Review

Negative regulation of cytokine signaling and immune responses by SOCS proteins

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Abstract

Immune and inflammatory systems are controlled by multiple cytokines, including interleukins and interferons. Many of these cytokines exert their biological functions through JAKs (Janus tyrosine kinases) and STAT (signal transduction and activators of transcription) transcription factors. CIS (cytokine-inducible SH2 (Src homology 2) protein) and SOCS (suppressor of cytokine signaling) are a family of intracellular proteins, several of which have emerged as key physiological regulators of cytokine-mediated homeostasis, including innate and adaptive immunity. In this review we focus on the molecular mechanism of the action of CIS/SOCS family proteins and their roles in immune regulation and inflammatory diseases including rheumatoid arthritis.

Introduction

Cytokines regulate many physiological responses and homeostasis, influencing the survival, proliferation, differentiation and functional activity of cells of the immune system, as well as those of most other organ systems [1]. Cytokines, including interleukins, IFNs and hemopoietins, activate the Janus kinases (JAK1, JAK2, JAK3 and Tyk2) that associate with their cognate receptors. Activated JAKs phosphorylate the receptor cytoplasmic domains that create docking sites for Src homology 2 (SH2)-containing signaling proteins. Among the substrates of tyrosine phosphorylation are members of the signal transducers and activators of transcription family of proteins (STATs) [2,3]. For example, IFN-γ uses JAK1 and JAK2, which activate mainly STAT1, whereas IL-6 binding to the IL-6 receptor α chain and gp130 activates primarily JAK1 and STAT3. Interestingly, the anti-inflammatory cytokine IL-10 also activates STAT3. STAT4 and STAT6 are essential for T helper (Th)1 and Th2 development, Because these are activated by IL-12 and IL-4, respectively. STAT5 is activated by many cytokines including IL-2, IL-7, erythropoietin and growth hormones. These are summarized in Fig. 1.

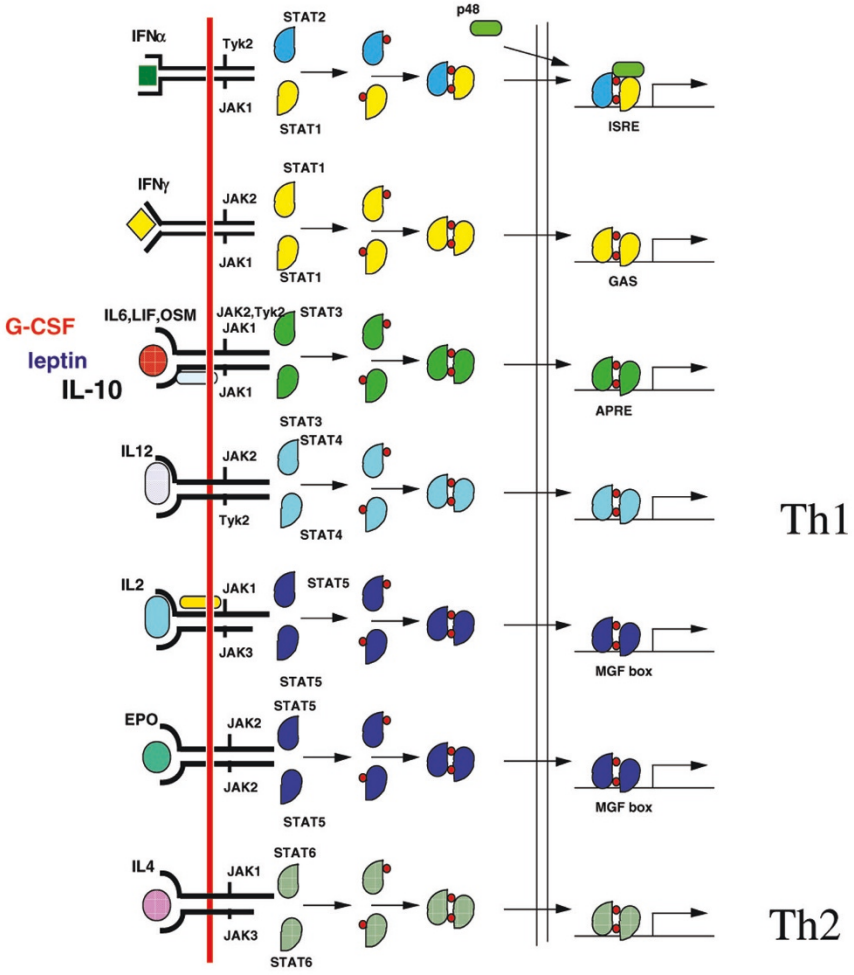
It has been recognized that sustained and/or excessive action of cytokines can be harmful to organisms. Accordingly, several mechanisms have been reported to modulate cytokine signaling to prevent this overaction of cytokines. For example, soluble forms of cytokine receptors that lack intracellular domains can inhibit the action of cytokines by simple competition for cytokine binding. Endocytosis of receptors and proteasomal degradation of signaling molecules after initial ligand stimulation is thought to have a role in preventing continuous cytokine signaling. In addition, several molecules that actively function as negative regulators of cytokine signaling, including SH2-containing phosphatase SHP-1, protein tyrosine phosphatase 1B (PTP1B), CD45 and T cell protein tyrosine phosphatase (TCPTP) [4] have also been reported to inhibit cytokine signaling as JAK phosphatases. The PIAS (protein inhibitors of activated STATs) family of proteins can inhibit the function of STATs by binding directly [5]. Moreover, recently accumulating evidence suggests that another family of proteins, suppressor of cytokine signaling (SOCS) proteins, is an important negative regulator for cytokine signaling [6,7].

CIS/SOCS family: structure and action mechanism

SOCS and cytokine-inducible SH2 protein (CIS) are a family of intracellular proteins, several of which have been shown to regulate the responses of immune cells to cytokines [6–10]. The discovery of the SOCS proteins seemed to have defined an important mechanism for the negative regulation of the cytokine–JAK–STAT pathway; however, recent studies using gene-disrupted (knockout; KO) mice have unexpectedly

CIS = cytokine-inducible SH2 protein; DC = dendritic cell; G-CSF = granulocyte colony-stimulating factor; GH = growth hormone; HCC = hepatocellular carcinoma; IFN = interferon; IL = interleukin; IRS = insulin receptor substrate; KIR = kinase inhibitory region; JAK = Janus kinase; KO = knockout; LPS = lipopolysaccharide; NF = nuclear factor; NK = natural killer; RA = rheumatoid arthritis; SH2 = Src homology 2; siRNA = short interfering RNA; SOCS = suppressor of cytokine signaling; STAT = signal transduction and activators of transcription; Th = T helper; TLR = Toll-like receptor; TNF = tumor necrosis factor.

Figure 1



The JAK/STAT (Janus family kinase/signal transduction and activators of transcription) pathway. EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor; IFN, interferon; IL, interleukin; JAK, Janus kinase; OSM, oncostatin M; STAT, signal transduction and activators of transcription; Th, T helper.

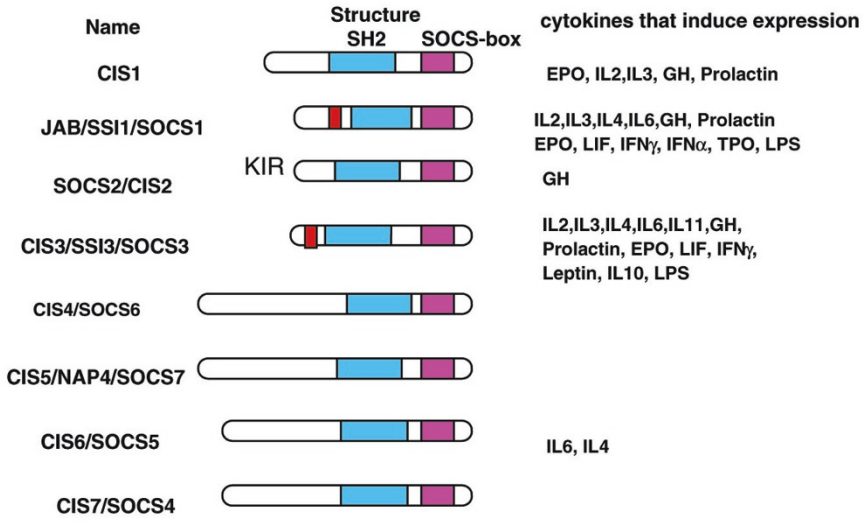
revealed profound roles of SOCS proteins in many immunological and pathological processes.

There are eight CIS/SOCS family proteins: CIS, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6 and SOCS7; each has a central SH2 domain, an amino-terminal domain of variable length and sequence, and a carboxy-terminal 40-amino-acid module known as the SOCS box (Fig. 2). The SOCS box has also been found in ASBs (ankyrin repeat-containing proteins with a SOCS box), SSBs (SPRY domain-containing proteins with a SOCS box) and WSBs (WD40 repeat-containing proteins with a SOCS box), as well as other miscellaneous proteins. The SOCS-family members best characterized so far are CIS, SOCS1, SOCS2 and SOCS3.

CIS was the first member identified in this family [11]. CIS and SOCS2 bind to phosphorylated tyrosine residues on activated (phosphorylated) cytokine receptors. Competition or steric hindrance for binding sites that are used to recruit and activate STATs (especially STAT5) has been proposed as the mechanism by which CIS and SOCS2 inhibit cytokine signaling [11,12]. CIS is induced by cytokines that activate STAT5 and bind to receptors that activate STAT5, namely erythropoietin, IL-2, IL-3, prolactin and growth hormone (GH) [11]. From an analysis of KO mice, SOCS2 has been shown to be a relatively specific negative regulator of GH–STAT5 [13,14]. SOCS5 has been shown to inhibit IL-4 signaling by interacting with the IL-4 receptor and inhibiting JAK1 binding to the receptor [15]. As mentioned below, receptor-CIS/SOCS complex is degraded by the ubiquitin–proteasome system, which could be an important inhibitory mechanism.

Both SOCS1 and SOCS3 can inhibit JAK tyrosine kinase activity because they have the kinase inhibitory region (KIR) in

Figure 2



Structures of suppressor of cytokine signaling (SOCS) family molecules. CIS, Src homology 2 (SH2)-containing protein; EPO, erythropoietin; JAB, JAK (Janus family kinase)-binding protein; KIR, kinase inhibitory region; NAP4, Nck/Ash-binding protein 4; SSI-1, STAT (signal transducer and activator of transcription)-induced STAT inhibitor-1.

their N-terminal domain, which is proposed to function as a pseudosubstrate [16] (Fig. 3). A three-dimensional structural model of the SOCS1/JAK2 complex has been proposed [17]. Whereas SOCS1 binds directly to the activation loop of JAKs through its SH2 domain, the SOCS3 SH2 domain binds the cytokine receptor (Fig. 3). The SOCS3 SH2 domain has been shown to bind to Tyr757 of gp130, Tyr985 of the leptin receptor and Tyr401 of the erythropoietin receptor, Tyr729 of the granulocyte colony-stimulating factor (G-CSF) receptor, Tyr800 of the IL-12 receptor and Tyr985 of the leptin receptor, most being the same binding sites for protein tyrosine phosphatase 2 (SHP-2) [18–22]. De Souza and colleagues [23] have mapped the phosphopeptide binding preferences of the SH2 domain from SOCS3 by using degenerate phosphopeptide libraries. They found that the consensus ligand-binding motif for SOCS3 was pTyr-(Ser/Ala/Val/Tyr/Phe)-hydrophobic-(Val/Ile/Leu)-hydrophobic-(His/Val/Ile/Tyr). The sequence around Tyr759 of gp130 (-pTyr-Ser-Thr-Val-Val-His-) almost completely matches this motif. Although SOCS3 binds with a much higher affinity to a gp130 phosphopeptide around Tyr759 than to phosphopeptides derived from other receptors, such as leptin and erythropoietin receptors, multiple SOCS3-binding sites are predicted to exist in these receptors, which might compensate for weaker binding to individual sites.

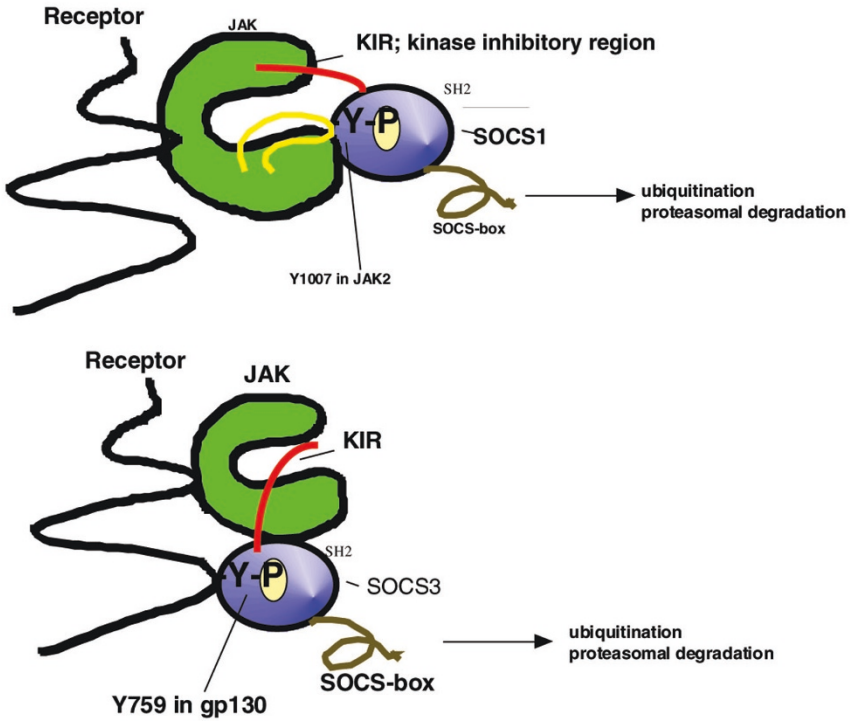
The function of the SOCS box is the recruitment of the ubiquitin-transferase system. The SOCS box interacts with Elongins B and C, Cullin-5 or Cullin-2, Rbx-1 and E2 [24–26]. Thus, CIS/SOCS family proteins, as well as other SOCS-box-containing molecules, probably function as E3 ubiquitin ligases and mediate the degradation of proteins associated through their N-terminal regions. SOCS proteins therefore seem to combine specific inhibition (that is, kinase inhibition by KIR) and a generic mechanism of targeting interacting proteins for proteasomal degradation. The importance of the SOCS box has been recognized from the following evidence: the SOCS box of SOCS1 is necessary for the suppression of the oncogenic activity of TEL-JAK2 by SOCS1 [27,28] as well as for the degradation of wild-type activated JAK2 [29], and mice that were genetically modified to lack only the SOCS box of SOCS1 exhibited inflammatory diseases similar to complete SOCS1-deficient mice with slower onsets [30]. SOCS1 is also suggested to be involved in the degradation of Vav [31] and in the ubiquitination and degradation of a papilloma virus oncoprotein, E7 [32]. SOCS1 and SOCS3 have also been shown to downregulate insulin signaling by inducing the degradation of insulin receptor substrate (IRS)-1 and IRS-2 [33,34]. However, the SOCS box is also known to be important for stabilization and/or degradation of the SOCS1 and SOCS3 proteins themselves [24]. The role of the SOCS box in the function of each of the SOCS proteins remains to be investigated further.

Physiological function of CIS/SOCS molecules defined by gene targeting

CIS1

CIS-transgenic mice exhibited growth retardation, impaired mammary gland development and reduced numbers of natural killer (NK) and NK T cells. These phenotypes in CIS-transgenic mice are remarkably similar to those observed in STAT5a KO and/or STAT5b KO mice [35], which is

Figure 3



The molecular mechanism by which suppressor of cytokine signaling 1 (SOCS1) and SOCS3 negatively regulate Janus kinase (JAK) activation. SOCS1 binds to the JAKs and inhibits catalytic activity; SOCS3 binds to JAK-proximal sites on cytokine receptors and inhibits JAK activity through KIR (kinase inhibitory region). These complexes may be degraded by ubiquitination and proteasomal degradation recruited through the SOCS box.

consistent with CIS having a specific role in the regulation of STAT5-mediated cytokine responses. Several reviews have mentioned that no obvious phenotype is observed in CIS KO mice but without showing any data. However, we have preliminary data suggesting that CIS is an important negative regulator of hematopoietic growth factors, including erythropoietin, IL-3 and thrombopoietin (A Yoshimura, unpublished data). These are consistent with our initial proposal that CIS is a negative regulator of STAT5 [11].

SOCS1

Although SOCS1 KO mice are normal at birth, they exhibit stunted growth and die within 3 weeks with a syndrome characterized by severe lymphopenia, activation of peripheral T cells, fatty degeneration and necrosis of the liver, and macrophage infiltration of major organs (acute SOCS1$^{-/-}$ disease) [36,37]. The neonatal defects exhibited by SOCS1$^{-/-}$ mice seem to occur primarily as a result of unbridled IFN-γ signaling, because SOCS1$^{-/-}$ mice that also lack the IFN-γ gene or the IFN-γ receptor gene do not die neonatally [38–40]. Constitutive activation of STAT1 and constitutive expression of IFN-γ-inducible genes were observed in SOCS1 KO mice. These data strongly suggest that the excess IFN-γ is derived from the abnormally activated T cells in SOCS1$^{-/-}$ mice. However, SOCS1 also has important regulatory functions in IFN-γ-independent inflammatory diseases; these are discussed later.

SOCS2

SOCS2 is known to bind to GH receptors and to inhibit the activation of STAT5b induced by GH. SOCS2-deficient mice at 12 weeks after birth exhibited a 30 to 40% increase in body weight compared with control mice; they also showed hypertrophy of the liver and other visceral organs related to the increase in weight [14]. Growth promotion by GH is dependent on the induction of insulin-like growth factor-1 (IGF-1) by GH, whereas SOCS2-deficient mice do not exhibit an increase in serum IGF-1. Expression of SOCS2 is not directly induced by IGF-1 but is directly induced by GH. In SOCS2-deficient mice GH-induced STAT5 activation, but not IGF-1 signaling, is mildly enhanced [13]. Furthermore, SOCS2$^{-/-}$STAT5b$^{-/-}$ double KO mice showed normal growth [13]. These data suggest that the action of SOCS2 is in the regulation of the GH signaling pathway.

SOCS3

SOCS3 KO mice die by placental function defects during the embryonic stage of development [41,42]. Deletion of SOCS3 causes an embryonic lethality that can be saved by a tetraploid rescue approach, which demonstrates an essential

role in placental development and a non-essential role in embryo development. Rescued SOCS3-deficient mice show a prenatal lethality with cardiac hypertrophy, suggesting that SOCS3 is essential for regulating LIF receptors or gp130 signaling [42]. Conditional KO mice studies demonstrated that SOCS3 is an important negative regulator of IL-6 [43–45] and G-CSF [46,47]. Mice in which the SOCS3 gene was deleted in all hematopoietic cells developed neutrophilia and a spectrum of inflammatory pathologies [47]. When stimulated with G-CSF *in vitro*, SOCS3-deficient cells of the neutrophilic granulocyte lineage exhibited prolonged STAT3 activation and enhanced cellular responses to G-CSF [46,47]. SOCS3-deficient mice injected with G-CSF displayed enhanced neutrophilia, progenitor cell mobilization and splenomegaly, but unexpectedly also developed inflammatory neutrophil infiltration into multiple tissues and consequent hindleg paresis [47]. Interestingly, conditional STAT3 deletion in neutrophils also exhibited hyper-responses to G-CSF [48], suggesting that a major role of STAT3 in neutrophils is the induction of SOCS3. It is probable that the ERK (extracellular signal-related kinase) pathway induced by G-CSF has a major function in the proliferation and differentiation of neutrophils.

Recently, the essential roles of SOCS3 in endocrine systems have also been clarified. Administration of leptin to neural cell-specific SOCS3 conditional KO mice greatly reduces their food intake and causes enhanced body weight loss compared with wild-type mice, indicating that SOCS3 in the brain negatively regulates leptin signaling [49]. Similar findings were observed in SOCS3 heterozygous mice [50]. Moreover, Socs3-deficient mice were resistant to weight gain and hyperleptinemia induced by a high-fat diet, and sensitivity to insulin was retained. These data indicate that SOCS3 is a key regulator of diet-induced leptin and also insulin resistance [49]. In addition, SOCS3-deficient adipocytes generated from SOCS3 KO fibroblasts are significantly protected from tumor necrosis factor (TNF)-α-induced insulin resistance, mainly due to reduced proteasomal degradation of IRS proteins by TNF-α, suggesting that SOCS3 is an important mediator of insulin resistance *in vivo* [51]. Taken together, these results indicate that SOCS3 can be a potential therapeutic target for many prevalent human metabolic disorders such as obesity and diabetes.

SOCS5

A study with SOCS5 transgenic mice suggested that SOCS5 inhibits Th2 differentiation by inhibiting IL-4 signaling [14]. SOCS5 is expressed preferentially in Th1 cells and SOCS5 can interact with the IL-4 receptor in the absence of tyrosine phosphorylation. This interaction of SOCS5 with the IL-4 receptor is likely to cause the reduction in IL-4-induced activation of STAT6 and thus to regulate Th2 polarization. In line with this finding, T cells from SOCS5 transgenic mice also exhibit reduced Th2 polarization. However, a recent analysis of SOCS5 KO mice failed to confirm the roles of SOCS5 in lymphocyte function [52]. $CD4^+$ T cells in SOCS5 KO mice showed a normal Th1/Th2 response both *in vitro* and *in vivo*. The conflicting findings in SOCS5 KO mice may be explained by SOCS5 being compensated for by other SOCS proteins such as SOCS4, because SOCS4 shares significant homology with SOCS5. Further analyses including those of SOCS4/SOCS5 double KO mice may be required to address the function of SOCS5 *in vivo*.

A *Drosophila* SOCS protein that is highly homologous to mammalian SOCS5 was cloned and named SOCS36E. Interestingly, ectopic expression of SOCS36E in transgenic flies results in phenotypes resembling those of flies defective in JAK/STAT or epidermal growth factor signaling [53]. This result could imply that mammalian SOCS5 is also involved in the regulation of JAK/STAT and/or epidermal growth factor signaling in mammals, but future studies are required to address this issue.

SOCS6

Mice lacking SOCS6 have been generated and develop normally with the exception of a 10% reduction in weight compared with wild-type littermates [54]. SOCS6 mRNA was expressed ubiquitously in murine tissues [54]. SOCS6 and SOCS7 SH2 domains interacted with a protein complex consisting of IRS-4, IRS-2 and the p85 regulatory subunit of phosphoinositide 3-kinase. However, there is no evidence so far to suggest that SOCS6 might be involved in the degradation of proteins [54].

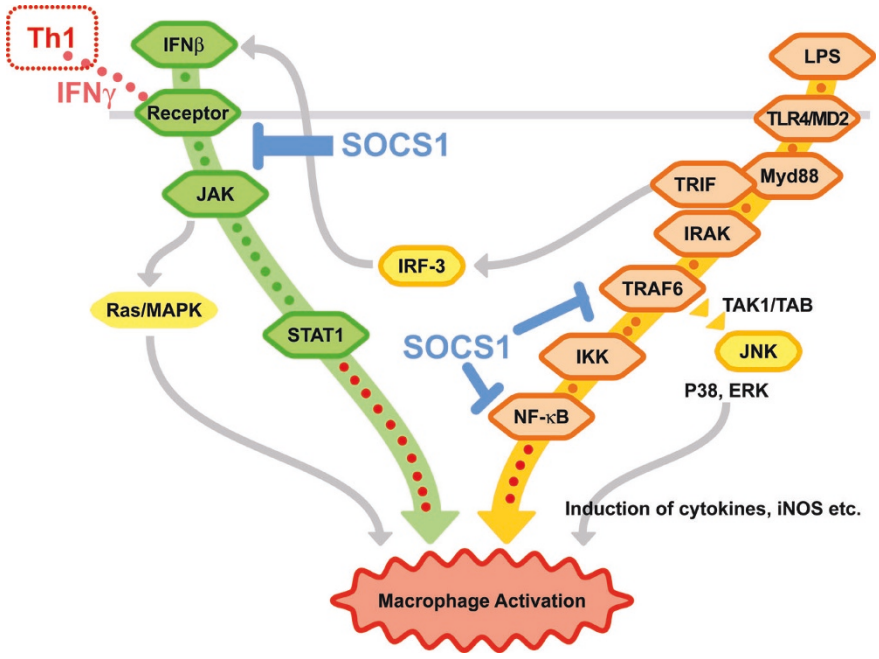
SOCS7

SOCS7 is highly expressed in the brain. $SOCS7^{-/-}$ mice were 7 to 10% smaller than their wild-type littermates, and within 15 weeks of age about 50% of the SOCS7-deficient mice died as a result of hydrocephalus that was characterized by cranial distortion, dilation of the ventricular system, reduced thickness of the cerebral cortex and disorganization of the subcommissural organ [55]. Thus, SOCS7 is important in the functioning of neuronal cells.

SOCS1 and innate immunity

SOCS1 deficiency in the hematopoietic compartment is thought to be sufficient to cause a $SOCS1^{-/-}$ disease, because transfer of $SOCS1^{-/-}$ bone marrow into irradiated JAK3-deficient recipients results in premature lethality [38,56]. $SOCS1^{-/-}$ rag-$2^{-/-}$ mice do not die prematurely [38], and $SOCS1^{-/-}$ NK T cells have been reported to be more numerous than normal in the liver and to be cytotoxic for syngenic liver cells [57]. T and/or NK T cells have therefore been suggested to have essential functions in $SOCS1^{-/-}$ diseases. However, mice lacking the SOCS1 gene, specifically in T and NK T cells, did not develop any of the inflammatory pathologies or neonatal death found in $SOCS1^{-/-}$ mice [58]. This indicates that other hematopoietic cells in addition to T and NK T cells are deeply involved in $SOCS1^{-/-}$ inflammatory diseases. Strong candidates are

Figure 4



Regulation of lipopolysaccharide (LPS) signaling by suppressor of cytokine signaling 1 (SOCS1). LPS stimulates NF-κB and the JNK/p38 pathway through Toll-like receptor (TLR)4/MD2 receptor. IFN-β is rapidly induced through the TRIF/IRF-3 pathway and activates the JAK/STAT1 (Janus kinase/signal transduction and activators of transcription 1) pathway. SOCS1 is probably induced by STAT1 and NF-κB, and then suppresses both STAT1 and NF-κB. One possible mechanism of NF-κB suppression is the induction of degradation of the p65 NF-κB subunit.

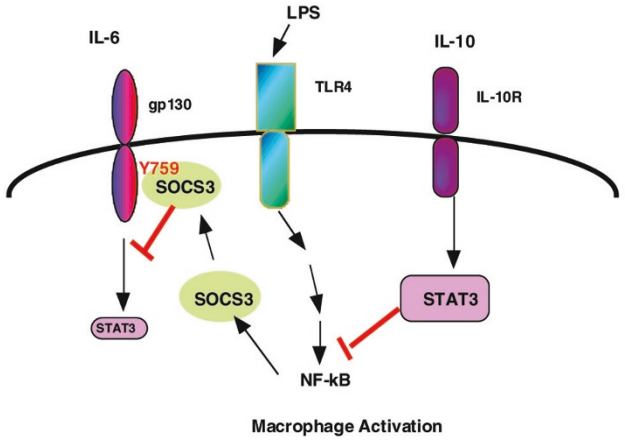
antigen-presenting cells including macrophages and dendritic cells (DCs).

Bacterial lipopolysaccharide (LPS) triggers innate immune responses through Toll-like receptor 4 (TLR4). Other bacterial pathogens including proteoglycans and CpG-DNA also activate other TLR-family receptors. Regulation of TLR signaling is a key step in inflammation, septic shock and innate/adaptive immunity. SOCS1 and SOCS3 were found to be induced by LPS or CpG-DNA stimulation in macrophages [59–61]. SOCS1 has been implicated in hyporesponsiveness to cytokines such as IFN-γ after the exposure of macrophages to LPS [61]. Furthermore, SOCS1-deficient mice are found to be more sensitive to LPS shock than wild-type littermates [62,63]. SOCS1$^{-/-}$ mice (before disease onset), SOCS1$^{+/-}$ mice and IFN-γ$^{-/-}$SOCS1$^{-/-}$ mice, as well as STAT1$^{-/-}$SOCS1$^{-/-}$ mice, have all been shown to be hyper-responsive to LPS and very sensitive to LPS-induced lethality. Macrophages from these mice produced increased levels of the pro-inflammatory cytokines, such as TNF-α and IL-12, as well as nitric oxide (NO), in response to LPS. One important mechanism of the suppression of LPS-induced macrophage activation by SOCS1 is the inhibition of IFN-β signaling indirectly activated by LPS [64,65]. However, a direct effect of SOCS1 on the TLR–NF-κB pathway has been also proposed [62,63]. Ryo and colleagues [66] showed that direct binding of SOCS1 to the p65 subunit of NF-κB induces proteasomal degradation of p65, which is one potential mechanism of TLR signal suppression by SOCS. Moreover, LPS tolerance was severely impaired in SOCS1$^{-/-}$ mice and SOCS1-deficient peritoneal macrophages [62,63]. However, Gingras and colleagues [64] did not observe enhanced LPS responses in SOCS1-deficient bone marrow-derived macrophages cultured with macrophage colony-stimulating factor (M-CSF). The nature of bone marrow-derived macrophages cultured in M-CSF *in vitro* is different from that of primary peritoneal macrophages [62–64]. SOCS1-deficient macrophages in tissue may be already affected by various environmental cytokines. Even so, SOCS1 is still deeply involved in the regulation of macrophage activation through regulating not only the JAK/STAT pathway but also the TLR–NF-κB pathway (Fig. 4).

SOCS1-deficient DCs are also hyper-activated and may be involved in the pathology found in SOCS1$^{-/-}$ mice [67]. We generated mice in which SOCS1 expression was restored in T and B cells on a SOCS1$^{-/-}$ background (SOCS1$^{-/-}$ transgenic mice). In these mice, DCs were abnormally accumulated in the thymus and spleen and produced high levels of BAFF/BLyS and APRIL, resulting in the aberrant expansion of B cells and autoreactive antibody production. SOCS1-deficient DCs efficiently stimulated B cell proliferation *in vitro* and autoantibody production *in vivo*. These results indicate that SOCS1 is essential to normal DC

functions and to the suppression of systemic autoimmunity that develops in SOCS1$^{-/-}$ transgenic mice [67]. Furthermore, we speculate that SOCS1$^{-/-}$ DCs are important in the onset of SOCS1$^{-/-}$ diseases, because SOCS1-deficient DCs can activate the proliferation not only of B cells but also of allogenic T cells [67]. We also observed that T cells produce higher amounts of Th1 cytokines such as IFN-γ and TNF-α in response to SOCS1$^{-/-}$ DCs than to wild-type DCs [68]. This nature of SOCS1-deficient DCs can be applied to anti-tumor immunity, because strong Th1 induction is believed to be important for DC-mediated vaccination. Shen and colleagues [69] reported that silencing the SOCS1 gene by using short-interfering-RNA (siRNA) technology in antigen-presenting DCs strongly enhances antigen-specific anti-tumor immunity. They showed that DCs transfected with SOCS1 siRNA were more responsive to LPS or IFN-γ than were DCs transfected with control siRNA, as indicated by an enhanced secretion of proinflammatory cytokines such as IL-6 and TNF-α and by the enhanced phosphorylation of STAT1, IκB and JNK upon stimulation. Antigen (ovalbumin) peptide-pulsed SOCS1-siRNA-treated DCs stimulated ovalbumin-specific cytotoxic T cell proliferation and functioned more strongly than did control DCs. These data indicate that SOCS1-deficient DCs can strongly activate CD4$^+$ (helper) and CD8$^+$ (cytotoxic) T cells.

Figure 5



Regulation of lipopolysaccharide (LPS) signaling by STAT3 (signal transduction and activators of transcription 3) and suppressor of cytokine signaling 3 (SOCS3) in macrophages. SOCS3 strongly suppresses STAT3 activated by IL-6/gp130 but not by IL-10, because SOCS3 does not bind to the IL-10 receptor. STAT3 is shown to suppress LPS signaling through STAT3, but the molecular mechanism of this process has not been clarified.

SOCS3 and innate immunity

IL-6 is a pro-inflammatory cytokine that has a progressive function in many inflammatory diseases, whereas IL-10 is an immunoregulatory cytokine that has potent anti-inflammatory activity. Although the transcription factor STAT3 is essential for the function of both IL-6 and IL-10 [70], it is not clear how these two cytokines exhibit such opposite functions. Recently, we demonstrated that at least in macrophages SOCS3 is a key regulator of the divergent action of these two cytokines. In macrophages lacking the SOCS3 gene, or carrying a mutation of the SOCS3 binding site (Y759F) in gp130, not only IL-10 but also IL-6 suppressed LPS-induced TNF-α production [43]. SOCS3 protein was strongly induced by both IL-6 and IL-10 in the presence of LPS, but selectively inhibited IL-6 signaling, because SOCS3 bound the IL-6 receptor, gp130 (Y759), but not the IL-10 receptor [43]. These data indicate that SOCS3 selectively blocks IL-6 signaling, interfering with its ability to inhibit LPS signaling (Fig. 5). Consistent with this is the observation that mice specifically lacking the SOCS3 gene in macrophages and neutrophils are resistant to acute inflammation as modeled by LPS shock. This phenotype is the complete opposite to macrophages in STAT3 conditional KO mice, which are more sensitive to LPS shock and produce more TNF-α in response to LPS [70]. We also found a similar opposite relationship between STAT3 and SOCS3 on DC activation (Y Matsumura and A Yoshimura, unpublished data).

Others have shown that IL-6 strongly activates STAT1 and induces the expression of IFN-responsive genes in SOCS3-deficient macrophages, implying that IL-6 might mimic the action of IFNs [44,45]. Interestingly, these reports also demonstrated that the absence of SOCS3 in macrophages changes the original function of IL-6. All three studies therefore indicate that SOCS3 is an important regulator to maintain a specific biological function on gp130-related cytokines *in vivo*. From such an interesting biochemical and biological function of SOCS3, we might be able to convert inflammatory cytokine IL-6 to an anti-inflammatory cytokine by suppressing the expression of SOCS3 in macrophages.

SOCS1 and inflammatory diseases

Given the wide range of immunoregulatory functions, SOCS1 might be implicated in the pathology of inflammatory diseases. In a murine model of autoimmune arthritis, joint inflammation and destruction was significantly enhanced in mice lacking SOCS1 [71,72]. Blood CD4$^+$ T cells from patients with rheumatoid arthritis (RA) contained higher levels of SOCS1 but lower levels of SOCS3 mRNA than control CD4$^+$ T cells, as determined by real-time polymerase chain reaction [73]. This higher expression of SOCS1 in T cells might explain the imbalance of the Th1/Th2 response or the resistance of T cells to IL-10 found in RA patients. In contrast, zymosan-induced arthritis was ameliorated in IL-6-deficient mice but exacerbated in STAT1-deficient mice [74], indicating that STAT1 is involved in the suppression of inflammation in this model. In STAT1$^{-/-}$ mice, gene expression of synovial SOCS1, but not that of SOCS3, was markedly reduced in STAT1-deficient mice. The expression of SOCS1 could be the underlying mechanism by which STAT1

controls joint inflammation. The important role of SOCS1 in joint inflammation and arthritis will probably be defined by conditional gene knockout.

SOCS1 has so far been shown to be deeply involved in hepatitis in humans. We found that SOCS1 gene silencing by DNA methylation is frequently observed in hepatitis induced by HCV infection [75], and SOCS1 gene methylation was well correlated with the severity of liver fibrosis, suggesting that decreasing SOCS1 gene expression by DNA methylation promotes liver inflammation.

SOCS3 and inflammatory diseases

There is accumulating evidence that SOCS3 could suppress inflammatory reactions in pathological situations in which IL-6-related cytokines have important progressive functions. This is because SOCS3 is a relatively specific inhibitor of gp130 as described above. STAT3 activation and high SOCS3 expression levels have been found in epithelial and lamina propria cells in the colon of IBD (inflammatory bowel disease) model mice, as well as in human ulcerative colitis and patients with Crohn's disease [76], and in synovial fibroblasts of patients with RA [77]. In a dextran sulfate sodium-induced mouse colitis model, a time-course experiment indicated that STAT3 activation was 1 day ahead of SOCS3 induction; STAT3 activation became apparent during days 3 to 5 and decreased thereafter, whereas SOCS3 expression was induced at day 5 and maintained high levels thereafter. In murine models of inflammatory synovitis, STAT3 phosphorylation preceded SOCS3 expression, which is consistent with the idea that SOCS3 is part of the STAT3 negative-feedback loop [76]. We have shown that overexpression of SOCS3 by adenoviral gene transfer could prevent the development of experimental arthritis [77]. The IL-6/STAT3 pathway therefore promotes the progression of the chronic status of diseases by contributing to cytokine and growth factor production, tissue hyperplasia, synovial fibroblast proliferation, fibrosis and osteoclast activation. On the basis of the evidence that forced expression of SOCS3 can inhibit IL-6-mediated STAT3 activation, we propose that SOCS3 is a negative regulator of inflammatory diseases in synovial fibroblasts, especially in those in which IL-6 levels are very high. A mouse line of mutated gp130 in which the SHP-2/SOCS3-binding site was disrupted developed a RA-like joint disease with increased production of Th1-type cytokines and immunoglobulins of the IgG2a and IgG2b classes [78]. In another case, gastrointestinal inflammation and adenoma were observed in similar mutant mice [79]. SOCS3 is therefore also critical in the development of chronic inflammatory disease. These studies reinforce the idea that cytokines operating through gp130 are probably important in activating RA synovial fibroblasts. Modulation of the gp130–JAK–STAT pathway is therefore a reasonable strategy for the development of new anti-inflammatory drugs. Specific JAK kinase inhibitors might have a therapeutic role in treating this and other disorders of the immune system, especially if toxicity does not preclude their use.

In contrast, the enhanced action of SOCS3 may promote allergic responses, because a recent analysis indicated that transgenic SOCS3 expression in T cells inhibits Th1 development and promotes Th2 development [80]. Indeed, that report also describes that increased SOCS3 expression in T cells is correlated with the severity of human allergic diseases such as asthma and atopic dermatitis. Modulation of SOCS3 levels in T cells could be useful in regulating the Th1/Th2 balance for the treatment of autoimmune inflammatory diseases.

SOCS and human cancer

Anti-tumor activity of SOCS1 has been reported by several groups. SOCS1 may inhibit the development and/or progression of hepatocellular carcinoma (HCC), because SOCS1 expression is significantly reduced in HCC cells; this can be explained by the inactivation of the SOCS1 promoter due to hypermethylation of CpG islands [81,82]. Yang and colleagues [83] investigated the promoter methylation status of major tumor suppressor genes including SOCS1, GSTP (pi-class glutathione S-transferase), APC (adenomatous polyposis coli), E-cadherin, RAR (retinoic acid receptor)-β, p14, p15, p16 and p73 in 51 cases of HCC. Among these, SOCS1 was the most frequently methylated (65%). Methylation of SOCS1, APC and p15 was more frequently seen in hepatitis C virus-positive HCC than in hepatitis C virus/hepatitis B virus-negative HCC. These data suggest that promoter hypermethylation of SOCS1 is an important event in HCC development. In support of this, a recent experiment has shown that SOCS1 heterozygous mice are hypersensitive to dimethylnitrosamine-induced hepatocarcinogenesis [75]. SOCS1 could be a novel anti-oncogene that accelerates inflammation-induced carcinogenesis. DNA hypermethylation of the SOCS1 gene is also found in several solid tumors derived from the colon, stomach, ovary, lung and breast; however, the frequencies in these tumors are not as high as in HCC [84–91].

In addition, SOCS1 may inhibit the progression of hematopoietic malignancies, because SOCS1 *in vivo* is preferentially expressed in lymphoid organs. Recent reports have indicated that reduced expression and DNA methylation of SOCS1 are frequently found in myeloma and leukemia cells [92,93]. Interestingly, biallelic mutation in the SOCS box of the SOCS1 gene was found in 9 of 20 primary mediastinal B cell lymphoma cells. These mutations probably result in impaired JAK2 degradation and sustained JAK2 activation [94]. In most cases, SOCS1 overexpression in cell lines could inhibit tumor cell proliferation; SOCS1 could therefore be an important target of anti-tumor therapy.

Like SOCS1, SOCS3 may also be involved in the development and progression of malignancies. In chronic

myelogenous leukemia cells, especially in cells in blast crisis and T cell lymphoma, SOCS3 is expressed constitutively and may confer resistance to IFN therapy [95,96]. In contrast, silenced expression of SOCS3 due to hypermethylation has been observed in human lung cancers and may be associated with the progression of cancer cells [97].

Therapeutic application

A next important step of the study of SOCS is a clinical application. Although it is too early to discuss its application to humans, several interesting trials *in vitro* and *in vivo* are under way. A group in the University of Florida developed a tyrosine kinase inhibitor peptide, Tkip, that is a mimetic of SOCS1 [98]. This 12-mer peptide interacts specifically with the autophosphorylation site of JAK2 and inhibits IFN-γ signaling. The peptide also suppressed the proliferation of prostate cancer cell lines in which STAT3 is constitutively activated [99]. Further characterization of JAK inhibition by SOCS1-KIR and Tkip peptide may uncover a novel mechanism to suppress the action of a specific cytokine. Overexpression of SOCS3 by adenovirus can prevent mouse RA models [77]. The generation of SOCS proteins carrying membrane-permeable peptide by genetic engineering might be another way to introduce JAK inhibitor into specific cells. The reduction of SOCS expression is also a therapeutic target. siRNA technology made the reduction of SOCS1 expression in DCs possible, and also improved anti-tumor immunity [69]. A decrease in SOCS1 and SOCS3 by antisense RNA treatment in obese diabetic mice improved insulin sensitivity and ameliorated hepatic steatosis and hypertriglyceridemia [100]. These studies are encouraging for controlling cytokine-related pathological conditions by mimicking or modulating SOCS proteins.

Conclusion

SOCS proteins are regulators of cytokine signal transduction and are essential to normal immune physiology, but they also seem to contribute to the development of immunological disorders including inflammatory diseases. Recently accumulated evidence regarding the balance of positive and negative pathways is important for a better understanding of immune systems, and this acquired knowledge will provide new insights that will assist the development of novel therapeutic strategies for both immunological diseases and cancer.

Competing interests

The author(s) declare that they have no competing interests.

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