Summary: The recent elucidation of the multiple molecular mechanisms underlying severe combined immunodeficiency (SCID) is an impressive example of the power of molecular medicine. Analysis of patients and the concomitant generation of animal models mimicking these disorders have quickly provided great insights into the pathophysiology of these potentially devastating illnesses. In this review, we summarize the discoveries that led to the understanding of the role of cytokine receptors and a specific tyrosine kinase, Janus kinase 3 (Jak3), in the pathogenesis of SCID. We discuss how the identification of mutations of Jak3 in autosomal recessive SCID has facilitated the diagnosis of these disorders, offered new insights into the biology of this kinase, permitted new avenues for therapy, and provided the rationale for a generation of a new class of immunosuppressants.

SCID as a cytokine receptor defect

The spectrum of disorders characterized as severe combined immunodeficiency (SCID) has been recognized for more than 50 years (1, 2). Because of major defects in T- and B-cell function, these infants typically present with severe infections during the first few months of life (3–6). Due to the rapid progression of recurrent and opportunistic infections with potentially lethal outcome, SCID is considered a true pediatric emergency that necessitates prompt diagnosis and treatment. Prior to the development of hematopoietic stem cell-transplantation therapy, SCID patients typically died of infection before their second birthday. Fortunately, the majority of these children now can be successfully treated. The advances in treatment of these disorders have also been associated with improved understanding of the molecular bases of these conditions.

From the beginning, it was observed that SCID was more common in boys; thus, prior to the actual identification of the gene underlying this disorder, X-linked SCID (X-SCID) was appreciated as an entity. Initially recognized as a subunit of the
interleukin-2 receptor (IL-2R), the cloning of the IL-2RG gene and its mapping to the X-chromosome made it a candidate gene for X-SCID (7–10). Mutations of IL-2RG were subsequently identified and are now known to account for almost half of all known cases of SCID (3, 4, 8). This disorder is phenotypically characterized as a T⁺B⁻NK⁻ SCID, indicating that T cells and natural killer (NK) cells typically fail to develop. B cells develop in these patients, but their function is greatly impaired. Because the severity associated with mutations of IL-2RG exceeded what would be expected of defective IL-2 signaling, it was suspected that the receptor served other critical functions. Subsequently, a subfamily of cytokines comprising IL-2, IL-4, IL-7, and later IL-9, IL-15, and IL-21 was recognized as sharing this common receptor subunit (8, 11–15). As a result, the receptor encoded by IL-2RG is now designated the common γc chain (γc). The notion that mutation of this common receptor affected the signaling by all these cytokines helped in explaining the severity of this disorder.

Janus kinases and cytokine signaling

The cloning of various cytokine receptors was a great advance in understanding the molecular basis of cytokine action, but precisely how this family of receptors mediated signal transduction remained somewhat of a mystery. These receptors were structurally distinct from other classes of receptors but had no intrinsic enzymatic activity. Nonetheless, it was known that stimulation of cells with cytokines induced tyrosine phosphorylation of substrates. The mystery was solved when a new class of protein tyrosine kinases was discovered. The Janus family of kinases (Jaks), including Tyk2, Jak1, and Jak2, were first identified using polymerase chain reaction (PCR)-based strategies and low-stringency hybridization (16–21). Using the former approach, Janus kinase 3 (Jak3) was cloned in 1994 (22–25). The completion of the human genome project 8 years later verified that, in fact, only four Jaks are present in the human kinome (26).

Clear evidence of a critical role of Jaks in cytokine signaling first came from studies showing that Tyk2 is required for interferon (IFN) signaling (27). Using a series of mutagenized cell lines defective in IFN signaling, it was recognized that one complementation group lacked Tyk2. Reconstitution of the cells with this kinase restored signaling, thus demonstrating that cytokine signaling is dependent upon Jak kinases. It was then shown that Jak2 is involved in growth hormone and erythropoietin signaling, and both Jak1 and Jak2 are involved in IFN-γ signaling (28–33). Other mutant cell lines lacking various Jaks were used to demonstrate their criticality for the respective cytokines (34). Subsequently, all Type I and Type II cytokine receptors have been found to associate with Jaks, and their essential function in vivo has been established by generating knockout mice that lack the various Jaks (35–43). Parallel to emerging data on Jak kinases, the signal transducers and activators of transcription (Stat) family was also identified; together these data provided a new paradigm in cytokine signal transduction, what has come to be known as the Jak/Stat pathway (34, 44, 45) (Fig. 1). Several Stats are activated by γc cytokines including Stat1, Stat3 and Stat5; by contrast, Stat6 is particularly important for mediating IL-4 signaling (46–48).

Initially only two γc-cytokines, IL-2 and IL-4, were shown to activate Jak3 (25, 49). This identification quickly led to the investigation of the interaction of Jak3 binding and γc (Fig. 2), thereby explaining the pattern of Jak3 activation by all γc cytokines (50–52).

Jak3–SCID

The identification of Jak3 and its association with γc immediately suggested that loss of function mutations in the JAK3 gene might be responsible for some forms of autosomal recessive SCID. It was hypothesized that because of the monogamous...
interaction between Jak3 and \( \gamma_c \), mutations of Jak3 were likely to be associated with the same cellular phenotype as X-SCID, namely T\(^-\)B\(^+\)NK\(^-\) (Fig. 2). This speculation was confirmed with the identification of more than 35 reported cases of Jak3–SCID in both European and US populations (53–59) (Fig. 3). Currently, it is estimated that Jak3 deficiency accounts for approximately 7–14\% of heritable SCID. Jak3 mutations are seemingly sporadic, and neither preferential gene locations (i.e. gene ‘hot-spots’) nor founder effects have yet been documented. The majority of Jak3–SCID patients are compound heterozygotes, having inherited a distinct mutation from each parent, although some individuals are homozygous for their mutations as a result of parental consanguinity. These patients demonstrated that Jak3 is essential for the proper development and function of immune cells. Equally though, these patients also establish that Jak3 function is restricted; other than defects in immune cells, these patients were healthy. Moreover, following successful stem cell transplantation, these patients are essentially normal, except for B-cell function (see below). This information provided clear evidence that Jak3 is only essential and non-redundant with respect to its role in the immune response. The specificity of Jak3 function in the immune system has important implications for the development of a new class of immuno-suppressive drugs.

The immune abnormalities associated with Jak3 deficiency were confirmed with the generation of Jak3-knockout mice; similar to humans, these mice have SCID that resembles \( \gamma_c \) deficiency (38–40). These mice have small thymi, absence of lymph nodes, and reduced numbers of \( \alpha/\beta \) and \( \gamma/\delta \) T cells and NK cells. Analysis of the thymic precursors in Jak3\(^{-/-}\)mice showed that there was severe reduction in progenitor cells, cytokine receptors and also regulates catalytic activity. Mutations have been identified in all of these domains. Most patients are compound heterozygotes, inheriting one mutant allele from each parent. Occasionally, in consanguineous families, patients are homozygous for their Jak3 mutations. Most mutations have dramatic effects on protein expression of Jak3, but some missense mutations or small in-frame deletions allow for some protein expression. These mutations affect kinase activity, receptor binding, and intracellular trafficking.

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**Fig. 2.** The molecular basis of severe combined immunodeficiency. A number of cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) utilize the common \( \gamma \) chain (\( \gamma_c \)) in conjunction with a ligand-specific chain to form their receptors. These receptor subunits bind Jak3 and Jak1. Mutations of IL-7R, \( \gamma_c \), and Jak3 disrupt cytokine signaling and lead to severe combined immunodeficiency (SCID); in fact, mutations of these genes may account for two-thirds to three-quarters of the cases of SCID.

**Fig. 3.** Schematic of Jak3 structure and the mutations identified. Seven Janus homology (JH) domains have been identified. JH1 is the carboxy-terminal tyrosine kinase domain. Adjacent to the kinase domain is the JH2 or pseudokinase domain, which itself lacks catalytic activity but is essential for regulating normal kinase activity. Jak3 also has a src homology 2 (SH2) domain, although its function is poorly understood. The amino-terminus of the Jak3 (JH5–JH7) has homology to FERM domain-containing proteins. This region of the Jaks mediates binding to \( \gamma_c \). Most mutations have dramatic effects on protein expression of Jak3, but some missense mutations or small in-frame deletions allow for some protein expression. These mutations affect kinase activity, receptor binding, and intracellular trafficking.
and when irradiated recipients were reconstituted with wild-type and Jak3-deficient stem cells, the Jak3−/− progenitors were unable to reconstitute T-cell development (60). Crossing Jak3−/− mice with T-cell receptor (TCR)-transgenic mice resulted in low numbers of peripheral T cells that exhibited increased apoptosis, with CD8+ T cells being more affected than CD4+ T cells (61). Using a transgenic approach that permitted expression of Jak3 in thymus, but not in the periphery, it was shown that expression of Jak3 is not just required for development of T cells; Jak3 is also required in peripheral cells for cell survival. Jak3−/− T cells were also noted to express activation markers and had impaired TCR signaling. However, using TCR-transgenic Jak3−/− T cells, it was observed that TCR signaling was intact. This finding has been interpreted to suggest that with time, there is no intrinsic defect in TCR signaling, but rather the abnormalities are due to expansion of autoreactive T cells in Jak3−/− mice. In addition to having profound defects in thymocyte development, Jak3 (and γc)-deficient mice also have severely defective B-cell development (38–40, 62, 63); the reason for the difference between mice and humans with respect to the roles of γc and Jak3 in B-cell development is unclear at this time but presumably relates to species-specific cytokine usage.

No effect on the development of myeloid or erythroid cells was noted in Jak3-deficient mice, consistent with observations in humans and indicative of a specific effect on lymphoid precursors. However, mice lacking Jak3 develop splenomegaly, associated with an increase in the number of neutrophils and monocytic cells in peripheral blood. As the mice age, infiltration of mononuclear cells occurs in the kidneys, lungs, and liver. However, when Jak3−/− mice were crossed with recombination activating gene-1 (RAG-1)-deficient animals, no splenomegaly or myeloid expansion is apparent, indicating that this abnormality is T-cell dependent. Presumably, this outcome is a reflection of the autoimmune alternations that occur in these mice and may not represent intrinsic derangements in myeloid cell growth (64).

**Jak3 structure, function, and regulation**

The JAK3 gene comprises 23 exons, with an open reading frame of 3372 bp that is translated into a 1124 amino acid protein of approximately 125 kDa (Fig. 3). Most of the described Jak3 mutations abrogate or markedly reduce Jak3 protein expression and/or stability; in fact, assessment of Jak3 protein levels in Epstein–Barr virus (EBV)-transformed B cells has been one screening method used to make the diagnosis of Jak3-SCID (59). In some cases, however, the mutated Jak3 is expressed, and these mutated proteins have provided useful insights into the structural features of the Jaks.

The carboxy terminus of Jak3 and other Jaks contains the functionally essential kinase domain. This domain has extensive homology to other well-studied kinases in which the crystal structure has been solved, like the insulin receptor kinase and c-Src (65). In isolation, the kinase domain is catalytically active. Mutations in this domain inhibit kinase activity and/or protein expression (57).

The kinase domain is flanked by the enzymatically inactive pseudokinase domain (Fig. 3). This unique domain structural feature of Jaks is also the basis for their name; like the Roman god Janus, these kinases are two-faced with respect to these domains. The pseudokinase domain has sequence similarity to the kinase domain, but several residues required for phosphotransferase activity are altered from the canonical motifs. Even though the pseudokinase domain itself lacks catalytic activity, a number of patient-derived mutations of Jak3 within this domain abrogate kinase activity of the whole molecule (55, 66). This finding suggested that the pseudokinase domain is an essential regulator of the kinase domain activity, and it was further shown that these domains physically associate. It is noteworthy that Jak2 also has been shown to be regulated in the same manner (67). Because the kinase domain–pseudokinase architecture is a unique feature of the Jaks, there is little additional information that offers insights into how this regulation occurs. Interestingly, in the context of RAF–MEK–ERK signaling pathway, it has been shown that B-Raf mutants that have reduced activity in vivo can still activate downstream kinases in vitro by allosteric mechanisms/transphosphorylation of intact C-Raf (68). Accordingly, it is tempting to speculate that the inactive Jak pseudokinase could also modulate the activity of adjacent Jak molecules by direct binding to the kinase domain in a manner analogous to catalytically inactive B-Raf and C-Raf; clearly detailed crystallographic data are needed to advance the understanding of this interesting domain.

The amino-terminus of Jaks contains a FERM (band four point one, ezrin, radixin, moesin) domain, and mutations identified in this region have established that the function of this domain is to mediate Jak3 binding to the cognate cytokine receptor as well as to regulate kinase activity (69–71). Using recombinant proteins, the FERM domain has been shown to directly bind the kinase domain and enhance kinase activity. Only one other family of protein tyrosine kinase has a FERM domain, the focal adhesion kinase (FAK). Analogously, point mutations in the FAK FERM domain interfere with the regulation of FAK kinase activity as well as with its association with its substrates (72–74). Again, our understanding of this regulation is hampered by our superficial notions of Jak structure.
The FERM domain segment has also been found to mediate binding of Jaks to cytokine receptors (70, 71, 75, 76). Recently though, it has become clear that there is additional complexity in the in vivo association of Jak3 with its cognate receptor, γc. Using live cell imaging and fluorescent fusion proteins, it is apparent that the FERM domain is necessary for receptor binding but not sufficient for the appropriate trafficking of Jak3 with γc (77) (Fig. 4). In fact, full-length Jak3 was required for proper localization and association, although kinase activity was dispensable. Interestingly, multiple patient mutations in different domains disrupt proper trafficking. Of particular interest was a mutation in the Src homology-2 (SH2)-like domain. This domain is not known to be functional in Jaks, although for other kinases this domain is a key feature. Nonetheless, mutation of a conserved residue in this domain abrogated proper trafficking of Jak3. Therefore, Jak3 mutations can result not only in defective protein expression and kinase activity but also can interfere with appropriate receptor association and intracellular localization of Jak3.

Because the crystal structure of the Jaks has not been solved, we are largely ignorant of how Jak kinase activity is truly regulated. Nonetheless, we know that Jak phosphorylation is important in positively regulating kinase activity. Jaks are also regulated by tyrosine phosphatases including the following: SHP1, SHP2, CD45, protein tyrosine phosphatase 1B (PTP1B), and T-cell PTP (TCPTP) (78). Like other protein tyrosine kinases, tyrosine residues in the Jak kinase domain activation loop are important for regulating catalytic activity. For Jak3, Y980 is a major site of autophosphorylation that positively regulates kinase activity. In contrast, phosphorylation of Y981 seems to inhibit kinase activity as mutation of this residue results in a hyperactive kinase; however, the mechanism underlying this activation is unclear (79). Phosphorylation in the Jak-activation loop allows one member of a family of negative regulators termed suppressors of cytokine signaling (SOCS), SOCS1, to bind and inhibit Jak activity. Deficiency of SOCS1 can enhance signaling by γc cytokines (80–82). In addition, SOCS1 forms an E3 ubiquitin ligase complex with elongins B and C, Cullin-5 (Cul-5), and Rhx1 to mediate the ubiquitination of Jak2, which may promote proteasome-dependent degradation of this and possibly other Jaks (83, 84).

Jaks can undergo another modification, ISGylation. IFN-stimulated gene 15 (ISG15) is a ubiquitin-like protein, which is one of the most highly IFN-inducible genes (78). Jak1 and Stat1 have been shown to be conjugated by ISG15 in a manner similar to ubiquitin and other ubiquitin-like modifiers like SUMO. UBP43, a ubiquitin-specific protease (also known as USP-18), removes ISG15 from ISGylated proteins, and mice lacking UBP43 are hypersensitive to IFN stimulation (85). A role for ISGylation of Jak3 has not been reported.

Jaks are also phosphorylated on other sites that appear to be important for the regulation of catalytic activity. For instance, Y785 and Y813 in Jak3 and Jak2, respectively, are other prominent sites of autophosphorylation (86). These sites serve to recruit SH2 domain-containing adapter molecules such as SH2Bβ. For Jak2, this mechanism enhances catalytic activity. In contrast, another related adapter, APS (adapter protein with Pleckstrin homology and Src homology 2 domains), also binds Jak2, but it negatively regulates activity (87). The role of SH2Bβ and APS in regulating Jak3 activity is less clear. Jak2 and Jak3 have also been reported to complex with STAM-associated molecule with the SH3 domain of STAM (AMSH), hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) proteins (88–90). The STAM/EAST/Hbp family of proteins comprises eight members that are well conserved from yeast to mammals. This family of proteins becomes tyrosine-phosphorylated by a variety of cytokines and growth factors. They are thought to play a role in intracellular protein trafficking and the regulation of actin cytoskeleton; however, the precise role of these adapters in cytokine signaling has not been elucidated (91, 92).

The levels of Jak3 protein and mRNA are highly regulated. Jak3 is preferentially expressed in hematopoietic cells and is upregulated upon cell differentiation and activation in T cells, B cells, and myeloid cells (22, 93, 94). The core Jak3-promoter activity resides in a 267-bp fragment 5′ to the transcription-initiation site, which contains putative Sp-1, activator protein-1 (AP-1), Ets, Stat, and other binding sites (95). Histone acetylation of this region correlates well with mRNA expression. Ets family transcription factors bind this region, and mutation of the Ets motifs abrogates promoter activity, suggesting that this family of DNA binding proteins is likely to be very important in regulating Jak3 gene expression. The Stat-binding site also appears to be important in cytokine-dependent regulation of Jak3 expression (96). It is also notable that like cytokines and cytokine receptors, the Jak3 gene has AU-rich elements (AREs) in its 3′ untranslated region. Presumably these motifs serve to destabilize the Jak3 mRNA, allowing for fine tuning of Jak3 expression, but this aspect of Jak3 regulation has not been studied.

**SCID as disorders of cytokine signaling**

In light of the identification of γc and Jak3 mutations, mutations of other cytokines, receptors, and signaling pathways were considered as additional candidates for causing SCID. Based
Fig. 4. Complexity of γc/Jak3 association and subcellular distribution. In the absence of γc, Jak3 is localized to the cytosol, whereas γc alone is localized to endosomes and the plasma membrane. When Jak3 and γc are both expressed, Jak3 localizes with γc in endosomes and enhances plasma membrane expression. The requirement for Jak3/γc colocalization is surprisingly stringent. The Jak3 FERM domain is necessary but not sufficient for localization. A number of mutations including patient-derived mutations in multiple domains disrupt localization (Figure from reference 77).
on its fundamental role in T-cell function and development, IL-7 and its receptor emerged as particularly strong candidates; indeed, mutations of IL-7R have now been identified to underlie about 10% of autosomal recessive SCID patients (97). In aggregate, mutations within the IL-7R/γc/Jak3 account for the majority (approximately 67–74%) of all cases of SCID, but because signaling by only one of the γc cytokines is blocked with IL-7R mutations, the phenotype of this form of SCID is distinct from Jak3–SCID and X-SCID. IL-7 activates Stat5A and Stat5B among other Stats. It is therefore notable that a patient has been recently identified with mutation of Stat5B (98). This child had multiple viral infections, including several episodes of Herpes zoster, as well as Pneumocystis pneumonia, but detailed studies of lymphoid function were not performed. IL-7 and other γc cytokines also activate Stat5A, so presumably this latter transcription factor compensates to some extent for the mutations in Stat5B. Stat5B is also important for growth hormone signaling and this child had profound growth retardation. It will be important to determine how common these patients are and to compare and contrast their deficits with IL-7R-, γc-, and Jak3-deficient patients.

Given these data, it is useful to relate the action of γc cytokines with the clinical phenotypes seen in SCID due to γc, Jak3, and IL-7R mutations (Fig. 5). In gene-targeted mice, mutations in the IL-7R gene result in marked reduction in thymocytes and peripheral T cells, including γδ T cells, entirely consistent with what is seen in humans with mutations of this receptor subunit (97, 99–104). Interference in IL-7 signaling disrupts thymocyte development at the double-negative (CD4−CD8−) stage prior to productive TCR rearrangement (105). The small numbers of T cells developing in the knockout mice have impaired survival and poor proliferation in response to mitogens. It is now clear that IL-7’s role in T-cell biology is not limited to lymphopoiesis, and it is also important for the homeostasis of mature peripheral lymphocytes (103, 104, 106). However, IL-7 is not uniquely responsible for maintaining the peripheral lymphocyte pool; it acts in concert with IL-15 in survival of CD8+ memory cells (107, 108). The survival of CD4+ T cells is thought to be less dependent upon both cytokines, with both IL-7 and TCR signals contributing to their homeostasis (109). If TCR and IL-7 signals are blocked, CD4+ T memory cells do not

![Fig. 5. Cytokine receptors that share γc subunit and their functions.](image-url)

The lists of actions are purposefully abbreviated and illustrate the major functions of these cytokines as they relate to the pathogenesis of severe combined immunodeficiency (SCID). In addition, the actions of these cytokines, especially IL-7, are not identical in humans and mice; the actions in humans are emphasized.
proliferate and fail to survive. However, IL-7 is not required for generation of CD4\(^+\)CD44\(^hi\) T cells. In summary, the major developmental defects in T-cell function seen with \(\gamma_c\), IL-7R, and Jak3 deficiency are readily attributable to the absence of IL-7 signaling. It is clear that IL-7 is important for the proper function of both CD4\(^+\) and CD8\(^+\) T cells, although defects in Jak3 and \(\gamma_c\) would be more severe because of defective IL-15 signaling.

B cells are present in patients with Jak3, \(\gamma_c\), and IL-7R mutations, indicating that IL-7/IL-7R are dispensable for human B-cell development and function. However, this is not the case in mice (97). In fact, IL-7 is a critical regulator of B-cell development, based in part upon the induction of the transcription factor Pax-5 (110). Nonetheless, in patients with X-SCID and Jak3–SCID, the function of mature B cells is significantly impaired. Not only is T-cell help absent but defective B-cell activation, maturation, and immunoglobulin class switching are thought to result directly from interruption of IL-4 and IL-21 signaling; these cytokines work in concert to regulate B-cell function (111). It is noteworthy therefore that patients with IL-7R mutations differ from Jak3- and \(\gamma_c\)-deficient patients with regard to B-cell function. The former patients generally have better B-cell function following transplantation, as would be predicted based on the restricted function of this cytokine versus the broader functions of \(\gamma_c\) and Jak3 (112).

IL-15 is also a critical cytokine for the development and survival of NK cells; thus, the lack of NK development observed in X-SCID and Jak3–SCID patients is due to defective signaling by this cytokine (107, 108, 113, 114). This explains the difference in phenotypes associated with IL-7R mutations versus Jak3 and \(\gamma_c\) mutations. The absence of IL-7 does not affect the NK-cell lineage, as can be seen with the T\(^-\)B\(^+\)NK \(^+\) phenotype found in SCID based on mutated IL-7R (97).

What about IL-2? As the prototypic T-cell growth factor, one would have expected this cytokine to be a major contributor to the development and function of T cells, but this is not the case. It appears to be considerably less critical in vivo than was expected based on in vitro studies. Mice lacking either IL-2 or IL-2R (IL-2R\(\alpha\) and \(\beta\)) are normal with respect to thymus development and peripheral T-cell subset composition (115–117). Paradoxically with respect to IL-2’s role in T-cell proliferation, knockout mice exhibit a severe lymphoproliferative disorder, presumably due to the loss of IL-2-dependent maintenance of self-tolerance. While the mechanisms underlying this function remain elusive, several possibilities have been proposed. IL-2 has been shown to modify activation-induced cell death and thymic selection (118, 119). IL-2 is also suggested to serve as the primary growth factor for T-regulatory cells (120, 121). Accordingly, patients with mutations of the IL-2R\(\alpha\) subunit have extensive lymphocytic infiltration and inflammation (122, 123). In principle, lack of IL-2 signaling in Jak3–SCID patients could result in autoimmunity. While most patients are severely immunodeficient, not all patients with \(\gamma_c\) and Jak3 mutations have profound lymphopenia (124–127). If T cells are generated, it is possible that autoimmune manifestations could occur, and in fact, a Jak3-deficient patient has been described who had a mixed picture of immunodeficiency and autoimmunity. It is possible that the lack of IL-2 signaling could be one contributor to this clinical presentation (125).

The disruption of signaling by \(\gamma_c\) cytokines (IL-7, IL-15, IL-4, and IL-21) nicely explains the clinical phenotype associated with Jak3 and \(\gamma_c\) mutations. The extent to which defective IL-2 signaling in SCID patients with residual T cells will be clinically meaningful needs to be considered in the future. One \(\gamma_c\) cytokine not mentioned thus far is IL-9, but the absence of this cytokine does not have major developmental effects on immune cells (128, 129). Another cytokine that deserves mentioning is thymic stromal lymphopoietin (TSLP). This cytokine exerts its effect via a receptor comprising the IL-7R\(\alpha\) chain and a distinctive subunit, TSLP receptor (TSLPR), which structurally resembles the \(\gamma_c\) chain. Recently generated TSLPR-deficient mice show that absence of TSLP signaling has little effect on lymphocyte development and function (130, 131). However, mice deficient in both TSLPR and \(\gamma_c\) have poorer lymphoid development than mice lacking just \(\gamma_c\). TSLP, therefore, likely accounts for some of the residual lymphoid development in \(\gamma_c\)-deficient mice and possibly patients with X-SCID. Notably, the effects of this cytokine on human cells are somewhat perplexing in that TSLP has major effects on CD11c\(^+\) dendritic cells rather than lymphoid cells (132). The function of this cytokine on human cells needs further clarification.

Thus far we have focused on the roles of \(\gamma_c\) and Jak3 in cytokine signaling in lymphocytes and related this information to the pathogenesis of SCID. As detailed above, Jak3 is not exclusively expressed in lymphocytes. It is also expressed in myeloid cells and has even been reported to be present in non-hematopoietic cells. A number of studies that have used non-selective inhibitors have attributed functions of Jak3 in dendritic cells, platelets, mast cells, and chondrocytes. A role for Jak3 has been proposed in signaling by a wide range of receptors such as chemokine receptors, CD40, TCR, Fc receptor, and thrombin receptor. In a number of cases, the role for Jak3 implied by the inhibitor has not been substantiated using
Jak3-deficient cells. These experiments need to be interpreted with caution. Although Jak3 might be expressed at some level, it is not clear that it has essential functions in non-lymphoid cells. For instance, Jak3 is highly inducible in monocytes, and monocytes appear to be largely normal in their function in Jak3 SCID patients (133).

These issues remain controversial but are important and will be critical to resolve if a selective Jak3 inhibitor is to come into wide use; however, it bears reiterating that Jak3–SCID patients are normal following stem cell transplant, even when they do not receive chemotherapeutic conditioning. This outcome indicates that non-lymphoid cells (myeloid and other hematopoietic lineages, as well as non-hematopoietic cells) are host-derived and lack Jak3. The present information suggests that non-lymphoid cells function normally without Jak3. The lack of Jak3 is, evidently, non-consequential, strongly arguing for very restricted roles of Jak3. Nonetheless, this is an area that deserves further investigation.

Clinical features of Jak3–SCID

Lymphopenia, the hallmark of other forms of SCID, may or may not be present in all patients with Jak3–SCID. Lymphocyte immunophenotyping, however, will show profound T-cell lymphopenia in the majority of patients; this directly relates to most of the signs and symptoms of this disease, namely the propensity for the development of severe infections (3, 57–59). Jak3- and γc-deficient patients can have normal or increased numbers of B cells (which can result in normal numbers of total lymphocytes), but patients present with functional impairment of humoral immune responses that also contributes to recurrent infections. Patients lack NK cells, and this deficiency may also contribute to the susceptibility to pathogens, especially viruses. Additional laboratory features include hypogammaglobulinemia, which may be variable due to persistence of maternal immunoglobulin, although there is a lack of specific antibody responses to immunization. Jak3-deficient lymphocytes also fail to proliferate normally in responses to mitogens, antigens, or allogeneic cells. Most Jak3–SCID patients are diagnosed during the first few months of life as they present with oral candidiasis, recurrent, severe sinopulmonary infections, intractable diarrhea, and failure to thrive. They can also develop opportunistic infections with organisms such as Pneumocystis cariini, and Candida. Viral infections from organisms like varicella, adenovirus, respiratory syncytial virus, parainfluenza, cytomegalovirus and EBV may be extremely severe and life-threatening.

The clinical diagnosis of Jak3-deficient SCID is based on recurrent, severe infections from opportunistic agents, in the context of profound, characteristic T-cell lymphopenia, conserved presence of B lymphocytes, and lack of NK cells (T−B+NK−). As indicated above, SCID is a pediatric emergency and the proper evaluation and treatment is critical, as prompt stem cell transplant is associated with improved immune reconstitution and clinical outcome (134). It is obviously important to complete the clinical diagnosis of T−B+NK− SCID with definition of the specific syndrome at the molecular level. Although X-SCID and Jak3–SCID patients share prognosis and indication for therapy with allogeneic transplantation if an optimal donor is available, knowledge of the specific molecular defect is of critical importance for genetic counseling and early or prenatal diagnosis in relatives of affected subjects, as well as for the implementation of specific forms of therapy based on gene transfer. The molecular diagnosis of Jak3− and γc−SCID can be made presumptively by assessing protein expression levels in EBV-transformed patient B cells. It should be noted, however, that IL-7R levels are highly variable on these cell lines. One drawback of assessing protein expression in EBV–B cells is that their establishment usually takes approximately 4 weeks. Unfortunately, due to the low levels of expression of the Jak3 protein in circulating B cells, Western blot analysis on fresh peripheral blood mononuclear cells (PBMCs) is not a reliable assay. EBV–B cells have also proven useful to study the defects of cytokine signaling in T−B+NK− patients, thus helping the final diagnosis (59). The definitive judgment, however, rests on the demonstration of Jak3 (or γc or IL-7R) mutations. The lack of genetic hot spots forces one to sequence the entire coding region and the adjacent intronic sequences. Analysis of single-strand conformation polymorphisms (SSCP) (135) can be used to help guide genotyping, and the improvements in automated sequencing have reduced the barrier to sequencing a large gene such as Jak3. The presence of circulating NK cells in T-lymphopenic patient (T−B+NK+) is grounds to look for mutations in IL-7R. It is tempting to speculate that ‘resequencing chips’ may eventually become available to analyze SCID patients (136). Such chips are available for other common mutations (e.g. BRCA1); as this technology becomes more common, this approach may become more economical and simpler than dideoxynucleotide sequencing.

Although Jak3-deficient γc-deficient SCID patients generally exhibit profound T-cell lymphopenia, this phenotype can change over the course of the disease. Moreover, patients with relatively normal to high numbers of circulating, yet often poorly functioning T cells have also been described. In most cases, T cells found in a Jak3–SCID patient can be
explained as engraftment of maternal T cells transplacentally, although in some cases some T-cell development occurs in the absence of Jak3 or $\gamma_c$ (124, 125, 127). In some cases, Jak3 deficiency is associated with only mild immunodeficiency characterized by extensive, but transitory, cutaneous warts (125). It is noteworthy that the T cells generated in Jak3-deficient patients do not have a normal TCR repertoire and their response to mitogens is abnormal. The prevalence of such forme fruste variants of Jak3–SCID is unknown, but the improved ability to detect this form of SCID should facilitate the identification of such patients.

**Treatment of Jak3–SCID**

The prognosis for Jak3-deficient SCID patients is the same as for all B T-SCID. Thus, SCID due to Jak3 deficiency is a lethal disorder. The advent of hematopoietic stem cell transplant (HSCT) revolutionized the outcome of SCID, and at present it is still the treatment of choice for Jak3–SCID. Optimal results (up to 95% survival rate) have been obtained with bone marrow transplantation from human leukocyte antigen (HLA)-matched siblings, whereas the survival rate is lower (approximately 70%) when HLA-mismatched family donors are used (137–139). It is of interest that despite their vestigial thymi, T-cell development occurs when normal hematopoietic stem cells are provided. The early block of thymic development of Jak3–SCID host T cells favors thymic repopulation with donor T-cell progenitors after transplantation, thus allowing the donor cells to thrive with continued maturation, even in the absence of conditioning the host with myeloablative regimens. However, particularly when no conditioning regimen is given, engraftment of donor-derived T cells is associated with the persistence of autologous B cells (4, 138). This is presumably due to competition between host and recipient which results in variable donor B-cell reconstitution and ineffective humoral responses, making post-transplantation treatment with intravenous infusion of immunoglobulins (IVIG) necessary on a chronic basis. Use of pretransplant conditioning has been claimed to favor the engraftment of donor-derived B cells (139–141). However, it will be important to validate this assumption through the prospective analysis of the outcome of bone marrow transplantation in mutation-proven Jak3-deficient infants. In a series of 10 Jak3-deficient patients transplanted at Duke University Medical Center, Durham, NC, USA, two received HLA-identical sibling marrow and eight received maternal haploidentical transplantation without pretransplant cytoreductive chemotherapy. Among the latter patients, one died and a subsequent transplantation of cord blood (preceded by chemoablation) was necessary in one patient (59). The nine surviving patients showed development of normal T-cell immunity. Donor B cells, however, were detected only in the patient who received chemoablation, and six patients continued to require IVIG therapy. NK-cell activity was not reconstituted in seven of the nine survivors (59). As a related finding, a very recent retrospective analysis of a group of 41 patients with SCID treated with HSCT at the Necker-Enfants Malades Hospital in Paris and who were alive 10 or more years after treatment showed that nine patients had extensive chronic human papillomavirus (HPV) disease limited to the skin. All nine patients had either X-SCID or Jak3–SCID. Patients with other forms of SCID did not have HPV disease. These observations suggest that NK cells or $\gamma_c$/Jak3-dependent signaling in keratinocytes may play a role in anti-HPV immunity (142).

Despite the success of STHC, a suitable donor is not always available for all Jak3–SCID patients; therefore, much experimental work has been aimed at developing alternative gene therapy approaches for treatment of both $\gamma_c$- and Jak3-deficient SCID patients (143, 144). In fact, retroviral $\gamma_c$ and Jak3 gene transfer has been shown to correct developmental and functional defects in vivo and in vitro in a number of preclinical studies (145–154). The gene-corrected cells showed reconstitution of a normal cytokine signaling. More importantly though, retroviral-mediated gene transfer of Jak3 and $\gamma_c$ into deficient mouse progenitor stem cells completely restored T-cell function and humoral immunity when the transduced progenitors were transplanted into SCID recipients. The efficacy of gene therapy approach is presumably due to the intense selective advantage of gene-corrected stem cells.

Clinical trials using gene therapy to reconstitute $\gamma_c$ expression in X-SCID were initiated in 1999 in France. Ten classical X-SCID patients received autologous CD34+ stem cells transduced with a replication-defective Moloney-based retroviral vector containing the $\gamma_c$ transgene, without prior myeloablation (155). This procedure was successful in nine patients with expression of transgene being detected in circulating T and NK cells already 30–40 days after treatment and eventually, normal numbers of NK as well as $\alpha\beta$ and $\gamma\delta$ T-cells being reconstituted. After the therapy normal T-cell function was achieved; T cells proliferated in response to mitogens and demonstrated a normal response to vaccination with tetanus toxoid and polioviruses. In addition, NK cells were observed to exhibit in vitro lytic activity. Although the $\gamma_c$ transgene was minimally expressed in circulating B cells, the patients had normal levels of IgM and IgG with restoration of class-switching; in this respect, gene therapy was more effective than STHC. A similar trial was also open in UK with comparable results in four patients.
As the findings from the preclinical studies of the X-SCID gene therapy trial were encouraging, Jak3 gene correction of bone marrow CD34+ cells was also attempted in a single Jak3–SCID patient who had failed HSCT. However, two consecutive attempts of the genetic correction of this patient’s CD34+ bone marrow hematopoietic progenitors were unsuccessful. There is no evidence from preclinical studies suggesting a reason why Jak3 gene therapy would be less likely to work than γc gene therapy. Therefore, as hypothesized by the investigators, it is possible that preceding long-term viral infections may have compromised thymic function in this particular patient (156).

Based on its efficacy, gene therapy for different forms of SCID may ultimately be a viable and possibly better alternative to HSCT; presumably, the selective pressure for gene-corrected cells is an advantage in this setting. Unfortunately, a complication has arisen with what is otherwise a remarkable success story. Thirty months after infusion of retrovirally corrected CD34+ stem cells, two of the youngest X-SCID patients developed a leukemic-like process with expanded clonal populations of T cells (155). Remarkably, both patients exhibited insertion of retroviral transgene in proximity to the LMO-2 (LIM-only domain 2) promoter, resulting in the overexpression of the protein encoded by this gene. LMO-2 is a transcription factor, known to be a central regulator of hematopoiesis. Translocation of LMO-2 is found in childhood T-cell leukemia, and transgenic overexpression of LMO-2 is transforming (157–160). This finding suggests that insertion of the retroviral vector in the proximity of the LMO-2 gene had caused activation of gene expression by well-known mechanisms of insertional oncogenesis. In this case, the expression of γc (a proliferation signal) may have provided the ‘second hit’ causing neoplastic transformation. One would have expected that insertion is a stochastic event, but the occurrence of leukemia in two of 10 patients raises the possibility that this is not random. If the LMO-2 locus is a frequent site for insertion, retroviral constructs encoding Jak3 might also have the propensity to insert in the same locus. Therefore, the retroviral gene therapy for Jak3– and X-SCID needs to be carefully reevaluated, and trials in the US are only allowed to treat patients who have failed a previous therapeutic attempt with HSCT.

The development of a selective Jak3 antagonist

If Jak3 is essential for immune cell function, it raises an interesting possibility. Specifically, if one intentionally interfered with Jak3 function, this approach could be the basis of a novel class of immunosuppressants or anticancer drugs. This application has particular appeal in that Jak3–SCID patients suggest that a highly selective Jak3 inhibitor should also have very limited and specific effects; after all, patients have immunodeficiency but they do not have abnormalities outside the immune system. Furthermore, stem cell transplantation of Jak3–SCID patients is corrective, indicative of very cell-selective functions of Jak3. In contrast, the most widely used immunosuppressive drugs (calcineurin inhibitors or corticosteroids) target ubiquitously expressed molecules. These drugs are very efficacious, but due to their widespread actions on diverse tissues, they can have broad metabolic toxicities. The adverse effects of these drugs remain a significant problem in the treatment of transplant rejection and autoimmune disease, especially as these disorders require lifelong treatment. In principle, a potent and truly selective Jak3 inhibitor might have significant advantages over current regimens.

Until recently, the generation of a selective protein tyrosine kinase inhibitor has not been recognized a realistic goal, because most kinase inhibitors are adenosine triphosphate (ATP) antagonists. In a cell, there are hundreds if not thousands of ATP-dependent proteins, suggesting that designing a selective kinase inhibitor could be impossible or at least extremely difficult. However, this pessimism vanished with the successful generation of imanitib (Gleevec), the very successful inhibitor of the BCR-Abl kinase, used to treat chronic myelogenous leukemia (CML) (161). Targeting kinases is now one of the most appealing approaches in pharmaceutical development. While this approach is attractive, does targeting Jak3 have specific challenges? One immediate issue is the extent to which inhibitors are selective among the Jaks. Some Jak3 inhibitors have been reported. For instance, pyridone-containing tetracycle compounds and the tyrphostin AG-490 inhibit Jak3 (162–164). However, these compounds also block Jak2, which is essential for many hematopoietic cytokines. Jak2 mediates signaling erythropoietin, macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage (GM)-CSF, and thrombopoietin, and Jak2 deficiency is embryonically lethal due to impaired erythropoiesis (37). Significant pharmacologic inhibition of Jak2 in vivo could be expected to result in anemia, thrombocytopenia, and leukemia; obviously this outcome would be less than desirable. In addition, tyrphostins also inhibit other classes of protein tyrosine kinases (165). Similarly, another Jak3 inhibitor has been reported, and it inhibits a variety of kinases and signaling by diverse receptors (165–167).

Despite these concerns, the development of a highly selective Jak3 antagonist, designated CP-690 550, has recently been
reported (165). Unlike other reported Jak3 inhibitors, CP-690 550 has nanomolar potency in in vitro kinase assays, with approximately 30–100× less potency for Jak2 and Jak1, respectively. The in vivo efficacy of the drug was established by its prevention of transplant rejection in a murine heterotopic heart transplant model as well as non-human primate renal transplant model. The prolongation of graft survival correlated well with the inhibition of cytokine-inducible genes in vivo. The total number of T lymphocytes did not diminish in animals treated with CP-690 550, but there was a trend in the reduction of CD8+ T cells, consistent with the documented effects of γc cytokines on CD4+ and CD8+ lymphocytes. A modest decline in NK cells was also observed in treated animals, presumably due to inhibition of IL-15 signaling. Mild anemia was observed with highest doses, but importantly CP-690 550 did not cause granulocytopenia or thrombocytopenia. This finding suggests that Jak2 antagonism in vitro and in vivo is not a major problem for this compound. Interestingly, CP-690 550 effectively blocked cytokine signaling, but had no effect on TCR signaling. This effect is relevant, because it raises the possibility that the Jak3 inhibitor might have synergistic effects when used in combination with calcineurin inhibitors. Potentially, this synergism could reduce doses needed for immunosuppression and possibly side-effects associated with these drugs; this possibility will need to be tested directly.

In view of these preclinical results, the clinical conditions where T and B lymphocytes are key players would be the logical target diseases for Jak3-inhibitor treatment trials. Blocking transplant rejection would be an obvious application, especially in the settings where patients have had unacceptable toxicities due to their present therapies. In addition, autoimmune diseases in which lymphocytes play a central role are candidates for the use of a Jak3 antagonist; diseases like psoriasis, multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus, and rheumatoid arthritis are all possibilities. Given the central role of Jak3 in immunity, suppression of its activity would not be expected to be risk-free; like other immunosuppressants, one would anticipate that there would some risk of infection. Exactly how immunosuppressive this drug is and how reversible its effects are remain to be determined.

Conclusions

The discovery that the IL-7R/γc/Jak3 axis accounts for the majority of SCID is an important breakthrough for a number of reasons. Clearly this knowledge facilitates the diagnosis of this disorder and may permit the identification of patients with non-classical presentations. While historically, most patients have been treated with STHC in the absence of a molecular diagnosis, establishing the etiology of SCID can help with selection of the appropriate treatment. This being said, it needs to be emphasized that SCID is a life-threatening emergency, and patients with a T^B_ SCID should receive STHC, even if a molecular diagnosis is not readily obtainable. The insights into pathogenesis also provide new treatment options in terms of gene therapy, which appears to be highly efficacious. Unfortunately, the complication of insertional oncogenesis and resulting malignant transformation remains an issue. Finally, establishing that Jak3 is essential for immune cell function has provided strong rationale for the development of a selective Jak3 antagonist as a novel class of immunosuppressive drugs. One such inhibitor has been successfully developed and is being tested.

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