JA, Detter JC, Blaydes SM, Brandt SJ, Chotai D, Hodgman C, Solari RC, Lovett M, Kingsmore SF (1996) Identification of the homologous beige and Chediak-Higashi syndrome genes. Nature 382:262–265
8. Baud O, Goulet O, Canioni D, Le Deist F, Radford I, Rieu D, Dupuis-Girod S, Cerf-Bensussan N, Cavazzana-Calvo M, Brousse N, Fischer A, Casanova JL (2001) Treatment of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. N Engl J Med 344:1758–1762
9. Bennett CL, Brunkow ME, Ramsdell F, O'Briant KC, Zhu Q, Fuleihan RL, Shigeoka AO, Ochs HD, Chance PF (2001) A rare polyadenylation signal mutation of the FOXP3 gene (AAUAAA→AAUGAA) leads to the IPEX syndrome. Immunogenetics 53:435–439
10. 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 13:533–538
11. Benoit L, Wang XX, Pabst HF, Dutz J, Tan R (2000) Cutting edge: Defective NK cell activation in X-linked lymphoproliferative disease. J Immunol 165:3549–3553
12. Benson KF, Li FQ, Person RE, Albani D, Duan Z, Wechsler J, Meade-White K, Williams K, Acland GM, Niemeyer G, Lothrop CD, Horwitz M (2003) Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. Nat Genet 35:90–96
13. Bettelli E, Dastrange M, Oukka M (2005) Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. Proc Natl Acad Sci USA 102:5138–5143
14. Betts MR, Brenchley JM, Price DA, De Rosa SC, Douek DC, Roederer M, Koup RA (2003) Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. J Immunol Methods 281:65–78
15. Binder D, Fehr J, Hengartner H, Zinkernagel RM (1997) Virus-induced transient bone marrow aplasia: major role of interferon-alpha/beta during acute infection with the noncytopathic lymphocytic choriomeningitis virus. J Exp Med 185:517–530
16. Bindl L, Torgerson T, Perroni L, Youssef N, Ochs HD, Goulet O, Ruemmele FM (2005) Successful use of the new immune-suppressor sirolimus in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). J Pediatr 147:256–259
17. Bjorses P, Halonen M, Palvimo JJ, Kolmer M, Aaltonen J, Ellonen P, Perheentupa J, Ulmanen I, Peltonen L (2000) Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. Am J Hum Genet 66:378–392
18. Bjorses P, Pelto-Huikko M, Kaukonen J, Aaltonen J, Peltonen L, Ulmanen I (1999) Localization of the APECED protein in distinct nuclear structures. Hum Mol Genet 8:259–266
19. Bleesing JJ, Brown MR, Straus SE, Dale JK, Siegel RM, Johnson M, Lenardo MJ, Puck JM, Fleisher TA (2001) Immunophenotypic profiles in families with autoimmune lymphoproliferative syndrome. Blood 98:2466–2473
20. Bobe P, Bonardelle D, Benihoud K, Opolon P, Chelbi-Alix MK (2006) Arsenic trioxide: A promising novel therapeutic agent for lymphoproliferative and autoimmune syndromes in MRL/lpr mice. Blood 108:3967–3975
21. Bohn G, Allroth A, Brandes G, Thiel J, Glocker E, Schaffer AA, Rathinam C, Taub N, Teis D, Zeidler C, Dewey RA, Geffers R, Buer J, Huber LA, Welte K, Grimbacher B, Klein C (2007) A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein p14. Nat Med 13:38–45
22. Bolitho P, Voskoboinik I, Trapani JA, Smyth MJ (2007) Apoptosis induced by the lymphocyte effector molecule perforin. Curr Opin Immunol 19:339–347
23. Brandau O, Schuster V, Weiss M, Hellebrand H, Fink FM, Kreczy A, Friedrich W, Strahm B, Niemeyer C, Belohradsky BH, Meindl A (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). Human Mol Genet 8:2407–2413
24. Bryceson YT, Rudd E, Zheng C, Edner J, Ma D, Wood SM, Bechensteen AG, Boelens JJ, Celkan T, Farah RA, Hultenby K, Winiarski J, Roche PA, Nordenskjold M, Henter JI, Long EO, Ljunggren HG (2007) Defective cytotoxic lymphocyte degranulation in syntaxin-11-deficient familial hemophagocytic lymphohistiocytosis 4 (FHL4) patients. Blood 110:1906–1915
25. Cannons JL, Yu LJ, Hill B, Mijares LA, Dombroski D, Nichols KE, Antonellis A, Koretzky GA, Gardner K,

Schwartzberg PL (2004) SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappa B1. Immunity 21:693–706
26. 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 119:482–487
27. Chan B, Lanyi A, Song HK, Griesbach J, Simarro-Grande M, Poy F, Howie D, Sumegi J, Terhorst C, Eck MJ (2003) SAP couples Fyn to SLAM immune receptors. Nat Cell Biol 5:155–160
28. Chediak MM (1952) New leukocyte anomaly of constitutional and familial character. Rev Hematol 7:362–367
29. Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM, Dale JK, Puck J, Davis J, Hall CG, Skoda-Smith S, Atkinson TP, Straus SE, Lenardo MJ (2002) Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature 419:395–399
30. Chung B, Aoukaty A, Dutz J, Terhorst C, Tan RS (2005) Cutting edge: Signaling lymphocytic activation molecule-associated protein controls NKT cell functions. J Immunol 174:3153–3157
31. Clark LB, Appleby MW, Brunkow ME, Wilkinson JE, Ziegler SF, Ramsdell F (1999) Cellular and molecular characterization of the scurfy mouse mutant. J Immunol 162:2546–2554
32. Clark R, Griffiths GM (2003) Lytic granules, secretory lysosomes and disease. Curr Opin Immunol 15:516–521
33. Cocks BG, Chang CCJ, Carballido JM, Yssel H, Devries JE, Aversa G (1995) A novel receptor involved in T cell activation. Nature 376:260–263
34. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, Cahn AP, Durham J, Heath P, Wray P, Pavitt R, Wilkinson J, Leversha M, Huckle E, Shaw-Smith CJ, Dunham A, Rhodes S, Schuster V, Porta G, Yin L, Serafini P, Sylla B, Zollo M, Franco B, Bolino A, Seri M, Lanyi A, Davis JR, Webster D, Harris A, Lenoir G, St Basile GD, Jones A, Behloradsky BH, Achatz H, Murken J, Fassler R, Sumegi J, Romeo G, Vaudin M, Ross MT, Meindl A, Bentley DR (1998) Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nature Genet 20:129–135
35. Czar MJ, Kersh EN, Mijares LA, Lanier G, Lewis J, Yap G, Chen A, Sher A, Duckett CS, Ahmed R, Schwartzberg PL (2001) Altered lymphocyte responses and cytokine production in mice deficient in the X-linked lymphoproliferative disease gene SH2D1A/DSHP/SAP. Proc Natl Acad Sci USA 98:7449–7454
36. De Benedetti F, Insalaco A, Diamanti A, Cortis E, Muratori F, Lamioni A, Carsetti R, Cusano R, De Vito R, Perroni L, Gambarara M, Castro M, Bottazzo GF, Ugazio AG (2006) Mechanistic associations of a mild phenotype of immunodysregulation, polyendocrinopathy, enteropathy, x-linked syndrome. Clin Gastroenterol Hepatol 4:653–659
37. de Saint BG, Fischer A (2001) The role of cytotoxicity in lymphocyte homeostasis. Curr Opin Immunol 13:549–554
38. de Saint BG, Fischer A (2003) Defective cytotoxic granule-mediated cell death pathway impairs T lymphocyte homeostasis. Curr Opin Rheumatol 15:436–445
39. Del Rey M, Ruiz-Contreras J, Bosque A, Calleja S, Gomez-Rial J, Roldan E, Morales P, Serrano A, Anel A, Paz-Artal E, Allende LM (2006) A homozygous Fas ligand gene mutation in a patient causes a new type of autoimmune lymphoproliferative syndrome. Blood 108:1306–1312
40. 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 beta 3A subunit of the AP-3 adaptor. Mol Cell 3:11–21
41. Di Rocco M, Marta R (1996) X linked immune dysregulation, neonatal insulin dependent diabetes, and intractable diarrhoea. Arch Dis Child Fetal Neonatal Ed 75:F144
42. Dianzani U, Bragardo M, DiFranco D, Alliaudi C, Scagni P, Buonfiglio D, Redoglia V, Bonissoni S, Correra A, Dianzani I, Ramenghi U (1997) Deficiency of the Fas apoptosis pathway without Fas gene mutations in pediatric patients with autoimmunity/lymphoproliferation. Blood 89:2871–2879
43. Domachowske JB (2006) Infectious triggers of hemophagocytic syndrome in children. Pediatr Infect Dis J 25:1067–1068
44. Donohue WL, Bain HW (1957) Chediak-Higashi syndrome; a lethal familial disease with anomalous inclusions in the leukocytes and constitutional stigmata: report of a case with necropsy. Pediatrics 20:416–430
45. Dupre L, Andolfi G, Tangye SG, Clementi R, Locatelli F, Arico M, Aiuti A, Roncarolo MG (2005) SAP controls the cytolytic activity of CD8(+) T cells against EBV-infected cells. Blood 105:4383–4389
46. Dutz JP, Benoit L, Wang XX, Demetrick DJ, Junker A, de S, Tan RS (2001) Lymphocytic vasculitis in X-linked lymphoproliferative disease. Blood 97:95–100
47. Eberl G, Lowin-Kropf B, MacDonald HR (1999) Cutting edge: NKT cell development is selectively impaired in Fyn-deficient mice. J Immunol 163:4091–4094
48. Eckelman BP, Salvesen GS, Scott FL (2006) Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. EMBO Rep 7:988–994
49. Enders A, Zieger B, Schwarz K, Yoshimi A, Speckmann C, Knoepfle EM, Kontny U, Muller C, Nurden A, Rohr J, Henschen M, Pannicke U, Niemeyer C, Nurden P, Ehl S (2006) Lethal hemophagocytic lymphohistiocytosis in Hermansky-Pudlak syndrome type II. Blood 108:81–87
50. Engel P, Eck MJ, Terhorst C (2003) The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. Nat Rev Immunol 3:813–821
51. Farquhar JW, Claireaux AE (1952) Familial haemophagocytic reticulosis. Arch Dis Child 27:519–525
52. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, Lambert N, Ouachee-Chardin M, Chedeville G, Tamary H, Minard-Colin V, Vilmer E, Blanche S, Le Deist F, Fischer A, de Saint BG (2003) Munc13–4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell 115:461–473
53. Feldmann J, Menasche G, Callebaut I, Minard-Colin V, Bader-Meunier B, Le Clainche L, Fischer A, Le Deist F,

Tardieu M, de Saint BG (2005) Severe and progressive encephalitis as a presenting manifestation of a novel missense perforin mutation and impaired cytolytic activity. Blood 105:2658–2663
54. Ferguson PJ, Blanton SH, Saulsbury FT, McDuffie MJ, Lemahieu V, Gastier JM, Francke U, Borowitz SM, Sutphen JL, Kelly TE (2000) Manifestations and linkage analysis in X-linked autoimmunity-immunodeficiency syndrome. Am J Med Genet 90:390–397
55. Filipovich AH (2006) Hemophagocytic lymphohistiocytosis and related disorders. Curr Opin Allergy Clin Immunol 6:410–415
56. Fischer A, Latour S, de Saint BG (2007) Genetic defects affecting lymphocyte cytotoxicity. Curr Opin Immunol 19:348–353
57. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, Puck JM (1995) Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 81:935–946
58. Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4:330–336
59. Fuss IJ, Strober W, Dale JK, Fritz S, Pearlstein GR, Puck JM, Lenardo MJ, Straus SE (1997) Characteristic T helper 2 T cell cytokine abnormalities in autoimmune lymphoproliferative syndrome, a syndrome marked by defective apoptosis and humoral autoimmunity. J Immunol 158:1912–1918
60. Gadue P, Morton N, Stein PL (1999) The Src family tyrosine kinase Fyn regulates natural killer T cell development. J Exp Med 190:1189–1196
61. Gagnaire MH, Galambrun C, Stephan JL (2000) Hemophagocytic syndrome: A misleading complication of visceral leishmaniasis in children – a series of 12 cases. Pediatrics 106:E58
62. 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 15:430–435
63. Gaspar HB, Sharifi R, Gilmour KC, Thrasher AJ (2002) X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. Br J Haematol 119:585–595
64. Gautam R, Novak EK, Tan J, Wakamatsu K, Ito S, Swank RT (2006) Interaction of Hermansky-Pudlak Syndrome genes in the regulation of lysosome-related organelles. Traffic 7:779–792
65. Gilmour KC, Cranston T, Jones A, Davies EG, Goldblatt D, Thrasher A, Kinnon C, Nichols KE, Gaspar HB (2000) Diagnosis of X-linked lymphoproliferative disease by analysis of SLAM-associated protein expression. Eur J Immunol 30:1691–1697
66. Grierson H, Purtilo DT (1987) Epstein-Barr virus infections in males with the X-linked lymphoproliferative syndrome. Ann Intern Med 106:538–545
67. Grierson HL, Skare J, Hawk J, Pauza M, Purtilo DT (1991) Immunoglobulin class and subclass deficiencies prior to Epstein-Barr virus infection in males with X-linked lymphoproliferative disease. Am J Med Genet 40:294–297
68. Griscelli C, Durandy A, Guy-Grand D, Daguillard F, Herzog C, Prunieras M (1978) A syndrome associating partial albinism and immunodeficiency. Am J Med 65:691–702
69. Gross TG, Filipovich AH, Conley ME, Pracher E, Schmiegelow K, Verdirame JD, Vowels M, Williams LL, Seemayer TA (1996) Cure of X-linked lymphoproliferative disease (XLP) with allogeneic hematopoietic stem cell transplantation (HSCT): report from the XLP registry. Bone Marrow Transplant 17:741–744
70. Haddad E, Sulis ML, Jabado N, Blanche S, Fischer A, Tardieu M (1997) Frequency and severity of central nervous system lesions in hemophagocytic lymphohistiocytosis. Blood 89:794–800
71. Hambleton G, Cottom DG (1969) Familial lymphoma. Proc R Soc Med 62:1095
72. Harada S, Sakamoto K, Seeley JK, Lindsten T, Bechtold T, Yetz J, Rogers G, Pearson G, Purtilo DT (1982) Immune deficiency in the X-linked lymphoproliferative syndrome. I. Epstein-Barr virus-specific defects. J Immunol 129:2532–2535
73. Harrington DS, Weisenburger DD, Purtilo DT (1987) Malignant lymphoma in the X-linked lymphoproliferative syndrome. Cancer 59:1419–1429
74. Heelan BT, Tormey V, Amlot P, Payne E, Mehta A, Webster AD (2002) Effect of anti-CD20 (rituximab) on resistant thrombocytopenia in autoimmune lymphoproliferative syndrome. Br J Haematol 118:1078–1081
75. Henning G, Kraft MS, Derfuss T, Pirzer R, Saint-Basile G, Aversa G, Fleckenstein B, Meinl E (2001) Signaling lymphocytic activation molecule (SLAM) regulates T cellular cytotoxicity. Eur J Immunol 31:2741–2750
76. Henter JI, Elinder G, Ost A (1991) Diagnostic guidelines for hemophagocytic lymphohistiocytosis. The FHL Study Group of the Histiocyte Society. Semin Oncol 18:29–33
77. Henter JI, Elinder G, Soder O, Hansson M, Andersson B, Andersson U (1991) Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. Blood 78:2918–2922
78. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G (2007) HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 48:124–131
79. Henter JI, Samuelsson-Horne A, Arico M, Egeler RM, Elinder G, Filipovich AH, Gadner H, Imashuku S, Komp D, Ladisch S, Webb D, Janka G (2002) Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. Blood 100:2367–2373
80. Higashi O (1954) Congenital gigantism of peroxidase granules; the first case ever reported of qualitative abnormity of peroxidase. Tohoku J Exp Med 59:315–332
81. Hoffmann T, Heilmann C, Madsen HO, Vindelov L, Schmiegelow K (1998) Matched unrelated allogeneic bone marrow transplantation for recurrent malignant lymphoma in a patient with X-linked lymphoproliferative disease (XLP). Bone Marrow Transplant 22:603–604
82. Holzelova E, Vonarbourg C, Stolzenberg MC, Arkwright PD, Selz F, Prieur AM, Blanche S, Bartunkova J, Vilmer E, Fischer A, Le Deist F, Rieux-Laucat F (2004) Autoimmune lymphoproliferative syndrome with somatic Fas mutations. N Engl J Med 351:1409–1418

83. Horak I, Lohler J, Ma A, Smith KA (1995) Interleukin-2 deficient mice: a new model to study autoimmunity and self-tolerance. Immunol Rev 148:35–44
84. Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061
85. Hugle B, Suchowerskyj P, Hellebrand H, Adler B, Borte M, Sack U, Overberg-Schmidt US, Strnad N, Otto J, Meindl A, Schuster V (2004) Persistent hypogammaglobulinemia following mononucleosis in boys is highly suggestive of X-linked lymphoproliferative disease – report of three cases. J Clin Immunol 24:515–522
86. Huizing M, Scher CD, Strovel E, Fitzpatrick DL, Hartnell LM, Anikster Y, Gahl WA (2002) Nonsense mutations in ADTB3A cause complete deficiency of the beta3A subunit of adaptor complex-3 and severe Hermansky-Pudlak syndrome type 2. Pediatr Res 51:150–158
87. Imashuku S, Kuriyama K, Sakai R, Nakao Y, Masuda S, Yasuda N, Kawano F, Yakushijin K, Miyagawa A, Nakao T, Teramura T, Tabata Y, Morimoto A, Hibi S (2003) Treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) in young adults: a report from the HLH study center. Med Pediatr Oncol 41:103–109
88. Imashuku S, Kuriyama K, Teramura T, Ishii E, Kinugawa N, Kato M, Sako M, Hibi S (2001) Requirement for etoposide in the treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. J Clin Oncol 19:2665–2673
89. Janka GE (2007) Hemophagocytic syndromes. Blood Rev 21:245–253
90. Jonas MM, Bell MD, Eidson MS, Koutouby R, Hensley GT (1991) Congenital diabetes mellitus and fatal secretory diarrhea in two infants. J Pediatr Gastroenterol Nutr 13:415–425
91. Jordan MB, Hildeman D, Kappler J, Marrack P (2004) An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder. Blood 104:735–743
92. Jung J, Bohn G, Allroth A, Boztug K, Brandes G, Sandrock I, Schaffer AA, Rathinam C, Kollner I, Beger C, Schilke R, Welte K, Grimbacher B, Klein C (2006) Identification of a homozygous deletion in the AP3B1 gene causing Hermansky-Pudlak syndrome, type 2. Blood 108:362–369
93. Kagi D, Ledermann B, Burki K, Zinkernagel RM, Hengartner H (1996) Molecular mechanisms of lymphocyte-mediated cytotoxicity and their role in immunological protection and pathogenesis in vivo. Annu Rev Immunol 14:207–232
94. Kawa K, Okamura T, Yasui M, Sato E, Inoue M (2002) Allogeneic hematopoietic stem cell transplantation for Epstein-Barr virus-associated T/NK-cell lymphoproliferative disease. Crit Rev Oncol Hematol 44:251–257
95. Khattri R, Cox T, Yasayko SA, Ramsdell F (2003) An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 4:337–342
96. Klein C, Philippe N, Le Deist F, Fraitag S, Prost C, Durandy A, Fischer A, Griscelli C (1994) Partial albinism with immunodeficiency (Griscelli syndrome). J Pediatr 125:886–895
97. Kobayashi I, Imamura K, Yamada M, Okano M, Yara A, Ikema S, Ishikawa N (1998) A 75-kD autoantigen recognized by sera from patients with X-linked autoimmune enteropathy associated with nephropathy. Clin Exp Immunol 111:527–531
98. Kobayashi I, Nakanishi M, Okano M, Sakiyama Y, Matsumoto S (1995) Combination therapy with tacrolimus and betamethasone for a patient with X-linked auto-immune enteropathy. Eur J Pediatr 154:594–595
99. Kronenberg M, Gapin L (2002) The unconventional lifestyle of NKT cells. Nat Rev Immunol 2:557–568
100. Lankester AC, Visser LF, Hartwig NG, Bredius RG, Gaspar HB, van der BM, van Tol MJ, Gross TG, Egeler RM (2005) Allogeneic stem cell transplantation in X-linked lymphoproliferative disease: two cases in one family and review of the literature. Bone Marrow Transplant 36:99–105
101. Latour S, Gish G, Helgason CD, Humphries RK, Pawson T, Veillette A (2001) Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. Nat Immunol 2:681–690
102. Latour S, Veillette A (2003) Molecular and immunological basis of X-linked lymphoproliferative disease. Immunol Rev 192:212–224
103. Le Deist F, Emile JF, Rieux-Laucat F, Benkerrou M, Roberts I, Brousse N, Fischer A (1996) Clinical, immunological, and pathological consequences of Fas-deficient conditions. Lancet 348:719–723
104. Levy-Lahad E, Wildin RS (2001) Neonatal diabetes mellitus, enteropathy, thrombocytopenia, and endocrinopathy: Further evidence for an X-linked lethal syndrome. J Pediatr 138:577–580
105. Lim MS, Straus SE, Dale JK, Fleisher TA, Stetler-Stevenson M, Strober W, Sneller MC, Puck JM, Lenardo MJ, Elenitoba-Johnson KS, Lin AY, Raffeld M, Jaffe ES (1998) Pathological findings in human autoimmune lymphoproliferative syndrome. Am J Pathol 153:1541–1550
106. Lucas KG, Ungar D, Comito M, Bayerl M, Groh B (2007) Submyeloablative cord blood transplantation corrects clinical defects seen in IPEX syndrome. Bone Marrow Transplant 39:55–56
107. Ma CS, Hare NJ, Nichols KE, Dupre L, Andolfi G, Roncarolo MG, Adelstein S, Hodgkin PD, Tangye SG (2005) Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4+ T cells. J Clin Invest 115:1049–1059
108. Ma CS, Pittaluga S, Avery DT, Hare NJ, Maric I, Klion AD, Nichols KE, Tangye SG (2006) Selective generation of functional somatically mutated IgM+CD27+, but not Ig isotype-switched, memory B cells in X-linked lymphoproliferative disease. J Clin Invest 116:322–333
109. Malbran A, Belmonte L, Ruibal-Ares B, Bare P, Massud I, Parodi C, Felippo M, Hodinka R, Haines K, Nichols KE, de Bracco MM (2004) Loss of circulating CD27+ memory B cells and CCR4+ T cells occurring in association with elevated EBV loads in XLP patients surviving primary EBV infection. Blood 103:1625–1631
110. Marcenaro S, Gallo F, Martini S, Santoro A, Griffiths GM, Arico M, Moretta L, Pende D (2006) Analysis of natural killer-cell function in familial hemophagocytic lymphohistiocytosis (FHL): defective CD107a surface expression heralds Munc13–4

defect and discriminates between genetic subtypes of the disease. Blood 108:2316–2323
111. Mazzolari E, Forino C, Fontana M, D'Ippolito C, Lanfranchi A, Gambineri E, Ochs H, Badolato R, Notarangelo LD (2005) A new case of IPEX receiving bone marrow transplantation. Bone Marrow Transplant 35:1033–1034
112. Menasche G, Feldmann J, Fischer A, de Saint BG (2005) Primary hemophagocytic syndromes point to a direct link between lymphocyte cytotoxicity and homeostasis. Immunol Rev 203:165–179
113. Menasche G, Ho CH, Sanal O, Feldmann J, Tezcan I, Ersoy F, Houdusse A, Fischer A, de Saint BG (2003) Griscelli syndrome restricted to hypopigmentation results from a melanophilin defect (GS3) or a MYO5A F-exon deletion (GS1). J Clin Invest 112:450–456
114. Menasche G, Pastural E, Feldmann J, Certain S, Ersoy F, Dupuis S, Wulffraat N, Bianchi D, Fischer A, Le Deist F, de Saint BG (2000) Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. Nat Genet 25:173–176
115. Migliorati R, Castaldo A, Russo S, Porta F, Fiorillo A, Guida S, Poggi V, Guarino A (1994) Treatment of EBV-induced lymphoproliferative disorder with epipodophyllotoxin VP16–213. Acta Paediatr 83:1322–1325
116. Milone MC, Tsai DE, Hodinka RL, Silverman LB, Malbran A, Wasik MA, Nichols KE (2005) Treatment of primary Epstein-Barr virus infection in patients with X-linked lymphoproliferative disease using B cell-directed therapy. Blood 105:994–996
117. Morra M, Simarro-Grande M, Martin M, Chen AS, Lanyi A, Silander O, Calpe S, Davis J, Pawson T, Eck MJ, Sumegi J, Engel P, Li SC, Terhorst C (2001) Characterization of SH2D1A missense mutations identified in X-linked lymphoproliferative disease patients. J Biol Chem 276:36809–36816
118. Mroczek EC, Weisenburger DD, Grierson HL, Markin R, Purtilo DT (1987) Fatal infectious mononucleosis and virus-associated hemophagocytic syndrome. Arch Pathol Lab Med 111:530–535
119. Myers AK, Perroni L, Costigan C, Reardon W (2006) Clinical and molecular findings in IPEX syndrome. Arch Dis Child 91:63–64
120. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalioti MD, Mullis PE, Antonarakis SE, Kawasaki K, Asakawa S, Ito F, Shimizu N (1997) Positional cloning of the APECED gene. Nat Genet 17:393–398
121. Nakamura H, Zarycki J, Sullivan JL, Jung JU (2001) Abnormal T cell receptor signal transduction of CD4 Th cells in X-linked lymphoproliferative syndrome. J Immunol 167:2657–2665
122. Neeft M, Wieffer M, de Jong AS, Negroiu G, Metz CH, van Loon A, Griffith J, Krijgsveld J, Wulffraat N, Koch H, Heck AJ, Brose N, Kleijmeer M, van der SP (2005) Munc13–4 is an effector of rab27a and controls secretion of lysosomes in hematopoietic cells. Mol Biol Cell 16:731–741
123. Nichols KE, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, Tangye SG, Schwartzberg PL, Koretzky GA, Stein PL (2005) Regulation of NKT cell development by SAP, the protein defective in XLP. Nat Med 11:340–345
124. Nieves DS, Phipps RP, Pollock SJ, Ochs HD, Zhu Q, Scott GA, Ryan CK, Kobayashi I, Rossi TM, Goldsmith LA (2004) Dermatologic and immunologic findings in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Arch Dermatol 140:466–472
125. Nistala K, Gilmour KC, Cranston T, Davies EG, Goldblatt D, Gaspar HB, Jones AM (2001) X-linked lymphoproliferative disease: three atypical cases. Clin Exp Immunol 126:126–130
126. Ohadi M, Lalloz MR, Sham P, Zhao J, Dearlove AM, Shiach C, Kinsey S, Rhodes M, Layton DM (1999) Localization of a gene for familial hemophagocytic lymphohistiocytosis at chromosome 9q21.3–22 by homozygosity mapping. Am J Hum Genet 64:165–171
127. Okano M, Bashir RM, Davis JR, Purtilo DT (1991) Detection of primary Epstein-Barr virus infection in a patient with X-linked lymphoproliferative disease receiving immunoglobulin prophylaxis. Am J Hematol 36:294–296
128. Okano M, Gross TG (1996) Epstein-Barr virus-associated hemophagocytic syndrome and fatal infectious mononucleosis. Am J Hematol 53:111–115
129. Oliveira JB, Bidere N, Niemela JE, Zheng L, Sakai K, Nix CP, Danner RL, Barb J, Munson PJ, Puck JM, Dale J, Straus SE, Fleisher TA, Lenardo MJ (2007) NRAS mutation causes a human autoimmune lymphoproliferative syndrome. Proc Natl Acad Sci USA 104:8953–8958
130. Opferman JT, Korsmeyer SJ (2003) Apoptosis in the development and maintenance of the immune system. Nat Immunol 4:410–415
131. Ouachee-Chardin M, Elie C, de Saint BG, Le Deist F, Mahlaoui N, Picard C, Neven B, Casanova JL, Tardieu M, Cavazzana-Calvo M, Blanche S, Fischer A (2006) Hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis: a single-center report of 48 patients. Pediatrics 117:e743–e750
132. Parolini S, Bottino C, Falco M, Augugliaro R, Giliani S, Franceschini R, Ochs HD, Wolf H, Bonnefoy JY, Biassoni R, Moretta L, Notarangelo LD, Moretta A (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 192:337–346
133. Pasquier B, Yin L, Fondaneche MC, Relouzat F, Bloch-Queyrat C, Lambert N, Fischer A, Saint-Basile G, Latour S (2005) Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. J Exp Med 201:695–701
134. Peake JE, McCrossin RB, Byrne G, Shepherd R (1996) X-linked immune dysregulation, neonatal insulin dependent diabetes, and intractable diarrhoea. Arch Dis Child Fetal Neonatal Ed 74:F195–F199
135. Perheentupa J (2006) Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Clin Endocrinol Metab 91:2843–2850
136. Peter ME, Dhein J, Ehret A, Hellbardt S, Walczak H, Moldenhauer G, Krammer PH (1995) APO-1 (CD95)-dependent and -independent antigen receptor-induced apoptosis in human T and B cell lines. Int Immunol 7:1873–1877

137. Powell BR, Buist NR, Stenzel P (1982) An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. J Pediatr 100:731–737
138. Pracher E, Panzer-Grumayer ER, Zoubek A, Peters C, Gadner H (1994) Successful bone marrow transplantation in a boy with X-linked lymphoproliferative syndrome and acute severe infectious mononucleosis. Bone Marrow Transplant 13:655–658
139. Punnonen J, Cocks BG, Carballido JM, Bennett B, Peterson D, Aversa G, de Vries JE (1997) Soluble and membrane-bound forms of signaling lymphocytic activation molecule (SLAM) induce proliferation and Ig synthesis by activated human B lymphocytes. J Exp Med 185:993–1004
140. Purtilo DT, Cassel CK, Yang JP, Harper R (1975) X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). Lancet 1:935–940
141. Purtilo DT, Grierson HL, Davis JR, Okano M (1991) The X-linked lymphoproliferative disease: from autopsy toward cloning the gene 1975–1990. Pediatr Pathol 11:685–710
142. Rao A, Kamani N, Filipovich A, Lee SM, Davies SM, Dalal J, Shenoy S (2007) Successful bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. Blood 109:383–385
143. Rao VK, Carrasquillo JA, Dale JK, Bacharach SL, Whatley M, Dugan F, Tretler J, Fleisher T, Puck JM, Wilson W, Jaffe ES, Avila N, Chen CC, Straus SE (2006) Fluorodeoxyglucose positron emission tomography (FDG-PET) for monitoring lymphadenopathy in the autoimmune lymphoproliferative syndrome (ALPS). Am J Hematol 81:81–85
144. Rao VK, Dugan F, Dale JK, Davis J, Tretler J, Hurley JK, Fleisher T, Puck J, Straus SE (2005) Use of mycophenolate mofetil for chronic, refractory immune cytopenias in children with autoimmune lymphoproliferative syndrome. Br J Haematol 129:534–538
145. Rieux-Laucat F, Fischer A, Deist FL (2003) Cell-death signaling and human disease. Curr Opin Immunol 15:325–331
146. Rieux-Laucat F, Le Deist F, Fischer A (2003) Autoimmune lymphoproliferative syndromes: genetic defects of apoptosis pathways. Cell Death Differ 10:124–133
147. Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IA, Debatin KM, Fischer A, de Villartay JP (1995) Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science 268:1347–1349
148. Rigaud S, Fondaneche MC, Lambert N, Pasquier B, Mateo V, Soulas P, Galicier L, Le Deist F, Rieux-Laucat F, Revy P, Fischer A, de Saint BG, Latour S (2006) XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. Nature 444:110–114
149. Roberts J, Searle J (1995) Neonatal diabetes mellitus associated with severe diarrhea, hyperimmunoglobulin E syndrome, and absence of islets of Langerhans. Pediatr Pathol Lab Med 15:477–483
150. Roifman CM (2000) Human IL-2 receptor alpha chain deficiency. Pediatr Res 48:6–11
151. Rudd E, Goransdotter EK, Zheng C, Uysal Z, Ozkan A, Gurgey A, Fadeel B, Nordenskjold M, Henter JI (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:e14
152. Sakamoto K, Seeley JK, Lindsten T, Sexton J, Yetz J, Ballow M, Purtilo DT (1982) Abnormal anti-Epstein Barr virus antibodies in carriers of the X-linked lymphoproliferative syndrome and in females at risk. J Immunol 128:904–907
153. Satake N, Nakanishi M, Okano M, Tomizawa K, Ishizaka A, Kojima K, Onodera M, Ariga T, Satake A, Sakiyama Y,. (1993) A Japanese family of X-linked auto-immune enteropathy with haemolytic anaemia and polyendocrinopathy. Eur J Pediatr 152:313–315
154. Savoldo B, Heslop HE, Rooney CM (2000) The use of cytotoxic t cells for the prevention and treatment of epstein-barr virus induced lymphoma in transplant recipients. Leuk Lymphoma 39:455–464
155. Schubert LA, Jeffery E, Zhang Y, Ramsdell F, Ziegler SF (2001) Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. J Biol Chem 276:37672–37679
156. Schuster V, Terhorst C (2007) X-linked lymphoproliferative disease due to defects of SH2D1A. 2nd:470–484
157. Seemayer TA, Gross TG, Egeler RM, Pirruccello SJ, Davis JR, Kelly CM, Okano M, Lanyi A, Sumegi J (1995) X-linked lymphoproliferative disease: twenty-five years after the discovery. Pediatr Res 38:471–478
158. Seidman EG, Lacaille F, Russo P, Galeano N, Murphy G, Roy CC (1990) Successful treatment of autoimmune enteropathy with cyclosporine. J Pediatr 117:929–932
159. Sharifi R, Sinclair JC, Gilmour KC, Arkwright PD, Kinnon C, Thrasher AJ, Gaspar HB (2004) SAP mediates specific cytotoxic T cell functions in X-linked lymphoproliferative disease. Blood 103:3821–3827
160. Shlapatska LM, Mikhalap SV, Berdova AG, Zelensky OM, Yun TJ, Nichols KE, Clark EA, Sidorenko SP (2001) CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. J Immunol 166:5480–5487
161. Shotelersuk V, Dell'Angelica EC, Hartnell L, Bonifacino JS, Gahl WA (2000) A new variant of Hermansky-Pudlak syndrome due to mutations in a gene responsible for vesicle formation. Am J Med 108:423–427
162. Shotelersuk V, Gahl WA (1998) Hermansky-Pudlak syndrome: models for intracellular vesicle formation. Mol Genet Metab 65:85–96
163. Slatter MA, Bhattacharya A, Abinun M, Flood TJ, Cant AJ, Gennery AR (2005) Outcome of boost haemopoietic stem cell transplant for decreased donor chimerism or graft dysfunction in primary immunodeficiency. Bone Marrow Transplant 35:683–689
164. Sleight BJ, Prasad VS, DeLaat C, Steele P, Ballard E, Arceci RJ, Sidman CL (1998) Correction of autoimmune lymphoproliferative syndrome by bone marrow transplantation. Bone Marrow Transplant 22:375–380
165. Sneller MC, Dale JK, Straus SE (2003) Autoimmune lymphoproliferative syndrome. Curr Opin Rheumatol 15:417–421
166. Sneller MC, Straus SE, Jaffe ES, Jaffe JS, Fleisher TA, Stetler-Stevenson M, Strober W (1992) A novel lymphoproliferative/autoimmune syndrome resembling murine lpr/gld disease. J Clin Invest 90:334–341

167. Solomou EE, Gibellini F, Stewart B, Malide D, Berg M, Visconte V, Green S, Childs R, Chanock SJ, Young NS (2007) Perforin gene mutations in patients with acquired aplastic anemia. Blood 109:5234–5237
168. Soresina A, Lougaris V, Giliani S, Cardinale F, Armenio L, Cattalini M, Notarangelo LD, Plebani A (2002) Mutations of the X-linked lymphoproliferative disease gene SH2D1A mimicking common variable immunodeficiency. Eur J Pediatr 161:656–659
169. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, Henter JI, Bennett M, Fischer A, de Saint BG, Kumar V (1999) Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science 286:1957–1959
170. Stinchcombe J, Bossi G, Griffiths GM (2004) Linking albinism and immunity: the secrets of secretory lysosomes. Science 305:55–59
171. Stinchcombe JC, Griffiths GM (2007) Secretory Mechanisms in Cell-Mediated Cytotoxicity. Annu Rev Cell Dev Biol 23:495–517
172. Strahm B, Rittweiler K, Duffner U, Brandau O, Orlowska-Volk M, Karajannis MA, Stadt U, Tiemann M, Reiter A, Brandis M, Meindl A, Niemeyer CM (2000) Recurrent B cell non-Hodgkin's lymphoma in two brothers with X-linked lymphoproliferative disease without evidence for Epstein-Barr virus infection. Br J Haematol 108:377–382
173. Sullivan JL (2004) X-linked lymphoproliferative syndrome. 1:516–520
174. Sumegi J, Huang D, Lanyi A, Davis JD, Seemayer TA, Maeda A, Klein G, Seri M, Wakiguchi H, Purtilo DT, Gross TG (2000) Correlation of mutations of the SH2D1A gene and epstein-barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. Blood 96:3118–3125
175. Sumegi J, Seemayer TA, Huang D, Davis JR, Morra M, Gross TG, Yin L, Romco G, Klein E, Terhorst C, Lanyi A (2002) A spectrum of mutations in SH2D1A that causes X-linked lymphoproliferative disease and other Epstein-Barr virus-associated illnesses. Leuk Lymphoma 43:1189–1201
176. Suzuki H, Kundig TM, Furlonger C, Wakeham A, Timms E, Matsuyama T, Schmits R, Simard JJ, Ohashi PS, Griesser H,. (1995) Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. Science 268:1472–1476
177. Taguchi O, Takahashi T (1996) Administration of anti-interleukin-2 receptor alpha antibody in vivo induces localized autoimmune disease. Eur J Immunol 26:1608–1612
178. Tangye SG, Lazetic S, Woollatt E, Sutherland GR, Lanier LL, Phillips JH (1999) Cutting edge: human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein SAP. J Immunol 162:6981–6985
179. Taniguchi M, Harada M, Kojo S, Nakayama T, Wakao H (2003) The regulatory role of Valpha14 NKT cells in innate and acquired immune response. Annu Rev Immunol 21:483–513
180. Teachey DT, Manno CS, Axsom KM, Andrews T, Choi JK, Greenbaum BH, McMann JM, Sullivan KE, Travis SF, Grupp SA (2005) Unmasking Evans syndrome: T cell phenotype and apoptotic response reveal autoimmune lymphoproliferative syndrome (ALPS). Blood 105:2443–2448
181. Teachey DT, Obzut DA, Axsom K, Choi JK, Goldsmith KC, Hall J, Hulitt J, Manno CS, Maris JM, Rhodin N, Sullivan KE, Brown VI, Grupp SA (2006) Rapamycin improves lymphoproliferative disease in murine autoimmune lymphoproliferative syndrome (ALPS). Blood 108:1965–1971
182. Teis D, Taub N, Kurzbauer R, Hilber D, de Araujo ME, Erlacher M, Offterdinger M, Villunger A, Geley S, Bohn G, Klein C, Hess MW, Huber LA (2006) p14-MP1-MEK1 signaling regulates endosomal traffic and cellular proliferation during tissue homeostasis. J Cell Biol 175:861–868
183. van der Werff Ten Bosch, Schotte P, Ferster A, Azzi N, Boehler T, Laurey G, Arola M, Demanet C, Beyaert R, Thielemans K, Otten J (2002) Reversion of autoimmune lymphoproliferative syndrome with an antimalarial drug: preliminary results of a clinical cohort study and molecular observations. Br J Haematol 117:176–188
184. Vowels MR, Tang RL, Berdoukas V, Ford D, Thierry D, Purtilo D, Gluckman E (1993) Brief report: correction of X-linked lymphoproliferative disease by transplantation of cord-blood stem cells. N Engl J Med 329:1623–1625
185. Wang J, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, Yao X, Puck JM, Straus SE, Lenardo MJ (1999) Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell 98:47–58
186. Wei ML (2006) Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. Pigment Cell Res 19:19–42
187. Wildin RS, Freitas A (2005) IPEX and FOXP3: clinical and research perspectives. J Autoimmun 25 Suppl:56–62
188. Wildin RS, Smyk-Pearson S, Filipovich AH (2002) Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet 39:537–545
189. Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW (1995) Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. Immunity 3:521–530
190. Williams LL, Rooney CM, Conley ME, Brenner MK, Krance RA, Heslop HE (1993) Correction of Duncan's syndrome by allogeneic bone marrow transplantation. Lancet 342:587–588
191. Wu C, Nguyen KB, Pien GC, Wang N, Gullo C, Howie D, Sosa MR, Edwards MJ, Borrow P, Satoskar AR, Sharpe AH, Biron CA, Terhorst C (2001) SAP controls T cell responses to virus and terminal differentiation of TH2 cells. Nat Immunol 2:410–414
192. 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 98:1107–1113
193. Wu Y, Borde M, Heissmeyer V, Feuerer M, Lapan AD, Stroud JC, Bates DL, Guo L, Han A, Ziegler SF, Mathis D, Benoist C, Chen L, Rao A (2006) FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell 126:375–387

194. Yin L, Ferrand V, Lavoue MF, Hayoz D, Philippe N, Souillet G, Seri M, Giacchino R, Castagnola E, Hodgson S, Sylla BS, Romeo G (1999) SH2D1A mutation analysis for diagnosis of XLP in typical and atypical patients. Hum Genet 105:501–505
195. Zhu S, Hsu AP, Vacek MM, Zheng L, Schaffer AA, Dale JK, Davis J, Fischer RE, Straus SE, Boruchov D, Saulsbury FT, Lenardo MJ, Puck JM (2006) Genetic alterations in caspase-10 may be causative or protective in autoimmune lymphoproliferative syndrome. Hum Genet 119:284–294
196. Ziegner UH, Ochs HD, Schanen C, Feig SA, Seyama K, Futatani T, Gross T, Wakim M, Roberts RL, Rawlings DJ, Dovat S, Fraser JK, Stiehm ER (2001) Unrelated umbilical cord stem cell transplantation for X-linked immunodeficiencies. J Pediatr 138:570–573
197. Zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter JI, Kabisch H, Schneppenheim R, Nurnberg P, Janka G, Hennies HC (2005) Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet 14:827–834