

MOLECULAR DEFECTS IN HUMAN SEVERE COMBINED IMMUNODEFICIENCY AND APPROACHES TO IMMUNE RECONSTITUTION

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■ **Abstract** Mutations in nine different genes have been found to cause the human severe combined immunodeficiency syndrome. The products of three of the genes—*IL-2RG*, *Jak3*, and *IL-7R α* —are components of cytokine receptors, and the products of three more—*RAG1*, *RAG2*, and *Artemis*—are essential for effecting antigen receptor gene rearrangement. Additionally, a deficiency of CD3 δ , a component of the T-cell antigen receptor, results in a near absence of circulating mature CD3+ T cells and a complete lack of γ/δ T cells. Adenosine deaminase deficiency results in toxic accumulations of metabolites that cause T cell apoptosis. Finally, a deficiency of CD45, a critical regulator of signaling thresholds in immune cells, also causes SCID. Approaches to immune reconstitution have included bone marrow transplantation and gene therapy. Bone marrow transplantation, both HLA identical unfractionated and T cell–depleted HLA haploidentical, has been very successful in effecting immune reconstitution if done in the first 3.5 months of life and without pretransplant chemotherapy. Gene therapy was highly successful in nine infants with X-linked SCID, but the trials have been placed on hold due to the development of a leukemic process in two of the children because of insertional oncogenesis.

INTRODUCTION

Human severe combined immunodeficiency (SCID) was first reported by Swiss workers more than 50 years ago (1). Infants with the condition were profoundly lymphopenic and died of infection before their first or second birthdays. In the ensuing years, differences in inheritance patterns were noted, indicating that there was more than one cause for this condition. In many families there was clearly X-linked recessive inheritance, whereas in others there was autosomal recessive inheritance. The first discovered molecular cause of human SCID, adenosine deaminase deficiency, was reported in 1972 (2). However, it was not until 21 years later that a second fundamental cause of the condition was found, i.e., the molecular

TABLE 1 Abnormal genes known to cause SCID

Chromosome	Disease
1q31–32	SCID caused by CD45 deficiency*
5p13	SCID due to IL-7 receptor alpha chain deficiency*
10p13	SCID (radiation sensitive; Athabaskan) due to mutations in the Artemis gene*
11p13	SCID caused by RAG1 or RAG2 deficiencies*
11q23	SCID caused by CD3 delta chain deficiency
19p13.1	SCID caused by Jak3 deficiency*
20q13.11	SCID caused by adenosine deaminase (ADA) deficiency*
Xq13.1	X-linked SCID caused by common gamma-chain (γ_c) deficiency*

*Gene cloned and sequenced; gene product known.

basis of X-linked SCID (3, 4). Since then, remarkable progress has been made in elucidating several other causes of this syndrome. It is now known that SCID can be caused in humans by mutations in at least nine different genes (Table 1) (5–9a), and the likelihood is that there are other causes yet to be discovered. Regardless of the underlying defect, infants with this syndrome are lymphopenic (Figure 1) and have profound deficiencies of T and B cell numbers and function (Figures 2a, 3a). In certain types, there are also marked deficiencies of natural killer (NK) cells and NK cell function (Figure 2a).

CLINICAL PRESENTATION

Affected infants begin to have problems with oral moniliasis, diarrhea, and failure to thrive in the first few months of life. Although the diagnosis of SCID can be made much earlier, it is frequently not made until serious infections develop, with the average age at referral for immune testing being approximately 6 months. Persistent infections with opportunistic organisms, such as *Candida albicans*, *Pneumocystis carinii*, varicella, adenovirus, respiratory syncytial virus, parainfluenza 3, cytomegalovirus, Epstein-Barr virus (EBV), and bacillus Calmette-Guerin (BCG), lead to death (Table 2) (10, 11). These infants also lack the ability to reject allografts, leaving them at risk for fatal GVHD. This condition is uniformly fatal in the first two years of life unless immune reconstitution can be accomplished (12–14).

Recognition of the characteristic lymphopenia (Figure 1) can result in early diagnosis—even at birth (11, 13). Their lymphocytes fail to proliferate in vitro in response to mitogens, antigens, or allogeneic cells (Figure 3a). Serum immunoglobulins and antibodies are diminished to absent. The thymuses are very small (usually less than 1g) and lack thymocytes, corticomedullary distinction, and Hassall's corpuscles. However, recent studies have shown that these thymuses are capable of

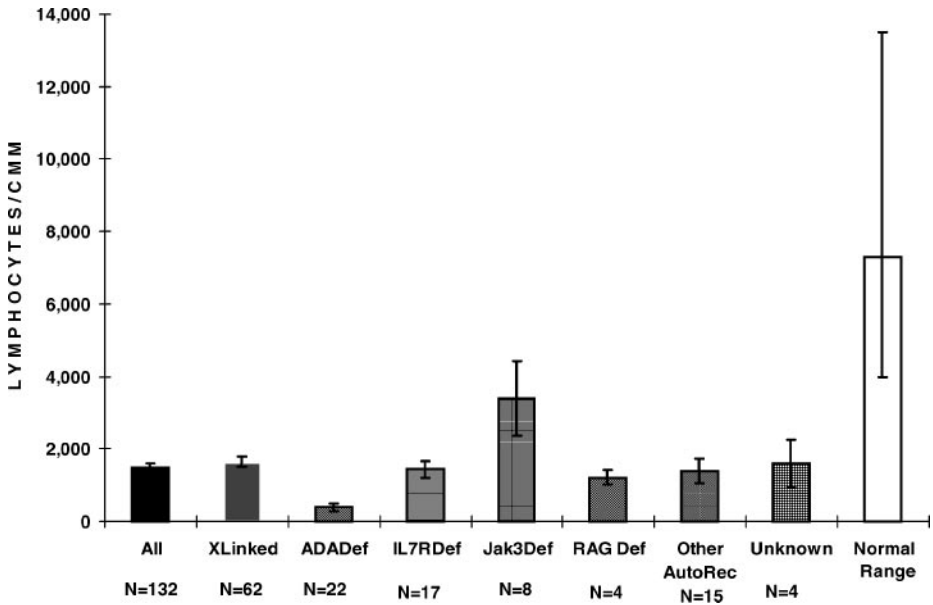


Figure 1 Means \pm SEM of absolute lymphocyte counts in 132 SCID infants at presentation, showing the characteristic of lymphopenia in all forms of SCID.

supporting T cell development when normal stem cells are provided (13, 15). Thymus-dependent areas of the spleen are devoid of lymphocytes, and lymph nodes and tonsils are absent. Flow cytometric studies have shown that there are unique lymphocyte phenotypes for the various genetic forms of SCID (Table 3), with some having B cells and no NK cells (so-called $T^{-}B^{+}NK^{-}$ SCID), others having no T cells but normal or elevated numbers of B and NK cells ($T^{-}B^{+}NK^{+}$ SCID), others having no B cells but many NK cells ($T^{-}B^{-}NK^{+}$ SCID), and others having extremely low numbers of all types of lymphocytes ($T^{-}B^{-}NK^{-}$ SCID) (Figure 2a) (6, 11, 12, 16).

SCID is a pediatric emergency (11–13). Nearly all cases could be diagnosed at birth if routine blood counts and manual differentials were done and flow cytometry and T cell functional studies performed when lymphocyte counts are below the newborn normal range (2000–11,000/mm³) (11, 17). Treatment could then be given shortly after birth (13).

MOLECULAR CAUSES OF SCID

Enormous strides have been made in identifying the molecular causes of SCID, with all but one of these discoveries having been made within the past 10 years (Table 1) (4–9, 18, 19). The products of three of the mutated genes that cause SCID

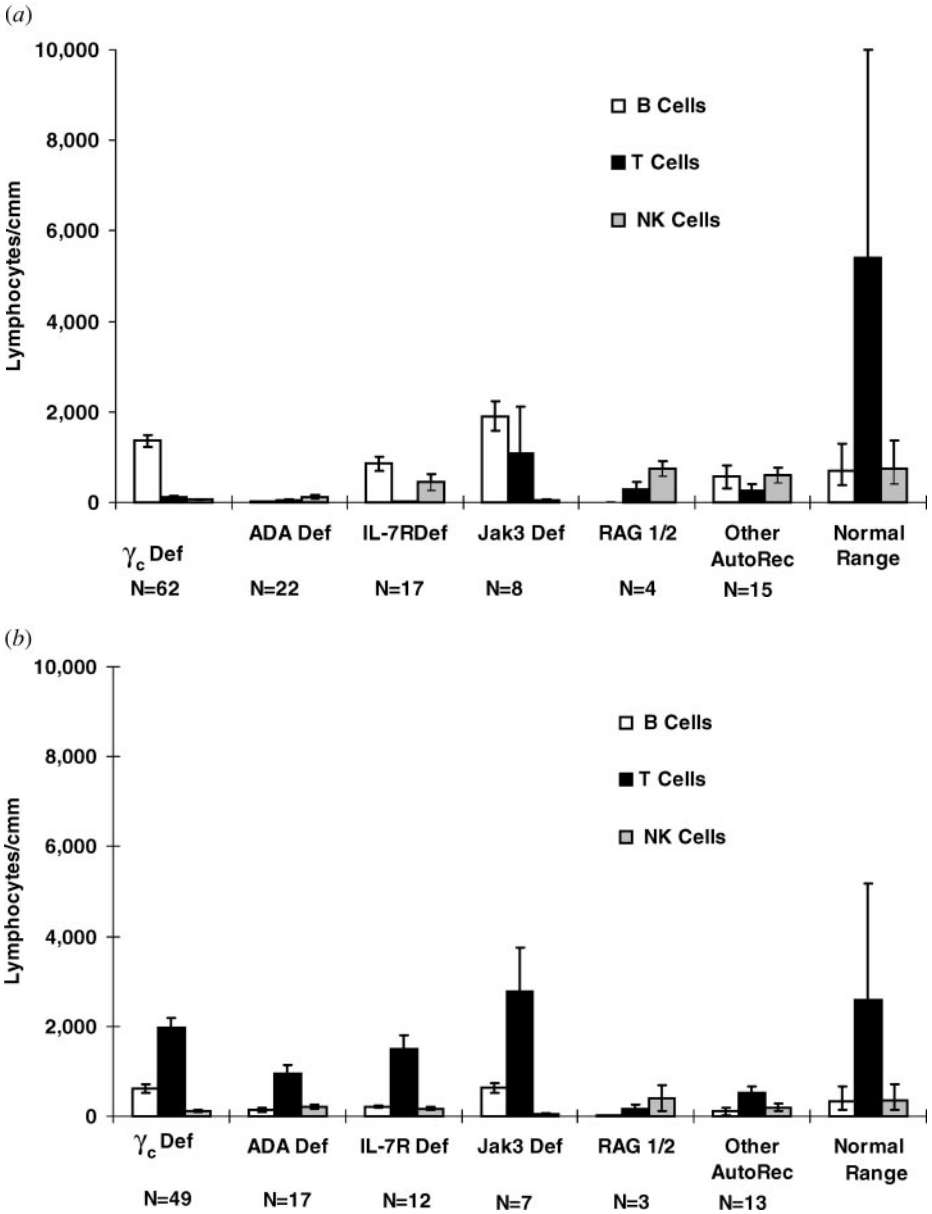


Figure 2 Means \pm SEM of CD20+ B cells, CD3+ T cells, and CD16+ NK cells in 132 SCID patients before (a) and most recently after transplantation (b) in the 102 survivors according to genetic type, as compared with ranges for normal controls.

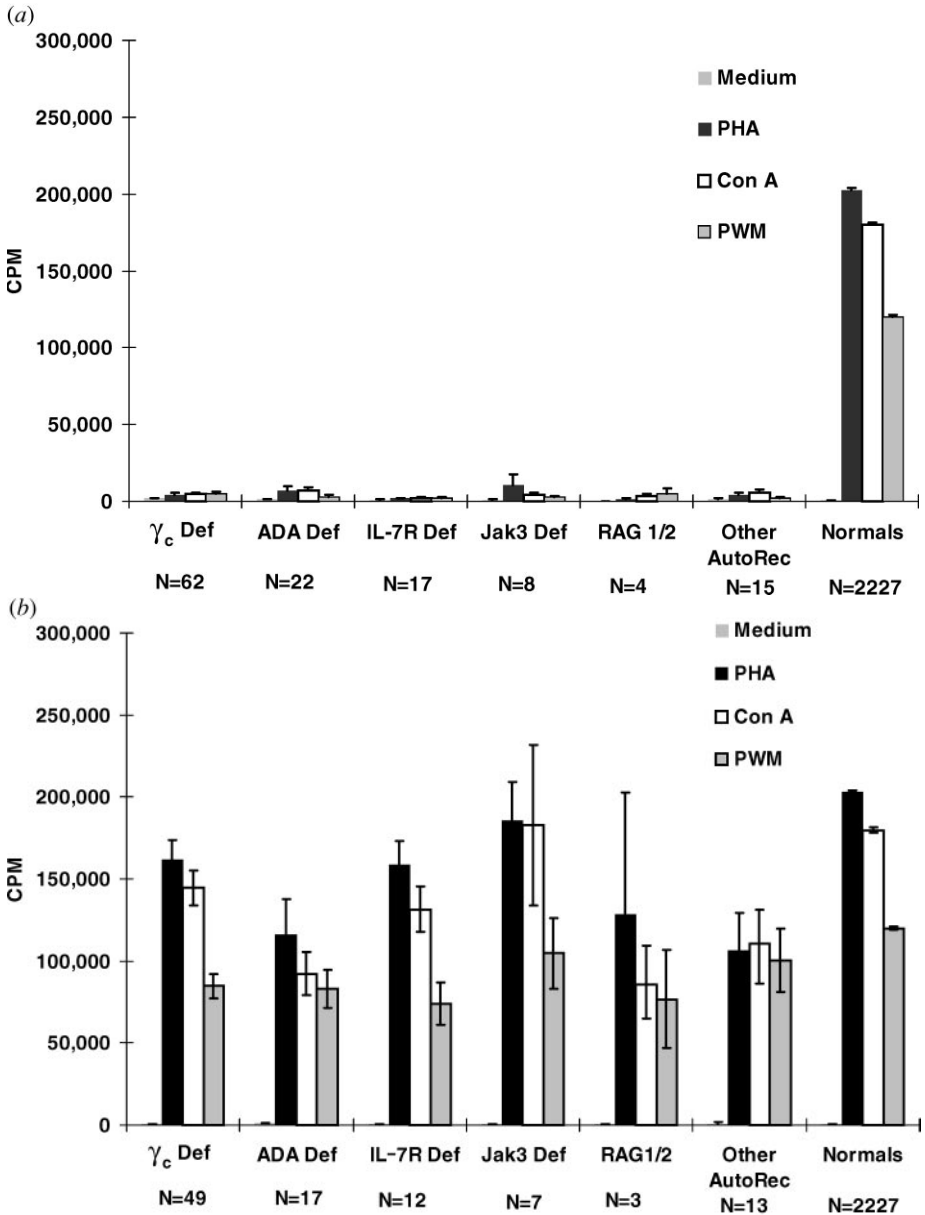


Figure 3 Means \pm SEM cpm [3 H] thymidine incorporation by proliferating lymphocytes from the 132 SCIDs before (a) and most recently after transplantation in the 102 survivors (b) according to genetic type in response to the mitogens, PHA, Con A, and PWM, as compared with means \pm SEM for normal controls.

TABLE 2 Causes of death in 30 SCID infants post-transplantation

Cause of death	Number of infants affected
Cytomegalovirus infection	7
Adenovirus infection	7
Epstein Barr virus infection/lymphoma	3
Enterovirus/Rotavirus infections	3
Parainfluenza 3 infection	2
Varicella infection	2
Herpesvirus infection	1
Respiratory syncytial virus infection	1
Candida sepsis	2
Mitochondrial defect	1
Nephrotic syndrome due to chemotherapy	1
Pulmonary hypertension	1
Veno-occlusive disease	1

are components of cytokine receptors. The products of three others are essential for rearrangement of antigen receptor genes, and the product of another is a component of the T-cell antigen receptor that appears essential for T cell development. The product of one gene is necessary to prevent toxic accumulation of metabolic wastes that lead to lymphocyte apoptosis. Finally, the product of the remaining mutated gene is the common leukocyte antigen, CD45, a phosphatase critical for regulating signaling thresholds in immune cells.

TABLE 3 Lymphocyte phenotypes of the different molecular types of SCID

Lymphocyte phenotype	Type of SCID
T ⁻ B ⁺ NK ⁻	X-linked (γ_c deficiency) Jak 3 deficiency CD45 deficiency
T ⁻ B ⁺ NK ⁺	IL-7R alpha chain deficiency CD3 delta chain deficiency
T ⁻ B ⁻ NK ⁻	Adenosine deaminase deficiency
T ⁻ B ⁻ NK ⁺	RAG1 or RAG2 deficiency Artemis deficiency

Adenosine Deaminase Deficiency

An absence of the enzyme adenosine deaminase (ADA) was identified by Giblett and coworkers in 1972 as a cause of SCID (2); this defect accounts for approximately 17% of patients with the condition (Figure 4) (12, 18). The gene encoding ADA was mapped to chromosome 20q13.2–q13.11, cloned, and sequenced (20). The ADA deficiency caused by mutations in this gene results in marked accumulations of adenosine, 2'-deoxyadenosine and 2'-O-methyladenosine. The latter directly or indirectly lead to lymphocyte apoptosis, resulting in the absence of T cell function. There are certain distinguishing features of ADA deficiency, including multiple skeletal abnormalities of chondro-osseous dysplasia on radiographic examination. The latter include flaring of the costochondral junctions and a bone-in-bone anomaly in the vertebral bodies. ADA-deficient patients have a more profound lymphopenia than do infants with other types of SCID, with mean absolute lymphocyte counts of less than 500/mm³ and a deficiency of all three types of immune cells (T⁻B⁻NK⁻ SCID) (Figure 2a, Table 3) (11, 12). Milder forms of this condition have been reported, leading to delayed diagnosis of immunodeficiency even to adulthood (21). The diagnosis should be suspected in any patient with recurrent infections who has severe lymphopenia.

Common Gamma Chain Deficiency

X-linked recessive severe combined immunodeficiency (SCID-X1) is the most common form of SCID, accounting for approximately 46% of U.S. cases (Figure 4) (11, 12). The abnormal gene in SCID-X1 was mapped to the Xq13 region

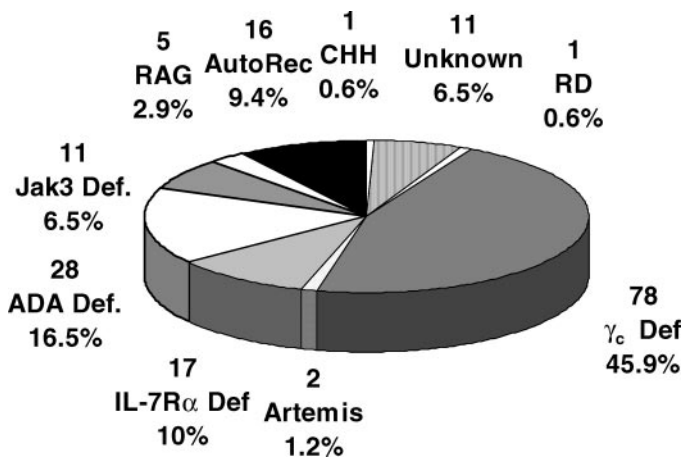


Figure 4 Relative frequencies of the different genetic types of SCID among 170 patients seen consecutively by the author over 3.5 decades.

and later identified as the gene encoding the common gamma chain (γ_c) shared by cell surface receptors for various interleukin molecules (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) (Table 1; Figure 5) (3, 4, 22, 23). Among the first 136 SCID-X1 patients studied, 95 distinct mutations were identified, resulting in abnormal γ_c chains in two thirds of the cases and absent γ_c protein in the remainder (24). The finding that the mutated gene results in faulty signaling through several cytokine receptors explains how T, B, and NK cells can be affected by a single mutation (19, 25). This was the initially discovered example of $T^-B^+NK^-$ SCID (Table 3). Humans with γ_c deficiency differ from the γ_c murine knockouts in that they do have B cells, whereas the mice do not (26). Even though B cells are the dominant lymphocyte type present in the circulation, the B cells do not undergo class switch recombination. An exception to SCID being invariably fatal without marrow transplantation or gene therapy was seen in a patient with SCID-X1 who underwent spontaneous clinical improvement and was found to have reversion of a documented mutation in the gene encoding γ_c , presumably in a T cell precursor (27).

Janus Kinase 3 Deficiency (Jak3-Deficient SCID)

SCID patients with autosomal recessive SCID caused by Jak3 deficiency resemble all other types in their susceptibility to infection and to graft-versus-host disease from allogeneic T cells. However, they have lymphocyte characteristics most closely resembling those of patients with X-linked SCID, including an elevated percentage of B cells and very low percentages of T and NK cells (Figure 2a; Table 3) (11, 12). Because Jak3 is the only signaling molecule known to be associated with γ_c (28), it was a candidate gene for mutations leading to autosomal recessive $T^-B^+NK^-$ SCID, the identical lymphocyte phenotype seen in SCID-X1 (Figures 2a and 5). This proved to be the case and thus far more than 30 patients have been identified who lack Jak3 (5, 11, 29, 30). As in the case of γ_c deficiency, B cells are present in humans with Jak3 deficiency but they do not undergo isotype switching, whereas B cells are absent in the Jak3 murine knockout (26). Naturally occurring Jak3 mutations that result in SCID can serve as valuable tools for delineation of the phenotype and clinical course of Jak3-deficient SCID following bone marrow transplantation, and for Jak3 structure-function analysis. A report of one such evaluation of Jak3 mutations has been made for a series of 23 Jak3-deficient patients from Europe, where it was noted that the clinical phenotype can vary greatly with this condition, ranging from classical SCID to almost normal (31). The author and her associates have found novel Jak3 mutations in 11 Jak3-deficient SCID patients, the first reported series from the United States, and have summarized the immunologic and clinical data as well as the results of bone marrow transplantation in these patients (Figure 4) (31a). Like SCID-X1 patients, Jak3-deficient SCID infants have very low numbers of NK cells, even after successful marrow transplantation (Figure 2a,b) (12). Moreover, in further similarity to SCID-X1 patients, they often fail to develop normal B cell function after transplantation despite their high

numbers of B cells. Their failure to develop NK cells or B cell function is believed to be due to these host B cells' abnormal cytokine receptors. The impaired IL-4 and IL-21 cytokine receptor signaling is thought to contribute to the host B cell dysfunction even though adequate T cell help is provided by the donor-derived T cells (32). Based on findings from IL-15 deficient mice, the NK cell deficiency in both SCID-X1 and Jak3-deficient SCIDs is thought to be due to failure to signal through the IL-15 receptor (33).

IL-7 Receptor Alpha Chain Deficiency (IL-7R α -Deficient SCID)

Several of the author's SCID patients who had previously been shown not to have either γ_c or Jak3 deficiency had a T⁻B⁺NK⁺ phenotype. Because mice whose genes for either the alpha chain of the IL-7 receptor (34) or of IL-7 itself (35) have been mutated are profoundly deficient in T and B cell function but have normal natural killer cell function, mutations in these genes were sought in human SCID (Figure 2a). Mutations in the gene for IL-7R α on chromosome 5p13 have been found thus far in 17 of the author's patients, as well as in 3 others (36), making it the third most common cause of human SCID in the United States (Figure 4) (6; J. Roberts, S. Brown, R. Buckley, submitted). Thus far, no humans who have SCID because of IL-7 deficiency have been found. The finding that mutations in IL-7R α alone result in T cell deficiency but not B cell or NK cell deficiency implies that the T cell but not the NK cell defect in SCID-X1 and Jak3-deficient SCID results from an inability to signal through the IL-7 receptor (Figure 5) (6, 37). Unlike IL-7R α mutant mice, humans with IL-7R α mutations not only have B cells, but these B cells appear to function normally after T cell immune reconstitution is effected by allogeneic bone marrow transplantation.

Recombinase-Activating Gene Deficiencies (RAG1- or RAG2-Deficient SCID)

Infants with autosomal recessive SCID caused by mutations in recombinase-activating genes, *RAG1* and *RAG2*, resemble all others in their infection susceptibility and complete absence of T or B cell function. However, their lymphocyte phenotype differs from those of patients with SCID caused by γ_c , Jak3, IL-7R α , or ADA deficiencies in that they lack both B and T lymphocytes and have primarily NK cells in their circulation (T⁻B⁻NK⁺ SCID) (Table 3; Figure 2a). This particular phenotype suggested a possible problem with their antigen receptor genes, leading to the discovery of mutations in *RAG1* and *RAG2* in approximately half of such SCID infants (7, 38, 39). These genes, on chromosome 11p13, encode proteins necessary for somatic rearrangement of antigen receptor genes on T and B cells. The proteins recognize recombination signal sequences (RSSs) and introduce a DNA double-stranded break, permitting V, D, and J gene rearrangements. *RAG1* or *RAG2* mutations result in a functional inability to form antigen receptors through genetic recombination. This genetic type of SCID is more common in Europe than in the United States. Only 5 such patients have been

found among the 170 SCID patients evaluated by the author (Figure 4). *RAG1*- or *RAG2*-deficient SCIDs frequently fail to develop B cells after bone marrow transplantation.

In addition to causing the SCID phenotype, some mutations in *RAG1* or *RAG2* genes lead to partially impaired V(D)J recombinational activity resulting in Omenn's syndrome (39, 40). Omenn's syndrome is characterized by the development soon after birth of a generalized erythroderma and desquamation, diarrhea, hepatosplenomegaly, hypereosinophilia, and markedly elevated serum IgE levels but very low levels or absence of the other immunoglobulin isotypes. The absolute lymphocyte count is elevated due to circulating, activated, and oligoclonal T lymphocytes that do not respond normally to mitogens or antigens in vitro (41, 42). Circulating B cells are not found, and lymph node architecture is abnormal due to a lack of germinal centers (43). Omenn's syndrome is fatal unless corrected by bone marrow transplantation. Unlike the situation for SCID infants, pretransplant chemotherapy is necessary for bone marrow graft acceptance in Omenn's syndrome.

CD3 δ Chain Deficiency (CD3 δ -Deficient SCID)

The most recently discovered cause of human autosomal recessive SCID is CD3 δ chain deficiency (9a). Infants with mutations in the gene encoding the delta chain of CD3 resemble all others in their infection susceptibility and complete absence of T cell function. Mutations in the human genes encoding CD3 ϵ and CD3 γ chains result in only a partial arrest of T cell maturation and, therefore, only moderate immunodeficiency (43a, 43b). By contrast, a homozygous stop codon mutation in the region of CD3 δ that encodes the extracellular domain of CD3 δ resulted in a profound deficiency of mature circulating CD3+ T cells, no CD4+ or CD8+ T cells, and a total absence of γ/δ T cells in three Mennonite first cousins (9a). The number of B cells was either normal or increased, and NK cells were normal in all. Thus, their lymphocyte phenotype resembled that of IL-7R α deficiency. Lymphocyte responses to mitogens were absent. In distinction from the other eight molecular types of human SCID, these infants with CD3 δ deficiency each had a nearly normal sized thymus on chest radiography. Histopathologically, there were moderate populations of T cell precursors but no typical corticomedullary distinction and no Hassell's corpuscles. These findings suggest that CD3 δ is essential for human T cell development (9a).

CD45 Deficiency

Another autosomal recessive cause of human SCID is a mutation in the gene encoding the common leukocyte surface protein CD45 (9, 44, 45). This hematopoietic-cell-specific transmembrane protein tyrosine phosphatase functions to regulate Src kinases required for T- and B-cell antigen receptor signal transduction (46). A 2-month-old male infant presented with a clinical picture of SCID and was found to have very low numbers of T and NK cells but an elevated number of B cells

(Table 3). The T cells failed to respond to mitogens, and serum immunoglobulins diminished with time. He was found to have a large deletion at one CD45 allele and a point mutation causing an alteration of the intervening sequence 13 donor splice site at the other (9). A second case of SCID due to CD45 deficiency has been reported (44, 45).

Artemis Deficiency

Another recently discovered cause of human SCID is a deficiency of a novel V(D)J recombination/DNA repair factor that belongs to the metallo- β -lactamase superfamily. It is encoded by a gene on chromosome 10p called Artemis (Table 1) (8, 47). A deficiency of this factor results in an inability to repair DNA after double-stranded cuts have been made by *RAG1* or *RAG2* gene products in rearranging antigen receptor genes from their germline configuration. Similar to *RAG1*- and *RAG2*-deficient SCID, this defect results in another form of T⁻B⁻NK⁺ SCID (Table 3), also called Athabascan SCID (48). In addition, there is increased radiation sensitivity of both skin fibroblasts and bone marrow cells of those affected with this type of SCID.

APPROACHES TO IMMUNE RECONSTITUTION

Bone Marrow Transplantation

Shortly after the discovery of HLA in 1968, (49, 50) immune function was transferred to an infant with SCID by transplantation of bone marrow from his HLA-identical sister (51). Over the ensuing decade, however, lethal graft-versus-host disease (GVHD) was a major problem when marrow from HLA-mismatched donors was used (52). In the late 1970s, studies in rats (53) and mice (54) revealed that allogeneic marrow or spleen cells that were depleted of T cells rescued the recipient from lethal irradiation without causing fatal GVHD, despite differences in MHC antigens between donor and host. Techniques developed in the early 1980s for the depletion of T cells from human marrow made it possible to restore immune function in all forms of SCID by marrow transplantation (10–14, 55–65). Because the defect in infants with SCID leads to a complete absence of T cell function, they cannot reject allografts. Therefore, successful marrow transplantation in SCID does not require pretransplant chemotherapeutic conditioning. Moreover, prophylaxis for GVHD is also not necessary after transplantation of HLA-identical or even rigorously T cell–depleted HLA haploidentical marrow because in the latter case the T cells that cause GVHD have been removed. These circumstances provide a unique opportunity to study the development of T cells from donor hematopoietic stem cells in an unmanipulated recipient because less than 1 million T cells per kilogram are given when one uses rigorous T cell–depletion techniques (66). At the time of this writing the author and her colleagues had performed hematopoietic stem cell transplantation in 132

consecutive infants with SCID at our institution over 21.3 years, and 102 of them survived. The outcome in all but 30 of these transplants has been previously reported (12, 13).

PATIENTS The 132 patients ranged in age from newborn to 21 months at diagnosis. They fulfilled the criteria of the World Health Organization for diagnosis of SCID (67). The patients were from 117 families and fit eight categories of the disease based on family history, sex, other clinical features, and results of enzyme analyses or molecular studies (11). The largest number of patients—62 boys from 50 families or 47%—had x-linked SCID due to mutations in the gene encoding the common γ chain (γ_c) (3, 4, 11, 68). Eight patients from 8 families had SCID due to mutations in the gene encoding Jak3 (5, 11, 29). Seventeen patients from 16 families had SCID caused by mutations in the gene encoding IL-7R α (6). Twenty-two infants from 19 families had SCID due to a deficiency of ADA (69). Fifteen patients from 15 families had autosomal recessive inheritance but unknown mutations; and 4 boys with no family history had SCID of unknown type. Immunologic monitoring was done whenever feasible every 3 weeks until T cell function was established (usually at 3–4 months post-transplantation), then every 3 months for the next 9 months, every 6 months for the next 2 years, then once a year thereafter. The studies were done with the approval of the Duke University Committee on Human Investigations and written informed consent of the parents.

All 117 HLA-haploidentical and 7 of the 15 HLA-identical transplants from related donors were T cell depleted (12, 55, 57). Thirty-three patients received from one to three additional T cell–depleted marrow transplants, from either the original donor or another haploidentical relative. None of the marrow recipients received any pretransplant conditioning or post-transplant prophylaxis against GVHD. Two patients were given cyclosporine for 1 month because of cutaneous GVHD from transplacentally transferred maternal T cells at presentation. Five of the infants who received haploidentical marrow transplants also received unrelated placental blood transplants. Four of the latter received pretransplant conditioning and were also given post-transplant prophylaxis against GVHD.

Results of Bone Marrow Transplantation at Duke

FACTORS INFLUENCING SURVIVAL Of the 132 SCID patients, 102 (77%) are alive. None show any evidence of susceptibility to opportunistic infections and most are in good general health. The follow-up ranges from 2 months to 21.3 years after transplantation. Of these 102 patients, 96 have survived 1 or more years after transplantation, 68 have been alive 5 or more years, and 37 for 10 or more years. Median follow-up of surviving patients is 5.4 years. All 15 recipients of marrow from HLA-identical donors, 87 of the 117 recipients given T cell–depleted haploidentical bone marrow from a related donor, and 2 of 5 infants from the latter group also given unrelated placental blood transplants are among the survivors. The survival rates are similar for the different genetic types of SCID, except that only 1

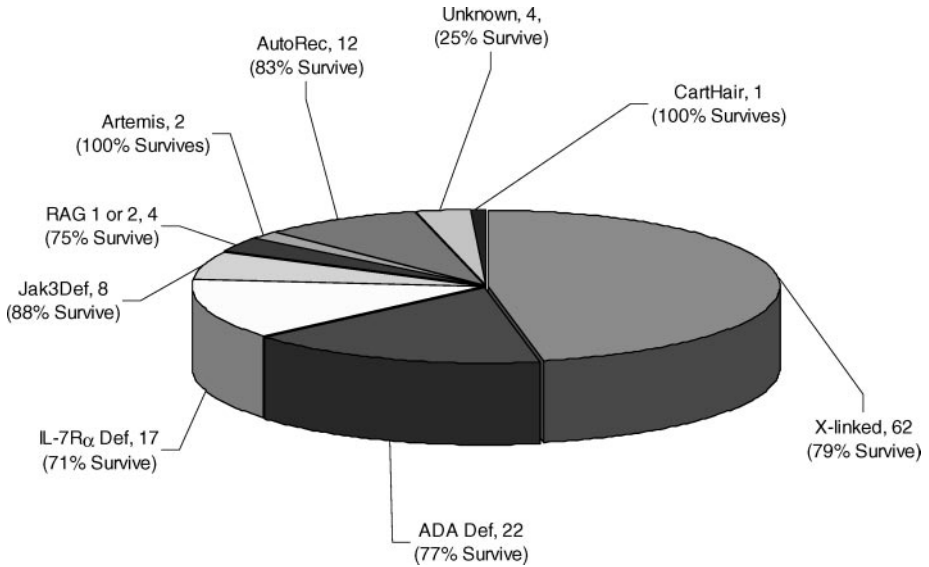


Figure 6 Survival following bone marrow transplantation of 102 of 132 SCIDs according to genetic type of SCID.

of the small group of 4 male infants with SCID of unknown type survive (Figure 6). Influences on survival include race (more who were Caucasian survive, $p < 0.001$), sex (all but three of the females transplanted survive, $p < 0.05$), and age at the time of transplant. Of the 36 infants transplanted during the first 3.5 months of life, 35 (97%) survive, compared to 67/96 (70%) who were transplanted after that age (Figure 7). Twenty-four of the 30 deaths occurred from viral infections: CMV, 7; EBV, 3; adenovirus, 7; enteroviruses, 2; parainfluenza 3, 2; Herpes zoster, 2; and Herpes simplex, 1 (Table 2). Two infants died of candida sepsis. One died from an unrelated mitochondrial defect after stable marrow engraftment, and another died of the nephrotic syndrome after chemotherapy had been given for what had been mistakenly diagnosed elsewhere as a malignancy. One ADA-deficient patient who had been treated with polyethylene glycol modified bovine ADA for 18 years died of pulmonary hypertension. None of the patients died from GVHD, despite the fact that 87 of the surviving patients received haploidentical bone marrow transplants.

GRAFT-VERSUS-HOST DISEASE GVHD occurred in 40/117 patients given T cell-depleted haploidentical parental marrow, 6/15 given unfractionated HLA-identical marrow, and 4/5 given placental blood. In 35/49 cases, this complication occurred when there was persistence of transplacentally transferred maternal T cells. Most of the GVHD after administration of T cell-depleted marrow was mild (Grade I or II) and required no treatment (70). Ten patients from the entire group had Grade III

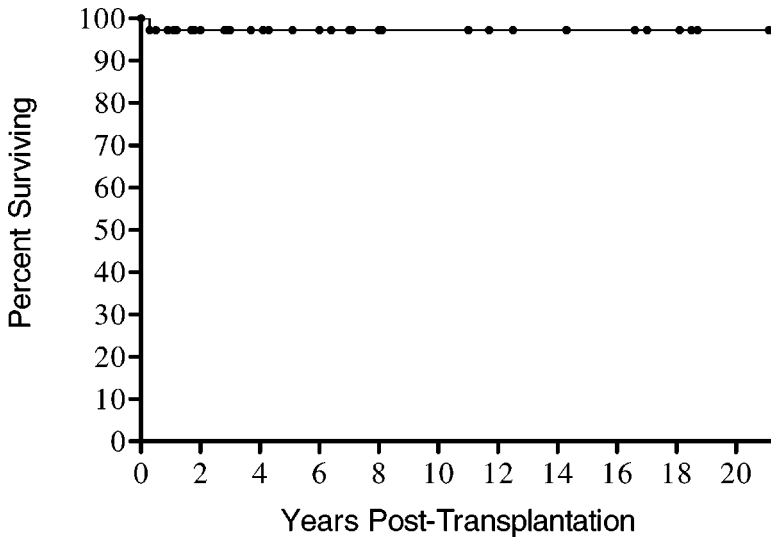


Figure 7 Kaplan Meier plot of 36 SCID infants transplanted in the first 3.5 months of life. Thirty-five survive from 3 months to 21.3 years post-transplantation; only 5 had HLA-identical donors.

GVHD involving the skin, gastrointestinal tract, and/or marrow; 9/10 were given corticosteroids and cyclosporine as treatment; one was given corticosteroids alone and two were given tacrolimus. One neonate with autosomal recessive SCID who received a 3-antigen mismatched transplant developed Grade 4 GVHD with autoimmune hemolytic anemia, bone marrow suppression, diarrhea, and cholestatic liver disease. He subsequently received a living-related liver transplant and remains on a low dose of tacrolimus 1.5 years later. His mother was the donor for both the bone marrow and the liver segment. He is a complete hematopoietic chimera and now has normal liver function. No patients died of GVHD, but one of the recipients of unrelated placental blood had severe acute GVHD and now has chronic GVHD, requiring continuous cyclosporine therapy.

ENGRAFTMENT AND CHIMERISM Genetic analyses of blood lymphocytes at the survivors' latest evaluations have shown that all of the T cells in 95/102 are of donor origin. In one γ_c -deficient patient, four T cell-depleted transplants—two from each parent—failed to engraft, but the patient is still alive at 10 years of age despite very low T cell function. Of note, 13/18 ADA-deficient patients given T cell-depleted haploidentical marrow and all 4 of those given HLA identical marrow, all without pretransplant chemotherapy or post-transplant GVHD prophylaxis, are alive at 0.3–18.6 years after transplantation, with hematopoietic chimerism in 10. Two of the 6 infants who failed to become chimeric received successful gene therapy in Italy (71), and the other 5 are receiving polyethylene

glycol-modified bovine ADA after rejecting haploidentical parental hematopoietic stem cell transplants.

By contrast to the development of donor T cells in all but the above 8 patients, the B cells in most cases remain those of the recipient. However, 5/15 recipients of HLA-identical marrow and 32/117 haploidentical marrow recipients have some donor B cells (range 3%–100% of all B cells, mean 55.8 \pm SEM 8.3%).

LYMPHOCYTE PHENOTYPES Patients with the various genetic types of SCID had distinct lymphocyte phenotypes before transplantation (Figure 2a) (11, 12). All patients had a profound deficiency of T cells and, when T cells were present, they were usually transplacentally transferred maternal T cells. In one Jak3-deficient patient, there were 8268 circulating maternal T cells/mm³ at presentation (72). B cells were elevated in γ_c -deficient, IL-7R α -deficient, and Jak3-deficient SCIDs, normal in autosomal recessive SCIDs of unknown molecular type, and absent in *RAG1*- and *RAG2*-deficient SCIDs. Numbers of NK cells were lowest in γ_c -deficient, Jak3-deficient (T⁻B⁺NK⁻) SCIDs, and in ADA-deficient (T⁻B⁻NK⁻) SCIDs, but were normal in IL-7R α -deficient (T⁻B⁺NK⁺) SCIDs, *RAG1*- or *RAG2*-deficient SCIDs (T⁻B⁻NK⁺), in other autosomal recessive SCIDs of unknown molecular type, and in the four males with unknown inheritance (Figure 2a) (11, 12). At the most recent evaluation following transplantation, the mean number of T cells in the 102 surviving patients was within the normal range for the γ_c -deficient, ADA-deficient, IL-7R α -deficient, and Jak3-deficient SCIDs and below the normal range in the *RAG1*- or *RAG2*-deficient SCIDs and in the autosomal recessive SCIDs of unknown molecular cause (Figure 2b). The mean number of B cells was still elevated in γ_c -deficient and Jak3-deficient SCIDs, very low in the *RAG1*- or *RAG2*-deficient SCIDs, but normal in all others. The mean number of NK cells remained low in the γ_c -deficient and Jak3-deficient groups but was normal in the others.

T CELL FUNCTION Figure 3 shows in vitro responses to the mitogens (phytohemagglutinin, concanavalin A, and pokeweed mitogen) by T cells from patients with the various types of SCID, before (3a) and after (3b) transplantation, as compared to such responses by T cells from normal adults. Remarkably, mean responses to all three mitogens were normal in all groups after transplantation as compared with extremely low responses before transplantation. Moreover, T cells from all patients with SCID responded poorly to allogeneic cells before transplantation; however, after transplantation T cells from all groups responded normally to allogeneic cells, candida, and tetanus antigens (not shown).

ROLE OF THE THYMUS Because very few T cells were present in the T cell-depleted donor marrow, it was initially surprising that genetically donor T cells emerged in these SCID infants at 90–120 days post-transplantation, considering that they all had vestigial thymi and no T cells prior to transplantation. To clarify whether the donor stem cells actually developed into T cells within the infants'

thymuses, we analyzed signal joint T cell–receptor recombination excision circles (TRECs) in 83 of the patients with SCID given marrow transplants (15). We found that T cell numbers were low before and early after transplantation, with a predominance of CD45RO⁺ T cells (primarily due to transplacentally transferred maternal cells), and TRECs were undetectable in blood mononuclear cells. A majority of infants given either T cell–depleted marrow or unfractionated HLA-identical marrow developed genetically donor TREC⁺, CD45RA⁺, CD62L⁺ T cells (recent thymic emigrants) by 3–6 months post-transplantation. Thymic output peaked (5525 ± 1502 TRECs/ μg PBMC DNA) 1–2 years after transplantation and declined to low ($<100/\mu\text{g}$ DNA) levels over 14 years, compared to over 80 years in normal individuals. CD45RA⁺ T cell numbers peaked at year 1 after transplantation (1513 ± 324 cells/ μl blood), but declined to 107 ± 35 cells/ μl at 14 years after transplantation. From these studies, we concluded that the vestigial thymus of infants with SCID can produce enough genetically donor T cells to confer T cell function by 3–6 months after transplantation, regardless of whether the donor is HLA-identical or haploidentical (15).

T CELL DIVERSITY Immunoscope analysis of the TCR V β repertoire was performed on 15 SCID patients given bone marrow transplants (BMT) (73). Before and within the first 100 days after BMT, patients' PBMC displayed an oligoclonal or skewed T cell repertoire, low TREC values, and a predominance of CD45RO⁺ T cells. In contrast, the presence of high numbers of CD45RA⁺ cells in the circulation of SCID patients >100 days post-BMT correlated with active T cell output by the thymus, as revealed by high TREC values, and a polyclonal T cell repertoire demonstrated by a Gaussian distribution of V β -specific peaks (Figure 8). Ten years after BMT we observed a decrease of the normal polyclonal T cell repertoire and an increase of a more skewed T cell repertoire. A decline of TREC levels and a decrease in the number of CD45RA⁺ cells beyond 10 years of BMT was concomitant with the detection of oligoclonal CD3⁺CD8⁺CD45RO⁺ cells. The switch from a polyclonal to a more skewed repertoire, observed in the CD3⁺CD8⁺CD45RO⁺ T cell subset, is a phenomenon that occurs normally with decreased thymic output during aging, but not as rapidly as in this patient population. We conclude that a normal T cell repertoire develops in SCID patients as a result of thymic output, and the repertoire remains highly diverse for the first 10 years after BMT. The TCR diversity positively correlated with TREC levels (73).

Figure 8 Immunoscope profile of TCR V β families. Each V β family was examined by PCR amplification and run-off reaction. Results are shown for each V β family as a density peak histogram. CDR3 sizes are shown on the *x*-axis and the peak fluorescence intensity is shown on the *y*-axis. (A) Immunoscope profile of a normal subject. (B) Immunoscope profile of a Jak-3 deficient SCID patient (J-1) before BMT. (C) Immunoscope profile of TCR V β families in an X-SCID patient at 218 days and (D) at 1984 days after BMT. [Figure reprinted with permission from (73).]

B CELL FUNCTION B cell function did not develop to the extent that T cell function did. Serum IgG prior to transplantation was in most cases maternal or from intravenous immunoglobulin, but paraproteins were present in some. One of the IL-7R α -deficient patients had both IgG and IgA paraproteins prior to transplantation, as has been noted previously in SCID (74–76). At their latest evaluation, 58 patients have normal serum IgA, 80 have normal IgM, and 48 have isohemagglutinins appropriate for host red blood cell type. Sixty-two of the 102 (61%) patients are currently receiving immunoglobulin replacement to prevent bacterial and common viral infections. All patients who are not receiving immunoglobulin infusions have demonstrated the capacity to produce antibodies to one or more vaccine antigens (data not shown). The best B cell function is in the IL-7R α -deficient SCIDs and in the ADA-deficient SCIDs, despite the fact that most of the children in those groups do not have donor B cells. This indicates that the molecular type of SCID is the principal determinant as to whether good B cell function will develop.

NK CELL FUNCTION Before engraftment, NK cell numbers and function were lowest in γ_c -deficient and Jak3-deficient SCIDs ($p < 0.001$) (Figure 2a), whereas they were higher than normal in all other types except ADA deficiency. Following transplantation, many γ_c - and Jak3-deficient SCIDs continued to have low NK function, whereas this function was normal in all other types. The NK cells in the IL-7R α -deficient SCID patients did not interfere with engraftment of T cell-depleted parental bone marrow stem cells despite no pretransplant chemotherapy. The NK cells in the RAG- and Artemis-deficient patients may have been responsible for the longer time course to development of T cell function but did not prevent engraftment even though no chemotherapy was given.

TRANSPLANTS PERFORMED IN THE FIRST 3.5 MONTHS OF LIFE As already noted, survival of the SCID patients who were transplanted in the first 3.5 months of life was superior to that in those performed later (Figure 7). We hypothesized that the kinetics of immune reconstitution would be different for those transplanted in the neonatal period when compared to those transplanted after that time. We compared immune function in 21 SCID patients transplanted in the neonatal period with that in 70 SCIDs transplanted after that (13). We measured lymphocyte phenotypes, proliferative responses to mitogens, immunoglobulin levels, and T-cell antigen receptor excision circles (TRECs) pretransplantation and sequentially post-transplantation. Infants transplanted in the newborn period developed higher lymphocyte responses to phytohemagglutinin and higher numbers of CD3+ and CD45RA+ T cells in the first 3 years of life than those transplanted late ($p < 0.05$) (Figures 9 and 10). TRECs peaked earlier and with higher values ($p < 0.01$) in the neonatal transplants (181 days–1 year) than in the late transplants (1–3 years) (Figure 11). Thus, SCID recipients of allogeneic, related hematopoietic stem cells in the neonatal period had higher levels of T cell reconstitution and thymic output and a higher survival rate than those transplanted after 28 days of life (13).

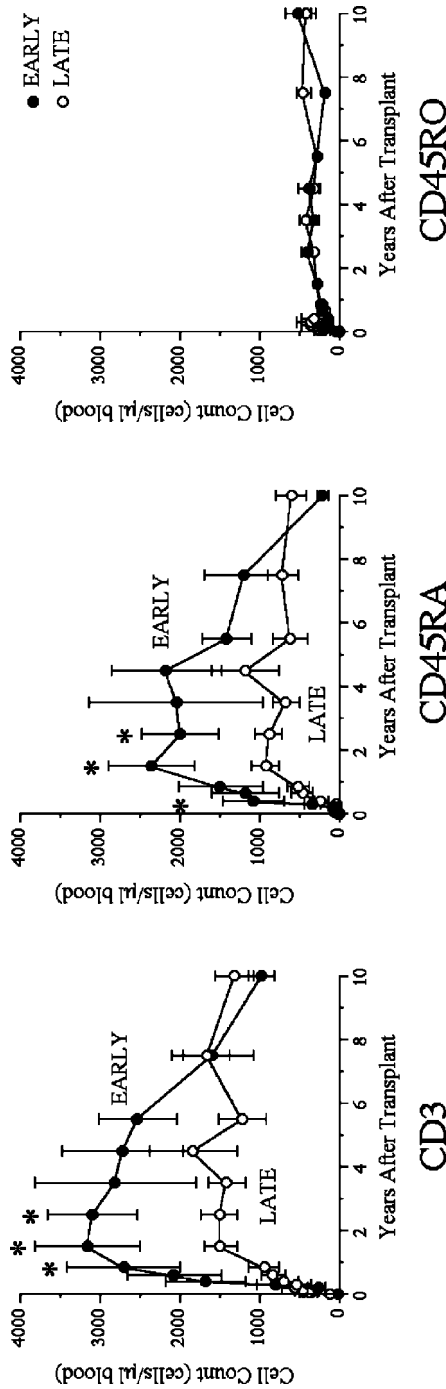


Figure 9 Increased numbers of circulating naive T cells in the early (newborn) versus late transplantation groups. Shown are the mean (+/-SEM) numbers of CD3+, CD45RA+, and CD45RO+ cells in patients receiving transplants early (n = 20) compared with those receiving transplants late (n = 66). The early group had increased numbers of CD3+ cells at 271 days to 1 year, 1-2 years, and 2-3 years after transplantation (P < .05). These numbers gradually declined and were comparable to the late group by 6 years after transplantation. [Figure reprinted with permission from (13).]

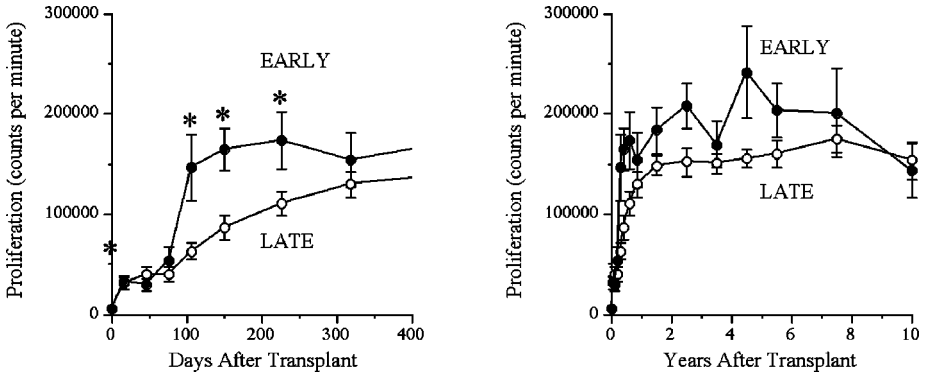


Figure 10 Comparison of proliferative responses to PHA in the early and late transplantation groups. Shown are the mean (+/-SEM) counts per minute of [³H] thymidine incorporation. Infants receiving transplants within the first 28 days of life (n = 20) had increased T cell proliferation to PHA at 91 through 120 days, 121 through 180 days, and 181 through 270 days after transplantation compared with those receiving transplants late (n = 69) (P < .05); n = total number of individuals analyzed in each group over 19.2 years. [Figure reprinted with permission from (13).]

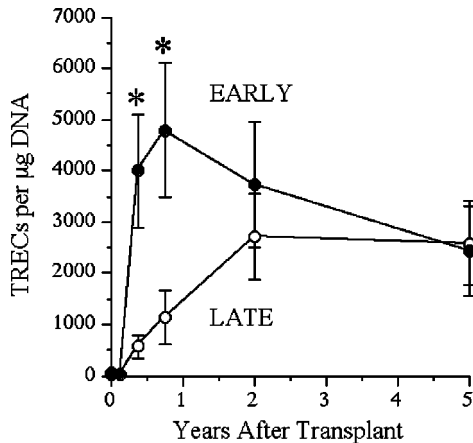


Figure 11 Faster and quantitatively higher thymic output in the early- versus late-transplantation groups. Shown are the mean (+/-SEM) number of TRECs for patients receiving early (n = 19) and late (n = 55) transplantations. Patients receiving transplants early had higher TREC values at 91 through 180 days and 181 days to 1 year after transplantation (P < .01). The mean TREC value peaked at 181 days to 1 year in those receiving transplants early and at 1 to 3 years in those receiving transplants late. [Figure reprinted with permission from (13).]

BOOSTER TRANSPLANTS In an attempt to overcome poor B or T cell function or resistance to engraftment, booster transplants were performed in 33 (25%) of the 132 patients, and 21 (64%) of them survived. All of these were done without pretransplant chemotherapy and with the same T cell depletion method as used for the initial transplants. Twenty-two patients received booster transplants from the same parental donor; and eight of them died of opportunistic viral infections. Nine received booster transplants from the other parent; four of those died. One patient received a blood transfusion from an identical twin SCID donor who had accepted a marrow transplant from their father, and another received a cord blood transplant. Immune function improved in all but four of the survivors who received booster transplants.

Overview

The above studies clearly demonstrate that transplantation of T cell–depleted HLA-identical or HLA-haploidentical bone marrow is highly effective in reconstituting T cell immunity in all of the known genetic types of SCID. No chemotherapeutic conditioning is required to achieve engraftment because the recipient is virtually devoid of T cells at the time of transplantation. This advantage eliminates adverse effects caused by these toxic agents, including neutropenia, red cell and platelet transfusion-dependency, mucositis, veno-occlusive disease, busulfan lung disease, growth suppression, sterility, and a 15% risk of later malignancy (77). Although GVHD prophylaxis was not used except for 1 month of cyclosporine given to two infants who presented with GVHD and for the placental blood transplants, clinically significant GVHD was seldom seen. The omission of GVHD prophylaxis with cyclosporine permitted the infants to develop T cell function without hindrance.

Normal T cell function appeared within 2 weeks after transplantation of unfractionated HLA-identical marrow because of the adoptive transfer of mature donor T cells. By contrast, it did not develop until 3–4 months after administration of T cell–depleted marrow, whether HLA-identical or haploidentical (12, 57). The latter is the average time required for the donor stem cells to become phenotypically and functionally mature T cells in the recipient (12, 57). T cell function often developed much earlier in neonatal recipients (13) and in patients in whom transplacental transfer of maternal T cells had occurred, but it developed later in some patients who had high numbers of NK cells at presentation. In the case of the unrelated placental blood transplants, T cells were present immediately, but T cell function was suppressed by the large doses of corticosteroids and cyclosporine needed to prevent or treat GVHD.

As for B cells, only 6/12 survivors of HLA-identical and 21/76 survivors of haploidentical transplants have some donor B cells (2%–100% of total B cells); 62/102 are currently receiving immunoglobulin replacement therapy to prevent bacterial and common viral infections because the capacity to produce protective antibodies has not yet been demonstrated. However, several of these 62 patients may be able

to discontinue immunoglobulin treatment because they are now producing IgA and isohemagglutinins.

Recent progress in identifying the molecular causes of SCID permitted us to study SCID mutations in relation to the outcome of hematopoietic stem cell transplantation. Most of the γ_c -deficient and Jak3-deficient patients who do not have any evidence of donor-derived B cells continue to have poor B cell function, as demonstrated by failure of isotype-switching following immunization with bacteriophage ϕ X174 and the inability to produce IgA, IgM, and IgE or isohemagglutinins normally *in vivo*. Thus, normal stem cells that mature in γ_c - and Jak3-deficient patients develop into normal T cells, but much less frequently into normal B cells; the host B cells in these patients most likely fail to function because they lack normal cytokine receptors. By contrast, a majority of the ADA- and IL-7R α -deficient patients and those with autosomal recessive SCID of unknown molecular cause have good host B cell function, indicating that those mutations do not adversely affect B cell function.

Before transplantation, NK cell numbers and function were also lowest in γ_c - and Jak3-deficient patients, whereas they were higher than normal in most other types of SCID. Following transplantation, profoundly low NK cell numbers and function persisted in most γ_c - and Jak3-deficient patients, whereas these were normal in all other types of SCID.

Although the ability to give half-matched T cell-depleted parental marrow to patients with SCID has been a remarkable therapeutic advance, it is not a perfect treatment. During the 3–4 months needed for donor stem cells to develop into mature functioning T cells, the infant is susceptible to viral infections. Pretransplant chemotherapy fails to accelerate immune reconstitution, heightens the susceptibility of the recipient to infection, and necessitates the use of cyclosporine, which prolongs the T cell deficiency (78). The poor B cell function in γ_c -deficient and Jak3-deficient patients in whom donor B cells do not develop has led some to use pretransplant conditioning. However, chemotherapy does not guarantee that donor B cells will develop, and the risks outweigh the potential for development of B cell function. Indeed, in a study from Europe, there was no better B cell function in those who received pretransplant chemotherapy than in those who did not (78).

Finally, resistance to engraftment is a problem that was overcome in all but six cases by booster or second parent T cell-depleted transplants, again without conditioning.

Placental blood transplantation from unrelated donors is fraught with problems because of GVHD in patients with SCID. In most institutions performing placental blood transplants for SCID, pretransplant chemotherapy is given, and prolonged (6 months) GVHD prophylaxis with cyclosporine is required (79–81). All of this heightens the risk of infection. *In utero* stem cell transplants from related donors do not appear to offer any advantage over such transplants done soon after birth. The mother would probably not be used as a donor for an *in utero* transplant because of the risks of anesthesia during pregnancy. The invasive procedures required in

in utero stem cell administration carry risks, and one would also not be able to detect or treat either a graft-versus-graft reaction or GVHD in utero (82, 83).

SCID is a pediatric emergency, and the potential exists to diagnose this condition routinely at birth (11). Cord blood white cell counts and manual differentials can detect the lymphopenia that is almost invariably present, and appropriate immunologic tests could then be done. Prenatal diagnosis can often be made when there is a family history of SCID. If a stem cell transplant from a relative can be done in the first 3.5 months of life, before infections develop, there is a high probability (>95%) of success.

Results of Bone Marrow Transplantation at Other Centers

Most of the published results of bone marrow transplantation for SCID at other centers have come from the European Group for Blood and Marrow Transplantation and the European Society for Immunodeficiency (14, 78, 84). The transplants were done at 37 different centers in Europe and, therefore, the approaches were not all the same. In a report of a long-term retrospective study of immune reconstitution in 193 SCIDs transplanted in Europe with T cell-depleted HLA-nonidentical marrow between January 1, 1983 and December 31, 1993, the overall survival at the time of publication in 1998 was 92 patients, or 47%. Eighty-nine of the 116 (77%) patients who survived for at least 6 months had been given pretransplant chemotherapy. Seventy-seven of the deaths occurred within 6 months after transplantation, and 24 more occurred after 6 months (78). A more recent publication from that group covered 1082 transplants in 919 immunodeficient patients in Europe during the 32 year period from January 1968 through December of 1999 (14). Of the 475 SCID patients transplanted, pretransplant conditioning was given to 275 or 57% of the patients. Three-year survival rates with sustained engraftment were better for HLA-identical (77%) than for mismatched transplants (54%). There was significant overall improvement in the outcomes of SCID transplants over time; the improvement went from approximately 40% survival of mismatched transplants performed from 1968–1985 to approximately 78% in those performed from 1996–1999. The latter was attributed to the development of rigorous T cell-depletion techniques, more effective antibiotics, and perhaps earlier recognition of SCID. Another finding was that the outcome was significantly better in the B cell⁺ SCIDs than in the B cell⁻ SCIDs (14, 84). The population reported was not characterized at a molecular level.

In the United States, 16 infants with Athabascan SCID due to Artemis mutations were given bone marrow transplants at the University of California in San Francisco between 1984 and 1999 (85). Seven of them received HLA-identical sibling marrow, and nine received T cell-depleted parental marrow. All but two of the infants received pretransplant chemotherapy. All seven of those who received HLA-identical marrow survived, whereas only five of the nine who received parental marrow survived. Three of the four children who died received radiation or busulfan, and two of the eight long-term survivors who were also

recipients of cytotoxic chemotherapy failed to develop secondary teeth. Thus, children with this radiation-sensitive form of SCID had a poor outcome if they were given pretransplant chemotherapy (85). At Children's Hospital of Los Angeles, between November of 1984 and December of 1997, 48 SCID infants were given bone marrow transplants (86). Eleven received HLA-identical related bone marrow transplants, and 37 received T cell-depleted haploidentical parental marrow. All recipients received pretransplant conditioning except one who received HLA-identical sibling marrow. At the time of the report, all of the 11 who received HLA-identical marrow were surviving, but only 17 of the 37 (47%) who received T cell-depleted parental marrow were surviving (86).

Although the approaches used to attempt immune reconstitution by bone marrow transplantation in SCID infants have differed from center to center, much has been learned about what factors influence success or failure. In general, the mortality has been much higher at centers that use pretransplant chemotherapy.

Gene Therapy

Until the past year, there was great optimism that primary immunodeficiency diseases for which the molecular defects have been identified could be corrected by gene therapy. From 1999–2002, 11 patients with SCID-X1 were administered autologous bone marrow cells into which a normal γ_c cDNA had been successfully transduced by retroviral gene transfer (87, 88). In 9 of the 11 patients, molecular studies demonstrated normal transgene expression in circulating T and NK cells by approximately 30–40 days after gene therapy, but it was minimal in B cells. Two of the patients did not express the transgene and were given allogeneic bone-marrow transplants. The nine infants with transgene expression developed normal T cell function at between 90 and 120 days after the treatments, similar to the kinetics after T cell-depleted allografts. Despite the fact that the transgene was minimally expressed in B cells, the nine who developed normal T cell function also did not require intravenous immunoglobulin infusions and were at home off of all medications. Thus, the efficacy of gene therapy in conferring immune function in those infants with SCID-X1 seemed to be far superior to that of allogeneic marrow stem-cell transplantation.

Tragically, however, serious adverse events occurred in the fourth and fifth patients treated by the French group (88a). Both children developed leukaemic-like processes, with expanded clonal populations of T cells. The clones carry the inserted γ_c cDNA, and the leukemias are considered to have been induced by the retroviral gene therapy by a process called insertional oncogenesis. The positions of insertion in both children are in or near a gene on chromosome 11 called *LMO-2*. The product of *LMO-2* is crucial for normal hematopoiesis and serves a regulatory function (89). However, *LMO-2* is also an oncogene that is aberrantly expressed in acute lymphoblastic leukemia of childhood. Both children were treated with chemotherapy and responded to it, but there was a recurrence in one, who was then given a matched unrelated donor transplant after chemoablation, thus destroying

the gene corrected cells. The other patients who were given gene therapy are being monitored closely and one has been found to have a similar insertion near the LMO-2 gene but has not yet developed a clonal proliferation. In view of these serious adverse events, retroviral gene therapy trials are currently on hold.

Because of the above events, the treatment of genetically determined immunodeficiency disorders remains a problem, with allogeneic stem-cell transplantation seeming to be the current best option for those defects that are invariably fatal early in life (12, 14). Efforts are being made to improve this therapy by giving higher numbers of affinity-purified allogeneic stem cells in preparations nearly devoid of T cells (90). If the imperfect results seen with allogeneic stem-cell therapy in the past were due to an insufficient number of stem cells, this approach should result in better immune reconstitution. The fact that such cell suspensions are virtually devoid of T cells should also circumvent the problem of GVHD (90). The only remaining obstacle would then be to ensure that diagnosis is made early before untreatable infections develop. However, this obstacle remains formidable because there is no currently no screening for any primary immunodeficiency disease at birth or during childhood or adulthood in any country. Thus, most patients are not diagnosed until they develop a serious infection, which will certainly adversely affect the ultimate outcome of definitive therapy (11, 12).

In summary, T cell-depleted haplo-identical marrow transplantation provides life-saving therapy for all forms of SCID. The remaining prospect of gene therapy offers hope that the remaining defects in these chimeras will eventually be correctable by that means.

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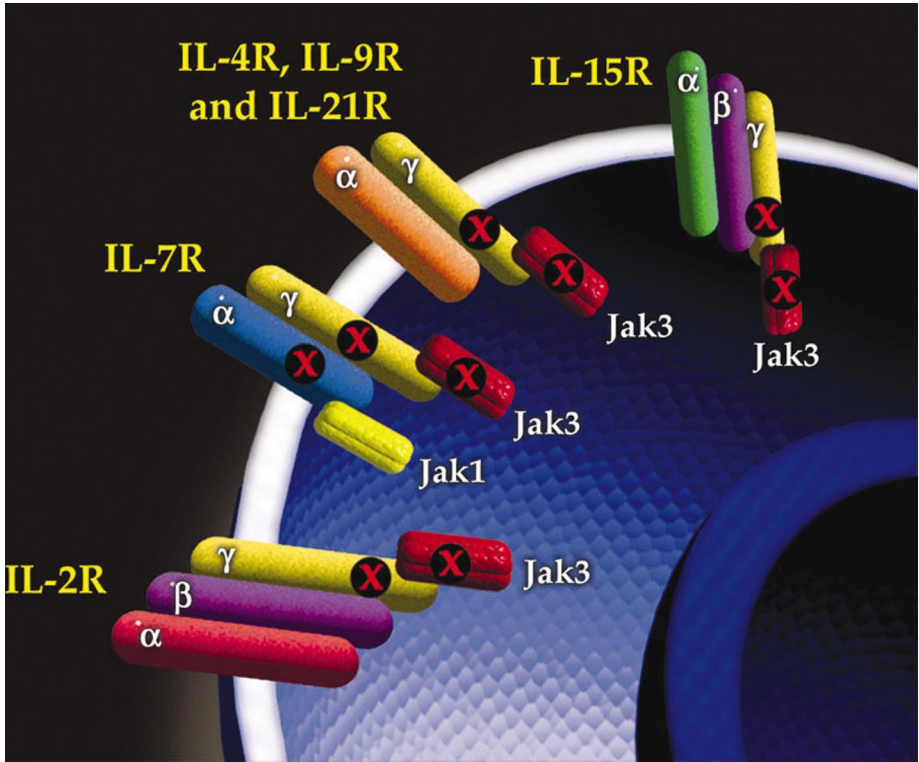


Figure 5 Diagram showing that Janus kinase 3 (Jak3) is the major signal transducer for the common gamma chain (γ_c) shared by multiple cytokine receptors. Mutations in the *IL-2RG* gene cause X-linked SCID, whereas mutations in the *Jak3* gene result in a form of autosomal recessive SCID that mimics X-SCID in lymphocyte phenotype (i.e., $T^-B^+NK^-$). Mutations in the alpha chain of the IL-7 receptor also cause SCID, but unlike X-linked and Jak3-deficient SCID, IL-7R α chain-deficient SCID infants have both B and NK cells (i.e., are T^-B^+,NK^+). [Figure reprinted with permission from (27a).]