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Cytokines and immunodeficiency diseases: critical roles of the γ_c-dependent cytokines interleukins 2, 4, 7, 9, 15, and 21, and their signaling pathways

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Summary: In this review, we discuss the role of cytokines and their signaling pathways in immunodeficiency. We focus primarily on severe combined immunodeficiency (SCID) diseases as the most severe forms of primary immunodeficiencies, reviewing the different genetic causes of these diseases. We focus in particular on the range of forms of SCID that result from defects in cytokine-signaling pathways. The most common form of SCID, X-linked SCID, results from mutations in the common cytokine receptor γ-chain, which is shared by the receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21, underscoring that X-linked SCID is indeed a disease of defective cytokine signaling. We also review the signaling pathways used by these cytokines and the phenotypes in humans and mice with defects in the cytokines or signaling pathways. We also briefly discuss other cytokines, such as interferon-γ and IL-12, where mutations in the ligand or receptor or signaling components also cause clinical disease in humans.

Overview of immunodeficiency diseases

Primary immunodeficiencies are a diverse group of more than 100 syndromes characterized by defects in the immune system function and heightened susceptibility to infections (1–3). In many of these immunodeficiencies, the defective genes have been identified, whereas in others, the genetic defects still remain to be determined. Functional defects in different cell types can result in immunodeficiency. For example, mutations in T-cell-specific genes such as CD3ε, CD3γ, or ζ-associated protein of 70 kDa (ZAP-70) result in severe T-cell defects, whereas mutation of the B-cell-specific Bruton's tyrosine kinase (Btk) results in Bruton's X-linked agammaglobulinemia. Interestingly, mutations in CD40 ligand, which is produced by T cells, causes hyper-immunoglobulin M (IgM) syndrome, where the main defect is in B cells, resulting from a lack of engagement of CD40 on the B-cell surface. Some

primary immunodeficiencies involve non-lymphoid cell types. For example, mutations in cytochrome b or β2-integrin cause defects mainly in phagocyte function.

The most severe primary immunodeficiencies are the various forms of severe combined immunodeficiency (SCID) (1, 2, 4). SCID consists mainly of inherited disorders characterized by defects in both T- and B-lymphocyte function, with variable defects in natural killer (NK) cell cytolytic activity as well. SCID is a relatively rare syndrome with an average incidence of about 1/80 000 live births (3). Patients with SCID suffer from severe opportunistic infections such as adenovirus, Epstein–Barr virus, *Candida albicans*, and *Pneumocystis carinii*, and they typically die within the first year of life if not treated. However, bone marrow transplantation fortunately can 'cure' most SCID patients (4, 5). Although the clinical manifestations of SCID are somewhat homogeneous, the molecular causes leading to SCID are diverse. Both autosomal and X-linked forms of SCID are known (6). In this review, we focus on defective cytokine signaling as the cause of immunodeficiency, in particular on the relationship to SCID. As mutations in the common cytokine receptor γ-chain (γ_c) result in X-linked SCID (XSCID), the most common form of SCID, we will discuss the cytokines that share this receptor chain, namely interleukins (ILs) 2, 4, 7, 9, 15, and 21.

Causes of SCID

The first autosomal recessive defect causing SCID was identified through positional cloning. The defect was in the gene encoding adenosine deaminase (ADA) gene, which results in the cellular accumulation of adenosine and its derivates, leading to lymphocyte death by apoptosis (7). The most common form of SCID is XSCID (SCIDX1). It is also known as the 'Bubble Boy' disease because of an XSID patient with this disease who lived for 12 years in a sterile protective environment prior to receiving a bone marrow transplant; unfortunately, the graft failed and he succumbed to an Epstein–Barr virus-associated lymphoma (8). The XSCID genetic locus was mapped to chromosome Xq13 (9). An entirely different line of research had identified and mapped the IL-2 receptor γ-chain to the same chromosomal region, leading to the hypothesis and subsequent confirmation that mutations in IL-2Rγ were the cause of XSCID (10). Identification of mutations in a chain of the IL-2 receptor as the cause of XSCID was most unexpected, as humans (11, 12) and mice (13) deficient in IL-2 were known to have normal lymphoid development. The explanation to this enigma was that IL-2Rγ turned out to be a shared receptor component for other cytokines as well. IL-2Rγ was initially shown to be shared by the receptors for IL-4 and IL-7 (14–16), and, accordingly, was renamed as the γ_c (15, 16). Since then, γ_c has been shown to also be a component of the receptors for IL-9, IL-15, and IL-21 (17–20). As discussed in greater detail below, a variety of data indicates that the lack of T cells and NK cells in XSCID are explained by the lack of IL-7 and IL-15 signaling, respectively (2), whereas defective signaling by IL-4 and IL-21 contributes to an intrinsic B-cell defect (21).

The genetic defects in most cases of SCID are now known. In a recent retrospective survey of 170 patients with SCID, mutations in the *IL2RG* gene, which encodes γ_c and thus results in XSCID, accounted for 46% of cases of SCID (4). ADA deficiency was the second most common cause (17%). Some of the other defined molecular defects leading to SCID include inactivating mutations in Janus kinase-3 (Jak3), a γ_c-associated tyrosine kinase (7%), IL-7Rα, which is a component of the receptors for both IL-7 and thymic stromal lymphopoietin (TSLP) (10%) (22), and the T- and B-cell receptor rearrangement-associated ARTEMIS (1.2%) and *RAG* (2.9%) genes (4). *Table 1* lists the major causes of SCID.

SCID can be divided into different forms based on the lymphocyte subsets affected and on the type of mechanism (*Table 1*). For example, some SCID patients have developmental defects in T cells, B cells, and NK cells ($T^-B^-NK^-$SCID), whereas others have defects only in T cells and NK cells ($T^-B^+NK^-$SCID), in T and B cells ($T^-B^-NK^+$SCID), or only in T cells ($T^-B^+NK^+$SCID). ADA deficiency is an example of $T^-B^-NK^-$SCID, whereas mutations in γ_c or Jak3 result in $T^-B^+NK^-$SCID. $T^-B^-NK^+$SCID is usually caused by mutations affecting antigen receptor rearrangements (in ARTEMIS, Rag1, or Rag2), whereas $T^-B^+NK^+$SCID can result from mutations in the *IL7R* or *CD45* genes (1, 2, 4, 6) (*Table 1*). Murine models of human SCID syndromes have been created by the use of knockout mice. In some cases, there are apparent differences in the phenotypes in humans and mice. Mice lacking either γ_c or Jak3 have a $T^-B^-NK^-$ phenotype in contrast to the $T^-B^+NK^-$ phenotype in humans (23–26), a point that is discussed below. Although SCID and γ_c-dependent cytokines are the

Table 1. **Known molecular causes of severe combined immunodeficiency**

		Phenotype T, B, or NK	
Gene defect	Mechanism	Human	Mouse
IL2RG	Cytokine signaling	$T^-B^+NK^-$	$T^-B^-NK^-$
JAK3	Cytokine signaling	$T^-B^+NK^-$	$T^-B^-NK^-$
IL7R	Cytokine signaling	$T^-B^+NK^+$	$T^-B^-NK^+$
RAG1	Antigen receptor recombination	$T^-B^-NK^+$	$T^-B^-NK^+$
RAG2	Antigen receptor recombination	$T^-B^-NK^+$	$T^-B^-NK^+$
ARTEMIS	Antigen receptor recombination	$T^-B^-NK^+$	$T^-B^-NK^+$
ADA	Metabolism	$T^-B^-NK^-$	$T^-B^-NK^-$

major focus of this review, we wish to also briefly discuss other immunodeficiencies resulting from mutations in other cytokine receptors or signaling molecules.

Other cytokine-related immunodeficiencies

Defects in non-γ_c-sharing cytokines can also result in immunodeficiency (1–3, 27). Patients with defective interferon (IFN)-γ signaling due to mutations in the genes encoding IFNGR1 (28, 29), IFNGR2 (30), or signal transducer and activator of transcription-1 (Stat1) (31) are extremely susceptible to mycobacterial infections, such as bacillus Calmette–Guérin and *Mycobacterium tuberculosis*, as well as to other intracellular bacteria (27). Stat1 is a critical signal-transducing molecule in the IFN-γ pathway (32, 33). The differentiation of T-helper (Th) cells into IFN-γ-secreting Th1-type cells is regulated in part by cytokines, particularly IL-12 (34). Accordingly, mutations in IL-12p40 subunit or its receptor IL12-Rβ1 also result in decreased immunity to intracellular infections (35–37). Thus, mutations in genes that control Th1 development and effector functions can result in immunodeficiency, even if they do not cause SCID.

The defective gene encodes XSCID is γ_c, a component of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21

γ_c was first identified as the third component of the receptor for IL-2 and known as IL-2Rγ (38). Earlier, two other components of the receptor for IL-2, IL-2Rα (39–42) and IL-2Rβ (43–46), had been identified. IL-2Rα can mediate low-affinity IL-2 binding ($K_d = 10^{-8}$ M) but cannot transduce signals (47–49). IL-2Rβ and γ_c together form intermediate affinity IL-2 receptors ($K_d = 10^{-9}$ M), which can signal in the presence of relatively high concentrations of IL-2 and which are expressed on NK cells and resting T cells (44, 45, 50). High-affinity IL-2 receptors ($K_d = 10^{-11}$ M) contain IL-2Rα, IL-2Rβ, and γ_c and can signal in response to very low concentrations of IL-2. γ_c was shown to be a critical component of receptors for IL-4, IL-7, IL-9, IL-15, and IL-21 (14–20). Together, these cytokines are sometimes referred to as IL-2-family or γ_c-dependent cytokines. Note, however, that in addition to IL-4 acting through a receptor containing IL-4Rα and γ_c in hematopoietic cells, in some cell types, it can also act through a receptor containing IL-4Rα and IL-13Rα1, referred to as the type 2 IL-4R (22). In this review, we refer to IL-4 as a γ_c-dependent cytokine as many of its actions require γ_c. TSLP and IL-13 are members of an extended family, because TSLP shares the IL-7Rα with IL-7 and IL-13 shares the IL-4Rα with IL-4 (22). See *Fig. 1* for a schematic of γ_c-dependent cytokines and their receptors. Mice deficient in different γ_c-dependent cytokines and their receptors have been created, and the phenotypes of these mice are summarized in *Table* 2.

The biology of γ_c-sharing cytokines

Given that the gene encoding γ_c is defective in XSCID and that γ_c is a component of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, an understanding of XSCID requires that we know more about the biology of these cytokines. The major known actions of these cytokines are summarized below.

IL-2 is a T-cell growth factor that also controls peripheral tolerance and affects NK cell cytolytic activity

The *in vitro* T-cell growth-promoting properties of IL-2 were well established at the initial discovery of IL-2 as 'T-cell

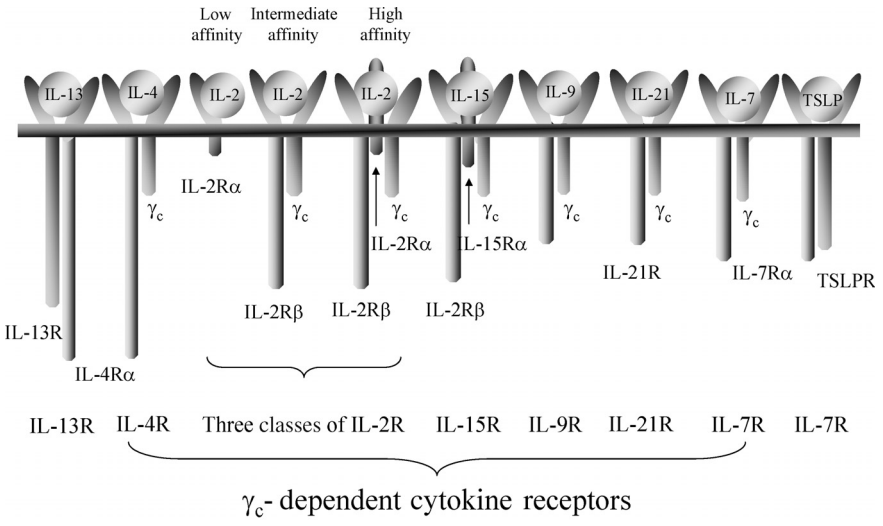

Fig. 1. A schematic representation of γ_c-sharing cytokines and their receptors. TSLPR, which shares IL-7Rα with IL-7, and IL-13, which shares IL-4Rα with IL-4, are also included as related cytokines. TSLPR is the protein in databases that is most related to γ_c.

Table 2. **Knockout phenotypes of γc-cytokines, their receptors, and associated signaling molecules**

	Affected γc-cytokines							
Molecule	IL-2	IL-4	IL-7	IL-9	IL-15	IL-21	Other	Major immunological phenotype
IL-2	+							Age-dependent $CD4^+$ T-cell expansion and autoimmunity
IL-4		+						Defective IgE (and IgG1) class-switch and Th2 differentiation
IL-7			+					Defective T- and B-cell development*
IL-9				+				Defective goblet cell hyperplasia and mastocytosis in the lung
IL-15					+			Decreased number of $CD8^+$ T cells and NK cells
IL-2Rα	+							$CD4^+$ cell expansion and autoimmunity
IL-2Rβ	+					+		Like IL-2 plus absent NK cells (IL-15)
IL-4Rα		+					(IL-13)	Like IL-4 but somewhat more severe†
IL-7Rα			+				TSLP	Like IL-7 but somewhat more severe*‡
IL-15Rα					+			See IL-15
IL-21Rα						+		Defective immunoglobulin production§
γc	+	+	+	+	+	+		Defective T, B, and NK cell differentiation*
Jak1	+	+	+	+	+	+	Many	Embryonic lethal
Jak3	+	+	+	+	+	+		Defective T, B, and NK cell differentiation*
Stat1						(+)	IFN-γ	Defective anti-viral responses
Stat5a	+		+	+	+	(+)	Many	Decreased T-cell proliferation
Stat5b	+		+	+	+	(+)	Many	Decreased T-cell proliferation, decreased number of NK cells
Stat3	(+)		(+)	(+)	(+)	+	Many	Embryonic lethal
Stat6		+					IL-13	See IL-4, IL-4Rα, defective B-cell proliferation†

IL, interleukin; NK, natural killer; TSLP, thymic stromal lymphopoietin; IFN, interferon.
*Although IL-7-, IL-7Rα-, γ_c-, and Jak3-deficient mice show defective T-cell development, they do have some $CD4^+$ T cells indicating that γc-independent signal can support some $CD4^+$ development.
†IL-13 receptor consists of IL-13Rα1 and IL-4Rα, and IL-13 signaling is abrogated in IL-4Rα knockout.
‡TSLP receptor consists of TSLP-specific chain and IL-7Rα, thus IL-7Rα knockout is defective in IL-7 and TSLP signaling.
§The role of IL-21 in immunoglobulin production is more greatly revealed in combination with IL-4 deficiency (21).

growth factor' (51). In IL-2-deficient mice, lymphoid development is essentially normal, with no obvious abnormalities of T, B, or NK cells (13). However, *in vitro* T-cell responses to polyclonal stimuli were abnormal, and elevated serum levels of IgG1 and IgE suggested some immune system malfunction (13). Nevertheless, *in vivo* responses to vaccinia, lymphocytic choriomeningitis virus, and vesicular stomatitis virus were not impaired (52). Aging $Il2^{-/-}$ animals manifested autoimmune symptoms, including autoimmune hemolytic anemia and inflammatory bowel disease (53) that were associated with the development of adenopathy and accumulation of activated lymphocytes (53, 54). Similar phenotypes are also seen in IL-2Rα-deficient mice (55) and in an IL-2Rα-deficient patient (56). These autoimmune phenomena are consistent with the fact that IL-2 and IL-2Rα (and thus high-affinity IL-2 receptors) play very important roles in the maintenance of peripheral tolerance *in vivo*.

The mechanism by which IL-2 controls T-cell homeostasis and maintains peripheral tolerance is not fully understood, but at least two important mechanisms have been implicated (57). First, IL-2 can sensitize T cells to death via a phenomenon known as activation-induced cell death (AICD). AICD involves the Fas–Fas ligand death pathway and is triggered by secondary antigen stimulation (58), and mutations in Fas or Fas ligand result in uncontrolled T-cell lymphoproliferation and a lupus-like autoimmune disease (59). IL-2 is critical for priming cells to AICD (58), as culturing cells in IL-2 *in vitro* sensitizes them to AICD (60), and T cells from $Il2r\alpha^{-/-}$ mice exhibit defective AICD (61). Furthermore, IL-2 induces Fas ligand expression-activated T cells in a Stat5-dependent manner (62, 63). Second, over the past few years, considerable evidence has accumulated to suggest that $CD4^+CD25^+$ regulatory T cells are involved in suppressing potentially autoreactive T-cell clones (64–66) and that IL-2 is required for the development of these cells (67). These cells are either missing or decreased in mice lacking expression of IL-2, IL-2Rα, or IL-2Rβ (68–71), as well as in Stat5a/b knockout mice (72, 73), suggesting that IL-2 and Stat5 are needed for regulatory T-cell development. Interestingly, thymus-restricted transgenic expression of IL-2Rβ can restore functional $CD4^+CD25^+$ regulatory T cells, suggesting that IL-2 signaling in the thymus is critical for the development of regulatory T cells (71). Whether regulatory T cells represent a developmentally distinct lymphocyte population or a functionally distinct subset of $CD4^+$ T cells is still unresolved. IL-2 also has other major actions such as increasing cytolytic activity of NK cells (51) and affecting the differentiation of Th2-type T cells (74).

IL-7 regulates lymphocyte development and homeostasis

IL-7 has effects on both T- and B-cell biology (75–77). An important role for IL-7 in T- and B-cell development was

first suggested by experiments in which B- and T-cell development was abrogated when mice were injected with antibodies to either IL-7 or IL-7Rα (78). The critical role for IL-7 in lymphocyte development was confirmed by the generation of mice deficient in IL-7Rα and IL-7 (79, 80). Interestingly, SCID patients with defective IL-7Rα expression have been identified, and these individuals have a $T^-B^+NK^-$ form of SCID (81). This finding underscores the non-redundant role of human IL-7Rα for T-cell but not B-cell development. Whether IL-7 contributes at all to human B-cell development is not clear; moreover, the cytokines or other molecules that contribute to B-cell development in humans, analogous to the role of IL-7 in the mouse, are also unknown. IL-7 is produced by thymic and bone marrow stromal cells (82–84). In the thymus, IL-7-dependent signals are critical at the double-negative stage ($CD4^-CD8^-CD44^-CD25^+$), and T-cell development beyond this point is blocked in IL-7- and IL-7Rα-deficient mice. In these animals, thymocyte and peripheral T-cell numbers are reduced, and T-cell responses to polyclonal stimuli are abnormal. IL-7 likely provides several distinct signals necessary for T-cell development, including effects on survival, proliferation, and T-cell receptor (TCR) rearrangements (75–77). The B-cell defect in IL-7- and IL-7Rα-deficient mice appears early in development at the pre-pro B-cell stage (79, 80), and correspondingly, transgenic expression or injection of IL-7 augments the expansion of early B cells *in vivo* (85–87). The role for IL-7 in murine B-cell development probably relates to a combination of effects on early B-cell proliferation, survival, and Ig gene rearrangement(s) (77, 88). Humans lacking γ_c or Jak3 also have normal B-cell numbers and a lack of T-cell help, but in these cases, it seems clear that there is an intrinsic B-cell defect as well. In XSCID in particular, this defect is indicated by the non-random X-inactivation patterns that are seen in terminally differentiated B cells (89). Moreover, post-bone marrow transplantation, both XSCID and Jak3-deficient patients require chronic intravenous gamma globulin if the donor B cells do not engraft, a situation that is not the case in IL-7R deficiency (3).

IL-7 also contributes to the regulation of lymphocyte homeostasis (90–93). Homeostatic proliferation of naïve T cells requires 'space' (lymphopenia) and low-avidity self-major histocompatibility complex (MHC) interactions. Physiologic homeostatic lymphocyte expansion that normally occurs in the neonate (94, 95) is markedly diminished for both naïve $CD4^+$ and $CD8^+$ T cells in IL-7-deficient hosts, suggesting that IL-7 is essential for the homeostatic expansion of naïve T cells (96–98). Correspondingly, transgenic overexpression of IL-7 results in expansion of T cells (99). This effect is not only based on increased survival, as transgenic expression of Bcl-2 (which is induced by IL-7) does not compensate for IL-7 in these models (97). IL-7 also contributes to the expansion of effector T cells and the homeostatic proliferation of $CD8^+$ memory cells (96, 100), although other cytokines such as IL-15 may be more important (101). In fact, although IL-7Rα is highly expressed on resting T cells, it is rapidly downregulated by TCR stimulation as well as by IL-2, further supporting the idea that other cytokines are more relevant for effector functions (102).

IL-9 is a mast cell growth factor

IL-9 was first identified as a late-acting T-cell growth factor and mast cell growth factor. IL-9-deficient mice have also been generated, and the lymphoid compartment develops normally in these animals. However, these mice exhibit excessive mucus production and mast cell proliferation (103). Such abnormalities have not been reported in humans with XSCID. Interestingly, IL-9 transgenic mice develop thymic lymphomas, consistent with the presence of IL-9 receptors in the thymus and with the ability of thymocytes to respond to IL-9 (104).

IL-15 regulates NK cell development and memory cell homeostasis

IL-15 was first identified as a T-cell growth factor activity (105, 106). Similar to IL-2, IL-15R consists of three chains: IL-15Rα, IL-2Rβ, and γ_c (101, 107, 108). Many of the biological actions attributed to IL-2 can also be induced by IL-15. Both IL-2 and IL-15 are T-cell growth factors *in vitro*, and they can both stimulate the proliferation of NK cells as well as induce NK cell cytolytic activity. However, important *in vivo* differences in the actions of these two cytokines have emerged, particularly in regard to NK cell development and $CD8^+$ T-cell homeostasis (108). As noted above, IL-2- and IL-2Rα-deficient mice show generally normal T, B, and NK cell development. However, IL-2Rβ-deficient mice have profoundly decreased numbers of NK cells and γ/δ T cells, suggesting that IL-15 but not IL-2 is necessary for the development/differentiation of these cells (109). Indeed, mice deficient in either IL-15 or IL-15Rα lack NK cells, confirming the distinctive role for IL-15 in NK cell development (110, 111).

IL-15- and IL-15Rα-deficient mice also exhibit decreased numbers of $CD8^+$ T cells and almost a total lack of memory phenotype $CD8^+$ T cells, suggesting that IL-15 is critical for

the homeostasis of naïve and memory $CD8^+$ T cells and NK cells (110, 111). Indeed, vesicular stomatitis virus and lymphocytic choriomeningitis virus infection models have revealed that IL-15 is important for the generation and expansion of virus-specific effector $CD8^+$ T-cell clones (112, 113). The findings from IL-15 and IL-15Rα knockout animals are supported by transgenic models, which suggest that IL-15 is involved in the generation and proliferation of $CD8^+$ T cells. Specifically, transgenic expression of IL-15 increases $CD8^+$ T-cell numbers (114), and overexpression of a modified stable form of IL-15 mRNA causes $CD8^+$ T-cell lymphomas (115). Interestingly, overexpression of either Stat5a or Stat5b, the STAT proteins that are activated by IL-15, in the lymphoid compartment results in the expansion of the peripheral $CD8^+$ T cells, with dramatic increases in $CD8^+$ memory T cells (116). The molecular basis for the role of Stat5 proteins in $CD8^+$ T-cell homeostasis is not clear yet, but as both IL-7 and IL-15 activate Stat5, one possibility is that the overexpressed Stat5 protein amplifies the effects of naturally occurring cytokines, particularly IL-15.

Roles of IL-4 and IL-21 in Th-cell differentiation and Ig synthesis

$CD4^+$ T cells can be divided into Th1- and Th2-lymphocyte subsets based on their cytokine production profiles (34, 117, 118). Human Th1 cells produce IFN-γ, whereas Th2 cells produce IL-4, IL-5, IL-9, IL-6, and IL-13. Th1-type cytokine polarization is involved in cell-mediated immune responses against intracellular pathogens such as *Toxoplasma gondii* and *Leishmania*. Th2-type cytokine expression is involved in antibody responses and in protection against parasites such as intestinal helminths. Th1-type responses have been indicated in autoimmunity, whereas Th2-type responses have been linked to the development of asthma and allergy. Cytokines are critical mediators of both Th1 and Th2 polarization, with IL-12 and IL-4 being major mediators of Th1 and Th2 differentiation, respectively. Targeted deletion of IL-4 or IL-4Rα (119, 120) or of Stat6 (the major STAT protein activated by IL-4) has severely compromised Th2 differentiation (119–123). IL-13 shares IL-4Rα with IL-4 as a receptor component, and IL-13 can also activate Stat6 and contribute to Th2 responses (22, 124).

Gene knockout models of different γ_c-dependent cytokines have revealed roles for IL-4 and IL-21 in the regulation of Ig production. Early work had indicated a role for IL-4 in B-cell Ig class-switch to IgG1 and IgE (125). This was verified by gene-targeting experiments in mice, and $Il4^{-/-}$, $Il4r^{-/-}$, and $Stat6^{-/-}$ mice all show decreased serum levels of IgG1 and IgE, demonstrating the essential role for IL-4 and Stat6 in regulation of class switch to IgG1 and IgE. IL-21R was originally identified, based on bioinformatics-related approaches, as a novel member of the type I cytokine receptor family that had significant homology to IL-2Rβ (126, 127). IL-21 was identified using a functional cloning approach (127). IL-21R was observed to be present on T, B, and NK cells, and IL-21 was suggested to play roles in NK cell cytotoxicity and differentiation, and B-cell and T-cell proliferation. $Il21r^{-/-}$ mice have normal T, B, and NK cell numbers (21, 128). Strikingly, these mice have diminished IgG1 production yet elevated IgE, and these changes are more marked after immunization with keyhole limpet hemocyanin or ovalbumin (21). This discordant response for IgG1 and IgE was surprising, as production of these two Igs is usually coordinately regulated. The increased IgE is dependent on IL-4, as Il4/Il2Ir double-knockout mice do not produce IgE. Moreover, these animals not only lose the production of IgG1 but also of IgG2a, IgG2b, and IgG3, whereas IgM is diminished but substantial levels are still produced (21). These results indicate that IL-4 and IL-21 cooperate in the regulation of IgG and IgE class-switching and Ig synthesis, and that together IL-4 and IL-21 serve as global regulators of Ig production. As noted above, γ_c-deficient mice lack B cells, preventing the analysis the B-cell defect in XSCID in these animals. In the IL-4/IL-21R-double knockout mice, IL-7 signaling is left intact, allowing B-cell development. Thereby, findings in this distinct setting enabled the conclusion that defective signaling by IL-4 and IL-21 together may explain the intrinsic B-cell defect in humans with XSCID (21). The effect of IL-21 on Th-cell differentiation is still unclear. Although IL-21 was reported to be a Th2 cytokine (129), unpublished observations from our lab show that Th1 cells also produce it. IL-21 has been reported to induce the expression of Th1-related genes (130) but also to inhibit Th1 differentiation (129). Interestingly, both IL-4 and IFN-γ can be induced in $IL\text{-}21R^{-/-}$ mice on mixed background (21), but IFN-γ production was increased in IL-21R knockout mice on C57B/6 background (128). Obviously, more work is needed to clarify these findings.

γ_c is defective in XSCID and is a component of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Of these cytokines, defective IL-7 signaling appears to explain the T-cell defect, defective IL-15 signaling appears to explain the NK cell defect, and defects in signaling mediated by the combination of IL-4 and IL-21 appear to explain the intrinsic B-cell defect in XSCID.

γ_c-dependent cytokines activate multiple signaling pathways

As discussed above, IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 mediate the oligomerization of γ_c and the appropriate cytokine receptor-specific chain (IL-2Rβ, IL-4Rα, IL-7Rα, IL-9R, or IL-21R) (*Figs 1 and 2*). This leads to Jak activation and phosphorylation of critical tyrosine residues in the receptor-specific chains. These tyrosine residues serve as docking sites for phosphotyrosine-binding proteins such as the Shc adapter protein, Insulin Receptor Substrate (IRS) proteins, and STAT proteins. These proteins couple proximal signaling events to the activation of downstream signaling pathways that contribute to cytokine-dependent gene expression programs and biological actions. There are three well-characterized major signaling pathways activated by γ_c-dependent cytokines; the Jak-STAT, phosphoinositide 3-kinase (PI3K)/Akt, and RAS-mitogen-activated protein kinase (MAPK) pathways (*Fig. 2*). The contribution of these pathways to T-cell function and cytokine-induced gene expression are discussed below.

Jak kinases are critical for the signaling of γ_c-dependent cytokines

Identification of γ_c as a component of the receptors for six cytokines led to a much greater understanding of the basis for the lymphoid developmental and functional abnormalities that are found in XSCID. However, elucidation of the molecular mechanisms that regulate signaling by γ_c-dependent cytokines was essential to understand the pathogenesis of XSCID in a better way. Like other type I cytokines, γ_c-family cytokines associate with Jak family tyrosine kinases. There are four different Jak kinases, denoted Jak1, Jak2, Jak3, and Tyk2. Jak1, Jak2, and Tyk2 are ubiquitously and constitutively expressed, whereas Jak3 expression is restricted to hematopoietic cells and is inducible (32, 131, 132). Jak kinases were originally identified based on their homology to other tyrosine kinases. However, their biological significance was appreciated only when the Jaks were shown to be activated in the context of signaling by IFNs and type I cytokines.

Jaks are cytoplasmic tyrosine kinases that constitutively associate with cytokine receptors. Among γ_c-dependent cytokines, the more 'cytokine-specific' type I cytokine receptor molecules (IL-2Rβ, IL-4Rα, IL-7Rα, IL-9R, and IL-21R) associate with Jak1, whereas γ_c associates with Jak3 (2, 17, 133, 134). Jak3 can also interact with IL-2Rβ (135). Discovery of the association between γ_c and Jak3 led to the hypothesis that mutations in Jak3 would also result in a form of SCID that was clinically and immunologically indistinguishable from XSCID (17). As discussed above, some patients with a $T^-B^+NK^-$ autosomal form of SCID have mutations in Jak3 (136, 137), with Jak3-deficient SCID representing approximately 7% of all cases of SCID (4). Mutations in Jak1 have not been described in human patients. However, Jak1 deficiency in mice results in perinatal lethality (138), making it likely that mutations in human Jak1 would be lethal as well. The basis for

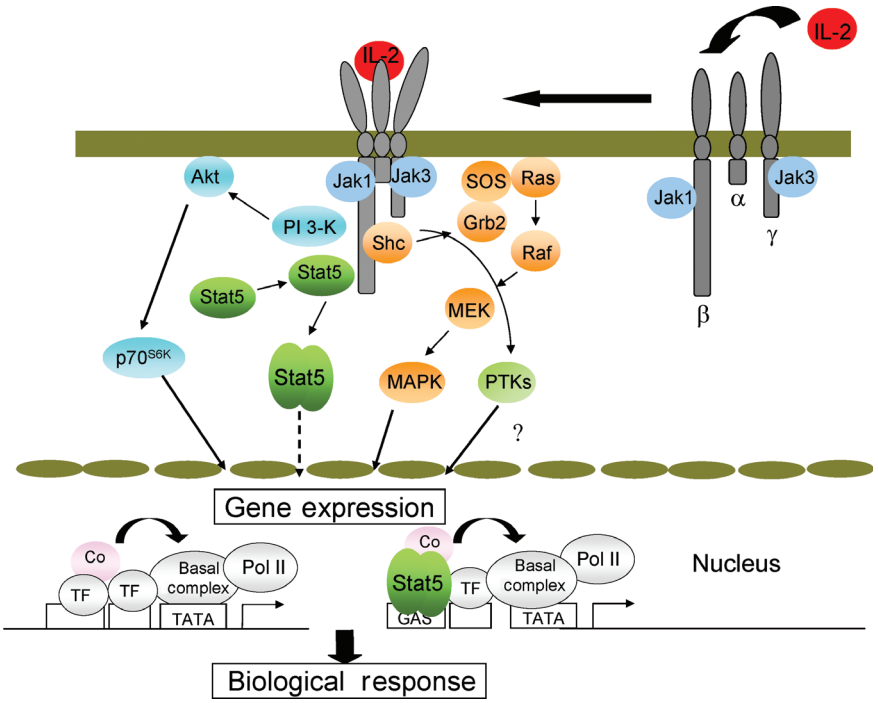

Fig. 2. A schematic representation of the major signaling pathways activated by IL-2. 'PTK' stands for protein tyrosine kinases activated by IL-2. Some of the PTKs reported to be activated by IL-2 include Syk, Pyk2, $p56^{lck}$, $p53/p56^{lyn}$, and $p59^{fyn}$. TF, transcription factor, Co, coactivator, Pol II, RNA polymerase II, GAS, γ-interferon-activated site.

the lethality is not specifically known, but is consistent with Jak1 being used by multiple cytokines and interferons, not limited to the γ_c-family of cytokines.

Activation of Jaks in response to cytokine stimulation is critical for cytokine signaling. Cytokine binding brings together receptor subunits and their associated Jaks. In the case of γ_c-dependent cytokines, this binding results in the catalytic activation of Jak1 and Jak3 (32). Jaks associate with cytokine receptors through membrane proximal Box1 and Box2 sequences, and deleting these domains abrogates cytokine signaling (32). Mutations within this region in human γ_c result in XSCID (48). The critical substrates of Jak1 and Jak3 have not been fully characterized, but they include tyrosine residues in the cytokine receptor unique chains, such as tyrosines 392 and 510 in IL-2Rβ, which induce IL-2-mediated activation of Stat5 proteins (49). Sequence comparisons of Jaks have identified conserved regions, denoted JH1-JH7 (for Jak homology regions 1 though 7) (32, 139). The JH regions do not necessarily correspond to functional domains. However, the JH1 region is at the C-terminus and corresponds to the kinase domain. Catalytic activation of Jaks is important for their function, and SCID patients with mutations in JH1 that abrogate Jak3 activation have been described (140). The critical nature of the catalytic domain is indicated by the observation that complementation of Jak1-deficient cells with catalytically inactive Jak1 does not restore IFN-α function (141). However, Jak kinase activation does not appear to be strictly obligatory for all cytokine signaling, as kinaseinactive Tyk2 has been shown to be able to partially restore IFN-α/β signaling in Tyk2-deficient cells (142). The pseudokinase domain (JH2) is just N-terminal to the catalytic JH1 domain. The function of the JH2 domain is not fully known, but it appears to play a role in regulating the catalytic activity of the kinase (143–145). In this regard, the Hopscotch mutation in the *Drosophila* Jak kinase is 'activating' and resides within the JH2 domain (146), and mutation of this region in Jak3 can cause SCID (137).

Jaks are constitutively associated with cytokine receptors. This association is dependent on the N-terminal Jak JH6 and JH7 domains (147–150). The N-terminus has been also denoted as the FERM domain, based on homology to band four point one protein, ezrin, radixin, and moesin. N-terminal mutations that abrogate Jak3 binding to γ_c have also been observed in SCID patients (140, 151, 152). The FERM domain is involved not only in cytokine receptor binding but also may regulate Jak kinase activity (152, 153).

Multiple STAT proteins are activated by γ_c-dependent cytokines

STAT proteins were initially identified in the context of IFN-α signaling as factors that bind to IFN-stimulated response elements in the promoters of interferon response genes (154). A total of seven mammalian STAT proteins are known, including Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6 (*Table* 3). STATs are latent cytoplasmic transcription factors that are recruited to phosphorylated cytokine receptors through their phosphotyrosine-binding carboxy-terminal SH2-domains. Receptor-associated STAT proteins are phosphorylated at critical C-terminal conserved tyrosine residues, allowing STAT homodimerization (e.g. Stat1, Stat3, Stat4, Stat5a, Stat5b, and Stat6) and heterodimerization (Stat1 and Stat2, Stat5a and Stat5b, and Stat1 and Stat3). Dimerized STATs translocate into the nucleus, although the mechanism is not fully understood, importin-α has been implicated in the nuclear import of Stat1 (155). Nuclear STAT proteins can interact with transcriptional coactivators such as CBP or p300, bind to STAT-binding motifs, and regulate the transcription of cytokine-target genes (156).

Different γ_c-family cytokines activate overlapping and in part distinct sets of STATs (2, 157) (*Table* 2). Stat5a and Stat5b are activated by most γ_c-sharing cytokines, as well as other cytokines including IL-3, IL-5, granulocyte–macrophage colony-stimulating factor, prolactin, growth hormone,

***Table* 3. γ_c-cytokines, their receptors, and activated signaling molecules and major biological functions**

Cytokine	Receptor	Jak- and STAT-signaling molecules		Biological function
IL-2	γ_c, IL-2Rβ, IL-2Rα	Jak1 Jak3	Stat5*, Stat3	Regulation of cell T-cell growth and peripheral tolerance; Boosting of NK cytolytic activity; important for Th2 differentiation and actions on B cells
IL-4	γ_c, IL-4Rα	Jak1 Jak3	Stat6	Th2 differentiation, B-cell differentiation
IL-7	γ_c, IL-7Rα	Jak1 Jak3	Stat5, Stat3	T-cell homeostasis, T- and B-cell (mouse) development
IL-9	γ_c, IL-9Rα	Jak1 Jak3	Stat5, Stat3	Airway mucus production, mast cell proliferation
IL-15	γ_c, IL-2Rβ, IL-15Rα	Jak1 Jak3	Stat5, Stat3	$CD8^+$ T-cell homeostasis, NK cell development
IL-21	γ_c, IL-21Rα	Jak1 Jak3	Stat3, Stat5, Stat1	B-cell differentiation, potential effects on T and NK cells

*Stat5 refers to both Stat5a and Stat5b.

thrombopoietin, and erythropoietin (132). Mice deficient in both Stat5a and/or Sta5b have been created (158–160) (*Table 2*). The immune function in different Stat5-deficient animals has been studied in detail. In *Stat5a*$^{-/-}$ mice, the lymphoid development is only modestly affected (161). Thymic cellularity is normal as is the distribution of lymphocyte subsets within the thymus. However, a small but significant decrease in splenocyte numbers is observed. T-cell proliferation in response to antigen stimulation in conjunction with low concentrations of IL-2 is impaired, but the growth response is normal at high concentrations of IL-2. The decreased responsiveness to IL-2 likely results from decreased IL-2-induced IL-2Rα expression and thus reduced high-affinity IL-2 receptor expression (161). Correspondingly, *Stat5a* knockout mice have decreased staphylococcal enterotoxin B-induced *in vivo* expansion of Vβ8^{+} T cells and deletion of Vβ6^{+} T cells, suggesting a functional role for Stat5a in antigen-driven T-cell proliferation *in vivo* (161). The immunological phenotype of *Stat5b*$^{-/-}$ mice is similar but more severe than that of *Stat5a*$^{-/-}$ mice (162). Thymocyte numbers are slightly decreased, and peripheral T-cell numbers are more reduced than in *Stat5a*$^{-/-}$ mice. NK cell numbers are also diminished in *Stat5b*$^{-/-}$ mice, as is IL-2-induced functional activity of NK cells. These findings suggest that even though Stat5a and Stat5b have overlapping roles in the immune system, Stat5b may be functionally more important, at least for T and NK cell function. As expected, *Stat5a/Stat5b* double-knockout mice show a more severe phenotype than either of the single-knockout mice (163). Importantly, lymphocytes from these *Stat5a/Stat5b* double-knockout mice proliferate very poorly in response to antigen even in the presence of high doses of IL-2, and they lack NK cells.

The different knockout models have demonstrated that Stat5 proteins have critical roles in the immune system and that they are important mediators of actions of γ_c-sharing cytokines. However, the physiological functions of Stat5 proteins are only partially understood. Earlier studies using cell lines with mutant IL-2Rβ chains that were unable to mediate Stat5 activation suggested that Stat5 is essential for IL-2-dependent cell proliferation (164–166). This conclusion is supported by the different Stat5 knockout phenotypes, which all show defects in T-cell proliferation, with the most severe defect being in Stat5a/Sta5b double-knockout mice (161–163). Other lines of evidence also support a role for Stat5 in lymphocyte proliferation. For example, a constitutively active mutant of Stat5 has been reported to confer growth factor independence to factor-dependent cell lines (167), and constitutive Stat5 activation has been observed in human T-cell lymphoma/leukemia virus (HTLV)-1-transformed T cells (168). Recently, activating retroviral insertions in the *Stat5* locus have been shown to cause B-cell lymphomas (169), and transgenic overexpression of Stat5a or Stat5b in the lymphoid compartment results in thymic lymphoblastic lymphomas (170). Collectively, the available data strongly suggest that Stat5 is involved in cell proliferation, as suggested by Friedmann *et al.* (166). Stat5-target genes have not been identified comprehensively. However, it is known that certain promitogenic genes or oncogenes, such as IL-2Rα and Pim-1, are regulated by Stat5 (171–174). There is also evidence that cyclin D1 and D2 promoters have functional Stat5-binding sites (175, 176). Thus, one critical function of Stat5 is to mediate lymphocyte proliferation, although many other important functions including support of Th2 differentiation have been suggested (156, 177).

IL-4 is the only γ_c-dependent cytokine that activates Stat6 (*Table 3*). Stat6 is critical for IL-4 and IL-13 function, and the phenotype of Stat6-deficient mice (121–123) is similar to that of mice deficient in IL-4 or IL-4Rα (119, 120). *Stat6*$^{-/-}$ T cells show defective differentiation along the Th2 lineage, and *Stat6*$^{-/-}$ B cells exhibit severely defective Ig class-switching, particularly to IgE. However, IL-4-dependent proliferation is only partially inhibited in these animals, suggesting a redundant role for other IL-4-regulated signaling pathways for proliferation, such as IRS-related proteins (125, 178).

The other STAT proteins known to be activated by γ_c-sharing cytokines are Stat3 and Stat1 (132). Stat3 is activated by IL-2, IL-7, IL-9, IL-15, and IL-21 (20, 165, 179–181). The role of Stat3 in the biology of γ_c-sharing cytokines is still only partially known. Stat3 deficiency is embryonic lethal, which likely results from defects in signaling of cytokines outside the hematopoietic system such as IL-6 family cytokines (182). Using conditional gene targeting, many different cell types deficient in Stat3 have been created and analyzed (183). T cells deficient in Stat3 develop normally, and IL-7-mediated T-cell proliferation is unaffected. However, IL-2-dependent proliferation is partially affected, which has been attributed to decreased IL-2-mediated IL-2Rα expression (184), analogous to the phenotype found in Stat5-deficient mice. As expected, IL-6-dependent T-cell proliferation requires Stat3 (184). Although Stat1 can be activated by γ_c-dependent cytokines (132), Stat1 deficiency has been reported to affect mainly IFN functions and anti-viral responses (31, 185, 186).

PI3K and Akt mediate survival and mitogenic signals

Of γ_c-dependent cytokines, at least IL-2, IL-4, and IL-7 activate PI3K and Akt (76, 125, 187). There is evidence that Akt is

important for cell survival in IL-2-signaling. An activated form of Akt can rescue cells from IL-2 deprivation-induced apoptosis (188, 189), and overexpression of a dominant negative Akt promotes T-cell apoptosis (189). The exact mechanism of how Akt controls cell survival/death in the IL-2 system has not been fully clarified, but it may involve Akt-dependent inhibition of pro-apoptotic Bad, induction of anti-apoptotic Bcl-2 and Bcl-X_L (188, 190, 191), and negative regulation of forkhead transcription factor FOXO3 (192, 193). PI3K and Akt may also be involved in mitogenic signaling in T cells through cell-cycle regulation via E2F (194, 187). The role of PI3K in signaling through other γ_c-sharing cytokines has been studied in less detail. However, activation of PI3K via IL-4Rα has been linked to a motif (the I4R motif) in the IL-4Rα cytoplasmic domain that mediates association with Shc and IRS molecules with PI3K and Akt, and these have been implicated in IL-4-dependent growth and survival signals (125). Mutating this motif *in vivo* resulted in decreased $CD4^+$ T-cell proliferation in response to IL-4 (178). In murine B-cell progenitors, PI3K is involved in the regulation of IL-7-dependent cell proliferation (195), and studies using fetal thymic organ cultures have shown that PI3K and Akt mediate thymocyte survival and proliferation in response to IL-7 (196). Thus, PI3K and Akt are essential mediators of γ_c-dependent survival and mitogenic signals.

The adapter protein Shc mediates IL-2-, IL-15- and IL-4-dependent growth and survival signals. Shc contributes to the recruitment of PI3K and Akt, but Shc also couples the Ras-MAPK pathway to cytokine receptor signaling. The role of RAS-MAPK pathway in T-cell biology has been recently reviewed extensively (197–199). Transgenic models using activating or dominant negative mutants of Ras and MEK1 have established a critical role for this pathway in positive selection and development of single-positive $CD4^+$ or $CD8^+$ T cells (200). Similarly, mice lacking extracellular signal-related kinase 1(Erk1) show a selective defect in thymic T-cell maturation with decreased numbers of single-positive lymphocytes (201). Accordingly, mice deficient in RASGRP1, a Ras guanine nucleotide exchange factor, show defective MAPK signaling with no Erk-1/Erk-2 phosphorylation in response to TCR stimulation and associated defective positive selection (202, 203). Overall, the role of MAPK in the signaling of γ_c-dependent cytokines is poorly understood. The availability of mouse models with attenuated or enhanced MAPK activity will hopefully help to resolve this issue.

Genes regulated by γ_c-dependent cytokines

As discussed earlier, γ_c-dependent cytokines mediate an array of biological functions. These functions are regulated by cytokine-initiated signaling pathways that regulate gene expression programs, and an understanding of the actions of γ_c-regulated genes will further elucidate the pathogenesis of XSCID. The characterization of cytokine-activated genes, including genes regulated by γ_c-dependent cytokines, has long been an area of considerable interest, although only few systematic studies have been conducted. A limited number of IL-2-regulated immediate early genes were identified in the early 1990s by using subtractive cDNA libraries (204); however, recent technological advances such as microarray and serial analysis of gene expression methodologies have provided the tools to simultaneously monitor changes in the expression of thousands of genes at a time, including at the genomic scale. These approaches have now allowed the identification of gene expression programs involved in complex biological processes including studies regarding genes induced by γ_c-dependent cytokines (205–207) and of changes in gene expression that take place during T-cell differentiation either in Th1 or Th2 conditions (208–211).

The microarray studies have generally confirmed the findings of earlier studies as well as identified a number of novel target genes. Some more general observations and conclusions can also be drawn from these initial studies. It appears that immune receptor stimulation, such as TCR or B-cell receptor stimulation, regulates the expression of high number of genes. More than one thousand genes have been reported regulated by TCR stimulation alone or in combination with the costimulatory CD28 receptor (212, 213). Similarly, IL-2, IL-7, and IL-15 were reported to regulate a highly overlapping set of more than one hundred genes in activated T cells (207). Similar number of IL-2- and IL-15-regulated genes has been reported by others (205, 206). The gene expression patterns triggered by distinct signals are surprisingly overlapping. For example, TCR stimulation regulates a relatively wide array of genes, but costimulation through the CD28 receptor mainly increases the amplitude of the TCR signal but does not significantly change the diversity of genes regulated by TCR (212, 213). In activated T cells, genes induced by IL-2, IL-7, and IL-15 are also highly overlapping with those regulated by TCR/TCR-like signals, with approximately 73% of γ_c-dependent genes being regulated also by the combination of phorbol 2-mysistate 3-acetate and ionomycin (207). Similarly, most genes regulated by IL-15 in memory T cells are also regulated by TCR stimulation (206). Only a minor fraction (<20%) of the total number of genes regulated by IL-02, IL-7, or IL-15 is relatively unique to cytokine stimulation. This observation fits well with the concept that γ_c-dependent cytokines can function as progression

factors that maintain signals initiated by antigen receptor stimulation. It also suggests that most γ_c-dependent genes have both cytokine and antigen response elements, analogous to what has been shown to be the case for the *IL-2Rα* gene (156).

The role of different cytokine-dependent signaling pathways in the regulation of gene responses is still unresolved. As mentioned earlier, IL-2, IL-7, and IL-15 regulate virtually identical sets of genes in T cells, whereas IL-4 induces partly overlapping but at the same time clearly distinct set of genes (207). This finding correlates well with the set of STAT proteins that are regulated by the different cytokines. IL-2, IL-7, and IL-15 activated mainly Stat3 and Stat5 proteins, whereas IL-4 activates Stat6 in T cells (*Table* 3). Thus, it is likely that the genes regulated by a cytokine are at least in part determined by the STAT proteins that are activated. The role of STATs in γ_c-dependent gene responses has been evaluated more directly in IL-4 signaling. About half of the genes regulated by IL-4 in B cells required Stat6 for their expression (214), whereas in T cells about one-fifth of IL-4-regulated genes were Stat6 dependent when the cells had been cultured in Th2-polarizing conditions (215). Interestingly, certain genes in both B and T cells were upregulated in the absence of Stat6, suggesting that Stat6 directly or indirectly possesses negative regulatory functions (214, 215).

Even if most target genes regulated by the IL-2, IL-4, IL-7, and IL-15 cytokines have been identified, only a few of these genes have been functionally evaluated, and even fewer have been shown to be critical for the biology of γ_c-dependent cytokines. Among the most potently induced genes are negative feedback regulators of cytokine signaling. The best characterized of these include the SOCS (suppressors of cytokine signaling) gene family, which consists of eight family members (CIS and SOCS1–7) (216, 217). CIS and SOCS1 are potently induced by IL-2, IL-4, IL-7, and IL-15 (207). The SOCS family members inhibit cytokine signaling at least via two distinct mechanisms. SOCS1 and SOCS3 can associate with JAKs and inhibit their catalytic activity, whereas CIS and SOCS2 more specifically inhibit STATs by preventing their association with cytokine receptors (216). Mice deficient in SOCS1, SOCS2, SOCS3, and SOCS4 have been reported, and SOCS1-deficient animals exhibit defective cytokine signaling related in part to amplified IFN-γ signaling, but they also demonstrate enhanced responsiveness to IL-2, IL-4, and IL-15, indicating a critical feedback role for SOCS1 (218–220). Mice defective in SOCS2, SOCS3, and SOCS6 have also been generated, but no obvious abnormalities related to γ_c-dependent cytokines have been reported (216).

Another family of negative regulators of cytokine signaling is the family of dual specificity phosphatases (DUSPs). DUSPs are a family of at least 40 phosphatases that can hydrolyze proteins on both phosphotyrosine and phosphoserine residues (221, 222). DUSPs are known to negatively regulate the MAPK pathway. *DUSP5* was recently identified as a gene regulated by IL-2, IL-7, and IL-15 but not IL-4. Functional analysis in a cell line model indicated a negative feedback role for *DUSP5* in the regulation of IL-2-dependent Erk1/2 regulation (207). *DUSP6*, which is also an Erk-1/2 phosphatase, was also identified as a gene induced by IL-2, IL-15, and IL-4 (207). Interestingly, *DUSP6* has also been identified as an IL-4-regulated gene in Th2-polarizing conditions (215). There is currently little information available on the *in vivo* role of Erk1/2 in the regulation γ_c-dependent functions. However, decreased Erk-1/2 activation in *Rasgrp1*-deficient mice has been shown to correlate with decreased TCR- and IL-2-dependent proliferation and defective homeostatic proliferation in T cells (203). Thus, DUSPs could have a negative feedback regulatory role in T-cell homeostasis.

Novel approaches to treatment of XSCID and other immunodeficiencies

Identification of mutations of γ_c as the cause of XSCID provided the ability to nearly immediately perform better prenatal and postnatal diagnosis as well as to identify XSCID heterozygous carrier females, allowing prenatal counseling (10, 48). In addition, it provided the basis for gene therapy for this disease. The success rates for both human leukocyte antigen-identical and haploidentical hematopoietic stem cell transplantations in SCID are relatively high (approximately 90 versus 80%, respectively) in SCID patients (223, 224). However, the B-cell defect is not always corrected by bone marrow transplantation, and in those cases, the patients require continuous Ig-replacement therapy (4). Thereby, XSCID provided a well-founded target for human gene therapy, where initially only individuals lacking suitable donors were treated. Initial success was reported in the treatment of XSCID using *ex vivo IL2RG* gene transfer into hematopoietic stem cells using a recombinant retrovirus (225). The gene therapy was extremely successful, representing the first curative gene therapy for a human disease. However, two of nine patients subsequently developed leukemia that were correlated with retroviral insertion within the *LMO2* proto-oncogene (226). Thus, gene therapy for XSCID clearly succeeded, but the two extremely serious adverse events have resulted in re-evaluating if treatment can be modified to retain the beneficial effects while minimizing the chance of serious adverse events.

Concluding remarks

The most common form of SCID is XSCID, which is caused by mutations in the γ_c, making this a disease of defective cytokine signaling. Jak3 directly associates with the γ_c, and mutations in the *JAK3* gene result in a similar form of $T^-B^+NK^-$ SCID that differs primarily in that it is an autosomal recessive disease. Defects in IL-7 and IL-15 signaling appear to explain the T-cell and NK cell defects in this disease, and strikingly, patients with mutations in the *IL7R* gene have been identified, having a $T^-B^+NK^+$ form of SCID. It is reasonable to speculate that mutations in the *IL7* gene will cause a similar clinical syndrome, but so far, they have not been identified. Mutations in the *IL15* or *IL15R* genes would be predicted to have defect in NK cell development and $CD8^+$ T-cell homeostasis, but neither of these has been reported in humans. Mutations in Jak1, which like Jak3, is activated by γ_c-dependent cytokines, have not been found, but as noted above, based on the fetal lethality in the murine knockout model, it is likely that humans with mutations in Jak1 would not be viable. Mutations in Stat5 and Stat3 proteins, the major STAT proteins downstream of IL-7 and IL-15, have also not been found as causes of immunodeficiency. It is possible that eliminating both Stat5a and Stat5b might be required to have a significant effect, an event that would statistically be unlikely to occur very often, whereas mutations in Stat3 would likely result in fetal lethality.

One of the striking features of the discovery of the genetic defect in XSCID is that this discovery came from work on the IL-2 receptor that was not at all oriented toward XSCID. The clinical discovery then resulted in elucidation of the sharing of γ_c by multiple cytokine systems and more about their biology. This most recently culminated with the discovery of the IL-21 system as a γ_c-dependent system that cooperates with IL-4 for Ig production, a finding that explains why the defect in IgG1 production in the absence solely of IL-4 is so modest. In the end, the study of the IL-2 receptor and then XSCID has blossomed into an arena of studies that has helped to greatly expand our knowledge of human and murine T, B, and NK cell biology. Studies using DNA arrays have further advanced our fund of knowledge, by elucidating the genes regulated by γ_c-dependent cytokines, thus allowing us to begin to connect gene activation profiles to cytokine-induced biological functions.

In addition to SCID, mutations in cytokine systems have been found to result in other immunodeficient states. As briefly discussed, mutations in the genes encoding IFNGR1, IFNGR2, IL-12 p40, IL-12Rβ1, and Stat1 have all been identified. These mutations result in defects related to the ability to clear mycobacterial infections. It is an exciting time period as other mutations continue to be identified in other components of cytokine systems, which not only help with the prospect of diagnosis and future therapy but also expand our knowledge of these critical cytokine systems.

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