 Suppressors of Cytokine Signaling (SOCS) Proteins and JAK/STAT Pathways
Regulation of T-Cell Inflammation by SOCS1 and SOCS3

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Abstract—Various cytokines are involved in the regulation of the immune system and inflammation. Dysregulation of cytokine signaling can cause a variety of diseases, including allergy, autoimmunity, inflammation, and cancer. Most cytokines use the so-called janus kinase/signal transducer and activator of transcription (STAT) pathway.1 In this pathway, cytokine binding results in receptor oligomerization, which initiates the activation of JAK kinases (JAK1, JAK2, JAK3, and Tyk2). The activated JAKs phosphorylate the receptor cytoplasmic domains, which creates docking sites for sec-homology-2 (SH2)-containing signaling proteins. Members of the signal transducers and activators of transcription family of proteins (STATs) are major substrates of JAKs. It is now known that a large number of cytokines, growth factors, and hormonal factors also activate JAK and STAT proteins; for example, proinflammatory cytokine IL-6 binds to the IL-6 receptor α chain and to gp130, both of which mainly activate JAK1 and STAT3. IFNγ receptors use JAK1 and JAK2, although it mainly activates STAT1. Interestingly, antiinflammatory cytokine IL-10 also activates STAT3. For helper T (Th) cell development, IL-6/IL-23, IL-12, and IL-4 activate STAT3, STAT4, and STAT6 respectively (Figure 1B). STAT3, STAT1/4, and STAT6 are essential for Th1, Th1, and Th2 differentiation, respectively.2 STAT5 is essential for regulatory T cell (Treg) development.2 The action of STAT5 also appears to be very direct, as STAT5 binds the Foxp3 gene, the master regulator of Tregs (see Figure 2).

Although our understanding of the intracellular signaling molecules that mediate the functional outcome of cytokine-receptor activation has increased profoundly, the most recent research has placed increasing emphasis on the mechanisms for the termination of signals and cross-talks with other signaling pathways. The cytokine-inducible SH2-containing (CIS)/suppressors of cytokine signaling (SOCS) family proteins are a major mechanism for such regulation.3–5 At the time of their discovery, the SOCS proteins were recognized as an important mechanism for the negative regulation of the cytokine-JAK-STAT pathway, but recent studies using gene-disrupted mice have revealed that they play additional, unexpected, and profound roles in many immunologic processes.4,5 Because space is limited, we will focus on the recent progress of SOCS1 and SOCS3 studies on inflammation and Th cell differentiation.

The CIS/SOCS Family
Suppressor of cytokine signaling (SOCS) proteins and CIS (also known as CISH) protein molecules make up a family of intracellular proteins.3–5 There are 8 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 (Figure 1A). The SOCS box is also found in other miscellaneous proteins. The SOCS box interacts with elongin B and elongin C, Cullins, and the RING-finger-domain-only protein RBX2 (which recruits E2 ubiquitin–transferase).6 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 N-terminal regions (Figure 1A).

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. The SH2 domains of CIS, SOCS2, and SOCS3 bind to phosphorylated tyrosine residues on 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 and Tyr985 of the leptin receptor. SOCS molecules bind to several tyrosine-phosphorylated proteins, including Mal (toll-like receptor signaling) and IRS1/2 (insulin signaling).

In addition, both SOCS1 and SOCS3 can inhibit JAK tyrosine kinase activity directly through their kinase inhibitory regions (KIR). The KIR has been proposed to function as a pseudosubstrate, and it is essential for the suppression of cytokine signals (Figure 2). The SH2 domain of SOCS3 does not have a high affinity to the activation loop of JAKs, yet the KIR of SOCS3 has a higher affinity to the kinase domain of JAK2 than the KIR of SOCS1 has. Because the receptors to which SOCS3 binds mostly activate STAT3, SOCS3 is an inhibitor relatively specific to STAT3. SOCS3 also inhibits STAT4, which is activated by IL-12 (Figure 1B).

**Overview of Th-Cell Differentiation**

Th cells play essential roles in adaptive immune responses and chronic inflammation. After emigrating from the bone
Th1 polarization is primarily driven by IL-12 and IFNγ, whereas Th2 polarization is primarily driven by IL-4. These respective cytokines signal via STAT4, STAT1, and STAT6 to directly control the transcription factors T-bet and GATA3, which, in turn, determine Th1 and Th2 differentiation, respectively. The Th17 differentiation of naïve T cells is initiated by IL-6 and transforming growth factor-β (TGF-β). In addition, IL-23, as well as IL-21, is thought to be a key cytokine for the maturation and maintenance of Th17 cells. IL-6, IL-21, and IL-23 all activate STAT3, which is thought to be essential for Th17 differentiation. It has also been reported that STAT3 plays a critical role in the induction of the orphan nuclear receptor RORγt, which directs Th17 cell differentiation by inducing the IL-23 receptor. The critical role of STAT3 in Th17 differentiation was also confirmed in human patients lacking functional STAT3. TGF-β also induces differentiation of naïve T cells into Foxp3+ Tregs in the peripheral immune compartment, and induced Treg and Th17 are reciprocally regulated.

**SOCS1 and Th-Cell Differentiation and Functions**

SOCS1 knockout (KO) mice die within 3 weeks of birth, 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. The neonatal defects exhibited by SOCS1−/− mice appear to occur primarily as a result of unbridled IFNγ signaling, because SOCS1−/− mice that also lack the IFNγ gene or the IFNγ receptor gene do not die neonatally. Constitutive activation of STAT1, as well as constitutive expression of IFNγ-inducible genes was observed in SOCS1 KO mice and cells. Because transgenic (tg) expression of the SOCS1 gene in T and B cells on a SOCS1-deficient background (SOCS1-KO-tg mice) can rescue neonatal death, lymphocytes (prob-
ably T cells) play a major role in early pathogenesis of SOCS1 deficiency.22

By using T-cell–specific conditional KO mice, we demonstrated that most SOCS1−/− CD4 naïve T cells differentiate into Th1, even under nonskewing conditions, whereas Th17 differentiation is strongly suppressed.23 As a result, T-cell–specific SOCS1 deficient mice are very sensitive to dextran sulfate sodium–induced colitis (Th1 type disease)24 but resistant to experimental autoimmune encephalitis, a typical Th17 type disease.23 Th17 suppression by SOCS1 deficiency is probably due to hyperproduction and signal transduction of IFNy. Indeed, STAT1 activation in SOCS1−/− T cells is upregulated, and strong Th1 skewing is corrected under STAT1+/− conditions (unpublished data).23 Interestingly, STAT3 activation is rather reduced in SOCS1-deficient T cells, mostly because of upregulation of SOCS3 gene expression, which can account for reduced IL-6 responses and Th17 differentiation.23 In addition, SOCS1−/− T cells are less responsive to TGF-β, although the mechanism has not been clarified yet. Reduced STAT3 activation and TGF-β signaling may explain suppression of Th17 differentiation in SOCS1-deficient T cells.

SOCS1 also plays an important role in the regulation of Tregs. Higher numbers of Tregs are observed in the thymus and spleen of T-cell–specific SOCS1-deficient mice.23 This is probably due to higher IL-2 responses, because IL-2 enhances proliferation of Tregs. Importantly, SOCS1 has been shown to be a target of miRNA-155 in Tregs. Lu et al showed that during thymic differentiation, upregulation of Foxp3 drives high expression of miRNA-155, which in turn promotes the competitive fitness and proliferative potential of Treg cells by inducing SOCS1 downregulation.25 However, SOCS1 has recently been found to have more profound functional roles in Tregs. Lu et al observed that SOCS1 deletion specifically in Tregs induces the development of spontaneous dermatitis, splenomegaly, and lymphadenopathy, suggesting a defective Treg function in these mice.25 The defective suppressive activity of SOCS1-deficient Tregs was confirmed through the failure to suppress colitis in Rag2−/− mice by the cotransfer of naive T cells and Tregs. Heightened STAT1 activation in SOCS1−/− Tregs leads to production of IFNy from transferred Tregs, and suppressor functions are restored under STAT1+/− conditions.26 Thus, SOCS1 is a “guardian” of Tregs, because SOCS1 inhibits Tregs to secrete IFNy by regulating STAT1.

Role of SOCS3 in Th Cells

The degree to which SOCS3 expression in T cells is increased correlates with the severity of human allergic diseases, such as asthma and atopic dermatitis.27 Enhanced action of SOCS3 may promote allergic responses, because a recent analysis has indicated that transgenic SOCS3 expression in T cells inhibits Th1 development and promotes Th2 development.27 Enhanced Th2 development could be due to suppression of Th1 because IL-12 mediated Th1 differentiation is impaired by SOCS3 overexpression. In contrast, mice lacking SOCS3 in T cells (produced by crossing SOCS3-floxed mice with Lck-Cre transgenic mice) have reduced allergen-induced eosinophilia in their airways.28 SOCS3 silencing with small interfering RNA in primary CD4+ T cells attenuated Th2 response in vitro.29 Adoptive transfer of SOCS3–small interfering RNA T cells exhibited markedly suppressed airway hyperresponsiveness and eosinophilia in an asthma model.29 These findings also suggest that the therapeutic modulation of SOCS3 expression in CD4+ T cells might be effective in preventing the development of allergic asthma.

T-cell–specific SOCS3-tg mice are also resistant to experimental autoimmune encephalitis.23 This resistance is mostly due to suppression of STAT3 by SOCS3 for Th17 differentiation. In contrast, SOCS3 deficiency promotes Th17 differentiation in T cells.30 Using VavCre-SOCS3 conditional KO mice, Wong et al reported that the IL-1-induced inflammatory joint disease model is severely deteriorated in the absence of SOCS3 accompanying with enhanced IL-17 production from CD4+ T cells.31 We have shown that overexpression of SOCS3 by adenovirus gene transfer can prevent the development of an experimental arthritis model, which is dependent on Th17.32 The absence of SOCS-3 therefore has dramatic proinflammatory effects by promoting Th17 development.

However, SOCS3 deficiency in T cells showed different effects on Th1 and Th2 cells. T-cell–specific SOCS3–conditional KO mice are resistant to Th1 and Th2 disease models.28 This resistance is mostly due to higher production of IL-10 and TGF-β from SOCS3-deficient T cells. It has been shown that IL-10 production is strongly upregulated by STAT3.33 We have shown that the TGF-β promoter contains potential STAT3 binding sites and is positively regulated by STAT3.28,34 In addition, SOCS3 deficiency in dendritic cells results in the enhanced induction of Foxp3+ Tregs, mostly dependent on higher production of TGF-β from SOCS3−/− dendritic cells.35 The paradoxical effect of SOCS3 on T-cell regulation is mostly due to a dual function of STAT3: it promotes production of both inflammatory IL-17 and antiinflammatory IL-10 and TGF-β. STAT3 also inhibits inflammatory cytokine production through suppression of nuclear factor-κB activation, and SOCS3 negatively regulates this process.36,37

SOCS and Vascular Inflammatory Diseases

SOCS proteins also play important regulatory roles in cardiovascular diseases, including intravascular coagulation, heart failure, and cardiovascular atherosclerosis. Inflammatory cytokines, such as tumor necrosis factor-α and IFNy, promote intravascular coagulation and tissue damages. It has been shown that SOCS1 deficiency in macrophages enhances endotoxin-mediated disseminated intravascular coagulation because of hyperproduction of tumor necrosis factor-α and IL-12.37,38 On the other hand, SOCS3 deficiency in macrophages protects mice from endotoxemia because of reduced production of inflammatory cytokines, which is due to the enhanced antinflammatory effect of STAT3.37

Inflammation is now known to promote atherosclerosis. Whereas normal vessels harbor only a few leukocytes, large numbers of both innate and adaptive immune cells accumulate during vascular inflammation, both in chronic forms, such as atherosclerosis, and in acute vasculitis. Because postnatal blocking of IFNy by overexpression of soluble IFNy-receptor prevented atherosclerotic plaque formation in
apolipoprotein E−/− mice, SOCS1 may also prevent atherosclerosis by suppressing IFNγ signaling.39 In human lesions, increased levels of STAT3 phosphorylation and IL-17 are associated with a stable plaque phenotype.40 Antisense oligodeoxynucleotides targeting SOCS3 exacerbate the atherosclerotic process in apolipoprotein E−/− mice by increasing the size, leukocyte content, and chemokine expression in the lesions.41 On the other hand, SOCS3 deficiency in T cells reduces atherosclerotic lesion development and vascular inflammation, which is dependent on IL-17, whereas overexpression of SOCS3 in T cells reduces IL-17 and accelerates atherosclerosis.42 In vivo administration of IL-17 reduces endothelial vascular cell adhesion molecule-1 expression and vascular T cell infiltration and significantly limits atherosclerotic lesion development. These results identify novel SOCS3-controlled IL-17 regulatory pathways in atherosclerosis and may have important implications for the understanding of the increased susceptibility to vascular inflammation. However, other reports suggest that IL-17 has a positive role in chronic vascular inflammation of atherosclerosis.43,44 At present, a mechanistic explanation for the discrepancies remains to be defined.

SOCS proteins may also interact with inflammatory pathways known to affect cardiac function. Accumulating evidence indicates that members of the IL-6 family of cytokines promote cardiac hypertrophy through the activation of STAT3.45 SOCS3 deficiency in the heart promotes cardiac hypertrophy by enhancing the JAK/STAT activity. Angiotensin II (Ang II) also exerts a potent growth stimulus on the heart and vascular wall. Calegari et al reported that angiotensin II at a physiological concentration enhances the expression of SOCS3 mRNA and protein, mainly via AT1 receptors.46 After induction, SOCS3 associates with JAK2 and impairs further activation of the JAK2/STAT pathway. Pre-treatment of rats with a specific phosphothioate antisense oligonucleotide to SOCS3 reverses the desensitization to angiotensin signaling. Thus, SOCS3 induced by angiotensin II in the heart probably prevents cardiac hypertrophy. The SOCS1-STAT pathway has recently been shown to modulate prostaglandin E2–mediated macrophage/dendritic cell suppression.47 SOCS1 may also play a role in cross-talk between cytokines and prostaglandin E2 in endothelial cells.

Concluding Remarks
In the past decade, following the discovery of the SOCS protein families, we have extended our understanding of the structure and function of these proteins. SOCS proteins not only act as simple negative-feedback regulators but also play a part in the fine tuning of the immune response and inflammation. JAK inhibition by small compounds is a promising therapeutic for various inflammatory diseases.48,49 Therapeutic trials using SOCS antisense oligonucleotide, small hairpin RNA, and peptide mimetics are under investigation in animal models.3,4 Especially, SOCS1 and SOCS3 are ideal therapeutic targets for autoimmune diseases; inflammatory diseases, including cardiovascular diseases; and cancer.

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Disclosures
None.

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