Abstract. Suppressors of cytokine signaling (SOCS) proteins have been identified as important mediators of negative regulatory circuits within cytokine receptor signaling. They are induced upon stimulation by an increasing set of cytokines as well as further immunological stimuli and are capable to inhibit Janus kinases and signal transducer and activator of transcription signaling. Inhibition is mediated by interfering directly with signal transduction at the receptor as well as targeting of associated molecules for proteosomal degradation. Targeted gene deletion approaches have revealed the importance of SOCS mediated termination of cytokine signaling during normal cellular activation. In addition to their function as classical feedback inhibitors SOCS proteins display a broad panel of inhibitory activity thereby mediating cross-talk modulation between different stimuli. The consequences for regulation of innate and adaptive immune responses are thus obvious. Finally, there are emerging data showing involvement of SOCS proteins in various immune diseases. Modulating SOCS activity could be a promising new approach for molecular therapeutic strategies.

Key words: cytokine receptor signaling; immune system; inhibition; suppressors of cytokine signaling (SOCS).

Introduction

The physiological function of the immune system is the defense against invading foreign organisms and pathogens and the maintenance of the organism’s integrity. Therefore the immune system makes use of specialized cells as well as soluble factors. In functional terms the immune system can be divided into an innate and an adaptive limb. While innate immunity serves as the earliest first line defense against pathogens and mainly recognizes conserved microbial structures, the adaptive immune system represents a system of highly specific recognition molecules and effector functions. The activation of the latter is a tightly organized process involving multiple steps including clonal expansion which in contrast to the innate immune defense takes a longer time. However, adaptive immunity possesses the feature of immunological memory thus enabling more efficient defense reactions upon repeated microbial encounters.

As both systems rely on different cells and as both systems are not organized solely within localized organs the intercellular communication via cell contacts and especially via soluble factors is of highest importance for the function of the system in general. Thus, cytokines and their receptors as mediators of cellular communication have been studied intensively throughout the last years. Although the receptors belonging to the superfamily of cytokine receptors lack intrinsic kinase activity, binding of the respective li-
gands induces tyrosine phosphorylation of various cellular proteins. This is due to the association of intracellular portions of cytokine receptors with cytoplasmatic tyrosine kinases. Janus kinases (JAKs) have been shown to play a critical role in this setting. As a general mechanism binding of cytokines to their receptors leads to oligomerization of the receptor chains. This in turn brings receptor bound JAKs into close proximity resulting in cross-phosphorylation of tyrosine residues within JAKs as well as the receptor itself. Tyrosine phosphorylated receptor chains now associate with proteins of the family of signal transducers and activators of transcription (STATs) via src-homology 2 (SH2) domains. STATs are phosphorylated through JAKs and subsequently dimerize and translocate into the nucleus where they initiate transcription. In addition to STAT activation further cellular signaling pathways as for example mitogen activated protein kinases (MAPK) and phosphatidylinositol 3 kinase are activated. However, studies using genetic deletion approaches have revealed an essential role of JAK or STAT molecules in cytokine receptor signaling.

Although activation of cytokine receptors only transiently activates JAKs and STATs the mechanisms which are involved in negative regulation have just recently begun to be exploited in detail. Negative feedback mechanism involve receptor internalization and dephosphorylation of signaling molecules through cellular phosphatases. Furthermore, protein inhibitor of activated STAT proteins specifically interact with STATs and inhibit DNA binding. A few years ago another family of negative regulators has been discovered which are called suppressors of cytokine signaling (SOCS) and which have now been shown to facilitate termination of cytokine receptor signaling. These proteins are induced upon activation of JAK-STAT signaling through a wide variety of cytokines and in turn inhibit further signaling via the JAK-STAT pathway. They therefore represent a prototypical intra-cellular negative feedback mechanism. In contrast to the former inhibitors SOCS are not or only marginally expressed in resting cells and are readily up-regulated upon cellular activation induced by JAK-STAT cascades. The significance of SOCS for regulation of innate and adaptive immune responses is just now beginning to be considered and is focus of this review.

The SOCS Family

There are eight SOCS proteins identified by date. Due to the initial discovery of SOCS1 by independent groups further nomenclatures exist. However, in this review we will refer to the proteins as SOCS because this nomenclature is the most widespread and we will only here shortly mention the additional names. The SOCS protein family consists of cytokine-inducible SH2 containing protein (CIS or CIS1), SOCS1 (JAK-binding protein, STAT-induced STAT inhibitor 1 – SS1), SOCS2 (CIS2, SS12), SOCS3 (CIS3, SS13), SOCS4 (CIS7), SOCS5 (CIS6), SOCS6 (CIS4) and SOCS7 (CIS5, Nck-Ash and phospholipase-C binding protein). All of the family members are characterized by a central SH2 domain which is able to bind to phosphorylated tyrosine residues. Furthermore SOCS proteins contain a carboxy-terminal 40 amino acid motif which is called SOCS box. The amino terminus varies considerably between the different family members. In addition SOCS1 and SOCS3 contain a kinase inhibitory region (KIR) just amino-terminal to the SH2 box which confers JAK inhibitory activity. The SOCS box itself is also found in further protein families as has been shown for proteins also containing WD-40 repeats, SPRY domains, ankyrin repeats and some small GTPases. SOCS1-3 and CIS which are beginning to be studied for their potential role in regulating immune responses are encoded by genes which are small in size and which contain only few introns (SOCS1, SOCS3: one; CIS, SOCS2: two). Thus these SOCS proteins bear characteristics of immediate-early genes which are rapidly inducible.

Mode of Action of SOCS

SOCS proteins as mentioned above contain protein domains which contribute to the proposed mechanisms of inhibiting cytokine receptor signaling. In general, SOCS proteins play an important role as classical feedback inhibitors. Thus, SOCS are rapidly induced upon cellular stimulation with cytokines and then negatively regulate further cytokine signaling. Indeed, STAT-response elements have been found in the promoter regions of CIS, SOCS1 and SOCS3. Deletion of the STAT1/STAT3 element in the murine SOCS3 promoter resulted in abrogated responses towards leukaemia inhibitory factor in reporter gene assays. In parallel inhibition of JAK/STAT signaling either by genetic approaches or in vitro studies also resulted in loss of SOCS induction.

An additional mode of regulation has been reported for SOCS1. It has been shown that SOCS1 is also regulated by translational repression. This was mediated by the presence of additional start codons upstream in
the 5' untranslated region and probably contributes to minimize endogenous levels of SOCS1 in unaffected cells. Concerning the mode of inhibition of JAK/STAT signaling there are differences between the family members. SOCS1 has been shown to bind directly to JAKs and to inhibit the kinase activity. Binding involved interactions of the SOCS' SH2 domain with Y1007 within the activation loop of JAK2, however in addition the KIR within SOCS1 was necessary to confer complete inhibition. The KIR may function as a pseudosubstrate and bind to the catalytic site of JAKs. In parallel SOCS3 also is able to inhibit kinase activity of JAKs. Therefore SOCS3 also makes use of the KIR. However, SOCS3 only weakly binds to JAKs themselves but interacts with phosphorylated tyrosine residues within the cytokine receptor chains. Thus, SOCS3 also inhibits JAK activity in a manner analogous to SOCS1, yet the recruitment towards the receptor-kinase complex differs. In addition it has been shown that SOCS3 is able to bind to cytokine receptors within docking sites for the signaling molecule SH2-domain containing protein tyrosine phosphatase 2 (SHP2). As a consequence MAPK signaling via SHP2 in glycoprotein 130 (gp130) mediated signal transduction is inhibited. Interestingly, it has been reported recently that SOCS3 itself can be phosphorylated and that this leads to the interaction with RasGAP resulting in enhanced Ras signaling after interleukin 2 (IL-2) triggering. Tyrosine phosphorylation did not alter the ability of SOCS3 to inhibit STAT signaling. Thus, inducible modifications of SOCS3 dissected IL-2 receptor (IL-2R) signaling with positive as well as negative regulatory effects. Underlining the findings that SOCS proteins may be able not only to exert inhibitory effects it has been shown that SOCS2 expression differentially modulates some genes having inhibitory functions at low levels of expression but strikingly increases cytokine signaling at higher expression levels. Thus, SOCS2 not only is an inhibitor but can have opposing effects.

In contrast neither SOCS2 nor CIS are able to inhibit JAKs. These molecules however rely on STAT competition by binding to activated receptors. Binding occurs via interactions of the SH2 domain with phosphotyrosine residues within the receptor chains and results in an inhibition of STAT recruitment.

Finally the SOCS box is thought to contribute to the inhibition of cytokine receptor signaling by SOCS proteins. It has been shown that the SOCS box interacts with elongins B and C which are participants of the E3 ubiquitin ligase complex. This in turn leads to ubiquitinylation and proteasomal degradation of SOCS and probably the bound receptor complexes. Indeed inhibition through SOCS proteins was diminished by usage of proteasome inhibitors. In addition, interaction of CIS with the erythropoietin receptor resulted in degradation of the complex. Furthermore, SOCS1 mediated inhibition of activated translocated ets leukemia (TEL)-JAK fusion proteins which are found in some leukaemias was also dependent on proteasomal degradation. However it has also been shown that proteasomal degradation directly affects JAK2 and that SOCS1 mediated downregulation of JAK2 was dependent on JAK2 degradation. Recently, the importance of the SOCS box has been addressed by targeted deletion. Mice lacking the SOCS box of SOCS1 showed increased responsiveness to interferon γ (IFN-γ) and slowly developed an inflammatory disease leading to death. The disease resembled that of SOCS1−/− mice albeit with delayed kinetics and slightly milder symptoms. In functional terms it could be shown that IFN-γ caused a prolonged activation of STAT1.

Conversely, it has been reported that the Pim serine/threonine kinases family can phosphorylate SOCS1 which subsequently decreases the binding between SOCS1 and elongin BC. Pim phosphorylation of SOCS1 resulted in a prolonged half-life and increased inhibition of IL-4 signaling. It has also been suggested that the interaction of the SOCS box with Elongin BC complex increases the stability of SOCS thereby enhancing inhibitory effects.

Genetic Models

Initially it was thought that SOCS proteins show a tissue or cytokine specificity, however it is now clear that no such clear specificity exists. Multiple cytokines induce a set of different SOCS proteins and in turn the distinct SOCS proteins are able to inhibit a bewildering variety of different cytokines. In vitro studies have made their substantial contribution to define the potential of different SOCS for their inhibitory activities, yet it is to genetic models to assess the biological valence of the distinct family members.

In this respect SOCS1−/− mice have prototypically revealed that although the ability of SOCS1 to inhibit cytokines is widespread, the lack of inhibition of IFN-γ is most prominent. Thus, SOCS1−/− mice die within three weeks after birth due to an fatal inflammatory disease. Fatty liver degeneration and multiple infiltrations of organs with hematopoietic cells are observed. In addition lymphopenia through accelerated apoptosis via Bax and aberrant T cell activation are
reported\textsuperscript{61}. The disease resembles that of IFN-\(\gamma\) administration in neonatal mice and indeed mice deficient for SOCS1 as well as IFN-\(\gamma\) show decreased pathology and no neonatal lethality anymore\textsuperscript{5, 61}. However, these mice eventually succumb to death at later time points with a more complex inflammatory syndrome and polycystic kidneys\textsuperscript{67}. Disease in SOCS1\textsuperscript{\textminus/\textminus} mice is due to increased sensitivity to IFN-\(\gamma\) as well as elevated levels of this cytokine. This results in an increased capacity of SOCS1\textsuperscript{\textminus/\textminus} macrophages to cope with viral infections and clearing of \textit{Leishmania}\textsuperscript{6}.

It is now also clear that the disease of SOCS1\textsuperscript{\textminus/\textminus} mice is dependent on lymphoid cells as it can be transferred by transplantation of hematopoietic tissue in recombination-activating gene 2 (Rag2) deficient recipients\textsuperscript{61}. Moreover depletion of natural killer (NK) cells and natural killer T (NKT) cells resulted in diminished hepatotoxicity in SOCS1\textsuperscript{\textminus/\textminus} mice\textsuperscript{53}. T cell targeted overexpression of SOCS1 led to the inhibition of multiple cytokine signaling and resulted in a disturbed T cell development with increase in CD4\textsuperscript{+} cells and spontaneous T cell activation\textsuperscript{37}. The observations strikingly resemble the phenotype of common \(\gamma\) chain or JAK3 deficient mice.

However, observations that double deficient mice for SOCS1 and STAT6 which mediates IL-4 signaling also show an altered onset of disease indicate that despite of the predominant role of SOCS1 for IFN-\(\gamma\) regulation further cytokines are also regulated aberrantly\textsuperscript{73}. SOCS2 gene deficient mice show gigantism with deregulated growth hormone and insulin-like growth factor 1 signaling\textsuperscript{66}. SOCS3\textsuperscript{\textminus/\textminus} mice are embryonic lethal\textsuperscript{60}. Initially it was reported that a marked erythrocytosis causes lethality, but now placental insufficiency has also been shown. Overexpression also resulted in embryonic lethality with anemia. However SOCS3 deficient fetal liver cells were able to reconstitute hematopoiesis in lethally irradiated adults and JAK3 deficient mice\textsuperscript{60}. Due to the early death of SOCS3\textsuperscript{\textminus/\textminus} mice during mid-gestation no further studies concerning a role of SOCS3 in regulation of immune responses have been performed \textit{in vivo}. On the other hand these results clearly indicate the importance of SOCS3 and in addition show that early cytokine signaling defects markedly alter the phenotype, a fact that has also to be obeyed when interpreting the phenotypes of other SOCS deficient mice.

Genetic deletion of CIS did not show any abnormalities. In contrast overexpression resulted in a reduction of the number of \(\gamma\)\(\delta\) T cells, NK cells and NKT cells\textsuperscript{64}. In addition IL-2 signaling involving STAT5 was inhibited. Together with alterations within the liver and lactation failure the disease has similarities with disease in STAT5a or STAT5b deficient mice. Therefore CIS has been assigned to play a special role in STAT5 signaling.

Recently SOCS5 transgenic mice have been generated and showed impaired IL-4 mediated T helper cell 2 (Th2) development, possibly through the unconventional association of SOCS5 with the IL-4R\(\alpha\) chain irrespective of tyrosine phosphorylation\textsuperscript{90}.

Taken together the experiments with genetically altered mice have gained valuable information concerning specific roles of the single SOCS proteins. However it has to be considered that mice genetically engineered always had alterations within multiple cytokines and that the clear phenotypes with inhibition of specific cytokines possibly mask other important effects especially during immunological challenges. In addition altered cytokine signaling during development of the mice can shift the phenotypes in distinct directions.

**Feedback and Cross-Talk Inhibition**

SOCS proteins have been reported to be induced by a huge variety of different cytokines. In contrast to earlier speculations it is now well accepted that no specificity between different SOCS family members and the inhibition of certain cytokines exists. Indeed there is an increasing number of reports showing the induction of SOCS proteins by many different cytokines in various tissues and cells (Table 1). Concerning the mode of action two modes are operative (Fig. 1). Firstly, cytokine triggering can lead to the induction of various SOCS proteins which in turn inhibit the respective signaling pathway and this is referred to as classical negative feedback inhibition. Corroborating this mode of action STAT responsive elements have been found in the promoter regions of CIS, SOCS1 and SOCS3\textsuperscript{6}. Also interferon regulatory factor 1 which is activated in response to IFN-\(\gamma\) is able to bind to the SOCS1 promoter\textsuperscript{83}. However, in a second way SOCS induction through a defined trigger subsequently leads to the inhibition of other cytokine signaling which is called cross-talk inhibition. In general, inhibition of further cytokines also affects JAK/STAT signaling, but recently it was also reported that possibly the inhibitory potential of SOCS proteins extends classical JAK/STAT signaling pathways (see below). As SOCS do not display a specificity for defined cytokines the fact of cross-talk inhibition is not entirely surprising, yet has important implications for the biological valence of...
Concerning the mode of induction LPS and proteins are key mediators in these negative regulatory
hibited IFN-γ to induce a set of various SOCS proteins CpG-DNA as well as IL-1 administration were reported apparently unrelated signaling pathways and that SOCS pathways different than JAK/STAT signaling were able to induce SOCS proteins. Thus, Toll-like receptor (TLR) triggering via lipopolysaccharide (LPS) and CpG-DNA as well as IL-1 administration were reported to induce a set of various SOCS proteins. Furthermore tumor necrosis factor α (TNF-α) and direct activation of MAPK via PMA also were able to increase the expression of SOCS proteins. SOCS proteins induced via these pathways were functional as they inhibited IFN-γ granulocyte-macrophage colony stimulating factor (GM-CSF) or IL-6 signaling. In addition SOCS3 has been reported to inhibit CXC chemokine receptor-4 signaling. These observations clearly indicate that cross-talk inhibition can also occur by apparently unrelated signaling pathways and that SOCS proteins are key mediators in these negative regulatory circuits. Concerning the mode of induction LPS and CpG-DNA were able to induce SOCS1, SOCS3 and CIS independent of intermediate protein synthesis which implicates a direct induction. In contrast other groups reported the induction of SOCS via autocrine or paracrine cellular activation after TNF-α administration or stimulation with Listeria monocytogenes. In these situations type I IFNs played a crucial role. However autocrine stimulation via type I IFNs was not operative after TLR induced SOCS expression in macrophages. Probably, depending on the experimental setting, direct as well as indirect effects contribute to SOCS induction via TLRs, IL-1 and TNF-α. In addition it has also been reported that IL-10 can induce SOCS3 independent of STATs in human neutrophils.

Most strikingly two groups recently showed that SOCS induction through LPS directly inhibited LPS signaling and contributes to so called LPS tolerance. Thus, SOCS1 deficient mice showed increased responses to LPS and furthermore co-transfection of SOCS1 and nuclear factor κB reporter constructs showed in-

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**Table 1. Induction and activity of SOCS proteins**

<table>
<thead>
<tr>
<th>Type and II receptor cytokines (JAK-STAT dependent)</th>
<th>TIR domain-containing receptor ligands, further stimulii</th>
<th>Signaling pathways that are sensitive to SOCS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CIS</strong></td>
<td>IL-2, IL-3, GM-CSF, EPO, GH, LH, PRL, IL-6, IL-13, IFN-γ, TPO</td>
<td>TNF-α, IL-1, TCR stimulation, LPS, Cpg-DNA, SCF, leptin, insulin</td>
</tr>
<tr>
<td><strong>SOCS 1</strong></td>
<td>IL-2, IL-3, GM-CSF, IFN-γ, IFN-β, LH, PRL, IL-6, IL-13, IFN-γ, IL-1, EPO, PRL</td>
<td>LPS, Cpg-DNA, SCF, leptin, insulin, IL-1, leptin, IL-10, CNTF</td>
</tr>
<tr>
<td><strong>SOCS 2</strong></td>
<td>GH, IL-3, IL-6, IFN-γ, EPO, PRL, IL-10, IL-2, CNTF, LPS</td>
<td>IL-1, IL-2, IL-4, IL-13, IL-16, IL-22, LIF, oncostatin M</td>
</tr>
<tr>
<td><strong>SOCS 3</strong></td>
<td>EPO, GM-CSF, IL-2, IL-3, IL-4, IL-6, IL-13, IFN-γ, IL-2, IL-6, IL-13, IFN-γ, IL-1, IL-6, IL-10, IL-11, IL-15, IL-22, LIF, PRL, IL-6, IL-7, leptin, LIF, insulin</td>
<td></td>
</tr>
<tr>
<td><strong>SOCS 4</strong></td>
<td>EPO, GM-CSF, IL-2, IL-3, IL-4, IL-6, IL-13, IFN-γ, IL-2, IL-6, IL-13, IFN-γ, IL-1, IL-6, IL-10, IL-11, IL-15, IL-22, LIF, PRL, IL-6, IL-7, leptin, LIF, insulin</td>
<td></td>
</tr>
<tr>
<td><strong>SOCS 5</strong></td>
<td>IL-2, EPO, GH</td>
<td>IL-1, IL-2, IL-4, IL-13, IL-16, IL-22, LIF, oncostatin M</td>
</tr>
<tr>
<td><strong>SOCS 6</strong></td>
<td>IL-6</td>
<td>EPO, GH</td>
</tr>
<tr>
<td><strong>SOCS 7</strong></td>
<td>IL-6, PRL, GH</td>
<td>IL-1</td>
</tr>
</tbody>
</table>

hibitory potential. Moreover it has been suggested that SOCS3 contributes in mediating the down-regulatory effects of IL-10 on LPS activation of macrophages. These results would broaden the potential targets of SOCS action to JAK/STAT independent signaling pathways. However, proofs for direct inhibition of signaling molecules within the TLR pathway through SOCS are still elusive. Only one group was able to show immuno-precipitation of SOCS1 with the TLR signaling molecule IL-1R-associated kinase but not with MyD88. Thus it remains still possible that SOCS mediated inhibition of TLR signaling is due to the inhibition of JAK/STAT dependent autocrine and paracrine amplification loops. Such a mechanism has been reported for example for type I IFNs which are secreted rapidly and which increase the cell’s sensitivity towards further TLR triggering. In parallel this signal is sensitive to inhibition through SOCS. Nevertheless, the fact of increased responses towards LPS in SOCS1 deficient mice clearly indicates a more complex potential of SOCS proteins in regulating the network of intracellular signal transduction mediators.

Role of SOCS in Innate Immunity

As mentioned above the role of SOCS in negative regulation of cytokines is clearly established. Moreover there is increasing evidence arguing for a broader role of SOCS even in the down-regulation of TLR signaling. Taken together this indicates a role of SOCS proteins in the regulation of innate immune responses. Innate immunity recognizes microbial pathogens through the recognition of conserved pathogen associated molecular patterns (PAMP) via pattern recognition receptors (PRR). This enables the system to recognize the whole complement of pathogens by a limited set of receptors. PRRs comprise soluble components like the complement system as well as cellular receptors (e.g. mannose receptor, TLRs, peptidoglycan recognition proteins). Activation of these receptors in general results in the induction of an anti-microbial response including soluble effector mechanisms like secretion of cytokines, anti-microbial peptides or radicals as well as cellular components including phagocytosis and increased antigen presentation. However, activation is
also followed by negative-regulatory events possibly to avoid overshooting reactions. In this respect LPS tolerance, standing for the inability of cells to respond similarly to a subsequent secondary stimulus of LPS, is a well known phenomenon. In this setting it has now been proposed that SOCS proteins participate in this effect. Indeed SOCS1 deficient mice showed impaired LPS tolerance. Given the fact that SOCS1 is inducible by TLR signals this suggests a role of SOCS within regulation of prototypical TLR signaling. In addition the generation of an orchestrated anti-microbial innate immune response requires the spatio-temporal interaction and activation of multiple cells. In this respect IFN-γ plays a crucial role as activator of macrophages to increase their anti-microbial capacity (IFN-γ mediated priming). This in turn is a process that is regulated via SOCS proteins and a tight regulation is indispensable. Indeed SOCS1 deficient mice have multi-cellular infiltrations especially of macrophages in various organs. Interestingly, SOCS1 induction by IFN-γ is slightly differently regulated from other STAT dependent effector molecules. Thus it has been reported that sub-threshold concentrations of IFN-γ increased sensitivity to subsequent IFN-γ stimulation in macrophages (sensitization) by elevating STAT1 levels. However these concentrations were not able to mount an ongoing SOCS1 feedback inhibition which indicates that fine-tuning of the innate immune systems can occur by dissecting positive and negative regulatory effects.

SOCS proteins seem to contribute to the mounting of an appropriate innate immune response by tightly regulating the activation status. During a physiological response the temporal and spatial development assures the contemporaneous presence of un-activated, primed, activated and tolerized macrophages and dendritic cells. However during sepsis this process could be disturbed and deregulated. During the initial phase of sepsis a synchronized activation of a huge and almost complete fraction of innate immune cells due to the high amounts of pathogens and their PAMPs may lead to an initially overshooting reaction which is then followed by tolerization and subsequent functional anergy. It is well possible that SOCS induction participates in the pathogenesis of sepsis as SOCS proteins could be an internal mediator of this anergic stage. Indeed it has been shown that SOCS induction through bacterial components is able to inhibit further signaling and to prevent activation of these cells by IFN-γ, IL-6 or GM-CSF. Thus, modulating SOCS activity could represent a novel approach for treating sepsis.

**Regulation of Adaptive Immunity through SOCS**

The important role of SOCS1 in lymphocytes has been demonstrated by the observations that hematopoietic stem cell transplantation was capable to transfer the disease seen in SOCS1−/− mice into Rag2−/− mice. In addition, SOCS1−/− Rag2−/− double deficient mice lacked the neonatal disease. T cell restricted SOCS1 over-expression revealed a role for thymocyte development and peripheral activation as transgenic mice showed a block during the triple-negative stage within the thymus and spontaneous peripheral T cell apoptosis. Also, retro-viral mediated over-expression of SOCS1 in fetal liver-derived hematopoietic progenitors prevented progression within T cell development and abrogated pre-T cell receptor induced proliferation. As SOCS1 is an important negative regulator of IFN-γ and as T lymphocytes are sensitive towards action of this cytokine it is consistent that T cell development is disturbed in SOCS1 deficient mice. However observations that T cell development remains disturbed in SOCS1−/− IFN-γ−/− mice which otherwise survive the neonatal stage indicate a possibly more broadened role of SOCS1 within T lymphocytes. Accordingly, defects are not merely a function of increased and prolonged IFN-γ signaling. SOCS1 deficiency results in altered T cell development as well as aberrant activation with blast morphology and increased expression of activation markers. In addition it has been shown that while normal mice require both IL-2 and T cell receptor (TCR) activation for proliferation of spleen cells in SOCS1 deficient mice IL-2 alone is sufficient. Furthermore thymocytes from SOCS1 deficient mice showed increased proliferation to IL-4 and this phenotype was reduced in STAT6−/− mice. Regarding the role of further SOCS family members the knowledge of modulating adaptive immunity is less. CIS transgenic mice have been shown to skew immune responses towards a Th2 type due to diminished IL-2 signaling. Interestingly, one report indicates a role for CIS in positive regulation of TCR mediated MAPK activation, possibly through interaction with protein kinase C-θ. Thus transgenic expression of CIS in CD4+ T cells increased TCR signaling. In contrast SOCS1 has been shown to inhibit nuclear factor of activated T cells (NFAT) activation in 293T cells expressing CD8 and Syk. Moreover, also SOCS3 is induced upon TCR ligation and SOCS3 delivery by retrovirus is able to inhibit NFAT mediated IL-2 production in primary murine T cells. Taken together it is highly probable...
that SOCS proteins also participate notably in direct positive and negative modulation of TCR signaling.

The above findings also indicate a possible role of SOCS proteins for regulation of Th1 and Th2 development. However it has to be distinguished whether different SOCS induction profiles primarily contribute to Th differentiation or merely influence cytokine signaling. In this respect initial reports suggested a specific role of SOCS1 in the inhibition of Th1 development by IL-6\textsuperscript{26}. IL-6 induced SOCS1, thereby mediating negative regulation of IFN-\(\gamma\) signaling and Th1 development. In contrast SOCS1 induction after IL-6 did not influence IL-4 signaling and Th2 development. Moreover in SOCS1\textsuperscript{−/−} CD4\textsuperscript{+} cells administration of IL-6 failed to inhibit the activities of IFN-\(\gamma\) and subsequent Th1 differentiation\textsuperscript{26}. However, observations that neonatal lethality as well as thymic and splenic atrophy and T cell activation are less severe in STAT1\textsuperscript{−/−} SOCS1\textsuperscript{−/−} and STAT6\textsuperscript{−/−} SOCS1\textsuperscript{−/−} double knockout mice indicate that SOCS1 participates in IFN-\(\gamma\) as well as IL-4 signaling respectively\textsuperscript{73}. Furthermore this report also clearly shows a missing cross-talk inhibition of IFN-\(\gamma\) and IL-4 under SOCS1 deficiency. Thus, SOCS1 does not exclusively mediate inhibition of Th1 signaling. In contrast another report showed an inhibitory role of SOCS1 in IL-4 induced epsilon germline promoter activation after IFN-\(\gamma\) stimulation \textit{in vitro} and thus linked SOCS1 expression with inhibitory effects on Th2 development\textsuperscript{105}. In parallel IFN-mediated IL-4 repression via SOCS1 has also been observed in human monocytes\textsuperscript{75}. Striking differences in SOCS1 and SOCS3 expression during Th1/Th2 development have been reported. Thus, Th2 expressed high levels of SOCS3 and inhibited IL-12 mediated STAT4 activation while Th1 had higher levels of SOCS1 and inhibited IL-4 mediated STAT6 activation\textsuperscript{25}. Recently, SOCS5 was reported to be exclusively detectable in Th1 and SOCS5 associated with IL-4R\(\alpha\) chain independently of tyrosine phosphorylation thus inhibiting IL-4 signaling\textsuperscript{80}. T cells from mice transgenic for SOCS5 had impaired IL-4 mediated Th2 development.

However, it has recently been shown that SOCS1 can inhibit IL-4\textsuperscript{41, 58} \textit{in vivo} as well as IL-12\textsuperscript{31} signaling \textit{in vitro}. Corroborating these findings, it has been reported that SOCS1\textsuperscript{−/−} CD4\textsuperscript{+} T cells upon challenge with anti-CD3 produce higher amounts of IFN-\(\gamma\) and IL-4\textsuperscript{38}. Apparently these T cells had already differentiated into Th1 as well as Th2 \textit{in vivo}. In parallel infections with \textit{Listeria monocytogenes} and \textit{Nipponstrongyulus brasiliensis} led to increased amounts of IFN-\(\gamma\) and IL-4. The effects were mediated by a failure to terminate IL-12 dependent STAT4 or IL-4 induced STAT6 signaling.

Overall these results indicate a general aberrant responsiveness of SOCS1 deficient mice in terms of Th1/Th2 differentiation.

Although the number of B lymphocytes is also reduced in SOCS1 deficient mice by now the exact mechanism has not been elucidated. Apparently no specific defect within B cells is operative, yet it seems that increased apoptosis is due to the prolonged action of IFN-\(\gamma\).

Another important aspect of SOCS within adaptive immunity is the modulation of communication with innate immunity. IFN-\(\gamma\) represents a most important positive activating signal for innate immune cells and in turn multiple cytokines evolved from innate immunity modulate adaptive immune responses. Temporal control of cytokine actions via induction of SOCS clearly interferes with the cell’s subsequent responsiveness to further stimulation. Thus SOCS could play an important role as intracellular short-term memory of recent stimulation. In the setting of intracellular communications it is immediately obvious that temporal control of cytokines can be mediated by such intracellular regulators. Interfering within these communicatory circuits by modulating SOCS could be a promising new approach for immune intervention.

**Role of SOCS in Diseases of the Immune System**

Our understanding of the role of SOCS proteins in various diseases of the immune system as well as possible therapeutic potential of modulating SOCS is still at the beginning. However given the fact that SOCS proteins are able to inhibit a wide variety of different cytokines this implies an important role within immune diseases. First studies now have shown that SOCS3 expression is elevated in human ulcerative colitis and an animal model of colitis\textsuperscript{80}. These diseases were associated with increased STAT3 activation. Inhibition of the activity of SOCS1 and SOCS3 through introduction of a mutant SOCS resulted in a more severe experimental disease in mice. Also, increased expression of SOCS1-3 in the epidermis from patients with psoriasis and allergic contact dermatitis has been observed underlining the role of SOCS in pro-inflammatory situations\textsuperscript{35}. Over-expression of SOCS1 or SOCS3 in keratinocytes reduced IFN-\(\gamma\) mediated STAT activation and expression of HLA-DR as well as intercellular adhesion molecule 1. However reports evaluating the potential of modulating SOCS activity are still erratic. In this respect it has been shown that in patients with rheumatoid arthritis in which STAT3 dependent
signaling plays a crucial role elevated SOCS3 can be observed. In experimental models of antigen- and collagen-induced arthritis adenoviral gene transfer of SOCS3 was able to mitigate the proliferative responses of synovial fibroblast and IL-6 secretion in vitro. Moreover onset and progression of the disease were altered. In parallel studies using mice with deleted STAT-binding sites in gp130 lacked STAT activation yet showed no alterations in SHP2-Ras-ERK activation. These mice had a severe joint disease which was attributable to sustained SHP2-Ras-ERK signaling due to impaired STAT mediated SOCS1 induction.

Finally, hematopoietic malignancies can be associated with increased or constitutively activated JAK/STAT signaling. In this respect the inhibition of leukemia associated TEL-JAK fusion protein mediated kinase activity and cell growth through SOCS1 indicates a potential benefit of evaluating SOCS within these kind of diseases. Also, lack of IFN responsiveness during treatment of some subsets of patients with hepatitis C virus and chronic myelogenous leukemia (CML) has been proposed to be potentially caused by differing individual levels of SOCS. Indeed blasts of patients with progressing CML had high levels of SOCS and forced SOCS3 expression in otherwise sensitive CML cell lines abrogated IFN-α responsiveness. The full complexity and therapeutic potential of modulating SOCS however is still elusive.

**Concluding Remarks**

Negative regulation of cytokine signaling under normal as well as pathologic conditions has gained increasing interest. SOCS proteins have been identified as inducible negative regulators of JAK/STAT dependent cytokine signaling. SOCS proteins firstly act as classical negative feedback inhibitors. However, secondly the panel of cytokines being sensitive to SOCS inhibition is very broad and thus these proteins in addition are capable of mediating cross-talk inhibition between different cytokine signaling pathways. Moreover it has been established that SOCS can also be induced by signals different from classical JAK/STAT signaling pathways. As a consequence SOCS proteins are important intracellular regulators of the cell’s responsiveness towards a variety of extracellular signals. This in turn has implications on regulation of innate as well as adaptive immune responses. Manipulating SOCS activity is a promising new approach for therapy and modulation of various inflammatory diseases and is just at the beginning of being deciphered.

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