



Suppressors of cytokine signaling and immunity

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The suppressors of cytokine signaling (SOCS) and cytokine-inducible SH2 protein are key physiological regulators of the immune system. Principally, SOCS1 and SOCS3 regulate T cells as well as antigen-presenting cells, including macrophages and dendritic cells. Here we review the function of SOCS1 and SOCS3 in innate and adaptive immunity, with particular emphasis on the relationship between immune regulation and SOCS.

Cytokines regulate the survival, proliferation, differentiation and function of immune cells as well as cells from most other organ systems¹. Cytokines—including interleukins, interferons and hemopoietins—activate the Janus kinases (JAK1, JAK2, JAK3 and Tyk2), which associate with their cognate receptors. Activated JAKs phosphorylate the cytoplasmic domain of the receptor, creating a docking site for SH2-containing signaling proteins. Among the substrates of JAK tyrosine kinases, members of the signal transducer and activator of transcription (STAT) family of proteins are most important for cytokine actions^{2,3}. For example, interferon- γ (IFN- γ) activates JAK1 and JAK2, which mainly induce STAT1 phosphorylation, whereas binding of the proinflammatory cytokine interleukin 6 (IL-6) to the IL-6 receptor α -chain and gp130 mainly activates STAT3 through JAK1. The anti-inflammatory cytokine IL-10 also activates STAT3. T helper type 1 (T_H1) and T_H2 development are controlled by IL-12-dependent STAT4 and IL-4-dependent STAT6 activation, respectively. Finally, STAT5 is activated by many cytokines, including IL-2, IL-7, erythropoietin and growth hormone.

Several members of the SOCS and cytokine-inducible SH2 protein (CIS) family of intracellular proteins regulate the responses of immune cells to cytokines^{4–6}. The discovery of the SOCS proteins seemed to explain how the cytokine-JAK-STAT pathway was negatively regulated. However, studies of gene-disrupted mice have shown unexpected and profound functions for SOCS proteins in many immunological processes.

CIS and SOCS family

Detailed descriptions of the CIS and SOCS family have been reviewed in depth elsewhere^{4–6}. There are eight CIS and SOCS family proteins (CIS, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6 and SOCS7), each of which has a central SH2 domain; an N-terminal domain of

variable length and sequence; and a C-terminal 40-amino-acid module called the SOCS box^{4–6}. SOCS1 was identified independently by three laboratories^{7–9}. The best characterized SOCS family members are CIS, SOCS1, SOCS2 and SOCS3. CIS and SOCS2 bind to phosphorylated tyrosine residues on activated (phosphorylated) cytokine receptors (Fig. 1). We have summarized the genetic modification studies of SOCS genes and their implications for function (Table 1).

CIS and SOCS2 compete with STATs or can sterically hinder the STAT binding sites of receptors, inhibiting STAT activation, as in the case of STAT5^{10,11}. CIS is induced by cytokines that activate STAT5, such as erythropoietin, IL-2, IL-3, prolactin and growth hormone¹⁰. The inhibitory activity of SOCS2 is not as strong as that of CIS *in vitro*. Unexpectedly, however, very high SOCS2 expression somehow enhances growth hormone-induced activation of STAT5 (refs. 12–14). Nevertheless, from an analysis of SOCS2-deficient mice, SOCS2 seems to be a relatively specific negative regulator of the growth hormone-STAT5 pathway¹⁵. SOCS5 has been proposed to inhibit IL-4 signaling by interacting with the IL-4 receptor and inhibiting binding of JAK1 to the receptor¹⁶.

Both SOCS1 and SOCS3 can inhibit JAK tyrosine kinase activity. They have a kinase inhibitory region in their N-terminal domain, which probably functions as a pseudosubstrate¹⁷ (Fig. 1). SOCS1 uses its SH2 domain to directly bind the activation loop of JAKs and binds the catalytic pocket of JAKs through its kinase inhibitory region. A three-dimensional model of the SOCS1-JAK2 complex¹⁸ supports this. In contrast, the SOCS3 SH2 domain binds the cytokine receptor (Fig. 1). The SOCS3 SH2 domain binds to Y759 of gp130, Y985 of the leptin receptor and Y401 of the erythropoietin receptor. Y759 of gp130 and Y401 of the erythropoietin receptor are also the binding sites for the protein tyrosine phosphatase 2 (SHP-2)^{19–23}. As SHP-2 can promote gp130 signaling through the activation of mitogen-activated protein kinases, it is possible that SOCS3 might also suppress aspects of gp130 signaling by competing with SHP-2 for receptor binding. Alternatively, SHP-2 may also negatively regulate gp130 signaling by dephosphorylating JAKs. Mapping of the SH2-domain binding preferences using degenerate phosphopeptide libraries²⁴ showed the consensus ligand binding motif for SOCS3

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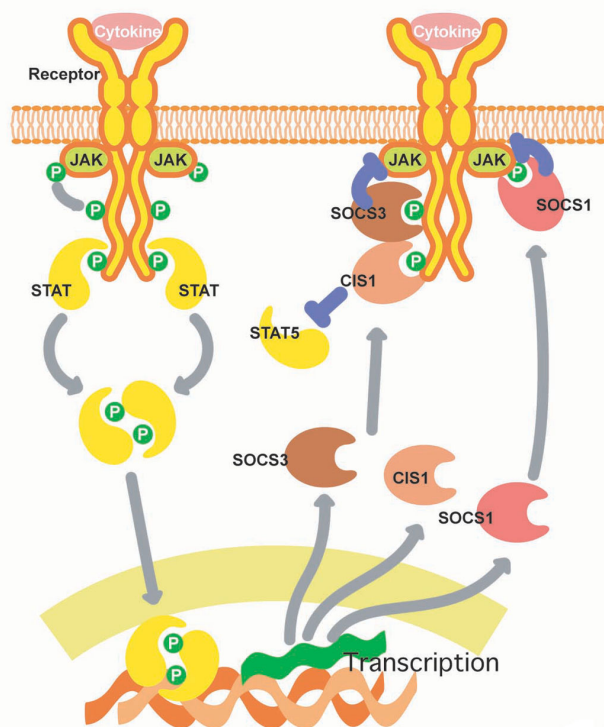


Figure 1 The molecular mechanism by which SOCS proteins negatively regulate cytokine signaling. Cytokine stimulation activates the JAK-STAT pathway, leading to the induction of CIS, SOCS1 and/or SOCS3. CIS, SOCS1 and SOCS3 seem to inhibit signaling by different mechanisms: SOCS1 binds to the JAKs and inhibits catalytic activity; SOCS3 binds to JAK-proximal sites on cytokine receptors and inhibits JAK activity; and CIS blocks the binding of STATs to cytokine receptors. Both SOCS1 and SOCS3 contain a kinase inhibitory region (KIR) for the suppression of JAK tyrosine kinase activity. P (in green circles), phosphorylated.

was pY-(S/A/V/Y/F)-hydrophobic-(V/I/L)-hydrophobic-(H/V/I/Y). The sequence around Y759 of gp130 (pYSTVH) almost completely matches this motif. Although SOCS3 binds with much higher affinity to a gp130 phosphopeptide around Y759 than to phosphopeptides derived from other receptors such as leptin and erythropoietin receptors, multiple SOCS3 binding sites are predicted to exist in these receptors and may compensate for weaker binding to these 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, cullins, Rbx-1 and E2 (refs. 25,26). Thus, CIS and 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. Therefore, SOCS proteins seem to combine specific inhibition (kinase inhibition by kinase inhibitory region) with a generic mechanism of targeting interacting proteins for proteasomal degradation. The fact that SOCS1-mediated suppression of TEL-JAK2 oncogenic activity^{27,28} requires both kinase inhibitory region and the SOCS box demonstrates the importance of these regions. The phenotype of SOCS box-deficient *Socs1* 'knock-in' mice demonstrates a requirement of the SOCS box for full SOCS1 activity²⁹. However, the SOCS box is also important for the stabilization and/or degradation of the SOCS1 and SOCS3 proteins themselves²⁵. Interaction of the SOCS box with elongin C stabilizes SOCS3

protein expression, whereas phosphorylation of SOCS box tyrosine residues disrupts the complex and enhances proteasome-mediated degradation of SOCS3 (ref. 30). The involvement of the SOCS box in the function of the other SOCS proteins remains to be investigated.

Diseases associated with SOCS1 deficiency

Although SOCS1-deficient mice are normal at birth, they show stunted growth and die within 3 weeks of age with a syndrome characterized by severe lymphopenia, activation of peripheral T cells, fatty degeneration and necrosis of the liver, as well as macrophage infiltration of major organs (acute *Socs1*^{-/-} disease)^{31,32}. The neonatal defects of *Socs1*^{-/-} mice occur mainly as a result of unbridled IFN- γ signaling, as *Socs1*^{-/-} mice lacking IFN- γ (*Ifng*^{-/-}) or the IFN- γ receptor do not die neonatally³³⁻³⁵. Constitutive STAT1 activation as well as constitutive expression of IFN- γ -inducible genes are found in SOCS1-deficient mice. These data strongly indicate that the excess IFN- γ is derived from abnormally activated T cells in *Socs1*^{-/-} mice. However, although neonatal or early adult disease can be avoided by the removal of IFN- γ , the lifespan of the *Socs1*^{-/-}*Ifng*^{-/-} mice is much shorter than that of *Ifng*^{-/-} mice³⁶. The main cause of premature death is associated with the development of polycystic kidneys, pneumonia, chronic skin ulcers and chronic granulomas in the gut and various other organs (chronic *Socs1*^{-/-} disease)³⁶. Lymphocyte-specific *Socs1*-transgenic mice on a *Socs1*^{-/-} background (*Socs1*^{-/-} transgenic) as well as *Socs1*^{-/-}*CD28*^{-/-} double-deficient mice show systemic lupus erythematosus-like autoimmune diseases with high serum concentrations of autoantibodies³⁷. Therefore, disease induced by SOCS1 deficiency is a complex syndrome consisting of acute and chronic inflammatory diseases and autoimmune-like diseases. The pathology of SOCS1-deficient mice raises challenging and profound questions of how these abnormalities occur and how such abnormalities induce development of acute and chronic *Socs1*^{-/-} diseases.

Part of the SOCS1-deficient phenotype might be explained by abnormal signaling by IFN- γ and other inflammatory cytokines, including IL-2 (refs. 38,39), IL-6 (ref. 40), IL-12 (ref. 41) and IL-15 (refs. 42,43). SOCS1 might also regulate signaling of tumor necrosis factor (TNF)⁴⁴, lipopolysaccharide (LPS)^{45,46}, insulin receptor substrate⁴⁷ and c-Kit⁴⁸. Here we will discuss how aberrant signaling of these cytokines, as well as T cell receptor (TCR) signaling, may induce such complex diseases. We have tabulated the known phenotypes of genetically modified mice on the *Socs1*^{-/-} background (Table 2).

SOCS1 and T cell activation

Early studies of SOCS1-deficient mice showed that SOCS1 deficiency induced aberrant T and natural killer T (NKT) cell activation^{31,32,49}. Although T and B lymphocyte numbers are below average in these mice, T lymphocytes, particularly CD8 cells, express cell surface

Table 1 Phenotype of CIS and SOCS gene deletion

Gene	Phenotype	Possible cytokines	Refs.
CIS	Increase in hematopoietic progenitors	EPO, IL-3	Unpublished ^a
SOCS1	Severe inflammation, neonatal death	IFN- γ	32,33
SOCS2	Gigantism	GH	15
SOCS3	Embryonic lethal	LIF, gp130	62,63
SOCS6	Mild growth retardation	IGF?	81

EPO, erythropoietin; GH, growth hormone. ^aT.H. and A.Y., unpublished data.

activation markers, and there is some evidence associating T and NKT cells as initiators of *Socs1*^{-/-} disease. For example, *Socs1*^{-/-} mice also deficient in recombination activation gene 2 (*Rag2*^{-/-}) do not die prematurely³³, and *Socs1*^{-/-} NKT cells are cytotoxic for syngeneic liver cells⁴⁹. Furthermore, SOCS1 deficiency in the hematopoietic compartment is sufficient to cause a *Socs1*^{-/-} disease, as shown by the transfer of *Socs1*^{-/-} bone marrow cells into irradiated recipients, which results in premature lethality of the mice^{33,50}. Unexpectedly, however, mice with a T cell-specific *Socs1* conditional deletion do not develop the inflammatory pathologies or neonatal death found in *Socs1*^{-/-} mice⁵¹. This indicates that SOCS1 deficiency causes multiple effects *in vivo* and requires other hematopoietic cell lineages. Same candidates would be antigen-presenting cells (APCs), including macrophages and dendritic cells, because APCs are essential in T cell activation.

In a recent study, irradiated adult syngeneic recipients were reconstituted with SOCS1-deficient bone marrow cells⁵⁰. Moribund mice did not have the acute or chronic diseases associated with *Socs1*^{-/-} mice, but developed a pathology characteristic of graft-versus-host disease, with typical chronic inflammatory lesions in the liver, skin, lungs and gut. This indicates cells derived from identical genetic backgrounds, with identical major histocompatibility complexes (MHCs), are autoactivated and raises two possibilities: activation of *Socs1*^{-/-} T cells does not require MHC-TCR signaling, or immunological tolerance is broken by self peptide-MHC-induced TCR activation of the *Socs1*^{-/-} T cells. Probably both TCR-dependent and TCR-independent pathologies are involved in *Socs1*^{-/-} diseases. However, the requirement of other hematopoietic-derived cells, such as APCs, for *Socs1*^{-/-} T cell activation^{50,51} indicates that TCR-dependent mechanism may be more important in the development of *Socs1*^{-/-} diseases. We have summarized the proposed functions of SOCS1 in T cell development and functions (Fig. 2).

SOCS1 and immunological tolerance

The thymus is the only organ that expresses SOCS1 in amounts sufficiently high to be detected by RNA hybridization and immunoblotting in wild-type mice in normal conditions. The main SOCS1-expressing cells in the thymus are immature double-positive thymocytes (CD4⁺CD8⁺). SOCS1 expression diminishes as double-positive

Table 2 Lethality and phenotype of double-deficient mice with *Socs1*^{-/-} background

Gene	Lifespan	Phenotype	Refs.
–	<3 weeks		
<i>Ifng</i> ^{-/-}	>1 year	Inflammatory diseases	33,34
<i>Rag2</i> ^{-/-}	>3 months		33
<i>Cd28</i> ^{-/-}	>1 year	SLE, inflammation	37
<i>Rag1</i> ^{-/-}	>3 months		52
<i>Rag1</i> ^{-/-} TCR-Tg	<3 months		52
TCR-Tg	<1 month		52
SOCS1-Tg in T, B cells	>1 year	SLE, inflammation	37

Tg, transgenic; SLE, systemic lupus erythematosus.

thymocytes differentiate into CD4⁺ or CD8⁺ single-positive cells. The sequential change of SOCS1 expression during thymic differentiation seems to be stochastic rather than being directed by cytokines^{33,51}. Strong thymic atrophy and few double-positive thymocytes are found in *Socs1*^{-/-} mice, and peripheral *Socs1*^{-/-} T cells are hyperactivated, as mentioned before. The possibility is thus raised that SOCS1 regulates TCR signaling (directly or indirectly) during the positive and negative selection process, resulting in both acute and chronic *Socs1*^{-/-} disease through disruption of central tolerance.

To examine the contribution of TCR-antigen specificity in the emergence of autoaggressive T cells, SOCS1-deficient mice have been generated that express a transgenic TCR specific for the exogenous antigen ovalbumin on a *Rag1*^{+/+} or *Rag1*^{-/-} background⁵² (Table 2). Although TCR-transgenic *Socs1*^{-/-} mice on a *Rag1*^{+/+} background have a longer lifespan than nontransgenic *Socs1*^{-/-}*Rag1*^{+/+} mice, they still die as young adults with inflammatory disease, and TCR-transgenic *Socs1*^{-/-} T cells seem to be activated despite the absence of OVA. Unexpectedly, TCR-transgenic *Socs1*^{-/-}*Rag1*^{-/-} mice die early, whereas nontransgenic *Socs1*^{-/-}*Rag1*^{-/-} mice remain healthy and do not die prematurely (Table 2). The difference between TCR-transgenic *Socs1*^{-/-}*Rag1*^{-/-} mice and nontransgenic *Socs1*^{-/-}*Rag1*^{-/-} mice is limited to the presence of mature T cells, indicating that mature T cells are a key factor for the development of acute *Socs1*^{-/-} diseases. These findings also indicate that SOCS1 deficiency induces the failure of self MHC-TCR recognition during thymic differentiation. This raises the possibility that the weak TCR-MHC interaction that is necessary for positive selection might be modified and impaired in the absence of SOCS1, resulting in escape of autoreactive T cells. This idea is supported by findings showing that *Socs1*^{-/-}*Cd28*^{-/-} mice survive more than 6 months without acute inflammatory disease, but develop autoimmune-like diseases³⁷. This escape mechanism could be mediated by excess cytokine signaling (for example, by IL-2 or IL-4) in *Socs1*^{-/-} mice during negative selection. That is, SOCS1 may be necessary for limiting the excess cytokine signals that prevent the apoptosis of self-reactive T cells. This idea is consistent with data showing that forced IL-4 expression in thymocytes results in aberrant T cell differentiation⁵³. Like *Socs1*^{-/-} mice, these transgenic mice have few CD4⁺CD8⁺ double-positive thymocytes and increases in the number of CD4⁺ and CD8⁺ single-positive thymocytes.

SOCS1 could also be involved in peripheral tolerance. Again, the long-term survival of *Socs1*^{-/-}*Cd28*^{-/-} mice supports this possibility, as TCR signaling without costimulation through CD28 is believed to generate an anergic state in peripheral T cells, a main mechanism of peripheral tolerance. In *Socs1*^{-/-}*Cd28*^{-/-} mice, most of the peripheral T cells show a nonaggressive CD44^{lo} phenotype due to insufficient T cell priming. Furthermore, T cells from *Socs1*^{-/-} mice can proliferate after anti-TCR stimulation in the absence of IL-2 or anti-CD28

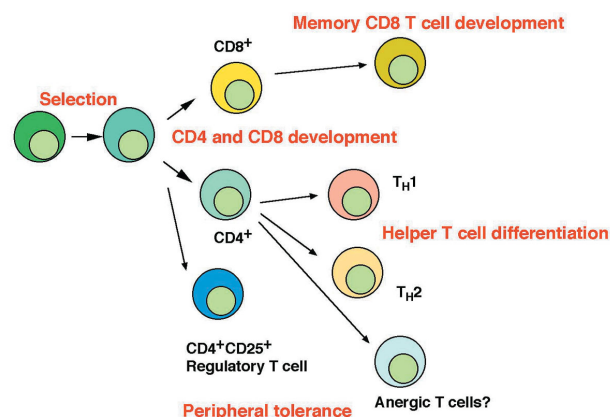


Figure 2 SOCS1 and T cell regulation. Possible stages at which SOCS1 could be involved: SOCS1 could be involved in early T cell development in the thymus (selection and CD4-CD8 determination), peripheral tolerance (suppression of the function of CD4⁺CD25⁺ regulatory T cells or maintenance of the anergic phenotype of peripheral T cells) and T_H1-T_H2 differentiation.

costimulatory signals³³. Thus, SOCS1 may be necessary for maintaining the anergic status of T cells. In this situation, SOCS1 may suppress reversal of the anergic status of T cells by cytokines. SOCS1 expression is very high in CD4⁺CD25⁺ regulatory T cells, which has led to the possibility that SOCS1 is involved in CD4⁺CD25⁺ regulatory T cell function^{54,55}. IL-6 produced from dendritic cells can disrupt the regulatory function of these cells⁵⁶. Therefore, SOCS1 may be necessary for the maintenance of CD4⁺CD25⁺ T cells regulatory function by inhibiting IL-6 signaling. Indeed, the suppressive activity of SOCS1-deficient CD4⁺CD25⁺ regulatory T cells is less than that of wild-type CD4⁺CD25⁺ regulatory T cells⁵⁵. Elucidating the molecular mechanism by which SOCS1 regulates immunological tolerance could open a new field for understanding the relationships between tolerance, cytokines and intracellular signaling.

SOCS1 in T cell lineage development and differentiation

SOCS1 is also profoundly involved in early CD4 and CD8 T cell development. Although SOCS1 is constitutively expressed in CD8⁺ single-positive thymocytes, its expression in CD4⁺ single-positive thymocytes is inducible by the γ_c -chain family of cytokines⁴². Using *Socs1*^{-/-}*Ifng*^{-/-} mice, as well as mice with a T cell-specific conditional deletion of *Socs1*, it was shown that lack of SOCS1 skews thymocyte development toward the CD8⁺ single-positive lineage. In addition, *Socs1*^{-/-}*Ifng*^{-/-} mice show increased CD8⁺ T cells with increased expression of surface activation markers in lymphoid tissues⁵². *Socs1*^{-/-} fetal thymic organ cultures and intrathymic transfer of CD4-CD8⁻ double-negative precursors from *Socs1*^{-/-} mice into *Rag1*^{-/-} mice showed that the lineage skewing in *Socs1*^{-/-} mice is a T cell-autonomous defect⁴². This has led to the suggestion that CD8 skewing is due to the hypersensitivity of SOCS-deficient CD8 T cells to IL-15 (refs. 42,43), which upregulates Bcl-x_L and CD44. In contrast, CD4⁺CD8⁺ double-positive thymocytes have high expression of SOCS1, and SOCS1-deficient peripheral CD8⁺ T cells show a CD44^{hi}CD25^{lo}CD69^{lo} memory phenotype⁵¹. Both of these phenotypes correlate with hypersensitivity to the γ_c family of cytokines, especially IL-7, indicating that acquisition of IL-7 signaling in the transit stage from double-positive to CD8 single-positive cells might regulate the maintenance of the naive phenotype in CD8⁺ T cells⁵¹. Thus, SOCS1 could regulate the naive phenotype of CD4⁺ and CD8⁺ T cells, the CD4/CD8 ratio and the memory phenotype of peripheral CD8 T⁺ cells.

SOCS1 is important in helper T cell differentiation. The mutually exclusive functions of IL-12 and IL-4 signals in T_H1 and T_H2 differentiation have been well characterized, but little is known about how each cytokine inhibits reciprocal T helper cell differentiation. Negative regulation by SOCS members possibly is involved in this balance. SOCS-deficient CD4⁺ T cells produce relatively higher amounts of IFN- γ and IL-4 than do wild-type CD4⁺ T cells in response to anti-CD3 restimulation⁵⁷, and *Socs1*^{-/-}*Ifng*^{-/-} double-deficient mice are more sensitive in T_H1-type arthritis models than are wild-type mice⁵⁸. SOCS1 inhibits IFN- γ as well as IL-12 signaling; thus, T_H1 skewing in SOCS1-deficient CD4⁺ T cells might be explained simply by hypersensitivity to IL-12 (ref. 41). However, controversial evidence indicates SOCS1 is expressed mainly in T_H1 cells derived from hen egg lysozyme-specific TCR-transgenic T cells⁵⁹. Thus, the function of SOCS1 in T_H1-T_H2 differentiation is still contentious. Another possibility to be considered is that IL-6-induced SOCS1 expression might be involved in inhibiting T_H1 responses⁴⁰. IL-6 is secreted by T_H2 cells and promotes T_H2 cell differentiation by inhibiting T_H1 polarization. Therefore, in T_H2-skewing conditions, IL-6 secreted by T_H2 cells would induce SOCS1 expression, resulting in the blocking of the IFN- γ signaling pathway, leading to accelerated T_H2 differentiation⁴⁰. In this case, SOCS1 expression skews T_H2 differentiation.

SOCS1 has also been proposed as an important effector molecule for cross-suppression of IFN- γ and IL-4. Usually, IFN- γ and IL-4 inhibit T_H2 and T_H1 development, respectively. In the absence of SOCS1, this cross-suppression is impaired⁵⁷. However, this is a complex situation, as SOCS1 can inhibit all cytokine signaling, including IFN- γ , IL-12 and IL-4, that are important for T_H1-T_H2 differentiation. Thus, the question of whether SOCS1 is a key factor in regulating the direction of T_H differentiation remains unresolved. Nevertheless, the findings noted here reveal multiple functions for SOCS1 in cytokine receptor signaling and emphasize an uncharacterized function of SOCS1 in the regulation of T_H1 and T_H2 cell differentiation.

SOCS3 in helper T cell differentiation and allergy

SOCS3-deficient mice die as a result of placental defects during embryonic development^{60,61}. Embryonic lethality can be rescued by replacing SOCS3-deficient placenta with *Socs3*^{+/+} placenta, demonstrating an essential function for SOCS3 in placental development and a nonessential function in embryo development. Rescued SOCS3-deficient mice show a prenatal lethality with cardiac hypertrophy⁶¹.

Like SOCS1, SOCS3 is also involved in T cell differentiation. SOCS3 is selectively expressed in murine T_H2 cells^{59,62}. Furthermore, SOCS3 expression in peripheral T cells from patients with T_H2 type diseases, such as atopic asthma and dermatitis, tightly correlates with the severity of the disease. Thus, the more severe the disease pathology, the higher the SOCS3 expression in T cells⁶². Serum immunoglobulin E (IgE) concentrations, which have been linked to the pathogenesis of allergic diseases, are also increased in people with high SOCS3 expression. T_H2 development and ovalbumin-induced airway hyper-responsiveness is notably enhanced in transgenic mice in which T cells constitutively express SOCS3 (ref. 62). The amounts of IgE, T_H2 cytokines and infiltration of eosinophils into bronchoalveolar lavage fluid are also enhanced in *Socs3*-transgenic mice, compared with that of control mice. Therefore, high SOCS3 expression skews helper T cell differentiation toward T_H2 cells.

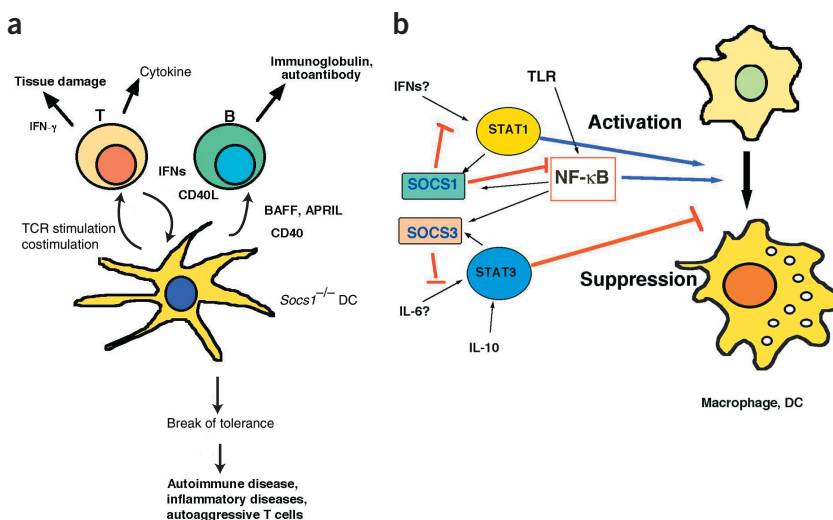
Preliminary evidence shows that SOCS3 can specifically bind to the cytoplasmic region of IL-12 receptor β (Try800; M.K., T.H. and A.Y., unpublished data) and inhibit IL-12-mediated STAT4 activation in T cells⁶². These results indicate that SOCS3 may regulate the onset and development of asthmatic diseases through inhibition of IL-12-mediated T_H1 development.

SOCS3 also regulates T cell function by negatively regulating IL-2 signaling⁶³ and inhibiting TCR and CD28 signaling pathways. Specifically, SOCS3 binds the calmodulin-dependent protein serine/threonine phosphatase calcineurin, thereby inhibiting TCR-mediated functions of the transcription factor NFAT⁶⁴. In addition, binding of SOCS3 to the PI3 kinase binding site on CD28 diminishes CD28-mediated production of IL-2 (ref. 65). Thus, SOCS3 may regulate T cell development and activation by multiple mechanisms.

SOCS1 and APC regulation

SOCS1 has an important regulatory function in macrophages and dendritic cells. Bacterial LPS triggers innate immune responses through the Toll-like receptor 4 (TLR4). Other bacterial pattern recognition motifs, including CpG-DNA, activate different TLR family receptors. Regulation of TLR signaling is a key step for inflammation, septic shock and innate and adaptive immunity. The SOCS proteins may be involved in regulating TLR signaling. For example, SOCS1 and SOCS3 are induced by LPS or CpG-DNA stimulation in macrophages⁶⁶⁻⁶⁹, and SOCS1 has been linked to the hyporesponsiveness of macrophages to cytokines such as IFN- γ after exposure to LPS. Furthermore, SOCS1-deficient mice are more sensitive to LPS shock than are their wild-type

Figure 3 Macrophage and dendritic cell regulation by SOCS. **(a)** SOCS1-deficient dendritic cells may disrupt central and peripheral tolerance. SOCS1-deficient dendritic cells (DCs) are hyperactivated and efficiently stimulate B cell proliferation and autoantibody production as well as T cell proliferation and strong cytokine production. **(b)** Functions of STAT1, STAT3, SOCS1 and SOCS3 in macrophage and dendritic cell activation. SOCS1 suppresses STAT1 (and NF- κ B by TLRs) and SOCS3 suppresses STAT3 activated by gp130-related cytokines. STAT1 activates macrophages and dendritic cells, whereas STAT3 suppresses them; thus, SOCS1 is a negative and SOCS3 is a positive regulator of APCs. IL-10 also suppresses macrophage activation through STAT3, but SOCS3 does not inhibit this pathway, as it does not bind the IL-10 receptor. However, there seems to be a cross-induction of SOCS1 and SOCS3 by STAT1 and STAT3. Therefore, the real situation is much more complicated.



littermates^{45,46}. *Socs1*^{+/-} mice and *Socs1*^{-/-}*Ifng*^{-/-} mice, as well as *Stat1*^{-/-}*Socs1*^{-/-} mice, are hyper-responsive to LPS and are very sensitive to LPS-induced lethality^{45,46}. Macrophages from these mice produce increased amounts of proinflammatory cytokines, such as TNF and IL-12, as well as nitric oxide, in response to LPS. LPS tolerance is severely impaired in *Socs1*^{-/-} mice and macrophages. Conversely, SOCS1 overexpression in a macrophage cell line suppresses LPS signaling, indicating that SOCS1 negatively regulates not only the JAK-STAT pathway but also the TLR-NF- κ B pathway. Although the molecular mechanism of the suppression of the NF- κ B pathway by SOCS1 has yet to be elucidated, the phenotype of SOCS1-deficient macrophages could provide new insights into TLR signaling regulation.

SOCS1-deficient dendritic cells are also hyper-responsive to IFN- γ and IL-4 (ref. 37). Dendritic cells from mice in which SOCS1 expression has been restored in T and B cells on a *Socs1*^{-/-} background (*Socs1*^{-/-} transgenic mice) accumulate abnormally in the thymus and spleen, where they produce large amounts of the cytokines BAFF (also called BlyS) and APRIL. This induces aberrant expansion of B cells and autoreactive antibody production (Fig. 3a). SOCS1-deficient dendritic cells efficiently stimulate B cell proliferation and immunoglobulin class switching *in vitro*. These results indicate that SOCS1 is essential in normal dendritic cell function and in the suppression of the systemic autoimmunity that develops in *Socs1*^{-/-} transgenic mice³⁷ (Fig. 3a,b).

Socs1^{-/-} dendritic cells may also mediate the onset of other *Socs1*^{-/-} diseases, because SOCS1-deficient dendritic cells not only induce B cell proliferation but also can trigger allogenic T cell expansion³⁷ (Fig. 3a). T cells also produce higher amounts of T_H1 cytokines such as IFN- γ and TNF in response to *Socs1*^{-/-}, rather than to wild-type dendritic cells (M.K., T.H. and A.Y., unpublished data). We conclude that SOCS1 deficiency in both T cells and dendritic cells is important for the development of autoaggressive T cells in *Socs1*^{-/-} disease.

SOCS3 and TLR signal modulation

IL-6 is a proinflammatory cytokine that has a progressive function in many inflammatory diseases, including Crohn disease and rheumatoid arthritis, 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 (ref. 69), it has not been clarified how these two cytokines have such opposite

functions. In macrophages, SOCS3 has been shown to regulate the divergent action of these two cytokines. In macrophages lacking *Socs3* or carrying a mutation in the SOCS3-binding site (Y759F) of gp130, not only IL-10, but also IL-6, suppresses LPS-induced TNF production⁷⁰. SOCS3 was strongly induced by both IL-6 and IL-10 in the presence of LPS, but selectively inhibited IL-6 signaling by binding the IL-6 receptor gp130 (Y759) but not the IL-10 receptor⁷⁰. Thus SOCS3 selectively blocks IL-6 signaling, interfering with its ability to inhibit LPS signaling. Consistent with this, mice specifically lacking *Socs3* in macrophages and neutrophils are resistant to acute inflammation, as modeled by LPS-induced shock. This phenotype is the complete opposite of that of macrophages in mice with a conditional *Stat3* deletion, which are more sensitive to LPS shock and produce more TNF in response to LPS⁶⁹. A similar 'bipolar' relationship between STAT3 and SOCS3 has been noted in dendritic cells. STAT3-deficient dendritic cells are hyperactivated⁷¹, whereas SOCS3-deficient dendritic cells are less capable of activating T cells than are wild-type dendritic cells (M.K., T.H. and A.Y., unpublished data). Thus, STAT3 prevents macrophage as well as dendritic cell activation, whereas SOCS3 suppresses this activity (Fig. 3b).

Others have shown that IL-6 strongly activates STAT1 and induces the expression of IFN-responsive genes in SOCS3-deficient macrophages, indicating that IL-6 might mimic the action of interferons^{72,73}. These results are seemingly contradictory, but interferons do have some immunosuppressive activities⁷⁴. Thus, IL-6 may induce an interferon-like anti-inflammatory action through STAT1 (and STAT3) activation in the absence of SOCS3. Unfortunately, the physiological function of SOCS3 during inflammation *in vivo* has not been examined well using SOCS3-deficient mice so far^{72,73}. Nevertheless, all studies agree that SOCS3 deficiency induces sustained STAT3 activation in response to IL-6 *in vitro* and *in vivo*. Thus, SOCS3 seems to be a specific negative regulator for gp130-related cytokines *in vivo*.

Defects in SOCS3 expression and function in APCs and T cells may be related to certain immunological diseases. For example, a mouse line with a mutated gp130 (Y759F), to which SOCS3 cannot bind, develop a rheumatoid arthritis-like joint disease with increased production of T_H1-type cytokines and immunoglobulins of the IgG2a and IgG2b subclass⁷⁵. Gastric adenoma has been reported in similar gp130 mutant mice⁷⁶. Whether SOCS3 is important in the development of these diseases needs to be determined.

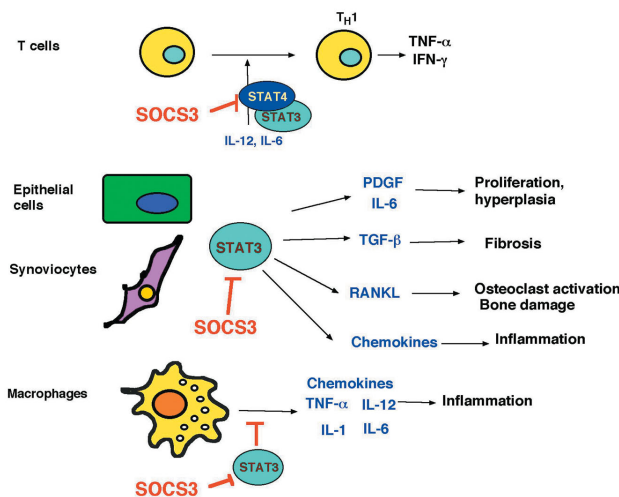


Figure 4 SOCS3 regulates inflammatory diseases by suppressing IL-6-related cytokines. The function of cytokines and main participants in rheumatoid arthritis models is shown. In T cells, SOCS3 inhibits T_H1 skewing by suppressing IL-12 and IL-6 signaling, resulting in suppression of TNF and IFN- γ production. In epithelial cells and synovial fibroblasts, SOCS3 inhibits IL-6-gp130-STAT3 signaling. This results in suppression of tissue hyperplasia by inhibition of autocrine IL-6 production and platelet-derived growth factor (PDGF) production, and the suppression of fibrosis through inhibition of TGF- β production, and production of the receptor activator of NF- κ B ligand (RANKL). Chemokines are also promoters of inflammation. Production of these inflammatory cytokines and chemokines are directly or indirectly stimulated through STAT3. In macrophages, however, SOCS3 functions are anti-inflammatory, as shown in **Figure 3b**.

SOCS3 and inflammatory diseases

In the preceding section, we presented evidence showing SOCS3 functions as a proinflammatory mediator by suppressing IL-6-gp130 signaling in macrophages (**Fig. 3b**). However, in pathological situations, evidence is accumulating that indicates SOCS3 can also suppress inflammatory reactions in which IL-6-related cytokines have an important progressive function. For example, STAT3 activation is found in epithelial and lamina propria cells in the colons of mice with inflammatory bowel disease, as well as in human ulcerative colitis and Crohn disease⁷⁷, and in the synovial fibroblasts of people with rheumatoid arthritis⁷⁸. 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 3–5 and decreased thereafter, whereas SOCS3 expression was induced at day 5 and was maintained⁷⁶. In murine models of inflammatory synovitis, STAT3 phosphorylation also precedes SOCS3 expression⁷⁸. Forced expression of either SOCS3 or a dominant negative form of STAT3 in mouse arthritis models suppresses the induction and/or development of the disease⁷⁸. These are consistent with the idea that the IL-6 and IL-6-related cytokine-mediated STAT3 pathways promote chronic disease progression, and that SOCS3 is part of this negative feedback loop^{77,78}. STAT3 contributes to progression of chronic inflammatory diseases by directly or indirectly inducing cytokine and growth factor production, which promotes tissue hyperplasia, synovial fibroblast proliferation, fibrosis and osteoclast activation (**Fig. 4**). Given the evidence that forced expression of SOCS3 can inhibit IL-6-mediated STAT3 activation, it seems likely that SOCS3 is a negative regulator of inflammatory diseases, especially in diseases associated with high IL-6 production.

Other SOCSs and immunity

Other CIS and SOCS molecules are involved in immune regulation. *Socs5*-transgenic mice have shown that SOCS5 inhibits T_H2 differentiation by inhibiting IL-4 signaling¹⁶. However, because SOCS5-deficient mice are not available, the *in vivo* functions of SOCS5 remain to be clarified. From *in vitro* analysis, CIS is characterized as a specific negative regulator of STAT5. CIS-transgenic mice clearly show that CIS specifically acts as a negative regulator for IL-2 signaling⁷⁹. Further analyses indicate that CIS is also important for NK and NKT cell development in the liver. As IL-15 is important for NK and NKT cell development, these data indicate that CIS also regulates IL-15 signaling. Preliminary data of CIS-deficient mice indicate hyperactivation of T cells in response to IL-2 (M.K., T.H. and A.Y., unpublished data). *In vitro* assays indicate that CIS also has an ability to skew T cell development in favor of T_H2 cells⁷⁹. This phenotype is remarkably similar to that of STAT5a- and/or STAT5b-deficient mice, which is again consistent with a specific function for CIS in the regulation of STAT5-mediated cytokine responses. Unexpectedly, a separate study has shown CIS is also associated with TCR signaling through binding to protein kinase C- θ . In CIS-expressing CD4⁺ T cell-transgenic mice, T cells show enhanced proliferative responses after TCR stimulation *in vitro*⁸⁰. Therefore, the uncharacterized functions of CIS in immunity might be better defined by an analysis of the mice with gene deletions.

Conclusion

SOCS proteins are regulators of cytokine signal transduction and are essential for normal immune physiology, but also seem to contribute to the development immunological disorders. Further understanding of the actions of SOCS proteins in immunity, as well as their applications for therapeutics and drug discovery, will depend on defining the physiological functions of each SOCS protein and the relative interaction affinities of specific SOCS partner proteins *in vivo*. In general, SOCS1 suppresses STAT1 and SOCS3 suppresses STAT3. STAT1 activates macrophages and dendritic cells, whereas STAT3 suppresses them; thus, SOCS1 is a negative and SOCS3 is a positive regulator of APCs. The molecular mechanism of APC regulation by STAT and SOCS, in addition to T cell regulation, is mostly unknown but represents a previously unknown regulatory pathway of T cell regulation by cytokine signaling. More insight into how the positive and negative regulatory pathways of the immune system are balanced will undoubtedly lead to a better understanding of inflammatory and autoimmune diseases and could lead to the development of new therapeutic strategies for these diseases.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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