NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Pediatr Transplant. Author manuscript; available in PMC 2012 November 1.

Published in final edited form as:
Pediatr Transplant. 2011 November ; 15(7): 733–741. doi:10.1111/j.1399-3046.2011.01563.x.

Genotype, Phenotype and Outcomes of Nine Patients with T-B +NK+ SCID

Grace P Yu[1,a], **Kari C Nadeau**[1,a], **David R Berk**[2], **Geneviève de Saint Basile**[3,4,5], **Nathalie Lambert**[5], **Perrine Knapnougel**[3], **Joseph Roberts**[6], **Kristina Kavanau**[7], **Elizabeth Dunn**[7], **E. Richard Stiehm**[8], **David B Lewis**[1], **Dale T Umetsu**[9], **Jennifer M Puck**[10], and **Morton J Cowan**[7]

[1]Division of Immunology and Allergy, Department of Pediatrics, Stanford University School of Medicine and Lucile Packard Children's Hospital at Stanford

[2]Departments of Medicine and Pediatrics, Divisions of Dermatology, Washington University School of Medicine

[3]Inserm, U768, Paris, F-75015 France

[4]Université Paris Descartes, IRNEM (IFR95), Paris, F-75015 France

[5]AP-HP, Hôpital Necker Enfants-Malades, Unité d'Immunologie-Hématologie Pédiatrique, Paris, F-75015 France

[6]Department of Pediatrics and Immunology, Duke University Medical Center

[7]Division of Blood and Marrow Transplantation, Department of Pediatrics, University of California San Francisco Children's Hospital

[8]Divison of Immunology, Allergy and Rheumatology, Department of Pediatrics, Mattel Children's Hospital at the University of California Los Angeles

[9]Division of Allergy and Immunology, Department of Pediatrics, Children's Hospital Boston

[10]Department of Pediatrics, Institute for Human Genetics, University of California San Francisco Children's Hospital

Abstract

There are few reports of clinical presentation, genotype, and hematopoietic cell transplant (HCT) outcomes for T-B+NK+ SCID patients. Between 1981 and 2007, 8 of 84 SCID patients who received and/or were followed after HCT at UCSF had the T-B+NK+ phenotype. One additional T-B+NK+ SCID patient was identified as the sibling of a patient treated at UCSF. Chart reviews were performed. Molecular analyses of *IL7R, IL2RG, JAK3* and the genes encoding the CD3 T-cell receptor components δ (CD3D), ε (CD3E), and ζ (CD3Z) were done. IL7R mutations were documented in 4 patients and CD3D mutations in 2 others. Three patients had no defects found. Only 2/9 patients had an HLA-matched related HCT donor. Both survived, and neither developed graft-versus-host disease. Five of 7 recipients of haploidentical grafts survived. Although the majority of reported cases of T-B+NK+ SCID are due to defects in IL7R, CD3 complex defects were also found in this series and should be considered when evaluating patients with T-B+NK+ SCID. Additional genes, mutations in which account for T-B+NK+ SCID, remain to be found.

Address correspondence and reprints to: Morton J Cowan, M.D., Chief, Division of Blood and Marrow Transplantation, University of California San Francisco Children's Hospital, Room M659, 505 Parnassus Ave, San Francisco, CA 94143-1278, Phone: 415-476-2188 Fax: 415-502-4867 mcowan@peds.ucsf.edu.
[a]Both authors contributed equally

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Better approaches to early diagnosis and HCT treatment are needed for patients lacking an HLA-matched related donor.

Introduction

Classic SCID patients have a profound defect in T cell numbers and T and B cell function, while B cell and NK cell numbers are variably affected. Severe combined immunodeficiency (SCID) is lethal early in life unless the cellular immune system is reconstituted. Over 14 disease genes for SCID are known (1-29) and a classification of cellular phenotypes has been established that correlates lymphocyte subset profile with the effects of impairment of particular immune cell developmental pathways.

The T-B+NK+ SCID phenotype has been reported for patients with mutations of genes encoding IL-7Rα, CD3δ, CD3ε, and CD3ζ although *IL2RG* (encoding the common γ chain γc defective in X-linked SCID) and *JAK3* mutations as causes of this phenotype have also been seen. (27, 30) Currently, the literature contains 49 cases of the IL-7Rα chain deficiency (12-15, 28, 31-38), 8 cases of CD3δ chain deficiency (39, 40), 3 cases of CD3ε chain deficiency (40), and 2 cases of CD3ζ chain deficiency. (41, 42)

Controversy exists regarding the need for conditioning prior to hematopoietic cell transplantation (HCT) in all forms of SCID. In particular, limited numbers of reports describe the clinical outcome of T-B+NK+ SCID patients after HCT, and it is not clear whether the presence of host NK cells has an impact on their outcome. (12, 13, 20, 32-34, 36, 43-50) Most published series of these patients were transplanted when the molecular cause of their SCID was still undiscovered. From 1981 to 2007, 8 T-B+NK+ SCID patients were transplanted (n=7) or followed shortly after transplant (n=1) at the University of California San Francisco (UCSF). An additional SCID patient was identified as a sibling of a patient who was treated at UCSF. The aim of our study was to study the incidence of the genetic defects leading to the T-B+NK+ SCID phenotype and evaluate the long-term engraftment and survival of these patients.

Patients and Methods

Subjects

All studies were approved by the UCSF Institutional Review Board. From a database of 84 SCID patients transplanted at UCSF between 1981 and 2007, 8 T-B+NK+ patients were identified. An additional T-B+NK+ patient was identified as a sibling of a patient treated at UCSF. A retrospective chart review was performed to summarize their presentations and clinical courses.

Cell Function

Peripheral blood lymphocyte subsets were determined by flow cytometry. T-cell function was assayed using lymphocyte mitogen responses to phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM). A $CD3^+$ T-lymphocyte count less than 300/μL and PHA response <10% of control were considered diagnostic of classic SCID when there was no evidence of maternal engraftment. (46) Serum immunoglobulin levels were analyzed via standard methods.

Transplantation methods

Seven of the 9 hematopoietic cell grafts were T-cell depleted using standard methods to prevent graft versus host disease (GVHD). (46) Patient 1a received donor stem cells

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

depleted of mature T cells by an anti-CD2 monoclonal antibody and complement, while patient 1b received an undepleted graft from her post-BMT sibling. (48) Both of these patients received cyclosporine for GVHD prophylaxis.

Post-HCT chimerism was determined by *in situ* hybridization for sex-disparate recipients and donors, quantitative PCR using short tandem repeats (STR) of peripheral blood mononuclear cells (PBMC), or specific lineages separated by magnetic beads. (51, 52) For each leukocyte lineage, mixed chimerism was defined as the presence of 5-95% donor-derived cells.

Mutational analysis

DNA was prepared from pre-HCT blood or skin fibroblasts from patients and blood from parents if available. Mutational analysis of *IL7R* was performed for all patients by sequencing the flanking and coding regions of exons 1-8 of the gene via previously published primers. (28) All patients who tested negative for *IL7R* mutations were subsequently tested for mutations in the genes encoding CD3δ, CD3ε, and CD3ζ chain via previously published methods. (40, 42) All male patients who tested negative for mutations in the *IL7R* or *CD3* subunit genes were tested for mutations in *IL2RG* via previously published methods. (11, 27) The remaining patients who tested negative for the above mutations were tested for *JAK3* mutations.

Results

Demographics

The patients included 6 females and 3 males from 6 unrelated families. (Table 1) Parental consanguinity occurred in 1 family (P2). The mean age at diagnosis of SCID was 8.9 months (range 2 days to 16 months). Two children (P1b, P4b) were diagnosed soon after birth because of a family history of SCID. Two sisters (P5a and 5b) presented with autoimmune hemolytic anemia (AIHA), one of whom also had immune thrombocytopenic purpura (ITP). The remaining patients presented with typical clinical features of SCID including failure to thrive, diarrhea, and recurrent and/or opportunistic infections.

Immunologic features prior to HCT

The primary immunologic characteristics of the patients at presentation are shown in Table 2. All patients presented with lymphopenia for their age. (53) The number of circulating CD3+ T lymphocytes was markedly depressed in all patients, ranging from undetectable to 238/μL. Maternal T-cell engraftment occurred in one patient (P5b). All patients had a decreased *in vitro* response to PHA of less than 10% of normal control except for one patient (P1b) where it was not measured. B cell numbers were normal to increased in all patients (79/μL to 1818/μL). As expected due to maternal transfer, Immunoglobulin levels showed variability in IgG levels. Circulating NK lymphocyte numbers were normal (range 63/μL to 728/μL).

Genetic Defects

Four patients from 2 families (1 and 5) were found to have *IL7R* mutations (Table 3). All had the previously published single nucleotide change in exon 5 cDNA 638C>T, causing the formation of a premature stop codon R206X. (13) The brother and sister pair P4a, P4b had a previously published nonsense mutation in exon 2 of CD3δ, cDNA 202C>T leading to the formation of a premature stop codon R68X. (39, 40) There were no mutations found in the coding or splice regions of the *CD3E, CD3Z, IL2RG*, or *JAK3* genes. Adenosine deaminase and purine nucleoside phosphorylase levels were normal in 5 patients (P1a, P1b, P2, P4a, and P5a) in whom these were tested.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Outcomes of HCT in T-B+NK+ SCID patients

Overall—Despite significant infections or autoimmune disease in 7 of the 9 patients, all were treated with HCT (Table 3). The mean age at transplantation was 10.2 months (range 5 weeks to 16 months). From 1987 to 1994, the source of cells for transplant was bone marrow. After 1994, peripheral blood stem cells mobilized with G-CSF were used for transplantation. The only exceptions were one HCT with marrow from an HLA-identical sibling (P5b) and one marrow from an HLA-matched sibling affected with SCID who had previously been reconstituted by paternal haploidentical bone marrow (P1b). (48) No patients experienced graft-versus-host disease.Seven patients were treated with haploidentical related HCTs (Table 3). Two of the 7 patients did not receive conditioning prior to HCT.

Individual Subject Treatment courses and Outcomes

Subject P2 had evidence of engraftment but died 5 months after transplant from respiratory failure secondary to RSV infection.

Subject P1a had decreasing T cell reconstitution requiring a boost without conditioning 23 months later and is currently alive and healthy.

Subject P5b received a bone marrow transplant from an HLA-identical sibling without conditioning. This subject is currently engrafted and has mixed chimerism of T, B, and NK lineages.

Subject P1b received a bone marrow transplant without conditioning from an HLA-matched sibling with SCID who had previously received a paternal haploidentical bone marrow transplant. This patient is durably engrafted with mixed chimerism of the T, B, and NK lineages.

Subject P4a suffered from cytomegalovirus (CMV) pneumonia and hepatitis at presentation. An immunosuppressive conditioning regimen of anti-thymocyte globulin (ATG) was used prior to maternal haploidentical BMT. The conditioning regimen was attenuated to minimize likelihood of disseminated CMV infection. However, the patient did not engraft despite the use of 1.5×10^{8}cells/kg of SBA-SRBC+ bone marrow cells. A subsequent conditioning regimen with ATG and cyclophosphamide for a second T cell depleted HCT, this time from his father, led to only transient engraftment. His third HCT consisted of a more aggressive myeloablative conditioning regimen of ATG, cyclophosphamide, and 700 cGy of fractionated total body irradiation (TBI). However, 1 month after this transplant, he succumbed to CMV pneumonia. His donor chimerism studies at that time did not show evidence of T or B cell engraftment.

Subjects P4b and P5a had haploidentical related HCTs with a conditioning regimen of cyclophosphamide and ATG. Both showed declining T-cell reconstitution after the first BMT and subsequently received a booster HCT 3 months and 19 months later (respectively) that led to durable engraftment. P4b has evidence of mixed T cell and myeloid chimerism while P5a has evidence of both T and B cell chimerism.

Subject P6 successively engrafted with a myeloablative conditioning regimen of busulfan, ATG, and cyclophosphamide prior to haploidentical related HCT and has evidence of T cell, B cell, NK cell, and myeloid mixed chimerism

Subject P3 had a conditioning regimen of anti-thymocyte globulin, cyclophosphamide and 700 cGY of TBI and is alive with evidence of T cell mixed chimerism.

Seven of the 9 patients (78%) remain alive a mean of 16 years post-HCT (range 6 to 23 years) (Table 3). All survivors have normal CD3 counts except for P1b and P4b. The *in vitro* response to PHA improved or normalized in all survivors. In addition to one patient who did

NIH-PA Author Manuscript

not receive a conditioning regimen(P1a), the other 4 patients [who received conditioning regimens (P3, P4b, P5a, P6)] have full B-cell reconstitution. All survivors have been followed between 6-23 years post transplant. All are healthy except for P3, who has short stature, osteochondromas and bilateral cataracts, presumably from the TBI he received 22 years ago.

Discussion

The majority of cases of T-B+NK+ SCID are reportedly caused by deficiency of the IL-7 receptor α chain, encoded by the *IL7R* gene. We found 4 of our 9 patients in 2 families had the IL-7 receptor alpha chain deficiency. Less commonly reported causes of T-B+NK+ SCID are defects in the genes encoding the CD3δ, CD3ε, and CD3ζ chains involved in the intracellular transmission of signals after TCR recognition. We discovered that 2 siblings in another family had the CD3δ chain deficiency. Sequencing for X-linked SCID, which is due to mutations in *IL2RG*, was also performed because it is the single most common genetic form of this disease and leaky phenotypes with substantial numbers of NK cells have been reported. Although no *IL2RG* defects were found in this series, sequencing for *IL2RG* along with *IL7R* and *CD3D, CD3E*, and *CD3Z* should be a considered when evaluating children with this immune phenotype. If these genes do not harbor defects, defects in *JAK3* and *CD45* should be considered. Although autosomal *JAK3* defects are a less common cause of SCID than X-linked *IL2RG*, these genotypes are phenotypically similar in all other respects. Determining the genetic defect is important for genetic counseling and possibly in the approach to transplant.

The role of conditioning in HCT for SCID remains controversial. Engraftment without pretransplant chemotherapy may be possible because SCID patients lack T-cell immune function and cannot easily reject their grafts. (32) This is certainly true for children with HLA matched sibling donors and both patients (P1b and P5b) in this study with HLA matched HCT did not receive pretransplant chemotherapy and durably engrafted. However, engraftment may be more problematic for mismatched alternative donor transplants. A European analysis of 178 SCID patients treated with HLA non-identical T-cell depleted BMT showed that a conditioning regimen of busulfan and cyclophosphamide resulted in higher engraftment rates. (49) However, a large European experience with 475 SCID patients did not show significant differences in survival between regimens that included conditioning and those that did not. (47) A U.S. center with 48 SCID patients saw a trend towards increased survival in the group that received conditioning, although statistical significance was not achieved, possibly because of the small patient numbers. (35) It is believed that NK cells mediate engraftment resistance. (54-56) Recently, a prospective pilot study of 15 consecutive patients undergoing haplocompatible transplants for SCID without conditioning showed that megadoses of CD34+ cells with a fixed dose of CD3+ cells resulted in 87% survival at a median of 39 months post transplant. (57) All of the NK- SCID patients engrafted but only 43% of the NK+ SCID patients who did not have detectable maternal cells pre-transplant engrafted without conditioning, further suggesting that host NK cells are capable of mediating donor hematopoietic stem cell rejection. In our experience (M. Cowan, unpublished data), the presence of NK cells in patients with SCID is associated with normal NK function in vitro.

Our patient numbers were too small to yield statistical conclusions regarding conditioning in haploidentical transplants. However, analysis of the individual patients receiving T-cell depleted haploidentical transplants showed that those treated with conditioning had a higher survival rate than those treated without conditioning. This is despite the fact that the patients with conditioning presented on average with more pre-transplant morbidity, were transplanted later that those patients without conditioning, and received similar doses of CD34+ stem cells. Furthermore, the one NK+ SCID patient who died (P4a with CD3δ

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

deficiency) was resistant to engraftment and required 2 additional HCTs with successively more myeloablative conditioning regimens. This suggests that transplant without conditioning would have been unsuccessful as well. However, our sample size was small and larger numbers of patients are needed to address the issues of whether host NK cells mediate graft resistance and whether conditioning improves survival and long-term engraftment in haploidentical transplants for NK+ SCID patients.

Analysis of the surviving patients who successfully engrafted showed evidence of mixed T chimerism with variable amounts of B and NK mixed chimerism. Our sample size was too small to determine whether the conditioning regimen affected donor chimerism.

It is well known that hematopoietic cell transplantation can cure SCID, with the highest success rates occurring with HLA identical related donors. Our outcome data for T-B+NK+ SCID patients at UCSF is similar to that from other centers; our patients have had 100% survival following HLA identical related transplants and 71% survival following haploidentical transplants. (32, 35, 47) However, HLA identical related donors are frequently not available and active infection often prevents a prolonged search for unrelated donors.

Determining the genetic defect is important for genetic counseling and possibly in the approach to transplant. For example, in patients with IL-7Ra deficient SCID, B cells can function once T cell immunity is restored so that myeloablative therapy may not be needed in order to restore both T and B cell immunity. (58) Consistent with this, patients with IL-7Ra deficient SCID in this case series (P1a, P1b, P5a, P5b) appeared to require less conditioning for engraftment compared to patients with other genetic defects and yet all had restoration of B cell function post transplant.

In order to improve the survival of SCID patients, it is important to determine optimal transplant regimens. Randomized, controlled, multi-center studies of treatment protocols are needed to find the optimal approach for children with SCID. However, such studies have not yet been done, in part due to the rarity of SCID and the multiplicity of different infectious exposures and underlying mutations and phenotypes that characterize SCID. Nonetheless, collaboration in this effort is imperative in order to give these children the best possible outcomes.

Those patients who were diagnosed at a young age because of family history of SCID (P1b, P4b) had no infectious complications, improved survival, and far fewer complications post HCT compared to patients who were diagnosed later and suffered from infectious complications prior to their diagnosis and treatment of SCID. The two patients who died in this case series after haploidentical HCT succumbed to infectious complications. P2 received a haploidentical transplant at 12.5 months without conditioning but, despite evidence of engraftment, succumbed to RSV infection. P4a presented with cytomegalovirus (CMV) pneumonia and hepatitis at 14 months of age. As a result his conditioning regimen was attenuated to prevent disseminated CMV infection. Unfortunately he did not engraft despite 3 haploidentical transplants, each with successively more myeloablative conditioning regimens. He ultimately succumbed to disseminated CMV infection. Our experience reinforces the published data that survival is improved if SCID is diagnosed at an early age, prior to infectious complications, and transplant occurs before 3.5 months of age. (20, 47, 49) As a result, it is critical to diagnose infants with SCID via newborn screening programs (59).

NIH-PA Author Manuscript

Acknowledgments

We are grateful to Gabriel Tsao M.D. and Irene H. Jun, M.D. and for their invaluable work in reviewing patient charts and Thierry Giffon Ph.D. for valuable assistance in the genetic analyses of our patients. This work was made possible by the American Academy of Pediatrics Residency Research Grant and the Stanford University School of Medicine Medical Scholars Grant. MJC and JP are supported by NIH U54 AI082973-01.

References

1. Villa A, Santagata S, Bozzi F, et al. Partial V(D)J recombination activity leads to Omenn syndrome. Cell. 1998; 93:885–896. [PubMed: 9630231]
2. Notarangelo LD, Santagata S, Villa A. Recombinase activating gene enzymes of lymphocytes. Curr Opin Hematol. 2001; 8:41–46. [PubMed: 11138625]
3. de Saint-Basile G, Le Deist F, de Villartay JP, et al. Restricted heterogeneity of T lymphocytes in combined immunodeficiency with hypereosinophilia (Omenn's syndrome). J Clin Invest. 1991; 87:1352–1359. [PubMed: 2010548]
4. Ochs HD, Davis SD, Mickelson E, Lerner KG, Wedgwood RJ. Combined immunodeficiency and reticuloendotheliosis with eosinophilia. J Pediatr. 1974; 85:463–465. [PubMed: 4443853]
5. Cederbaum SD, Niwayama G, Stiehm ER, Neerhout RC, Ammann AJ, Berman W Jr. Combined immunodeficiency presenting as the Letterer-Siwe syndrome. J Pediatr. 1974; 85:466–471. [PubMed: 4443854]
6. Omenn GS. Familial Reticuloendotheliosis with Eosinophilia. The New England journal of medicine. 1965; 273:427–432. [PubMed: 14328107]
7. Moshous D, Callebaut I, de Chasseval R, et al. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. Cell. 2001; 105:177–186. [PubMed: 11336668]
8. Moshous D, Li L, Chasseval R, et al. A new gene involved in DNA double-strand break repair and V(D)J recombination is located on human chromosome 10p. Hum Mol Genet. 2000; 9:583–588. [PubMed: 10699181]
9. Li L, Drayna D, Hu D, et al. The gene for severe combined immunodeficiency disease in Athabascan-speaking Native Americans is located on chromosome 10p. Am J Hum Genet. 1998; 62:136–144. [PubMed: 9443881]
10. Villa A, Sobacchi C, Notarangelo LD, et al. V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. Blood. 2001; 97:81–88. [PubMed: 11133745]
11. Lebet T, Chiles R, Hsu AP, Mansfield ES, Warrington JA, Puck JM. Mutations causing severe combined immunodeficiency: detection with a custom resequencing microarray. Genet Med. 2008; 10:575–585. [PubMed: 18641513]
12. Butte MJ, Haines C, Bonilla FA, Puck J. IL-7 receptor deficient SCID with a unique intronic mutation and post-transplant autoimmunity due to chronic GVHD. Clin Immunol. 2007; 125:159–164. [PubMed: 17827065]
13. Giliani S, Mori L, de Saint Basile G, et al. Interleukin-7 receptor alpha (IL-7Ralpha) deficiency: cellular and molecular bases. Analysis of clinical, immunological, and molecular features in 16 novel patients. Immunol Rev. 2005; 203:110–126. [PubMed: 15661025]
14. Roifman CM, Zhang J, Chitayat D, Sharfe N. A partial deficiency of interleukin-7R alpha is sufficient to abrogate T-cell development and cause severe combined immunodeficiency. Blood. 2000; 96:2803–2807. [PubMed: 11023514]
15. Puel A, Leonard WJ. Mutations in the gene for the IL-7 receptor result in T(-)B(+)NK(+) severe combined immunodeficiency disease. Curr Opin Immunol. 2000; 12:468–473. [PubMed: 10899029]
16. Roberts JL, Lengi A, Brown SM, et al. Janus kinase 3 (JAK3) deficiency: clinical, immunologic, and molecular analyses of 10 patients and outcomes of stem cell transplantation. Blood. 2004; 103:2009–2018. [PubMed: 14615376]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

17. Mella P, Schumacher RF, Cranston T, de Saint Basile G, Savoldi G, Notarangelo LD. Eleven novel JAK3 mutations in patients with severe combined immunodeficiency-including the first patients with mutations in the kinase domain. Hum Mutat. 2001; 18:355–356. [PubMed: 11668621]
18. Frucht DM, Gadina M, Jagadeesh GJ, et al. Unexpected and variable phenotypes in a family with JAK3 deficiency. Genes Immun. 2001; 2:422–432. [PubMed: 11781709]
19. Schumacher RF, Mella P, Badolato R, et al. Complete genomic organization of the human JAK3 gene and mutation analysis in severe combined immunodeficiency by single-strand conformation polymorphism. Hum Genet. 2000; 106:73–79. [PubMed: 10982185]
20. Buckley RH, Schiff SE, Schiff RI, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. The New England journal of medicine. 1999; 340:508–516. [PubMed: 10021471]
21. Buckley RH, Schiff RI, Schiff SE, et al. Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. J Pediatr. 1997; 130:378–387. [PubMed: 9063412]
22. Bozzi F, Lefranc G, Villa A, et al. Molecular and biochemical characterization of JAK3 deficiency in a patient with severe combined immunodeficiency over 20 years after bone marrow transplantation: implications for treatment. Br J Haematol. 1998; 102:1363–1366. [PubMed: 9753072]
23. Candotti F, Oakes SA, Johnston JA, et al. Structural and functional basis for JAK3-deficient severe combined immunodeficiency. Blood. 1997; 90:3996–4003. [PubMed: 9354668]
24. Russell SM, Tayebi N, Nakajima H, et al. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science. 1995; 270:797–800. [PubMed: 7481768]
25. Macchi P, Villa A, Giliani S, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature. 1995; 377:65–68. [PubMed: 7659163]
26. Russell SM, Johnston JA, Noguchi M, et al. Interaction of IL-2R beta and gamma c chains with Jak1 and Jak3: implications for XSCID and XCID. Science. 1994; 266:1042–1045. [PubMed: 7973658]
27. Puck JM, Pepper AE, Henthorn PS, et al. Mutation analysis of IL2RG in human X-linked severe combined immunodeficiency. Blood. 1997; 89:1968–1977. [PubMed: 9058718]
28. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. Nat Genet. 1998; 20:394–397. [PubMed: 9843216]
29. Schwarz K, Gauss GH, Ludwig L, et al. RAG mutations in human B cell-negative SCID. Science. 1996; 274:97–99. [PubMed: 8810255]
30. Notarangelo LD, Mella P, Jones A, et al. Mutations in severe combined immune deficiency (SCID) due to JAK3 deficiency. Hum Mutat. 2001; 18:255–263. [PubMed: 11668610]
31. Jo EK, Kook H, Uchiyama T, et al. Characterization of a novel nonsense mutation in the interleukin-7 receptor alpha gene in a Korean patient with severe combined immunodeficiency. International journal of hematology. 2004; 80:332–335. [PubMed: 15615257]
32. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Annu Rev Immunol. 2004; 22:625–655. [PubMed: 15032591]
33. Ponda P, Schuval SJ, Kaplan B, Logalbo P, Roberts JL, Bonagura VR. Interleukin 7 receptor alpha-chain-mutation severe combined immunodeficiency without lymphopenia: correction with haploidentical T-cell-depleted bone marrow transplantation. Ann Allergy Asthma Immunol. 2006; 97:755–758. [PubMed: 17201233]
34. Ahmed N, Leung KS, Rosenblatt H, et al. Successful treatment of stem cell graft failure in pediatric patients using a submyeloablative regimen of campath-1H and fludarabine. Biol Blood Marrow Transplant. 2008; 14:1298–1304. [PubMed: 18940685]
35. Patel NC, Chinen J, Rosenblatt HM, et al. Outcomes of patients with severe combined immunodeficiency treated with hematopoietic stem cell transplantation with and without preconditioning. J Allergy Clin Immunol. 2009; 124:1062–1069. e1061–1064. [PubMed: 19895994]
36. Neven B, Leroy S, Decaluwe H, et al. Long-term outcome after hematopoietic stem cell transplantation of a single-center cohort of 90 patients with severe combined immunodeficiency. Blood. 2009; 113:4114–4124. [PubMed: 19168787]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

37. Rossberg S, Schwarz K, Meisel C, et al. Delayed onset of (severe) combined immunodeficiency (S)CID (T-B+NK+): complete IL-7 receptor deficiency in a 22 months old girl. Klin Padiatr. 2009; 221:339–343. [PubMed: 19890784]
38. Zhang ZY, Zhao XD, Wang M, Yu J, An YF, Yang XQ. A compound heterozygosity mutation in the interleukin-7 receptor-alpha gene resulted in severe combined immunodeficiency in a Chinese patient. Zhonghua Er Ke Za Zhi. 2009; 47:691–695. [PubMed: 20021794]
39. Dadi HK, Simon AJ, Roifman CM. Effect of CD3delta deficiency on maturation of alpha/beta and gamma/delta T-cell lineages in severe combined immunodeficiency. The New England journal of medicine. 2003; 349:1821–1828. [PubMed: 14602880]
40. de Saint Basile G, Geissmann F, Flori E, et al. Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3. J Clin Invest. 2004; 114:1512–1517. [PubMed: 15546002]
41. Rieux-Laucat F, Hivroz C, Lim A, et al. Inherited and somatic CD3zeta mutations in a patient with T-cell deficiency. The New England journal of medicine. 2006; 354:1913–1921. [PubMed: 16672702]
42. Roberts JL, Lauritsen JP, Cooney M, et al. T-B+NK+ severe combined immunodeficiency caused by complete deficiency of the CD3{zeta} subunit of the T cell antigen receptor complex. Blood. 2006
43. Grunebaum E, Mazzolari E, Porta F, et al. Bone marrow transplantation for severe combined immune deficiency. JAMA. 2006; 295:508–518. [PubMed: 16449616]
44. Friedrich W, Honig M, Muller SM. Long-term follow-up in patients with severe combined immunodeficiency treated by bone marrow transplantation. Immunol Res. 2007; 38:165–173. [PubMed: 17917023]
45. Roifman CM, Grunebaum E, Dalal I, Notarangelo L. Matched unrelated bone marrow transplant for severe combined immunodeficiency. Immunol Res. 2007; 38:191–200. [PubMed: 17917025]
46. Dror Y, Gallagher R, Wara DW, et al. Immune reconstitution in severe combined immunodeficiency disease after lectin-treated, T-cell-depleted haplocompatible bone marrow transplantation. Blood. 1993; 81:2021–2030. [PubMed: 8471764]
47. Antoine C, Muller S, Cant A, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. Lancet. 2003; 361:553–560. [PubMed: 12598139]
48. Stiehm ER, Roberts RL, Hanley-Lopez J, et al. Bone marrow transplantation in severe combined immunodeficiency from a sibling who had received a paternal bone marrow transplant. The New England journal of medicine. 1996; 335:1811–1814. [PubMed: 8943163]
49. Bertrand Y, Landais P, Friedrich W, et al. Influence of severe combined immunodeficiency phenotype on the outcome of HLA non-identical, T-cell-depleted bone marrow transplantation: a retrospective European survey from the European group for bone marrow transplantation and the european society for immunodeficiency. J Pediatr. 1999; 134:740–748. [PubMed: 10356144]
50. Patel NC, Chinen J, Rosenblatt HM, et al. Long-term outcomes of nonconditioned patients with severe combined immunodeficiency transplanted with HLA-identical or haploidentical bone marrow depleted of T cells with anti-CD6 mAb. J Allergy Clin Immunol. 2008; 122:1185–1193. [PubMed: 19084111]
51. Ozyurek E, Cowan MJ, Koerper MA, Baxter-Lowe LA, Dvorak CC, Horn BN. Increasing mixed chimerism and the risk of graft loss in children undergoing allogeneic hematopoietic stem cell transplantation for non-malignant disorders. Bone Marrow Transplant. 2008; 42:83–91. [PubMed: 18391990]
52. Geha RS, Notarangelo LD, Casanova JL, et al. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol. 2007; 120:776–794. [PubMed: 17952897]
53. Comans-Bitter WM, de Groot R, van den Beemd R, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr. 1997; 130:388–393. [PubMed: 9063413]
54. Kamel-Reid S, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. Science. 1988; 242:1706–1709. [PubMed: 2904703]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

55. Murphy WJ, Kumar V, Bennett M. Rejection of bone marrow allografts by mice with severe combined immune deficiency (SCID). Evidence that natural killer cells can mediate the specificity of marrow graft rejection. J Exp Med. 1987; 165:1212–1217. [PubMed: 3549961]
56. Hamby K, Trexler A, Pearson TC, Larsen CP, Rigby MR, Kean LS. NK cells rapidly reject allogeneic bone marrow in the spleen through a perforin- and Ly49D-dependent, but NKG2D-independent mechanism. Am J Transplant. 2007; 7:1884–1896. [PubMed: 17617852]
57. Dvorak CC, Hung GY, Horn B, Dunn E, Oon CY, Cowan MJ. Megadose CD34(+) cell grafts improve recovery of T cell engraftment but not B cell immunity in patients with severe combined immunodeficiency disease undergoing haplocompatible nonmyeloablative transplantation. Biol Blood Marrow Transplant. 2008; 14:1125–1133. [PubMed: 18804042]
58. Railey MD, Lokhnygina Y, Buckley RH. Long-term clinical outcome of patients with severe combined immunodeficiency who received related donor bone marrow transplants without pretransplant chemotherapy or post-transplant GVHD prophylaxis. J Pediatr. 2009; 155:834–840. e831. [PubMed: 19818451]
59. Routes JM, Grossman WJ, Verbsky J, et al. Statewide newborn screening for severe T-cell lymphopenia. JAMA. 2009; 302:2465–2470. [PubMed: 19996402]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Clinical Presentation of 9 Patients with T-B+NK+ SCID

Patient	Sex	Age at Diagnosis	Presentation	Parental consanguinity
1a	F	7 mo	*Pneumocystis jiroveci* pneumonia, failure to thrive, oral candidiasis	No
1b	F	2 dys	Family history of sister with SCID, no thymic shadow on chest X-ray, lymphopenia	No
2	M	12 mo	*Pneumocystis jiroveci* pneumonia, diarrhea, failure to thrive, otitis media,	Yes
3	M	15 mo	Diarrhea, failure to thrive, chronic upper respiratory infection, pneumonia, recurrent otitis media, aphthous ulcers	No
4a	M	13 mo	Otitis media, pneumonia, oral thrush, *Candida* diaper dermatitis, diarrhea, hepatosplenomegaly, fever, anorexia, pulmonary aspergillosis, CMV hepatitis and pneumonitis, *Clostridium difficile* infection	No
4b	F	6 dys	Family history of brother with SCID	No
5a	F	13 mo	Diarrhea, failure to thrive, autoimmune hemolytic anemia, pneumococcal sepsis, meningococcal meningitis, parainfluenza upper respiratory tract infection	No
5b	F	12 mo	Diarrhea, autoimmune hemolytic anemia, immune thrombocytopenic purpura	No
6	F	8 mo	Jejunal atresia s/p resection, failure to thrive, diarrhea, oral and perineal candidiasis, seizures secondary hypocalcemia	No

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 2

Presenting immunological features of 9 T-B+NK+ SCID patients

Patient	Lymphocyte Absolute Counts (per µL)	CD3 Absolute Count (per µL)	CD4 Absolute Count (per µL)	CD8 Absolute Count (per µL)	CD 19 Absolute Count (per µL)	CD16/56 Absolute Count (per µL)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	PHA (%)	Maternal Engraftment
1a	2300	20	10	10	855	n.d.	42	<7	51	0%	No
1b	340	110	2	7	1140	728	1560	<7	8	n.d.	No
2	1620	<20	<20	<20	1818	121	1780	<7	32	0%	No
3	1150	46	35	23	196	127	346	9.8	98	0.4%	No
4a	1190	238	71	71	726	238	531	48	46.5	0.1%	No
4b	1530	<20	<20	<20	995	505	723	<6.7	25.2	1%	No
5a	880	88	106	18	79	651	1890	<5	600	0%	No
5b	1000	10	0	10	507	486	1540	86	388	1%	Yes (25% CD3+ cells maternal)
6	320	103	58	36	292	63	53	12.2	52	8%	No

Abbreviations: PHA = proliferation in response to phytohemagglutinin (% of control value), n.d. = not done

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 3

Hematopoietic Stem Cell Transplantation Regimens, Genetic Defects, and Outcomes in 9 patients with T-B+NK+ SCID

Patient	Gene Defect	HCT Age	Conditioning	Cells /kg	Donor Cell Type	Engraftment	Complications	Status; Time post HCT	Latest Donor Chimerism, %			
									T	B	NK	Other
1a	*IL7R* (cDNA 638C>T)	8 mo	None	1.3×10^8	Paternal T depleted BM	Yes, T-cell loss	None	Alive, well 20 yr	92	0	n.d.	XY (donor) PBMCs: 31.6
		Boost 31 mo	None	1.6×10^8	As above	Yes	None					
1b	*IL7R* (cDNA 638C>T)	5 wk	None	4.8×10^8	SCID sib [post HCT] BM	Yes	None	Alive, well 14 yr	95	100	n.d.	XY (donor) PBMCs: 95
2	Unk	12.5 mo	None	1.6×10^8	Maternal T depleted PBSC	Yes	RSV, respiratory failure, chronic lung disease, pulmonary shunting	Died 5 mo	n.d.	0	n.d.	XX (donor) PBMCs: 74
3	Unk	15 mo	ATG, cyclophosphamide, TBI (700 cGy)	5.3×10^8	Paternal T depleted BM	Yes	FTT, poor growth, slipped capital femoral epiphyses, cataracts, osteochondromas	Alive, well 23 yr	100	0	n.d.	n.d.
4a	*CD3D* (cDNA 202C>T)	14 mo	ATG	1.5×10^8	Maternal T depleted BM	No	CMV esophagitis, hepatitis, and retinitis; pseudomonas sinusitis	Died 1 mo	0	0	n.d.	Non-T non-B cells: 0
		Repeat 19 mo	ATG cyclophosphamide	2.5×10^8	Paternal T depleted BM	Yes, late rejection	Sepsis, thrombocytopenia, anemia					
		Repeat 28 mo	ATG, cyclophosphamide, TBI (700 cGy)	1.4×10^8	Paternal T depleted BM	Yes, early	Disseminated CMV infection including CMV pneumonia leading to respiratory failure					
4b	*CD3D* (cDNA 202C>T)	2mo	ATG cyclophosphamide	5.0×10^9	Maternal T depleted BM	Declining T cells	None	Alive, well 16 yr	81	0	4	CD14/15:100
		Boost: 5mo	None	8.0×10^7	As above	Yes	Persistent B cell deficiency requiring IVIG until 12 y/o					
5a	*IL7R* (cDNA 638C>T)	16 mo	ATG, cyclophosphamide	1.2×10^8	Maternal T depleted BM	T-cell loss	Diarrhea, FTT, Coombs positive hemolytic anemia, thrombocytopenia, Hepatitis C	Alive, well 18 yr	Donor HLA detected	Donor HLA detected	n.d.	Non-T non-B cells: Donor HLA detected
		Boost: 35 mo	None	1.8×10^8	As above	Yes	Sinusitis					
5b	*IL7R* (cDNA 638C>T)	13 mo	None	5.7×10^8	HLA-matched sibling BM	Yes	CMV treated with gancyclovir	Alive, well 6 yr	98	5	15	CD14/15: 1
6	Unk	10 mo	Busulfan, cyclophosphamide, ATG	5.6×10^8	Maternal T depleted PBSC	Yes	Enterococcus, *C. tropicalis*, and *Enterobacter cloacae* sepsis, G-tube Pseudomonas cellulitis, oral candidiasis	Alive, well 13 yr	77	65	76	CD14/15: 83

Abbreviations: BM = bone marrow; Unk = unknown; ATG = anti-thymocyte globulin; CMV = cytomegalovirus; TBI = total body irradiation; PBSC = peripheral blood stem cell; RSV = respiratory syncytial virus; FTT = failure to thrive; n.d., not done.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 4

Immunological reconstitution in the 7 surviving T-B+NK+ SCID patients after HCT

Patient	Lymphocyte Absolute Counts (per µL)	CD3 Absolute Count (per µL)	CD4 Absolute Count (per µL)	CD8 Absolute Count (per µL)	CD19 Absolute Count (per µL)	CD16/56 Absolute Count (per µL)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	PHA (%)
1a	704	169	120	99	338	ND	1130	87	90	9%
	Post boost: 1200	1071	452	621	355	127	1020	151	122	90%
1b	1976	439	178	277	1344	68	1060	123	66	90%
3	2680	1876	938	911	429	214	1220	88	168	100%
4b	Post BMT: 2300	<23	<23	<23	2070	207	n.a.	n.a.	n.a.	1%
	Post boost: 1000	370	270	70	190	420	1100	183	91.3	100%
5a	Post BMT: 750	195	120	75	248	262	1050	54.1	80.4	1%
	Post Boost: 1370	1445	673	693	218	297	n.a.	27.5	69	100%
5b	1700	888	273	546	512	256	658	60	211	35%
6	2330	1701	862	629	468	140	n.a.	35	278	64%

Abbreviations: PHA = proliferation in response to phytohemagglutin (% of control value), n.a. = not available