**Minireview**

Inhibitors of Cytokine Signal Transduction*

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Samuel Wormald‡ and Douglas J. Hilton

From the Walter and Eliza Hall Institute of Medical Research and the Cooperative Research Centre for Cellular Growth Factors, Parkville, Victoria 3050, Australia

Cytokines are secreted proteins that regulate diverse biological functions by binding to receptors at the cell surface to activate complex signal transduction pathways including the Janus kinase-signal-transducer and activator of transcription (JAK-STAT) pathway. Stringent mechanisms of signal attenuation are essential for ensuring an appropriate, controlled cellular response. Three families of proteins, the SH2-containing phosphatases (SHP), the protein inhibitors of activated STATs (PIAS), and the suppressors of cytokine signaling (SOCS), inhibit specific and distinct aspects of cytokine signal transduction. The analysis of mice lacking genes for members of the SHP and SOCS families has shed much light on the roles of these proteins in vivo. In recent in vitro studies, the protein modifiers ubiquitin and SUMO (small ubiquitin-like modifier) have emerged as key players in the strategies employed by SOCS and PIAS to repress signaling.

Cytokines regulate many cellular processes, often in concert and often via similar signal transduction pathways (1). Many cytokines are recognized by members of the hematopoietin family of transmembrane cell surface receptors, which oligomerize upon ligand binding, permitting the juxtaposition, cross-phosphorylation on tyrosine residues, and activation of receptor-associated Janus kinase (JAK) family members. JAKs then phosphorylate tyrosine residues in the cytoplasmic domain of the receptor, creating recognition sites for signaling proteins with Src homology 2 (SH2) or other phosphotyrosine binding domains. Members of the signal transducers and activators of transcription (STAT) family are latent transcription factors with SH2 domains that are phosphorylated by JAKs upon binding to the receptor, enabling them to dimerize and enter the nucleus where they regulate gene transcription (for reviews see Refs. 2 and 3) (Fig. 1A).

Rampant cytokine signal transduction can have disastrous biological consequences, and for this reason, signaling pathways are tightly controlled at multiple points (Fig. 1B). SH2-containing phosphatase (SHP) proteins are constitutively expressed and can attenuate cytokine signal transduction by dephosphorylating signaling intermediates such as JAK and its receptor. Members of the protein inhibitors of activated STATs (PIAS) family are also constitutively expressed and attenuate signal transduction by repressing STAT activity. The process of sumoylation has been implicated recently in PIAS-mediated repression of STAT activity. To date, the only known inducible inhibitors of cytokine signaling are the suppressor of cytokine signaling (SOCS) proteins, of which there are eight family members: SOCS1–SOCS7 and the cytokine-inducible SH2-domain-containing protein (CIS). SOCS proteins can recognize cytokine receptors or the associated JAKs and attenuate signal transduction both by direct interference with signaling and by targeting the receptor complex for ubiquitin-mediated proteasomal degradation.

**SHP**

There are two members of the SHP family in mammals, SHP-1 and SHP-2. SHPs were first implicated in the negative regulation of cytokine signaling when analyses of *motheaten* (me) mice mapped the causative mutation to the *Hcph* locus, which encodes the SHP-1 protein (4–6). Mice that are homozygous for the *me* mutation suffer from severe immunological defects including enhanced proliferation of macrophages and neutrophils in the lungs, which leads to a fatal pneumonitis, and in the skin, which leads to patchy dermatitis and the “motheaten” appearance (for reviews see Refs. 7 and 8).

SHP-1 and SHP-2 consist of two consecutive N-terminal SH2 domains and a C-terminal protein-tyrosine phosphatase domain (Fig. 2). In pathways of signal transduction, phosphorylation of tyrosine, serine, or threonine residues is a common mechanism by which signaling intermediates become activated. Both SHPs bind with their SH2 domains to phosphorytrosine residues of a number of cytokine receptors. SHP-1 negatively regulates cytokine signal transduction by dephosphorylating signaling components such as the interleukin-4 (IL4) receptor (9), the stem cell factor receptor c-kit (10), the erythropoietin receptor (11, 12), and JAK2 (12–14). SHP-1 also associates in an SH2-independent manner with the insulin receptor (15) and with JAK2 (16). SHP-2, on the other hand, appears to function mainly as a positive regulator of signaling (for review see Ref. 17), although there is evidence to suggest that it can inhibit cytokine signaling via the gp130 receptor (18).

**PIAS**

PIAS1 was originally identified in a yeast two-hybrid screen for STAT1-interacting proteins and was later characterized as a specific inhibitor of STAT1-mediated transcriptional activation (19, 20; for review see Ref. 21). The PIAS family members PIAS3, PIASx, and PIASy were identified based on sequence similarity to PIAS1 and have been shown to inhibit STAT3 (19), STAT1 (22), and STAT4 (23), respectively. The mechanisms by which the various PIAS family members inhibit STAT signaling appear to differ; PIAS1 and PIAS3 bind STAT1 and STAT3, respectively, to inhibit association with DNA, whereas...
neither PIASx nor PIASy prevents their target STATs from associating with DNA and must function in some other manner.

Recently, several very interesting experiments have begun to cast some light on the mechanisms by which PIAS proteins may inhibit STAT activity. In a yeast two-hybrid screen for proteins that interact with the ubiquitin-like protein modifier SUMO1, the *Saccharomyces cerevisiae* protein Siz1p was identified (24, for review see Ref. 25). Johnson and Gupta (26) showed that Siz1p is necessary for covalent attachment of SUMO1 (sumoylation) to *S. cerevisiae* septin proteins and that it functions in a manner analogous to the role of E3 ubiquitin ligase in ubiquitination, acting in conjunction with the E1-like Aos1/Ubs2 and E2-like Ubc9 enzymes in an ATP-dependent reaction (26–28, for reviews see Refs. 29 and 30). Johnson and Gupta (26) also noticed that a variant RING domain in Siz1p is similar to a conserved region in the PIAS family (Fig. 2), suggesting that PIAS proteins could function as E3 SUMO ligases in mammals (Fig. 3). E3 SUMO ligase activity has since been demonstrated for all members of the PIAS family, and many targets of this activity have been identified, with STATs proving no exception. PIAS1, PIAS3, and PIASx can all sumoylate STAT-1 at Lys-703, close to the site at which it is phosphorylated by JAKs (Tyr-701) (31, 32), and mutation of Lys-703 to Arg results in an increased response to interferon-γ (IFNγ) (32).

How sumoylation of STAT1 inhibits its activation is not understood. One possibility is that SUMO modification may serve as a targeting signal for STAT1, as has been demonstrated in the case of the LEF1 transcription factor. Upon PIASy-mediated sumoylation, LEF1 is sequestered into nuclear bodies, and LEF1 transcriptional activity is repressed (33). Neither of these scenarios, however, explains the observation that PIASx and PIASy can inhibit STAT activity in association with DNA. The recent finding that PIASx is a transcriptional co-repressor of STAT4 and that an inhibitor of histone deacetylase can disrupt PIASx-mediated repression suggests that PIASx may be involved in the regulation of chromatin structure.

**SOCS**

SOCS1 (also known as JAB and SSI-1) was cloned simultaneously by three different groups based on its ability, when overexpressed, to suppress macrophage differentiation in response to IL6 signaling (34), its association with the kinase domain of JAK2 (35), and structural similarities between its SH2 domain and that of STAT3 (36). SOCS family members have a central SH2 domain that is flanked by a variable length N-terminal domain and a 40-amino acid C-terminal domain,
Fig. 3. Mechanisms of covalent protein attachment that have been implicated in the negative regulation of cytokine signaling. A, ubiquitin (Ub) conjugation occurs in a three-step process that begins with the ATP-dependent formation of a thiol ester linkage between ubiquitin and the ubiquitin-activating enzyme, E1. E1 adenylates ubiquitin, and ubiquitin is then transferred to a conjugating enzyme, E2. E2 associates with an E3 ubiquitin ligase complex that catalyzes the transfer of ubiquitin to a lysine residue from the substrate. SOCS1 forms an E3 ubiquitin ligase complex with elongins B and C, Cullin-5 (Cul-5), and Rbx1 to mediate the ubiquitination of JAK2 and probably other proteins as well. Ubiquitin itself is also a substrate for E3 activity, allowing the formation of long ubiquitin chains. The ubiquitinated substrate is subsequently recognized and degraded by the proteasome. B, the process of SUMO (Su) attachment is similar to that of ubiquitin. The Uba2/Aos1 heterodimer and Uba9 fulfill the roles of the E1 activating enzyme and the E2 conjugating enzyme. PIAS1 and several other proteins possess E3 SUMO ligase activity, although in some cases sumoylation can occur independently of an E3 ligase. Unlike ubiquitin, polymerization of SUMO has not been observed in vivo. The mechanism by which sumoylation inhibits STAT1 activity is unclear.

The SOCS box (Fig. 2) (37, 38). SOCS proteins bind with their SH2 domains to phosphotyrosine residues in cytokine receptors (in the case of SOCS2, SOCS3, and CIS) (39–41) or JAKs (in the case of SOCS1) (35) and can suppress cytokine signaling either by binding to and inhibiting the activity of JAKs, by competing with STATs for phosphorylated binding sites on receptors, or by targeting bound signaling proteins for proteasomal degradation (38, 42, for reviews see Ref. 43).

A role for SOCS in mediating proteasomal degradation was first suggested when the SOCS box of SOCS proteins was found to associate with elongins B and C (44, 45), two proteins that form an E3 ubiquitin ligase complex with Cullin-5 and Rbx-1 (46). Because ubiquitination can target a protein for degradation by the proteasome, this raises the possibility that SOCS proteins may inhibit signaling by functioning as adaptors for an E3 ubiquitin ligase complex, which could mediate the ubiquitination of SOCS binding partners (Fig. 3A). Consistent with this idea, SOCS1 has been shown to target TEL-JAK2, a fusion protein with constitutive JAK2 activity, and wild-type JAK2 for ubiquitination and proteasomal degradation in a SOCS box-dependent manner in vitro (42, 47, 48).

Discerning the roles of the various SOCS proteins in regulating signaling by the multitude of cytokines that induce them has proven difficult in vitro due to a functional redundancy that emerges between different SOCS proteins when overexpressed (for review see Ref. 49). The analysis of mice lacking the genes coding for SOCS1 or SOCS2 has revealed critical roles for these proteins in negatively regulating certain cytokines. Socs1−/− mice exhibit increased sensitivity to the inflammatory cytokine IFNγ and prolonged STAT1 activation and die before 3 weeks of age from a complex disease, whereas Socs1−/− Ifnγ−/− mice are healthy (50). Socs2−/− mice have enhanced signaling by growth hormone and insulin-like growth factor I and are significantly larger than their wild-type littermates (51). Socs3−/− mice die midgestation due to placental insufficiency (52), a fate similar to that of Stat3−/− mice (53). Three groups recently generated mice lacking SOCS3 in specific tissues and demonstrated that SOCS3 is important for attenuating signaling by IL6, a cytokine that regulates inflammatory and acute phase responses (54–56). Croker et al. (54) and Lang et al. (56) observed prolonged IL6-induced activation of STAT1 and STAT3 in hepatocytes and macrophages lacking SOCS3 and, surprisingly, the induction of many genes normally associated with signaling by IFNγ. Prolonged activation of STAT1 and induction of IFNγ-inducible genes in response to IL6 has also been described in STAT3-deficient mouse embryo fibroblasts (57), suggesting that STAT3-mediated induction of SOCS3 could be important for preventing an IFNγ-like response to IL6 signaling. Yasukawa et al. (55), however, found that the activity of IL6 became immunosuppressive in the absence of SOCS3, a characteristic that is not normally attributed to either IL6 or IFNγ. Clearly SOCS3 is playing an important role in sculpting the cellular response to IL6, although the exact nature of this role remains to be determined.

Conclusions

Negative regulation of signal transduction pathways is necessary for an appropriate cellular and physiological response to cytokine stimulation. Over the past few years, several different mechanisms by which cytokine signaling is attenuated have been identified. The details, however, in determining the functions of specific inhibitors of cytokine signaling within particular cytokine signal transduction pathways have often been difficult to elucidate. The generation of mice lacking genes coding for some members of the SOCS family has illustrated a few dramatic examples of the crucial functions of particular SOCS proteins in attenuating signaling by particular cytokines. For other cytokines the picture appears to be not so simple, and in many cases multiple functionally redundant inhibitors are probably responsible for attenuating signal transduction. Sorting out the roles of negative regulators of cytokine signaling in the plethora of networks that are activated in response to cytokine will certainly prove a challenge for the future.

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