Negative Regulation of Cytokine Signaling in Immunity

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Cytokines are key modulators of immunity. Most cytokines use the Janus kinase and signal transducers and activators of transcription (JAK-STAT) pathway to promote gene transcriptional regulation, but their signals must be attenuated by multiple mechanisms. These include the suppressors of cytokine signaling (SOCS) family of proteins, which represent a main negative regulation mechanism for the JAK-STAT pathway. Cytokine-inducible Src homology 2 (SH2)-containing protein (CIS), SOCS1, and SOCS3 proteins regulate cytokine signals that control the polarization of CD4⁺ T cells and the maturation of CD8⁺ T cells. SOCS proteins also regulate innate immune cells and are involved in tumorigenesis. This review summarizes recent progress on CIS, SOCS1, and SOCS3 in T cells and tumor immunity.

There are four types of the cytokine receptors: (1) receptors that activate nuclear factor (NF)- κ B and mitogen-activated protein (MAP) kinases (mainly p38 and c-Jun amino-terminal kinase [JNK]), such as receptors for the tumor necrosis factor (TNF)- α family, the interleukin (IL)-1 family, including IL-1 β , IL-18, and IL-33, and the IL-17 family; (2) receptors that activate the Janus kinase and signal transducers and activators of transcription (JAK-STAT) pathway—most cytokines belong to this family; (3) transforming growth factor (TGF)- β receptors carrying a serine/threonine kinase that activates Smad-family transcription factors; and (4) growth factor receptors in which cytoplasmic domain contains the tyrosine kinase domain. This latter family typically signals via the Ras extracellular signal-regulated kinase

(ERK) pathway (see Fig. 1). Any receptor that activates intracellular signaling pathways has multiple negative feedback systems, which ensures transient activation of the pathway and downstream transcription factors. Typical negative regulators are shown in Figure 1. Lack of such negative regulators results in autoimmune diseases, autoinflammatory diseases, and sometimes-fatal disorders, including cancer. Thus, negative feedback is essential for homeostasis.

Cytokine receptor signal regulators can be classified into three types: (1) proteins that physically suppress signal generation, (2) protein phosphatases, and (3) proteins recruiting degradation systems or processes such as proteasomes, autophagy, and endocytosis. All are multidomain proteins that bind to the receptors and/or signaling molecules through an Src ho-

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Figure 1. The cytokine signaling and their major negative regulators. There are four types of cytokine receptors: (1) receptors that activate nuclear factor (NF)- κ B and mitogen-activated protein (MAP) kinases; (2) receptors that activate the Janus kinase and signal transducers and activators of transcription (JAK-STAT) pathway; (3) transforming growth factor (TGF)- β receptors; and (4) growth factor receptor family. Typical negative regulators are also shown. TNF, Tumor necrosis factor; TNFR, TNF receptor; FGF, fibroblast growth factor; FGFR, FGF receptor; IKK, I κ B kinase; SOCS/CIS, suppressor of cytokine signaling/cytokine-inducible Src homology 2 (SH2)-containing protein; Sprad, Sprouty-related protein with an EVH1 domain.

mology 2 (SH2) domain or other binding motifs and then suppress the signaling via other domains. For example, A20/TNFAIP3 is an important negative regulatory protein for the NF- κ B pathway that interacts with NF- κ B essential modulator (NEMO)/I κ B kinase (IKK) γ and functions as a deubiquitinase (Shembade and Harhaj 2012). Sprouty-related protein with an EVH1 domain (Sprad) family proteins suppress the Ras-ERK pathway by bridging the growth factor/cytokine receptors and NF-1, a Ras-GTPase-activating protein (Wakioka et al. 2001; Nonami et al. 2003; Yoshida et al. 2006; Hirata et al. 2016). Some negative-regulator proteins have two or more inhibitory domains; for example, suppressor of cytokine signaling (SOCS)1 and SOCS3 have an amino-terminal kinase-inhibitory region (KIR) that inhibits JAK tyrosine kinase activity and a carboxy-ter-

минаl SOCS-box that recruits the ubiquitin-transferase complex. Because of space limitations in this review, we focus on proteins, especially SOCS proteins, which regulate signal transduction, but not on molecules interacting with the extracellular domain of the receptors or on transcription factors.

THE JAK-STAT PATHWAY

Cytokines play several essential roles in the development, differentiation, and function of myeloid and lymphoid cells. Some of them, including ILs, interferons (IFNs), and hematopoietic growth factors, activate the JAK-STAT pathway (O'Shea et al. 2002). In this pathway, cytokine binding results in receptor oligomerization, which initiates the activation of JAK kinases (JAK1, JAK2, JAK3, and TYK2). JAK3

is associated with IL-2 receptor γ (common cytokine receptor γ chain), and is activated by IL-2-related cytokines. The activated JAKs phosphorylate the receptor cytoplasmic domains, which creates docking sites for SH2-containing signaling proteins. The STAT proteins are the major substrates for JAKs. A large number of cytokines, growth factors, and hormonal factors activate the JAK-STAT pathway. For example, IFN- γ receptors activate JAK1 and JAK2, which then mainly phosphorylate and activate STAT1, whereas IL-6 binds to the IL-6 receptor α (IL-6R α) chain and to gp130, both of which mainly activate JAK1 and STAT3 (Guschin et al. 1995). The anti-inflammatory cytokine IL-10 also activates STAT3 (Weber-Nordt et al. 1996). T helper (Th)1, Th2, and Th17 are induced by IL-12, IL-4, and IL-6/IL-23, and thus STAT4, STAT6, and STAT3 are essential

for Th1, Th2, and Th17 differentiation, respectively. IL-4 in combination with TGF- β has been shown to induce Th9 in vitro (Tamiya et al. 2013). IL-2/STAT5 is essential for regulatory T-cell (Treg) development, and IL-21/STAT3 is essential for follicular helper T (Tfh) cell differentiation (Vogelzang et al. 2008). IL-21 also regulates CD8 $^{+}$ T cells (Gagnon et al. 2007) and Th17 cell differentiation (Bettelli et al. 2007).

THE CIS/SOCS FAMILY: MOLECULAR MECHANISMS

SOCS proteins and cytokine-inducible SH2-containing protein ([CIS] also known as CISH) molecules comprise a family of intracellular proteins (Fig. 2) (Yoshimura et al. 1995, 2007; Endo et al. 1997; Matsumoto et al. 1997; Tamiya

Figure 2. Structure and function of the suppressor of cytokine signaling (SOCS) family. The SOCS family consists of eight family members. All eight members share a central Src homology 2 (SH2) domain and a carboxy-terminal SOCS box. In addition, SOCS1 and SOCS3 possess a kinase inhibitory region (KIR) that inhibits Janus kinase (JAK) activity. (Right) The general mechanism of the action of cytokine-inducible SH2-containing protein (CIS), SOCS1, and SOCS3. STAT, Signal transducers and activators of transcription; JAB, Janus kinase-binding protein.

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et al. 2011). There are eight CIS/SOCS family proteins: CIS, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, and SOCS7, each of which 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) (Hilton et al. 1998; Kamizono et al. 2001; Kamura et al. 2004). The SOCS box interacts with elongin B/C, Cullins, and the Really Interesting New Gene (RING)-finger domain-only protein RBX2, which recruits E2 ubiquitin transferase (Kamura et al. 2004). CIS/SOCS family proteins, as well as other SOCS-box-containing molecules, function as E3 ubiquitin ligases and mediate the degradation of proteins that are associated with these family members through their amino-terminal regions (Fig. 2).

The central SH2 domain determines the target of each SOCS and CIS protein. The SH2 domain of SOCS1 directly binds to the activation loop of JAKs (Yoshimura et al. 1995; Yasukawa et al. 1999). The SH2 domains of CIS, SOCS2, and SOCS3 bind to phosphorylated tyrosine residues of the activated cytokine receptors. SOCS3 binds to gp130-related cytokine receptors, including the phosphorylated tyrosine 757 (Tyr757) residue of gp130, the Tyr800 residue of IL-12 receptor β 2, and the Tyr985 residue of the leptin receptor (Fig. 2). SOCS molecules bind to several tyrosine phosphorylated proteins and promote their degradation. SOCS1 binds to Mal, thereby negatively regulating Toll-like receptor (TLR) signaling (Mansell et al. 2006). SOCS3 has been shown to be an important regulator of insulin signaling, although binding to IRS1/2 (Shi et al. 2004; Torisu et al. 2007). SOCS3 also regulates chemokine signaling in B cells by interacting with focal adhesion kinase (FAK) (Le et al. 2007). SOCS2 binds active Pyk2 via pY402 and ubiquitinates it in natural killer (NK) cells (Lee et al. 2010). SOCS6 promotes p56Lck degradation via the proteasome in T cells (Choi et al. 2010).

In addition to general SOCS-box function in this family, both SOCS1 and SOCS3 have a unique amino-terminal motif that can inhibit JAK tyrosine kinase activity directly through

their KIR. KIR has been proposed to function as a pseudosubstrate, and it is essential for the suppression of cytokine signals (Fig. 2) (Yasukawa et al. 1999; Kershaw et al. 2013). Recent study of the ternary cocrystal structure between mouse SOCS3, JAK2 kinase domain, and a fragment of gp130 supported this hypothesis (Fig. 3). The kinase-inhibitory region of SOCS3 occludes the substrate-binding groove on JAK2, and biochemical studies show that it blocks substrate association. SOCS3, and probably SOCS1, inhibits the catalytic activity of JAK1, JAK2, and TYK2, but not JAK3, because the SH2-KIR domain interacts with an evolutionarily conserved “GQM” sequence that is present in all vertebrate forms of JAK1, JAK2, and TYK2, but not JAK3, where it lines one edge of the substrate-binding groove (Babon et al. 2012). We have shown that a KIR-mutant SOCS1 functions as a dominant negative form not only for SOCS1 but also for SOCS3 (Hanada et al. 2001). Thus, the interaction between KIR and GQM motif is essential for the tight binding and for the inhibition of the tyrosine kinase activity. The SH2 domain of SOCS3 does not bind to JAKs with high affinity, but it is required for receptor binding to inhibit JAKs, whereas the SOCS1 SH2 domain has been shown to inhibit JAK kinase activity through direct binding of its SH2 domain to the activation loop of JAKs. Regulation of type I IFN signaling by SOCS1 was shown not to require any of the phosphorylation sites in the IFNAR1 receptor. Because receptors to which SOCS3 binds mostly activate STAT3, SOCS3 is an inhibitor that is relatively specific to STAT3. However, SOCS3 also inhibits STAT4, which is activated by IL-12 (Fig. 2, right) (Yamamoto et al. 2003).

A recent study suggested that alveolar macrophages secrete SOCS1 and SOCS3 in exosomes and microparticles, respectively, for uptake by alveolar epithelial cells and subsequent inhibition of STAT activation (Bourdonnay et al. 2015). Secretion and transcellular delivery of vesicular SOCS proteins was diminished by cigarette smoking, suggesting a novel mechanism of dysregulated inflammation by smoking.

Figure 3. Structure of the complex of Janus kinase (JAK)2 and suppressor of cytokine signaling (SOCS)3, and gp130 phosphopeptide. SOCS3 binds the kinase domains of JAK1, JAK2, and TYK2 and inhibits its catalytic activity by blocking the substrate-binding site with its kinase inhibitory region. SOCS3 remains bound to gp130 while in complex with JAK (beige) and adenosine triphosphate (ATP) binding is unaffected. (Based on Kershaw et al. 2013, with permission, from the authors.)

CIS: INHIBITOR OF CYTOKINE AND T-CELL RECEPTOR (TCR) SIGNALING

CIS (also called CISH) was discovered as a rapid-inducible gene in response to various cytokines, including erythropoietin (EPO), IL-2, IL-3, and IL-5, which mostly activate STAT5 (Yoshimura et al. 1995; Matsumoto et al. 1997). CIS does not possess the KIR and cannot inhibit JAK tyrosine kinase activity directly. However, CIS binds to phosphorylated cytokine receptors, such as the EPO receptor, IL-2 receptor, murine IL-3 receptor β chain, prolactin receptor, and the growth hormone (GH) receptor, which mostly activate STAT5 (Yoshimura et al. 1995; Matsumoto et al. 1997; Aman et al. 1999; Ram and Waxman 2000; Endo et al. 2003). Thus, CIS is believed to suppress STAT5 by masking STAT5-binding phosphotyrosine motifs on the receptors, and also by inducing ubiquitin/proteasome-dependent degradation of the activated receptors (Fig. 2, right) (Okabe et al. 1999). In zebrafish, expression of *cish.a*

(there are two *cish* genes, *cish.a* and *cish.b* in zebrafish) was regulated by the JAK2-STAT5 pathway via conserved tetrameric STAT5-binding sites (TTCN₃GAA...TTCN₃GAA) in its promoter, and knockdown of *cish.a*, but not *cish.b*, resulted in enhanced embryonic erythropoiesis, myelopoiesis, and lymphopoiesis. This study showed conserved CIS functions for the control of hematopoiesis through STAT5 (Lewis et al. 2014).

However, recent studies using *Cish*^{-/-} mice challenged this theory (Yang et al. 2013). *Cish*-deficient mice >10 months of age showed spontaneous allergic lung inflammation, including mucus impaction and more eosinophil infiltrates. T cells from *Cish*^{-/-} mice expressed significantly higher IL-4 and IL-9 levels than wild-type (WT) cells, whereas IFN- γ and IL-17 levels were comparable. In vitro differentiation experiments indicated that *Cish* deficiency skewed T-cell differentiation toward Th2 and Th9 cells in the presence of IL-4. Higher amounts of STAT5 and STAT6 were recruited

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to the IL-4, IL-9, and GATA-3 promoters in *Cish*^{-/-} T cells as compared with WT T cells. These data suggest that CIS is a negative feedback regulator of IL-4, although the precise biochemical mechanism remains to be clarified.

CIS has been shown to be involved in regulation of TCR signaling. CIS expression is induced by TCR stimulation in T cells, and T-cell activation from *Cish*-transgenic mice showed enhanced proliferative responses and prolonged survival following TCR stimulation (Li et al. 2000). Subsequent research showed that there was an interaction between CIS and activated protein kinase C (PKC) α , β , and θ , followed by promotion of the ERK pathway (Chen et al. 2003). Paradoxically, recent research indicates that genetic deletion of *Cish* in CD8⁺ T cells enhances their expansion and function, resulting in pronounced and durable regression of established tumors (Palmer et al. 2015). In this study, CIS was shown to bind to the TCR intermediate PLC- γ 1, targeting it for proteasomal degradation after TCR stimulation. The reason why *Cish* knockdown and overexpression show similar phenotypes in T cells has not been clarified, but similar paradoxical phenomena were observed in the regulation of GH signaling by SOCS2 (Greenhalgh et al. 2002). CIS and SOCS2 expression levels may be critical for regulation of signaling or they have different functions for various targets.

Another recent paper suggests that CIS is a critical negative regulator of IL-15 signaling in NK cells and that deletion of *Cish* enhances antitumor immunity (Delconte et al. 2016). CIS was rapidly induced in response to IL-15, and deletion of *Cish* rendered NK cells hypersensitive to IL-15, as evidenced by enhanced proliferation, survival, IFN- γ production, and cytotoxicity toward tumors. In this study, CIS has been shown to selectively interact with JAK1 and targeting JAK1 for proteasomal degradation. *Cish*-deficient mice were resistant to melanoma, prostate, and breast cancer metastasis in vivo, and this was intrinsic to NK cell activity. Although the mechanisms are different, these studies suggest possibilities for new cancer immunotherapies directed at blocking CIS function.

Human genetic linkage studies of *CISH* have shown an association between *CISH* genetic variants and susceptibility to bacteremia, malaria, and tuberculosis (Khor et al. 2010; Sun et al. 2014), as well as persistent hepatitis B virus (HBV) infection or clearance of HBV (Tong et al. 2012; Hu et al. 2014; Song et al. 2014). The molecular mechanism of *CISH*-related infection regulation remains to be clarified.

SOCS1 AND T CELLS

SOCS1 can inhibit almost all cytokines using JAKs because it binds to and directly inhibits JAKs. Thus, SOCS1 specificity is regulated by its induction. *Socs1* knockout (KO) mice and conditional KO (cKO) mice showed that SOCS1 plays an essential negative regulatory role in IFN- γ , IL-2, IL-4, and IL-7 functions (Yoshimura et al. 2012). Because *Socs1*-deficient mice showed an aberrant CD4/CD8 ratio, SOCS1 has been implicated in T-cell development in the thymus (Catlett and Hedrick 2005). SOCS1, SOCS3, and CIS were shown to be critical targets of ThPOK, which is essential for the CD4⁺ lineage fate, thereby inhibiting CD8⁺ lineage programming (Luckey et al. 2014). Transgenic SOCS1 expression in thymocytes, however, rescued defects in CD4⁺ T-cell development in ThPOK-deficient mice (Luckey et al. 2014). SOCS1 and other SOCS molecules may be related to IL-7 sensitivity that determines the CD4/8 lineage decision.

SOCS1 plays critical roles in Th subset differentiation. *Socs1*^{-/-} CD4-naïve T cells differentiated into Th1 cells, even under non-skewing conditions, whereas Th17 differentiation was strongly suppressed (Tanaka et al. 2008). Th17 suppression by *Socs1* deficiency is probably a result of hyperproduction and signal transduction of IFN- γ . STAT3 activation was reduced in *Socs1*-deficient T cells, mostly because of up-regulation of SOCS3 gene expression, which can account for reduced IL-6 responses and Th17 differentiation (Tanaka et al. 2008). In addition, *Socs1*^{-/-} T cells were less responsive to TGF- β , although the mechanism has not been clarified (Tanaka et al. 2008).

SOCS1 AND TREGs

SOCS1 also plays an important role in the regulation of Tregs. Several reports have suggested that Tregs lose the expression of their master transcription factor, Foxp3, under certain inflammatory conditions. Thus, inflammatory cytokine signaling, including IFN- γ and IL-6 signaling, play important roles in the pathogenic conversion of Tregs (Takahashi and Yoshimura 2014; Sekiya et al. 2016). SOCS1 has been reported to play an important role in Treg-cell integrity and function by protecting Tregs from excessive inflammatory cytokines (Takahashi et al. 2011). *Socs1* deficiency in Tregs did not affect in vitro suppression activity, but it impaired suppressive Treg function in vivo despite the increase in Tregs (Lu et al. 2009). *Socs1*-deficient Tregs lose Foxp3 expression and convert into Th1- or Th17-like cells, probably because of STAT1 and STAT3 hyperactivation. The increase in Treg number may be explained by hypersensitivity to IL-2/STAT5 signaling in *Socs1*-deficient Tregs. Recently, Ubc13 has been reported to be involved in suppressive activity by controlling Treg effector cytokine signaling molecules, including SOCS1 (Chang et al. 2012). Smad2/3-deficient Treg phenotypes were similar to those observed in *Socs1*-deficient Tregs (Takimoto et al. 2010). This may be because hyperactivation of STAT1 inhibits the TGF- β /Smad pathway, which is important for Treg maintenance (Tanaka et al. 2008). Thus, interactive suppression of these molecules by STAT1 may be a mechanism of Foxp3 instability. Similarly, dysregulated STAT3 and STAT6 are thought to induce Treg instability (Feng et al. 2014). However, the role of SOCS1 in Tregs remains to be clarified. In humans, negative correlation between SOCS3 and Foxp3 levels was reported (Lan et al. 2013).

Socs1 is a target of miR-155 and miR-146a in Tregs (Lu et al. 2009, 2010, 2015). Lu et al. showed that, during thymic differentiation, up-regulation of Foxp3 is associated with high miR-155 expression, which in turn promotes the competitive fitness and proliferative potential of Treg cells by inducing *Socs1* down-regulation. miR-155 deficiency also attenuates liver

ischemia-reperfusion injury through up-regulation of *Socs1*, which was associated with promotion of M2 macrophage polarization and suppression of Th17 differentiation (Tang et al. 2015). The importance of *Socs1* as the target gene for miR-155 was shown by disrupting the miR-155 binding site in the *Socs1* 3' UTR in a murine germline, which shows that this axis is important for Treg and NK cell function (Lu et al. 2015). Conversely, miR-146a targets STAT1, thereby regulating SOCS1 expression (Lu et al. 2010).

SOCS1 AND ANTITUMOR IMMUNITY

Immune checkpoints are molecules in the immune system, which down-regulate the activation of T cells. They have garnered great interest in immunology and cancer science because immune-checkpoint molecules are involved in antitumor immunity and they are therapeutically important. The best known of these molecules are PD-1 and CTLA4, which negatively regulate TCR and costimulatory signals, respectively. Because SOCS1 is an important negative regulator of cytokine signaling, especially for IFN- γ and IL-12, which are essential cytokines for antitumor immunity, SOCS1 is now considered to be an immune checkpoint molecule for antitumor immunity. Previously, we and others showed that SOCS1-silenced dendritic cells (DCs) induce stronger antitumor immunity (Shen et al. 2004; Hanada et al. 2005; Chen et al. 2015). Myeloid-cell-specific *Socs1*-deficient mice were resistant to tumor growth in an IFN- γ -dependent manner (Hashimoto et al. 2009). In CD8⁺ T cells, even though *Socs1* deficiency caused defective expansion following in vivo antigen stimulation (Ramathan et al. 2010), *Socs1*-silenced CD8⁺ T cells showed stronger antitumor activity (Dudda et al. 2013). Because SOCS1 is an important target of miR-155, miR-155 overexpression enhanced the antitumor response, and enforced *Socs1* expression in CD8⁺ T cells phenocopied the miR-155 deficiency, whereas SOCS1 silenced augmented tumor destruction (Dudda et al. 2013). Similarly, miR-155 facilitates tumor growth modulation of myeloid-derived

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suppressive cells (MDSCs) through *Socs1* repression (Chen et al. 2015). These observations indicate that SOCS1 is a key regulator of anti-tumor immunity in both DCs and CD8⁺ T cells. Thus, a SOCS1 inhibitor that suppresses SOCS–JAK interaction could be a potent enhancer of antitumor immunity (Ahmed et al. 2015; Chikuma et al. 2017).

SOCS1 AND TUMORIGENESIS

SOCS1 is a unique tumor-suppressor gene that regulates inflammation-related tumorigenesis (Hanada et al. 2006; Inagaki-Ohara et al. 2013). Silencing of *SOCS1* was frequently observed in hepatocellular carcinoma (HCC) (Yoshikawa et al. 2001) and also in premalignant hepatitis C virus (HCV)-infected patients (Yoshida et al. 2004). Subsequent studies showed increased susceptibility of *Socs1*^{+/-} mice to the hepatocarcinogen diethylnitrosamine (DEN) (Yoshida et al. 2004). Liver injury is associated with hyperactivation of STAT1 and reduced activation of STAT3 (Ogata et al. 2006). Therefore, reduced expression of SOCS1 might enhance tissue injury and inflammation by hyperactivation of STAT1, promoting the turnover of epithelial cells and enhancing their susceptibility to oncogenesis. However, recent studies suggest a new role for SOCS1 in cancer. SOCS1 may regulate IFN- γ , IL-6, and hepatic growth factor signaling in the liver (Gui et al. 2015). In addition, SOCS1 has been shown to promote activation of the p53 tumor suppressor by a direct interaction (Mallette et al. 2010; Bouamar et al. 2015) and regulate p21^{CIP1/WAF1} protein (p21) expression and stability (Yeganeh et al. 2016). SOCS1 interacts with p21 and promotes its ubiquitination and proteasomal degradation.

Decreased *SOCS1* gene expression could be a mechanism involved in promoter hypermethylation in human. *SOCS1* promoter hypermethylation is detected in various cancers. SOCS1 DNA hypermethylation is also frequently found in certain types of lymphomas and myelodysplastic syndrome (MDS). In these cases, the silencing of *SOCS1* leads to dysregulation of JAK-STAT signal transduction, and

therefore contributes to growth factor hypersensitivity. *SOCS1* gene loss-of-function mutations have been frequently observed in classical Hodgkin lymphoma (cHL) (Lennerz et al. 2015) and primary mediastinal and diffuse large B-cell lymphomas (DLBCL) (Schif et al. 2013). *SOCS1* deletion resulted in sustained JAK2-STAT5 activation, which may lead to dysregulated proliferation, whereas SOCS1 overexpression prevented tumor growth (Kamio et al. 2004; Tagami-Nagata et al. 2015).

SOCS1: GENOME-WIDE ASSOCIATION STUDIES

Recent genome-wide association studies (GWASs) revealed that *SOCS1* single-nucleotide polymorphisms (SNPs) are found in various diseases, including primary biliary cirrhosis (PBC) (Dong et al. 2015), multiple sclerosis (Disanto et al. 2014; de Lapuente et al. 2015; Leikfoss et al. 2015), leprosy (Liu et al. 2015), Crohn's disease (Ellinghaus et al. 2012), celiac disease (Dubois et al. 2010), and serum IgE levels (Mostecky et al. 2011). These data strongly suggest a role for SOCS1 in immune regulation and in human immunological disorders.

SOCS3: ESSENTIAL REGULATOR FOR STAT3-RELATED CYTOKINES

SOCS3 is highly specific for several key cytokines that are related to the gp130 family, because the SOCS3-SH2 domain has a high affinity for phosphorylated gp130. Conditional tissue deletion of SOCS3 showed a nonredundant ability to inhibit signaling from IL-6 and also from leukemia inhibitory factor (LIF), leptin, and granulocyte colony-stimulating factor (G-CSF) (Yoshimura et al. 2007). In macrophages, *Socs3* deficiency resulted in the conversion of the effects of IL-6 to those of IL-10, which is a potent inhibitor of macrophages and DCs (Yasukawa et al. 2003). This is probably because of sustained activation of STAT3 in the absence of *Socs3*. Macrophages expressing mutant gp130 that is unable to bind SOCS3 displayed similar sustained STAT3 activation

and anti-inflammatory effects. However, mice lacking *Socs3* in the skin or mice carrying gp130 mutant develop exacerbated inflammation, chronic disease, and cancer (Inagaki-Ohara et al. 2013). Thus, the biological functions of the IL-6/STAT3 pathway are totally dependent on cell types.

ROLE OF SOCS3 IN HELPER T CELLS

SOCS3 in T cells regulates Th1/2/17 differentiation. SOCS3 expression in T cells is shown to positively correlate with the severity of human allergic diseases such as asthma and atopic dermatitis, because SOCS3 inhibits IL-12/Th1 development (Seki et al. 2003). SOCS3 also suppresses Th17 development because SOCS3 inhibits STAT3, which is essential for Th17 development (Chen et al. 2006; Tanaka et al. 2008). *Socs3* deficiency in T cells reduced atherosclerotic lesion development and vascular inflammation, which was dependent on IL-17, whereas SOCS3 overexpression in T cells reduces IL-17 and accelerates atherosclerosis (Taleb et al. 2009). SOCS3 overexpression by gene transfer could prevent the development of experimental arthritis and severe aortic aneurysm formation, which are highly dependent on Th17 (Shouda et al. 2001; Romain et al. 2013). However, *Socs3* deficiency in T cells showed different effects on Th1 and Th2 cells. T-cell-specific *Socs3*-cKO mice were resistant to Th1 and Th2 disease models. This is mostly because of higher IL-10 and TGF- β production from *Socs3*-deficient T cells (Kinjyo et al. 2006). *Socs3* deficiency in DCs also promotes induction of Foxp3⁺ Tregs, which is dependent on higher production of TGF- β from *Socs3*^{-/-} DCs (Kinjyo et al. 2006; Matsumura et al. 2007). A paradoxical effect of SOCS3 on T-cell regulation is mostly because STAT3 has a dual function—it promotes production of both inflammatory IL-17 and anti-inflammatory IL-10 and TGF- β .

SOCS3 AND CANCER

SOCS3 might also be involved in the development and progression of malignancies. SOCS3

expression levels were lower in HCV-positive tumors compared with nontumor regions (Ogata et al. 2006). Reduced SOCS3 messenger RNA (mRNA) and protein expression has been observed in various human cancers and is associated with constitutive STAT3 activation (Inagaki-Ohara et al. 2013). Recently, we reported that stomach tissue-specific deletion of *Socs3* resulted in gastric tumors, and this was dependent on leptin (Inagaki-Ohara et al. 2014). A SOCS3 SNP was reported to be associated with human gastric cancer (Wang et al. 2016). Similarly, gp130 mutant mice carrying the Y757F mutant, which lost binding affinity to SOCS3, developed gastric tumors (Jenkins et al. 2005). In this case, IL-11 and TGF- β have been shown to play important roles (Judd et al. 2006). Loss of *Socs3* also promoted pancreatic cancer driven by the oncogenic Ras mutation (Lesina et al. 2011). SOCS3 overexpression suppressed growth of malignant fibrous histiocytoma cell lines by inhibiting STAT3 and IL-6 production. In addition, this study raised the possibility that small molecule inhibitors of JAK-STAT could be therapeutic for IL-6-producing tumors (Shouda et al. 2006). SOCS3 mutation (or variant) in the SH2 domain was discovered in a patient with polycythemia vera (Suessmuth et al. 2009).

SOCS3 AND GWAS

GWAS studies have identified many SNPs of SOCS3 underlying variations in plasma-lipid levels (Asselbergs et al. 2012), asthma (Hao et al. 2012), hypospadias (Karabulut et al. 2013), nonalcoholic fatty liver disease (Grigoryev et al. 2015), nonalcoholic steatohepatitis (Sharma et al. 2015), type 2 diabetes (Chambers et al. 2015), and body mass index (BMI) (Al Muftah et al. 2016). These studies suggest that SOCS3 in humans is important for adaptive immunity and for tissue injury and metabolism. SOCS3 has been shown to regulate IL-6 and tyrosine kinase receptors such as insulin and hepatocyte growth factor (HGF) (Sun et al. 2005; Tokumaru et al. 2005; Torisu et al. 2007).

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CONCLUDING REMARKS

Over the past two decades, following the discovery of the SOCS family proteins, we have extended our understanding of the structure and function of these proteins. SOCS proteins act as simple negative-feedback regulators, and play a role in the fine-tuning of the immune response, inflammation, and metabolism, and also in cancer. Therapeutic trials using SOCS antisense oligonucleotide, short hairpin RNA (shRNA), and peptide mimetics are under investigation in animal models. Development of SOCS mimetics, based on structural analysis of the JAK–SOCS complex, is highly desirable.

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Negative Regulation of Cytokine Signaling in Immunity

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