

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

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