Review

Severe Combined Immunodeficiency (SCID): from molecular basis to clinical management

Ivonne Sponzilli[1, 2], *Luigi D. Notarangelo*[1, 3]

[1] Division of Immunology, Children's Hospital, Harvard Medical School, Boston, MA, USA; [2] Department of Pediatrics, University of Parma, Italy; 3 The Manton Center for Orphan Disease Research, Children's Hospital, Boston, MA, USA

Abstract. Primary immune deficiency diseases (PID) comprise a genetically heterogeneous group of disorders that affect distinct components of the innate and adaptive immune system, such as neutrophils, macrophages, dendritic cells, complement proteins, natural killer cells, as well as T and B lymphocytes. Severe combined immunodeficiency (SCID) is a group of disorders characterized by increased susceptibility to severe infections and early death. The diagnosis of SCID is supported by the demonstration of low absolute lymphocyte count and T cell lymphopenia (variably associated with numerical defects of B and NK cells). In the last two decades, advances in the characterization of the molecular pathophysiology of SCID, have permitted the development of novel diagnostic assays based on analysis of the expression of the disease-associated proteins and mutation analysis. More recently, pilot newborn screening programs for the identification of infants with SCID have been initiated in the United States. Prompt and aggressive treatment of infections, antimicrobial prophylaxis (in particular against *Pneumocystis jiroveci*) and regular administration of immunoglobulins are essential to reduce the risk of early death. However, survival ultimately depends on reconstitution of immune function, that is usually achieved by means of hematopoietic cell transplantation (HCT). Gene therapy and enzyme replacement therapy have also been used successfully is selected forms of SCID. Here we review the molecular and cellular pathophysiology and the mainstay of treatment of SCID. (www.actabiomedica.it)

Key words: Severe Combined Immunodeficiency, newborn screening, hematopoietic cell transplantation, gene therapy.

Abbreviations

ADA: Adenosine deaminase
ALC: Absolute lymphocyte count
BCR: B-cell receptor
CMV: Cytomegalovirus
dATP: deoxyadenosine triphosphate
GvHD: Graft-versus-host disease
HCT: Hematopoietic cell transplantation
HIV: Human immunodeficiency virus
HLH: Hemophagocytic lymphohistiocytosis
ICOS: Inducible T-cell costimulator
IPEX: Immune dysregulation–polyendocrinopathy–enteropathy–X-linked
JAK: Janus kinase
LMO: LIM domain only 2 (rhombotin-like 1)
NHEJ: Nonhomologous end-joining
NK: Natural killer
PID: Primary immunodeficiency
RAG: Recombinase-activating gene
RSV: Respiratory syncityal virus
SCID: Severe combined immunodeficiency
SCIDX1: X-linked severe combined immunodeficiency
TAP: Transporter of antigenic peptide
TCR: T-cell receptor
TLR: Toll-like receptor
TREC: T-cell receptor excision circle

Introduction

Primary immunodeficiencies (PIDs) comprise more than 150 different disorders that affect the development, function, or both of the immune system (1). Most forms of PID are monogenic disorders that follow a simple mendelian inheritance; however, some PIDs are more complex. In such cases, genetic abnormalities confer susceptibility, but manifestation of the clinical phenotype often depends also on environmental factors or on the effect of other disease modifying genes. Furthermore, the heterogeneity of mutations even within the same SCID-causing genes may result in significant variability of the clinical and immunological phenotype (2).

Among PIDs, SCID comprises a heterogeneous group of disorders with impaired development and/or function of T lymphocytes, associated with defective antibody responses that may result from intrinsic defects in B lymphocytes or from lack of helper T cell activity (3).

Typically, SCID is characterized by severe T cell lymphopenia. In some cases, there is a residual number (and/or function) of circulating T lymphocytes. These cases are also referred to as "atypical SCID" (3).

Until now, mutations in fifteen different genes have been shown to account for SCID.

In spite of this extensive genetic heterogeneity, patients with SCID present with a rather uniform clinical phenotype, characterized by early-onset infections (often due to opportunistic pathogens) and chronic diarrhea with failure to thrive. Skin rash, autoimmune manifestations, microcephaly, and sensorineural deafness are other features that may associate with specific gene defects.

Severe infections and dystrophy are the major causes of death, the usually occurs within the first two years of life, unless immune reconstitution is achieved, mainly by means of hematopoietic cell transplantation (HCT) (3-5).

Pathogenesis and classification of SCID

The pathogenesis of SCID reflects distinct mechanisms that affect various steps in T-cell development (Fig. 1).

a) Increased death of early lymphocyte progenitor cells

Adenosine deaminase deficiency (ADA) results in an accumulation of adenosine, deoxyadenosine, and their phosphorylated derivatives, among which deoxyadenosine triphosphate (dATP) is toxic and causes cell death by apoptosis. Lymphocyte precursors are exquisitely sensitive to the effect of dATP (6). Accordingly, ADA deficiency is characterized by virtual absence of circulating T, B and natural killer (NK) lymphocytes.

b) Defective signaling through the common γ-chain-dependent cytokine receptors

Several cytokine receptors [interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15, and IL-21R] share the common γ-chain (γc) subunit (7). I The most frequent form of SCID, X-linked SCID (SCID-X1), is caused by mutations in the γc-encoding gene (8). SCID-X1 is characterized by the absence of both T and NK lymphocytes. This phenotype is accounted for by impaired signaling through IL-7R and IL-15R. In particular, interaction of IL-7 with its receptor (IL-7Rα/γc heterodimer) induces survival, proliferation, and differentiation of thymocyte progenitors. Consistent with this, mutations of IL7R (encoding for IL-7Rα chain) also results in SCID with absence of peripheral T cells (9). NK cell deficiency in SCID-X1 is thought to result from the defective IL-15 interaction with IL-15R that is composed of a heterotrimer including IL2Rα/IL-2Rβ/γc chains (7). B cell development is unaffected in patients with SCID-X1, indicating that in humans none of the cytokines that signal through γc-containing receptors is required for B cell development. However, terminal differentiation of B lymphocytes to antibody-secreting plasmablasts is impaired, reflecting poor signaling through IL-21R.

All of the γc-containing cytokine receptors signal through Janus Associated Kinases (JAKs); in particular, γc is physically and functionally coupled to JAK3.

Consistent with this, *JAK3* gene mutations result in a SCID phenotype indistinguishable from SCID-X1 (10, 11); however, JAK3 deficiency is inherited as autosomal recessive trait.

c) Defective V(D)J recombination

A major step in T- and B-lymphocyte differentiation is the somatic rearrangement of the genes en-

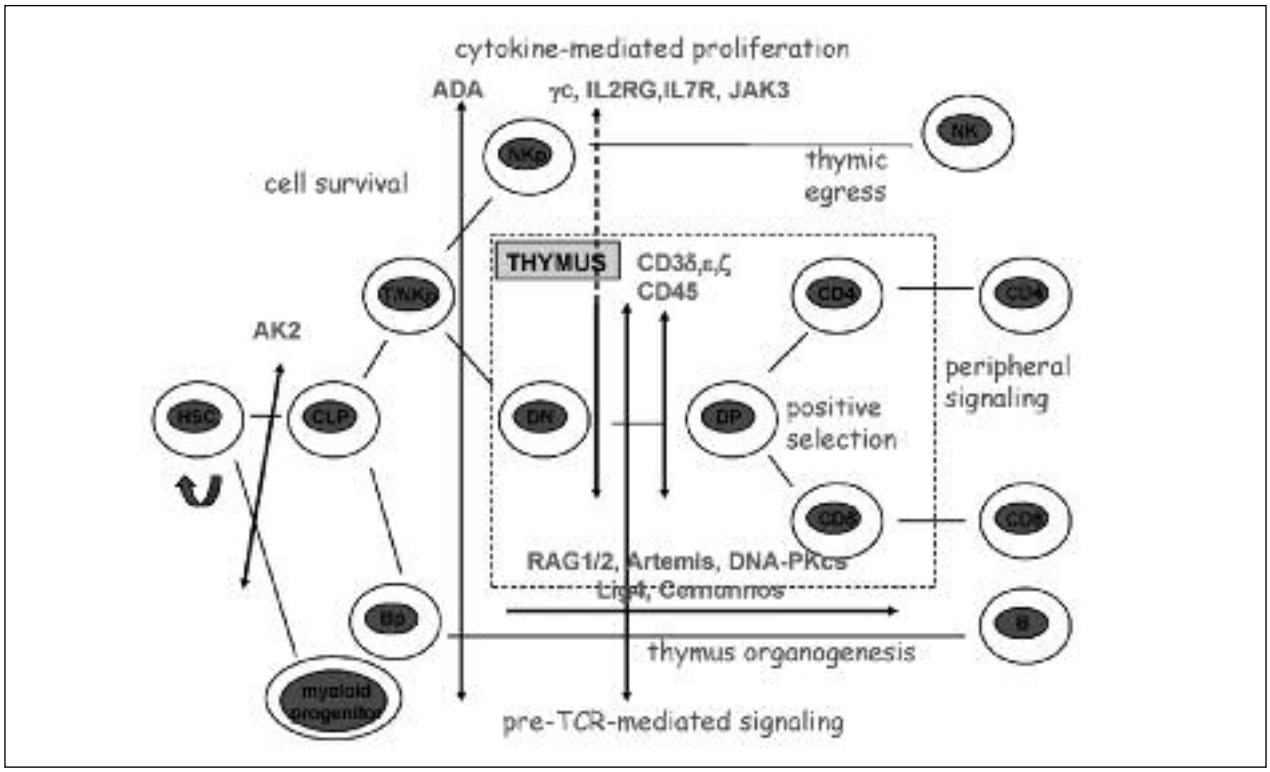

Figure 1. Blocks in T-and B-cell development associated with SCID

coding for the T-cell antigen receptor (TCR) and the B-cell antigen receptor (BCR), that ultimately results in generation of a diversified repertoire of T and B lymphocytes. This process, known as V(D)J recombination, is initiated by the recombination-activating gene-1 (RAG-1) and RAG-2 proteins, which cleave DNA at specific sequences surrounding the V, D, and J elements of TCR and BCR genes. Mutations of either *RAG1* or *RAG2* genes result in a faulty development of both T and B cells, whereas NK cell differentiation is spared (12). Hypomorphic mutations in either gene may enable a limited generation of T cell clones that undergo clonal expansion in the periphery and tend to infiltrate tissues causing severe damage. This phenotype is also known as Omenn syndrome (13). In most cases, patients with Omenn syndrome are severely B cell lymphopenic, however a proportion of them develop autoantibodies, reflecting residual and aberrant B cell development.

A series of proteins involved in the non-homologous end-joining (NHEJ) pathway of DNA repair mediate the last steps of V(D)J recombination. Mutations of some of these proteins (Artemis, DNA-PKcs, DNA ligase IV, Cernunnos/XLF) are also responsible of SCID with lack of T and B cells, but preserved number of NK lymphocytes (T- B- NK+ SCID) (14,15). However, Cernunnos/XLF deficiency is usually characterized by a leaky phenotype (with residual presence of T and B lymphocytes), and heterogeneity of the immunological phenotype has been observed in patients with DNA ligase IV deficiency. Another distinctive feature of SCID associated with defects in NHEJ is the frequent occurrence of microcephaly and other extra-immune manifestations that reflect generalized cellular deficiency of DNA repair.

Defective signaling through pre-TCR/TCR

Rare cases of SCID consisting of pure T-cell deficiencies have been attributed to defects in key proteins involved in pre-TCR/TCR signaling. Following rearrangement of the TCR β locus, a TCR β chain is

expressed on the cell surface of thymocytes in combination with a pre-Tα molecule and with the subunits of the CD3 complex, forming the pre-TCR. Deficiency in the genes encoding for the CD3δ, CD3ε, or CD3ζ chains result in selective block of T cell development. Remarkably, complete deficiency of CD3γ, another subunit of the pre-TCR/TCR signaling complex, leads to a milder immunodeficiency (18), showing that the CD3 subunits exert different functions.

Signaling through the CD45 phosphatase is also important in lymphocyte development. CD45 deficiency has been reported in few cases of SCID in humans (16, 17).

Defects of the Zeta-Associated Protein of 70 kDa (ZAP-70) affect later stages in T cell development, with complete block in generation of CD8+ lymphocytes; CD4+ T cells are produced and released to the periphery, but are functionally impaired. Finally, genetic defects that affect expression of HLA class II molecules on the surface of thymic epithelial cells prevent positive selection of CD4+ thymocytes; these patients show a selective deficiency of CD4+ lymphocytes, usually associated with hypogammaglobulinemia and antibody deficiency. Since neither ZAP-70 or HLA class II deficiency result in complete arrest in T cell development, they are not classified under SCID, but are considered other forms of combined immunodeficiency (3).

Clinical presentation and diagnostic approach to SCID

Severe and/or recurrent infections, and chronic diarrhea with failure to thrive are typical manifestations of SCID. Respiratory infections due to opportunistic pathogens (*P. jiroveci*) and viruses (RSV, CMV, parainfluenzae virus type 3) are particularly common and may lead to rapid deterioration of oxygen saturation, requiring ventilation. Viral, bacterial and protozoan (Giardia, Cryptosporidium) infections are responsible for diarrhea. Of note, SCID infants may develop severe diarrhea and failure to thrive also following immunization with rotavirus vaccine. It is important to emphasize that a single episode of severe infection early in life, especially when associated with growth failure, is enough to consider SCID as a possible underlying cause. Persistent or recurrent oral and perineal candida infection is also a common finding.

The paucity of lymphoid tissue in patients with SCID is reflected by the absence of tonsils and lack of the thymic shadow at chest X-ray. Lymph nodes are unusually small; however, lymphadenopathy may be observed in patients with Omenn syndrome or with maternal T cell engraftment.

In addition to infections, infants with SCID may present also with various degrees of skin manifestations, ranging from generalized erythroderma to eczema. Alopecia or sparse hair may also be present.

Microcephaly is typically present in SCID due to defects of DNA repair (DNA ligase IV and Artemis gene defects). On the other hand, facial dysmorphisms (anteroverted nostrils, short philtrum, low-set ears, micrognatia, arched palate) characterize DiGeorge syndrome. In this disease, there is a variable degree of T cell lymphopenia because of a developmental defect that involves the thymus. However, only 1% of infants with DiGeorge syndrome have such a profound T cell lymphopenia to cause severe susceptibility to infections and a SCID-like phenotype.

A high level of suspicion by the primary care provider is key to a timely diagnosis of SCID. This is extremely valuable to maximize the chances of success of HCT and achieve permanent correction of the disease. Infants with SCID who undergo HCT in the first 3.5 months of life have a 97% survival rate, which is significantly higher than in those undergoing transplantation later in life (19). Not infrequently, it is the allergist, pulmonologist, gastroenterologist and/or the intensive care physician who first comes in contact with a child with SCID.

Medical history (with particular regard to type, location, age at onset, and severity of infections) and physical examination provide important hints to suspect SCID. Family history is also important in the approach to SCIDs because of the monogenic nature of most forms of these disorders (2). In particular, a history of early deaths in males on the maternal side is suggestive of X-linked SCID, the most common form of SCID in western countries. Parental consanguinity and/or belonging to genetically and/or geographically

restricted populations is often observed in patients with autosomal recessive forms of SCID.

The laboratory diagnosis of SCID is usually simple. T cells normally account for 70% of circulating lymphocytes. Because most infants with (20) they are typically lymphopenic. Correct interpretation of the absolute lymphocyte count (ALC) requires comparison to age-specific reference values (21). The ALC reference interval is 3,400 to 7,600 cells/mm^3 for healthy newborns. However, approximately 30% of SCID infants do not show lymphopenia. This may reflect the presence of genetic defect that spare B and NK cell development, associated with an expansion of these cells. Alternatively, a normal ALC in infants with SCID may reflect maternal T cell engraftment. While it is known that transplacental passage of maternal lymphocytes is common during pregnancy, the fetus is usually immunocompetent enough to reject such cells. In contrast, maternally-derived T cells that have crossed the placenta persist in infants with SCID, and may even expand. The fact that in most cases maternally-derived T lymphocytes are not HLA-identical to the fetus, justifies the occurrence of graft-versus-host disease (GvHD)-like manifestations (skin rash, diarrhea, cytopenias, liver abnormalities) that are often present in SCID infants with maternal T-cell engraftment. However, as many as 30-50% of these infants may not show any obvious signs of GvHD, and just present typical manifestations of SCID. Therefore, infants who present with clinical features of SCID and a normal ALC must be evaluated for the possible presence of maternally-derived T cells, which is virtually pathognomonic of SCID.

Immunological phenotype of circulating lymphocytes is very important to confirm the diagnosis of SCID, and may provide significant hints to identify the specific gene defect. Determination of T, B and NK lymphocytes can be easily achieved by flow cytometry, using monoclonal antibodies to CD3, CD19 and CD16/CD56, respectively. In most cases, $CD3^+$ cells are virtually absent or severely reduced in number. This may not be the case in infants with hypomorphic mutations that allow for some partial T cell development, or in SCID patients with maternal T cell engraftment. In these situations, however, circulating T cells have an aberrant phenotype, and largely co-express CD45R0, a marker of activated T cells, whereas early in life >80% of circulating $CD3^+$ cells express CD45RA, indicative of naïve T cells. Based on the presence or absence of B and NK lymphocytes, patients with SCID may be classified into various groups (B^+NK^-, B^+NK^+, B^-NK^+, and B^-NK^- SCID). Each of these groups may reflect some distinctive genetic defects (Table 1).

In the approach to patients with possible SCID, it is important to rule out other causes of severe T cell lymphopenia, in particular HIV infection by searching for HIV RNA.

Consistent with the severely reduced number of circulating T cells, in vitro lymphocyte proliferative responses to mitogens (phytoemagglutinin) are drastically diminished.

Although infants with SCID are often hypogammaglobulinemic and are unable to produce specific antibodies, determination of serum immunoglobulins has limited value to confirm the suspicion of SCID. In particular, SCID infants may have normal IgG levels early in life, because these are typically maternally-de-

Table 1. Fifteen abnormal genes in patients with SCID

	Lymphocyte phenotype
Cytokine-receptor-mediated signaling	
IL2RG	$T^-B^+NK^-$
JAK3	$T^-B^+NK^-$
IL7RA	$T^-B^+NK^+$
Assembly and expression of antigen receptor genes	
RAG1	$T^-B^-NK^+$
RAG2	$T^-B^-NK^+$
Artemis	$T^-B^-NK^+$
Ligase 4	$T^-B^-NK^+$
DNA-PKcs	$T^-B^-NK^+$
CD3δ	$T^-B^+NK^+$
CD3ε	$T^-B^+NK^+$
CD3ζ	$T^-B^+NK^+$
Cernunnos/XLF	$T^-B^-NK^+$
Other genes	
ADA	$T^-B^-NK^-$
CD45	$T^-B^+NK^+$
AK2	$T^-B^+NK^+$

AK2 Adelylate kinase 2; DNA-PKcs, DNA protein kinase catalytic subunit; IL7RA, IL-7 receptor a chain; NK, natural killer

rived IgG. Furthermore, some forms of SCID with B lymphocytes (T^-B^+ SCID) may allow for residual production of serum IgM. Finally, IgE are often elevated in infants with some forms of SCID due to hypomorphic mutations (eg, Omenn syndrome) or in patients with maternal T cell engraftment.

Antibody responses to immunizations are abrogated. Importantly, use of live vaccines should be strictly avoided in infants with possible SCID, as this might lead to disseminated infection. Once a possible diagnosis of SCID has been considered by the primary care provider or the specialist who has first seen the patient, the crucial next step is referral to an immune deficiency specialist who is expert in the diagnosis, treatment, and management of cellular immunodeficiencies. This individual will have the knowledge to quickly perform definitive and more sophisticated tests to rule in or out the presence of SCID (22).

Neonatal screening programs for SCID

Results of HCT for infants with SCID are particularly good if the transplant is performed in the neonatal period or within the first 3.5 months of life; in such cases, survival exceeds 95% (19, 22, 24).

This notion has prompted the development of newborn screening for SCID. At least 3 different methods of newborn screening for SCID have been proposed, including lymphocyte counts, quantitative polymerase chain reaction for T-cell receptor excision circles (TRECs), pieces of DNA produced only by T cells, and analysis of IL-7 levels in dried blood spots collected at birth.

Children with SCID have persistently low lymphocyte counts, which makes using such counts a potential way to identify newborns with SCID. However, the requirement for additional blood samples, the costs and the relatively limited sensitivity (60-70% at best) and poor specificity of the assay have prevented the use of absolute lymphocyte count at birth as a newborn screening assay for SCID. While sensitivity and specificity would be improved by the addition of flow-cytometric assessment of T lymphocytes, this would require sophisticated instrumentation and a significant increase in costs.

IL-7 is a key cytokine that promotes T cell development. Impairment of generation of T cells in infants with SCID is associated with markedly increased serum levels of IL-7. This has prompted the development of studies aiming to assess the possible use of measurement of IL-7 levels at birth as a screening assay for SCID. A 2-tiered program has been proposed, in which IL-7 levels are measured first on newborn dried blood spots (Guthrie's cards), followed by determination of T-cell receptor excision circles (TRECs), a byproduct of V(D)J recombination that is detectable in newly generated T lymphocytes that leave the thymus and hence represent a robust indicator of T-cell lymphopoiesis. In one study, samples from 13 children with SCID and 183 anonymized dried blood spots were analyzed for IL-7 and TREC levels. For the first tier (IL-7 measurement), the authors calculated 96.1% specificity and 85% sensitivity (confidence interval: 55%–98%); for the second tier (TREC count) they calculated a specificity of 92.3% and a sensitivity that approaches 100% (25).

Finally, assessment of TREC levels by polymerase chain reaction in DNA extracted from Guthrie's cards has been proposed as a newborn screening for SCID, with no need to measure also IL-7 levels. Preliminary studies have shown that no TRECs are detected in peripheral blood from infants with SCID (26). Based on this, a pilot study has been started in Wisconsin and Massachusetts (27). In both cases, infants with SCID have been successfully identified at birth (28, 29).

Additional States (California, New York) are now attempting to detect SCID at birth by measuring TREC levels.

Treatment of SCID

HCT represents a life-saving procedure in SCID, because it induces functional and long-lasting reconstitution of immunity. Since 1968, when HCT was successfully applied for the first time in humans in an infant with SCID-X1 (1), hundreds of patients with SCID have benefited worldwide from this procedure. Because of the inability of SCID patients to reject donor-derived cells (even when they are HLA-mis-

matched to the recipient), pre-transplant myeloablative therapy is not required in patients with SCID to enable donor T cell engraftment. This situation brings about a significant clinical advantage, because it permits to avoid a major toxicity (3).

Results of HCT for SCID are extremely good (>95% survival) in infants who have an HLA-matched family donor (30).

However, this option is available only to 15% of the patients. Development of methods that allow depletion of mature T cells from the bone marrow has permitted since the early 1980s to exploit use of T-cell depleted HCT from a haploidentical donor (typically, the mother or the father) in infants with SCID who do not have an HLA-matched sibling. This approach gives optimal results when the transplant is performed within the first 3.5 months of life (19, 22, 24). However, results are less favorable when T-cell depleted haploidentical HCT is performed later in life.

Improvements in prevention of GvHD and development of more effective preventive and therapeutic regimens against infections have resulted in progressive improvement of outcome of HCT for SCID (30). Nonetheless, significant differences remain in survival rate after HCT for SCID, depending on the degree of HLA matching between the donor and the recipient. This different outcome reflects different kinetics of T-cell reconstitution (23).

HCT from HLA-identical donors is performed without manipulating the graft. In these conditions, mature T cells that are contained in the bone marrow of the donor, undergo expansion in vivo after transplantation into the SCID patient, and provide some protection while donor-derived stem cells home to the thymus, initiate and complete their maturation into newly generated T lymphocytes. Therefore, T-cell reconstitution after HLA-identical HCT for SCID follows a bimodal pattern: (i) early (10-15 days) expansion of mature T cells resulting from both homeostatic and antigen-driven expansion and (ii) neo-thymopoiesis in host thymus leading to the late appearance (3-6 months) of mature naive T cells. In contrast, reconstitution following T-cell depleted HCT strictly depends on the ability of donor-derived stem cells to mature into T lymphocytes, a process that may take as many as 3-6 months. During this interval, the recipient remains highly susceptible to severe infections (3). HCT from matched unrelated donors has been recently used with increased frequency in patients with SCID (30,31). Results are intermediate between HLA-identical and haploidentical HCT.

Although HCT may allow prolonged survival in the majority of infants with SCID, several problems remain to be addressed. In particular, a significant proportion of patients develop complications, that may affect quality of life (32, 33). The quality of immune reconstitution and the nature of the underlying genetic defect have been identified as critical risk factors. Poor or delayed T cell reconstitution is associated with increased risk of infections, inflammatory and autoimmune complications, and frequent need of nutritional support following transplantation. SCID patients with defects of NHEJ are at risk for growth/developmental delay, teeth abnormalities, and poor nutritional status. Patients with ADA deficiency often develop hearing defects and behavioral abnormalities. Finally, patients with SCID-X1 or with JAK3 deficiency are highly prone to warts.

Besides HCT, other forms of treatment have been developed to treat specific forms of SCID. Patients with ADA deficiency may benefit from intramuscular administration of bovine ADA conjugated with polyethylenglycole (34). This treatment is usually effective in achieving detoxification, however it is very expensive and has to be continued indefinitely.

Following the identification of SCID-causing genes and the development of molecular tools to deliver genes into cells, gene therapy has become an attractive therapeutic option for patients with SCID who do not have an HLA-identical family donor. Initial attempts were performed in 1990 in patients with ADA deficiency, using retroviral-mediated ADA gene transfer into peripheral blood lymphocytes, but were soon directed towards targeting of CD34+ hematopoietic stem cells. With use of non myeloablative chemotherapy, gene therapy represents a very effective treatment for patients with ADA deficiency, as demonstrated by experience in Milan (35). Two groups, in Paris and London, have used retrovirus-mediated gene therapy in 20 patients with SCID-X1, without using any chemotherapy. Seventeen of these patients are currently alive and show good T-cell re-

constitution, confirming the efficacy of the procedure. However, 5 of the 20 patients have developed leukemic proliferation due to insertional mutagenesis; insertion of the retrovirus within a proto-oncogene (LMO-2 in 4 of the 5 cases) has resulted in deregulated expression of the oncogene and clonal proliferation. Four of these 5 patients have been successfully cured, however one patient has died of treatment-refractory leukemia (36). These serious adverse events have prompted the development of novel, hopefully safer vectors, in which the strong viral Long Terminal Repeat enhancer sequences have been removed, and expression of the γc-encoding gene is driven by a weaker promoter. A new multi-center trial for SCID-X1 with use of such self-inactivating retroviral vector is currently underway in London, Paris, Boston, Los Angeles and Cincinnati.

Sixty years after the first description (Glanzman and Riniker, 1950), the study of patients with SCID continues to inform our knowledge of the human immune system. Furthermore, SCID has represented an important clinical setting to test the efficacy of novel preventive and therapeutic approaches to infectious diseases, and has pioneered the development of gene therapy. A fatal group of disorders has been turned into a curable condition. However, important objectives remain to be addressed, including the need of novel strategies to speed-up and improve immune reconstitution after HCT to prevent long-term complications, and the development of innovative approaches to gene therapy, possibly based on true gene correction "in situ" or on targeting of regions of the genome that are devoid of oncogenes and may thus serve as "safe harbors" for integration of the therapeutic gene.

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Correspondence: Ivonne Sponzilli
Division of Immunology, Children's Hospital
Harvard Medical School
Boston, MA, USA
E-mail: ivonne.sponzilli@childrens.harvard.edu