# *Immunology Series*

# Severe Combined Immunodeficiencies of the Common γ-chain/JAK3 Signaling Pathway

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During the 50 years since the first description of a severe combined immunodeficiency [1] (the term SCID was coined 25 years later by Soothill), an increasing number of genes has been identified whose abnormalities account for this heterogenous group of diseases that are characterized by profound impairment in the development and/or function of both the cellular and the humoral part of the immune system. Affected patients develop severe and/or recurrent infections (often sustained by opportunistic pathogens) early in life. Chronic diarrhea and failure to thrive occur in most patients. SCID is usually fatal unless immune function is restored by successful hematopoietic stem-cell transplantation [2] or gene therapy. The typical immunologic phenotype of the most common form, which affects approximately 1/50–100,000 births and is thus responsible for almost 50% of SCID in humans, is characterized by a lack of circulating T (and natural killer) cells but a normal (to increased) number of B lymphocytes (T$^-$B$^+$ SCID). From a functional point of view, T cells fail to respond to *in vitro* stimulation with mitogens and to allogeneic cells, and B cells are unable to produce specific antibodies. Histologically, the thymus lacks a clear cortical-medullary differentiation, Hassall's corpuscles are absent, and there is usually a severe depletion of lymphoid tissues. This picture is due to defects in one of two genes encoding two proteins that play a crucial role in cytokine-induced signal transduction in lymphocytes: the common gamma chain [3] and JAK3 [4,5].
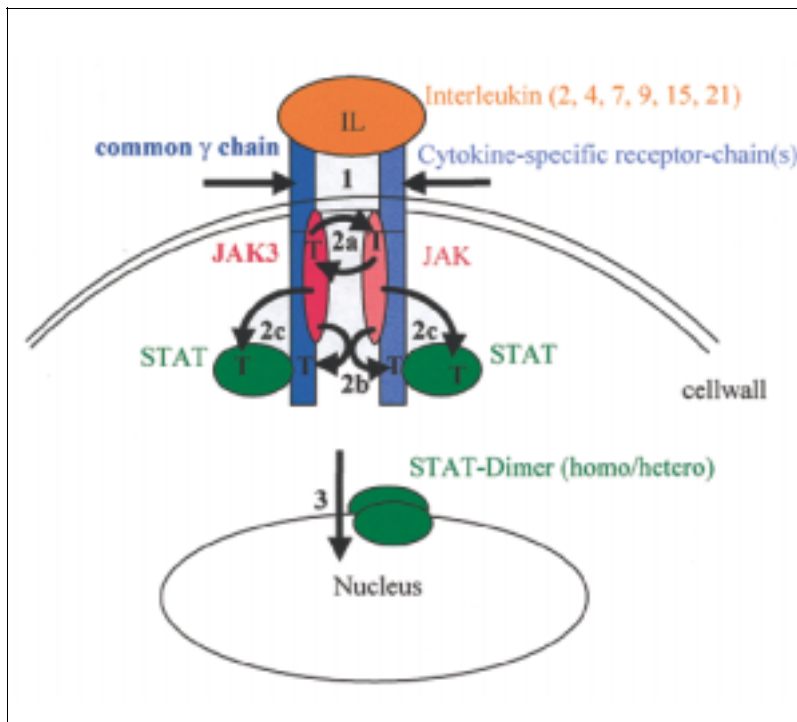
## Physiology

The development and immunologic activity of lymphocytes are regulated by cytokines, messenger proteins that circulate in the blood and initiate and govern a variety of inflammatory reactions, and cell differentiation/activation events, by interacting with the membrane-bound receptors on their target cells. Particularly interleukin-7 is important for early differentiation of

thymocyte precursors, whereas IL-2 is a powerful stimulator for peripheral blood T lymphocytes, and maintains T cell homeostasis. IL-15 (and probably IL-21 as well) is a differentiation factor for NK cells and IL-4 is important for terminal B cell differentiation and isotype switching.

Cytokine receptors of type 1 (to which most IL receptors belong) do not have intrinsic catalytic activity [6]. They are composed of two or more transmembrane subunits. The γc is such a cytokine receptor subunit that is shared by receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 [7]. As part of the hematopoietic cytokine receptor family, it is characterized by four conserved cysteines and the repeated WSXWS motif. To signal, those cytokine receptors that include γc as a component need an intracellular signal transducer. The primary transducer to the γc is JAK3, a member of the Janus-associated kinase family of protein tyrosine kinases. Different members of the JAK family show a similar structure, in that they present a COOH-terminal kinase domain, flanked by a non-identical non-catalytic pseudokinase domain. Four members of the JAK family have been identified in humans, and include JAK1, JAK2, TYK2, and JAK3. Another subunit of the same cytokine receptor will also have a member of the JAK family attached to the intracytoplasmic part of the chain next to the membrane. Upon binding of the cytokine to its receptor, a series of events that are crucial for signal transduction occur [Figure 1]: ligand-mediated heterodimerization of the receptor chains (one Interleukin-specific chain and the common gamma chain which transfers the signal) brings the associated JAKs close enough to each other to allow for reciprocal tyrosine phosphorylation (first phosphorylation event), resulting in activation of both JAKs. This activation leads within minutes to an increased enzymatic activity of JAKs that can then phosphorylate tyrosine residues in the more distal portion of the cytoplasmic tail of both receptor subunits (second phosphorylation), thus providing docking

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SCID = severe combined immunodeficiency
IL = interleukin

NK = natural killer
JAK = Janus-associated kinase

**Figure 1.** The common γ-chain / JAK3-signaling pathway upon specific IL binding
1. Heterodimerization of the receptor chains.
2. Three sequential tyrosine phosphorylations by JAK3:
   a. Reciprocal phosphorylation (activation) of both JAKs;
   b. Phosphorylation of both receptor subunits (docking sites);
   c. Phosphorylation of STATs (secondary messengers).
3. Homo- or heterodimerization of STATs allows them to reach the target genes in the nucleus.

sites for additional signaling proteins: the signal transducers and activators of transcription. Seven members of the STAT family are known in humans: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. Some specificity exists, so that different cytokine receptors recruit different STATs. This inter-action is mediated by the phosphorylated tyrosine residue on the cytokine receptor, and an SH2 (src homology 2) domain in the STAT molecules. As a result of this process, a receptorial super complex will be formed, which includes the cytokine receptor, the JAKs, and the STATs. Once bound to the cytokine receptor, STATs will themselves be phosphorylated by the JAKs on a single tyrosine residue close to the SH2 domain (third phosphorylation event). The phosphorylated STATs can then interact with each other, and form homo- or heterodimers. These dimers then cross the nuclear membrane and bind to regulatory sequences in the promoter of specific cytokine-inducible target genes.

Rapid regulation of JAK activity is achieved directly by differential autophosphorylation of tyrosine residues in the putative activation loop of the kinase domain and by inhibitory

proteins, such as JAB (JAK-binding protein). Indirect regulation via other molecules has also been described. Thus, a functioning γc/JAK3 signaling pathway is essential for ontogeny and homeostasis of the lymphoid system.

## Genetics

The high male to female ratio (3:1 up to 5:1) of patients affected with T$^-$B$^+$ SCID was the first clue that there might be more than one gene responsible for the disease. Indeed, in most cases, T$^-$B$^+$ SCID is inherited as an X-linked trait (and is also designated as SCIDX1, MIM#300400) and is due to γc defects, but a significant proportion of cases have an autosomal inheri-tance and are due to JAK3 defects.

The γc, a transmembrane protein composed of 369 amino acids, is encoded by *IL2RG*, a gene that maps to Xq12-13.1. The *IL2RG* mutation database (IL2RGbase, accessible on the inter-net at http://www.nhgri.nih.gov/dir/lgt/SCID/IL2RG base.html) lists over 150 different muta-tions in more than 220 unrelated patients [8,9]. For each generation a 30% rate for new muta-tions is expected by the Haldane rule for lethal X-linked diseases, as indeed confirmed in large case series. *De novo* mutations can occur not only in affected males but also in the maternal and most often in the grandfather's germ line, where they are difficult to diagnose and pose a risk for the offspring. SCIDX1-associated mutations oc-cur in any of the eight exons of the gene but are not uniformly distributed. Most defects occur in the extra-cellular portion (exon1–5), with the highest incidence in exon 5 that encodes the important WSXWS cytokine receptor motif. A genotype-phenotype correlation has been observed.

The *JAK3* gene has been identified as the defective gene in the second most common variant of T$^-$B$^+$ SCID, which is inherited as an autosomal recessive trait (JAK3 deficiency, MIM#600173). The *JAK3* gene (GenBank # U09607) maps on chromosome 19p12-13.1, the ORF of 3372 basepairs translates into a 1124 amino acid protein of approximately 125 kDa. The genomic organization of the human *JAK3* gene is composed of 23 exons [10]. To date, 23 JAK3-deficient patients from 21 different families have been described, whose mutations are accessible in the *JAK3* mutation database (posted on the internet at http://www.uta.fi/imt/bioinfo/JAK3base.html) [9,11]. Given the small number of mutations detected so far in this disease, definitive conclusions regarding distribution of the mutations in the seven structural domains and genotype/phenotype correlation are not yet possible. However, more recently, a few individuals have been identified who seem to be affected with a somewhat milder phenotype of the disease.

Since both proteins are adjacent in the same signaling

STAT = signal transducers and activators of transcription

pathway, defects in the *IL2RG* or in the *JAK3* genes result in an identical immunologic phenotype, which is due to the defect in all six involved functional cytokine/receptor units. Only IL-4 seems to also have a γc/JAK3 independent signaling pathway.

## Clinical features and immunology

Patients with X-linked or JAK3-deficient SCID are highly susceptible to severe infections from the first days of life. Profound lymphopenia, florid thrush in the oropharynx, persistent and severe pneumonia – often due to *Pneumocystis carinii* – and intractable diarrhea leading to failure to thrive, were already described in the first published cases 50 years ago [1]. Infections with viruses, especially from the herpes group, are also hazardous; in particular Epstein-Barr virus infection can induce B lymphocyte proliferative disorder, and cytomegalovirus is a leading cause of severe interstitial pneumonia and death in these patients.

An absolute lymphocyte count beneath the age-specific normal range is still the most simple laboratory hallmark for suspicion. The next diagnostic step in the evaluation of a child with suspected SCID is flow cytometry to enumerate the major lymphocyte subpopulations. Typically, patients with SCIDX1 or with JAK3 deficiency have a virtual absence of both T and NK cells, whereas the vast majority of circulating lymphocytes are represented by B cells. Serum immunoglobulin concentrations may be normal for IgG as long as maternal antibodies persist, but are low for IgA and IgM. Antigen (vaccine) stimulation results in lack of specific antibody production. Moreover, isoagglutinins are also undetectable [12].

Atypical immunologic presentations may occur and warrant careful evaluation. Infection with EBV may result in B lymphocyte proliferative disorder; in that case the lymphocyte count may be exceptionally high, but clonality of the expanded B cells and integration of the EBV-genome allow the correct diagnosis. Much more common (30–50% of all cases) is maternal T cell engraftment, which may vary in extent and severity. Occasionally, the number of circulating T cells may even be normal; in such cases however, there are specific abnormalities of the T cells, such as a skewed representation of CD4+ or of CD8+ cells, and a characteristic activation profile (CD45R0+, DR+) of the circulating T cells. From the clinical point of view, in most cases maternal T cell engraftment is asymptomatic but occasionally may cause features resembling graft versus host disease: namely skin rash, liver abnormalities, bone marrow aplasia, diarrhea. Importantly, viral infections (cytomegalovirus particularly) may activate maternal T cells, and hence contribute to exacerbating the clinical picture [12]. Even worse are the consequences of unirradiated blood transfusions in SCID infants, as they are followed within a few days by massive proliferation and activation of donor-derived T cells that cause a severe, and usually fatal, graft vs. host disease.

Ig = immunoglobulin
EBV = Epstein-Barr virus

This picture is reminiscent of the Omenn syndrome (and is in fact named Omenn-like presentation). However, while in Omenn syndrome the activated T cells are autologous, the allogeneic origin of the activated T cells in infants with T⁻B⁺ SCID and maternal T cell engraftment (or with post-transfusional graft vs. host disease) may be demonstrated by karyotyping, HLA typing, or with the use of polymorphic DNA markers.

Until the diagnosis of SCID is ruled out, the affected baby should be hospitalized in a protective environment with adequate antimicrobial prophylaxis, and administration of any live vaccine must be delayed. Especially bacille Calmette-Guerin vaccination can lead to disseminated mycobacterial infections that are very difficult to treat. Avoiding any infection is pivotal because once an infant becomes seriously infected – especially in the lungs [13] – the chances of survival diminish rapidly, even with hematopoietic stem-cell transplantation. Since the disease is fatal, unless immunologic reconstitution is achieved by transplantation of functional hematopoietic stem-cells, early diagnosis is of utmost importance, not only to prevent organ failure but also because young age at the time of the procedure is one of the best predictors for survival [14]. That makes SCID a pediatric emergency.

## Diagnosis

Diagnostic criteria for the diagnosis of SCID have been published by the World Health Organization [15], and updated by a joint committee of the PanAmerican Group for Immune Deficiencies and of the European Society for Immune Deficiencies [16]. Suspicion of T⁻B⁺ SCID is based upon analysis of the immunologic phenotype and function. A complete family history may help to discern the X-linked type from the autosomal recessive form (parental consanguinity, occurrence of typical clinical signs also in females). Diagnosis is then performed by biochemical and molecular assays. In most cases of SCIDX1, the γc is not expressed on the surface and the diagnosis can be established by flow cytometry (best performed on B cells). However, this very specific test lacks sensitivity, in that missense mutations that abolish function or lead to expression of a protein with a truncated intracytoplasmic tail may well allow for surface membrane expression of a mutant γc that is recognized by monoclonal antibodies. Expression of the γc may also be assessed by Western blot analysis. On the other hand, patients with T⁻B⁺ SCID due to JAK3 defects express a normal γc but in most cases show very low to undetectable levels of the JAK3 protein in lymphocytes and lymphoblastoid B cell lines, as assessed by Western blotting. In some cases of T⁻B⁺ SCID with residual expression of mutant γc or JAK3 proteins, functional assays may be required to detect a defect in the signal transduction cascade. In that case *in vitro* stimulation with appropriate cytokines fails to induce tyrosine-phosphorylation of JAK3 and of its target STAT proteins.

Before the genes were sequenced, X chromosome inactivation was often used in the differential diagnosis between SCIDX1 and SCID due to JAK3 deficiency. In fact, obligate

carriers of SCIDX1 exhibit a skewed inactivation of the mutated X chromosome in both T and mature B cells, whereas a random X chromosome inactivation is observed in neutrophils and monocytes [17]. Conversely, carriers of JAK3 deficiency (an autosomal recessive disease) display a random X chromosome inactivation in all cell lineages. The observation of the skewed pattern of X inactivation in lymphocytes from carriers of SCIDX1 is another argument in favor of a strong selective advantage of lymphoid precursors expressing a normal γc.

Now that both the *IL2RG* and the *JAK3* genes have been cloned and sequenced, and their genomic organization is well known, direct mutation analysis represents a straightforward approach to definitive confirmation of the diagnosis. Moreover, it allows early prenatal diagnosis based on analysis of chorionic villi DNA [18,19]. Furthermore, this approach may also be applied to identify the gene defect in families in which biologic material from affected patients is not available.

## Treatment

Without treatment, SCIDX1 or JAK3-deficient SCID is usually fatal within the first year of life. The discovery of the HLA system made bone marrow transplantation possible, and in fact since 1968 blood stem-cell transplantation has successfully been used to treat SCID patients [20] and remains the mainstay of therapy [2,14,21]. But before that procedure can be performed, aggressive treatment of existing new infections and their avoidance are of utmost importance. Isolation of the patient in a protected environment, substitution therapy with intravenous Ig, prophylaxis of *Pneumocystis carinii* pneumonia, and adequate supportive therapy (such as parenteral nutrition) constitute the first line of treatment. Use of live vaccines and unirradiated blood products must be avoided. In the meantime, a donor must be sought in the family (HLA-identical siblings, haplo-identical parent) or in the national and international bone marrow donor registries. If no casual engraftment has occurred, bone marrow transplantation can be performed without myeloablative conditioning regimens, and no long-term immunosuppression is required to achieve persistent engraftment of donor T cells. When an HLA-identical donor is available, success is well over 90%. Haplo-identical transplantation from one of the parents is a life-saving and life-sustaining quick alternative if no identical donor is available and searching for matched unrelated donors in the registries takes too long. T cell depletion of the haplo-identical graft has yielded success rates of around 75%. The absence of NK cells observed in most γc- and JAK3-deficient patients facilitates engraftment (with respect to the T⁻B⁻ forms of SCID) especially after haplo-identical bone marrow transplantation. B cell engraftment is more difficult to achieve; in order to overcome this problem, the use of preparative chemotherapy has been advocated. However, even in the absence of B cell engraftment (and thus with persistence of functionally defective B cells), reconstitution of the immune function has frequently been observed after T cell-depleted hematopoietic stem-cell transplantation [21]. Defi-

nitely, humoral immune reconstitution is not a problem following HLA-identical transplant, indicating that collaboration between donor-derived T cells and autologous (genetically defective) B cells is sufficient to induce adequate antibody production. As an alternative to classic bone marrow transplantation, hematopoietic stem-cells can also be collected from the peripheral blood of the donor (with a better yield after stimulation with granulocyte-colony stimulating factor) or from cord blood, with good results.

*In utero* transplantation in SCIDX1 patients has also been performed successfully by us [22] and others [23], by injection of haplo-identical CD34+ cells into the fetal peritoneum at week 20–22 of gestation. However, this approach needs prenatal diagnosis and therefore is feasible only in fetuses belonging to families with previously affected members.

Given the severity of the disease, the fact that despite recent advances T cell-depleted stem-cell transplantation still carries a significant risk of failure, and the demonstration of a strong selective advantage of gene-corrected vs. mutant lymphoid precursors, points to SCIDX1 being an ideal candidate for a gene therapy-based approach. This speculation was further substantiated by the observation that a spontaneous reverse mutation in an SCIDX1 patient led to partial but sustained correction of his immunodeficiency. Finally, attempts at gene transfer in the murine knockout model have shown that development of T and B lymphocytes and reconstitution of both cellular and humoral immune function can be achieved with this approach. Similarly, biochemical correction of JAK3-deficient human B cell-derived cell lines has also been successfully accomplished *in vitro* [24], and full reconstitution of the immune function has been observed in murine JAK3 knockout mice following gene therapy [25].

Based on these premises, the group of Alain Fischer in Paris [26] recently performed, for the first time, gene therapy in three patients with SCIDX1 by transducing the *IL2RG* gene *ex vivo* into CD34+ cells using a Moloney retrovirus-derived vector. The transduced cells were then re-infused without any myeloablative or immunosuppressive therapy. Remarkable immunologic (increase in T and NK cells) and clinical (diarrhea and eczema disappeared) improvements were rapidly demonstrated [26], making SCIDX1 the first example of successful gene therapy in humans. Thus, 33 years after hematopoietic stem-cell transplantation, primary immune deficiencies have paved the way for a novel form of treating human diseases.

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