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Jak3, severe combined immunodeficiency, and a new class of immunosuppressive drugs

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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 γ chain (γ_c). 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 γ_c cytokines including Stat1, Stat3 and Stat5; by contrast, Stat6 is particularly important for mediating IL-4 signaling (46–48).

Initially only two γ_c-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 γ_c (*Fig. 2*), thereby explaining the pattern of Jak3 activation by all γ_c cytokines (50–52).

Jak3–SCID

The identification of Jak3 and its association with γ_c 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

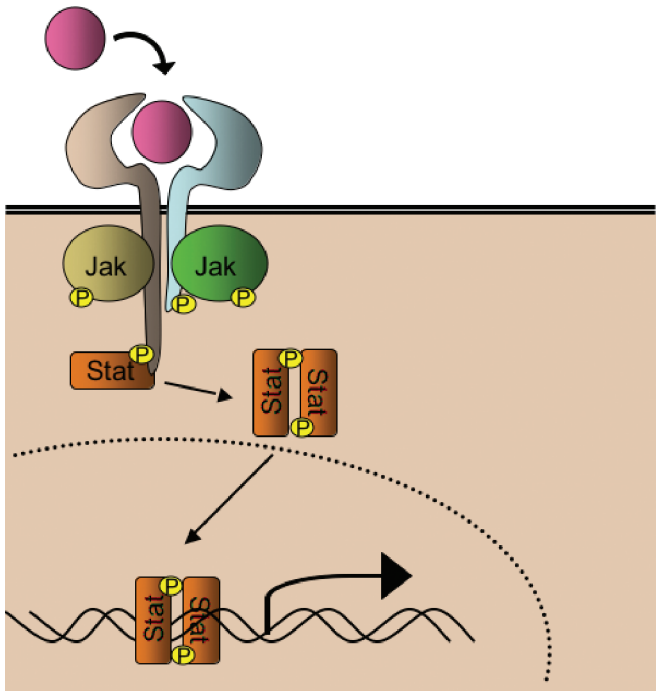

***Fig. 1.* Overview of cytokine signaling.** Upon ligand binding, cytokine receptor-associated Janus kinases (Jaks) are activated by transphosphorylation. Stat proteins bind the phosphorylated receptor chains allowing the Jaks to phosphorylate the Stat. Phosphorylated Stats translocate and accumulate in the nucleus where they regulate gene expression.

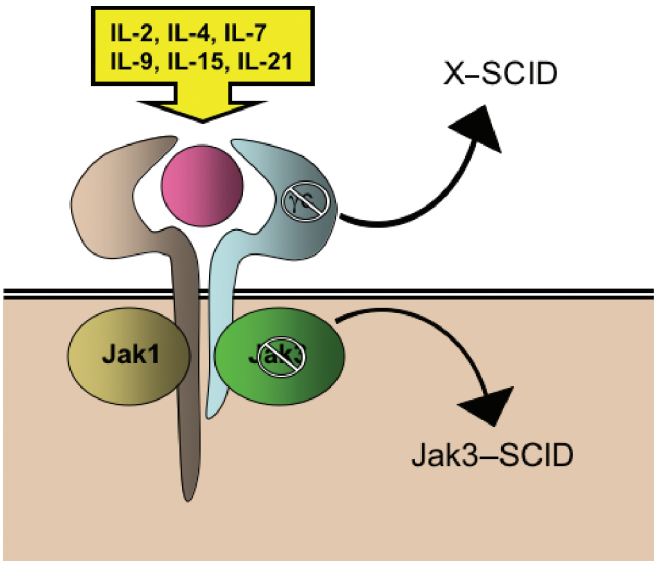

***Fig. 2.* The molecular basis of severe combined immunodeficiency.** A number of cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) utilize the common γ chain (γ_c) in conjunction with a ligand-specific chain to form their receptors. These receptor subunits bind Jak3 and Jak1. Mutations of IL-7R, γ_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.

interaction between Jak3 and γ_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 immunosuppressive 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 γ_c deficiency (38–40). These mice have small thymi, absence of lymph nodes, and reduced numbers of α/β and γ/δ T cells and NK cells. Analysis of the thymic precursors in $Jak3^{-/-}$ mice showed that there was severe reduction in progenitor cells,

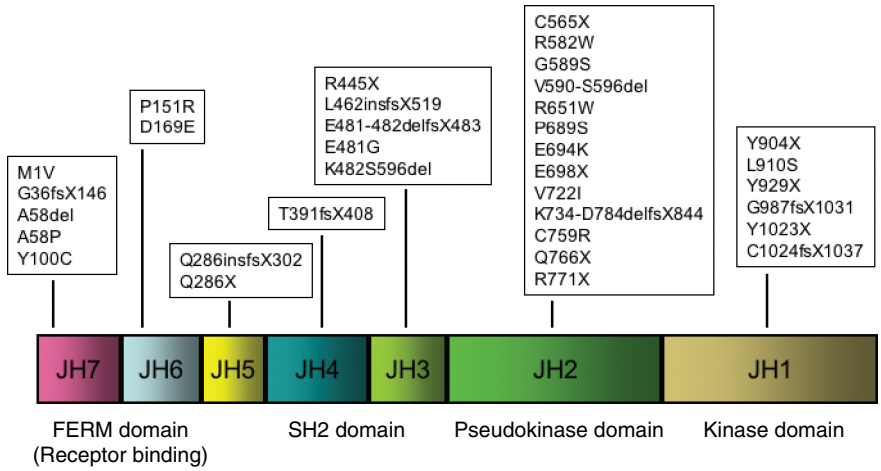

***Fig. 3.* Schematic of Jak3 structure and the mutations identified.** Seven Janus homology (JH) domains have been identified. JH1 is the carboxy-terminal tyrosine kinase domain. Adjacent to the kinase domain is the JH2 or pseudokinase domain, which itself lacks catalytic activity but is essential for regulating normal kinase activity. Jaks also have a src homology 2 (SH2) domain, although its function is poorly understood. The amino-terminus of the Jaks (JH5–JH7) has homology to FERM domain-containing proteins. This region of the Jaks mediates binding to 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.

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 γ_c)-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 γ_c 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 vitro* can still activate downstream kinases *in vivo* 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, γ_c. 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 γ_c (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 γ_c cytokines (80–82). In addition, SOCS1 forms an E3 ubiquitin ligase complex with elongins B and C, Cullin-5 (Cul-5), and Rbx1 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 destablilize 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 γ_c and Jak3 mutations, mutations of other cytokines, receptors, and signaling pathways were considered as additional candidates for causing SCID. Based

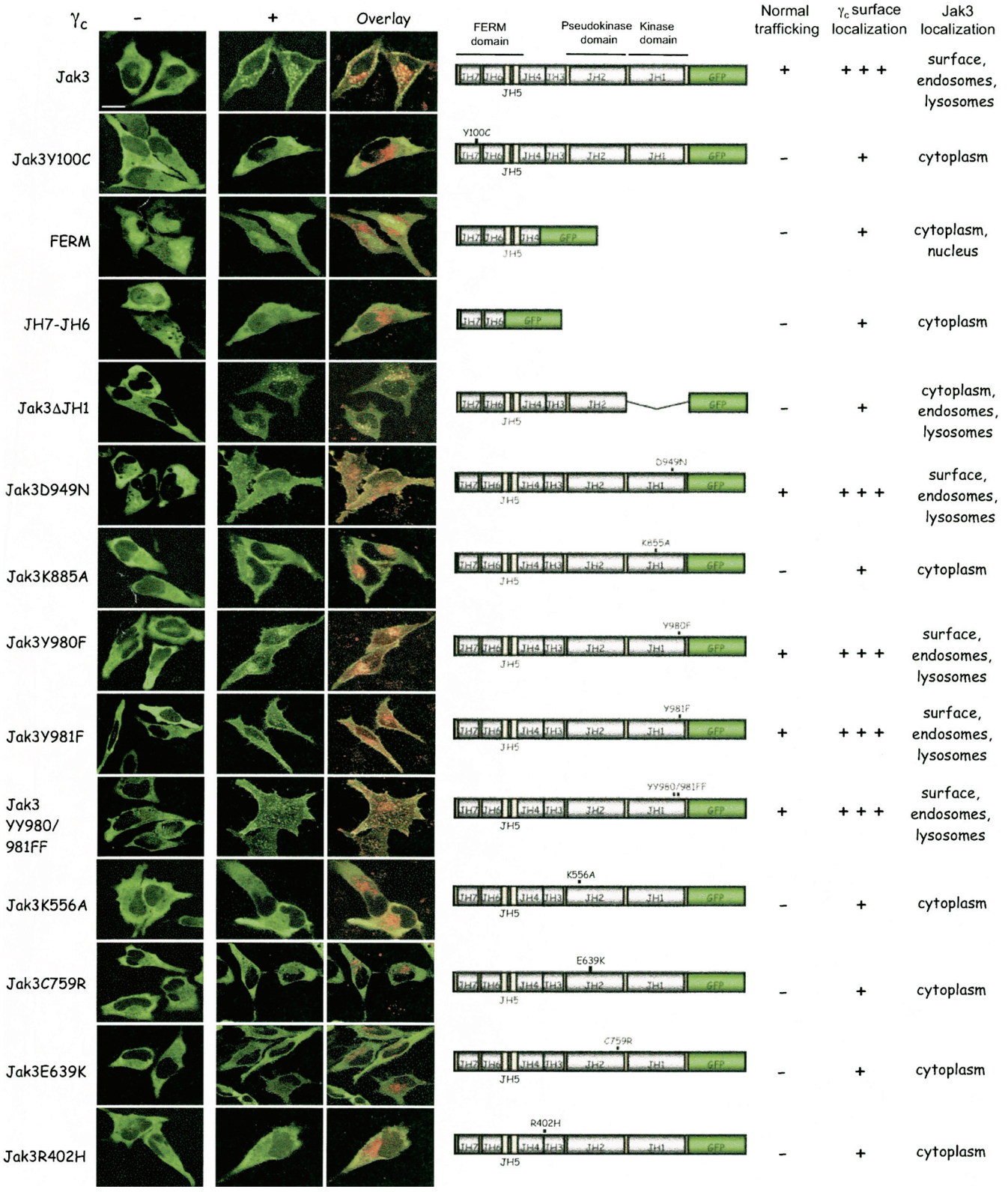

Fig. 4. Complexity of γ_c/Jak3 association and subcellular distribution. In the absence of γ_c, Jak3 is localized to the cytosol, whereas γ_c alone is localized to endosomes and the plasma membrane. When Jak3 and γ_c are both expressed, Jak3 localizes with γ_c in endosomes and enhances plasma membrane expression. The requirement for Jak3/γ_c colocalization is surprizingly 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/γ_c/Jak3 account for the majority (approximately 67–74%) of all cases of SCID, but because signaling by only one of the γ_c 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 γ_c 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-, γ_c-, and Jak3-deficient patients.

Given these data, it is useful to relate the action of γ_c cytokines with the clinical phenotypes seen in SCID due to γ_c, 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

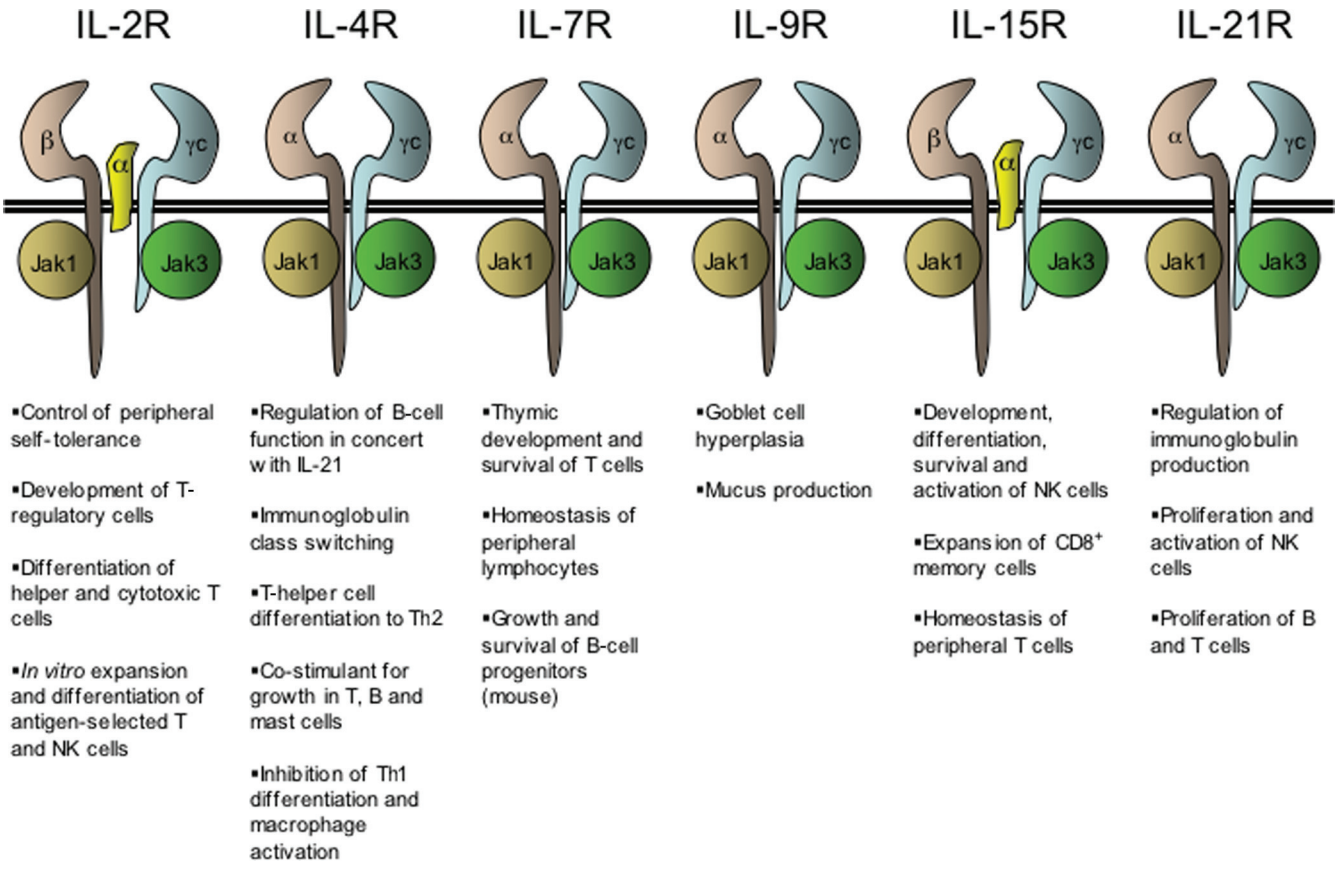

***Fig.* 5. Cytokine receptors that share γ_c subunit and their functions.** The lists of actions are purposefully abbreviated and illustrate the major functions of these cytokines as they relate to the pathogenesis of severe combined immunodeficiency (SCID). In addition, the actions of these cytokines, 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 γ_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 γ_c would be more severe because of defective IL-15 signaling.

B cells are present in patients with Jak3, γ_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 γ_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 γ_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 γ_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-2Rα and β) 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-2Rα 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 γ_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 γ_c cytokines (IL-7, IL-15, IL-4, and IL-21) nicely explains the clinical phenotype associated with Jak3 and γ_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 γ_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-7Rα chain and a distinctive subunit, TSLP receptor (TSLPR), which structurally resembles the γ_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 γ_c have poorer lymphoid development than mice lacking just γ_c. TSLP, therefore, likely accounts for some of the residual lymphoid development in γ_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 γ_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 γ_c-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 γ–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 γ_c 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 γ_c-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 γ_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^+ 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 transplant 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 γ_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 γ_c- and Jak3-deficient SCID patients (143, 144). In fact, retroviral γ_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 γ_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 γ_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 γ_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 γ_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 γ_c 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 γ_c (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 imanatib (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 leukopenia; 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 γ_c 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/γ_c/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.

References

1. Glanzmann E, Riniker P. Essentielle lymphocytophtose. Ein neues krankeitsbild aus der Sauglingspathologie. Ann Pediatr 1950;**174**:1–5.
2. Hitzig W, Bosch H, Huser HJ. Agammaglobulinemia & alymphocytosis with atrophy of lymphatic tissue. Helv Paediatr Acta 1958;**13**:551–585.
3. Buckley RH, et al. Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. J Pediatr 1997;**130**:378–387.
4. Buckley RH. Primary immunodeficiency diseases due to defects in lymphocytes. N Engl J Med 2000;**343**:1313–1324.
5. Candotti F, Notarangelo L, Visconti R, O'Shea J. Molecular aspects of primary immunodeficiencies: lessons from cytokine and other signaling pathways. J Clin Invest 2002;**109**:1261–1269.
6. Notarangelo LD, et al. Of genes and phenotypes: the immunological and molecular spectrum of combined immune deficiency. Defects of the gamma (c)-JAK3 signaling pathway as a model. Immunol Rev 2000;**178**:39–48.
7. Takeshita T, et al. Cloning of the gamma chain of the human IL-2 receptor. Science 1992;**257**:379–382.
8. Noguchi M, et al. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell 1993;**73**:147–157.
9. Puck JM, et al. The interleukin-2 receptor gamma chain maps to Xq13.1 and is mutated in X-linked severe combined immunodeficiency, SCIDX1. Hum Mol Genet 1993;**2**:1099–1104.
10. Markiewicz S, et al. Fine mapping of the human SCIDX1 locus at Xq12-13.1. Hum Mol Genet 1993;**2**:651–654.
11. Asao H, et al. Cutting edge: the common gamma-chain is an indispensable subunit of the IL-21 receptor complex. J Immunol 2001;**167**:1–5.

12. Leonard WJ, Noguchi M, Russell SM. Sharing of a common gamma chain, gamma c, by the IL-2, IL-4, and IL-7 receptors: implications for X-linked severe combined immunodeficiency (XSCID). Adv Exp Med Biol 1994;**365**:225–232.
13. Kondo M, et al. Functional participation of the IL-2 receptor gamma chain in IL-7 receptor complexes. Science 1994;**263**:1453–1454.
14. Kondo M, et al. Sharing of the interleukin-2 (IL-2) receptor gamma chain between receptors for IL-2 and IL-4. Science 1993;**262**:1874–1877.
15. Kimura Y, et al. Sharing of the IL-2 receptor gamma chain with the functional IL-9 receptor complex. Int Immunol 1995;**7**: 115–120.
16. Firmbach-Kraft I, Byers M, Shows T, Dalla-Favera R, Krolewski JJ. tyk2, prototype of a novel class of non-receptor tyrosine kinase genes. Oncogene 1990;**5**:1329–1336.
17. Harpur AG, Andres AC, Ziemiecki A, Aston RR, Wilks AF. JAK2, a third member of the JAK family of protein tyrosine kinases. Oncogene 1992;**7**:347–1353.
18. Krolewski JJ, Lee R, Eddy R, Shows TB, Dalla-Favera R. Identification and chromosomal mapping of new human tyrosine kinase genes. Oncogene 1990;**5**:277–282.
19. Wilks AF. Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc Natl Acad Sci USA 1989;**86**:1603–1607.
20. Wilks AF. Cloning members of protein-tyrosine kinase family using polymerase chain reaction. Methods Enzymol 1991;**200**:533–546.
21. Wilks AF, Harpur AG, Kurban RR, Ralph SJ, Zurcher G, Ziemiecki A. Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol Cell Biol 1991;**11**:2057–2065.
22. Kawamura M, et al. Molecular cloning of L-JAK, a Janus family protein-tyrosine kinase expressed in natural killer cells and activated leukocytes. Proc Natl Acad Sci USA 1994;**91**:6374–6378.
23. Rane SG, Reddy EP. JAK3: a novel JAK kinase associated with terminal differentiation of hematopoietic cells. Oncogene 1994;**9**: 2415–2423.
24. Takahashi T, Shirasawa T. Molecular cloning of rat JAK3, a novel member of the JAK family of protein tyrosine kinases. FEBS Lett 1994;**342**:124–128.
25. Witthuhn BA, et al. Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. Nature 1994;**370**:153–157.
26. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002;**298**:1912–1934.
27. Velazquez L, Fellous M, Stark GR, Pellegrini S. A protein tyrosine kinase in the interferon alpha/beta signaling pathway. Cell 1992;**70**:313–322.
28. Argetsinger LS, et al. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. Cell 1993;**74**:237–244.
29. Witthuhn BA, et al. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell 1993;**74**:227–236.
30. Muller M, et al. The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. Nature 1993;**366**:129–135.
31. Silvennoinen O, Ihle JN, Schlessinger J, Levy DE. Interferon-induced nuclear signalling by Jak protein tyrosine kinases. Nature 1993;**366**:583–585.
32. Silvennoinen O, Witthuhn BA, Quelle FW, Cleveland JL, Yi T, Ihle JN. Structure of the murine Jak2 protein-tyrosine kinase and its role in interleukin 3 signal transduction. Proc Natl Acad Sci USA 1993;**90**:8429–8433.
33. Watling D, et al. Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-gamma signal transduction pathway. Nature 1993;**366**:166–170.
34. Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994;**264**:1415–1421.
35. Rodig SJ, et al. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell 1998;**93**:373–383.
36. Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell 1998;**93**:397–409.
37. Parganas E, et al. Jak2 is essential for signaling through a variety of cytokine receptors. Cell 1998;**93**:385–395.
38. Nosaka T, et al. Defective lymphoid development in mice lacking Jak3. Science 1995;**270**:800–802.
39. Park SY, et al. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. Immunity 1995;**3**:771–782.
40. Thomis DC, Gurniak CB, Tivol E, Sharpe AH, Berg LJ. Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science 1995;**270**:794–797.
41. Shimoda K, et al. Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. Immunity 2000;**13**:561–571.
42. Karaghiosoff M, et al. Partial impairment of cytokine responses in Tyk2-deficient mice. Immunity 2000;**13**:549–560.
43. Boulay JL, O'Shea JJ, Paul WE. Molecular phylogeny within type I cytokines and their cognate receptors. Immunity 2003;**19**:159–163.
44. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. Annu Rev Immunol 1998;**16**:293–322.
45. O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell 2002;**109**:S121–S131.
46. Johnston JA, et al. Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15. Proc Natl Acad Sci USA 1995;**92**:8705–8709.
47. Lin JX, Leonard WJ. The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. Oncogene 2000;**19**:2566–2576.
48. Wurster AL, Tanaka T, Grusby MJ. The biology of Stat4 and Stat6. Oncogene 2000;**19**:2577–2584.
49. Johnston JA, et al. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. Nature 1994;**370**:151–153.
50. Boussiotis VA, et al. Prevention of T cell anergy by signaling through the gamma c chain of the IL-2 receptor. Science 1994;**266**:1039–1042.
51. Miyazaki T, et al. Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. Science 1994;**266**:1045–1047.
52. Russell SM, 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.
53. Macchi P, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature 1995;**377**:65–68.
54. Russell SM, et al. Mutation of Jak3 in a patient with SCID. essential role of Jak3 in lymphoid development. Science 1995;**270**:797–800.
55. Candotti F, et al. Structural and functional basis for JAK3-deficient severe combined immunodeficiency. Blood 1997;**90**:3996–4003.
56. Bozzi F, 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.

57. 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.
58. Notarangelo LD, et al. Mutations in severe combined immune deficiency (SCID) due to JAK3 deficiency. Hum Mutat 2001;**18**:255–263.
59. Roberts JL, 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.
60. Baird AM, Lucas JA, Berg LJ. A profound deficiency in thymic progenitor cells in mice lacking Jak3. J Immunol 2000;**165**:3680–3688.
61. Thomis DC, Lee W, Berg LJ. T cells from Jak3-deficient mice have intact TCR signaling, but increased apoptosis. J Immunol 1997;**159**:4708–4719.
62. Cao X, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity 1995;**2**:223–238.
63. DiSanto JP, Muller W, Guy-Grand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. Proc Natl Acad Sci USA 1995;**92**:377–381.
64. Grossman WJ, et al. Dysregulated myelopoiesis in mice lacking Jak3. Blood 1999;**94**:932–939.
65. Vihinen M, et al. Molecular modeling of the Jak3 kinase domains and structural basis for severe combined immunodeficiency. Clin Immunol 2000;**96**:108–118.
66. Chen M, et al. Complex effects of naturally occurring mutations in the JAK3 pseudokinase domain: evidence for interactions between the kinase and pseudokinase domains. Mol Cell Biol 2000;**20**:947–956.
67. Saharinen P, Takaluoma K, Silvennoinen O. Regulation of the Jak2 tyrosine kinase by its pseudokinase domain. Mol Cell Biol 2000;**20**:3387–3395.
68. Wan PT, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;**116**:855–867.
69. Cacalano NA, et al. Autosomal SCID caused by a point mutation in the N-terminus of Jak3: mapping of the Jak3-receptor interaction domain. EMBO J 1999;**18**:1549–1558.
70. Chen M, et al. The amino terminus of JAK3 is necessary and sufficient for binding to the common gamma chain and confers the ability to transmit interleukin 2-mediated signals. Proc Natl Acad Sci USA 1997;**94**:6910–6915.
71. Zhou YJ, et al. Unexpected effects of FERM domain mutations on catalytic activity of Jak3: structural implication for Janus kinases. Mol Cell 2001;**8**:959–969.
72. Chen R, et al. Regulation of the PH-domain-containing tyrosine kinase Etk by focal adhesion kinase through the FERM domain. Nat Cell Biol 2001;**3**:439–444.
73. Cooper LA, Shen TL, Guan JL. Regulation of focal adhesion kinase by its amino-terminal domain through an autoinhibitory interaction. Mol Cell Biol 2003;**23**: 8030–8041.
74. Dunty JM, Gabarra-Niecko V, King ML, Ceccarelli DF, Eck MJ, Schaller MD. FERM domain interaction promotes FAK signaling. Mol Cell Biol 2004;**24**:5353–5368.
75. Radtke S, et al. Novel role of Janus kinase 1 in the regulation of oncostatin M receptor surface expression. J Biol Chem 2002;**277**:11297–11305.
76. Saharinen P, Silvennoinen O. The Janus kinase protein family. In: Sehgal PB, Levy DE, Hirano T, eds. Signal Transducers and Activators of Transcription (Stats) Activation and Biology. Dordrecht, the Netherlands: Kluwer Academic Publishers 2003:27–43.
77. Hofmann SR, et al. Jak3-independent trafficking of the common gamma chain receptor subunit: chaperone function of Jaks revisited. Mol Cell Biol 2004;**24**:5039–5049.
78. Shuai K, Liu B. Regulation of JAK-STAT signaling in the immune system. Nat Rev Immunol 2003;**3**:900–911.
79. Zhou YJ, et al. Distinct tyrosine phosphorylation sites in JAK3 kinase domain positively and negatively regulate its enzymatic activity. Proc Natl Acad Sci USA 1997;**94**:13850–13855.
80. Chong MM, et al. Suppressor of cytokine signaling-1 is a critical regulator of interleukin-7-dependent $CD8^+$ T cell differentiation. Immunity 2003;**18**:475–487.
81. Sporri B, Kovanen PE, Sasaki A, Yoshimura A, Leonard WJ. JAB/SOCS1/SSI-1 is an interleukin-2-induced inhibitor of IL-2 signaling. Blood 2001;**97**:221–226.
82. Cornish AL, et al. Suppressor of cytokine signaling-1 regulates signaling in response to interleukin-2 and other gamma c-dependent cytokines in peripheral T cells. J Biol Chem 2003;**278**:22755–22761.
83. Ungureanu D, Saharinen P, Junttila I, Hilton DJ, Silvennoinen O. Regulation of Jak2 through the ubiquitin-proteasome pathway involves phosphorylation of Jak2 on Y1007 and interaction with SOCS-1. Mol Cell Biol 2002;**22**:3316–3326.
84. Kamizono S, et al. The SOCS box of SOCS-1 accelerates ubiquitin-dependent proteolysis of TEL-JAK2. J Biol Chem 2001;**276**: 12530–12538.
85. Malakhova OA, et al. Protein ISGylation modulates the JAK-STAT signaling pathway. Genes Dev 2003;**17**:455–460.
86. Kurzer JH, Argetsinger LS, Zhou YJ, Kouadio JL, O'Shea JJ, Carter-Su C. Tyrosine 813 is a site of JAK2 autophosphorylation critical for activation of JAK2 by SH2-B beta. Mol Cell Biol 2004;**24**:4557–4570.
87. O'Brien KB, O'Shea JJ, Carter-Su C. SH2-B family members differentially regulate JAK family tyrosine kinases. J Biol Chem 2002;**277**:8673–8681.
88. Asao H, et al. Hrs is associated with STAM, a signal-transducing adaptor molecule. Its suppressive effect on cytokine-induced cell growth. J Biol Chem 1997;**272**:32785–32791.
89. Takeshita T, et al. STAM, signal transducing adaptor molecule, is associated with Janus kinases and involved in signaling for cell growth and c-myc induction. Immunity 1997;**6**:449–457.
90. Endo K, et al. STAM2, a new member of the STAM family, binding to the Janus kinases. FEBS Lett 2000;**477**:55–61.
91. Lohi O, Lehto VP. STAM/EAST/Hbp adapter proteins – integrators of signalling pathways. FEBS Lett 2001;**508**:287–290.
92. Lohi O, Poussu A, Mao Y, Quiocho F, Lehto VP. VHS domain – a longshoreman of vesicle lines. FEBS Lett 2002;**513**:19–23.
93. Musso T, et al. Regulation of JAK3 expression in human monocytes: phosphorylation in response to interleukins 2, 4, and 7. J Exp Med 1995;**181**:1425–1431.
94. Tortolani PJ, et al. Regulation of JAK3 expression and activation in human B cells and B cell malignancies. J Immunol 1995;**155**:5220–5226.
95. Aringer M, et al. Characterization and analysis of the proximal Janus kinase 3 promoter. J Immunol 2003;**170**:6057–6064.
96. Mangan JK, Rane SG, Kang AD, Amanullah A, Wong BC, Reddy EP. Mechanisms associated with IL-6-induced up-regulation of Jak3 and its role in monocytic differentiation. Blood 2004;**103**:4093–4101.
97. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in T(−)B(+)NK(+) severe combined immunodeficiency. Nat Genet 1998;**20**:394–397.
98. Kofoed EM, et al. Growth hormone insensitivity associated with a STAT5b mutation. N Engl J Med 2003;**349**:1139–1147.
99. 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.

100. Peschon JJ, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med 1994;**180**:1955–1960.
101. Maki K, et al. Interleukin 7 receptor-deficient mice lack gammadelta T cells. Proc Natl Acad Sci USA 1996;**93**:7172–7177.
102. Candeias S, Peschon JJ, Muegge K, Durum SK. Defective T-cell receptor gamma gene rearrangement in interleukin-7 receptor knockout mice. Immunol Lett 1997;**57**:9–14.
103. Fry TJ, Mackall CL. Interleukin-7: master regulator of peripheral T-cell homeostasis?. Trends Immunol 2001;**22**:564–571.
104. Fry TJ, Mackall CL. Interleukin-7: from bench to clinic. Blood 2002;**99**:3892–3904.
105. Perumal NB, et al. TCR-gamma genes are rearranged but not transcribed in IL-7R alpha-deficient mice. J Immunol 1997;**158**:5744–5750.
106. Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nat Immunol 2000;**1**:426–432.
107. Becker TC, et al. Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. J Exp Med 2002;**195**:1541–1548.
108. Goldrath AW, et al. Cytokine requirements for acute and basal homeostatic proliferation of naive and memory $CD8^+$ T cells. J Exp Med 2002;**195**:1515–1522.
109. Seddon B, Tomlinson P, Zamoyska R. Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. Nat Immunol 2003;**4**:680–686.
110. Nutt SL, Urbanek P, Rolink A, Busslinger M. Essential functions of Pax5 (BSAP) in pro-B cell development: difference between fetal and adult B lymphopoiesis and reduced V-to-DJ recombination at the IgH locus. Genes Dev 1997;**11**:476–491.
111. Ozaki K, et al. A critical role for IL-21 in regulating immunoglobulin production. Science 2002;**298**:1630–1634.
112. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Annu Rev Immunol 2004;**22**:625–655.
113. Cooper MA, et al. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. Blood 2002;**100**:3633–3638.
114. Fehniger TA, Cooper MA, Caligiuri MA. Interleukin-2 and interleukin-15: immunotherapy for cancer. Cytokine Growth Factor Rev 2002;**13**:169–183.
115. Schorle H, Holtschke T, Hunig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature 1991;**352**:621–624.
116. Kundig TM, Schorle H, Bachmann MF, Hengartner H, Zinkernagel RM, Horak I. Immune responses in interleukin-2-deficient mice. Science 1993;**262**:1059–1061.
117. Nelson BH. Interleukin-2 signaling and the maintenance of self-tolerance. Curr Dir Autoimmun 2002;**5**:92–112.
118. Refaeli Y, Van Parijs L, Abbas AK. Genetic models of abnormal apoptosis in lymphocytes. Immunol Rev 1999;**169**: 273–282.
119. Bassiri H, Carding SR. A requirement for IL-2/IL-2 receptor signaling in intrathymic negative selection. J Immunol 2001;**166**:5945–5954.
120. Shevach EM. Regulatory T cells in autoimmmunity. Annu Rev Immunol 2000;**18**:423–449.
121. Malek TR. The main function of IL-2 is to promote the development of T regulatory cells. J Leukoc Biol 2003;**74**:961–965.
122. Sharfe N, Dadi HK, Shahar M, Roifman CM. Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. Proc Natl Acad Sci USA 1997;**94**:3168–3171.
123. Roifman CM. Human IL-2 receptor alpha chain deficiency. Pediatr Res 2000;**48**:6–11.
124. Sharfe N, Shahar M, Roifman CM. An interleukin-2 receptor gamma chain mutation with normal thymus morphology. J Clin Invest 1997;**100**:3036–3043.
125. Frucht DM, et al. Unexpected and variable phenotypes in a family with JAK3 deficiency. Genes Immun 2001;**2**:422–432.
126. Mella P, et al. Development of autologous T lymphocytes in two males with X-linked severe combined immune deficiency: molecular and cellular characterization. Clin Immunol 2000;**95**:39–50.
127. Brugnoni D, et al. Development of autologous, oligoclonal, poorly functioning T lymphocytes in a patient with autosomal recessive severe combined immunodeficiency caused by defects of the Jak3 tyrosine kinase. Blood 1998;**91**:949–955.
128. Townsend JM, Fallon GP, Matthews JD, Smith P, Jolin EH, McKenzie NA. IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. Immunity 2000;**13**:573–583.
129. McMillan SJ, Bishop B, Townsend MJ, McKenzie AN, Lloyd CM. The absence of interleukin 9 does not affect the development of allergen-induced pulmonary inflammation nor airway hyperreactivity. J Exp Med 2002;**195**:51–57.
130. Carpino N, et al. Absence of an essential role for thymic stromal lymphopoietin receptor in murine B-cell development. Mol Cell Biol 2004;**24**:2584–2592.
131. Al-Shami A, et al. A role for thymic stromal lymphopoietin in $CD4^+$ T-cell development. J Exp Med 2004;**200**:159–168.
132. Watanabe N, et al. Human thymic stromal lymphopoietin promotes dendritic cell-mediated $CD4^+$ T cell homeostatic expansion. Nat Immunol 2004;**5**:426–434.
133. Villa A, et al. Monocyte function in a severe combined immunodeficient patient with a donor splice site mutation in the Jak3 gene. Blood 1996;**88**:817–823.
134. Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. Blood 2002;**99**:872–878.
135. Schumacher RF, 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.
136. Hacia JG. Resequencing and mutational analysis using oligonucleotide microarrays. Nat Genet 1999;**21**:42–47.
137. Patel DD, Gooding ME, Parrott RE, Curtis KM, Haynes BF, Buckley RH. Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. N Engl J Med 2000;**342**:1325–1332.
138. Antoine C, 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.
139. Haddad E, et al. Long-term immune reconstitution and outcome after HLA-nonidentical T-cell-depleted bone marrow transplantation for severe combined immunodeficiency: a European retrospective study of 116 patients. Blood 1998;**91**:3646–3653.
140. Wijnaendts L, Le Deist F, Griscelli C, Fischer A. Development of immunologic functions after bone marrow transplantation in 33 patients with severe combined immunodeficiency. Blood 1989;**74**:2212–2219.
141. Dror Y, et al. Immune reconstitution in severe combined immunodeficiency disease after lectin-treated, T-cell-depleted haplocompatible bone marrow transplantation. Blood 1993;**81**:2021–2030.

142. Laffort C, et al. Severe cutaneous papillomavirus disease after haemopoietic stem-cell transplantation in patients with severe combined immune deficiency caused by common gammac cytokine receptor subunit or JAK-3 deficiency. Lancet 2004;**363**:2051–2054.
143. Cavazzana-Calvo M, Hacein-Bey S, Yates F, de Villartay JP, Le Deist F, Fischer A. Gene therapy of severe combined immunodeficiencies. J Gene Med 2001;**3**:201–206.
144. Fischer A, Hacein-Bey S, Cavazzana-Calvo M. Gene therapy of severe combined immunodeficiencies. Nat Rev Immunol 2002;**2**:615–621.
145. Candotti F, Johnston JA, Puck JM, Sugamura K, O'Shea JJ, Blaese RM. Retroviral-mediated gene correction for X-linked severe combined immunodeficiency. Blood 1996;**87**:3097–3102.
146. Candotti F, Oakes SA, Johnston JA, Notarangelo LD, O'Shea JJ, Blaese RM. In vitro correction of JAK3-deficient severe combined immunodeficiency by retroviral-mediated gene transduction. J Exp Med 1996;**183**:2687–2692.
147. Candotti F, O'Shea JJ, Villa A. Severe combined immune deficiencies due to defects of the common gamma chain-JAK3 signaling pathway. Springer Semin Immunopathol 1998;**19**:401–415.
148. Oakes SA, et al. Signaling via IL-2 and IL-4 in JAK3-deficient severe combined immunodeficiency lymphocytes: JAK3-dependent and independent pathways. Immunity 1996;**5**:605–615.
149. Otsu M, Anderson SM, Bodine DM, Puck JM, O'Shea JJ, Candotti F. Lymphoid development and function in X-linked severe combined immunodeficiency mice after stem cell gene therapy. Mol Ther 2000;**1**:145–153.
150. Hacein-Bey H, et al. gamma-c gene transfer into SCID X1 patients' B-cell lines restores normal high-affinity interleukin-2 receptor expression and function. Blood 1996;**87**:3108–3116.
151. Hacein-Bey S, Basile GD, Lemerle J, Fischer A, Cavazzana-Calvo M. gammac gene transfer in the presence of stem cell factor, FLT-3L, interleukin-7 (IL-7), IL-1, and IL-15 cytokines restores T-cell differentiation from gammac (-) X-linked severe combined immunodeficiency hematopoietic progenitor cells in murine fetal thymic organ cultures. Blood 1998;**92**:4090–4097.
152. Bunting KD, Sangster MY, Ihle JN, Sorrentino BP. Restoration of lymphocyte function in Janus kinase 3-deficient mice by retroviral-mediated gene transfer. Nat Med 1998;**4**:58–64.
153. Bunting KD, Flynn KJ, Riberdy JM, Doherty PC, Sorrentino BP. Virus-specific immunity after gene therapy in a murine model of severe combined immunodeficiency. Proc Natl Acad Sci USA 1999;**96**:232–237.
154. Bunting KD, Lu T, Kelly PF, Sorrentino BP. Self-selection by genetically modified committed lymphocyte precursors reverses the phenotype of JAK3-deficient mice without myeloablation. Hum Gene Ther 2000;**11**:2353–2364.
155. Hacein-Bey-Abina S, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med 2002;**346**:1185–1193.
156. Sorrentino BP, Lu T, Ihle JN, Buckley RH, Cunningham JM. A clinical attempt to treat JAK3-deficient SCID using retroviral-mediated gene transfer to bone marrow $CD34^{+}$ cells. Mol Ther 2003;**7**:S449.
157. Warren AJ, Colledge WH, Carlton MB, Evans MJ, Smith AJ, Rabbitts TH. The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. Cell 1994;**78**:45–57.
158. Yamada Y, Warren AJ, Dobson C, Forster A, Pannell R, Rabbitts TH. The T cell leukemia LIM protein Lmo2 is necessary for adult mouse hematopoiesis. Proc Natl Acad Sci USA 1998;**95**:3890–3895.
159. Boehm T, Foroni L, Kaneko Y, Perutz MF, Rabbitts TH. The rhombotin family of cysteine-rich LIM-domain oncogenes: distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. Proc Natl Acad Sci USA 1991;**88**:4367–4371.
160. Neale GA, Rehg JE, Goorha RM. Disruption of T-cell differentiation precedes T-cell tumor formation in LMO-2 (rhombotin-2) transgenic mice. Leukemia 1997;**11**:289–290.
161. Druker BJ. Perspectives on the development of a molecularly targeted agent. Cancer Cell 2002;**1**:31–36.
162. Thompson JE, et al. Photochemical preparation of a pyridone containing tetracycle: a Jak protein kinase inhibitor. Bioorg Med Chem Lett 2002;**12**:1219–1223.
163. Meydan N, et al. Inhibition of acute lymphoblastic leukaemia by a Jak-2 inhibitor. Nature 1996;**379**:645–648.
164. Kirken RA, et al. Tyrphostin AG-490 inhibits cytokine-mediated JAK3/STAT5a/b signal transduction and cellular proliferation of antigen-activated human T cells. J Leukoc Biol 1999;**65**:891–899.
165. Changelian PS, et al. Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. Science 2003;**302**:875–878.
166. Sudbeck EA, et al. Structure-based design of specific inhibitors of Janus kinase 3 as apoptosis-inducing antileukemic agents. Clin Cancer Res 1999;**5**:1569–1582.
167. Saemann MD, et al. Suppression of early T-cell-receptor-triggered cellular activation by the Janus kinase 3 inhibitor WHI-P-154. Transplantation 2003;**75**:1864–1872.