Core Messages

- Combined immunodeficiency comprises a heterogeneous group of disorders, characterized by lack of T cell-mediated immunity and impaired B cell function.
- Combined immunodeficiency has most often an early onset, frequently with protracted diarrhea, interstitial pneumonia, recurrent or persistent candidiasis, and failure to thrive.
- Infections are sustained by intracellular pathogens (especially *Pneumocystis jiroveci*), viruses, bacteria, and fungi.
- Use of live vaccines and of unirradiated blood products is highly contraindicated in infants with severe combined immunodeficiency (SCID).
- Maternal T cell engraftment is common in SCID. It may cause “graft-versus-host”-like features, but may also occur without specific symptoms.
- Family history is of paramount importance. Some combined immunodeficiencies inherited as an X-linked trait, but several autosomal recessive forms exist, which altogether comprise the majority of cases.
- Lymphopenia is a laboratory hallmark of SCID. However, normal lymphocyte count may be observed in some forms of SCID, especially if maternal T cell engraftment is present or if the defect allows for residual T cell development.
- Hypomorphic defects may lead to leaky forms of SCID, with residual T cell development. Autoimmune manifestations are common in this setting.
- Hematopoietic stem cell transplantation is the treatment of choice for SCID, and can cure more than 70% of the affected patients. Promising results have been achieved with gene therapy, although long-term safety remains an issue.

2.1 Introduction

The first description of a child with a deficiency in cellular immunity was made by Glanzmann and Riniker in 1950 [162]. Some years later, Hitzig et al. identified patients with a combined deficiency of the cellular and humoral immunity, the so-called “Swiss Type” agammaglobulinemia with the clinical triad of mucocutaneous candidiasis, intractable diarrhea and interstitial pneumonia [187]. As immunodeficiencies with autosomal recessive and also X-linked transmission were observed subsequently, a heterogeneous etiology was soon suspected.

In the meantime, about 150 different primary immunodeficiency diseases (PID) have been described [133, 152], and more than 120 genes could be identified, whose mutations generate inborn immunodeficiencies [152, 258]. The analysis of the molecular basis of the different PID has contributed to a better understanding of the physiological development of the immune system, allowing a precise molecular diagnosis, genetic counseling and prenatal diagnosis, and is the basis for the development of innovative therapeutical options like gene therapy.

The overall incidence of all inborn immunodeficiencies is 1:10,000 newborns. Even today, in some patients, the diagnosis of PID is made only late or not at all. Recurrent infections with often severe and protracted evolution lead to growth delay and organ damage, and can, if untreated, lead promptly to death in the case of severe PID. Combined immunodeficiencies are a heterogeneous group of immunodeficiencies (Table 2.1) that are characterized by defects of the T cell development and/or T cell function, and that can be associated to variable defects of B or natural killer cells. Due to the missing T cell help, the B cell function is altered even in case of normal B cell maturation.
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<th>Diseases</th>
<th>Subtype</th>
<th>Genetic defects</th>
<th>Associated features</th>
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<tbody>
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<td>T-B+ Severe combined</td>
<td>γc deficiency</td>
<td>IL2RG</td>
<td>Decreased serum levels of all immunoglobulin isotypes; markedly decreased T and NK cells; normal or increased B cells</td>
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<td>immunodeficiency</td>
<td>JAK3 deficiency</td>
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<td>Decreased serum levels of all immunoglobulin isotypes; markedly decreased T cells; normal number of B cells; normal or decreased NK cells.</td>
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<td>CD45 deficiency</td>
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<td>DNA ligase IV deficiency</td>
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<td>Decreased serum levels of all immunoglobulin isotypes; decreased T and B cells; microcephaly; radiation sensitivity</td>
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<td>Cernunnos/XLF deficiency</td>
<td>NHEJ1</td>
<td>Decreased serum levels of all immunoglobulin isotypes; decreased T and B cells; microcephaly; radiation sensitivity; growth retardation</td>
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<td>CD40 deficiency</td>
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<td>Normal or decreased serum levels of immunoglobulin isotypes; decreased number of CD4+ T cells</td>
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<td>Defective TCR mediated activation; autoimmunity; myopathy</td>
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<td>Winged-helix nude (WHN) deficiency</td>
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<td>Decreased serum levels of all immunoglobulin isotypes; markedly decreased T cells; normal number of B cells; alopecia; abnormal thymic epithelium</td>
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<td>CD25 deficiency</td>
<td>IL2RA</td>
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<td>Normal or decreased T cells; decreased number of CD4+ T cells; autoimmunity; lymphoproliferation</td>
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<td>STAT5B deficiency</td>
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<td>Decreased T cells; growth hormone-insensitive dwarfism; dysmorphic features</td>
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Severe combined immunodeficiency (SCID) is the most severe form of PID, which are characterized in most cases by complete absence of T cell-mediated immunity and by impaired B cell function [137]. The overall incidence is about 1:50,000–1:100,000 newborns. Possibly, there may be a higher incidence due to early lethality in undiagnosed cases in the case of patients who succumb in the course of overwhelming infections before the diagnosis of immunodeficiency is made. The differential diagnostic course of overwhelming infections before the diagnosis is made. The differential diagnostic:

### 2.2.1 Definition

T-B+ SCID (OMIM#600802) are characterized by impaired development of mature T cells while B cells are present but nonfunctional. This form presents the most frequently observed SCID phenotype and can be observed in 30–50% of all cases [47, 393]. T-B+ SCID can be further distinguished according to the presence or absence of natural killer (NK) cells.

In the case of γc deficiency and JAK3 deficiency, NK cells are virtual absent (T-B+NK- SCID), whereas NK cell development is intact in SCID patients with T-B+SCID phenotype. While NK cells are present in normal number in the IL-7 receptor α deficiency and in defects of the different subunits of the TCR, the CD3 γ, δ, ε, and ξ-chain, NK cells could be reduced in number in the CD45 deficiency.

**γc deficiency.** De Saint Basile et al. mapped the XL-SCID to the proximal long arm of the chromosome X (Xq12-13.1) [93]. After the cloning of the gamma c gene (IL2RG, OMIM*308380) [402] and its localization in the same region on the X-chromosome, mutations in the gamma c gene have been identified in XL-SCID patients [292, 324].

The gene IL2RG covers 4.5 kb of genomic DNA in Xq13.1 and contains a coding sequence of 1,124 nucleotides distributed into eight exons. It is constitutively expressed in lymphoid cells including both T, B and NK cell-lineages [239] and encodes the gamma c chain of the interleukin-2 receptor. The gamma c is a type 1 transmembrane protein which is transported to the cell membrane after cleavage of a signal peptide.

Defective production of interleukin-2 has been observed in an immunodeficient patient who had detectable circulating T cells [440]. The observation that the knockout mouse for IL-2 shows disturbed peripheral T cell homeostasis and autoimmunity, but does not display a SCID phenotype [370], already suggested that XL-SCID is not caused exclusively by impaired IL-2 mediated signaling. This hypothesis has been further confirmed by the identification of mutations in the IL-2RA gene encoding the interleukin-2 receptor alpha chain (CD25), a subunit of the tripartite high-affinity receptor for interleukin, in a patient who showed decreased numbers of peripheral

**JAK3 deficiency.** Patients with mutations in JAK3 (OMIM*600173) present with an autosomal recessive form of T-B+SCID [253, 299, 361].

**IL7-Rα deficiency.** A selective impairment of T cell development is found in deficiency of the Interleukin-7 receptor alpha (IL7R-ALPHA, OMIM*146661), also known as CD127: B and NK cells are present and patients may show elevated B cell counts. This condition is due to mutations in the Interleukin-7 receptor alpha (IL-7Rα) gene located on chromosome 5p13 [251]; it follows an autosomal recessive inheritance.

**CD45 deficiency.** CD45 deficiency generates T-B+NK+ SCID due to mutations in the CD45 (OMIM+151460) tyrosine phosphatase.

**CD3/TCR complex deficiencies.** Some rare cases of T-B+ SCID may be due to mutations affecting the CD3/T cell receptor (TCR) complex (γ: CD3G, OMIM*186740; δ: CD3D, OMIM*186790; ε: CD3E, OMIM*186830; ξ: CD3Z, OMIM*186780) [135].
T cells and abnormal T cell proliferation but normal B cell development and autoimmune features [375] like the murin IL-2 knockout. The complex XL-SCID phenotype can be explained by the fact that the interleukin-2 receptor gamma chain is not only part of the interleukin-2 receptor but also of the IL-4, IL-7, IL-9, IL-15 and IL-21 receptors [255] [110], and has therefore also been designated "common gamma chain" [239]. Multiple cytokine-mediated pathways are thus abrogated in the gamma c deficiency giving rise to the pronounced defect in T cell maturation. Exceptionally, patients with gamma c deficiency may develop some autologous T cells which may be associated with a milder clinical phenotype [103, 270, 360].

**JAK3 deficiency.** The human JAK3 gene maps to chromosome 19p12-13.1 [189, 362] and is organized in 23 exons. Its cDNA is composed of 4,064 nucleotides encoding for a protein of 1,124 amino acids [371]. JAK3 is a lymphoid tissue-specific tyrosine kinase and belongs to the Janus family of protein kinases [210]. JAK3 is a lymphoid tissue-specific tyrosine kinase and belongs to the Janus family of protein kinases [210]. It is involved in the signal transduction pathway of several cytokines, such as IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 [22, 206]; thus, the same cytokines which are affected by the lack of the common gamma chain in the case of XL-SCID. In fact, JAK3 interacts intracellularly with the common gamma c chain. This explains why the clinical manifestations of XL-SCID and JAK3 deficiency are virtually identical, besides the fact that JAK3 deficiency can be observed in both female and male patients, as it follows an autosomal recessive inheritance. Upon association of the gamma c with JAK3, crossphosphorylation of the JAK proteins and of the cytokine receptor takes place. The STAT proteins are then recruited and themselves phosphorylated. This allows their dimerization, nuclear translocation and binding to regulatory elements in the nucleus. Finally the transcription of target genes is induced [296].

**IL7-Rα deficiency.** An important step during lymphoid development is the interaction between IL-7 and the γ-c containing IL-7 receptor complex. This is underscored by the fact that IL-7 or the IL-7 receptor α chain deficiency generates impaired lymphoid maturation with a SCID phenotype in mice [102, 317]. Whereas IL-15 is important for NK-cell development [224] and IL-21 is implicated in innate and adaptive immune functions [174], the physiological significance of IL-4 or IL-9 impairment during lymphoid maturation is not yet fully elucidated.

IL-7 provides survival and proliferative signals through the IL-7 receptor and thus plays a critical role in early T cell development. Severe combined immunodeficiency with T+B+NK+ phenotype in humans due to mutations in the Interleukin-7 receptor alpha gene was first described by Puel et al. in 1998 in two patients with failure to thrive, diarrhea, recurrent otitis, viral infections and candidiasis [327]. Other patients with defects in the IL-7Rα have subsequently been described [44, 161, 326, 350].

**CD45 deficiency.** The cell-surface coreceptor CD45, or common Leukocyte Surface Protein, is a hematopoietic-cell-specific transmembrane protein that is implicated in the regulation of src kinases involved in T and B cell antigen receptor signaling. Mice with a CD45 deficiency display a profound immunodeficiency. The thymocyte maturation is blocked at the transitional stage from immature CD4+CD8+ to mature CD4+ or CD8+ cells, and only a few T cells are detected in peripheral lymphoid organs [216].

Up to now, few cases of CD45 deficiency have been identified. A 2-month-old infant with mutations in the CD45 tyrosine phosphatase gene was described by Kung et al. [228]. This patient presented with low CD4 numbers while B cell counts were normal and NK cells were found albeit in reduced number. The TCR αβ T cells were lacking, but γδ T cells were present. The second case was reported by Tchilian et al. in 2001 [404]. CD45 deficiency has thus to be examined in T-B+NK+ SCID phenotype when the more common etiologies have been ruled out.

**CD3/TCR complex deficiencies.** The antigen specificity of the T cell receptors (TCR) is based on a heterodimer composed of either the αβ- or γδ-chain. This heterodimer is associated to four polypeptide chains: the CD3 γ, δ, ε and ξ chains. Mutations of each of these transmembrane proteins may occur and may generate an abnormal or absent expression of the TCR causing moderate to severe immunodeficiency [9]. The phenotypic expression may be variable and depends on the degree of the residual expression of the defective TCR-subunit. Patients thus display variable susceptibility to infection and autoimmunity. They have very few or completely undetectable circulating CD3+ T cells, poor responses to T cell mitogens and various levels of immunoglobulins.

CD3γ deficiency has been described in Turkish and Spanish patients [18, 316]. A defect of the δ chain has been found in a Canadian patient [82]. A French patient presented a CD3ε deficiency [387, 406]. Complete CD3δ and γ-deficient patients who present with SCID-symptoms have been described [94, 401]. A 4-month-old boy with primary immunodeficiency was reported to have a homozygous germ-line mutation of the gene encoding the CD3ξ subunit of the T cell receptor–CD3 complex [341]. Interestingly, the CD3ξ-deficiency was partially corrected by somatic mutations resulting in
a milder phenotype and in decreased numbers of circulating T cells. A second patient with complete CD3γ deficiency resulting in T-B+NK+ SCID was described recently [344].

2.2.3 Clinical Manifestations

Despite the huge heterogeneity on the molecular level, the clinical manifestations of the different SCID forms are comparable, as shown by the observations in large cohorts of SCID patients in Europe and the United States of America, which have revealed that the clinical presentation with regard to the infectious events is quite similar [47, 393]. The onset of manifestations is characteristically early, often before the third month of life. Despite the protection through maternal antibodies, SCID patients develop recurrent infections with protracted course and unexpected complications. Before the age of 6 months, the SCID patients develop chronic diarrhea, interstitial pneumonia and/or therapy-resistant mucocutaneous candidiasis. Infections with opportunistic germs like Pneumocystis jiroveci (previously Pneumocystis carinii) or cryptosporidium are currently present. But intracellular microorganisms like listeria, Salmonella typhi, toxoplasma and mycobacteria can also be found. Other manifestations are due to infections by Aspergillus sp or viral infections like adenovirus, respiratory syncytial virus (RSV), cytomegalovirus (CMV), herpes simplex virus (HSV) or Epstein-Barr Virus (EBV). The suspicion of SCID is always to be considered as a “pediatric emergency” with the risk of a rapidly fatal evolution if the immunodeficiency remains undetermined.

The clinical alarm signs in an infant which should direct our attention to a possible immunodeficiency are failure to thrive or loss of weight (often observed between the 3rd and 6th months of age), chronic diarrhea, atypical eczematous skin manifestations, absence of adequate response to current antibiotics, recurrent candidiasis, and persistent respiratory symptoms (chronic cough, chronic respiratory obstruction, progressive tachypnea or dyspnea). The clinical examination of a “classical” SCID patient reveals a hypoplasia of the lymphatic tissues (lymph nodes, tonsils); there is no thymic shadow in the chest radiography. Consanguineous setting is in favor of an inborn error of the immune system as many deficiencies follow an autosomal recessive inheritance-pattern and are thus more frequently observed in consanguineous families. Lymphopenia and hypogammaaiglobulinemia are additional factors that should lead to further immunological investigations.

Vaccination with live vaccines is contraindicated in SCID patients. Bacille Calmette-Guérin (BCG) vaccination in SCID patients causes disseminated infections that may be fatal. Infiltrating and ulcerating lesions at the impact of the vaccination and in the regional lymph nodes, but also systemic propagation, with popular cutaneous lesions, osteolytic lesions and organ impairment of liver, spleen, lymph-node and lung, may occur. As the BCG vaccination is no longer generally recommended in many countries, it should be checked if a patient has been exposed to BCG vaccination and, if so, adequate antibiotic treatment should be initiated even in the absence of any clinical manifestation. In the case of oral live polio vaccine or upon contact with recently vaccinated persons, central nervous poliomyelitis-infections and carditis may occur.

Other SCID manifestations concern in rare cases chronic hepatitis or sclerosing cholangitis. Cutaneous manifestations interests consist in recurrent warts, molluscum contagiosum, atypical eczematous skin lesions, alopecia, seborrhoic skin manifestations as well as cellulitis.

The maternal alloreactive T cells may lead to the clinical picture of “graft versus host disease” (GVHD). Habitually asymptomatic, the so-called “materno-fetal” may touch different organs. Maculopapular rash and hypereosinophilia frequently exist, while more rarely liver involvement with disturbed liver enzymes, profuse diarrhea or pancytopenia are found. Transfusion of nonirradiated blood products can generate a fatal GVHD; thus, only irradiated products should be used.

γc deficiency. XL-SCID is characterized by early onset of severe infections starting during the first months of life, typically between 3 and 6 months of age. The clinical manifestations do not differ substantially from the general presentation of SCID patients. Milder phenotypes exist.

JAK3 deficiency. While most JAK3-deficient patients present with a clinical phenotype virtually indistinguishable from boys affected by XL-SCID, some JAK3 patients reveal an unexpected clinical heterogeneity, emphasizing the need for adequate investigations in order to rule out JAK3 deficiency even in atypical clinical presentations [297].

IL7-Rα deficiency, CD45 deficiency. Patients present the same clinical phenotype as the other SCID patients.

CD3/TCR complex deficiencies. Recio et al. recently studied two new Turkish patients with complete CD3γ deficiency. The comparison with three formerly described CD3γ-deficient patients of Spanish and Turkish origin revealed for all patients a similar
immunological phenotype with a partial TCR/CD3 expression defect, mild αβ- and γδ-T lymphocytopenia, poor in vitro proliferative responses to antigens and mitogens at diagnosis, and very low TCR rearrangement excision circles and CD45RA(+) αβ T cells [332]. Interestingly, an important intrafamilial and interfamilial clinical variability was observed in patients with the same CD3G mutations, two of them reaching the second or third decade, respectively, in healthy conditions, whereas the other three died early in life with typical SCID features associated to enteropathy. In contrast, all reported patients with complete CD3δ (or CD3ε) deficiencies show clearly the life-threatening SCID phenotype with very severe αβ and γδ T lymphocytopenia. These data confirm the observation of Roifman et al., who showed that the absence of CD3δ in humans results in a complete arrest in thymocyte development at the stage of double negative to double positive transition, and in impaired development of γδ T cell receptor-positive T cells [348]. Interestingly, the three studied patients with CD3δ deficiency showed a normal sized thymus shadow on chest radiography, but biopsy revealed abnormal thymus structure [348].

2.2.4 Diagnosis

Anamnesis is a central element in the establishment of diagnosis and allows the identification of those children for whom immediate immunological explorations are indicated. As in most cases SCID follows autosomal recessive or X-linked inheritance, it is very important to perform an exact inquiry of family history and to analyze the genealogical background of the patient. Attention has to be paid to any other family member presenting infectious susceptibility, autoimmune manifestation or tumor-disease. Cases of unidentified infant death have to be reported. Obviously, autosomal recessive inborn errors are more frequent in a consanguine setting.

Basic investigations should contain a complete white blood count. Eosinophilia can be frequently observed in SCID patients. Absolute lymphocyte counts are often less than 1,000/µl, but normal lymphocyte counts do not exclude SCID, as some forms of SCID present with absolute lymphocyte counts which may be within normal range. This may be the case on one hand in SCID-forms in which T cell maturation is only impaired in a limited way (e.g., PNP deficiency), and on the other hand in patients with “leaky” or atypical SCID who present hypomorphic mutations, which allow a residual function of the defective protein.

A special situation is the persistence of maternal T cells after transplacental materno-fetal transfusion. In these cases, the presence of maternal T cells should be eliminated through chimerism analysis; in male patients by in situ XX/XY hybridization of the CD3 positive cells, and in girls by molecular biological methods (HLA or VNTR analysis of CD3 positive cells). In some cases, skin, liver or intestinal biopsies may be necessary to rule out a materno-fetal GVHD. HIV-infection should be ruled out systematically in all cases of suspected SCID.

Analysis of humoral immunity should be performed by dosage of immunoglobulins IgG, IgA and IgM. Antibody production in SCID patients is deeply reduced or completely abolished. In the first months of life, a normal IgG-level may be observed due to the transmission of maternal antibodies during pregnancy, whereas a reduced IgM level is more significant. A detailed exploration of humoral immunity through analysis of specific antibody-levels following vaccination, allohemagglutinins or IgG-subclass is not useful before the second year of life, but should be done in older infants with suspected immunodeficiency. In case of enteropathy it is important to determine values for albumin in order to rule out an exudative enteropathy that may generate a “secondary” hypogammaglobulinemia through enteral protein loss. Sometimes, intestinal biopsies may be justified, as lymphopenia may be observed in the context of lymphangiectasia.

In order to perform precise immunological diagnostic, a center for pediatric immunology should be contacted promptly. The characterization of the lymphocyte subpopulations can be achieved by flow cytometry and allows in most cases a first diagnostic classification of the SCID type with regard to the presence or absence of the different lymphocyte-populations (CD4+ and CD8+ T cells, CD19+ B cells and CD3-CD16/56+ NK cells). It is important to determine at the same time the absolute lymphocyte count. Normal ranges of the different lymphocyte subpopulations are age-dependant [77, 98, 376].

T cell function can be assessed in specific laboratory assays in vitro by testing the lymphocyte proliferation upon stimulation through so-called mitogens or through specific antigens; the latter is only meaningful after vaccination (e.g., tetanus, tuberculin) or after infection (e.g., Candida, CMV or Varicella zoster virus). T cell receptor excision circles or TREC are episomal DNA circles that are generated during V(D)J recombination by endjoining of the removed genomic
DNA segments; they attest continuing thymic output. These TREC can be analyzed by polymerase chain reaction (PCR) [106]; patients with impaired T cell maturation lack TREC.

Depending on the characterization of the specific immunophenotype of the patient, different diagnostic hypothesis can be formulated. A molecular diagnosis should be achieved based on the identification of the underlying gene defect, but in no case should the adequate treatment be postponed because the definitive diagnosis is pending. Enzymatic determination of adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) should always be performed in distance to eventual blood transfusions.

Ultrasound of the thymus or chest radiography allows the evaluation of the size of the thymus which is generally reduced in the SCID patients. In the case of ADA-deficient patients, an alteration of the anterior rips may be observed. Additional imaging may be necessary in the context of infectious complications. In all cases, a detailed microbiological workup should be performed. Direct identification through culture or with the help of PCR should be privileged, as serological analysis is not significant in immunodeficient patients with abolished antibody production. Bronchoalveolar lavage or digestive endoscopy with biopsies may be necessary in order to attempt microbiological documentation.

γc deficiency. γc deficiency is suspected in male patients with or without positive family history upon immunophenotyping of peripheral blood. Typically, but not always, patients display a T-B+NK- phenotype and lack the expression of the γc chain on peripheral blood lymphocytes as analyzed with the help of monoclonal γc antibodies [202]. Some patients may express a nonfunctional γc chain which may be detected by the monoclonal antibody. Maternal T cells can also complicate the interpretation of the results. While XL-SCID patients usually present with absent or low NK cell counts and poor NK cell cytotoxicity, there have been observations of patients with confirmed mutations in the IL2RG gene who possess NK cells with certain NK cytotoxicity [315].

Theoretically, γc deficiency could be present exceptionally in females in the case of Turner syndrome (45X0), and in the very rare females with constitutionally unbalanced X-chromosome inactivation. Diagnosis should be confirmed by genetic analysis of the IL2RG. IL2RG mutations have been reported in different ethnical groups. IL2Rgbase [323], a database of identified mutations, is available on the web http://www.genome.gov/DIR/GMBB/SCID. The majority of mutation concerns single nucleotide changes leading to nonsense and missense-mutations, but there are also insertions, deletions and splice mutations. Mutations are not evenly distributed within the gene. There exist recurrent mutations at several positions, so-called “hot-spots”, most mutations concern the exon 5, followed by exon 3 and 4 [323]. Prenatal diagnosis at 11 weeks of gestational age is possible once the mutation is identified in a given family.

Female carriers remain healthy, showing nonrandom X-inactivation in T, B and NK cells with the nonmutated X-chromosome being the active X-chromosome in their lymphocytes [78, 325], whereas myeloid cells show random inactivation. This underlines the important function of the common γc for the development of the lymphoid cell-lineages. This nonrandom X-inactivation in lymphoid cells has been used for the diagnosis of the carrier status.

JAK3 deficiency. Diagnosis is based on immunophenotyping and molecular diagnosis. The mutations found in JAK3 deficiency have been collected in a database, the “JAK3base” that is accessible at http://bioinf.uta.fi/JAK3base.

IL7-Rα deficiency. IL-7R alpha deficiency should be looked for in patients with a T-B+NK+ phenotype. Confirmation of the diagnosis can be achieved by identification of the mutation.

CD45 deficiency. Diagnostic procedures are the same as for other SCID-forms.

CD3/TCR complex deficiencies. Diagnosis is confirmed by sequencing of the genes coding for the different transmembrane subunits of the CD3 complex (the CD3 γ, δ, ε and ξ chains).

### 2.2.5 Management

At the slightest suspicion of SCID, adequate prophylaxis and treatment has to be initiated immediately, with the aim of treating acute infections and preventing their recurrence. It is essential to isolate any suspected SCID patient in a sterile environment and to apply drastic hygienic measures. Suspicion of SCID is always a “pediatric-immunological emergency”, as a rapid and adequate treatment in specialized centers allows the initiation of a curative therapy. The preparations for hematopoietic stem cell transplantation (HSCT) should be launched immediately on diagnosis of SCID, a specialized center should be contacted, and the patient should be transferred promptly. HLA-typing
of the patient, his eventual siblings and his parents has to be performed as soon as possible.

As soon as the blood drawing for the exploration of the humoral immunity has been performed, the substitution of immunoglobulins should be started. Residual levels of IgG > 8 g/l should be obtained. Aggressive antibiotic treatment of acute infectious complications has to be started. A Pneumocystis jiroveci pneumonia must be ruled out or treated respectively; a prophylactic treatment with Sulfamethoxazol/Trimethoprim has to be initiated. If necessary, antifungal treatment has to be started. Antiviral therapy may be indicated in the case of CMV- or adenovirus-infection. In a case of RSV-infection, Palivizumab may be useful. Attention has to be paid to children who were vaccinated with the BCG vaccine, and in these children a treatment by Isoniazid and Rifampicin has to be initiated. In the case of signs of BCGitis, antituberculosis treatment including four or more drugs is necessary. Systemic BCGitis can be fatal.

Exclusively irradiated blood products should be transfused; CMV negative patients should receive only CMV negative blood products.

At diagnosis, SCID patients are often in poor nutritional condition and present chronic intestinal infection and inflammation which lead to impaired intestinal absorption. A high caloric parenteral nutrition is justified to cover the energetic requirement especially as due to infections energy requirement is higher in SCID patients than in age-matched controls. The parenteral nutrition and anti-infectious intravenous therapy requires a central venous line. During central venous line placement tracheal secretions for additional microbiological analysis should be obtained in children with respiratory symptoms. In some cases, a fibroblast biopsy for further genetic or functional investigations with regard to the underlying immunodeficiency can be justified.

Except for infants with complete Di George syndrome who lack a HLA identical donor and who need a cultured allogenic thymic transplantation, all children with PID may be cured by allogenic HSCT, which is actually the treatment of choice for SCID.

Up to now, only a few patients were treated by somatic gene therapy in clinical studies. The first successful bone marrow transplantations (BMT) were performed in 1968 [24, 150] shortly after the description of the “major human histocompatibility system” [12]. Since then, more than 1,300 patients with PID have been transplanted worldwide. In the beginning, only unfractionated HSCT with HLA identical donors could be performed. Only about 20% of the patients dispose of an HLA identical sibling. The development of T cell depletion techniques starting at the beginning of the 1980s [334] allowed the transplantation from haploidentical parental donors. Bone marrow, peripheral blood stem cells (PBSC) harvested by cytapheresis or cord blood can be used as source for HSCT.

Best results with regard to survival and immune reconstitution can be observed when using HLA-identical sibling donors. In some cases, the search for an HLA identical unrelated donor can be justified, if the patient’s HLA-type allows the identification of an HLA-matched unrelated donor in a reasonable time span. In clinically critic situations or in the case of a rare HLA-type in the patient, no time should be wasted with an unrelated donor search, and haploidentical HSCT with one of the parents should be prepared.

Considerable progress has been observed with regard to survival rates: the first report in 1977 on the outcome of SCID patients showed survival with functional graft in only 14 out of the 69 transplanted patients [36]. In 2004, Buckley et al. report survival rates of 84% in the case of HLA identical siblings, 71% in HLA-matched unrelated donors and 63% in haploidentical donors [41, 42]. The most frequent reasons of death concern infectious complications, veno-occlusive disease and GVHD. In isolated cases, in utero transplantation has been reported, but there seems to be no advantage in comparison to HSCT performed soon after birth.

The first successful treatment by gene therapy was observed in the case of XL-SCID, which was the proof of principle that gene therapeutic correction of the hematopoetic stem cell is feasible [64] and results in sustained immune reconstitution [175]. However, the occurrence of severe adverse effects has been observed subsequently [176, 177], with the appearance of leukemic transformation in 4 patients out of 10 in the French patient group and in 1 patient treated at the Great Ormond Street Hospital. Gene therapy for other SCID-forms is under development [63].

Allogenic HSCT thus remains currently the treatment of choice for SCID until the problems about safety of gene therapy due to insertional oncogenesis are resolved.

yc deficiency. Unless treated, XL-SCID is usually lethal in the first year of life. In very rare cases, mild courses have been observed, so that exceptionally the diagnosis may be made after 2 years of age. Rare isolated cases have been reported in which a particular mutational profile seems to be responsible for an atypical mild phenotype [103].
Allogeneic HSCT is a curative treatment for XL-SCID patients and shows good success with regard to survival [16, 41]. The best results are achieved with an HLA identical related donor. In the case of haploidentical donors, the immune reconstitution with regard to humoral immunity might be mediocre as patients often present only partial chimerism after HSCT with persistence of autologous B lymphocytes, so that immunoglobulin-substitution has to be continued after HSCT [16]. Two isolated cases have been reported of successful in utero BMT, in which fetuses between 17 and 20 weeks of gestation received haploidentical T-depleted BMT via intraperitoneal infusion [138, 444]. In the follow-up, both patients showed adequate immune reconstitution and independence from immunoglobulin substitution [26, 27].

The observation in a single patient that spontaneous reversion of the genetic defect may occur in vivo, probably within a T cell progenitor, and can generate functional T cells [394] and a stable T cell repertoire [37], was a powerful argument for the selective advantage of the corrected cell, and opened the way for the development of gene therapy, an innovative therapy option for PID. In 1999, a first clinical gene therapy trial was initiated in the Necker Hospital in Paris with inclusion of XL-SCID patients who lacked HLA-identical donor. The XL-SCID was the first disease in humans which was treated successfully by gene therapy. It could be demonstrated that the retroviral-mediated gene transfer of the γc gene allowed sustained restoration of the patients’ immune function [64, 175]. This was the proof of principle that gene transfer in hematopoietic stem cells can restore the development of the immune system. The appearance of severe adverse events due to insertional oncogenesis with development of uncontrolled T cell proliferation was first observed in two patients [176, 177], while at the time of this writing, in total four patients have been identified with leukemic transformation which appeared after gene therapy.

Additional gene therapy trials for XL-SCID were launched by Thrasher et al. at the Great Ormond Street Hospital [149]. Until recently, no severe adverse events have been documented in this trial in which a similar protocol to the French one is used; the differences regard essentially the culture conditions and the vector design. However, Thrasher et al. reported a case of leukemia caused by the gene therapy in December 2007. Chinen et al. also reported on gene therapy for XL-SCID [72].

**JAK3 deficiency.** Treatment options are similar to the ones available for γc SCID patients, and allogeneic HSCT is the treatment of choice. The specific interaction of JAK3 and γc represents the biochemical basis for the similarities between these two immunodeficiencies and thus it is not surprising, that the rationale for feasibility of gene therapy is the same for both disorders. Candotti et al. reported on in vitro retro viral-mediated gene correction for JAK3-deficiency [56], Bunting et al. showed the restoration of lymphocyte function in JAK 3-deficient mice by retroviral mediated gene transfer [49]. Clinical trials are though not yet available.

**IL7-Rα deficiency, CD45 deficiency.** Therapeutic procedures are the same as for other forms of SCID.

**CD3/TCR complex deficiencies.** Therapeutic procedures depend on the degree of immunodeficiency and are substantially the same as for other SCID-forms.

**Prognosis.** Without treatment, SCID patients will succumb to infections early in life, usually within the first year. The prognosis of SCID patients depends particularly on the moment of diagnosis that is the time at which adequate treatment is initiated to treat and limit deleterious infectious complications. Thus, early diagnosis is crucial for prognosis. Today it can be considered that about two-thirds of the SCID patients will survive. No general newborn screening has been available, but has been repeatedly discussed in the past [41, 42]. The Department of Health and Family Services of Wisconsin, USA, recently approved that screening for SCID is added to the current panel for newborn screening starting from January 2008. This collaborative effort from the Jeffrey Modell Foundation, the Wisconsin State Laboratory of Hygiene and Children’s Hospital of Wisconsin opens the way for to prompt identification of SCID patients, allowing fast access to life saving treatment, and will allow evaluation of effectiveness and outcome of this early testing for SCID.

### 2.3

**T-B- Severe Combined Immunodeficiency (RAG1/2 Deficiencies, Artemis Deficiency, ADA Deficiency)**

#### 2.3.1 Definition

As has been explained in Sect. 2.2, SCID is a heterogeneous group of diseases that affect cellular and humoral immune function. 20–30% of all SCID patients have a phenotype where circulating T cells and B cells are almost entirely absent but NK cells are present (T-B-NK+ SCID, OMIM#601457) [134]. This
particular form of SCID has an autosomal recessive pattern of inheritance and is most commonly caused by a defect in the recombination activating genes (RAG1, OMIM*179615; RAG2, OMIM*179616) [152, 295]. There are also some types of T-B-NK+ SCID with sensitivity to ionizing radiation (OMIM#602450), which are caused by mutations in the gene encoding Artemis (DCLRE1C, OMIM*605988). T-B-NK– SCID (OMIM#102700) is caused by mutation in the adenosine deaminase gene (ADA, OMIM*608958).

2.3.2 Etiology

The immune system encounters a vast array of foreign antigens, the recognition of which is facilitated by antigen-specific immunoglobulins (Ig)/B cell receptors (BCR), or T cell receptors (TCR). Igs and BCR control humoral immunity, recognizing soluble antigens, while TCR are responsible for binding and reacting against antigens presented via cells using the human leukocyte antigen molecule. The diversity in the variable region of antigen receptors is created through random somatic recombination of genetic elements, forming a contiguous coding segment for a functional unit. This receptor also serves as a checkpoint in lymphocyte development; lack of it causes T cells to be blocked at the CD4, CD8 double negative stage and B cells do not mature past the pro B compartment [447]. T cells lacking receptors cannot undergo selection in the thymus to become CD4+ or CD8+ immunocompetent cells, and IgM+ B cells are not exported from the bone marrow, resulting in T-B- SCID.

The principle genes that control the mechanism responsible for recombination of the antigen receptors are called recombination activating genes 1 and 2 (RAG1 and RAG2). The RAG genes are convergently expressed specifically in lymphocytes and the RAG proteins that are produced act as a heterodimer, targeting the variable (V), diversity (D) and joining (J) components of TCR and Igs which are then randomly selected from pre-existing gene segments and joined together through a process of recombination.

There are seven antigen receptor loci in mammals: TCR α, β, γ and δ loci along with Immunoglobulin receptors H, κ and λ loci. The N-terminal variable part of TCR β and δ, and Ig heavy chain (H) are assembled through V, D and J recombination, while TCR α and γ and the Ig light chains are produced from V and J segments only (Fig. 2.1). These gene fragments are recombined together and then joined, through RNA splicing, to a constant (C) region to produce a functional receptor. Because each loci comprises numerous copies of each V, D or J segment, random joining of these different regions of DNA can produce in excess of...
of $10^{14}$ possible receptor combinations which are capable of recognizing the array of antigens encountered.

Each V, D and J gene is flanked by a recombination signal sequence (RSS) which is recognized by the RAG complex. Each RSS comprises a conserved palindromic seven base pairs (bp), followed by an AT-rich nine base pair motif, separated by either 12 or 23 bp of weakly conserved DNA. The length of the spacer is vital for producing functional receptors because recombination occurs only between RSS with 12 and 23 bp spacers [410]. Hence, V and J regions are flanked by RSS with different spacers so that V–J recombination occurs in preference to a nonfunctional V–V or J–J arrangement. If the D segment is involved, such as for the IgH antigen receptor loci, appropriate spacers flank it to ensure the regions are joined in the correct order (Fig. 2.1).

As demonstrated by experiments in vitro [265], RSS with unlike spacers are joined when the RAG complex produces a double strand break at the border of the palindromic heptamer motif, leaving a 3’ hydroxyl group that is then covalently joined to the same nucleotide position on the opposite strand. This results in DNA with a conserved coding sequence and a hairpin structure on the coding terminus. This action also excises the DNA between the recognition sites to produce a blunt 5’ phosphorylated signal terminus on the section that is looped out. The RAG proteins remain associated with all the cleaved ends of DNA [5]. The blunt signal ends are then ligated, typically without any modification [245], to form an excision circle with an exact signal joint (Fig. 2.2) [416]. These DNA circles are generally lost from the genome through dilution during cell division.

The second stage of V(D)J recombination requires the resolution of the hairpin ends to form a functional, rearranged reading frame. The ligation of the coding joint is imprecise compared to that of the signal ends with the loss or addition of approximately 15 nucleotides. This adds further variation to the receptor domain, although it does carry the risk of producing nonfunctional genes through frameshift mutations or

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**Fig. 2.2** RAG1 and RAG2 recognise the V and J regions of light chains and recombine them together randomly to produce an array of antigen receptors. In germline DNA, Igκ comprises approximately 40 V and 5 J segments, while Igλ has about 30 V and 4 J segments (a). The RAG complex randomly selects a V and J region, bringing them into close proximity and most commonly, loops out the intervening DNA (b). The V and J genes are then recombined together, and joined with an imprecise coding joint, while the blunt ends of the excised DNA are ligated together to form a signal joint (c). The DNA is then transcribed and the recombined V–J region is spliced to the constant or C region to form the mature message RNA (d). After translation, a leader sequence at the start of the V region enables transport of the light chain to the endoplasmic reticulum. The process is very similar for heavy chain and TCR β/δ recombination, only the additional D segments separating V and J are firstly recombined with a J region, before V is randomly joined to the D–J segment produced initially.
introduction of premature stop codons. The addition or loss of nucleotides arises firstly by the random opening of the hairpin within the coding region, rather than exactly at the covalently closed terminus [357, 461]. If the hairpin is opened asymmetrically, the overhang can be filled in by the addition of short palindromic (P) repeat nucleotides upon resolution of the structure [242]. RAG1/2 can mediate hydrolysis of hairpins in vitro [31, 378], but while their presence appears to be required [207, 357, 456], Artemis (DCLRE1C) is the most likely candidate to open the RAG-generated coding hairpin [278]. This protein is phosphorylated by the DNA protein kinase catalytic subunit (DNA-PKcs) activating an endonuclease capable of cleaving hairpin DNA [109, 252]. Coding ends are also modified through template-independent addition of random N (GC rich) nucleotides by terminal deoxynucleotidyl transferase (TdT) [160, 221, 356]. Joining of homologous regions or truncation of random nucleotides at the ends of the free DNA are further mechanisms implicated in producing additional junctional diversity [358].

The loose ends of the modified coding signal are joined by ubiquitous proteins involved in the nonhomologous end joining (NHEJ) pathway. DNA-dependant protein kinase (DNA-PK) recognises open DNA ends, mediated by the DNA-binding subunits Ku70 and Ku80 and catalytic subunit DNA-PKcs. The final joining of the double strand breaks of RAG-associated cleavages is probably due to a complex of several factors [167]: a novel protein, XRCC4 [244] associates with DNA ligase IV [80, 167] and the protein Cernunnos or XLF [6, 39, 53] to ligate double strand breaks. Mutations of these NHEJ factors can lead to immunodeficiency [39, 236] (see Sect. 2.6 for more details). RAG1 and RAG2 are located on chromosome 11p13, 8 kb apart. The proteins are the only lymphoid specific factors required for recombination of RSS sites. When the genes are artificially expressed in nonlymphoid cells where rearrangement does not normally occur, a test substrate is recombined [304, 369], suggesting that the remaining required factors are available in all cell lineages. Equally, lack of either RAG1 or RAG2 in humans or mice [275, 377] leads to an absence of mature T and B cells with no other defects, implying that RAG genes function only in lymphoid cells.

As homozygous or compound heterozygous mutation cause disease, this form of SCID follows an autosomal recessive pattern of transmission. RAG1 and RAG2 are arranged in an unusual tail-to-tail configuration, sharing a 3’ untranslated region and both lacking introns [4]. There is no homology between the genes, but they are highly conserved in animals, emphasising their importance. In addition to this genomic configuration, the close arrangement of genes suggests that they may have appeared at the same time in early vertebrates through an insertion of a mobile genetic element [30, 408].

In addition to mutations of RAG1 or RAG2, T-B-SCID in humans has been caused by aberrant expression of Artemis [278], Ligase IV (see Sect. 2.5 for more details) [30, 40, 302, 337] and cernunnos/XLF (see Sect. 2.6 for more details) [39]. Because these genes are also involved in DNA double strand break repair, SCID caused by their disruption is also associated with radiosensitivity [386].

A mutation in the adenosine deaminase gene, which normally breaks down toxic products of the purine scavenging pathway, causes apoptosis in lymphocytes. As such this also results in a T-B-SCID, but patients also lack NK cells [159]. Patients with the very rare disease, reticular dysgenesis, have markedly decreased circulating T and NK cells and defective maturation of B and myeloid cells. This rare disease is due to a stem cell defect with unknown molecular basis [97].

Out of 174 cases of SCID examined at one American medical center, 3.4% were due to RAG mutations, 1.1% due to Artemis and 16.1% caused by ADA deficiency [46], although worldwide, RAG mutations account for approximately 50% of T-B-SCID [45].

Mutations of a given gene can generate a multitude of clinical phenotypes depending on the type of mutation and additional somatic mutations, environmental and regulatory factors. Hypomorphic mutations in the genes RAG1 or RAG2 have been shown to generate an oligoclonal T cell repertoire which, in the case of Omenn syndrome, will expand and display self-reactivity [92, 424] (see Sect. 2.4 for more details). The observation that identical mutations in RAG1 or RAG2 can be observed in Omenn syndrome but also in typical and atypical SCID patients [293, 372, 424], sometimes in the same kindred [79], suggests the involvement of one or more modifying factors.

An interesting phenotype of hypomorphic RAG1 mutations has recently been described in several patients with TCRβ T cell lymphopenia, severe CMV infection and autoimmunity [96, 115]. T cells have been shown to be of autologous origin. De Villartay et al. described four unrelated patients from consanguineous families who presented hypomorphic mutations in RAG1; three of the four identified mutations have already been described in patients with Omenn syndrome (del T631, del 368-369 and R841W). The missense mutation Q981P found in the fourth patient involves amino acids within the minimal core of RAG1 leading to a protein with residual RAG1 activity [96]. The remaining patient developed
EBV-associated lymphoproliferation and presented a R561H RAG1 mutation [115] which had also been described previously in Omenn syndrome patients.

It can be speculated that in these patients due to hypomorphic mutations in RAG1, a limited T cell repertoire is generated. The early occurrence of CMV infection may then induce a huge expansion of oligoclonal non γδ T cell clones.

Patients with attenuated forms of T-B-NK+SCID have been described, for example, a patient who survived for 6 years without HSCT carrying mutations in RAG1, a R559S substitution on one allele and a R897X substitution on the second allele [225]. This patient presented maternal derived T cells and autologous peripheral B cells which were shown to be functional as specific anti HSV antibodies were observed. In fact, it has become obvious that the clinical spectrum for RAG1/RAG2 defects comprises not only complete abolition of V(D)J recombination leading to typical T-B-NK+SCID patients and hypomorphic mutations giving rise to Omenn syndrome: more and more “atypical” SCID forms are identified [425, 426]. Thus genetic analysis of the RAG1 and RAG2 genes should also be considered in atypical clinical presentations.

Hypomorphic mutations in the Artemis gene may be found in patients that show clinical and immunological features that are indistinguishable from Omenn syndrome due to mutations in RAG1 or RAG2 [114] (see Sect. 2.4 for more details).

In the Necker Hospital study, four patients of two different kindreds showed a combined immunodeficiency with profound B and T lymphopenia and severe hypogammaglobulinemia generated by mutations in the last exon leading to truncation of the Artemis-protein, and thus leaving intact the metallo-beta-lactamase domain [279, 280]. These “hypomorphic” mutations display a partial V(D)J recombination activity, as assessed in the functional V(D)J assays in patients’ fibroblasts, and have an incomplete complementation of the sensitivity to ionizing radiation compared with a cell line fully deficient in Artemis. The patients present polyclonal T and B lymphocyte populations albeit in low number. Interestingly, two out of the four patients developed EBV-associated B cell lymphomas; in three of the four patients a general genomic instability was found. It has thus been hypothesized that Artemis may play an important role in genome stability. According to the hypothesis of Kinzler and Vogelstein [214], Artemis may be considered as genomic “caretaker” involved in the repair of genomic lesions and thus guaranteeing genomic stability. This hypothesis was emphasized by the observations of chromosomal fragments, fusion and detached centromers in different cell lines of Artemis knockout mice [353] indicating genomic instability in these mice [351]. Artemis/p53-deficient mice succumb to progenitor B cell tumors [352]. Furthermore, it has recently been described that tumorigenesis in several tissues is accelerated in Artemis deficient mice in a Trp 53 heterozygous setting, emphasizing the tumor suppression role for nonhomologous end-joining in lymphoid and non lymphoid cells [303, 452]. These findings suggest that Artemis deficient patients may be at risk for the development of lymphoid and nonlymphoid malignancies.

### 2.3.3 Clinical Manifestations

Symptoms of T-B-SCID are similar to all other SCID and are generally manifested as early opportunistic infections with impaired growth by the second or third month after birth. Patients often present with candidiasis, chronic persistent infections of the airways, and local or systemic bacterial infections. These most commonly cause rhinitis, otitis, mastoiditis, abscesses, conjunctivitis and meningitis. Chronic diarrhea associated with gram-negative enteric bacterial sepsis causes a failure to thrive.

Maternal T cells are engrafted in half of all patients and NK cells are present in this form of SCID. After decline of maternal immunoglobulins, no antibodies circulate in the peripheral blood and the lack of mature B and T cells is often accompanied by an absence of a thymus, tonsils and cervical lymph nodes.

### 2.3.4 Diagnosis

To diagnose T-B-SCID, a full lymphocyte count and flow cytometry should be performed on peripheral blood, including markers for B, T and NK cells. RAG and Artemis deficient patients will generally lack T cells and B cells with NK cells present. For full T-B-SCID, patients generally lack a thymus on X-ray or ultrasound imaging. Once an initial diagnosis has been determined based on physical examination, further investigation can be performed to establish the molecular basis of disease. DNA sequencing can reveal the mutation responsible and if parental mutation has been previously determined, prenatal diagnosis can be offered [400, 423].

### 2.3.5 Management

Upon presentation, management of life-threatening infection is the immediate concern and is treated with antibiotics...
and antifungal drugs specific for the pathogen (often *Candida* spp, *Pneumocystis jiroveci* or *Staphylococcus aureus*). Antiviral agents should be used only if necessary.

Isolation of the patient with meticulous skin and mucosal hygienic practice is essential to prevent further infection. Prophylactic antibiotics, antifungal agents and intravenous immunoglobulins are usually required. Parenteral or enteral nutrition is an option when patients have severe diarrhea and are failing to thrive through malnutrition.

Patients should not be immunized with live viral vaccines, as they can cause fatal symptoms.

If left untreated, all forms of T-B-SCID are fatal. HSCT is the only curative therapy available, although the mortality rate with this treatment is higher when compared to other types of SCID [16]. For ADA deficiency replacement therapy is available. Gene therapy is a possibly future option for treating SCID, and up to date successful treatment was observed in ADA-deficient patients [7], while animal models for gene therapy in Rag2 deficiency have been developed [63, 457].

Advances in treating other types of SCID have been made [7, 64, 149, 175, 306], using retroviruses to deliver functional copies of the affected gene to patients' stem cells ex vivo. The treated cells can then be reimplanted and give rise to an effective immune system. Gene therapy vectors to treat T-B-SCID are currently being tested [231, 281, 457] and may soon provide an alternative treatment in situations when bone marrow donors are unavailable.

### 2.4 Omenn Syndrome

#### 2.4.1 Definition

Omenn syndrome (OS, OMIM#603554) is a related disease first described by Gilbert Omenn in 1965 after observing a consanguineous family with an unusual skin disorder [305].

#### 2.4.2 Etiology

Omenn syndrome is caused primarily by missense mutations in *RAG1* or *RAG2*, which do not entirely abrogate V(D)J recombination [424, 430]. Partial activity of the recombination activating genes allows some T cell clones to develop and survive, but because of the oligoclonal nature of the population, patients remain immunodeficient. The severity of disease is variable and can be partially attributable to genotype although there are exceptions: identical mutations in *RAG* genes have been discovered in both T-B-SCID and OS patients [79]. As OS describes a heterogeneous range of symptoms and is not a molecular definition, the disease can be the result of mutations in genes other than the *RAGs* [156], such as Artemis [114] or IL-7Rα [152, 295].

#### 2.4.3 Clinical Manifestations

Symptoms are similar to other SCID but also characterized by lymphadenopathy and hepatosplenomegaly which are problems unusual in other types of SCID. Patients also suffer from alopecia and an exudative erythroderma that is associated with episodes of *Staphylococcus aureus* sepsis. This skin condition becomes apparent as pachydermia which progresses to desquamation, resulting in protein loss through the skin which, in conjunction with diarrhea, causes hypoproteinemia and edema. Normal to elevated levels of T cells can be present but these cells have a skewed T-helper-2 (Th2) profile [71] and due to their highly oligoclonal nature [92, 183], are poorly functional. Th2 cells produce elevated levels of IL-4 and IL-5 which lead to hypereosinophilia and despite the absence of B cells, increased serum levels of IgE.

#### 2.4.4 Diagnosis

To diagnose OS, lymphocyte count and flow cytometry should be performed on peripheral blood. An initial misdiagnosis of atopic dermatitis or a food allergy is possible in Omenn syndrome. Engraftment of maternal T cells in utero can cause a skin condition with a similar appearance to graft-versus-host-type illness, but OS can be differentiated by lack of T cell chimaerism and eosinophilia, where lymphadenopathy and hepatosplenomegaly are also hallmarks of the syndrome. In OS, B cells are absent but an oligoclonal population of T cells is present with an activated antigen stimulated Th2 cell profile, as shown by presence of CD30 of the T cell surface with a CD45RO positive phenotype. These cells are responsible for the increased IL-4 and IL-5 levels in serum.

Immunoglobulins A and M are absent whilst levels of IgE and maternal IgG will be elevated. OS T cell lymphocyte stimulation assays against concanavalin A (conA), pokeweed mitogen (PWM) and phytohemagglutinin (PHA) are absent or greatly decreased. Lymphocytes will, however, show some response to
stimulation with anti-CD3, superantigens and phorbol myristate acetate (PMA).

Patients with OS generally lack a thymus on X-ray or ultrasound imaging. DNA sequencing can reveal the mutation responsible and if parental mutation has been previously determined, prenatal diagnosis can be offered [400, 423].

2.4.5 Management

Therapeutic procedures are the same as for other forms of SCID. Dermatitis can be treated with immunosuppression and topical steroids. Immunosuppression of the patients’ oligoclonal T cells has decreased incidence of graft-versus-host disease [178].

2.5 DNA Ligase IV Deficiency

2.5.1 Definition

An atypical and rare form of autosomal recessive radiosensitive SCID is due to DNA ligase IV deficiency (OMIM#606593) and is characterized by a profound but not complete defect in the development of T and B lymphocytes (T-B-NK+ SCID) associated with various degrees of microcephaly, developmental defects and growth delay. There is a high heterogeneity with regard to the level of immunodeficiency in DNA Ligase IV deficiency ranging from no immunodeficiency to profound SCID phenotypes.

2.5.2 Etiology

DNA ligase IV (LIG4, OMIM’601837) is located on chromosome 13.q22-q34: the cDNA encoding a polypeptide of 844 amino acids [439]. It is essential for embryonic development and its complete deficiency causes early lethality accompanied by defective lymphogenesis and defective neurogenesis in knockout mice [143, 148]. DNA ligase IV is a component of the nonhomologous end-joining and participates thus in the repair of DNA double strand breaks (dsb) that arise during DNA damage induced by ionizing radiation, but also in the context of endogenously induced DNA dsb during V(D)J recombination. As detailed in Sect. 2.3, V(D)J recombination is initiated by the lymphoidspecific proteins RAG1 and RAG2 that introduce a DNA dsb between a coding segment (V, J or D) and the specific recombination signal sequence (RSS). This generates four different extremities: two blunt signal ends and two hairpin sealed coding ends, which are then resolved by the NHEJ-DNA repair pathway composed of at least six factors: DNA-PKcs, Ku70, Ku80, Artemis, LIG4, and XRCC4. Whereas the signal ends can be directly ligated by the complex formed by DNA-ligase IV with XRCC4 [80, 166], giving rise to a precise signal joint, the coding ends have to be processed prior to their ligation which generates an imprecise coding joint. V(D)J recombination in patients’ fibroblasts shows only moderate impairment with an almost normal recombination frequency of coding- and signal joint formation, but the fidelity of the signal joint formation in DNA ligase IV deficient patients is highly compromised [40].

The LIG4 Y288C mouse strain presents hypomorphic mutations in the DNA ligase IV gene and is characterized by growth retardation and immunodeficiency. The diminished DNA double-strand break repair in LIG4 Y288C mice causes a progressive loss of hematopoietic stem cells and bone marrow cellularity during ageing [291], and thus it can be speculated that DNA ligase IV may be required beyond V(D)J recombination for lymphoid homeostasis, explaining why DNA ligase IV deficiency can cause profound immunodeficiency despite the fact that there is only moderate in vitro impairment of V(D)J recombination in DNA ligase IV deficient patients.

2.5.3 Clinical Manifestations

Hypomorphic mutations have been described in humans, first in a 14-year-old leukemia patient who overresponded to radiotherapy [337, 338]. The observed increased cellular sensitivity to ionizing radiation was the clue to the diagnosis of DNA ligase IV deficiency. Interestingly, this patient did not display developmental or immunological abnormalities before the onset of leukemia. Subsequently, DNA ligase IV deficient patients with a varying degree of T and B immunodeficiency, microcephaly, facial dysmorphe, growth retardation and developmental delay have been described [30, 40, 121, 302]. Some patients present exclusively a T-B-NK+ SCID phenotype without any growth or developmental defects [415].
After the first leukemia patient who had been reported to have a mutation in DNA Ligase IV, several other patients have been identified with DNA ligase IV deficiency and lymphoproliferation or lymphoid malignancy: EBV associated B cell lymphoproliferation in two patients [40, 409], and acute T cell leukemia in another patient [30].

### 2.5.4 Diagnosis

The immunophenotype of DNA ligase IV-deficient patients may be very heterogeneous, ranging from an almost complete T-B-NK+ SCID phenotype to milder presentation with various degrees of lymphopenia and hypogammaglobulinemia [155, 302]. Radiosensitivity and microcephaly are important clues to diagnosis, but there may be rare cases without the typical microcephaly. Other characteristic features that should lead to the suspicion of DNA ligase IV deficiency are developmental retardation and growth delay. Pancytopenia may be present in some patients. The diagnosis can be confirmed by sequencing of the \( \text{LIG}4 \) gene.

### 2.5.5 Management

HSCT has been proposed to cure immunodeficiency [40, 121, 169, 415]. HSCT outcome may be limited by complications due to increased sensitivity to conditioning regimens, even if “reduced intensity” conditioning is chosen, and more severe GVHD due to the DNA repair deficiency. It can be speculated that long-term outcome may be compromised by occurrence of secondary malignancies; nevertheless, the observation period after the few performed HSCT for DNA ligase IV deficiency is still too short to draw final conclusions.

### 2.6 Cernunnos Deficiency

#### 2.6.1 Definition

Patients with Cernunnos deficiency are characterized by severe T lymphopenia, progressive B lymphopenia and microcephaly [39]. This condition is a rare autosomal recessive primary immunodeficiency.

#### 2.6.2 Etiology

The observation of patients presenting a T-B-NK+ phenotype with increased sensitivity to ionizing radiation without mutations in the known factors involved in nonhomologous end joining (NHEJ) in mammals (Ku70, Ku80, DNA-dependent protein kinase catalytic subunit, XRCC4, DNA ligase IV, or Artemis) [83] indicated that there were still other NHEJ-repair-factors to be discovered. Recently, a new factor was identified through the study of five human SCID patients with severe progressive T and B cell lymphopenia and increased sensitivity to ionizing radiation: CERNUNNOS (OMIM*611290) or XRCC4-like factor (XLF), was cloned contemporarily via a complementation strategy in Cernunnos-deficient patients’ fibroblasts [39] and via its capacity to interact with XRCC4 [6], respectively. Cernunnos is located on the long arm of chromosome 2 (2q35) and its cDNA comprises 2063 nucleotides giving rise to a protein of 299 amino acids. Cernunnos shows homology to XRCC4 [53] and forms a complex with XRCC4 and DNA-ligase IV. Its precise molecular function remains to be elucidated, but it can be considered as a “new” factor of the NHEJ pathway. With regard to V(D)J recombination, the fidelity of signal joints is impaired in Cernunnos deficiency with various length of nucleotide deletions [39, 83].

#### 2.6.3 Clinical Manifestations

Cernunnos-deficient patients present recurrent bacterial, viral and/or parasitic infections like those observed in other SCID patients. Two patients out of five described succumbed to infections. Interestingly, like patients with DNA ligase IV deficiency, Cernunnos-deficient patients also display developmental defects and microcephaly. In humans, several conditions are characterized by the association of defective DNA repair and neurodegenerative disease [303, 330]. Other abnormal features observed in Cernunnos deficiency are bone and urogenital malformations. Three patients presented with a “bird-like face”.

#### 2.6.4 Diagnosis

Laboratory exploration of Cernunnos-deficient patients find mild to severe B and T lymphopenia...
whereas NK cell counts are normal. With age, the number of circulating B cells has been found to decline progressively [39]. This B cell deficiency is accompanied by hypogammaglobulinemia with low IgG and IgA but raised IgM in two patients, thus suggesting that Cernunnos may be involved in class switch recombination. Interestingly, circulating naive T cells were absent and T lymphocytes were found to display exclusively T cell memory phenotype (CD45RO+). T cell function is impaired when assessed by PHA mitogen induced proliferation assays. Patients show also increased sensitivity to ionizing radiation. Diagnosis can be confirmed by sequence analysis.

Other elements that may be present are autoimmune cytopenia, chromosomal instability or bone marrow aplasia [39].

### 2.6.5 Management

Treatment options depend on the severity of the immunodeficiency and are comparable to the management of other types of SCID. Opportunistic infections have to be treated and prevented. HSCT may be a curative therapeutic approach. With regard to the underlying DNA repair defect, attention has to be paid to eventual toxicity of conditioning regimens and associated medication.

### 2.7 Purine Nucleoside Phosphorylase (PNP) Deficiency

#### 2.7.1 Definition

PNP deficiency is a combined immunodeficiency caused by mutations in the enzyme PNP (OMIM+164050) and subsequent accumulation of purine metabolites such as deoxyguanosine. Patients typically present with recurrent infections, autoimmunity and ataxia. Presentation may be delayed beyond 1–2 years of life.

#### 2.7.2 Etiology

PNP is a key enzyme in the purine salvage pathway. PNP catalyzes the phosphorylation of inosine, deoxyinosine, guanosine and deoxyguanosine to yield guanine or hypoxanthine and ribose-1-phosphate or 2’-deoxyribose 1-phosphate. These ubiquitous purine metabolic pathways are responsible for the proper balance between the production of dephosphorylated purines, detoxification by further degradation to uric acid, and salvage by metabolism back to the nucleotide level. PNP is also responsible for catalyzing guanosine and deoxyguanosine back into the GTP pool. Maintenance of low and balanced intracellular deoxynucleoside triphosphate pools is critical for the fidelity of DNA synthesis and repair [75, 120, 271, 333].

The metabolic consequences of the PNP deficiency is the accumulation of all four PNP substrates; inosine, deoxyinosine, guanosine and deoxyguanosine [74]. Because PNP activity is obligatory to purine degradation, no uric acid is produced [74]. Of the four metabolites, only deoxyguanosine can be phosphorylated further in mammalian cells [122, 433]. As a result, cells from patients with PNP deficiency accumulate abnormally high levels of intracellular dGTP [74]. The high concentration of dGTP is believed to cause lymph toxicity in patients with PNP deficiency.

Much of these metabolic effects on the immune system were learned from animal models. Three mutant mice lines were generated with a single amino acid substitution and partial PNP enzymatic activity (1–5% of wild type) [385]. The PNP mutant mice developed partial immunodeficiency after 2–3 months consistent with the partial reduction in PNP enzymatic activity. The total number of thymocytes was reduced with a decrease in the number of CD4+CD8+ double positive cells and an increase in immature CD4-CD8-double-negative cells. In parallel spleen, T cells were reduced by 50% and their response to T cell mitogen was impaired partially. The overall conclusion of this study was that the progressive T cell defect is similar to the human disorder. It is likely that the partial nature of the mutations in the PNP may hinder direct comparison with the human disease and further insight into the mechanism of the immunodeficiency.

Arpaia et al. [19] generated a PNP-deficient mouse by gene targeting resulting in a complete absence of PNP enzymatic activity. The PNP-deficient mice develop severe immunodeficiency at an early age characterized by abnormal intrathymic T cell differentiation, progressively reduced peripheral T cells with impaired immune function, and minimal abnormalities of B lymphocytes or other tissues. The observed immune phenotype of the PNP-deficient mice is similar to clinical observations in patients with PNP deficiency.

The following observations of the immune phenotype of PNP-deficient mice shed light on the mechanism by which PNP deficiency may cause
immunodeficiency: (1) the development of T cells in PNP-deficient mice is affected at the CD4+CD8+ double-positive intrathymic stage of differentiation; (2) in PNP−/−mice, the double-positive thymocytes undergo enhanced apoptosis in vivo and markedly increased rates of activation induced apoptosis in vitro; and (3) apoptosis of double-positive thymocytes can be induced by inhibition of PNP in the presence of deoxyguanosine. The deoxyguanosine-induced apoptosis of double-positive thymocytes is inhibited by overexpression of Bcl-2 or by inhibition of caspase activity.

Together, the experimental evidence supports the following hypothesis explaining the mechanisms of the immunodeficiency caused by PNP deficiency:

1. The accumulation of a PNP lymphotoxic substrate, rather than the lack of the product of the enzymatic reaction, is responsible for the immunodeficiency [379, 381].
2. Deoxyguanosine is the only PNP substrate that is phosphorylated further and has been demonstrated to be lymphotoxic [171, 186].
3. To exert its lymphotoxicity, deoxyguanosine has to be phosphorylated first to dGTP, which in turn inhibits ribonucleotidase reductase activity, depletes dCTP, and inhibits DNA synthesis and repair [171].
4. There is evidence that deoxyguanosine-induced apoptosis is initiated in the mitochondria. There is a secondary loss of the mitochondrial deoxyguanosine kinase enzymatic activity in PNP mutant mice and in PNP-deficient mice [205, 310, 460]. Deoxyguanosine is produced or actively transported into the mitochondria [435, 436], phosphorylated by the mitochondrial deoxyguanosine kinase, and the end product dGTP likely destabilizes deoxyguanosine kinase protein. Mitochondrial dGTP is also likely to inhibit mitochondrial DNA repair and initiate apoptosis by way of cytochrome C release [243].
5. Any hypothesis explaining the biochemical mechanism of cytotoxicity of PNP deficiency must explain the lymphocyte and in particular T cell specificity of the disease. One explanation for the T lymphocyte specificity is the high deoxyguanosine phosphorylating activity in T lymphocytes as compared with lymphocytes or any other tissue [60, 211, 322].
6. A second explanation for the T cell specificity of PNP deficiency lies in the inherent susceptibility of immature thymocytes to apoptosis during T cell selection [266]. Immature double-positive T cells express low levels of Bcl-2 and are uniquely sensitive to apoptosis during negative selection [429]. Thymocytes at this stage of differentiation have been shown to be especially vulnerable to deoxyguanosine-induced apoptosis [52, 76]. According to this hypothesis, dGTP accumulation in PNP-deficient CD4+CD8+ thymocytes increases the proportion of thymocytes undergoing negative selection by increasing susceptibility to activation-induced apoptosis [429].

2.7.3 Clinical Manifestations

PNP deficiency is a rare disease with an estimated frequency of 4% among patients with SCID [52]. Patients with PNP deficiency typically have a triad of symptoms including neurologic abnormalities, autoimmune phenomena, and recurrent and unusual infections.

Similar to children with other types of severe immunodeficiency, PNP deficiency may come to medical attention during the first year of life because of prolonged diarrhea, oral thrush, or respiratory infections [84, 186]. Other infections include meningoencephalitis, recurrent otitis, sinusitis, mastoiditis, pharyngitis, pneumonia, and skin infection [75, 84, 139]. Patients are extremely susceptible to viral infections such as varicella, CMV, EBV, parainfluenza [52], and the polyoma JC virus [312]. There is a considerable heterogeneity both in age of presentation and severity of symptoms. In some cases, significant infections are delayed until later in life [75, 84, 113, 139, 312] or have only mild symptoms, which may be credited to residual PNP activity [180].

Neurologic abnormalities are common in PNP deficiency [84, 180], and more than 20% of cases seek medical consultation due to neurologic symptoms that can not be explained by infections or preceding signs of immunodeficiency [388]. The majority of neurologic manifestations are related to the motor system dysfunction, such as nonprogressive cerebral palsy, spastic paresis, or ataxia. Dysequilibrium characterized by hypotonia, pronounced difficulty in maintaining posture and upright position, associated with spastic diplegia and ataxia [180] or spastic paraplegia, have also been described [307, 399]. Other neurologic findings include tremor, developmental delay, hyperactivity, behavioral problems, and varying levels of mental retardation, some of which may be related to recurrent brain infarcts.

One-third of the patients manifest autoimmune phenomena, which may be the presenting feature [52, 139]. These include autoimmune hemolytic anemia (associated with autoantibodies to erythrocytes) [84], idiopathic thrombocytopenic purpura, autoimmune neutropenia, arthritis, pericarditis, and systemic lupus erythematosus [43]. Patients with autoimmune disorders may test positive for rheumatic factors and antinuclear antigens [59].
2.7.4 Diagnosis

PNP deficiency is an autosomal recessive disorder. The gene that encodes PNP is localized on chromosome 14q13.1 [448]; and several disease-causing mutations have been identified [14, 23, 84, 273, 308, 367, 413]. Different mutations in the PNP gene produce proteins with variable degrees of enzymatic activity that correlate with accumulation of nucleoside substrates and with the clinical course. Retention of partial enzyme activity may lead in some patients to less severe metabolic abnormalities, delayed presentation, milder clinical symptoms, and immune dysfunction [75].

All patients with PNP deficiency have purine nucleoside abnormalities (elevated inosine and deoxyinosine, and also guanosine and deoxyguanosine in blood and urine). Uric acid blood level is typically below 2.0mg/dl. Normal or slightly decreased uric acid levels are found in a few patients with partial enzyme activity [32, 180]. Low serum uric acid levels also may be caused by proximal renal tubular diseases (e.g., Fanconi syndrome) or xanthinuria, in which blood and urine levels are extremely low [191]. Other metabolic abnormalities found in patients with PNP deficiency include elevated dGTP, undetectable in normal individuals, and depletion of GTP in erythrocytes to about 10% of normal levels [186].

PNP activity can be determined by measuring the rate of conversion of radioactivity labeled inosine to hypoxanthine [52] or by spectrophotometry in which the coupled conversion of inosine to uric acid in the presence of xanthine oxidase is tested [343]. Normal PNP activity varies in different human cell and tissues extracts; the diagnosis of PNP deficiency is based commonly on enzyme activity in hemolysate [158]. Undetectable or lower than 1% activity is usually found in patients with PNP deficiency [59], but activity as high as 4.8% of normal control was associated with immunodeficiency, although with a mild course and delayed presentation [364]. Determination of PNP activity could be affected by recent erythrocyte transfusion [113]. It is advised in these instances to measure inosine, guanosine and their deoxy analogue concentrations in the urine, or PNP activity in mononuclear cells or peripheral blood T cells [180, 186].

Prenatal exclusion of PNP deficiency can be performed by measuring the enzyme activity in fetal red blood cells [139] and amniocytes or by determining the purine profile in amniotic fluid. The advantage of the latter is that purine levels are available within a short time after amniocentesis [59]. Assessing PNP activity in chorionic villi is an effective alternative that can be performed early in the course of pregnancy [59].

The only available cure for patients with PNP deficiency is HSCT. Recently, there have been a few reports of successful restoration of immune function in patients with PNP after HLA-matched sibling HSCT [25, 58]. Myeloablative conditioning is required in order to reduce the risk of rejection caused by residual immune function frequently documented in these patients. Conditioning regimens included cyclophosphamide and busulfan, without [25] or with anti-thymocyte globulin (ATG) [99], or alternatively busulfan and fludarabine [73]. In the absence of a matched related donor, cord blood has been recently used successfully in a patient with PNP deficiency [282]. Whether these patients can benefit from matched unrelated donor marrow or cord blood...
transplants remains to be determined in a larger group of patients. In addition, HSCT may not reverse neurological manifestations as previously observed [25].

When HSCT is unavailable, enzyme replacement using polyethylene glycol (PEG)-PNP could provide temporary remedy similar to the treatment of patients with ADA deficiency [186]. Its efficiency has been recently tested, demonstrating complete immune reconstruction of PNP-/- mice, but unfortunately PEG-PNP is not commercially available [19]. Other future therapies such as enzyme replacement with trans-activator of transcription (TAT)-PNP [411] or gene therapy are now undergoing pre-clinical studies.

In the past, several other modalities of therapy were proposed for PNP deficiency. Erythrocyte transfusions used as enzyme replacement were originally encouraging, but subsequently proved inefficient [390]. Other treatment including deoxycytidine and tetrahydrouridine [395, 437], guanine [437], adenine, uridine, and hypoxanthine [75, 390] showed no benefit. Attempts to restore immune function in patients with PNP deficiency with thymus transplant or with thymosine fraction 5 were also unsuccessful.

Supportive treatment is warranted in patients with PNP deficiency, as in all immunodeficiency states [139]. Immunoglobulin replacement therapy should be considered in cases who have antibody deficiency or autoimmune manifestation [388].

The life expectancy of individuals with PNP deficiency has been poor. Most of the patients who did not receive HSCT died during early childhood. The oldest reported patient reached the second decade of life [403]. Death has occurred from overwhelming infections, such as generalized chickenpox complicated by pneumonia and carditis, or pneumonia and chronic pulmonary disease. A high frequency of malignancy was also noted, including pharyngeal tumors, lymphoma, and lymphosarcoma [75, 267, 367].

### 2.8

**Immunoglobulin Class Switch Recombination Deficiencies (affecting CD40–CD40L)**

#### 2.8.1 Definition

Immunoglobulin class switch recombination deficiencies (Ig CSR deficiencies), previously termed “Hyper-IgM syndromes (HIGM)” and originally termed “dysgamma-globulinemia”, are rare immunodeficiency diseases characterized by defective production of Ig requiring a switch process, i.e., IgG, IgA and IgE, whereas the IgM concentration is either normal or increased. Although rare cases of HIGM with autosomal recessive inheritance have been reported recently, most cases are inherited as X-linked recessive trait and are due to a mutation in the CD40 ligand encoding gene [11, 21, 100, 145, 222]. The gene responsible for some autosomal recessive forms was identified as CD40 [125]. The clinical and biological characteristics of both HIGM syndromes associated with a defect in the CD40-CD40 ligand interaction are very similar and point to the importance of this interaction in the immune response. These characteristics distinguish them from other HIGM with Ig CSR deficiencies due to intrinsic B cell defects [123, 126, 298] (see Sect. 3.4 for more details).

#### 2.8.2 Etiology

The X-linked form of HIGM (XHIGM or HIGM1) syndrome (OMIM#308230) is due to a mutation in CD40 ligand (CD40L, also called CD154). The CD40L gene (OMIM*300386), also called tumor necrosis factor superfamily 5 (TNFSF5), maps on the X chromosome region q26 and is organized in five exons and four introns. CD40L is a type II transmembrane glycoprotein 261 amino acids long that is mainly expressed on activated CD4 T lymphocytes as a trimer. The crystal structure of the intracellular part of CD40L shows that hydrophobic and hydrophilic residues are crucial for CD40 binding [209]. Different mutations of the gene have been described in a large number of patients, including missense mutations, deletion, insertions, nonsense-mutations and splice site mutation [237, 300, 373]. Although the mutations described involve all parts of the gene, most of them are located in exon 5, affecting regions that are conserved in sequence analogy with tumor necrosis factor [300]. The majority of missense mutations described affect the folding and stability of the molecule rather than the CD40-binding site directly [209, 300]. There is no clear phenotype-genotype correlation, although some mutations allowing a residual binding of CD40 are associated with a less severe phenotype [85, 373]. Some rare cases of XHIGM have been described in girls secondary to a skewed X inactivation chromosome [95, 198].

In 2001, Ferrari et al. [125] identified CD40 mutation in three patients from two unrelated families with autosomal recessive HIGM syndrome (HIGM3, OMIM#606843), and a fourth case was reported in 2003 in a third family [230]. So far, all patients...
described present homozygous mutations. CD40 is a type I transmembrane protein 277 amino acids long and is included in the TNF-R superfamily. CD40 is constitutively expressed on B cells, monocytes, macrophages, dendritic cells and nonhematopoietic cells. The CD40 gene (OMIM’109535) displays nine exons. Two identified mutations affect CD40 splicing and the other one consists of amino acid substitution in the extracellular part of the protein. However, whatever the mutation involved, CD40 is not expressed at the membrane level.

The CD40–CD40L interaction plays a major role in the cross talk between immune cells. Engagement by CD40L induces CD40 signal transduction in B and dendritic cells. CD40 could already be trimerized independently of CD40L engagement by its pre-ligand-associated domain (PLAD) identified in the extracellular regions of TNFR members [69]. The CD40–CD40L interaction plays a crucial role in T cell-dependant B cell proliferation and differentiation in the presence of a second signal (such as IL-4 or IL-10). It is consequently critical for germinal center formation and for the generation of a secondary antibody repertoire. The latter results from two main processes. First, there is switch recombination that leads to the expression of different immunoglobulin isotypes. The second process consists of the somatic hypermutation characterized by a high rate accumulation of point mutations in the V regions of Ig genes and allows the selection of B cells bearing a high affinity antigen specific BCR. Altogether, these processes lead to high affinity antibody production and to the generation of memory B cells and of long-life plasma cells. Although rare somatic mutations can be detected in IgM-bearing B lymphocytes [442], the main consequence of a defect in CD40–CD40L interaction is the absence of generation of a secondary antibody repertoire. However, several sources of evidence indicate that HIGM1 and HIGM3 are not solely a humoral immunodeficiency. CD40 triggering also plays a central role in T cell-mediated activation of monocytes-dendritic cells [10, 62, 140, 204]. Engagement of CD40 on dendritic cells leads to their maturation and the secretion of IL-12 a cytokine with a major role in Th1 immunity. Failure to produce IL-12 and thereby interferon γ [65] is a likely event in the T cell immunity defect observed in HIGM affecting CD40–CD40L interaction.

2.8.3
Clinical Manifestations

This section summarizes the clinical manifestations observed in HIGM1 patients; the disorder has been recognized since 1993 and has been the object of many reports [237, 241, 449]. However, the clinical manifestations observed in the four patients with HIGM3 recently described are very similar.

In most cases, age at the time of diagnosis is between 3 months and 2 years and the clinical presentation evokes a combined immunodeficiency. However, it seems that variability in susceptibility to opportunistic infection in HIGM1-deficient patients could exist since some patients develop such infection early in life while others do not, at least not until adulthood.

The most common clinical manifestations observed in HIGM1 patients are infections, especially infections involving the respiratory tract. First of all, the pneumonias occur in more than 80% of patients, and Pneumocystis jiroveci accounts for most of the cases in infancy. It is noticeable that this infection is the first manifestation of the disease in over one-third of patients. The occurrence of such an infection in a young patient has to evoke this diagnosis, especially if hypogammaglobulinemia is associated. Lung infections can also be due to viruses including CMV, adenovirus, herpes simplex virus or bacteria such as pseudomonas or staphylococcus. Finally, mycobacteria including bacillus Calmette-Guérin (BCG) and fungi such as Histoplasmosis and Cryptococcus can be responsible for lower respiratory tract infections. Upper respiratory tract infections including sinusitis and otitis are also common and affect more than 40% of patients.

Gastrointestinal problems also affect over 50% of patients. These problems are often of infectious origin especially due to Cryptosporidium. Diarrhea associated with Giardia lamblia, Salmonella or Entameoba histolytica have been reported [241]. Inflammatory bowel disease and intestinal hyperplasia may cause chronic diarrhea in some patients. The intestinal problems follow a chronic course leading to failure to thrive, and parenteral nutrition is required. The liver is often affected. The common lesion is sclerosing cholangitis that is most often related to Cryptosporidium infection and that may require liver transplantation. Hepatitis has been reported either with or without a proven viral etiology. As with other immunodeficiencies, the risk of neoplasm, especially lymphoma, is increased. But in HIGM1, the risk of neoplasm also includes carcinomas affecting the liver, pancreas, biliary tree [185, 241, 283]. These observations suggest that physiological CD40 expression on regenerating or inflamed bile duct epithelium could play a role in triggering local immune response [185].

The most typical hematological abnormality is neutropenia that is observed in over 60% of patients. It is usually chronic and can be exacerbated by infectious
episodes and be associated with oral ulcers and gingivitis. Chronic infections can lead to anemias, but some of them are related to parvovirus B19 infection [33]. Neurologic problems including meningitis and encephalitis have also been reported. Despite the frequent absence of identification, several organisms are involved such as Toxoplasma, Cryptococcus, and Mycobacteria [241]. Moreover, viruses including enterovirus and JC virus are responsible for some neurological features [181, 398]. Some cases of arthritis, nephritis and hyperparathyroidism have been reported. The osteopenia observed in some patients suggests a regulatory role for CD40L in bone mineralization [248].

2.8.4 Diagnosis

The characteristic serum Ig profile observed in HIGM1 consists in markedly decreased serum IgG, IgA and IgE and normal to increased IgM levels. Indeed, a normal IgM level is frequently observed at the time of diagnosis in around 50% of patients, especially in young patients [241]. However, nearly 70% of patients will present a hyper IgM during their lifetime. In some cases, the level of IgG which is generally very low can reach normal values. In the same way, some patients present normal or high IgA level as well as IgE. These near-normal immunoglobulin profiles, sometimes associated with an antibody response to T cell-dependent antigens, could be associated with a milder phenotype [33, 85]. The serum IgG and IgA levels were undetectable in three patients presenting HIGM3, and in the fourth, only IgG was detectable at a very low level [125, 230].

In both HIGM1 and HIGM3, T cell counts were generally normal, although a low proportion of CD45R0 memory T cells is frequently observed [204]. Whereas total B cell count is normal in most cases, the B cell population is characterized by the lack of B cells that do not express IgD and that express CD27, which correlates with the failure of class switch recombination and of somatic hypermutation processes [3, 249].

The screening assay for diagnosis of HIGM1 is based on the absence of CD40 binding on the patient’s activated T cells. Usually, T cell activation is driven by the association of phorbol ester and ionomycin, and the expression of a functional CD40 ligand is revealed by binding fluorescent chimeric CD40-Ig molecules assessed in flow cytometry. Some monoclonal fluorescent anti-CD40L antibodies which recognize the binding site of CD40 can be used cautiously for the diagnosis [237]. However, some CD40L mutations associated with milder pheno-
types allow a residual CD40 binding and the level of fluorescent intensity has to be taken into consideration for a suitable interpretation. Moreover, when a defect of CD40 binding is detected, it is important to rule out a T cell activation defect which could lead to an absence of CD40 ligand expression without intrinsic defect in this molecule. The final diagnosis requires CD40L molecular analysis. Carrier detection in females has to be performed by direct sequencing when the searched-for mutation is known. Therefore, prenatal diagnosis can be offered by using a chorionic Villi biopsy taken at week 8 to 10 of pregnancy. Direct mutation identification, if known in the family at risk, or an intragenic polymorphic marker can be used [101].

The screening assay for the diagnosis of HIGM3 is founded on the absence of CD40 expression assessed by immunofluorescence since so far all patients described display a complete lack of CD40 expression on B cells as well as on monocytes. However, we cannot absolutely exclude that some mutant proteins could be expressed and recognized by monoclonal antibodies. Then, the diagnosis of CD40 deficiency requires genetic analysis.

2.8.5 Management

The treatment included immunoglobulin substitution that resulted in a marked decrease of upper and lower respiratory tract bacterial infections. In some cases, immunoglobulin replacement therapy also led to the resolution of lymphoid hyperplasia when it existed before treatment. Under intravenous immunoglobulin treatment, IgM level often drops to normal value. The neutropenia is also frequently corrected by this substitution. However, in some patients presenting severe and symptomatic neutropenia, treatment by granulocyte-colony-stimulating factor has been given, successfully in most cases. Depending on the frequency and the severity of opportunistic infection, especially by Pneumocystis jiroveci, a prophylactic antibiotic therapy using trimethoprimsulfamethoxazole is recommended, especially when the patient had presented a previous episode of opportunistic infection. In spite of these preventive measure the survival rate is still poor, although variable from one series to another. An important cause of death is still opportunistic infections, including Pneumocystis jiroveci, CMV and mycobacteria. But it is noticeable that severe liver disease is responsible for many deaths, particularly in the European cohort. Indeed, in the US registry, these complications seem to be less frequent. This
could reflect a lower incidence of *Cryptosporidium* infection. Neoplasm complications are also an important element in the prognosis. Consequently, more aggressive treatment such as HSCT has to be considered. Indeed, HSCT using either bone marrow from familial HLA identical [34, 203, 407] or matched unrelated donors [13, 157, 213, 241] or cord blood [462] has been successfully performed in patients with HIGM1 associated with CD40L deficiency. The European Group for Bone Marrow Transplantation, in association with the European Society for Immunodeficiencies, recommends a careful follow-up of the liver and biliary functions and regular screening for *Cryptosporidium* infection, with the goal of proposing HSCT to at-risk patients before liver alteration that constitutes a pejorative factor for the transplantation especially in mismatched situation [157]. According the CD40 expression on nonhematopoietic cells, stem cell transplantation as treatment in HIGM3 patients is more uncertain. However, one out of two patients with HIGM3 who received HSCT has been cured [229, 264].

### 2.9 MHC Class II Deficiency (CIITA Deficiency, RFX5 Deficiency, RFXAP Deficiency, RFXANK Deficiency)

#### 2.9.1 Definition

MHC class II deficiency (OMIM#209920) is a rare immunodeficiency in autosomal recessive transmission. To date, about 100 patients presenting this immunodeficiency have been described in the literature. Most patients are of North African origin (Tunisia, Morocco, Algeria). However, patients of various origins in Europe, United States, and Asia have been described. This syndrome is also called “Bare Lymphocyte syndrome”. It is characterized by the absence of expression of HLA class II molecules. This absence of expression is the result of a mutation in the genes encoding one of the four trans-acting elements that regulate the expression of HLA class II molecules.

#### 2.9.2 Etiology

MHC class II deficiency was initially subdivided into four functional complementation groups: A, B, C, D. These four complementation groups were confirmed when the four genes involved were identified, that is, the genes encoding the Class II transactivator (CTIA in group A, OMIM’600005) [392], the regulatory factor X associated protein containing ankyrin repeat (RFXANK also called RFX-B in group B, OMIM’603200) [259, 284], the fifth member of the regulatory factor X family (RFX5 in group C, OMIM’601863) [391] and the regulatory factor associated protein (RFXAP in group D, OMIM’601861) [112]. Identification of the molecular origin of this immunodeficiency contributed to the clarification of the respective roles of these factors in the regulation of the transcription of HLA class II molecules. HLA class II molecules DR, DP, DQ are α/β heterodimers. In humans, the genes encoding these different chains are located on chromosome 6. The molecules are expressed constitutively by thymic epithelial cells, by the antigen presenting cells (B lymphocytes, dendritic cells and monocytes/macrophages) and by activated T lymphocytes. Aside from this constitutive expression, the expression of HLA class II molecules can be induced specifically by interferon γ. HLA class II molecule expression is regulated by a proximal region promoter called S-Y comprised of 4 cis-acting DNA elements called the S, X, X2 and Y boxes [223, 335, 427]. The RFX ubiquitous complex composed of RFX5, RFXANK and RFXAP binds box X. CREB binds box X2, and NF-Y binds box Y. The totality of factors that bind the S-Y module constitute a complex called «enhanceosome». In case of a mutation in the gene encoding one of the components of RFX observed in patients presenting a MHC class II deficiency belonging to groups B C and D, the S-Y site is unoccupied [165, 208], proving that each of these components is indispensable for binding the enhanceosome on the S-Y site. Binding of the enhanceosome on the S-Y module is necessary for the transcription of molecule MHC class II genes, but it is not sufficient (Fig. 2.3). In fact, recruitment of the inducible CIITA coactivator, whose gene is mutated in patients with a MHC class II deficiency of group A, is indispensable.

In most patients (environ 60%), the affected gene is *RFXANK* (group B) and mutations modify the Ankyrin repeat region, a region whose integrity is required for RFXANK function. The *RFXANK* mutation 752del G-25, linked with a founding effect, is observed in almost all North African patients [450]. Mutations in the *RFXAP* gene (group D) account for about 20% of patients. These mutations result
in synthesis of truncated proteins. The mutations observed in group A patients (about 15%) involve the CIITA gene [259]. These mutations are diverse: missense mutations, nonsense mutations and splice site mutations. In the remaining patients (group C), mutations in the RFX5 gene generally lead to synthesis of truncated proteins [260]. Punctual mutations in RFX5 or CIITA are associated with milder phenotypes [288, 451].

2.9.3 Clinical Manifestations

Despite the heterogeneity of molecular origins responsible for the different groups of patients presenting MHC class II deficiency, clinical manifestations are similar [28, 119, 218, 365]. However, mild forms associated with certain mutations have been described [107, 184, 451].

Patients present recurrent infections characteristic of combined immunodeficiency. Susceptibility to bacteria, viruses and fungi testifies to the severity of this immunodeficiency. The first infection occurs in infancy, at an average age of 4 months, and exceptionally after the age of 1 year. These recurrent infections essentially involve the gastrointestinal tract, the lungs, the upper respiratory tract and the urinary tract.

Digestive problems are common. They take the form of diarrhea starting most often during the first year of life, becoming chronic and associated with malabsorption leading to delayed height–weight development. Histology findings commonly include villous atrophy associated with intraepithelial infiltration by lymphocytes and macrophages. These types of diarrhea are very often associated with Candida, Giardia lamblia and cryptosporidium infections. However, viruses (enterovirus species or adenoviruses),
gram-negative bacteria (*Escherichia coli*, *Salmonella* species, *Shigella*, *Pseudomonas*) and gram-positive bacteria (*Staphylococcus* and *enterococcus*) are also frequently involved.

Hepatic abnormalities take multiple forms. Sclerosing cholangitis secondary to chronic infection due to *Cryptosporidium* develops secondarily in over half the patients and constitutes a major factor in prognosis. Hepatitis cases are most often of viral origin. Cholangitis cases of bacterial origin (*Pseudomonas, Enterococcus* and *Streptococcus*) have also been observed.

Pulmonary infections occur in almost all patients. These can be interstitial affections caused by viral infections (adenovirus, CMV and RSV) or by *Pneumocystis jiroveci* which can cause major hypoxia leading to the death of the patient. Most patients present more than one episode of pulmonary infection of bacterial origin. The chronic nature of these pulmonary affections very frequently leads to bronchiectasis. Chronic upper respiratory tract infections such as sinusitis, rhinitis and otitis are common.

Meningitis and meningoencephalitis of viral origin can cause death in some cases. Enteroviruses including the polioviruses, the herpes simplex virus, the coxsackievirus and the adenovirus have been reported. Infectious pyelonephritis and septicemias can also occur. Autoimmune cytopenias, particularly hemolytic anemias and neutropenias are described in about 10% of patients.

Severity of clinical symptoms varies from one patient to another. In general, this variability cannot be clearly correlated with the mutated gene or the type of mutation. Specifically, this variability is observed among patients presenting an *RFXANK* mutation due to a founding effect.

### 2.9.4 Diagnosis

The immunological consequences of lack of MHC class II expression orient the diagnosis. These features can be accounted for by the lack of MHC class II expression on antigen presenting cells [119, 218]. The first characteristic is the inability to develop antigen-specific humoral and cellular responses. Delayed-type hypersensitivity skin tests and in vitro antigen-specific stimulation are negative in all patients. By contrast, responses to mitogens are normal. Humoral immunity is also always impaired. Hypogammaglobulinemia is variable from one patient to another, from agammaglobulinemia to a slight decrease in one immunoglobulin isotype (mainly IgA and IgG2). In all cases, specific antibody production is impaired. Patients display normal T cell count. However, most of them present CD4 lymphopenia. By analogy with MHC class II -/- mice, the latter could reflect the abnormal selection and maturation of CD4 T lymphocytes in the absence of MHC class II expression on the thymus [170]. However, some MHC class II expression has been detected on medullary thymus cells from dead children and from aborted fetuses [168]. This finding suggests leakiness of the defect or the presence of an alternative regulation pattern of MHC class II gene transcription in thymic cells that can account for partially preserved CD4 T cell differentiation and their normal repertoire building assessed by Vβ and Vα usage [232, 342].

The diagnosis is based on the lack of MHC class II expression assessed by immunofluorescence. In most patients, MHC class II molecules DR, DP, DQ are completely undetectable on blood B lymphocytes and monocytes as well as on in vitro activated T cells. In some cases, residual expression of these molecules has been reported on various cell types. At least in some cases, this leaky expression, always lower than expression observed in controls, seems to be associated with a less severe clinical phenotype. In most patients, low expression of MHC class I molecules, around 10–30% of controls, is also observed.

The final diagnosis requires mutation detection. The existence of the four different genes involved makes molecular analysis difficult. Different strategies can be proposed to direct the molecular analysis. First, in case of consanguinity, the study of polymorphic markers flanking the four genes involved can be useful. Second, according to the frequency of the mutation 752delG-25 in patients of North Africa origin, it is judicious to search for this mutation first in this population. In other cases, a functional identification of the gene affected could be helpful. Recently, a functional test based on direct correction of the genetic defect by transduction of cells from patients with lentiviral vectors encoding CIITA, RFXANK, RFX5 or RFXAP has been proposed as a valuable tool for the diagnosis and classification of new MHC class II-deficient patients [262]. Molecular characterization is a crucial step for proposing an appropriate prenatal diagnosis at 8–10 weeks of gestation in at-risk families.

### 2.9.5 Management

MHC class II deficiency has a very poor prognosis. Supportive care associating symptomatic and prophylactic treatment of infection can reduce the frequency and the severity of clinical problems.
lin replacement therapy is a part of this care. In some cases, parenteral nutrition is needed. However, this supportive care, as complete as possible, does not prevent progressive organ failure and death that occurs in most cases before 20 years of age.

The only radical treatment that can be proposed is HSCT for which some successful outcomes have been reported [16, 217, 365]. However, it appears that HSCT in MHC class II deficiency is associated with a lower survival rate than other immunodeficiencies. This is true whatever the compatibility between the donor and the recipient, i.e., HLA matched or HLA mismatched. The poor prognosis of HSCT is not limited to the non-HLA identical situations in which the survival rate is reported to be as low as 32% [16, 136]. Recently, a series has reported the outcome of HLA identical stem cell transplantation in 15 MHC class II deficiency patients [336]. Seven out of the 15 patients died early after transplantation, and a high rate of GVHD was observed. This occurrence of GVHD is clearly associated with viral infection status before stem cells transplantation. These observations suggest that stem cell transplantation could be improved in these patients in different ways. Performing the transplantation at the time of diagnosis would minimize the risk of viral infection. Careful detection of viral replication would make it possible to propose preemptive treatment of viral infection before and around the HSCT.

### 2.10 MHC Class I Deficiency (TAP1/2 Deficiencies, Tapasin Deficiency)

#### 2.10.1 Definition

MHC class I deficiency (OMIM#604571) is characterized by low expression of the MHC class I molecules. This is true whatever the molecular basis. In no case, a complete absence of MHC class I molecule expression has ever been described. To date, less than 20 patients with elucidated MHC class I deficiency have been reported and only one of them presented tapasin deficiency [455]. Others display a deficiency of either TAP1 or TAP2 [88, 91, 146, 261, 274, 446, 455]. However, some asymptomatic subjects present nonelucidated low expression of MHC class I molecule [314]. Only elucidated MHC class I deficiency is discussed in this section.

#### 2.10.2 Etiology

MHC class I molecules are expressed ubiquitously and present endogenous peptides to CD8+ T cells. Consequently, MHC class I molecules are designated as the central agents of antiviral immune response. The peptides, usually eight or nine amino acids in length, and binding MHC class I molecules result from the degradation of newly synthesized protein carried out by the proteasome. They are further translocated in the endoplasmic reticulum by the two transporters associated with antigen processing proteins (TAP1 and TAP2), where they are loaded onto the MHC class I heavy chain/β2-microglobulin heterodimer. This loading is dependant on the peptide-loading complex that contains the heterodimer TAP1/TAP2, the thiooxido-reductase ERp57 and the glycoprotein chaperone calreticuline and tapasin (Fig. 2.4) [219, 453]. The role of tapasin seems to be multiple and complex. However, it is clear that tapasin stabilizes the TAP1/TAP2 complex, links it to MHC class I molecules and facilitates loading of peptides with progressively higher affinity [51, 453]. The peptide-loaded MHC class I molecules are further transported to the cell membrane where expression takes place. Membrane expression of MHC class I molecules is dependant on their association with high affinity peptides. MHC class I molecules that do not bind high affinity peptides do not travel through the Golgi apparatus and the empty MHC class I molecules expressed at the membrane level are unstable. Consequently, a defect in either TAP1/TAP2 complex or in tapasin leads to low MHC class I expression.

TAP1 and TAP2 molecules include a core domain, 10 and 9 transmembrane domains, respectively and a catalytic nucleotide-binding domain. The genes encoding these two proteins, TAP1 (OMIM 170260) and TAP2 (OMIM 170261), are located in the HLA class II region [66, 233, 463]. So far, 12 families presenting a defect in TAP1/TAP2 complex have been reported. Homozygous TAP1 and TAP2 mutations have been found in seven and five families respectively [88, 91, 146, 261, 274, 446]. All these mutations lead a premature stop codon and consequently to a truncated nonfunctional protein.

Only one patient presenting a tapasin (TAPBP, OMIM 601962) mutation has been described [455]. The tapasin molecule contains a short cytoplasmic tail, a transmembrane region and an N terminal intraluminal region. The mutation described consists in a large deletion of 7.4 kb leading to a putative frame shifted and truncated protein that is not detectable.
Clinical Manifestations

The clinical consequences of TAP1/TAP2 deficiencies are variable from one subject to another. Some patients are asymptomatic [90, 309]. In most cases, symptoms, when they exist, occur late in childhood, at about 4–7 years of age. Despite the few patients described, no difference in clinical manifestation can be detected between TAP1 and TAP2 deficiency. Two typical features have been reported [66, 87, 147, 463]. The first consists of chronic infections affecting the respiratory tract and the second of skin granulomatous lesions.

In most cases, the respiratory tract is involved. Chronic infections of the upper respiratory tract are often the first manifestation and are responsible for purulent rhinitis, pansinusitis and otitis media. Frequent association with nasal polyposis has to be noted. Secondly, the infections extend to the lower respiratory tract and to chronic inflammatory lung diseases that progressively degrade the lung tissues, including bronchiolitis, bronchiectasis, and emphysema. These lesions inevitably evolve into a respiratory insufficiency. Death may be secondary to this degradation but may also occur during an acute infection. The pathogen most often involved in respiratory alteration is *Haemophilus influenza*, but others can be detected such as *Streptococcus pneumoniae*, *Klebsiella*, *Pseudomonas aeruginosa* and *Toxoplasma gondii*. Altogether, respiratory manifestations can mimic cystic fibrosis.

Skin lesions are present in half the patients and can be the only manifestation in patients without respiratory involvement [274]. They start with local inflammation that progressively extends, ulcerates, and evolves into chronic necrotizing granulomatous lesions mimicking Wegener disease [274, 321, 446]. In most cases, they are localized on the legs. However, some such lesions have been described on the
face, around the mouth and the nose, and in some cases are very mutilating, associated with perforation and destruction of the nasal cartilage. In some cases, these granulomatous lesions are related to vasculitis [274, 321, 446] associated with infiltration by NK cells and, to a lesser extent, TCRγδ T lymphocytes [274]. More recently, such skin lesions have been reported in association with *Toxoplasma gondii* infection [104]. Moreover, such granulomatous lesions can involve the upper respiratory tract, but have never been found in patient lung biopsies.

Recently, necrotizing retinochoroiditis related to *Toxoplasma gondii* has been reported as the only clinical manifestation in a 14-year-old patient [309].

In spite of the role of the MHC class I in the peptide presentation to CD8 T lymphocytes, it is noticeable that no patient presents severe viral infection and there is no evidence of a higher incidence of neoplasm in these subjects. This observation suggests that either other effectors such as NK cells and TCRγδ T lymphocytes could be efficient enough to eliminate virus infected cells in this situation, or independent TAP peptide presentation is sufficient to trigger TCRα/β CD8 lymphocytes. NK cells and TCRγδ T lymphocytes, beneficial in virus clearance, could however generate granulomatous and epithelial lesions, the lack of MHC class I dependent inhibition of their cytotoxic activity allowing the killing of uninfected cells [147, 466]. Epithelial lesions could favor bacterial colonization. Moreover, the TAP dependant MHC class I presentation of exogenous peptides of bacterial origin could play a more important role in the antibacterial defense than previously thought [87, 463].

Clearly, there is no correlation between mutation and clinical severity. The environmental context and/or genetic background could constitute determinant factors in the development of clinical manifestations.

The only patient presenting tapasin deficiency suffered from primary chronic glomerulonephritis for 10 years at time of diagnosis. This 54-year-old woman does not present any manifestation that can be related to an immunodeficiency, except herpes zoster virus infection [455].

### 2.10.4 Diagnosis

With the exception of two patients who present T cell lymphopenia, most patients have normal T cell count. However, most of them present a slight CD8 TCRα/β lymphopenia in contrast to the TAP−/− mouse model [417]. However, it seems that a more severe CD8 TCRα/β lymphopenia could exist early in life and be partially corrected later [87]. CD8 T lymphocytes display a diversified α/β repertoire [87] and cytotoxic activity, at least against EBV [89, 90]. In most patients, TCRγδ T lymphocyte count is increased, especially T lymphocytes bearing Vδ1 chain, and these lymphocytes can kill autologous cells [90, 274]. NK cells that are present in the normal range show poor spontaneous cytotoxic activity against MHC class I-deficient targets that is corrected after cytokine-mediated activation. Moreover, activated NK cells can kill autologous cells [261, 428, 464, 465]. The killing of autologous cells by TCRγδ T lymphocytes and activated NK cells could play a role in the pathogenesis of epithelial lesions.

In most cases, hypergammaglobulinemia involving different isotypes is observed. However, some patients present a hypogammaglobulinemia involving one or more isotypes [261, 321]. Antibodies to common viruses are present even in case of hypogammaglobulinemia, and often at high titer [105].

The diagnosis is based on low MHC class I expression assessed by immunofluorescence. Residual expression is 30- to 100-fold less than in controls [88, 91] [274]. Final diagnosis requires mutation detection. The involvement of TAP1/TAP2 or tapasin can be assessed by HLA typing in consanguineous families that confirms the linkage to the chromosome 6. A functional test based on direct correction of the genetic defect by infection of patient cell line with recombinant vaccinia virus expressing TAP1, TAP2 or both subunits could assist genetic diagnosis [87, 359].

### 2.10.5 Management

Chronic lung colonization evolves to respiratory failure which may lead to the patient’s death. Based on the similarity of these respiratory manifestations with those observed in cystic fibrosis, it is legitimate to propose to symptomatic patients with TAP deficiency management analogous with that recommended in cystic fibrosis, including prophylactic antibiotic therapy in association with physiotherapy [147]. In spite of the absence of humoral immunodeficiency, treatment using immunoglobulin replacement therapy has been reported useful in patients with severe pneumonia.

The lesions of the upper respiratory tract may require local medical treatment (local washing and topical steroids) or surgical (polypectomy) treatment. However, surgery has to be carefully considered.
because, in one patient, surgical intervention for chronic sinusitis has been reported to accelerate the nasal disease [147].

Treatment of skin granulomatous lesions is based only on optimal antiseptic topical care [147]. Immunosuppressive treatment, including steroids in combination with either cyclophosphamide, methotrexate, azathioprine or cyclosporin, has worsened skin lesions as well as lung manifestations and has to be avoided. In the same way, immunomodulatory intervention based on the use of Interferon α or γ is also disappointing, since it is associated with lesion progression [446].

A curative treatment has not been reported so far. Lung transplantation could be considered if the hypothesis concerning the role played by NK and TCRγδ cells in lesion pathogenesis is confirmed. The rationale of HSCT that would provided MHC class I positive hematopoietic cells could be debated.

### 2.11 CD8 Deficiency (CD8α Chain Defect, ZAP-70 Deficiency)

#### 2.11.1 Definition

Two immunodeficiencies characterized by the isolated absence of CD8 T cells have been identified, caused by a defect in either ZAP-70 [68, 117] or CD8α chain [86]. In spite of this shared feature, the clinical and biological consequences are very different. The ZAP-70 deficiency constitutes a severe combined immunodeficiency, while the CD8α defect is considered nonsevere and compatible with life. Both are inherited as an autosomal recessive trait.

#### 2.11.2 Etiology

The differentiation and activation of T lymphocytes require TCR-dependant signal transduction including tyrosine phosphorylation of many substrates. The tyrosine kinase Zeta associated protein-70 (ZAP70, OMIM*176947), belonging to the tyrosine kinase Syk family, plays a major role in this biochemical pathway. The antigen recognition is assured by the TCR, while the CD3 complex consisting of the γ, δ, ε, ζ, η chains transmits an intracytoplasmic signal by recruiting tyrosine kinases from the Src and Syk families. The CD3 complex contains, in its intracytoplasmic portion, a total of ten ITAM (immunoreceptor tyrosine-based activation motif), targeted for phosphorylation. Three of these motifs are carried by CD3ζ, and one by each of the other chains, CD3γ, CD3δ and CD3ε. Phosphorylation of these motifs by protein kinases of the Src family leads to recruitment by the ζ chain of ZAP-70, which is then phosphorylated and activated by the tyrosine kinase p56lck [67, 441]. ZAP-70 phosphorylates different substrates and consequently induces a calcium signal and MAPK activation leading to immune response [163] (Fig. 2.5). The normal thymic differentiation of CD4 positive T lymphocytes in ZAP-70 deficient patients proves that in humans, in contrast with the ZAP -/- mouse model, CD4 differentiation can occur in the absence of this tyrosine kinase [20, 286, 346, 349]. Syk, highly expressed in patient thymocytes, may compensate for the loss of ZAP-70 in CD4 but not in CD8 thymic selection [153, 294]. ZAP-70 is also expressed in NK cells.

To date, less than 15 patients and 8 different mutations have been reported in the literature, but it can be supposed that some ZAP-70-deficient patients are not reported. Most patients are born of related parents and present a homozygous mutation [20, 68, 116-118, 153, 263, 269, 294, 346, 412]. The mutations described include missense mutations, splice site mutation and deletion. Most mutations involve the catalytic domain but in fact affect protein stability. Two missense mutations found in a compound heterozygote patient, one affecting the first SH2 domain and the other affecting the kinase domain, are associated with a temperature-dependant instability of ZAP-70 [263].

CD8 molecules are expressed on the cell surface either as an αα homodimer in NK cells and TCRγδ T lymphocytes, or as an αβ heterodimer in TCRαβ T lymphocytes. However, surface expression of CD8β is dependent on expression of CD8α because CD8β polypeptides are otherwise retained in the endoplasmic reticulum and degraded. CD8 constitutes a coreceptor for TCR recognition of MHC class I-binding peptides and is necessary for the maturation, positive selection and activation of class I MHC restricted cytotoxic T lymphocytes. To date, two cases of CD8α deficiency have been reported in two families (CD8A, OMIM*186910) [86, 256]. Both are Spanish gypsy patients and present the same homozygous mutation. It is a missense mutation, Gly111Ser, affecting a very conserved position. This mutation is also disappointing, γα, because in one patient, surgical intervention for chronic sinusitis has been reported to accelerate the nasal disease [147].

Treatment of skin granulomatous lesions is based only on optimal antiseptic topical care [147]. Immunosuppressive treatment, including steroids in combination with either cyclophosphamide, methotrexate, azathioprine or cyclosporin, has worsened skin lesions as well as lung manifestations and has to be avoided. In the same way, immunomodulatory intervention based on the use of Interferon α or γ is also disappointing, since it is associated with lesion progression [446].
2.11.3 Clinical Manifestations

Patients with ZAP-70 deficiency present infections indistinguishable from those observed in other SCID patients. They occur in most cases within the first year of life and involve bacterial, viral and fungal pathogens. In some cases, opportunistic infections such as *Pneumocystis jiroveci*-related pneumonia or a CMV uncontrolled infection are the first manifestations of the disease. Frequently, *Candida* is responsible for cutaneous and oral infections and even for septicemia. Other infections due to various viruses including varicella zoster virus, rotavirus and parainfluenza have been reported, as well as lower and upper respiratory tract bacterial infections. These infections are often associated with a failure to thrive. Moreover, the patient presenting mutations associated with a thermo-sensitive ZAP-70 was affected by infiltrative erythematous skin lesions on his face and extremities [263]. In contrast with other SCID patients, ZAP-70-deficient patients display palpable lymph node and a normal sized thymus detected by chest radiology.

The severity of this later immunodeficiency contrasts with the late onset of clinical manifestations in both CD8α-deficient patients described so far. The age at diagnosis in the latter is 25 and 16 years [86, 256]. However, both patients suffered from recurrent respiratory infections very close to those observed in TAP deficiency since the childhood. In the first patient described, the pulmonary lesions led to death at 33 years of age. The main pathogens reported are *Pseudomonas aeruginosa* and *Haemophilus influenza*. Similarities with TAP deficiency are numerous. Some subjects who present the same CD8 deficiency are as healthy as the siblings of the first case described, and patients do not present high incidence of viral infection.

2.11.4 Diagnosis

ZAP-70 and CD8α deficiencies share a common feature: the lack of blood CD8 T lymphocytes. However, other biological findings are very different and are going to be described sequentially.

ZAP-70-deficient patients have a normal or high blood lymphocytes count. Except for the absence of CD8 TCR α/β T lymphocytes (in most cases, less than 3% of blood lymphocytes), other lymphocyte populations, including CD4 T lymphocytes, NK cells and TCRγδ T lymphocytes, are normally present. CD4 lymphocytes display a normal Vβ repertoire [349], suggesting that ZAP-70 is not indispensable for CD4 lymphocyte selection. However, peripheral CD4 lymphocytes function poorly. *In vitro* proliferation assays are useful to orient the final diagnosis. The proliferative as well as the IL-2 secretory responses to PHA and anti-CD3 antibody are
absent and restored in part by exogenous IL-2. Antigen-induced proliferations are also poor. In contrast, the association of a phorbol ester (PMA) with a calcium ionophore (ionomycin) that bypasses proximal TCR/CD3 signaling induces normal T cell proliferation. The lack of calcium mobilization and poor protein tyrosine phosphorylation after CD3 triggering confirm a defect in a proximal signal step [20, 153].

Humoral immunity is variably altered. Hypogammaglobulinemia involving all isotypes associated with a complete absence of specific antibodies observed in most patients contrasts with the normal or high level of immunoglobulins reported in others [374]. Some of these patients display normal antibody response after tetanus immunization. In any case, the hypogammaglobulinemia does not constitute an absolute diagnostic criterion.

Final diagnosis requires DNA sequencing in order to confirm and to characterize the **ZAP-70** mutation.

Blood T lymphocyte phenotype is characteristic of patients with CD8α deficiency. The patients present normal TCRα/β CD3, TCRγ/δ CD3 and CD4 T lymphocyte counts. Surprisingly, the lack of CD8 T cells is associated with an increased T cell population that expresses CD3 and TCRα/β, but expresses neither CD4 nor CD8 [86, 256]. This population is polyclonal and displays a normal Vβ repertoire. It probably represents a population of CD8 cytotoxic T lymphocytes, since it expresses a phenotype associated with effectors cytotoxic T lymphocytes (CD11b+, CD57+ and CD28-) and transcripts for CD8α and CD8β [86].

In contrast with the ZAP-70-deficient CD4 T lymphocytes, CD4 lymphocytes from CD8α-deficient patients are normally functional. Proliferative responses are normal whatever the mitogen or antigen tested. NK cells are normally present and are functional towards the K562 line as target. Humoral immunity is completely spared. The final diagnosis will be established by the detection of a CD8A mutation; to date, only one mutation has been described.

### 2.11.5 Management

Because of the very different severity of the clinical manifestations of the two types of immunodeficiency, Zap-70 deficiency and CD8α deficiency, prognosis and consequently management will be different as well.

By analogy with the other forms of SCID, the only treatment of Zap-70 deficiency is HSCT. Matched and mismatched transplantations were successful in most of the transplanted patients [118, 383, 412]. In contrast, the management of patients with CD8α deficiency consists of treatment of respiratory infections and prevention of bronchiectasis. One patient died when a lung transplantation was planned, after improvement with intravenous antibiotic therapy [86]. It can be supposed that early recognition allowing treatment at the time of the first clinical manifestations would lead to the best prognosis.

#### 2.12 CD4 Deficiency (p56lck Deficiency, Idiopathic CD4 Lymphopenia)

**2.12.1 Definition**

In 1993, the Centers for Disease Control (CDC) defined the condition of decreased CD4+ T cell count without HIV as idiopathic CD4+ T lymphocytopenia (ICL). This entity is characterized by (1) CD4+ T cell count <0.3 × 10^9/l in adults and <1 × 10^9/l in children above 23 months of age or <20% of the total T cell count on two occasions; (2) the absence of HIV-1, HIV-2 or human T cell lymphotrophic virus infection (HTLV); and (3) the absence of any known immunodeficiency disorder or therapy associated with reduced CD4+ T cell count. Most cases are adults but some ICL have been described in children. ICL has to be distinguished from secondary forms of CD4 lymphocytopenia. These secondary forms include infections (mycobacteria, viruses such as CMV, EBV, HBV) malignancies, and autoimmunes diseases [432].

**2.12.2 Etiology**

It is unlikely that a single pathophysiology will be operative in ICL. Some potential pathogenic mechanisms of ICL have been proposed. Defective cytokine related decreased bone marrow clonogenic capability has been involved in de novo T cell generation [200]. A disturbed thymic T cell maturation process may account for the decrease in naïve T cells [144]. Enhanced expression of Fas and Fas ligand in unstimulated cell populations might lead to spontaneous apoptosis of T lymphocytes [234, 345]. Impaired early biochemical events of the CD3-TCR pathway have been detected with reduction of T cell proliferation [193] and are related to low expression of p56lck in at least one case (LCK, OMIM*53390) (Fig. 2.5) [192].
Chapter 2 Combined T and B Cell Immunodeficiencies

2.12.3 Clinical Manifestations

In most cases, a diagnosis of ICL is made at the time of opportunistic infections such as Cryptococcus infection [226, 289, 301, 458], Pneumocystis jiroveci pneumonia [238, 382] or mycobacterium infection [201, 290, 384, 418]. These infections occur in patients without particular history and often constitute the first manifestation of the disease [384]. The most common pathogen involved is Cryptococcus neoformans with a central nervous system (CNS) localization in most cases [467]. Manifestations outside the CNS may be isolated or not [226, 458]. Other fungal infections are also frequent, and they include histoplasmosis, candidiasis (oral, vaginal and esophageal), and cerebral toxoplasmosis. Mycobacterial infections are also frequent. Typical [201] and atypical mycobacteria [290] are involved, with pulmonary and extra pulmonary localizations [418]. Viral infections are also frequent. The most frequently observed virus is the zoster virus which may lead to multidermatotomal localization. Oral or genital herpes simplex, human papillomavirus, molluscum contagiosum and CMV infections and HHV8 related Kaposi’s sarcoma are also reported, as well as bacterial infections such as nocardiosis and salmonellosis [111, 188, 257, 313, 328, 384, 389, 420, 434].

Some noninfectious clinical manifestations associated with ICL have been described [384]. They include autoimmune diseases such as Behcet’s disease [420], Sjogren syndrome [215], psoriasis [182], vasculitis [35], and thrombotic thrombocytopenic purpura [384].

Some patients already known to present CD4 lymphopenia have developed secondary malignancies. This observation suggests that idiopathic CD4 lymphopenia could favor malignancy occurrence. As in other immunodeficiencies, lymphomas, especially B cell non hodgkin's lymphomas, are often reported [50, 55, 172], as well as HHV8-related Kaposi’s sarcoma [132, 199, 340].

However, such CD4 lymphopenia has been reported in healthy subjects.

2.12.4 Diagnosis

CD4 deficiency is probably a heterogeneous disorder. Nontransient CD4 lymphopenia is the biological feature that defines this disease. CD4+ T cell counts are stable over time in contrast to the progressive loss of this subpopulation observed in the course of HIV disease. Naïve CD4 CD45RA T cells are more affected than the memory CD4 CD45RO T cells and the Vβ repertoire has been reported restricted [144]. High levels of plasma IL-7 were found and inversely correlate with CD4+ T cell counts [254].

In addition to CD4+ lymphocytopenia, several patients also display CD8+ lymphocytopenia [384]; low memory CD27+B or NK cell counts have also been reported in others [111, 188, 254, 384, 389].

A slight hypogammaglobulinemia involving IgG and IgA is often associated [188, 389].

Finally, the diagnosis is based upon the exclusion of known causes of CD4 lymphopenia, especially HIV infection. Moreover, secondary CD4 lymphopenia has also to be excluded before concluding to ICL.

2.12.5 Management

Because the similarity with the clinical manifestations observed in HIV patients, management can be based on the guidelines for these latter. However, because of the great clinical variability observed among patients, the clinical course of an individual patient has to be taken into account. Prophylactic treatment against Pneumocystis jiroveci can be proposed. The need for lifelong prophylaxis against cryptococcus is debated. Some authors recommend it. However, the absence of relapse associated with a better outcome of cryptococcosis in ICL than initially described, reported in a recent series, brings this prophylaxis into question [467]. Antiviral and antifungal prophylaxis may be proposed depending on the clinical history of the patient. Infection management has to include early diagnosis and appropriate treatment. Treatment by interferon γ, in association with antifungal treatment, has been useful in a patient who presented cryptococcosis [289].

Some treatments intended to increase CD4 lymphocytes have been reported occasionally. IL-2 treatment has improved CD4 count in the three patients treated [81, 434, 445]. However, one of them developed gastric aplastic large cell lymphoma more than
one year after treatment initiation, without a clear relationship between the treatment and the occurrence of malignancy [432].

Allogenic HSCT performed in a patient who had developed aplastic anemia has led to complete immune reconstitution [318].

2.13 CRAC Deficiency

2.13.1 Definition

Calcium++ release-activated calcium channels (CRAC) deficiency, which was identified in 1994, is characterized by the lack of intracytoplasmic calcium increase after immunoreceptor engagement. To date, only seven patients from four families presenting such an immunodeficiency are known; the molecular basis seems to be heterogenous while the functional characteristics are similar [128, 130, 131, 235, 311].

2.13.2 Etiology

Calcium signals are second messengers that play a crucial role in immune and in nonimmune cells. For example, in T lymphocytes, TCR/CD3 triggering leads to the kinase activation described in the CD8 deficiency section, and subsequently to ZAP-70-dependant phosphorylation and activation of the phospholipase Cγ (PLCγ) which then hydrolyses phosphatidylinositol-4,5 biphosphate (PtdIns(4,5)P2) to diacylglycerol (DAG) and Inositol-1,4,5-triphosphate (InsP3). The binding of InsP3 to the Ca2+ permeable ion channel, the InsP3 receptor at the endoplasmic reticulum (ER) membrane level, induces Ca2+ release from ER stores. Ca2+ depletion of ER stores results in store operated Ca2+ entry (SOCE) mainly mediated by the CRAC channel in plasma membrane (Fig. 2.5) [127].

Recently, the mechanism of CRAC channel activation by the Ca2+ depletion of ER stores has been at least partially elucidated by the identification of STIM1 by two independent RNAi screens [246, 354]. The ubiquitous protein STIM1 is localized in the ER and cytoplasmic membrane and acts as a Ca2+ sensor because a Ca2+ binding EF-hand motif is localized in its portion facing the ER lumen.

The structure of the CRAC channel was an enigma for a long time. Recently, two independent genetic analyses, that are genome-wide SNP analyses of two patients presenting a Ca++ channel deficiency and their relatives, and genome-wide RNA interference screen in Drosophila, allowed the identification of Orai1, a new component of the CRAC channel [130, 422, 459]. Orai1 is a ubiquitous transmembrane protein with four membrane domains, that constitutes the pore-forming subunits of the CRAC channel [173]. Orai1 colocalizes with STIM1 after ER store depletion, providing a physical basis for the activation of Ca2+ influx [250].

Homzygous missense mutation in exon 1 of human ORAI1 (OMIM+610277) leading to replacement of a highly conserved arginine residue by tryptophan at position 91 has been found in two patients from one family [130]. However, ORAI1 mutation would not account for all patients with a Ca++ channel deficiency.

2.13.3 Clinical Manifestations

In six out the seven patients, the diagnosis was made before 3 months of age, either because severe clinical manifestations or because of family history [128, 130, 131, 235, 311]. Clinical manifestations, including BCGitis, CMV dissemination, toxoplasmic encephalitis and candidiasis, are very close to those observed in SCID.

In the last patient, diagnosis was carried out later in childhood, and the infection was less severe while the patient benefited from early management (unpublished data).

The hallmark of this immunodeficiency is the association with a myopathy in four patients. In one case, association with a hypohydrotic ectodermal dysplasia has been reported [127].

2.13.4 Diagnosis

In these patients, T cell differentiation is unaffected. All blood lymphocyte populations, including CD4, CD8, TCRα/β and TCRγ/δ T cells, B lymphocytes and NK cells, are normally present. In some patients, a high percentage of CD45RO CD29 memory T cells has been noted [131, 235].

The diagnosis is based on poor proliferative response to mitogens including PHA and anti-CD3 monoclonal antibody that is partially restored by exogenous IL-2 [131, 235]. The expression of cytokines such as IL-2, IL-4, IL-10, IFN-γ and TNF-α is also altered. In some patients, proliferation induced by the association of
PMA and Ionomycin is also low [131]. Paradoxically, in one patient, specific antigen-induced proliferation is detectable [235].

Hypergammaglobulinemia is observed. It involves at least IgA and IgM, and in one case IgG. In one patient, IgG displayed restricted heterogeneity. The antibody response to immunization is absent in all cases. However, in one patient, anti-CMV antibodies of IgM isotype have been detected.

In all patients, activation-induced extracellular Ca2+ influx is absent, contrasting with normal Ca2+ release from ER stores. This calcium influx defect is seen not only after receptor triggering but also when thapsigargin, an inhibitor of the SERCA (sarcoplasmic endoplasmic reticulum calcium ATPase) which pumps calcium from the cytoplasm into the ER, is used to deplete internal Ca2+ stores. All these observations indicate a defect in SOCE. This SOCE defect is found in T and B cells as well as in nonhematopoietic cells such as fibroblasts [129, 235, 311]. That may account for the extra hematopoietic clinical manifestations reported. In one patient, neutrophils and platelets display the same defect without detectable functional consequences [235].

So far, ORAI1 mutations have only been reported in two patients from one family [130]. Further study of other patients with Ca2+ channel deficiency might elucidate the mechanisms of the SOCE and Ca2+ signaling pathways.

2.14.2 Etiology

In 1994, the genetic basis of the well-known “nude” mouse, associating hairlessness and congenital athymia, was reported for the first time. It involves a new gene, WHN (also called FOXN1), and consists of a single base deletion in exon 3. This frameshift mutation leads to a predicted aberrant protein. The protein whn is a member of the forkhead/winged-helix transcription factor family. It is mainly expressed in thymus and in skin [287] and plays a crucial role in the differentiation of thymic epithelial cells [396] as well as skin epithelial cells [268]. The mutation observed in nude mice leads to a protein deprived of the DNA binding domain.

Five years later, in 1999, Franck et al. identified a homozygous mutation of the human gene WHN (OMIM*600838), localized on the chromosome 17, in two siblings. This mutation, R255X, is a nonsense mutation and predicts complete absence of functional protein [142]. The two patients were born of consanguineous parents in a small community in southern Italy. It was secondarily shown that this mutation is present in 6.52% of this population, and is related to a single ancestral origin [2].

2.14.3 Clinical Manifestations

Only two patients from one family have been reported [319]. In both patients, alopecia affecting the scalp, the eyebrows and the eyelashes associated with nail dystrophy was noted at birth, as well as bilateral epicanthal fold.

Subsequently, at 2 months of age, they developed immunodeficiency manifestations. The first one presented with a clinical picture mimicking Omenn syndrome, including erythrodermia, diarrhea and hepatosplenomegaly, and died at 12 months of age following recurrent infection and severe failure to thrive. The second one also developed erythrodermia at 2 months of age. No thymic shadow was seen at radiologic examination.

2.14.4 Diagnosis

Both patients display a T cell lymphopenia affecting mainly the CD4 population. B and NK cell populations are present at normal or high level. Proliferations induced by PHA or anti-CD3 monoclonal antibody are low, in contrast to those induced by PMA + Ionomycin, which is normal.
Immunoglobulin levels reported in one patient are normal. The detection of allohemaglutinins in one patient contrasts with the absence of specific antibodies after immunization.

2.14.5 Management

One out of the two patients received nondepleted HLA identical BMT from her healthy heterozygous brother, with successful engraftment [320]. CD4 and CD8 T lymphocytes increased promptly and are stable 6 years later. However, the CD4 T population displays only a memory phenotype CD45RO. This suggests that, as expected, CD4 recovery mainly results from the expansion of graft T lymphocytes. Moreover, the Vβ repertoire of CD4 lymphocytes is similar in the donor and the engrafted patient. Conversely, the prompt recovery of naïve CD45RA CD8 population suggests extrathymic lymphopoiesis. However, the CD8 compartment reconstitution is poor as judged by restricted TCR-Vβ diversity. T cell lymphoproliferation restored early after transplantation has further decreased to reach 20% of the normal value. In spite of this incomplete immune T reconstitution, humoral immunity is restored as judged by the production of specific antibodies after immunization, especially with antigen unknown by donor. However, the patient is free of infections at 6 years follow-up.

2.15 CD25 Deficiency

2.15.1 Definition

Human IL-2 receptor α chain deficiency (CD25 deficiency, OMIM#606367), caused by mutation in the IL2RA gene (OMIM#147730), is a combined immunodeficiency characterized by invasive viral and bacterial sinopulmonary infections, as well as lymphoproliferation and severe multiorgan autoimmune disorders.

2.15.2 Etiology

The high affinity receptor for IL-2 is composed of three subunits: α (CD25), β (CD122) and γ (common γ) [272]. Whereas the β and γ chains are constitutively expressed on T cells, α chain expression is restricted to the early stages of thymocyte differentiation and to activated mature T cells. Although the β and γ chains together can form an IL-2 receptor of low affinity, the α chain cannot form a functional receptor in the absence of both the other chains [239]. The presence of the high affinity receptor on activated T cells is necessary for optimal proliferative responses to IL-2 after stimulation of the T cell receptor.

CD25 is also highly expressed on CD4+, naturally occurring, T regulatory cells [141, 190, 212]. These specialized cells play an important role in a complex regulatory system which maintains tolerance to self [363], controls lymphocyte homeostasis [15], and regulates immune responses to various pathogens [29]. Naturally occurring T regulatory cells express FOXP3, a transcription factor which is essential for the development of these cells. Genetic abnormalities in FOXP3 result in a low number of T regulatory cells which leads to IPEX (Immunodysregulation, polyendocrinopathy, enteropathy, X-linked) [70] (see Sect. 5.7 for more details).

In humans, a genetic defect in CD25 was described in two patients. These patients provided an opportunity to study CD25 role in thymic and extrathymic processes affecting T cell development. Examination of the first patient’s thymocytes and blood lymphocytes revealed no expression of CD25 [375]. The patient’s thymus was normal in size but displayed no Hassall’s corpuscles and loss of corticomedullary distinction. The expression of bcl-2 was elevated throughout the thymus and consequently apoptosis was dramatically diminished [347]. In a normal thymus, high bcl-2 levels are expressed only in cortical immature thymocytes and not in medullary more mature T cell progenitors. The reduction in Bcl-2 expression in medullary thymocytes enables apoptosis of autoreactive T cells.

In the periphery, dense lymphocyte infiltration was observed in the lung, gut, liver, bone and soft tissue causing chronic inflammation and tissue atrophy. In this patient, analysis of T cell repertoire revealed over-representation of certain Vβs families; those clones were found in tissue infiltrates [347].

The second patient was carefully studied for the effects of CD25 deficiency on peripheral tolerance [61]. This study clearly shows that, in humans, CD25 is required for the development of CD4+CD25+ T regulatory cells and the production of the immunosuppressive cytokine, IL-10. Together, these two patients show that in humans, CD25 has a critical role in the development of central as well as peripheral tolerance.
2.15.3 Clinical Manifestations

The patients described to date showed a combination of both immunodeficiency and autoimmune manifestations. The first patient, a male child of consanguineous parentage, presented at the age of 6 months. While the second patient presented earlier at the age of 6 weeks.

In both patients, severe viral infections such as CMV pneumonitis were part of the initial presentation, and they later on suffered persistent CMV disease and EBV infection. Evidence for the presence of these organisms was found in lung, gut and lymphoid tissues. Both patients had recurrent bacterial infections of the lungs, middle ear and sinuses. The first patient also experienced chronic oral thrush and candida esophagitis. Lymphoproliferation was markedly evident in both patients with lymphadenopathy and hepatosplenomegaly apparent at the ages of 8 months and 2 years, in patient 1 and patient 2, respectively. In one patient, lymphocytic infiltration was identified in multiple organs such as the lungs, liver, gut and bones.

Autoimmune manifestations were strikingly apparent in both patients who presented in infancy with severe autoimmune enteropathy causing chronic diarrhea and severe failure to thrive. Biopsy revealed chronic inflammation and villous atrophy, typical for autoimmune enteropathy. In addition, one patient had primary biliary chrosis (PBC), the first case to present at this young age. Antimitochondrial antibodies and antinuclear antibodies (reactive to sp100 and PML protein) were positive in this patient’s serum, while p-ANCA and c-ANCA antibodies were negative. Finally, the patient’s serum reacted with the human PDC-E2 (pyruvate dehydrogenase complex epitope) which is specific for PBC. The diagnosis of PBC at such an early age in this patient stresses the importance of both T regulatory cells and auto reactive T cells in the pathophysiology of PBC [17].

The patient described by Caudy et al. [61] showed autoimmune manifestations of the endocrine system as he presented at the age of 6 weeks with type I diabetes mellitus and, at the age of 3 years, he was diagnosed with hypothyroidism. At the age of 5 years he developed additional autoimmune manifestations including autoimmune hemolytic anemia and neutropenia with positive antigranulocyte antibodies.

Beyond the universal immunodeficiency described in these patients the role of CD25 in increased susceptibility to a variety of autoimmune diseases has been revealed. Vella et al. who studied a large population of 7,457 individuals with type I diabetes used a set of 20 tag SNPs in the CD25 gene and found linkage between affected patients to multilocus polymorphisms in the CD25 gene [419]. This was further supported by a recent study revealing two markers, rs706778 A/G and rs3118470 C/T in the 5’ end of intron 1 of the CD25 gene, which were associated with type 1 diabetes [329]. Similar genetic aberrations were found in patients with Grave’s disease suggesting it is also an important susceptibility gene for Grave’s disease [38].

Recent studies of risk alleles for multiple sclerosis also showed a correlation between specific polymorphisms in the CD25 gene (alongside with IL-7Rα and HLA-DRA) and the prevalence of the disease [179]. Two of the SNPs showing significant association with multiple sclerosis were found in intron 1 of the CD25 gene, rs12722849 risk allele C and rs 2104286 risk allele T. Altogether, these results imply that genetic abnormalities in the CD25 gene predispose individuals to autoimmune diseases.

2.15.4 Diagnosis

The diagnosis of CD25 deficiency should be considered in patients who present with autoimmunity and immunodeficiency. The combination of recurrent infections and chronic candidiasis with enteropathy, endocrinopathies, lymphadenopathy and other autoimmune manifestations may be suggestive of this deficiency. CD25-deficient patients share similar clinical features with other immunodeficiency such as IPEX (FOXP3 deficiency) and the autoimmune, polyendocrinopathy with ectodermal dystrophy (AIRE deficiency) (see Sects. 5.6 and 5.7 for more details).

The two patients described with CD25 deficiency had different mutations. The first patient had a homozygous deletion of 4bp (60–64) in the IL-2R alpha gene resulting in translational frame shift [375]. The second patient was carrying a single base pair insertion after position 692 in one allele and a C to T substitution at position 301 in the second allele resulting in a stop codon. Analysis of CD25 expression revealed no expression in the patients and intermediate expression in each of the parents.

In both cases, immune workup revealed immunoglobulin levels that were normal to somewhat high. The number of circulating CD3+ varied from reduced in the first patient to normal in the second patient. In both cases, the number of circulating CD4+ lymphocytes number was reduced. Surface expression of CD25 was negative in all lymphocytes.
populations, resting as well as activated. CD25 protein level measured by immunoblotting was markedly reduced. Lymphocyte proliferation assay demonstrates poor responses to PHA as well as to anti-CD3 antibody [375]. Expression of Foxp3 in CD4+ cells was normal but production of IL-10 was undetectable [61].

Analysis of T cell repertoire showed representation of all TCRVβ families with overrepresentation of several clones. These clones were found in multiple tissues with heavy lymphocytic infiltrates [347]. Serology studies may also be of value in this condition. Assessment of hormone levels as well as autoantibodies such as antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), and antineutrophil cytoplasmic antibodies (ANCA) may help to better define the various autoimmune manifestations which associate with CD25 deficiency.

2.15.5 Management

Early diagnosis and treatment is important since it may prevent the extent of damage caused by infections as well as autoimmune inflammatory processes. Symptomatic and supportive treatment with total parenteral nutrition may be required. Prompt antibiotic, antiviral and antifungal therapy should be administered when required, and hormonal replacement should be instituted and monitored.

Immunosuppressive treatment with corticosteroids or cyclosporin A may provide temporal relief from some autoimmune manifestations. The only known cure for this condition is HSCT. Engraftment is facilitated by myeloablative conditioning. Long-term survival and robust immune reconstitution has been observed in one patient [375].

2.16 STAT5B Deficiency

2.16.1 Definition

STAT5B deficiency is a newly described immuno-deficiency, caused by mutations in the STAT5B gene (OMIM’604260), resulting in respiratory disease as well as short stature due to impaired growth hormone (GH) signaling. The epidemiology and natural history of this disease is still not fully understood as only five patients have been described.

2.16.2 Etiology

Signal Transducers and Activators of Transcription (STATs) are latent transcription factors in the cellular cytoplasm [8]. Activation of the seven known mammalian STATs occurs in response to a wide variety of ligands such as cytokines, growth factors and hormones. Upon binding of these ligands to their receptors, tyrosine kinases such as JAKs (janus associated kinases) phosphorylate the receptor on tyrosine residues. This creates a high affinity binding site for the Src homology 2 (SH2) domain of STATs on the receptors which are subsequently phosphorylated by JAKs. STATs then dissociate from the receptor and form hetero- or homodimers that translocate into the nucleus, bind to DNA and activate transcription of various genes.

STAT 5 is composed of two highly homologous genes, STAT 5a and STAT 5b, which are closely linked on chromosome 11. Both STAT 5a and 5b are activated by similar hormones and hematopoietic and lymphocyte specific cytokines [8, 285]. While their actions may be partially redundant, they still share unique functions and differ in their COOH-terminal domains. From rodent models, STAT 5a deficient mice exhibit defective lactation and failed differentiation of the mammary gland [247]. However, while STAT 5b does display some response to prolactin, it is more important in normal cellular differentiation and proliferation particularly as a response to growth hormone [151, 331, 414].

Growth hormone-induced signaling occurs through the STAT pathways- STAT 1, 3, 5a and 5b [331]. Rodent models have demonstrated the need for STAT 5b in the generation of insulin-like growth factor 1 (IGF-1) in response to GH and for normal postnatal growth [151, 331, 414]. In rodents, STAT 5b is responsive to the pulsatile pattern of GH secretion and helps activate male expressed liver genes as well as regulate sexually dimorphic growth [151, 414]. STAT 5b-deficient males exhibit a female growth pattern and liver gene expression, but STAT 5b deficient females remain unaffected. This is in sharp contrast to the human cases of STAT 5b deficiency, in which four out of five are female.

Both STAT 5a and STAT 5b are important in immunity. Many lymphocytic cytokines use STAT 5 in their intracellular signaling cascade including IL-2 and IFN-γ [405]. STAT 5a deficient mice show defective GM-CSF induced proliferation [124]. Nakajama et al. suggested that STAT 5a deficient mice have impaired T cell proliferation likely due to a reduction in the expression of the IL-2Rα chain thereby affecting
However, high doses of IL-2 were found to normalize proliferative responses in these cells. In STAT 5b-deficient mice, thymocyte numbers are mildly diminished and, in the periphery, both STAT 5a- and STAT 5b-deficient mice have decreased numbers of splenocytes [197, 285]. Imada et al. showed that the proliferation of STAT 5b-deficient splenocytes was significantly affected and could not be overcome with IL-2 [197]. Although NK cell numbers were reduced in both STAT 5a- and STAT 5b-deficient mice, STAT 5b-deficient mice had a significant decrease in NK cell function. Moriggl et al. then determined that the profoundly impaired T cell proliferation in the presence of IL-2 noted in doubly STAT 5a- and 5b-deficient mice was due to an intrinsic defect in cell cycle progression rather than to decreased IL-2 receptor expression and that activation of STAT 5b occurs through cytokine receptors and not the TCR complex [276, 277]. In contrast to previous reports they showed that the lack of STAT 5a/5b does not alter the expression of IL-2Rα or β. These mice also had splenomegaly, lacked NK cells and had an activated T cell phenotype similar to mice deficient in IL-2Rα. Finally, STAT 5 proteins are not required for B cell proliferation [443].

STAT 5b has been implicated in malignant transformation and is involved in Src-mediated oncogenesis [48, 54, 240, 380]. A number of tumors possess elevated Src tyrosine kinase activity playing an important role in cell cycle control. STAT 5b is shown to accelerate v-Src oncogenic activity and tumorigenicity [54, 380]. For example, both STAT 5a and 5b are overexpressed in breast cancer, perhaps because of overexpression of other signaling molecules such as Src tyrosine kinases and the epidermal growth factor receptor (EGFR) [397, 438, 454]. As a result, STAT 5a and 5b have emerged as possible targets for cancer therapeutics [48]. Recently, STAT 5b was also found to have a role in vascular smooth muscle growth and motility and the pathogenesis of vessel wall diseases [57, 227].

2.16.3 Clinical Manifestations

The syndrome of growth hormone insensitivity (GHI) phenotypically mimics GH deficiency [355]. Levels of GH are normal to elevated and levels of IGF are low with no response to exogenous GH therapy. The majority of patients with this phenotype also have low levels of GH binding protein (GHBP), the extracellular portion of the GH receptor (GHR).

However, there are some patients with normal levels of both GH and GHBP. A few of these patients have been found to have mutations in the IGF-receptor, but they have normal to elevated levels of IGF-1, as well as growth retardation evident in the intrauterine period, and no evidence of immunodeficiency [1]. While the various etiologies for GHI are still largely uninvestigated, five human patients were found to have mutations in STAT5B [194, 195, 220, 421, 431].

The first was a female born to consanguineous parents of Argentinian descent [220]. Although she had evidence of growth restriction in the first 3 years of life, she presented to an endocrinologist at 7 years of age. She exhibited facial dysmorphism, was below the 5th percentile for height and weight and had severe respiratory difficulties with the eventual diagnosis of lymphoid interstitial pneumonia. A year later, she developed hemorrhagic varicella and several episodes of herpes zoster. She had a second lung biopsy demonstrating Pneumocystis jiroveci. The second patient was born to consanguineous parents of Turkish descent at a normal birth length [195]. She was initially referred to an endocrinologist at almost 8 years of age with both weight and height below the third percentile. Other aspects of her history included recurrent epistaxis as well as pruritic skin lesions and recurrent pulmonary infections. Chest imaging was consistent with pulmonary fibrosis. Although the mechanism of the fibrosis is not clearly understood, it may have been due to an infectious cause. The next described patient was a male born at a normal birth length in the Dutch Antilles with no reported consanguinity [421]. At 16 years of age, he presented with hemorrhagic varicella requiring treatment with acyclovir. His growth retardation was severe but he also showed evidence of delayed puberty. When reevaluated at the age of 30 years, he had not experienced recurrent infections of any kind but did have pseudogynecomastia. Finally, a STAT 5b defect was found in siblings for the first time in sisters born to consanguineous parents of Kuwaiti descent [194]. They both presented with short stature and recurrent pulmonary infections.

2.16.4 Diagnosis

STAT5b deficiency should be considered in the differential diagnosis of a patient who is born with a normal birth length but then acquires significant short stature and
recurrent infections, particularly pulmonary, although clinical evidence of immunodeficiency may not necessarily be present [421]. Interestingly, one patient also developed pulmonary fibrosis and had recurrent epistaxis [195]. Although STAT5b deficiency in mice only affects males, four of the five cases described in humans are female and therefore sex may not be a relevant consideration in making the diagnosis.

All described patients had biochemical evidence of GHI with deficient IGF-1 and IGFBP-3 production. Any trials of treatment with growth hormone failed and, if measured, GHBP levels were normal. The male patient also had biochemical and clinical evidence of hyperprolactinemia [421].

Some of the patients displayed evidence of immune dysfunction [194, 195, 220]. In the first case, the investigators showed that the patient’s cells could not produce STAT 5b on exposure to IFN-γ [220]. While this may not be practical for routine laboratory analysis, it suggests that cytokines important for a competent immune response such as IFN-γ require STAT 5b for efficient transcription of certain important genes, one of them being IGF [196]. STAT5b-deficient mice have been shown to have poor lymphocyte proliferation [197, 276, 277, 285, 405]. While the male patient had a normal reported immunologic assessment in terms of cytokine production, his episode of hemorrhagic varicella at the age of 16 years may nonetheless still be considered significant [421, 431]. Therefore, investigations such as lymphocyte markers as well as lymphoproliferative assays could be considered in the immunologic evaluation of such patients.

In the first patient, STAT5B sequence analysis revealed a homozygous missense mutation in exon 15 predicting an A630P (Alanine to Proline) substitution within the SH2 domain [220]. Although STAT 5b can be expressed with this mutation, its functionality is severely affected and it cannot be activated upon ligand binding. In the second patient, a frame shift mutation was identified at position 1,191 resulting in a truncated protein lacking the C-terminal half, the SH2 and transactivation domains [195]. The male patient also had a novel frameshift mutation resulting in a similarly truncated and inactive protein [421]. Finally, the female siblings had a homozygous deletion at the junction of exon 13-intron 13 [194]. While these are very different mutations, the clinical presentation of the patients is very similar suggesting that the final pathway of STAT 5b activation and subsequent gene transcription is similarly affected.

The observations of these five cases suggest that the overwhelming majority of GH action is through the GHR-JAK-2-STAT5b-IGF pathway.

### 2.16.5 Management

It is still unclear how to manage such patients as there have been only five described cases; two of them were treated but did not respond to GH. An evaluation by an immunologist with a thorough immune workup would be warranted. Patients should be closely monitored for infections such as severe varicella or recurrent pneumonias which should be aggressively treated to avoid adverse consequences such as bronchiectasis or pulmonary fibrosis. If fibrosis develops, immunosuppression such as steroids may be considered, although with caution as these patients are already immunosuppressed. Due to the paucity of clinical data, it is not known if HSCT would be beneficial, but perhaps could be a consideration in the future. It is important to understand STAT5b deficiency for the clinician that may encounter a child with significant short stature and evidence of immunodeficiency.

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Chapter 2: Combined T and B Cell Immunodeficiencies


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