Signaling by Type I and II cytokine receptors: ten years after

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Discovered during the past ten years, Janus kinases and signal transducers and activators of transcription have emerged as critical elements in cytokine signaling and immunoregulation. Recently, knockout mice for all the members of these families have been generated, with remarkably specific outcomes. Equally exciting is the discovery of a new class of inhibitors, the suppressor of cytokine signaling family. The phenotypes of mice deficient in these molecules are also striking, underscoring the importance of negative regulation in cytokine signaling.

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Abbreviations

CIS	cytokine-inducible SH2-domain-containing protein
Epo	erythropoietin
FERM	band 4.1 ezrin, radixin and moesin
FGF	fibroblast growth factor
GH	growth hormone
IL-2Rβ	IL-2 receptor β
Jak	Janus kinase
JH	Janus-homology domain
KIR	kinase-inhibitory region
LIF	leukemia inhibitory factor
PI3′-K	phosphatidylinositol 3′ kinase
PIAS	protein inhibitor of activated Stats
Prl	prolactin
SCID	severe combined immunodeficiency
SH3	Src-homology 3
SHP-2	SH2-domain-containing protein tyrosine phosphatase 2
SOCS1	suppressor of cytokine signaling 1
SSI1	Stat-induced Stat inhibitor 1
Stat	signal transducer and activator of transcription

Introduction

Cytokines that bind Type I and Type II receptor families are a diverse group of secreted factors involved in embryogenesis, growth, adiposity, fertility, lactation, hematopoiesis, immunoregulation and host defense. The understanding of the positive and negative signaling elements used by these two families has exploded over the past ten years. Consequently, a number of comprehensive reviews have been written on this subject [1–6]; the present review will emphasize recent immunologically relevant findings.

Table 1

Usage of Jaks and Stats by various cytokines.

Cytokines	Signaling molecules*	
Type I		
Cytokines utilizing γc		
IL-2, IL-7, IL-9, IL-15	**Jak1**, **Jak3**	**Stat5**
IL-4	**Jak1**, **Jak3**	**Stat6**
Cytokines utilizing βc		
GM-CSF, IL-3, IL-5	**Jak2**	Stat5
Cytokines utilizing gp130		
IL-6, IL-11, CNTF, LIF, OSM	**Jak1**, Jak2, Tyk2	**Stat3**
IL-12	Jak2, **Tyk2**	**Stat4**
Cytokines utilizing homodimeric receptor		
GH	Jak2	Stat5b
Prl	Jak2	**Stat5a**
Epo, TPO	**Jak2**	Stat5
G-CSF, leptin	Jak2	**Stat3**
Type II		
IFNs		
IFN-α/β	**Jak1**, Tyk2†	**Stat1**, **Stat2**
IFN-γ	**Jak1**, **Jak2**	**Stat1**
IL-10	**Jak1**, Tyk2†	**Stat3**
IL-20	?	Stat3

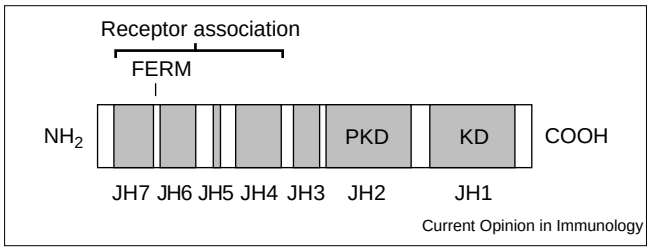
*Bold is used for signaling molecules that have proven to be essential for cytokine signaling. †Not essential, as proven by knockout.
OSM, oncostatin M; TPO, thrombopoietin.

Ligands and receptors: good at sharing

Type I receptors bind factors designated variously as hormones, interleukins, or colony-stimulating factors (Table 1) but, despite the different designations, the ligands all have a common four-α-helical structure [7]. The receptors are transmembrane proteins with conserved Cys residues and a WSXWS motif extracellularly. Intracellularly, the receptors are more divergent but have a conserved membrane-proximal domain including a hydrophobic α-helical segment that serves to bind Janus kinases (Jaks) [8]. The conserved features of the ligands and receptors permit computational database mining and the recent identification of new family members, including IL-20, IL-23 and the thymic stromal lymphopoietin (TSLP) receptor [9•,10•,11]. Type II receptors are structurally related to the Type I receptors and bind interferons and IL-10-family cytokines. The IL-10 family is rapidly expanding and now includes IL-19, IL-20, IL-TIF, MDA-7 and AKK-155 [9•].

An important feature of cytokines binding Type I/II receptors is their sharing of receptor subunits, which defines subfamilies (Table 1). For instance, IL-2, IL-4, IL-7, IL-9, IL-15 and possibly IL-21 all use γc — the common γ chain — in conjunction with ligand-specific chains, which helps explain both the specific and redundant actions of

Figure 1



Structure of Jaks. Kinases in this family contain seven common homology domains (JH1–7). The JH1 domain is a kinase domain (KD) and is responsible for the Jaks' catalytic activity whereas the JH2 domain is a pseudokinase domain (PKD) without catalytic activity although it is essential for normal Jak function. Jaks do not have any SH3 domains, but the JH3 domain has homology with SH2 domains. The amino termini of the Jaks contain a FERM domain, which is critical for their association with the receptor and for the kinase function.

these cytokines. Moreover, some cytokines, like IL-2 and IL-15, share two receptor subunits (i.e. IL-2 receptor β [IL-2Rβ] and γc) but have an additional ligand-specific subunit (IL-2Rα or IL-15Rα). Another level of complexity is that more than one type of receptor may exist: one IL-4 receptor consists of the IL-4R associated with γc and a second comprises IL-4R with the IL-13R. Recently, a third level of complexity has come to light, as illustrated by IL-12 and IL-23 [10•]. The p40 subunit, first identified as a component of IL-12, is not homologous to other cytokines but rather is homologous to cytokine receptors, having a WSXWS motif. It was first identified in association with p35 to form IL-12, which has critical functions in promoting cell mediated immunity. In contrast, IL-23 comprises p40 and a novel subunit, p19; the latter has homology to other cytokines. Like IL-12, IL-23 binds to IL-12Rβ1 and induces IFN-γ (i.e. type II IFN) production. Unlike IL-12, it does not bind IL-12Rβ2 but does induce proliferation of memory T cells, a property that IL-12 lacks.

Jaks

Jaks constitutively bind the membrane-proximal domains of Type I and II cytokine receptors and appear to be the major initiators of signaling induced by cytokines that use these receptors (Table 1). Four mammalian Jaks have been identified — Jak1, Jak2, Jak3 and Tyk2 — and they are a small but evolutionarily conserved family with orthologues in teleosts, birds and insects.

Jak structure

Three-dimensional structural data for any of the Jaks are still lacking but, based on primary sequence comparison, seven regions of homology — termed Janus homology domain 1–7 (JH1–7) — have been defined (Figure 1). JH1 comprises the kinase domain whereas JH2 comprises the pseudokinase domain. The latter segment lacks catalytic activity but is essential for a normal kinase activity and, thus, appears to have a regulatory function [12–14]. Jaks lack Src-homology 3 (SH3) domains but the JH3 domain shares homology with SH2 domains, although binding of phosphotyrosine to this domain has not been reported [15]. The amino termini of Jaks comprise a band 4.1 ezrin, radixin and moesin (FERM) domain, a feature shared with only one additional kinase family, the Fak family [16]. The amino termini of the Jaks are responsible for association with cytokine receptors (Figure 2). Interestingly, a human-patient mutation in the conserved basic/aromatic core of the FERM domain was found to result in failure of Jak3 to associate with γc [17]. Strikingly, this and other amino-terminal mutations inhibit catalytic activity, suggesting that this domain is also important for normal function of the kinase (Y-J Zhou *et al.*, unpublished data).

All Type I and II cytokines activate various Jaks in some combination but the pivotal function of the Jaks is best illustrated in mice or humans deficient in these kinases.

In vivo consequences of Jak deficiency

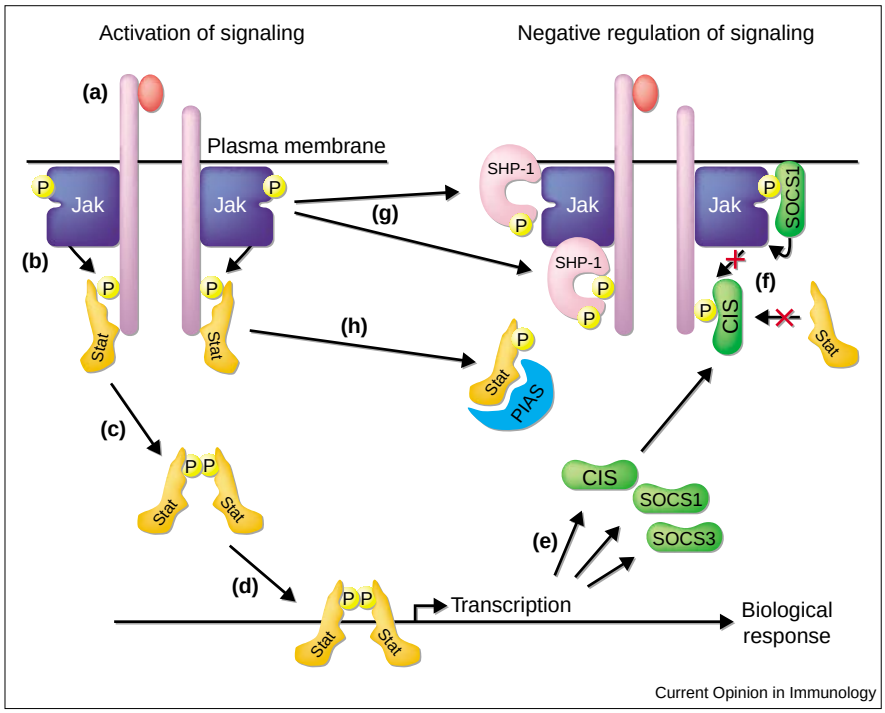
Jak3

Severe combined immunodeficiency (SCID) comprises heterogeneous disorders manifested by abnormalities in both T and B lymphocytes, the most common being X-linked SCID (X-SCID) due to mutations of γc. Because Jak3 binds γc, Jak3 mutations were sought and identified in SCID patients [18]. Jak3 knockout mice were also generated; they too have defects of T, B and NK cells [19]. The consequences of the lack of γc and the lack of Jak3 are identical, arguing that Jak3 is essential for γc-mediated signals and not important for cytokines other than those that use γc [20].

Jak3 deficiency and X-SCID are associated with impaired signaling via all the γc cytokines. The lack of NK development in γc or Jak3 deficiency is likely to be the result of defective IL-15 signaling [21] whereas the T and B lymphocyte abnormalities are largely the result of defective IL-7 signaling. Both mice and humans with IL-7R mutations develop SCID but have normal NK cell development [22]. Moreover, the underlying defect in Jak3$^{-/-}$, IL-7$^{-/-}$ or IL-7R$^{-/-}$ mice appears to be a profound diminution of early progenitor cells and increased apoptosis, though there are normal proportions of the major subsets of thymocytes [23]. The absence of Jak3 is associated with downregulation of Bcl-2 and upregulation of Bax [24], consistent with the findings in IL-7R- or γc-deficient mice and suggesting that a major role of IL-7, acting via Jak3, is to inhibit programmed cell death.

Though the numbers of thymic progenitors are small in Jak3 deficient mice, mature T cells develop, albeit with few CD8$^+$ T cells. However, the CD4$^+$ T cells are not normal in their function. These T cells express activation markers and activate poorly *in vitro*. The TCR repertoire type in Jak3$^{-/-}$ mice is also skewed. When TCR-transgenic mice (lacking antigen) are crossed with Jak3$^{-/-}$ mice, there is a reduction in the number of T cells and they no longer express activation markers, suggesting that the aberrant T cell phenotype is due to antigen-receptor-mediated activation [25].

Figure 2



Cytokine signaling and mechanisms of negative regulation. (a) Cytokine signaling is initiated by binding of cytokines (red) to cell surface receptors (purple), causing receptor dimerization. In turn, this results in juxtaposition, cross-phosphorylation and activation of Jaks, which are noncovalently bound to the receptors; phosphorylation is shown in yellow. (b) Activated Jaks phosphorylate tyrosine residues within the cytoplasmic domain of receptors, creating docking sites for proteins with SH2 and phosphotyrosine-binding (PTB) domains, such as the Stats. Once bound to receptors, Stats are themselves phosphorylated by Jaks and (c) dissociate from the receptor to form active homo- and hetero-dimers. (d) Stat dimers translocate to the nucleus and effect gene transcription. Several mechanisms contribute to negative regulation of cytokine signaling. (e) Cytokines, acting via Stats, increase the transcription of SOCS family genes. (f) SOCS family proteins can inhibit cytokine signaling by binding to phosphorylated receptors or Jaks, thus acting as a classic negative feedback loop to inhibit cytokine signaling. (g) Phosphatases such as SHP-1 are also phosphorylated by Jaks and inhibit signaling by dephosphorylation of Jaks and receptors. (h) Phosphorylated Stats can also be inhibited by binding to PIAS proteins.

In humans, depending upon the amount of Jak3 present, Jak3-deficient SCID patients may produce T cells. When they do, they too may have an activated phenotype and oligoclonal expansion. This may be associated with autoimmunity (DM Frucht *et al.*, unpublished data). The oligoclonal expansion of Jak3 deficient T cells may be the consequence of impaired IL-2 signaling. IL-2, IL-2Rα and IL-2Rβ deficient mice develop lymphoproliferative disease that is thought to be the result of inefficient activation-induced cell death [26]. This mechanism may underlie the abnormalities in Jak3 deficient T cells and, in fact, the Jak3 deficient individual with T cells failed to upregulate Fas ligand in response to IL-2. Thus, Jak3 deficiency, paradoxically, appears to be associated with both increased and impaired apoptosis.

In mice, but not in humans, B cell development is highly dependent upon Jak3 and, presumably, this reflects species-specific actions of cytokines involved in lymphoid development. Thymic stromal lymphopoietin, a newly cloned cytokine, also uses the IL-7R as a receptor subunit, probably explaining the greater severity of IL-7R deficiency versus IL-7 deficiency for mouse B cell development.

IL-21 reportedly activates Jak3; by inference it is reasonable to speculate it too is a γc cytokine. IL-21 also activates NK cells, so it will be of interest to determine what the consequences of the absence of IL-21 and how this relates to Jak3- and γc-deficiencies [27•,28].

Jak1

Like Jak3$^{-/-}$ mice, Jak1$^{-/-}$ mice have SCID because Jak1 binds to the ligand-specific receptor subunit of γc-using cytokines. Additionally, though, Jak1$^{-/-}$ mice die perinatally of an ill-characterized neurologic defect, presumably due to failure of signaling via cytokines that use gp130 (e.g. ciliary neurotrophic factor [CNTF], etc.). Using cells from these mice, Jak1 was found to be essential for signaling by type I (IFN-α/β) and type II (IFN-γ) IFNs, consistent with prior studies using a mutagenized cell line.

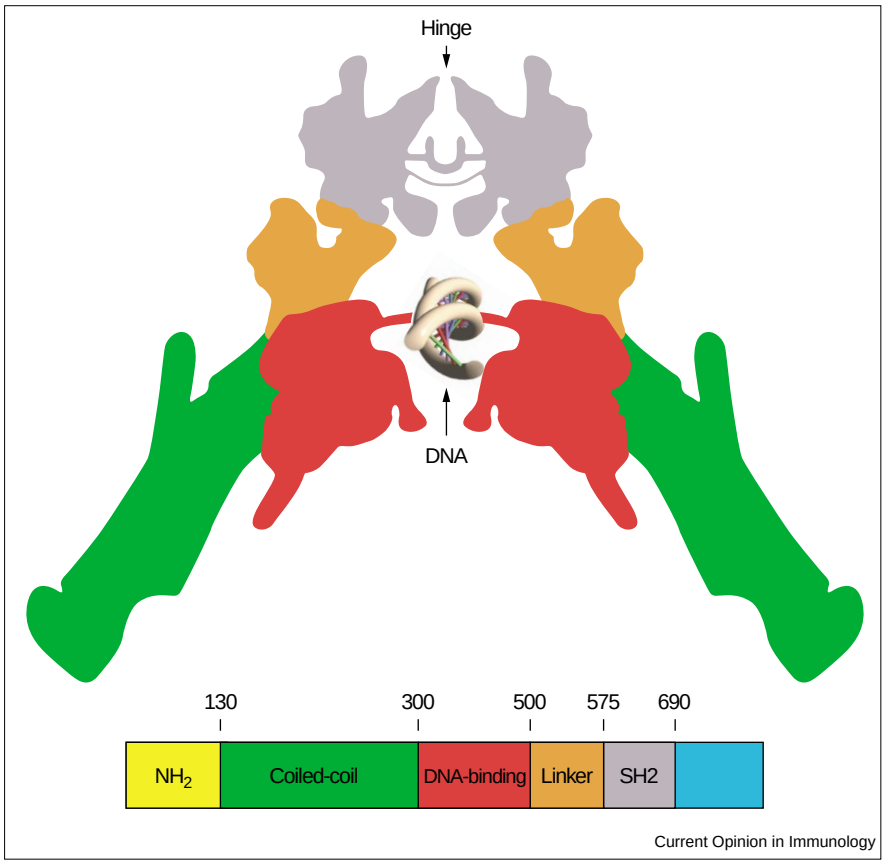
Jak2

Mice lacking Jak2 die during embryogenesis because of a failure of definitive erythropoiesis in the fetal liver, a phenotype that coincides with that of erythropoietin (Epo)$^{-/-}$ and EpoR$^{-/-}$ mice. Jak2 is also essential for thrombopoietin, IL-3, GM-CSF and IFN-γ responses whereas responses to G-CSF, type I IFNs and IL-6 are normal. Because of the lethality of Jak2 deficiency, the *in vivo* consequence of the absence of signaling by these latter cytokines has not been addressed; no doubt this will be studied following the generation of lineage-specific knockouts.

Tyk2

The demonstration that signaling by type I IFNs was abrogated in a cell line lacking Tyk2 launched the interest in Jaks as mediators of cytokine signaling [2,3,5]. Thus, the findings from two groups that generated Tyk2$^{-/-}$ mice were especially

Figure 3



Structure of Stats. The SH2 domain of Stats (shown in gray) serves two critical functions – binding to cytokine receptors and dimerizing Stat monomers. Dimeric Stats bind to DNA in a clamp-like structure, with the phosphotyrosine–SH2 interactions forming the hinge of the clamp. Stats have a carboxy-terminal transcriptional-activation domain (turquoise) and a conserved amino-terminal domain involved in Stat tetramerization. Stats also have an α-helical coiled-coil region that binds other transcription factors and co-activators. Modified from [34,35].

surprising because they differed from what was predicted [29••,30••]. Both studies found that neither IFN receptor expression nor signaling was greatly impaired, contrary to what was found in the Tyk2 deficient cell line. Indeed, the studies agreed that IL-12 signaling was affected more that IFN signaling, though IFN-γ-producing T cells could still be generated in these mice; neither study assessed the potent combination of IL-12 and IL-18 on IFN-γ production.

The consequence of Tyk2 deficiency on viral susceptibility depended upon the viral challenge. Tyk2 was judged to be not essential for signaling by IL-6, IL-10 and other cytokines. Thus, it is probably safe to conclude that, in contrast to other Jaks, which have clear and essential functions, Tyk2's functions are more redundant; perhaps Tyk2 evolved later to augment host defense against viruses. The discrepancies between the original findings in Tyk2 deficient cell lines and the knockout mice will need to be resolved; the level of expression of other Jaks and the degree to which they can compensate for each other will also need to be investigated. No doubt Tyk2 knockout mice will spawn a number of additional studies.

Stats

After activation, the Jaks phosphorylate receptor subunits on tyrosine residues to recruit proteins with SH2 domains or phosphotyrosine-binding (PTB) domains. These proteins, in turn, are also phosphorylated by Jaks, coupling cytokine stimulation to a number of pathways, including the Ras/Raf/MAPK and phosphatidylinositol 3′ kinase (PI3′-K)/Akt pathways.

Phosphorylation of cytokine receptors also generates docking sites for a class of SH2-domain-containing cytosolic molecules termed signal tranducers and activators of transcription (Stats); the first members of this class, Stat1 and Stat2, were isolated by the Darnell laboratory [1]. Shortly thereafter other family members were identified: Stat3, Stat4, Stat5a, Stat5b and Stat6 [31–33]. Stats have also been found in insects and even in *Dictyostelium*. Receptor-bound Stats are then phosphorylated, allowing them to dimerize via reciprocal SH2–phosphotyrosine interactions. This permits translocation to the nucleus and DNA binding. Generally, Stats bind two types of DNA motifs, ISREs (IFN-stimulated response elements [consensus: AGTTTNCNTTTCC]) and GAS (γ-activated sequence) elements (consensus: $TTCN_3GAA$). Other than Stat6, which binds $TTCN_4GAA$, there appears to be little specificity in Stat binding to DNA.

Stat structure

Dimeric Stats bind to DNA in a clamp-like structure, similar to NF-κB and p53 but with fewer direct contact sites

with the DNA backbone (Figure 3) [34–36]. Carboxy-terminal to the central DNA-binding domain (residues ~300–500) is a linker domain, an SH2 domain (residues ~575–690) and a conserved site of tyrosine phosphorylation; phosphotyrosine–SH2 interactions form the hinge of the clamp. Thus the SH2 domain is responsible for two key functions of the Stats: firstly, binding phosphorylated receptor subunits and, secondly, dimerization and DNA binding. Despite the importance of SH2–phosphotyrosine interactions, there may be Stat functions independent of dimerization. Via a mechanism yet to be elucidated, apoptotic abnormalities in a Stat1 deficient cell line were corrected by a Stat1 mutant lacking the conserved tyrosine residue; how a Stat monomer would bind DNA is unknown.

Amino-terminal to the central DNA-binding domain is the coiled-coil domain (residues 130–300), which is likely to mediate association with other transcription factors and coactivators, including p48, p300/CBP, Nmi, glucocorticoid receptor, c-Jun and NF-κB although CBP/p300 can also interact with the carboxyl termini of Stats.

Recently, it has been shown that Stat1-mediated IFN-α/β-induced transcription can be regulated by methylation of an arginine residue. Stat1 has been suggested to be a substrate of the protein arginine methyl-transferase, PMRT1, on arginine 31 in the amino terminus of the molecule. In the absence of arginine methylation Stat1 is associated with the inhibitor, protein inhibitor of activated Stats 1 (PIAS1), which inhibits Stat DNA-binding and transcription induction [37].

Since most Stats bind to the same consensus sequences, it is notable that multimerized, imperfect Stat-binding sites are present in some cytokine-inducible promoters. Although these sites individually bind Stats poorly, cooperative dimer–dimer interaction can occur at these sites. The conserved amino-termini of the Stats form a hook-like structure that facilitates these dimer–dimer interactions [38].

The carboxyl termini of the Stats are variable and contain a transcriptional activation domain. Stat1, Stat3, Stat4 and Stat5 have been observed to be serine phosphorylated in response to cytokine stimulation at a conserved proline-directed kinase phosphorylation site (PXSP) in the transcriptional activation domain [39]. In many cases, mutation of this conserved serine residue decreases transcriptional activation and gene regulation; however, this is not uniformly the case [40]. MAPK family members can phosphorylate these sites but exactly which kinases are responsible and how they are coupled to specific cytokine receptors is unclear [41,42]. Serine phosphorylation of Stats can also be influenced by stimuli other than cytokines, including ultraviolet irradiation, lipopolysaccharide and Gram-positive bacteria. Stat serine phosphorylation, therefore, can provide a means for crosstalk between different receptors and pathways [43–46]. The Stat2 activation domain differs from other family members and is rich in acidic amino acids. Alternatively spliced forms exist for many if not all the Stats; in most of these cases, the carboxy-terminal transcriptional activation domain is deleted and these truncated forms can act as inhibitors.

Stats lack a classical nuclear localization signal, so phosphotyrosine–SH2-domain-mediated dimerization is essential for nuclear translocalization but, in addition, the amino termini also appear to be involved [47]. However, whether regulation of nuclear entry or egress is the critical step in nuclear accumulation of Stat proteins has yet to be determined. The nuclear import of Stat1 has been shown to be dependent upon the activity of the small GTPase, Ran, whereas nuclear import signals are located in the DNA-binding domain [48].

Stat function

The critical functions of Stat1 and Stat2 in transmitting cytokine dependent signals were first established through the use of mutagenized cell lines defective in IFN-mediated responses. Now, mice deficient in each of the Stats have been generated and in some cases mice deficient in multiple Stats have been produced. These mice clearly demonstrate the nonredundant functions of specific Stats.

Stat1

A number of cytokines and growth factors activate Stat1, including IFNs, IL-2, IL-6 and even epidermal growth factor. It was notable therefore, that Stat1$^{-/-}$ mice develop normally but are highly susceptible to viral and some bacterial infections, a phenotype reminiscent of the defects observed in IFN-αR$^{-/-}$ and IFN-γR$^{-/-}$ mice and IFN-γR deficient humans. Many of the prototypic IFN-inducible genes are poorly expressed in these mice. Recently, it has also been shown that type I IFNs inhibit the production of IFN-γ early during viral infection [49,50]. Stat1 appears to be responsible for this effect, too, as, in its absence, type I IFNs mediate IFN-γ production; the mechanism responsible for this induction is not known, however.

IFNs, for the most part, are growth inhibitory and, accordingly, Stat1 also functions to restrain cell growth and upregulate the cdk inhibitor p21/waf1; in this respect its function is very different from other Stats. Interestingly, Stat1$^{-/-}$ mice develop tumors at a higher rate and this appears to be due to defective IFN-γ responses [51]. The lack of Stat1 is associated with defective apoptosis and reduced expression of caspases, so this may be another contributing factor to the emergence of tumors in Stat1 null mice.

One function of Stat1 that may be unrelated to IFN signaling is its possible role in fibroblast growth factor (FGF) signaling. Thanatophoric dysplasia type II dwarfism is associated with gain-of-function mutations of FGF3, leading to constitutive activation of Stat1 and consequently increased p21/waf1 expression in cartilage. Analysis of Stat1$^{-/-}$ mice suggests that Stat1 is required for the FGF-mediated growth inhibition of chondrocytes [52].

Stat2

Stat2 is only activated by type I IFNs and, unlike other Stats, requires Stat1 and p48 for interaction with DNA. Stat2$^{-/-}$ mice were recently reported and, as anticipated, they have defective responses to type I IFNs and are highly susceptible to viral infection [53]. Interestingly, responses were also blunted in heterozygous Stat2$^{+/-}$ mice, indicating a gene dosage effect. In addition, Stat1 levels were diminished in fibroblasts from Stat2$^{-/-}$ mice, revealing a type I IFN autocrine loop. However, cell-type-specific requirements for Stat2 were also apparent. Stat2 was found to be essential for fibroblasts and lymphocytes but macrophages were not dependent upon Stat2 for type I IFN responses.

Stat3

Stat3 is activated by cytokines signaling through gp130 (i.e. IL-6, leukemia inhibitory factor [LIF], ciliary neurotrophic factor, etc.). Gene targeting of Stat3 is embryonically lethal, perhaps due to impairment of LIF function. Because of this lethality, various conditional knockouts have been made [54]. Absence of Stat3 in T cells had no effect on lymphoid development and a modest effect on lymphocyte proliferation whereas targeting of Stat3 in myeloid cells resulted in exaggerated inflammatory response and death; the latter appears to be due to the failure of IL-10 signaling. Moreover, Stat3 appears to be required for normal G-CSF responses [55]. Targeting of Stat3 in the mammary gland decreased epithelial apoptosis and delayed involution upon forced weaning. In keratinocytes, the absence of Stat3 severely impaired wound-healing as a result of disruption of growth-factor-dependent migration of epidermal cells.

Interestingly, there are now a number of links between Stat3 activation and cancer. These include the observations that expression of a constitutively dimerized Stat3 is oncogenic, that transformation by other oncogenes is dependent upon Stat3 or other Stats (see below) and that constitutive Stat activation is found in a number of cancers [56,57]. It should be noted that, in addition to their function as transcription factors, Stats may also function in proliferative signaling via roles as adapter molecules. For example, Stat3 has been reported to bind PI3′-K [58].

Stat4

IL-12 is a major Stat4 activator and, accordingly, Stat4$^{-/-}$ mice have defective IL-12 responses and Th1 differentiation [33]. The phenotype is consistent with the abnormalities seen in IL-12$^{-/-}$ or IL-12R$^{-/-}$ mice, and in IL-12R deficient humans [59]. In humans, but not mice, IFN-α/β also activate Stat4. This is because Stat2 is apparently required for IFN-α/β-mediated Stat4 activation and mice have a microsatellite insertion in Stat2 that prevents this interaction [50,60,61]. This was an important finding because it helps explain why IFN-α/β can direct Th1 differentiation in humans but not in mice — a very important species-specific difference.

Stat4 is expressed at high levels in thymus, spleen, activated T cells and NK cells. It is also expressed at high levels in testes but Stat4 deficient mice have no defects in spermatogenesis or fertility. Recently, Stat4 has been found to be inducibly expressed in activated macrophages and dendritic cells [62]. In mice, the expression of Stat4 is required for the production of IFN-γ by these nonlymphoid cells [63]. The ability of nonlymphoid cells to autoactivate in response to IL-12 and IFN-γ may have important sentinel functions in host defense. In addition, this may be a mechanism by which these cells can promote Th1 differentiation.

Stat5

Encoded by two genes, Stat5a and Stat5b share 93% identity at the protein level. Moreover, both are activated by many cytokines, including prolactin (Prl), growth hormone (GH), Epo and IL-2. Despite their homology, Stat5a and Stat5b knockout mice established specific functions. Stat5a$^{-/-}$ mice have impaired mammary gland development and failure of lactation. In contrast, Stat5b$^{-/-}$ mice have defective sexually dimorphic growth and GH-dependent regulation of liver gene expression.

To evaluate whether Stat-5a and -5b serve redundant functions for other cytokines, Stat5a/Stat5b double-knockout mice were generated [64]. Some of these mice die perinatally but some mice survive and have features consistent with the lack of GH and Prl signaling; they are small and females are infertile.

A major surprise, however, was that lymphoid development is remarkably normal in Stat5 null mice. In contrast to Jak3$^{-/-}$ and IL-7R$^{-/-}$ mice, T and B cells development was unaffected by the absence of Stat5. By contrast, NK cell development was abrogated in these mice. [32,65••]. Though T cells develop in these mice, they are nonetheless abnormal. They fail to proliferate *in vitro* and the ability of IL-2 to induce cell cycle genes is lost. Like IL-2$^{-/-}$ and IL-2R$^{-/-}$ mice, Stat5$^{-/-}$ mice paradoxically develop lymphoproliferative disease.

The importance of Stat5 in cellular proliferation and transformation is supported by the fact that the transforming ability of the Tel-Jak2 oncogene is abrogated in Stat5 deficient mice [66]. Constitutive Stat5 phosphorylation is also noted in a number of cancers — both artificial and natural [57].

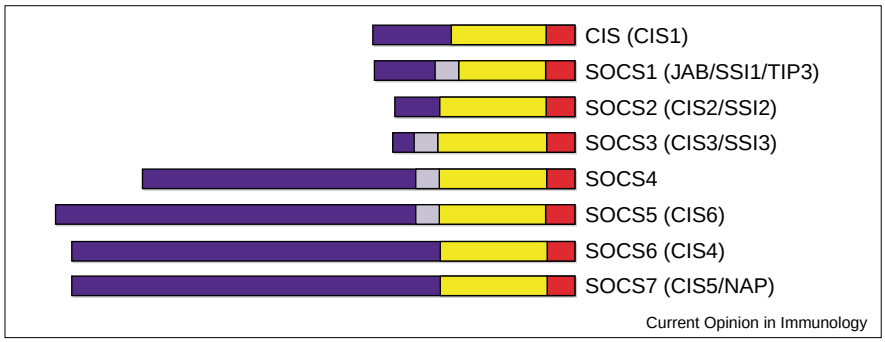
Stat6

Given that Stat6 is activated by IL-4, the finding that Stat6$^{-/-}$ mice fail to develop Th2 immune responses was expected but nonetheless striking [33]. Stat6 controls the expression of p27kip1 and, accordingly, IL-4-dependent proliferation was blocked in Stat6$^{-/-}$ mice. The mice have defective IgE responses, impaired regulation of IL-4-inducible genes and attenuated allergic and asthmatic disease [67]. Equally interesting was that Stat6 deficiency does not result in complete abrogation of IL-4 responses; antiapoptotic effects of IL-4 are preserved in Stat6 deficient cells.

The cytokine IL-13 shares a receptor subunit with IL-4. IL-13 also activates Stat6, and IL-13 responses are abrogated in

Figure 4

Structure and nomenclature of the SOCS family. The structures of CIS and SOCS1–7 are shown schematically, with alternative names shown in parentheses. SOCS boxes are shown in red, SH2 domains are shown in yellow, KIRs of SOCS are shown in grey and the amino-terminal regions are shown in blue.



Stat6$^{-/-}$ mice. Interestingly, Stat4/Stat6 double-knockouts develop default Th1 responses. Although Stat4 and Stat6 clearly play important roles in promoting the Th1 versus Th2 dichotomy, other transcription factors, such as GATA-3, c-Maf, IRF-1 and T-bet also play major roles in this process [68].

Mechanisms of attenuating cytokine signaling

Of equal importance to the positive mechanisms initiated by cytokine signals are the negative regulatory mechanisms that serve to dampen or terminate cytokine signals [69,70]. Accordingly, there are a number of mechanisms presently identified — their importance being vividly demonstrated by mice lacking these inhibitors.

The SOCS family – cytokine-inducible feedback inhibitors

CIS (cytokine-inducible SH2-domain-containing protein), the first member of this family, was identified as an immediate-early gene, induced by IL-3, that inhibited signaling and proliferation [70]. Using different strategies, three groups subsequently separately cloned another family member, termed SOCS1 (suppressor of cytokine signaling 1), JAB (JAK binding protein) and SSI1 (Stat-induced Stat inhibitor 1) [71,72]. The SOCS/CIS/SSI family now includes eight members, CIS and SOCS1–SOCS7 [73] (Figure 4). Although the final nomenclature of the family has not been settled, for simplicity we will use the designation 'SOCS'.

The mRNA and protein expression for SOCS members is rapidly induced by a variety of stimuli, including cytokines and growth factors. Importantly, SOCS expression is reduced in mice lacking various Stat genes but, in addition, there are examples of SOCS induction in response to stimuli that do not activate Stats, such as lipopolysaccharide or IL-1. Functioning as a classic negative feedback loop, SOCS proteins are structurally conserved but inhibit signaling by different mechanisms. SOCS-box-containing proteins can be divided into five subfamilies of proteins with SH2 domains, WD-40 repeats, ankyrin repeats, SPRY domains or GTPase domains [73]. The function of the SOCS-box has not been established but several lines of evidence point to a role in ubiquitin-mediated protein degradation, potentially through the binding of the elongin BC complex [74]. The elongins bind to E_3 ligase complex of CUL 2 and Skp1 and this may regulate protein turnover. The function of many SOCS proteins is unknown; only CIS and SOCS1–SOCS3 will be discussed further here.

CIS

CIS, the first member of the SOCS family to be identified, is induced by Epo, IL-3, GH, IL-2 and by Prl, and it competes with Stats for the receptor phosphotyrosine residues that serve as a docking site. CIS transgenic mice have low birth weight, stunted growth and defective mammary gland development, possibly due to a complete block of Prl signaling, a phenotype similar to that observed with Stat5$^{-/-}$ mice [75]. However, the lack of a clear phenotype in the CIS$^{-/-}$ mice suggests that, unlike other SOCS family members (see below), it may be redundant [28].

SOCS1

SOCS1 mRNA is induced by many cytokines, including IL-6, IL-2, IL-4, LIF, IFN-α/β, IFN-γ, GH and G-CSF. SOCS1 inhibits Jak1, Jak2 and Tyk2 but affects Jak3 kinase activity minimally. Mutational analysis of SOCS1 and SOCS3 has established that both the SH2 domain and an amino-terminal kinase-inhibitory region (KIR), but not the SOCS-box, are essential for blocking Jak activity and cytokine signaling; notably, the KIR is not found in other SOCS proteins [76–78] although SOCS4 and SOCS5 have amino-terminal regions with sequences similar to KIRs.

SOCS1$^{-/-}$ mice, though born healthy, rapidly succumbed to a complex, multiorgan disease characterized by leukocytic infiltration and necrosis with severe B cell lymphopenia. SOCS1$^{-/-}$ mice have activated Stat1 in the liver, have elevated expression of IFN-γ-inducible genes, are hypersensitive to IFN-γ and have elevated circulating levels of this cytokine [79••,80••]. Accordingly, mice lacking both SOCS1 and IFN-γ are healthy, as are doubly deficient SOCS1$^{-/-}$Rag2$^{-/-}$ mice.

SOCS2

SOCS2$^{-/-}$ mice are born at the expected Mendelian ratio, survive weaning and are healthy and fertile as adults [81••]. Male SOCS2$^{-/-}$ mice grow more rapidly than their wild-type counterparts; SOCS2$^{-/-}$ females are also heavier, but less dramatically so. With the notable exception of the brain,

most organs in SOCS2$^{-/-}$ animals are proportionately large and the long bones are 7–15% longer. The increased weight of SOCS2$^{-/-}$ animals is not due to increased fat; rather the animals are if anything leaner than normal, a phenotype similar to that of GH transgenic mice. The mechanism of SOCS2 action in regulating body size is not entirely clear but an important mechanism of GH action is the induction IGF1 (insulin-like growth factor 1). Of note, SOCS2$^{-/-}$ animals exhibit elevated expression of IGF1 mRNA in a number of tissues, including the heart, lung and spleen.

SOCS2 is unusual among the SOCS family: whereas SOCS2 mRNA expression is induced in response to many cytokines, its capacity to inhibit cytokine signaling in overexpression experiments was less impressive than SOCS1. Indeed, high concentrations of SOCS2 potentiated GH, Prl and IL-2 signaling. One explanation for this effect was that SOCS2 may antagonize the action of endogenous SOCS1, leading to a potentiated response [82,83]; whether this occurs *in vivo* has not yet been determined.

SOCS3

Like SOCS1, SOCS3 can inhibit Stat activation in response to many cytokines, including GH, leptin, IL-2, IL-4 and IL-10; SOCS3 is also induced in response to most four-α-helical cytokines tested. SOCS3 only partially inhibits Jak activation but its effect is enhanced in the presence of receptors, suggesting that SOCS3 inhibits cytokine responses by binding to phosphorylated components of the receptor complex [17,84]. For gp130, an SH2-domain-containing protein tyrosine phosphatase 2 (SHP-2)-interaction site (YEPY^{757}STV) is also a SOCS3 contact site; SOCS3 may compete for the SHP2–gp130 interaction site [85,86].

SOCS3 is also unusual in that phosphorylated SOCS3 binds RasGAP, maintaining Ras in the activated state [87,88]. Therefore, although SOCS3 can block Stat activation, it can activate the ERK pathway. This interesting observation suggests that SOCS proteins are not only inhibitors; SOCS3, at least, can also positively modulate specific survival pathways.

SOCS3$^{-/-}$ mice die during mid-gestation, (between day 11.5 and day 16.5) though the basis of this embryonic lethality is unclear. Some mice display marked erythrocytosis, consistent with the finding that a SOCS3 transgene results in severe anemia [28]. However, reconstitution of irradiated mice with SOCS3$^{-/-}$ fetal liver results in normal hematopoiesis, suggesting that the critical role of SOCS3 may be limited to embryogenesis. Clearly, many questions remain regarding the physiological role of SOCS3.

PIAS

PIAS are a family of constitutively expressed negative regulators of Stats and four members have been identified: PIAS-1, -3, -X and -Y. They share homology amongst themselves but have no previously characterized motifs [89]. PIAS proteins may not be specific for Stat interaction, as PIAS1 was previously described as a helicase 11 interacting protein; the *in vivo* relevance of this family of proteins will need to be assessed by the production of the relevant knockout mice.

Phosphatases in cytokine signaling

Given the importance of tyrosine kinases in initiating signaling, it comes as no surprise that tyrosine phosphatases are also important inhibitors. For instance, SHP-1 is an important negative regulator, as illustrated by the 'motheaten mice' phenotype. The lack of SHP-1 affects almost all hematopoietic lineages and results in the characteristic motheaten appearance of the coat and fatal pneumonitis resulting from unchecked neutrophil and macrophage proliferation [90]. Several hematopoietic receptors, including the IL-4R, EpoR, GHR and IL-2R have been shown to recruit SHP-1. The mechanism by which SHP-1 inhibits signaling has not been entirely elucidated but it presumably occurs via SH2–phosphotyrosine interaction. SHP-1 may directly dephosphorylate and inactivate Jaks or dephosphorylate other key tyrosines on the cytokine receptors that are involved in signaling. Loss of SHP-1 expression has been implicated in human lymphoproliferative disease and may be involved in malignant transformation, as reported with HTLV-1 associated leukemia and other tumors.

The action of SHP-2 is more complicated. Though it has been reported to inhibit signaling by some cytokines, it also acts as an adaptor protein, enhancing PI-3′K and Ras activation. SHP-2 binds the receptor and recruits Grb-2 and SOS, thus initiating Ras activation. SHP-2 does not bind all receptors directly; it also binds docking proteins such as Gab-2 [91,92].

Two phospholipid phosphatases, SHIP (SH2-containing inositol phosphatase) and PTEN, target PIP-3 but at different positions on the inositol ring [93]. Normally PTEN antagonizes the activation of PI3′-K and PKB/Akt by growth factors. Many tumors and cell lines have PTEN mutations, resulting in activation of the Akt/PKB and phosphatidylinositol PI3′-K pathway. This can antagonize the apoptosis induced by cytokine withdrawal.

Recently, it has been shown that the absence of CD45 leads to augmented Jak and Stat phosphorylation in hematopoietic cells. Whether the major role of CD45 is to statically influence Jaks or whether there is a dynamic component of regulation is not known. Whether other receptor phosphatases affect Jaks in nonhematopoietic cells will also need to be determined [94].

Finally, Stats are dephosphorylated over time and this presumably reflects the activity of a Stat phosphatase. The identity of the major Stat phosphatase and its intracellular location remains enigmatic at present.

Conclusions

Over the past 10 years, there has been enormous progress in determining how cytokine signaling is initiated and how it is attenuated. With the completion of the sequencing of the human genome and the use of bioinformatic tools, we will

soon know all of the cyokines and receptors in this family. However, the complexity of shared receptor subunits and alternative receptors will no doubt complicate the analysis of these new members.

Jaks have emerged as a small family of tyrosine kinases with rather specific functions, as illustrated by humans with mutations and by gene targeted mice. A major gap in our current knowledge, however, is the three-dimensional structure of the Jaks; this information is eagerly awaited. In addition, we only have cursory information regarding the basis of Jak/receptor association and the delineation of the function of Jak phosphorylation sites.

The Stats, too, appear to be a relatively small but evolutionarily conserved family of transcription factors with highly specific functions; remarkably it appears that at least four of the six mammalian Stats have major functions in regulating host defense and immune responses. We have much more detailed structural information on Stat molecules but clearly we need to know much more about cytokine-inducible genes and how Stats work with other transcription factors to regulate the expression of these genes; microarray technology is rapidly filling this void.

Three classes of negative regulatory proteins have been discovered: phosphatases, PIAS and SOCS proteins. Each of these protein families appears to act at a distinct point and at a particular time in the cytokine signaling cascade. Although many experiments examining the function of these negative regulators have been carried out *in vitro*, some of the most informative experiments have been carried out in animals with naturally occurring or engineered mutations in the genes encoding these proteins. Continued analysis of the signal transduction in primary cells expressing normal levels of the salient proteins will be important if the physiologically relevant controls on responses are to be uncovered. Additionally, mutations and polymorphisms in these negative regulators would be good candidates for autoimmune disease susceptibility genes in humans; it will be important to test this possibility.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Darnell JEJ, Kerr IM, Stark GR: Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994, 264:1415-1421.
2. Ihle JN: Cytokine receptor signalling. *Nature* 1995, 377:591-594.
3. Leonard WJ, O'Shea JJ: Jaks and STATs: biological implications. *Annu Rev Immunol* 1998, 16:293-322.
4. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD: How cells respond to interferons. *Annu Rev Biochem* 1998, 67:227-264.
5. Yeh TC, Pellegrini S: The Janus kinase family of protein tyrosine kinases and their role in signaling. *Cell Mol Life Sci* 1999, 55:1523-1534.
6. Kotenko SV, Pestka S: Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. *Oncogene* 2000, 19:2557-2565.
7. Nicola NA, Hilton DJ: General classes and functions of four-helix bundle cytokines. *Adv Protein Chem* 1998, 52:1-65.
8. Constantinescu SN, Huang J, Nam H, Lodish HF: The erythropoietin receptor cytosolic juxtamembrane domain contains an essential, precisely oriented, hydrophobic motif. *Mol Cell* 2001, 7:377-385.
9. • Blumberg H, Conklin D, Xu W, Grossmann A, Brender T, Carollo S, Eagan M, Foster D, Haldeman BA, Hammond A *et al.*: Interleukin 20. discovery, receptor identification, and role in epidermal function. *Cell* 2001, 104:9-19.

IL-20 is a recently described cytokine that belongs to the IL-10 family.

10. • Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K *et al.*: Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 2000, 13:715-725.

A newly discovered molecule, p19, heterodimerizes with IL-12 p40. The resulting novel cytokine, IL-23, shares some activity with IL-12 but has other activities that IL-12 does not show.

11. Pandey A, Ozaki K, Baumann H, Levin SD, Puel A, Farr AG, Ziegler SF, Leonard WJ, Lodish HF: Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. *Nat Immunol* 2000, 1:59-64.
12. Chen M, Cheng A, Candotti F, Zhou YJ, Hymel A, Fasth A, Notarangelo LD, O'Shea JJ: 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.
13. Saharinen P, Takaluoma K, Silvennoinen O: Regulation of the Jak2 tyrosine kinase by its pseudokinase domain. *Mol Cell Biol* 2000, 20:3387-3395.
14. Yeh TC, Dondi E, Uze G, Pellegrini S: A dual role for the kinase-like domain of the tyrosine kinase Tyk2 in interferon-alpha signaling. *Proc Natl Acad Sci USA* 2000, 97:8991-8996.
15. Kampa D, Burnside J: Computational and functional analysis of the putative SH2 domain in Janus kinases. *Biochem Biophys Res Commun* 2000, 278:175-182.
16. Girault JA, Labesse G, Mornon JP, Callebaut I: Janus kinases and focal adhesion kinases play in the 4.1 band: a superfamily of band 4.1 domains important for cell structure and signal transduction. *Mol Med* 1998, 4:751-769.
17. Cacalano NA, Migone TS, Bazan F, Hanson EP, Chen M, Candotti F, O'Shea JJ, Johnston JA: 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.
18. Notarangelo LD, Giliani S, Mazza C, Mella P, Savoldi G, Rodriguez-Perez C, Mazzolari E, Fiorini M, Duse M, Plebani A *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.
19. Baird AM, Thomis DC, Berg LJ: T cell development and activation in Jak3-deficient mice. *J Leukoc Biol* 1998, 63:669-677.
20. Suzuki K, Nakajima H, Saito Y, Saito T, Leonard WJ, Iwamoto I: Janus kinase 3 (Jak3) is essential for common cytokine receptor gamma chain (gamma[c])-dependent signaling: comparative analysis of gamma(c), Jak3, and gamma(c) and Jak3 double-deficient mice. *Int Immunol* 2000, 12:123-132.
21. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, Matsuki N, Charrier K, Sedger L, Willis CR *et al.*: Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med* 2000, 191:771-780.
22. Puel A, Leonard WJ: Mutations in the gene for the IL-7 receptor result in T(-)B(+)NK(+) severe combined immunodeficiency disease. *Curr Opin Immunol* 2000, 12:468-473.
23. Baird AM, Lucas JA, Berg LJ: A profound deficiency in thymic progenitor cells in mice lacking Jak3. *J Immunol* 2000, 165:3680-3688.
24. Wen R, Wang D, McKay C, Bunting KD, Marine JC, Vanin EF, Zambetti GP, Korsmeyer SJ, Ihle JN, Cleveland JL: Jak3 selectively regulates bax and bcl-2 expression To promote T-cell development. *Mol Cell Biol* 2001, 21:678-689.
25. Gozalo-Sanmillan S, McNally JM, Lin MY, Chambers CA, Berg LJ: Two distinct mechanisms lead to impaired T cell homeostasis in Janus kinase 3- and CTLA-4-deficient mice. *J Immunol* 2001, 166:727-730.

26. Van Parijs L, Refaeli Y, Lord JD, Nelson BH, Abbas AK, Baltimore D: Uncoupling IL-2 signals that regulate T cell proliferation, survival, and Fas-mediated activation-induced cell death. *Immunity* 1999, 11:281-288.

27. • Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, Johnston J, Madden K, Xu W, West J *et al.*: Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* 2000, 408:57-63.

Characterization of IL-21 and of its receptor complex suggests that IL-21 may belong to the cytokine family whose receptors share the common γ chain, γc.

28. Marine JC, McKay C, Wang D, Topham DJ, Parganas E, Nakajima H, Pendeville H, Yasukawa H, Sasaki A, Yoshimura A, Ihle JN: SOCS3 is essential in the regulation of fetal liver erythropoiesis. *Cell* 1999, 98:617-627.

29. •• Shimoda K, Kato K, Aoki K, Matsuda T, Miyamoto A, Shibamori M, Yamashita M, Numata A, Takase K, Kobayashi S *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.

See annotation to [30••].

30. •• Karaghiosoff M, Neubauer H, Lassnig C, Kovarik P, Schindler H, Pircher H, McCoy B, Bogdan C, Decker T, Brem G *et al.*: Partial impairment of cytokine responses in Tyk2-deficient mice. *Immunity* 2000, 13:549-560.

References [29••,30••] show that, although expected to have a dramatic effect on IFN signaling, Tyk2 deficiency *in vivo* had surprisingly modest consequences.

31. Hirano T, Ishihara K, Hibi M: Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene* 2000, 19:2548-2556.

32. Lin JX, Leonard WJ: The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. *Oncogene* 2000, 19:2566-2576.

33. Wurster AL, Tanaka T, Grusby MJ: The biology of Stat4 and Stat6. *Oncogene* 2000, 19:2577-2584.

34. Becker S, Groner B, Muller CW: Three-dimensional structure of the Stat3 beta homodimer bound to DNA. *Nature* 1998, 394:145-151.

35. Chen X, Vinkemeier U, Zhao Y, Jeruzalmi D, Darnell JEJ, Kuriyan J: Crystal structure of a tyrosine phosphorylated STAT-1 dimer bound to DNA. *Cell* 1998, 93:827-839.

36. Bromberg J, Darnell JE: The role of STATs in transcriptional control and their impact on cellular function. *Oncogene* 2000, 19:2468-2473.

37. Mowen KA, Tang J, Zhu W, Schurter BT, Shuai K, Herschman HR, David M: Arginine methylation of STAT1 modulates IFNα/β-induced transcription. *Cell* 2001, 104:731-741.

38. Vinkemeier U, Moarefi I, Darnell JEJ, Kuriyan J: Structure of the amino-terminal protein interaction domain of STAT-4. *Science* 1998, 279:1048-1052.

39. Decker T, Kovarik P: Serine phosphorylation of STATs. *Oncogene* 2000, 19:2628-2637.

40. Kovarik P, Mangold M, Ramsauer K, Heidari H, Steinborn R, Zotter A, Levy DE, Muller M, Decker T: Specificity of signaling by STAT1 depends on SH2 and C-terminal domains that regulate Ser727 phosphorylation, differentially affecting specific target gene expression. *EMBO J* 2001, 20:91-100.

41. Goh KC, Haque SJ, Williams BR: p38 MAP kinase is required for STAT1 serine phosphorylation and transcriptional activation induced by interferons. *EMBO J* 1999, 18:5601-5608.

42. Visconti R, Gadina M, Chiariello M, Chen EH, Stancato LF, Gutkind JS, O'Shea JJ: Importance of the MKK6/p38 pathway for interleukin-12-induced STAT4 serine phosphorylation and transcriptional activity. *Blood* 2000, 96:1844-1852.

43. Gollob JA, Schnipper CP, Murphy EA, Ritz J, Frank DA: The functional synergy between IL-12 and IL-2 involves p38 mitogen-activated protein kinase and is associated with the augmentation of STAT serine phosphorylation. *J Immunol* 1999, 162:4472-4481.

44. Su L, Rickert RC, David M: Rapid STAT phosphorylation via the B cell receptor. Modulatory role of CD19. *J Biol Chem* 1999, 274:31770-31774.

45. Beuvink I, Hess D, Flotow H, Hofsteenge J, Groner B, Hynes NE: Stat5a serine phosphorylation. Serine 779 is constitutively phosphorylated in the mammary gland, and serine 725 phosphorylation influences prolactin-stimulated ***in vitro*** DNA binding activity. *J Biol Chem* 2000, 275:10247-10255.

46. Stoiber D, Stockinger S, Steinlein P, Kovarik J, Decker T: ***Listeria monocytogenes*** modulates macrophage cytokine responses through STAT serine phosphorylation and the induction of suppressor of cytokine signaling 3. *J Immunol* 2001, 166:466-472.

47. Strehlow I, Schindler C: Amino-terminal signal transducer and activator of transcription (STAT) domains regulate nuclear translocation and STAT deactivation. *J Biol Chem* 1998, 273:28049-28056.

48. McBride KM, McDonald C, Reich NC: Nuclear export signal located within the DNA-binding domain of the STAT1 transcription factor. *EMBO J* 2000, 19:6196-6206.

49. Nguyen KB, Cousens LP, Doughty LA, Pien GC, Durbin JE, Biron CA: Interferon alpha/beta-mediated inhibition and promotion of interferon: STAT1 resolves a paradox. *Nat Immunol* 2000, 1:70-76.

50. O'Shea JJ, Visconti R: Type 1 IFNs and regulation of TH1 responses: enigmas both resolved and emerge. *Nat Immunol* 2000, 1:17-18.

51. Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, Schreiber RD: Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. *Proc Natl Acad Sci USA* 1998, 95:7556-7561.

52. Sahni M, Ambrosetti DC, Mansukhani A, Gertner R, Levy D, Basilico C: FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway. *Genes Dev* 1999, 13:1361-1366.

53. Park C, Li S, Cha E, Schindler C: Immune response in Stat2 knockout mice. *Immunity* 2000, 13:795-804.

54. Akira S: Roles of STAT3 defined by tissue-specific gene targeting. *Oncogene* 2000, 19:2607-2611.

55. McLemore ML, Grewal S, Liu F, Archambault A, Poursine-Laurent J, Haug J, Link DC: STAT-3 activation is required for normal G-CSF-dependent proliferation and granulocytic differentiation. *Immunity* 2001, 14:193-204.

56. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RD, Albanese C, Darnell JEJ: Stat3 as an oncogene. *Cell* 1999, 98:295-303.

57. Bowman T, Garcia R, Turkson J, Jove R: STATs in oncogenesis. *Oncogene* 2000, 19:2474-2488.

58. Pfeffer LM, Mullersman JE, Pfeffer SR, Murti A, Shi W, Yang CH: STAT3 as an adapter to couple phosphatidylinositol 3-kinase to the IFNAR1 chain of the type I interferon receptor. *Science* 1997, 276:1418-1420.

59. Wu C, Wang X, Gadina M, O'Shea JJ, Presky DH, Magram J: IL-12 receptor β2 (IL-12Rβ2)-deficient mice are defective in IL-12-mediated signaling despite the presence of high affinity IL-12 binding sites. *J Immunol* 2000, 165:6221-6228.

60. Farrar JD, Smith JD, Murphy TL, Murphy KM: Recruitment of Stat4 to the human interferon-alpha/beta receptor requires activated Stat2. *J Biol Chem* 2000, 275:2693-2697.

61. Farrar JD, Smith JD, Murphy TL, Leung S, Stark GR, Murphy KM: Selective loss of type I interferon-induced STAT4 activation caused by a minisatellite insertion in mouse Stat2. *Nat Immunol* 2000, 1:65-69.

62. Frucht DM, Aringer M, Galon J, Danning C, Brown M, Fan S, Centola M, Wu CY, Yamada N, El Gabalawy H, O'Shea JJ: Stat4 is expressed in activated peripheral blood monocytes, dendritic cells, and macrophages at sites of Th1-mediated inflammation. *J Immunol* 2000, 164:4659-4664.

63. Fukao T, Frucht DM, Yap G, Gadina M, O'Shea JJ, Koyasu S: Inducible expression of Stat4 in dendritic cells and macrophages and its critical role in innate and adaptive immune responses. *J Immunol* 2001, 166:4446-4455.

64. Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN: Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 1998, 93:841-850.

65. •• Moriggl R, Topham DJ, Teglund S, Sexl V, McKay C, Wang D, Hoffmeyer A, van Deursen J, Sangster MY, Bunting KD *et al.*: Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells. *Immunity* 1999, 10:249-259.

These studies [64,65••] are notable in that, in contrast to the deficiency of Jak3 and IL-7R, the absence of Stat5 did not abrogate T and B cell

development; NK cell development, however, was impaired. Additionally, though, lymphocytes from Stat5 deficient are quite abnormal.

66. Schwaller J, Parganas E, Wang D, Cain D, Aster JC, Williams IR, Lee CK, Gerthner R, Kitamura T, Frantsve J *et al.*: Stat5 is essential for the myelo- and lympho-proliferative disease induced by TEL/JAK2. *Mol Cell* 2000, 6:693-704.

67. Miyata S, Matsuyama T, Kodama T, Nishioka Y, Kuribayashi K, Takeda K, Akira S, Sugita M: STAT6 deficiency in a mouse model of allergen-induced airways inflammation abolishes eosinophilia but induces infiltration of CD8+ T cells. *Clin Exp Allergy* 1999, 29:114-123.

68. Glimcher LH, Murphy KM: Lineage commitment in the immune system: the T helper lymphocyte grows up. *Genes Dev* 2000, 14:1693-1711.

69. Hilton DJ: Negative regulators of cytokine signal transduction. *Cell Mol Life Sci* 1999, 55:1568-1577.

70. Yasukawa H, Sasaki A, Yoshimura A: Negative regulation of cytokine signaling pathways. *Annu Rev Immunol* 2000, 18:143-164.

71. Nicholson SE, Hilton DJ: The SOCS proteins: a new family of negative regulators of signal transduction. *J Leukoc Biol* 1998, 63:665-668.

72. Naka T, Fujimoto M, Kishimoto T: Negative regulation of cytokine signaling: STAT-induced STAT inhibitor. *Trends Biochem Sci* 1999, 24:394-398.

73. Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcalf D, Nicola NA: Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc Natl Acad Sci USA* 1998, 95:114-119.

74. Zhang JG, Farley A, Nicholson SE, Willson TA, Zugaro LM, Simpson RJ, Moritz RL, Cary D, Richardson R, Hausmann G *et al.*: The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. *Proc Natl Acad Sci USA* 1999, 96:2071-2076.

75. Matsumoto A, Seki Y, Kubo M, Ohtsuka S, Suzuki A, Hayashi I, Tsuji K, Nakahata T, Okabe M, Yamada S, Yoshimura A: Suppression of STAT5 functions in liver, mammary glands, and T cells in cytokine-inducible SH2-containing protein 1 transgenic mice. *Mol Cell Biol* 1999, 19:6396-6407.

76. Yasukawa H, Misawa H, Sakamoto H, Masuhara M, Sasaki A, Wakioka T, Ohtsuka S, Imaizumi T, Matsuda T, Ihle JN, Yoshimura A: The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. *EMBO J* 1999, 18:1309-1320.

77. Nicholson SE, Willson TA, Farley A, Starr R, Zhang JG, Baca M, Alexander WS, Metcalf D, Hilton DJ, Nicola NA: Mutational analyses of the SOCS proteins suggest a dual domain requirement but distinct mechanisms for inhibition of LIF and IL-6 signal transduction. *EMBO J* 1999, 18:375-385.

78. Narazaki M, Fujimoto M, Matsumoto T, Morita Y, Saito H, Kajita T, Yoshizaki K, Naka T, Kishimoto T: Three distinct domains of SSI-1/SOCS-1/JAB protein are required for its suppression of interleukin 6 signaling. *Proc Natl Acad Sci USA* 1998, 95:13130-13134.

79. •• Marine JC, Topham DJ, McKay C, Wang D, Parganas E, Stravopodis D, Yoshimura A, Ihle JN: SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. *Cell* 1999, 98:609-616.
See annotation to [80••].

80. •• Alexander WS, Starr R, Fenner JE, Scott CL, Handman E, Sprigg NS, Corbin JE, Cornish AL, Darwiche R, Owczarek CM *et al.*: SOCS1 is a critical inhibitor of interferon gamma signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell* 1999, 98:597-608.
These papers [79••,80••] vividly illustrated the essential function of SOCS1 in constraining IFN-γ production and response; the absence of this negative regulator causes catastrophic immunologic disease.

81. •• Metcalf D, Greenhalgh CJ, Viney E, Willson TA, Starr R, Nicola NA, Hilton DJ, Alexander WS: Gigantism in mice lacking suppressor of cytokine signalling-2. *Nature* 2000, 405:1069-1073.
The striking finding of this paper was the extreme specificity of the SOCS2 deficiency.

82. Favre H, Benhamou A, Finidori J, Kelly PA, Edery M: Dual effects of suppressor of cytokine signaling (SOCS-2) on growth hormone signal transduction. *FEBS Lett* 1999, 453:63-66.

83. Pezet A, Favre H, Kelly PA, Edery M: Inhibition and restoration of prolactin signal transduction by suppressors of cytokine signaling. *J Biol Chem* 1999, 274:24497-24502.

84. Hansen JA, Lindberg K, Hilton DJ, Nielsen JH, Billestrup N: Mechanism of inhibition of growth hormone receptor signaling by suppressor of cytokine signaling proteins. *Mol Endocrinol* 1999, 13:1832-1843.

85. Nicholson SE, De SD, Fabri LJ, Corbin J, Willson TA, Zhang JG, Silva A, Asimakis M, Farley A, Nash AD *et al.*: Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. *Proc Natl Acad Sci USA* 2000, 97:6493-6498.

86. Schmitz J, Weissenbach M, Haan S, Heinrich PC, Schaper F: SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130. *J Biol Chem* 2000, 275:12848-12856.

87. Cohney SJ, Sanden D, Cacalano NA, Yoshimura A, Mui A, Migone TS, Johnston JA: SOCS-3 is tyrosine phosphorylated in response to interleukin-2 and suppresses STAT5 phosphorylation and lymphocyte proliferation. *Mol Cell Biol* 1999, 19:4980-4988.

88. Cacalano NA, Sanden D, Johnston JA: Tyrosine-phosphorylated SOCS3 inhibits STAT activation but binds to p120 RasGAP and activates Ras. *Nat Cell Biol* 2001, 3:in press.

89. Shuai K: Modulation of STAT signaling by STAT-interacting proteins. *Oncogene* 2000, 19:2638-2644.

90. Zhang J, Somani AK, Siminovitch KA: Roles of the SHP-1 tyrosine phosphatase in the negative regulation of cell signalling. *Semin Immunol* 2000, 12:361-378.

91. Gadina M, Sudarshan C, Visconti R, Zhou YJ, Gu H, Neel BG, O'Shea JJ: The docking molecule gab2 is induced by lymphocyte activation and is involved in signaling by interleukin-2 and interleukin-15 but not other common gamma chain-using cytokines. *J Biol Chem* 2000, 275:26959-26966.

92. Gu H, Maeda H, Moon JJ, Lord JD, Yoakim M, Nelson BH, Neel BG: New role for Shc in activation of the phosphatidylinositol 3-kinase/Akt pathway. *Mol Cell Biol* 2000, 20:7109-7120.

93. Krystal G: Lipid phosphatases in the immune system. *Semin Immunol* 2000, 12:397-403.

94. Irie-Sasaki J, Sasaki T, Matsumoto W, Opavsky A, Cheng M, Welstead G, Griffiths E, Krawczyk C, Richardson CD, Aitken K *et al.*: CD45 is a JAK phosphatase and negatively regulates cytokine receptor signalling. *Nature* 2001, 409:349-354.