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Review

The suppressors of cytokine signalling (SOCS)

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Abstract. Members of the SOCS (suppressor of cytokine signalling) family of proteins play key roles in the negative regulation of cytokine signal transduction. A series of elegant biochemical and molecular biological studies has revealed that these proteins act in a negative feedback loop, inhibiting the cytokine-activated Janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling pathway to modulate cellular responses. Although structurally related, the precise mechanisms of SOCS-1, SOCS-3 and cytokine-inducible SH2-containing protein (CIS) action vary. Direct interaction of SOCS SH2 domains with the JAK kinases or cytokine receptors allows their recruitment to the signalling complex, where they inhibit JAK catalytic activity or block access of the STATs to receptor binding sites. The defining feature of the family, the C-terminal SOCS box domain, appears dispensable for these actions but is likely to play a key role in negative regulation of signalling by targeting molecules associated with the SOCS proteins for degradation. The relevance of SOCS-mediated regulation of cytokine responses has been brought into sharp focus by the dramatic phenotypes of mice lacking these regulators. Indispensable roles for members of this family have been identified in the regulation of interferon γ, growth hormone and erythropoietin, and the absence of SOCS-1 or SOCS-3 is lethal in mice.

Key words. JAK; STAT; SOCS.

Introduction

Four α-helical-bundle cytokines are secreted regulatory proteins that control the survival, growth, differentiation and function of cells. They do so by specifically binding and inducing multimerisation of members of the hemopoietin receptor family, cell surface proteins that lack intrinsic enzymatic activity and are defined by conserved sequence motifs in their extracellular domains [1]. Important transducers of signals from activated hemopoietin receptors are the JAKs, which are brought into close proximity upon receptor multimerisation, leading to cross-phosphorylation and activation [2]. The JAKs subsequently phosphorylate receptor cytoplasmic domains, creating docking sites for the recruitment of cytoplasmic proteins that contain SH2 or other phosphotyrosine-binding motifs, including the signal transducer and activator of transcription (STAT) family of transcription factors. Once bound to the receptor, the STATS are phosphorylated by the JAKS, dimerise and translocate to the nucleus, where they bind specific DNA elements and alter the expression of target genes in response to cytokine stimulation [3–6]. An appropriate cellular response depends not only on receptor/cytokine interaction and the initiation of intracellular signalling, but also on the timely and effective termination or modulation of signalling. In recent years, elucidation of the mechanisms that negatively regulate the JAK/STAT signalling pathway has progressed rapidly. Negative regulation is achieved by receptor downregulation, dephosphorylation of signalling intermediates by cytoplasmic protein tyrosine phos-

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phatases like SHP-1, and by direct inhibition of STAT action by members of the protein inhibitors of activated STAT (PIAS) family of proteins. In addition, members of the SOCS family of proteins appear to be induced by cytokine stimulation to act as negative regulators of signalling in a classical negative feedback loop. This review focuses on the SOCS, their biochemical and biological actions, and their potential function as part of a large family of E3 ubiquitin ligases.

Suppressors of Cytokine Signalling

The cytokine-inducible SH2-containing protein (CIS) was isolated in a screen for immediate-early genes induced by cytokine receptors, and its overexpression was shown to reduce the growth rate of interleukin (IL)3-dependent hemopoietic cell lines, implicating it in the negative regulation of cytokine signalling [7]. Subsequently, suppressor of cytokine signalling-1 (SOCS-1) was cloned independently by three groups, as a molecule that binds the tyrosine kinase JH1 domain of JAK2 [8], cross-reacts with a monoclonal antibody raised against the GTFLLRFS sequence motif found within the SH2 domain of STAT3 [9], and whose expression renders M1 cells refractory to IL-6-induced differentiation [10]. Database searches using SOCS-1 sequences revealed that CIS was highly related to SOCS-1, and that in addition to CIS and SOCS-1, six other members of the family, SOCS-2–7, comprise a novel protein family [10–12]. SOCS family proteins contain N-terminal regions of variable length and amino acid composition possessing no recognisable protein motifs, well-conserved central SH2 domains, and an ~40-amino acid C-terminal region of homology termed the SOCS box.

In addition to identifying CIS and SOCS-1 – SOCS-7, database searches employing a SOCS box consensus sequence revealed expressed squences tags (ESTs) encoding a number of other human and/or murine proteins that also possess C-terminal SOCS boxes [9, 11, 12]. Rather than SH2 domains, however, these proteins contain other recognisable motifs upstream of the SOCS box, and have been classified accordingly: the WD40 repeat-containing proteins with a SOCS box (WSBs), the SPRY domain-containing proteins with a SOCS box (SSBs), the ankyrin repeat-containing proteins with a SOCS box (ASBs), and the GTPase domain and SOCS box-containing proteins (the RAR family) [12] (fig. 1). Whereas chicken WSB-1, also known as cSWIP-1, has been implicated in the Sonic hedgehog signalling cascade [13], it is unclear whether the WSBs or these other families of SOCS box-containing proteins play a role in the negative regulation of signalling pathways or have other biological functions.

The SOCS proteins as inhibitors of cytokine signalling

The model of SOCS action is that of a classical feedback loop, whereby factor-induced activation of signalling pathways induces expression of effector genes, including those encoding SOCS proteins, the latter then functioning to attenuate the signal by inhibiting various components of the signalling pathway [9]. Typically, SOCS genes are not abundantly expressed in resting cell or tissues, but are induced by a wide range of cytokines, hormones and growth factors. The best-characterised members of the family in this respect are CIS, SOCS-1 and SOCS-3. The in vitro data have revealed that CIS expression is induced and can inhibit signalling by erythropoietin (EPO) [14], growth hormone (GH) [15–19], IL-2 [9, 10, 14, 20, 21], IL-3 [10, 14] and prolactin (PRL) [15, 22]. SOCS-1 expression is induced and can inhibit signalling by GH [16, 17, 23], interferons [9, 24, 25], IL-4 [8, 26, 27], IL-6 [8, 9], thrombopoietin (TPO) [9] and PRL [22, 28]. SOCS-3 expression is induced and can inhibit signalling by GH [16, 17, 23, 29], EPO [9, 11], GM-CSF [11], interferons [24, 25], IL-6 [9, 30], leptin [31], leukemia inhibitory factor (LIF) [11, 32, 33] and PRL [22, 28]. Whereas cytokine-induced STAT activation is one mechanism of SOCS induction [8, 29, 34–36], evidence is accumulat-

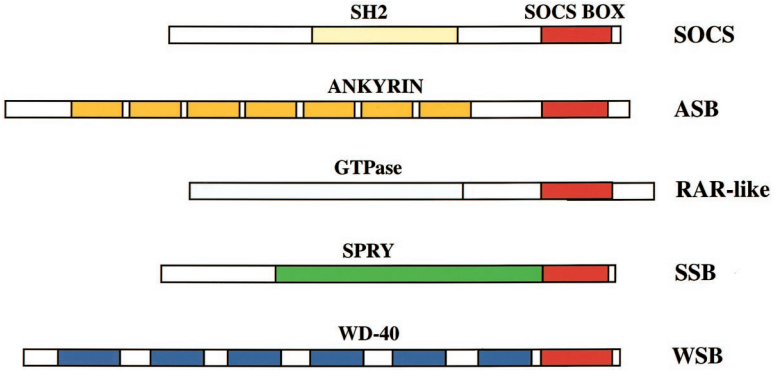

Figure 1. The SOCS box-containing protein families.

ing that SOCS expression can also be induced by stimuli that do not activate STATS, such as lipopolysaccharide (LPS) [37], and that do not signal via JAKs, including insulin and stem cell factor (SCF) [38, 39].

The overexpression data clearly indicate that CIS, SOCS-1 and SOCS-3 can inhibit the JAK/STAT signalling pathway, but their biochemical modes of action appear to differ. SOCS-1 can interact directly with JAK1, JAK2 and Tyk2, and inhibit their phosphorylation and catalytic activity, thereby downregulating JAK-dependent phosphorylation of receptors and STATS [7, 8, 30]. Structure/function studies have indicated that the SH2 domain and N-terminal region of SOCS-1 are essential for inhibiting JAK activity, but that the C-terminal SOCS box is not required [30, 40, 41]. The SH2 domain binds a phosphopeptide derived from the activation loop of JAK2, but high-affinity binding and inhibition of kinase activity requires an additional ~30-amino acids N-terminal of the SH2 domain, which has been termed the kinase inhibitory region (KIR). The KIR has sequence homology with the psuedosubstrate inhibitory region of JAK2, and can bind JAK2 independently, leading to the suggestion that SOCS-1 may inhibit JAK activity via an interaction between the KIR and the JAK catalytic loop which blocks kinase activation [30, 40]. Interestingly, in LIF-stimulation reporter assays in 293T cells, the N-terminal domains of SOCS-1 and SOCS-3 are functionally interchangeable, but not with those of other SOCS proteins, illustrating a functional overlap between SOCS-1 and SOCS-3 in in vitro experiments [30].

Unlike SOCS-1, CIS does not appear to interact directly with JAK kinases. CIS is induced by STAT5 in response to EPO and IL-3 stimulation, and binds EPO receptor and IL-3 receptor β chain in a phosphorylation-dependent manner [10, 14]. Overexpression of CIS inhibits EPO-dependent STAT5 activation, and has been suggested to inhibit signalling by competing for the phosphorylated receptor residues that act as docking sites for STATS [14]. Indeed, CIS does bind the second tyrosine residue of the intracellular domain of the EPO receptor, phosphotyrosine Y401, one of two docking sites for STAT5 [42]. However, mutation of Y401 to phenylalanine abolishes CIS binding but not STAT5 activation, which could still be achieved through Y343 of the EPO receptor. This suggests that CIS may act by a mechanism other than simple competition with STATS for receptor binding.

The mechanism of SOCS-3 action appears to share elements of that of CIS and SOCS-1. SOCS-3 inhibits many of the same signalling pathways as SOCS-1, and coimmunoprecipitates with JAK2; however, overexpression of SOCS-3 does not inhibit the in vitro kinase activity of JAK1 or JAK2 [30]. Like CIS, SOCS-3 binds to phosphorylated receptors, including the leptin receptor, GH receptor, gp130, EPO receptor and the IL-2 receptor β chain [20, 43–46]. SOCS-3 can inhibit GH signalling, but does not do so by competing for STAT5 docking sites on the GH receptor [43]. Optimal SOCS-3 inhibition of GH-induced JAK2 activation signalling requires high levels of the GH receptor, suggesting that SOCS-3 inhibits JAK2 when bound to activated receptor, an action which requires the N-terminal domain of SOCS-3. A similar mode of action has been inferred from the IL-2 receptor system, where the ability of SOCS-3 to inhibit JAK activity is enhanced in the presence of activated the IL-2R β chain [20]. Interestingly, a SOCS-3 chimera bearing the CIS N-terminal domain is unable to inhibit signalling [30, 43]. If the N-terminal domains of SOCS-1 and SOCS-3 are indeed functioning in a similar manner, then it may be that SOCS-3 can act to inhibit JAK activation, but only when recruited to the appropriate site on an activated receptor. In support of this idea is the observation that SOCS-3 binds preferentially to the SHP-2 binding site on the gp130 and leptin receptors [44, 46]. SHP-2 is a known substrate of JAKs, mediating induction of the Ras/MAPK signalling pathway, and as such would be in close proximity to JAK. In this way, SOCS-3 might overcome its relatively poor affinity for the JAKs. Thus, despite their structural similarity, CIS, SOCS-1 and SOCS-3 appear to inhibit signalling via distinct mechanisms.

The SOCS box as a mediator of ubiquitination

Although the function of the SH2 domain as a phosphotyrosine-binding motif and the potential of the N-terminal domain as a kinase inhibitory region have been established, the role of the C-terminal SOCS box remains unclear. The SOCS box is not essential for the inhibition of cytokine-induced JAK/STAT activation [30, 40, 41, 47]. Its presence in five distinct protein families has led to the suggestion that rather than participating directly in the negative regulation of cytokine signalling, this domain may function as an adaptor via which a disparate array of proteins are linked to a common process [9]. There is now evidence that the process in question is proteasomal degradation, and that the SOCS box is the key mediator. Proteins destined for degradation are targeted by their covalent linkage to chains of ubiquitin via a three-step biochemical pathway [48–50]. Initially, free ubiquitin is recruited to the pathway by an activating enzyme, E1 [51]. The C-terminal glycine residue of ubiquitin is adenylated in an ATP-dependent reaction, which results in the binding of ubiquitin to a cysteine residue of E1 via a thiolester linkage [52]. The next step involves the transfer of ubiquitin to a carrier enzyme, E2, again via thiolester linkage to a cysteine residue [53]. Finally, ubiquitin is transferred to a lysine residue ε-amino group from the target substrate in a reaction catalysed by a third enzyme, E3 [53]. Additional ubiquitin molecules are then added to the

first to form a chain, each linked to the next by an isopeptide bond between lysine48 and glycine76 [54]. The component of this pathway responsible for specific targeting of particular proteins is the E3, which may take the form of a single protein or a multisubunit complex. It is thought that SOCS box proteins function as part of the latter.

Two groups have shown that the SOCS box facilitates an interaction between the elongin BC complex and various SOCS box-containing proteins including SOCS-1, SOCS-3, ASB-2, WSB-1 and RAR-1 [47, 55]. Elongin BC also interacts with the von Hippel-Lindau (VHL) tumour suppressor protein [56], as a member of the VBC complex, which has been demonstrated to have E1- and E2-dependent ubiquitin ligase activity [57, 58]. Several groups have shown that VBC mediates the ubiquitination of VHL binding partners in vitro [59–62]. Elongin BC acts as a bridge between VHL and the rest of the VBC complex, which comprises the cullin family member Cul2, and the ring finger protein Roc1 [63, 64]. This arrangement is highly reminiscent of the well-characterised SCF ubiquitin ligase complex, which contains a relative of Cul1, Cul2, the ring finger protein Roc1 and a molecule with homology to elongin C, Skp1 [64–69]. An analogy has been drawn between the two complexes, especially given that Skp1 fulfils the same role as elongin BC, tethering adaptor molecules to the cullin/ring finger components in order that their binding partners may be ubiquitinated. Skp1 binds adaptor molecules via their conserved F boxes, a motif of ~40-amino acids which allows the SCF complex to interact with a number of adaptor proteins, and hence ubiquitinate a range of adaptor substrates. Elongin BC binds VHL and the SOCS box-containing proteins through a sequence element known as the BC box which forms part of the approximately 40 amino acid SOCS box, and has the consensus amino acid sequence (T,S,P)(L,M)XXX(C,S)XXX(V,L,I) [47, 55]. Thus, it appears the SOCS box may serve as an adaptor via which SOCS box-containing proteins recruit their binding partners to a core E3 ubiquitin ligase complex, targeting them for ubiquitination and, presumably, subsequent degradation.

In the case of the SH2 domain-containing SOCS family proper, this argument makes for an attractive model of SOCS regulation of cytokine signalling whereby the SOCS proteins not only inhibit the activation of various components of the signalling pathway, but also target them for degradation, terminating the signal (fig. 2). The proof that this model has physiological relevance has yet to emerge, but in vitro evidence is accumulating that suggests that the presence of the SOCS box does affect the stability of SOCS binding partners. Consistent with the idea that signalling components may be targets for proteasomal degradation, it has been demonstrated that signalling through the JAK/STAT pathway is sustained in

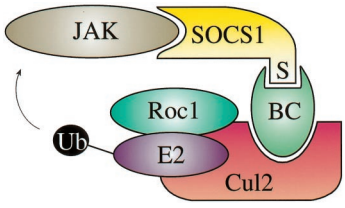

Figure 2. Model of SOCS participation in an E3 ubiquitin ligase complex. In the example shown, SOCS-1 acts as the specificity component of the E3, recruiting JAK kinase via SH2 domain binding. Interactions between the SOCS box (S) and the elongin BC complex (BC) tether SOCS-1 to the E3, which in turn recruits the E2 ubiquitin-conjugating enzyme, facilitating the ubiquitination of JAK.

the presence of proteasomal inhibitors [42, 70]. CIS appears to suppress erythropoietin signalling by binding to the activated erythropoietin receptor and targeting it for ubiquitin-dependent degradation [42]. In COS7 cells, SOCS-1 binds to the guanine nucleotide exchange factor Vav, apparently coupling it to the ubiquitination machinery [71]. Recently three groups have presented evidence that the SOCS box mediates ubiquitin-proteasome-dependent degradation of the oncoprotein TEL-JAK2 [72–74]. Expression of SOCS-1 in Ba/F3 cells transformed with TEL-JAK2 inhibits their IL-3-dependent growth, and SOCS-1 inhibits the kinase activity of the fusion protein. However, the latter was not sufficient to explain the suppression of TEL-JAK2 activity, and it appears that SOCS-1 expression decreases TEL-JAK2 protein levels. This decrease is associated with SOCS-box-dependent ubquitination of TEL-JAK2, and is reversed by treatment of cells with proteasomal inhibitors. Lethally irradiated mice reconstituted with bone marrow cell infected with a bicistronic retrovirus expressing TEL-JAK2 and SOCS-1 displayed a significantly longer latency period for TEL-JAK2-induced disease than those reconstituted with bone marrow cells infected with a retrovirus expressing TEL-JAK2 alone, indicating that SOCS-1 is an inhibitor of TEL-JAK2-mediated transformation in vivo [72]. Interestingly, the SOCS box of CIS can substitute for that of SOCS-1 in mediating TEL-JAK2 degradation, but not that of SOCS-3, which the authors suggest may indicate that the particular SOCS box must be in the proper orientation for TEL-JAK2 ubiquitination to occur [73].

Whether or not the SOCS box also mediates the ubiquitination of the SOCS proteins themselves is unclear. In the SCF analogy, there is evidence that the relatively unstable F box proteins are themselves ubiquitinated and degraded, potentially via an autocatalytic mechanism involving SCF itself [75-79]. It would be an elegant regulatory mechanism indeed if the SOCS proteins also mediate their own degradation, leaving the cell ready for another round of signalling. However, studies of VHL have indicated that VHL mutants unable to bind elongin

BC are degraded by the proteasome in VHL-deficient renal carcinoma cells, and that wild-type VHL is stabilised by the interaction with elongin BC [80]. Two groups have presented evidence that this is also true of SOCS-1, suggesting that the SOCS box may have the effect of protecting SOCS box proteins from proteolytic degradation [41, 47]. In contrast, both CIS and SOCS-3 have been shown to be unstable proteins that are degraded by the ubiquitin-proteasome system, and SOCS-3 is reported to be more stable in Ba/F3 cells without its SOCS box [55, 73]. This has led to the suggestion that the structure of individual SOCS boxes may determine whether a particular SOCS protein is targeted for ubiquitin-mediated degradation. The clarification of how the SOCS box performs awaits the outcome of studies undertaken in a physiological setting.

Physiological roles of the SOCS proteins

The participation of SOCS proteins in a cytokine-induced negative feedback loop that regulates JAK/STAT signal transduction implies that knowledge of the cytokines that induce expression of a given SOCS in particular tissues will be a strong predictor of its biological role. However, as studies investigating cytokine induction of SOCS accrue, a more complex picture is emerging. Specific SOCS genes, particularly SOCS-1 and SOCS-3, appear sensitive to induction by a bewilderingly diverse array of cytokines, and in many cases this is accompanied by evidence that SOCS expression inhibits signalling from these stimuli [81]. Often, these studies examined actions of SOCS proteins when ectopically expressed or present at levels greater than normally occur and may have exaggerated the true biological functions of these regulators. Indeed, the analysis of genetically modified mice, particularly those in which SOCS genes have been deleted, have identified more specific physiological roles for members of this family than might initially have been anticipated.

The SOCS-1 gene is essential for survival beyond the postnatal period. Mice in which this gene has been deleted by gene targeting are born in apparent good health and in the numbers predicted for normal survival throughout gestation. However, within 10 days, SOCS-$1^{-/-}$ mice are considerably smaller than their wild-type littermates, and they fail to thrive and die, typically before they reach 3 weeks of age [82–84]. The disease in SOCS-$1^{-/-}$ mice presents a complex pathological picture. Fatty degeneration of the liver associated with significant areas of necrosis is invariably evident and probably accounts for the death of the mice. Significant atrophy of the thymus and a marked reduction in lymphocytes in the lymphoid organs and circulation were also observed, and many tissues also exhibited inflammatory infiltrates, typically T-lymphocytes, granulocytes and macrophages [84]. Evidence of accelerated apoptosis of lymphocytes within the thymus and spleen with up-regulation of Bax expression was also reported [82].

The basis for the SOCS-$1^{-/-}$ disease emerged from experiments prompted by the observation that the phenotype in these mice was strikingly similar to that observed in mice injected with interferon [85]. Constitutive activation of STAT1 and over-expression of class I MHC and interferon-γ(IFNγ)-induced genes in SOCS-$1^{-/-}$ mice suggested that the lethal disease in these mice might result from excessive responses to IFNγ. The key role of IFNγ was unequivocally demonstrated by the absence of disease in SOCS-$1^{-/-}$ mice treated with neutralising anti-IFNγ antibodies or lacking the IFNγ gene. The gene dosage of IFNγ was critical, with SOCS-$1^{-/-}$ IFN$\gamma^{-/-}$ mice remaining disease free for the 6-month observation period, whereas SOCS-$1^{-/-}$ IFN$\gamma^{+/-}$ mice succumbed to a delayed and modified disease characterised by myocarditis and polymyositis [86]. Subsequent analyses suggested that heightened production of IFNγ and increased inherent sensitivity to the cytokine combine to induce disease in the absence of SOCS-1 [83, 87]. Excess IFNγ production is likely to be the result of anomalous T-cell activation in the absence of SOCS-1. Because a higher than normal proportion of T cells express activation markers in SOCS-$1^{-/-}$ IFN$\gamma^{-/-}$ mice as well as in the original SOCS-$1^{-/-}$ IFN$\gamma^{+/+}$ animals [83, 86], SOCS-1 appears to have an important role in T-lymphocyte development or activation that is independent of IFNγ and yet to be fully elucidated. Together, these studies establish that SOCS-1 is a critical physiological regulator of IFNγ action, allowing the beneficial immunological effects of this cytokine while controlling potentially damaging associated pathology.

A comprehensive analysis of hematopoiesis in SOCS-$1^{-/-}$ mice has revealed some additional subtle defects. The incorporation of a lacZ marker gene into the SOCS-1 locus in one gene-targeting study allowed a surrogate analysis of SOCS-1 gene expression in hematopoietic cells. Within the bone marrow, significant SOCS-1 transcription was identified, not only in lymphocytes as expected from the phenotype of SOCS-$1^{-/-}$ mice, but also in granulocytes and macrophages and a proportion of their immature progenitors [88]. In the SOCS-$1^{-/-}$ mice themselves, the frequencies of most hematopoietic progenitors is normal, although a mild increase in macrophage colony-forming cells was noted. Nevertheless, granulocyte-macrophage progenitor cells from SOCS-$1^{-/-}$ mice were more sensitive to GM-CSF in culture, raising the possibility that SOCS-1 may have a role in signalling by GM-CSF [88]. Whether such a role might be reflected in the granulocyte and macrophage infiltration of multiple organs in SOCS-$1^{-/-}$ mice has not been directly explored. Cells derived from SOCS-$1^{-/-}$ mice have also proven to

be more sensitive to tumour necrosis factor-α (TNFα) [89], although the significance of this observation to disease in SOCS-1$^{-/-}$ mice has also not yet been directly evaluated.

Transgenic mice overexpressing SOCS-1 in T cells [90] have provided in vivo confirmation of many in vitro studies showing that SOCS-1 can inhibit signalling from a large number of diverse cytokines, at least when ectopically or overexpressed. In this context, the apparently highly specific physiological defect in IFNγ signalling manifest in SOCS-1$^{-/-}$ mice is a conundrum. Although there appear to be no obvious anomalies in SOCS-1$^{-/-}$ mice indicative of defects in multiple signalling pathways, it remains possible that some of the functions of SOCS-1 are shared with other members of the SOCS family. Evidence of any redundant roles of SOCS-1 may emerge from analysis of mice lacking multiple SOCS genes.

Mice lacking SOCS-2 are indistinguishable from their normal littermates until weaning, but subsequently display accelerated growth, resulting in adult SOCS-2$^{-/-}$ mice that are 30 to 40% larger than wild-type mice. The increase in body weight reflects a generalised and uniform increase in the size of most organs, as well as increased muscle mass and skeletal size, with evidence suggesting cell number within organs rather than cell size is increased [91]. There is no significant increase in fat mass in these mice. The majority of organs in SOCS-2$^{-/-}$ mice show no obvious histological anomalies with the exception of the skin, in which markedly increased deposition of collagen occurs. This is particularly prevalent in male mice and is occasionally also observed in the lungs, liver, salivary glands and pancreas. This feature, as well as altered regulation of major urinary protein, which is also evident in SOCS-2$^{-/-}$ mice, was previously observed in mice with elevated growth hormone (GH) or insulin-like growth factor-I (IGF-I) and is often associated with acromegaly in humans. These data strongly imply that SOCS-2 has a key regulatory role in the GH/IGF-I system of growth control. Consistent with this hypothesis, elevated expression of IGF-I messenger RNA (mRNA) has been observed in some organs of SOCS-2$^{-/-}$ mice, although this was not uniform [91]. Unequivocal evidence that SOCS-2 controls GH or IGF-I signalling and the mechanism by which this is achieved has not yet been demonstrated. While SOCS-2 can be induced by GH and has been shown to associate with the GH and IGF-I receptors, such studies have yet to convincingly define the negative regulatory role for SOCS-2 in GH or IGF-I signalling [17, 19, 23, 92]. The analysis of GH and IGF-I signalling in SOCS-2$^{-/-}$ mice and cells derived from them should provide a valuable addition to these previous analyses in the dissection of the mechanism of SOCS-2 action in growth control. Although SOCS-2 expression can be induced by a range of hemopoietic cytokines, no hematopoietic abnormalities were evident in SOCS-2$^{-/-}$ mice [91]. The phenotype in SOCS-2$^{-/-}$ mice has been confirmed by the discovery that disruption of the SOCS-2 gene resulting in loss of expression accounts for the excessive growth of the high growth (hg) spontaneous mouse mutant [93].

Mice lacking SOCS-3 have been reported to die at mid-gestation with delayed development and excessive erythropoiesis [94]. Higher than normal proliferative capacity was observed in erythroid progenitor cells, although fewer of these immature cells were present in SOCS-3$^{-/-}$ fetal liver than in normal controls. Curiously, reconstitution of irradiated adult mice with SOCS-3$^{-/-}$ fetal liver did not result in excessive erythroid cell production. Complementary studies in which SOCS-3 was ectopically expressed in hematopoietic cells of transgenic mice yielded the converse phenotype to the SOCS-3 knockout: extreme anemia, also resulting in embryonic lethality [94]. As SOCS-3 has been shown to associate with the erythropoietin (EPO) receptor and can inhibit EPO signalling when overexpressed in EPO-responsive cell lines [45], these data suggest that SOCS-3 plays a key role in regulation of EPO signalling in vivo.

The widespread overexpression of CIS in transgenic mice results in a panoply of abnormalities [15]. Although these mice develop normally, their growth is retarded. Moreover, female mice carrying the transgene fail to effectively lactate and exhibit defective mammary gland differentiation. Finally, the numbers of specific subsets of T cells and natural killer cells are significantly reduced in CIS transgenic mice, and T lymphocytes exhibit a failure to properly proliferate in response to IL-2. Similar phenotypes characterise mice lacking STAT5A and/or STAT5B and an important role for CIS in the regulation of these signalling intermediates has been proposed, particularly in the control of GH, prolactin and IL-2 signalling. The observations that these cytokines can induce CIS expression via STAT5-responsive elements in the CIS promoter and that CIS appears to regulate STAT5 activity by competing for receptor binding sites [10] add credence to this conclusion. Paradoxically, however, disruption of the CIS gene in mice results in no phenotypic anomalies [94]. This result seems to imply that the phenotypes of the transgenic mice exaggerate CIS function as a result of excess or ectopic expression, or that the actions of CIS in the regulation of growth, lactation and T-lymphopoiesis can be functionally compensated in mice.

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