

Educational paper

The expanding clinical and immunological spectrum of severe combined immunodeficiency

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Abstract Severe combined immunodeficiency (SCID) is one of the most severe forms of primary immunodeficiency characterized by absence of functional T lymphocytes. It is a paediatric emergency, which is life-threatening when recognized too late. The clinical presentation varies from the classical form of SCID through atypical SCID to Omenn syndrome. In addition, there is a considerable immunological variation, which can hamper the diagnosis. In this educational review, we describe the immunopathological background, clinical presentations and diagnostic process of SCID, as well as the therapeutic possibilities.

Keywords Severe combined immunodeficiencies · Diagnosis · Lymphocytes · Therapy · Primary immunodeficiencies

Abbreviations

GVHD	Graft versus host disease
HSCT	Hematopoietic stem cell transplantation
NHEJ	Non-homologous end joining
PID	Primary immunodeficiency
SCID	Severe combined immunodeficiency

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Introduction

Severe combined immunodeficiency (SCID) is an inherited primary immunodeficiency, which is characterized by the absence or dysfunction of T lymphocytes affecting both cellular and humoral adaptive immunity [46]. It is one of the most severe forms of primary immunodeficiency (PID), which is life-threatening when recognized too late. Seven percent of PID patients suffer from a T cell deficiency, including SCID [18]. Depending on the genetic defect, B and natural killer (NK) cells may be present or absent. Conventionally, SCID can be classified as T–B+ and T–B– SCID with further subdivision based on the presence or absence of NK cells. However, the presentation is not always classic, and the presence or absence of NK cells may be misleading. Therefore, a phenotype describing NK cells no longer forms a part of the classification system of the International Union of Immunological Societies [47]. It has become clear that clinical presentation has wide phenotype variability with considerable immunological variation [43]. These aspects can impede the diagnosis of SCID. In this review, we address the immunological and clinical spectrum of SCID and provide some clues and tools for diagnosing SCID.

Clinical presentations of SCID

Classical SCID

A family history of unusual or fatal infective complications or unexplained infant death is important, particularly in consanguineous families; a history of affected male relatives suggests common gamma chain-deficient SCID. Affected infants generally appear well at birth, but within the first few months of life, demonstrate failure to clear infections and present with

persistent respiratory tract or gastrointestinal infections, failure to thrive and, sometimes, apparent food intolerance (Table 1) [20]. Persistent respiratory tract infection is common, with failure to clear viruses accompanying persistent bronchiolitic-like signs. Insidiously progressive respiratory disease with radiological evidence of interstitial pneumonitis and hyperinflation suggests *Pneumocystis jiroveci* infection, which may be a co-pathogen with respiratory viruses (Fig. 1) [4]. Persistent viral diarrhoea with failure to thrive is an important sign. Although patients with SCID are often initially well and growing normally, they fall away from the growth centile after a few months when infection occurs because of intestinal villous atrophy, leading to malabsorption, which in severe cases results in malnutrition.

Bacterial infections are less common in part because of the presence of maternal IgG in early infancy. However, prolonged otitis media and invasive bacterial infections, such as staphylococcal or pseudomonas septicaemia and pneumonia, may occur, which may respond poorly to appropriate treatment. However, patients with associated agranulocytosis, such as those with reticular dysgenesis due to adenylate kinase 2 (AK2) deficiency, generally present in the first few days of life with omphalitis or invasive bacterial sepsis [5].

Severe invasive fungal infection is rare, but often fatal. Extensive persistent superficial candidiasis is more common. Disseminated BCGosis occasionally may be the presenting feature in immunised infants. Skin lesions demonstrate acid fast bacilli on histological analysis. A mild reticular skin rash, which may be thickened and lichenoid, with or without slightly deranged liver function tests may be seen in maternofetal graft versus host disease. As SCID infants lack functional T cells, they cannot reject foreign lymphocytes acquired from the mother in utero, and so the skin is infiltrated by abnormal maternal T lymphocyte clones [41]. A similar clinical picture may occur in patients who have received an unirradiated blood transfusion, due to viable donor lymphocytes in the red cell donation, although in these cases, the

rash is more severe and lymphadenopathy and hepatosplenomegaly may be present.

Examination usually reveals a wasted child who has dropped through the weight centiles—head circumference is usually preserved. There may be abdominal distension and muscle wasting due to malabsorption and malnutrition. Respiratory signs may include tachypnoea, nasal flaring, subcostal and intercostal recession, with widespread crepitations and rales, and cyanosis. There may be evidence of oral or perineal candidiasis and other superficial infections. There is no clinically detectable lymphoid tissue, although detecting this in young infants is not easy because lymph nodes and tonsils in normal infants are often very small. There may be hepatomegaly, with or without splenomegaly, particularly when disseminated Bacille Calmette–Guerin (BCG) infection is present. Rare presentations include Hodgkin-like polymorphous lymphoproliferative disorder, with rapidly growing extranodal tumours [57]. Very rarely, erythrophagocytosis has been described, in association with maternal T lymphocyte engraftment.

Omenn syndrome

Omenn syndrome is characterised by a generalised thickened erythematous rash, often with scaling and erythematous exfoliating, protein-losing erythroderma, developing a “leathery” consistency (Fig. 2). Hair, including eyebrows and eyelashes, is usually lost as the rash evolves. The rash may be present at birth or evolve over the first few weeks of life. There is an associated lymphadenopathy, particularly of the axillary and inguinal nodes. Hepatosplenomegaly is a frequent finding. There are raised serum IgE levels with a marked eosinophilia and combined immunodeficiency [71]. Children usually suffer from diarrhoea, failure to thrive and persistent infection as seen in other forms of SCID—staphylococcal or pseudomonas skin infection are particularly common. Affected infants are often miserable because of the

Table 1 Presenting features of classical and atypical severe combined immunodeficiency and Omenn syndrome

Classical SCID	Omenn syndrome	Atypical SCID
Present in infancy	Present in infancy	Present >12 months of age
Persistent viral respiratory +/- gastrointestinal infection	Erythroderma	Recurrent, severe, prolonged viral infection
<i>Pneumocystis jiroveci</i> pneumonitis	Alopecia	bronchiectasis
Disseminated BCG infection	Hepatosplenomegaly	Autoimmune cytopenias
Failure to thrive	Massive lymphadenopathy	Failure to thrive
Superficial candidiasis	Inflammatory pneumonitis/enteritis	Granulomatous cutaneous lesions
Maternofetal graft versus host disease	Raised IgE	EBV-associated lymphoproliferation
Absent lymphoid tissue	Eosinophilia	Partial or restricted antigen-specific antibody responses
Absent immunoglobulins	Lymphocytosis	Lymphopenia
Absent T lymphocytes		



Fig. 1 Chest radiograph from a 5-month-old infant with severe combined immunodeficiency showing bilateral patchy shadowing secondary to interstitial pneumonitis due to infection with respiratory syncytial virus and *Pneumocystis jiroveci*. There is hyperinflation of the lungs, and the midline pleural borders of the upper lobes are visible because the thymic shadow is absent (courtesy of The Paediatric Immunology Unit, Great North Children's Hospital, Newcastle upon Tyne)

high levels of circulating inflammatory cytokines. Pneumonitis and enteritis may be predominantly inflammatory rather than infective. The clinical picture may resemble SCID with maternofetal engraftment; molecular genetic studies to identify the origin of the dermal infiltrative T lymphocytes can differentiate the two disorders [2]. Originally described in patients with mutations in recombination activation genes (RAG) 1 and 2, mutations in a number of different genes



Fig. 2 A newborn infant with Omenn's syndrome due to a mutation in the RAG 1 gene. Note the confluent erythematous exfoliating, thickened rash with a "leathery" consistency and loss of hair and eyebrows (courtesy of The Paediatric Immunology Unit, Great North Children's Hospital, Newcastle upon Tyne)

have subsequently been described, including artemis, IL7Ra, RMRP, 22q11 deletion, CHD7, DNA ligase IV (LIG4), adenosine deaminase (ADA) and interleukin 2 receptor, gamma (IL2RG) [71].

Atypical SCID

Patients may present with atypical forms of SCID or Omenn syndrome. Previously described as profound combined immunodeficiency, these patients usually survive beyond 12 months of age. Increasingly, hypomorphic mutations in genes normally associated with classical SCID are identified, thus retaining some protein function. Alternative mechanisms of demonstrating partial immunity include spontaneous gene reversion in early lymphoid progenitors [52, 59, 60, 74]. Such patients present with severe, prolonged infection, which may slowly resolve. Partial antibody responses can be demonstrated to restricted antigens. Other presentations include autoimmune manifestations, particularly with autoimmune cytopenias, and EBV-driven lymphoproliferative tumours. Rarely, cutaneous granulomatous lesions have been described [16, 19, 30, 32, 55]. It is important to consider atypical SCID presentations in children presenting beyond the first year of life so that appropriate antimicrobial treatment can be commenced and the patient considered for curative therapy (vide infra).

Other forms of SCID

In addition to SCID caused by developmental defects, SCID can also be caused by mutations affecting lymphocyte survival, as seen in patients with reticular dysgenesis due to mutations in AK2 [48] and in the enzyme deficiencies ADA and purine nucleoside phosphorylase (PNP), involved in nucleotide metabolism and salvage [3, 12]. As a result of the deficiency, toxic metabolites are formed, to which lymphocytes are exquisitely sensitive. Consequently, ADA and PNP deficiencies usually lead to profound lymphopenia [54]. Finally, several deficiencies have been described that can give rise to a clinical phenotype of SCID, but only affect a subset of T cells, e.g. MHC class II deficiency, ZAP 70 kinase deficiency [47] ([62] #1755). Additionally, defects in CD154 (CD40 ligand) and CD40 may present in infancy with *P. jiroveci* pneumonia. These types of SCID will not be further discussed in this review.

Stepwise diagnostics for SCID

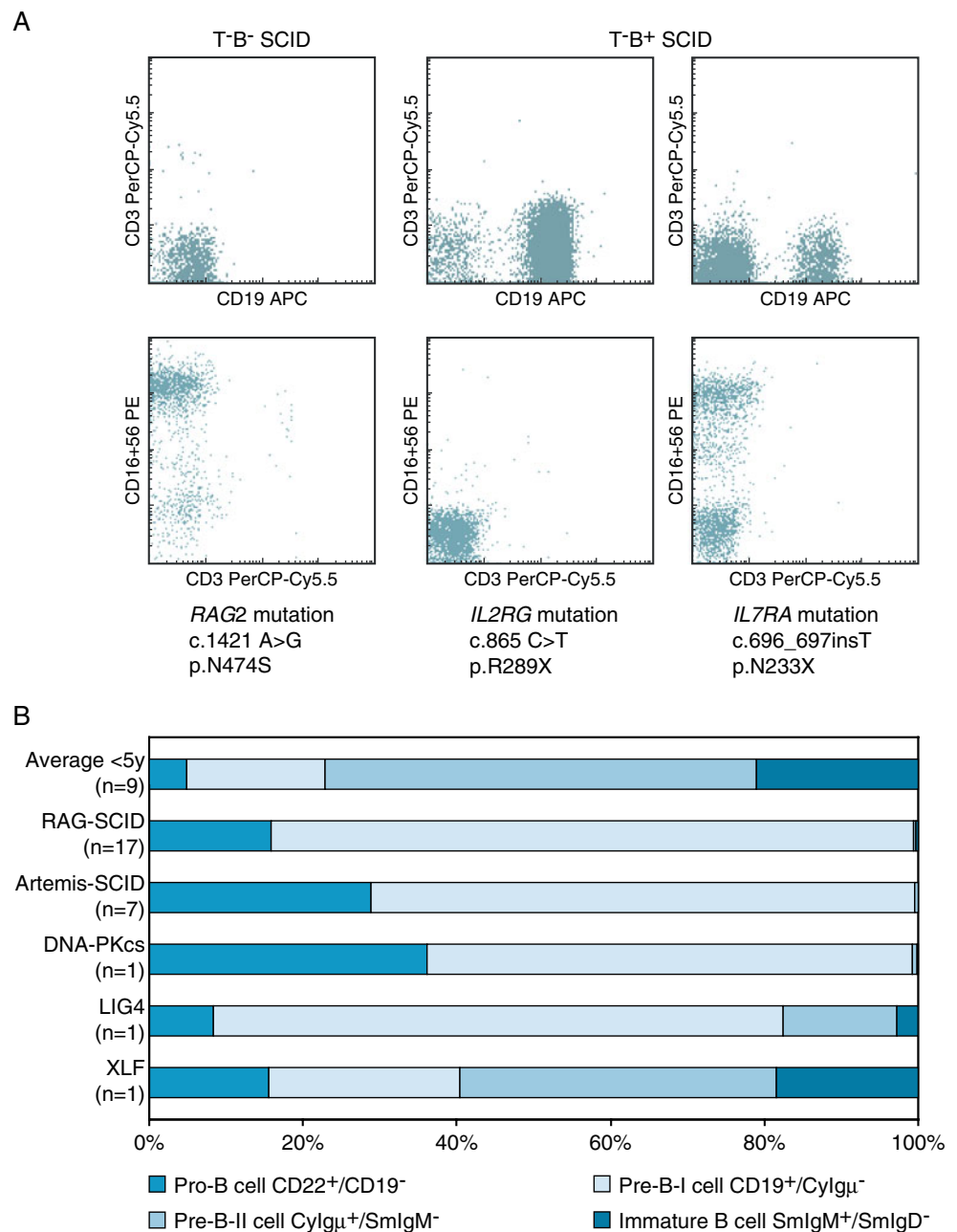
Flow cytometric immunophenotyping of peripheral blood

The first step in the diagnostic process in a patient presenting with features consistent with SCID is ruling

out HIV infection [17]. When HIV has been excluded, a blood smear differential may demonstrate lymphocytopenia, which is suggestive of SCID [28]. It should be noted, however, that a normal lymphocyte count on the differential white cell count does not exclude SCID because the absolute number of lymphocytes may be normal, but lymphocyte subsets may be severely reduced or absent. Therefore, flow cytometric immunophenotyping of lymphocyte subsets in peripheral blood is an important screening assay. In classical SCID, the various types can easily be discriminated (Fig. 3a) with a straightforward analysis of B, T and NK cells. For correct interpretation, reference values of age-matched controls should be used [13].

Fig. 3 Flow cytometric analysis of peripheral blood and bone marrow of SCID patients.

a Flow cytometric analysis of lymphocyte subsets in peripheral blood of SCID patients can be used for definition of the type of SCID and guides molecular diagnostics. **b** For B⁻ SCID patients, flow cytometric analysis of the bone marrow precursor B cell compartment delineates the precursor B cell differentiation block, which can be helpful in candidate gene selection



Interpretation of results is more complicated in Omenn syndrome or atypical SCID. These patients present with high numbers of oligoclonal T cells [14], the presence of which may be misleading, and so detailed analysis of T cells in patients clinically suspected for typical or atypical SCID is of utmost importance [71].

Gene defects and disease mechanisms in T-B⁺ SCID

T-B⁺ SCID is caused by mutations in cytokine-mediated signalling. The majority of patients have X-linked SCID caused by mutations in the *IL2RG* gene encoding the common γ chain (γ c). The γ c chain is shared by the IL2,

IL4, IL7, IL9, IL15 and IL21 cytokine receptors [31]. Cytokines mediate oligomerization of the γ c chain with the appropriate cytokine receptor chain, which leads to Janus kinase 1 (JAK1) and Janus kinase 3 (JAK3) activation and phosphorylation of critical tyrosine residues in the receptor chains (Fig. 4) [23, 36]. JAK1 and JAK3 phosphorylate each other and phosphorylate STAT5. Upon phosphorylation, STAT5 dimerizes and translocates to the nucleus where it activates multiple genes [37]. Autosomal recessive forms of T-B+ SCID are less frequent and have been shown to be caused by mutations in the *JAK3* or *IL7RA* genes [38, 50]. Mutations in the *IL7RA* gene abrogate T cell development, but do not interfere with NK cell development.

A separate category of T-B+ SCID patients have mutations in one of the four CD3 genes (*CD3G*, *CD3D*, *CD3E* and *CD3Z*) [21, 52]. The CD3 complex is composed of one CD3 γ , CD3 δ and CD3 ϵ chain and two CD3 ζ chains (Fig. 4b). The absence of one of the CD3 chains inhibits formation of the CD3 complex and consequently expression and signalling via (pre)T cell receptors.

Gene defects and disease mechanisms in T-B- SCID

Patients with T-B- SCID generally have a defect in V(D)J recombination [15]. This process takes place in developing B and T cells and is responsible for the rearrangement of the immunoglobulin and T cell receptor genes (Fig. 5). Different steps of V(D)J recombination can be discriminated. In the first step, proteins encoded by the recombination

activating genes (RAG1 and RAG2) form a heterodimer and make a single-stranded nick between a coding element (Variable (V), Diversity (D), or Joining (J) gene segment and the recombination signal sequence (RSS) [69], resulting in the formation of a hairpin-sealed coding end at the side of the coding element and a blunt signal end at the RSS side (Fig. 4). In the second phase, which is referred to as the processing phase, the DNA–protein kinases (PK) complex, composed of Ku70, Ku80 and DNA–PKcs, binds to the hairpin-sealed coding end and phosphorylates Artemis, which subsequently opens the hairpin. Further processing of the DNA ends takes place, i.e. nucleotide deletions, random non-templated insertions of nucleotides by TdT before the ends, are ligated by LIG4/XRCC4 in conjunction with Cernunnos/XLF. The first phase of V(D)J recombination is lymphoid specific, while the processing and ligation phase are carried out by the ubiquitously expressed components of the non-homologous end-joining pathway (NHEJ) of DNA double-strand breaks [70]. If a mutation occurs in one of the NHEJ factors, the patients not only have defective V(D)J recombination but also a general DNA DSB repair defect, which results in increased sensitivity to ionizing radiation. Mutations have been identified in RAG1, RAG2, Artemis and DNA–PKcs giving rise to typical and atypical SCID [40, 56, 64]. Mutations in LIG4 can give either rise to T-B- SCID or the LIG4 syndrome, which is characterized by microcephaly and growth retardation [26, 65]. Mutations in XLF (Cernunnos) also give rise to these manifestations [9].

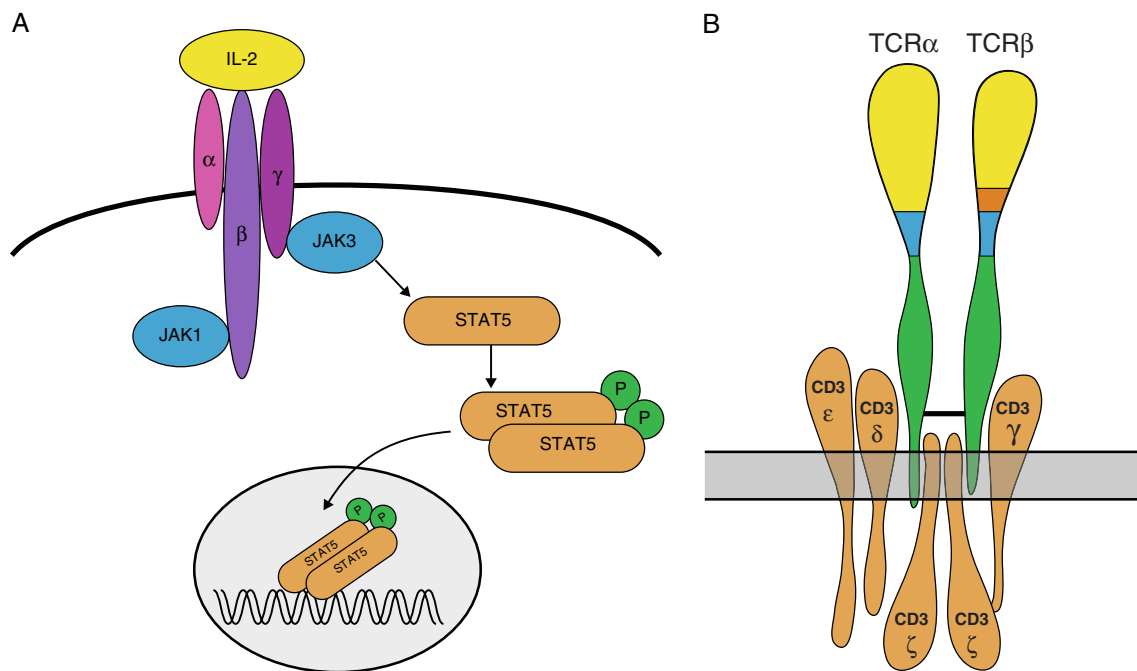
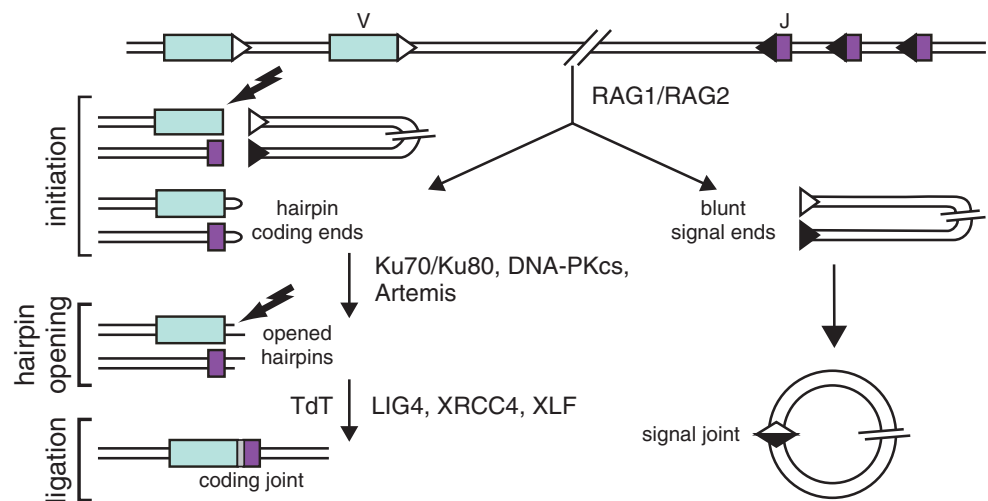


Fig. 4 **a** γ c/JAK3 signalling pathway (adapted from Gaspar et al. [23]). **b** T cell receptor with CD3 signalling complex

Fig. 5 A schematic representation of the three steps of the V(D)J recombination process and the involved molecules



Detailed analysis of T cells potentially present in patients suspected for having typical or atypical SCID

There are several reasons for the presence of T cells in SCID patients. First, T cells can be engrafted transplacentally from the mother. In 50% of B⁻ SCID and in 80% of B⁺ SCID, maternal T cells can be detected [41]. These T cells can be present at low frequencies, but can also exceed the upper limit of reference. The immunophenotype of these T cells can be diverse. Most have a mature (CD45RO⁺) phenotype, but this cannot be regarded as a golden rule. They may have a disturbed CD4/CD8 ratio or aberrant CD3 expression (van der Burg, unpublished observation). To prove that T cells are maternal, they can be analysed by human leukocyte antigen (HLA) typing or the origin determined by XY FISH in case of boys, or short tandem repeat analysis can be performed [41, 68].

Patients with Omenn syndrome have hypomorphic mutations resulting in the presence of T cells that expanded in the periphery [72]. These T cells are autologous and generally oligoclonal. The clonality of the T cells can be determined by flow cytometry, e.g. by using a V β analysis kit [63]. Molecular clonality assays by heteroduplex analysis or spectratyping are alternative methods which reliably determine whether the T cells present are oligoclonal, polyclonal or oligoclonal in a polyclonal background [34, 35]. The latter would be predominantly due to infections.

A much rarer explanation for the presence of autologous T cells in SCID patients is the occurrence of somatic reversion mutations [73]. These reversion mutations have been described in a few X-linked SCID cases, in a single RAG deficiency and in patients with a CD3Z deficiency [52, 59, 60, 74]. In these patients, somatic reversion mutation occurred, probably in early T cells, and corrected the genetic defect. If somatic reversion occurs, the T cells have a selective growth advantage and the potential to

develop normal function. The mechanism by which this somatic reversion arises is as yet unknown.

T cells in patients with suspected typical or atypical SCID should always be typed in detail. Analysis of TCR expression can be particularly helpful. In some patients with a partial V(D)J recombination defect, a high frequency of TCR $\gamma\delta$ T cells is detected [19].

Analysis of protein expression of candidate genes

In T-B⁺ SCID, analysis of CD132 expression on lymphocytes and measurement of STAT5 phosphorylation upon IL2 stimulations and informative screening tests are advanced [25, 75]. If CD132 expression is absent, this is indicative of X-linked SCID, and in virtually all cases without CD132 expression, a mutation is found in the *IL2RG* gene. The same holds true for the analysis of IL7RA expression. This is somewhat more complicated because IL7R α is mainly expressed on T cells, which are typically absent in these patients. Aberrant results in STAT5 phosphorylation [75] point toward defects downstream of the γc chain, and if aberrant, sequence analysis of *JAK3* is a logical choice for molecular analysis.

Flow cytometric analysis of precursor B cell compartment in bone marrow

In the case of T-B⁻ SCID, analysis of the precursor B cell compartment in bone marrow can give information whether or not there is an underlying defect in the V(D)J recombination process. A typical SCID patient with a V(D)J recombination defect due to mutations in *RAG1*, *RAG2*, *Artemis* or *DNA-PKcs* has a full block in precursor B cell differentiation before the cytoplasmic Ig μ -positive pre-B-II cell stage (Fig. 3b) [44, 45, 64]. In a hypomorphic mutation, the differentiation block can be incomplete,

implying that low frequencies of pre-B-II can be present. Alternatively, an incomplete precursor B cell differentiation block can be due to the type of gene defect. *LIG4* and *XLF* deficiencies give rise to the presence of pre-B-II and immature B cells and even mature B cells in XLF deficiency [30, 65].

Sequence analysis of candidate genes

Based on the clinical presentation and the immunophenotype, a candidate gene is selected, and the gene is sequenced to identify a mutation [66]. The frequency of mutations in T–B+ and T–B– SCID are depicted in Fig. 6. For some genes, e.g. *RAG1*, *RAG2* and *Artemis*, in vitro function tests can be used to determine the level of (reduced) enzymatic activity of the mutation [49, 67]. In T–B– SCID patients without a defect in the *RAG1* or *RAG2* gene, it is important to determine whether the patient is sensitive for ionizing radiation and consequently has a defect in the NHEJ pathway using a clonogenic survival assay on fibroblasts cultured from a skin biopsy [40, 56, 64]. Analysis of the coding joints of immunoglobulin gene rearrangements in bone marrow precursor B cells, in vivo V(D)J recombination studies, is a valuable tool in the diagnostic process of radiosensitive T–B– SCID patients because it can give a clue which step in the V(D)J recombination assay is affected [64, 65, 67]. The latter tests are not routinely done in a diagnostic setting, but can be of importance in more complicated cases.

Supportive management

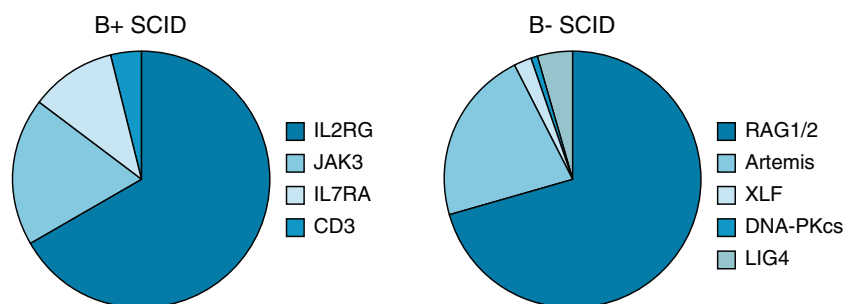
Infants suspected of having a severe immunodeficiency disorder should be placed in protected isolation, limiting the numbers of persons involved with care; specifically, individuals with respiratory or gastrointestinal symptoms of infection should avoid contact. If the mother is cytomegalovirus (CMV)-negative, breastfeeding should be encouraged—otherwise, it should be discontinued to prevent neonatal CMV infection from being transmitted through breast milk. Strict handwashing procedures are

critical to prevent infection. Blood products should be CMV-negative and irradiated to avoid the risk of transfusion GVHD [61]. Appropriate imaging of chest, abdominal organs and brain should be considered, guided by the clinical features. For those diagnosed later, particular attention needs to be paid to nutritional status and the management of dietary intolerances secondary to infectious or inflammatory gastrointestinal problems. Advice from paediatric gastroenterologists should be sought early, to minimise the impact of the disease on the gut and to institute modular formula milk feeds or parenteral nutrition as appropriate. Respiratory paediatricians should be consulted early to maximise supportive therapy and prevent further lung damage. Imaging to detect focal infiltration is important and may guide subsequent biopsy. Infection should be sought aggressively, and biopsy material may be required to demonstrate infection. Culture of appropriate tissue specimens, including bronchoalveolar lavage fluid, and PCR may be needed to identify infecting pathogens—serology is generally unhelpful. Infections should be vigorously treated—broad spectrum multi-agent antimicrobial therapy may be required. Co-trimoxazole as prophylaxis against *P. jiroveci* should be given. Antifungal prophylaxis should also be used, and antiviral prophylaxis with aciclovir is used in patients with a previous herpes simplex infection. Supporting the emotional needs of the family is also very important.

Curative therapy

Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for patients with SCID. If HSCT with conditioning chemotherapy is embarked upon, isolation in facilities with positive-pressure-filtered air supply is necessary, mainly to reduce the risk of aspergillosis and droplet-borne viral infections. European data regarding outcome of HSCT for SCID Patient data are collected in the Stem Cell Transplantation for Immunodeficiencies in Europe registry, giving data on almost 700 patients, and have recently been published [24]. A broad repertoire of stem cell sources are used, including stem cells from marrow, mobilised peripheral blood stem cells or those harvested from umbilical cord blood. Best results are

Fig. 6 Distribution of B+ SCID ($n=159$ patients) and B– SCID ($n=136$ patients) in Europe according to the ESID patient registry 2010 (<http://www.esid.org/statistics.php?sub=2>). ESID, European Society for Immunodeficiencies



obtained using HLA-matched sibling donors, with survival of around 90% in the best circumstances. The molecular defect has a bearing on outcome, with B⁻ SCID patients having an overall worse outcome than B⁺ forms of SCID [42]. The outcome is better in the absence of infection, arguing for the early identification of patients through neonatal screening programmes [8]. New chemotherapy conditioning regimens are increasingly utilised, with improved outcome. A successful procedure is generally curative, with patients leading normal lives off medication, but few long-term studies have demonstrated long-term sequelae for some patients [39, 58]. Particular problems relate to ongoing thymopoiesis, with failure leading to T lymphocyte senescence in the long term [7, 10]. Long-term immunoglobulin therapy is necessary for some B lymphocyte dysfunction or failure of donor engraftment. Chemotherapy may lead to infertility. Hypothyroidism, secondary to chemotherapy, affects about 10% of patients. Some sequelae relate to the specific genetic defect, for instance, human papillomavirus-associated warts in IL2RG/JAK3 SCID [33] and neurodevelopmental disorders in ADA deficiency [53].

For ADA deficiency, enzyme replacement therapy with polyethylene-glycosylated ADA is an alternative treatment [29]. Treatment is required lifelong, is expensive and results in only partial immune reconstitution. Sequelae include the development of autoimmunity, but in the short term, it may allow some immune reconstitution and clearance of infection before proceeding to definitive therapy.

Gene therapy has been used for ADA- and IL2RG-deficient SCID [1, 6, 11]. Advantages include removal of the necessity for chemotherapy conditioning and available treatment despite lack of a matched donor. Earlier ADA trials were only partially successful, and the majority of patients required ongoing PEG-ADA therapy. More recently, the procedure has been more successful, although low doses of chemotherapy give the best results [22]. Some patients have an ongoing requirement for immunoglobulin replacement. XL-SCID gene therapy does not require chemotherapy and has led to complete immune reconstitution, but insertion of the retroviral vector close to oncogenes has led to the development of lymphoproliferation in some patients [27]. Development of new, probably safer, vectors and directed insertion of the mutated gene away from oncogenes promise improved outcome, and clinical trials are ongoing [51]. Treatment of other forms of SCID is at a pre-clinical phase.

Concluding remarks

SCID is one of the most severe forms of primary immunodeficiency and is a paediatric emergency, which is life-threatening if recognized too late. Therefore, early diagnosis and good clinical management are crucial. The

clinical and immunological spectrum of SCID is broader than initially described, so (atypical) SCID should be considered as a potential diagnosis more often. Improved supportive care, detection of infection by molecular means and less toxic chemotherapy conditioning regimens have significantly improved survival, and patients should be referred urgently to centres specializing in the diagnosis and treatment of such patients to optimize outcome.

Learning points

- SCID is one of the most severe forms of PID and is a life-threatening paediatric emergency.
- The molecular basis of most forms of SCID is now recognized.
- Early careful liaison with the immunology laboratory will enable the most appropriate investigations to be performed.
- Atypical, later presentation of patients with partial gene function is increasingly described.
- Atypical SCID should be considered in patients presenting with unusual, severe or recurrent infections.

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