SPECIAL REPORTS AND REVIEWS

Suppressors of Cytokine Signaling: Relevance to Gastrointestinal Function and Disease

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Background & Aims: The suppressor of cytokine signaling (SOCS) proteins are a family of Src homology 2 domain-containing proteins. Currently, there are 8 members of the SOCS family, of which a number have been implicated strongly in the negative regulation of cytokine signal transduction pathways. *Methods:* This review focuses on recent discoveries about 4 SOCS family members, SOCS-1, -2, and -3, and cytokine-inducible SH2-domain containing (CIS), and provides more limited information about other SOCS family members. *Results:* A large number of cytokines and growth factors are now known to induce SOCS proteins. In turn, SOCS inhibit the actions of a growing number of cytokines and growth factors in vitro or in vivo. SOCS proteins exert their inhibitory effects at the level of activation of janus kinases (JAKs) or by competing with transcription factors for binding sites on activated cytokine receptors. SOCS proteins also may mediate the ubiquitination and subsequent degradation of the SOCS protein and its bound signaling complex. Genetic modification of SOCS genes in mice has revealed crucial roles in the negative regulation of a number of important physiologic parameters including interferon γ activity, growth, blood cell production, and placental development. *Conclusions:* Information about SOCS action in gastrointestinal function and disease is only just emerging, but available data indicate a role in growth of gastrointestinal tissues, inflammatory bowel disease, and cancer.

Cytokines are key mediators of appropriate physiologic functions within the gastrointestinal tract and accessory organs such as the liver and pancreas. The α4-helix bundle family of cytokines includes interferon (IFN)-γ, many interleukins (ILs), growth hormones (GHs), the colony stimulating factors, erythropoietin (EPO), and leukemia inhibitory factor (LIF). The phenotypes of a number of transgenic and gene knockout models have emphasized the dramatic role cytokines play in gastrointestinal function. For example, several mouse models of spontaneous inflammatory bowel disease (IBD) exhibit overproduction of T-helper (Th) cell–derived cytokines such as the Th-1–derived cytokine IFN-γ,[1–4] or the Th-2–derived cytokine IL-4.[5–8] Widespread overexpression of GH leads to overgrowth of the small and large intestines[9,10] and overgrowth and neoplastic changes in liver and spleen.[11,12] Overexpression of IL-6 in transgenic mice leads to plasma cell hyperproliferation and plasmacytoma,[13] whereas co-overexpression of a soluble IL-6 receptor in the IL-6 transgenics leads to hepatocellular hyperplasia and accelerated plasmacytoma development.[14] Other IL-6 transgenic lines show body growth retardation and reduced levels of circulating insulin-like growth factor I (IGF-I).[15] Clearly, cytokine action must be regulated tightly to prevent pathophysiological consequences.

Research in the past decade has led to major advances in our understanding of the pathways by which cytokines activate target cells (Figure 1). Each cytokine binds to a specific receptor that spans target cell membranes and induces dimerization or oligomerization, causing conformational changes in receptor structure. These receptor modifications allow for the recruitment of janus kinases (JAKs), enzymes that bind to the receptors and phosphorylate specific tyrosine residues on themselves and on the intracellular portion of the receptors. Tyrosine phosphorylated receptors then serve as docking sites for signaling proteins that contain Src homology 2 (SH2) domains or phosphotyrosine binding domains. Such

Figure 1. Cytokines bind to specific sites on the extracellular domains of their cognitive receptor, causing receptor dimerization. This allows the recruitment of JAKs to the receptors, which then cross-phosphorylate each other before phosphorylating the receptor on key tyrosine residues. STAT molecules bind to these phosphorylated docking sites, are in turn phosphorylated, dimerize, and enter the nucleus where they initiate transcription. Some of the genes transcribed by these factors include the SOCS genes. The SOCS proteins then act to negatively regulate activated receptor complexes by inactivating JAKs or blocking recruitment sites for STATs and also may target signaling complexes for ubiquitination and degradation.
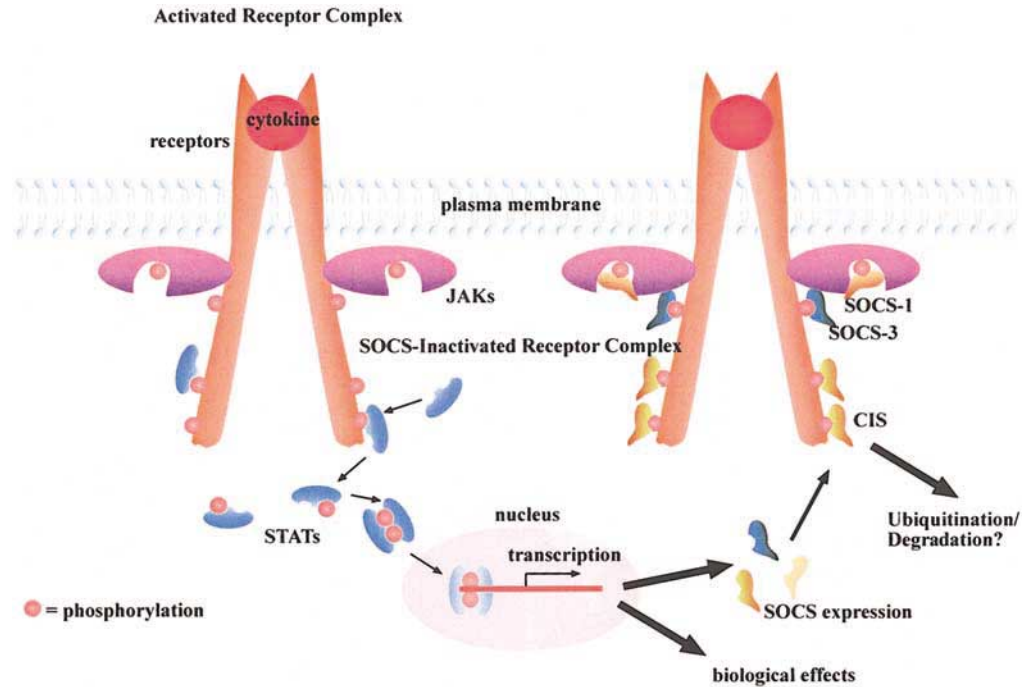
signaling proteins include RAS and phosphatidylinositol 3-kinase that, as well as being activated by cytokine receptors, also are associated with signal transduction by receptor tyrosine kinases that mediate the actions of growth factors such as the epidermal growth factor or IGFs. In addition, a family of transcription factors known as the signal transducers and activators of transcription (STATs) bind to activated cytokine receptors via SH2 domains and are key mediators of cytokine action. Once bound, the STATs themselves become a substrate target of the JAKs and are phosphorylated on key tyrosine residues. Activated STATs disassociate from the receptor and form homo- or heterodimers with other STAT molecules. These paired molecules cross the nuclear membrane and bind to DNA sequences in the promoter regions of target genes that are selective for particular STAT family members and mediate the transcription of cytokine-regulated genes.

Although our understanding of the intracellular signaling molecules that mediate the functional outcome of cytokine-receptor activation has increased profoundly, more recent research has placed increasing emphasis on the mechanisms by which cytokine signals are terminated. The first group of molecules implicated as negative modulators of cytokine signaling were phosphatases. Phosphatases such as signal transducers and activators of transcription (SHP-1) dephosphorylate cytokine receptors and JAKs, leading to termination of phosphorylation-dependent signaling cascades.[16] The cell surface marker CD45 also has phosphatase activity and has been

shown to regulate cytokine signaling by its ability to dephosphorylate JAKs.[17] The protein inhibitor of activated STAT proteins form a second and distinct family of molecules capable of inhibiting a number of cytokine pathways by their ability to bind and prevent activated
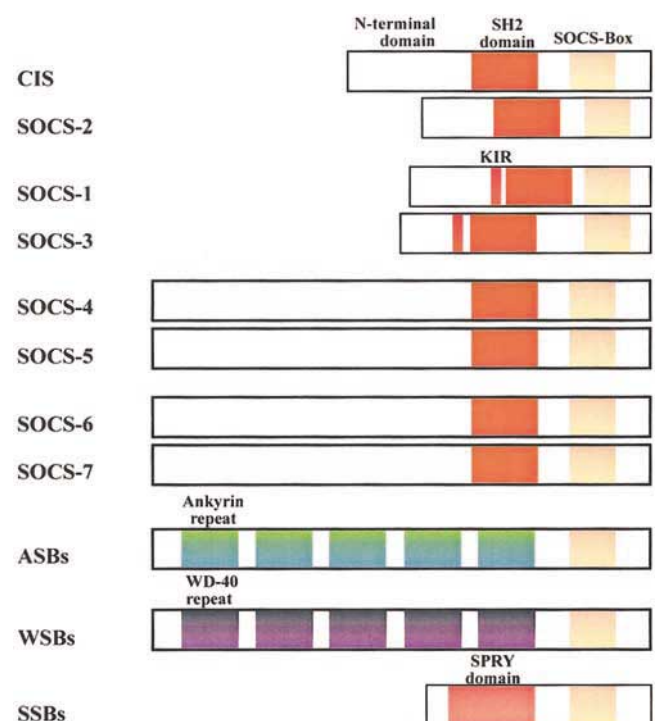
Figure 2. Schematic of SOCS box containing proteins. The SOCS proteins are paired based on the highest amino acid homologies. The domain structures of each molecule is detailed.

STAT protein dimers from entering the nucleus to initiate transcription.[18,19] A third family, and the topic of this article, is composed of the suppressors of cytokine signaling (SOCS), which have been shown to be important physiologic regulators of cytokine signaling.

Discovery

The first member of the SOCS family of proteins to be discovered was called cytokine-inducible SH2-domain containing (CIS) protein. CIS was found in a screen for negative regulators of IL-3 and EPO signaling.[20] Subsequent investigations using a number of methods detected a different but homologous protein called SOCS-1. SOCS-1 was discovered based on its ability to inhibit IL-6–mediated differentiation of the myeloid leukemia M1 cells into macrophages.[21] SOCS-1 also was detected in a yeast 2-hybrid screen for proteins capable of interacting with the kinase domain of JAK2[22] and by screening an expression library with antibodies directed against the SH2 domain of STAT3.[23] Given its discovery by different lines of investigation, SOCS-1 has also been termed JAK binding protein, or STAT-induced STAT inhibitor. Subsequent scans of genomic and expressed sequence tag databases revealed that CIS and SOCS-1 proteins were part of a larger family of 8 proteins (CIS and SOCS-1 through SOCS-7). As shown in Figure 2, each family member possesses a central SH2 domain and a conserved motif at the C-terminus called a SOCS-box, whereas the N-terminal regions of SOCS proteins have variable length and sequence homology.[21,24–26] Furthermore, a large number of other proteins contain a SOCS box but lack an SH2 domain. These SOCS-related proteins can be grouped based on the presence of homologous motifs within their N-terminal regions that include ankyrin repeats, WD-40 repeats, SPRY domains, and guanosine triphosphatase–like domains (Figure 2).[24]

Mechanisms of Suppressors of Cytokine Signaling Induction and Action

Much research has documented that SOCS proteins are induced by cytokines that use JAK/STAT signaling pathways (Figure 1). STATs are proposed as the direct mediators of SOCS induction, and SOCS in turn inhibit JAK kinase activation or the ability of JAKs to activate STATS, thus forming a classic negative feedback mechanism (Figure 1). In addition, considerable evidence supports a concept that one or more SOCS proteins may act to limit the duration of cytokine signaling by serving as adaptor molecules to link active signaling complexes with degradation pathways. Ubiquitin-dependent proteasome-mediated degradation is a major intracellular

pathway by which membrane-associated proteins are degraded. This proteasome-degradation complex involves a number of proteins including ubiquitin ligases, elongins, and cullin-2. It is hypothesized that the SOCS proteins and/or their bound signaling complexes become targets for ubiquitination, and subsequently are degraded by the proteasome. Evidence in support of the concept stems from findings that a number of the SOCS, and SOCS-box containing, proteins bind to elongin B and C, which in turn bind E3 ubiquitin ligase and cullin-2.[27,28]

As well as induction by cytokines that use JAK-STAT signaling pathways, it increasingly is clear that SOCS proteins are induced via STAT-independent mechanisms including stimuli such as stem cell factor,[29] lipopolysaccharide,[30] and methylated DNA.[31] Transcription factors that mediate STAT-independent SOCS induction are not well defined. SOCS mRNAs are expressed constitutively in multiple cell types and numerous tissues throughout the body including the gastrointestinal tract, liver, and pancreas (Miller, unpublished data, 2002),[21,26,32–35] but their expression is enhanced greatly by cytokines. The time course and duration of SOCS induction vary depending on the particular cytokine or SOCS protein as discussed in more detail later. The scope of the potential modulatory actions of SOCS is shown in Table 1, which summarizes much of the available information generated from initial in vivo and in vitro experiments about which cytokine pathways induce each SOCS protein and which cytokine pathways are inhibited by particular SOCS proteins. More detailed information is available now about SOCS-1, SOCS-2, SOCS-3, and CIS and this is reviewed later, as well as the more limited information about other SOCS family members. As yet, relatively little is known about the function of SOCS in the gastrointestinal tract but some published and preliminary findings support the concept that SOCS play a role in the regulation of growth of the gastrointestinal tract and in gastrointestinal disease.

Suppressors of Cytokine Signaling 1

SOCS-1 mRNA is induced by and inhibits a variety of cytokines, including IFNs, IL-4, IL-6, LIF, and EPO in vitro or in vivo (Table 1). Cytokine induction of SOCS-1 mRNA generally is rapid and transient, with detectable induction within 15 minutes, peak induction at 2 hours, and reversion to baseline by 4 hours. SOCS-1 inhibits cytokine action by inhibiting JAK activation in vitro or in vivo. The SH2 domain of SOCS-1 is thought to be a key mediator of JAK inhibition because it binds to the kinase domain of JAK2.[22] Phosphopeptide bind-

Table 1. SOCS Proteins Are Induced, Inhibited, and Associated With a Range of Growth Factor and Cytokine Signaling Systems In Vitro and In Vivo

SOCS protein	Growth factors and cytokines that induce SOCS	Signaling pathways inhibited by SOCS	SOCS protein associations
CIS	IL-2,[21,23,120] IL-3,[20,120] IL-6,[21] IL-9,[133] IFN-α,[134] TNF-α,[135] EPO,[20,120] LIF,[21] leptin,[136] GH,[35,78,137,138] OSM,[120] TSLP,[139] prolactin[68]	EPO,[20,120] IL-2,[140] IL-3,[20] GH,[87] and prolactin[69]	GH receptor,[83,85,87] EPO receptor,[20] IL-2 receptor,[140] PCKθ,[126] IL-3 receptor[20]
SOCS-1	IL-6,[21,52,109] IL-7,[57] IL-13[21] IFN,[69,141–144] G-CSF,[21] GM-CSF,[21,25] M-CSF,[145] TPO,[23] LIF,[21] CNTF,[146] cardiotrophin,[47] GH,[78,137] prolactin[68]	IFN,[21,47,144] EPO,[22] IL-3,[22] IL-4,[59,148,149] IL-6,[21] LIF,[21,25] GH,[78,83,85] prolactin,[67–69,150] TNFα,[135] Tpo,[151] TSLP[139]	JAK1-3,[22,152,153] IGF-1 receptor,[88] FGF receptor,[25] Kit receptor,[25,29] Grb-2,[29] VAV,[29] insulin receptor[127]
SOCS-2	IL-6,[21] IL-9,[133] IFN-α,[134] IFN-γ,[154] LIF,[21] GH,[35,78,137] prolactin,[68] insulin[155]	GH,[85,87] IL-6,[21] IGF-I[88]	IGF-I receptor,[88] GH receptor,[83,85] prolactin receptor [68]
SOCS-3	IL-1,[156] IL-2,[32] IL-6,[21] IL-9,[133] IL-10,[157,158] IL-13,[21] IFN-α,[134] IFN-γ,[144] GM-CSF,[25] EPO,[21,25] LIF,[21,25,26,159] CNTF,[146] Leptin,[160,161] insulin,[155,162] cardiotrophin,[147] GH,[35,78,137] prolactin[68]	EPO,[51,108] IL-2,[32] IL-3,[32] IL-4,[63,148] IL-6,[37] IL-9,[133] IL-11,[163] LIF,[21,25] IFNγ,[144] GH,[78,83,85] prolactin,[67–69] leptin,[160] insulin,[162] IGF-I[164]	GH receptor,[83,85] IGF-I receptor,[164] gp130,[110,111] leptin receptor,[165] Lck,[25] FGF receptor,[25] PYK2[25]
SOCS-4	Unknown	Unknown	Unknown
SOCS-5	IL-6[24]	IL-6[37]	Unknown
SOCS-6	Unknown	Unknown	Insulin receptor[127]
SOCS-7	Unknown	Unknown	Ash,[129] PLC γ,[129] Nck[129]

TNF, tumor necrosis factor; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; M-CSF, macrophage colony stimulating factor; TPO, thrombopoietin; CNTF, cilliary neurotrophic factor; PKC, protein kinase C; PLC, phospholipase C.

ing studies suggest that SOCS-1 inhibits JAK2 by binding to a key tyrosine (Tyr-1007) in the activation loop of its kinase domain,[36] thereby supporting a model in which SOCS-1 inhibits cytokine signaling by binding to the activation loop of JAK-2 and restricting access of other substrates to JAK-dependent phosphorylation and activation. Structure function studies defined the SH2 domain and 30 amino acids of the adjacent N-terminal region of SOCS-1 (called the kinase inhibitory region) as essential for SOCS-1 inhibition of IL-6 signaling. The entire N-terminal domains of SOCS-1 and SOCS-3 appear to be interchangeable in terms of their ability to inhibit signaling by LIF, which implies that SOCS-1 and SOCS-3 may have a shared or overlapping mechanism of inhibiting the actions of some cytokines.[37]

As well as JAK inhibition, there is considerable evidence that SOCS-1 mediates proteasomal degradation of a number of signaling proteins. SOCS-1 binds to the hematopoietic-specific guanine nucleotide exchange factor, VAV, and targets it for ubiquitin-mediated protein degradation.[38] The conserved C-terminal SOCS-box motif appears to mediate SOCS interaction with components of the proteasomal degradation machinery,[27,28] even though the SOCS box may protect the SOCS protein itself from degradation[39] and is dispensable for cytokine signaling inhibition in overexpression systems.[36,37,39] The strength of the argument that the SOCS box mediates degradation of signaling complexes recently has gained more credence with a number of studies showing a role for SOCS-1 in the regulation of the TEL-JAK oncogene, which is associated with human

leukemia. The TEL-JAK protein is a fusion protein caused by a chromosomal translocation from the 5′ end of the TEL gene on 12p13 to the 3′ end of JAK2 on 9p24 and is observed in some human lymphomas. Transfection of TEL-JAK expression constructs into Ba/F3 cells leads to cytokine-independent proliferation caused by hyperactivation of JAKs and STAT5.[40] Enforced overexpression of SOCS-1 blocks JAK activity and reduces the level of TEL-JAK expression.[41] Additionally, the SOCS box of SOCS-1 interacts with cullin-2 and promotes ubiquitination of TEL-JAK2, whereas overexpression of dominant-negative cullin-2 suppresses SOCS-1–dependent TEL-JAK2 degradation.[42] The biologic significance of this observation was shown by the finding that transplantation of murine bone marrow with retroviral constructs expressing both TEL-JAK2 and SOCS-1 prolongs the latency of disease and death compared with those expressing TEL-JAK2 alone.[43] The phenotype of SOCS-1△/△ mice, which lack only the SOCS box of SOCS-1 also supports an important role for this motif in SOCS-1 action (see later).[44]

Mice devoid of SOCS-1 (SOCS-1⁻/⁻ mice) have provided significant insight into the physiologic role(s) of this cytokine suppressor and validated the large amount of previously generated in vitro data. SOCS-1⁻/⁻ mice are born healthy and in expected Mendelian proportions, but die before weaning from a complex hematologic and pathologic phenotype.[45,46] Histologic examination revealed that SOCS-1⁻/⁻ mice exhibited fatty degeneration and necrosis of the liver, significant depletion of T- and B-lymphocyte numbers, deficits in the number of lym-

phoid follicles, and extensive macrophage infiltration in the liver, pancreas, heart, and lung.[46] The gut, bladder, kidney, and gonads of SOCS-1$^{-/-}$ mice are grossly normal, although these mice do suffer reduced thymus weight and lower thymic and splenic T-cell numbers, which has been postulated to reflect accelerated T-cell apoptosis.[45] The important role of SOCS-1 in regulating the immune response to infection was emphasized by the findings that SOCS-1$^{-/-}$ mice are hyperresistant to Semliki forest virus, and IFN-γ–treated bone marrow macrophages from SOCS-1$^{-/-}$ mice have a greatly enhanced capacity to kill *Leishmania major*.[47]

The phenotypic characteristics of SOCS-1$^{-/-}$ mice have striking similarity to those observed in neonatal mice injected with IFNs,[48] raising the possibility that SOCS-1$^{-/-}$ mice suffer from deregulated production or responsiveness to IFNs. Consistent with this possibility, SOCS-1$^{-/-}$ mice have more activated STAT1, a primary mediator of IFN action, and increased expression of STAT1-responsive genes.[47] SOCS-1$^{-/-}$ IFN-γ$^{-/-}$ double knockout mice survive weaning and are healthy until at least 6 months of age.[47] The pathology associated with absolute SOCS-1 deficiency also can be prevented by giving SOCS-1$^{-/-}$ mice twice-weekly injections of antibodies against IFN-γ. Together, these in vivo findings provide compelling evidence that SOCS-1 limits IFN action. SOCS-1$^{-/-}$ IFN-γ$^{-/-}$ mice are more acutely sensitive to IFN-γ injections and have more prolonged STAT1 phosphorylation in response to IFN-γ compared with IFN-γ$^{-/-}$ mice.[49] Interestingly, although SOCS-1$^{-/-}$ IFN-γ$^{+/-}$ mice appear normal from birth to adulthood, abnormalities appear later in life, characterized by polymyositis and myocarditis that kills all mice by 160 days of age.[50]

SOCS-1△/△ mice, which lack only the SOCS box of SOCS-1, are born healthy but die prematurely of a complex histopathologic disease similar to that of SOCS-1$^{-/-}$ mice, although the onset of the disease phenotype and eventual mortality is delayed in SOCS-1△/△ mice.[44] This intermediate phenotype provides strong support that the SOCS box is necessary for appropriate timing, duration, or magnitude of cytokine inhibition by SOCS-1, presumably by mediating proteasome degradation of SOCS-1–associated cytokine receptor signaling complexes. Direct evidence that the SOCS box of SOCS-1 plays an important role in SOCS-1 regulation of IFN-γ signaling derives from findings that IFN-γ–induced STAT1 activation is prolonged in SOCS-1△/△ hepatocytes compared with wild-type cells, but is not as extended as in SOCS-1$^{-/-}$ hepatocytes.[44]

The concept that the SOCS-1$^{-/-}$ phenotype is explained by hyperresponsiveness to IFN-γ may be only part of the story because these mice also have elevated levels of IFN-γ.[51] The cellular sites of this IFN-γ overproduction and the exact sites where absolute SOCS-1 deficiency leads to deregulated IFN-γ action are yet to be conclusively defined, but available evidence indicates deregulation of Th-1 cells. Diehl et al.[52] reported findings that suggest that SOCS-1 mediates IL-6 inhibition of Th-1 cell differentiation. Consequently, a lack of SOCS-1 could allow for enhanced Th-1 differentiation and Th-1 cells could be responsible for the increased IFN-γ expression in SOCS-1$^{-/-}$ mice.[52] JAK3-deficient mice suffer from B- and T-cell lymphopenia and significantly depleted thymocyte populations.[53–55] When JAK3-deficient mice are reconstituted with bone marrow from SOCS-1$^{-/-}$ mice they develop the same disease phenotype as observed in SOCS-1$^{-/-}$ mice.[56] This supports a concept that lymphocyte or myeloid cells from SOCS-1$^{-/-}$ mice can mediate expansion of IFN-γ–producing lymphocytes. The hypothesis that lymphocytes are the major source of the disease in SOCS-1$^{-/-}$ mice also was examined by crossing recombination activating gene (RAG)-2$^{-/-}$ mice and SOCS-1$^{-/-}$ mice. RAG-2$^{-/-}$ mice suffer from a lack of B and T cells owing to defective B- and T-cell receptor rearrangement, and so are not susceptible to disease mediated by activated B or T cells. SOCS-1$^{-/-}$ RAG-2$^{-/-}$ mice have a longer lifespan than SOCS-1$^{-/-}$ mice, providing strong evidence that T cells and/or B cells contribute to the abnormalities in SOCS-1$^{-/-}$ mice.[56]

Examination of transgenic mice that overexpress SOCS-1 in their T cells confirms many of the conclusions obtained in SOCS-1 null mice and also shows impaired responses to IL-6 and IL-7.[57]

Emerging evidence suggests that SOCS-1 may integrate the interactions between IFN-γ and IL-4.[58,59] This may be relevant especially to better understanding of the pathophysiology of Crohn's disease (CD) and ulcerative colitis (UC), which are associated with a predominance of IFN-γ–producing Th-1 cells and IL-4–producing Th-2 cells, respectively.[60] IFN-γ, a Th-1–derived cytokine, is known to antagonize the actions of IL-4 by repressing the expression of a number of IL-4–induced genes including 15 lipoxygenase and IL-1–receptor antagonist.[61,62] Intriguingly, neither the 15 lipoxygenase nor IL-1–receptor antagonist genes contain a classic IFN-γ responsive gamma activated sequence within their promoters; a DNA response element that mediates IFN-γ action by binding STAT1α. These genes do, however, contain a STAT6 binding element that generally mediates the

transcriptional effects of IL-4.[58] By using cultured human monocytes, Dickensheets et al.[59] found that pretreatment of cells with IFN-γ but not IL-1, IL-2, G-colony stimulating factor, IL-6, or transforming growth factor β suppresses the ability of IL-4 to activate STAT6. This inhibitory effect of IFN-γ correlates temporally with induction of SOCS-1. They also showed that forced expression of SOCS-1 in RAW264 macrophages suppressed transcriptional activation of IL-4–induced reporter genes[59] as has been observed in other cell lines.[63] The intriguing possibility that SOCS-1 may mediate the interplay between IFN-γ and IL-4 is further supported by recent in vivo studies by Naka et al.[64] They found that IL-4 injections accelerate disease development in SOCS-1^{−/−} mice, whereas deletion of STAT1 or STAT6 genes improves disease pathologies.[64] They also found that the hyperactivity of the IFN-γ and IL-4 signaling systems in SOCS-1^{−/−} mice caused abnormal activation of natural killer T cells that appeared to mediate much of the degenerative liver pathology.[64] Recent studies suggested that SOCS-1 predominates in mouse-derived Th-1 cells and limited IL-4 action in these cells,[65] further supporting the concept that SOCS-1 has a key role in Th-1 cell differentiation and selective cytokine responsiveness. Future studies of SOCS-1 regulation of IFN-γ/IL-4 actions and interactions in animal models of IBD, and in specific immune cell subtypes derived from animal models and patients with IBD, should lead to new insights into mechanisms that underlie aberrant or dysregulated T-cell responses in UC or CD.

SOCS-1 appears to have physiologic roles outside the hematopoietic compartment. SOCS-1^{−/−} mice exhibit low blood glucose levels and have sustained insulin-induced phosphorylation of insulin-receptor substrate-1, indicating inhibitory effects of SOCS-1 on insulin signaling.[66] SOCS-1 inhibits prolactin signaling in vitro[67–69] and inhibits the in vivo effects of prolactin on mammary gland development. SOCS-1^{−/−} IFN-γ^{−/−} mice show accelerated lobualveolar development, increased milk protein gene expression, and higher levels of phosphorylated STAT5 at day 18 of pregnancy. Deletion of one SOCS-1 allele is capable of rescuing a lactogenic defect in mice lacking one allele of the prolactin receptor, which clearly implicates SOCS-1 as a dose-dependent, negative regulator of prolactin signal transduction.[70] Gene dosage effects of SOCS-1 on prolactin receptor signaling raise the important possibility that relatively small quantitative differences in expression of SOCS-1, or other SOCS, may lead to aberrant cytokine action that could in turn underlie disease susceptibility or severity.

Heterozygote mouse models with targeted disruption of one SOCS allele could prove useful to delineate the consequences of quantitative differences in SOCS expression for cytokine action on growth, function, or disease in gastrointestinal tissues.

Given the role that cytokine signaling plays in development and control of cell growth it is perhaps not surprising that SOCS-1 has been implicated as a tumor suppressor. Two groups have shown recently that the CpG islands within the *SOCS-1* gene are methylated in a large proportion of human hepatocarcinomas, which results in silencing of SOCS-1 expression and constitutive JAK activation.[71,72] Re-introducing SOCS-1 back into hepatocellular carcinoma cells is sufficient to reduce their growth rate and inhibit anchorage-independent growth. These findings implicate constitutive activation of the JAK/STAT pathway as a key factor in the development of hepatocellular carcinoma. The tumor suppressor activity of SOCS-1 warrants further investigation as a potentially important factor in susceptibility in cancer of other gastrointestinal tissues such as the colon or pancreas.

Although low-level, constitutive expression of SOCS-1 has been reported in small and large intestines of humans and rodents,[26,33,34] the cellular sites of SOCS-1 expression in intestines in vivo have not been defined. Given the apparent role of SOCS-1 in integrating IL-4 and IFN-γ action, more detailed studies of the levels and sites of SOCS-1 expression in intestines of IBD patients and animal models of IBD are warranted. One published report[73] and our preliminary findings[33] do not show altered expression of SOCS-1 mRNA in resected, diseased intestines from patients with UC or CD compared with nondiseased controls, or in the intestines of a number of animal models of IBD.[33,73] However, in these studies, SOCS-1 expression was analyzed in total RNA extracted from entire intestines at times when disease was well established. Analyses of SOCS-1 at different phases of disease and in isolated subpopulations of cells such as intraepithelial or lamina propria lymphocytes, or epithelial cells, are required to definitively establish if SOCS-1 expression changes in the intestine during the course of IBD. Recent, preliminary studies indicate that SOCS-1 is expressed in lamina propria monocytes from patients with IBD.[74] It is important to point out that much of our information to date about SOCS-1 expression, especially in cells or tissues in vivo, is at the mRNA level. Levels of expression of SOCS-1 are, however, subject to regulation at the level of translation, where in-frame AUG codons within the 5′ untranslated region permit translational repression.[75,76] Recent studies have

revealed that the Pim family of serine/threonine kinases regulate the stability of SOCS-1 such that Pim-mediated phosphorylation stabilizes SOCS-1^{−/−} and Pim^{−/−} mice show reduced levels of SOCS-1.[77] Thus, it will be essential to examine SOCS-1 expression at the protein as well as mRNA level to fully define its sites and levels of expression in normal or diseased gastrointestinal tissues.

Suppressors of Cytokine Signaling 2

Compared with SOCS-1, fewer cytokines induce SOCS-2 and these include GH and prolactin (Table 1). On cytokine exposure in vivo or in vitro, initial low levels of SOCS-2 expression climb steadily over a period of 8 hours or longer,[35,78] which contrasts with the rapid, more transient mRNA induction observed for SOCS-1 and other SOCS such as SOCS-3. Initial studies examined whether SOCS-2, like SOCS-1, modulates cytokine action in M1 cells. SOCS-2 overexpression caused only a partial inhibition of IL-6 signaling, which contrasts with the more potent inhibition by SOCS-1.[37] No inhibitory effects of SOCS-2 overexpression have been noted in most hematopoietic cytokine assay systems, implying a limited role of SOCS-2 in immune function. Exceptions are chronic myeloid leukemia cells with Bcr-Abl mutation that show elevated SOC-2 expression.[79] Forced overexpression of SOCS-2 in these cells reduces growth and increases sensitivity to antagonists of the Bcr-Abl tyrosine kinase.[79]

A number of lines of evidence suggest that SOCS-2 may have a particular role in regulating the actions of GH. GH has a well-established role in mediating somatic and skeletal growth in the postnatal period as well as proportionate growth of many organs including the liver and small and large intestine.[80] Many, but not all, of the growth-promoting actions of GH are mediated by circulating IGF-I, which derives largely from hepatocytes[81] and by IGF-I, which is expressed in mesenchymal cells within nonhepatic tissues.[80,81] It recently has been shown that STAT5b is a key mediator of GH-induced IGF-I expression in the liver.[82] SOCS-2 binds to glutathione-S-transferase–GH–receptor fusion proteins in a phosphorylation-dependent manner and binds to a region of the intracellular domain of the GH receptor that contains 3 postulated STAT5 binding sites.[83–85] SOCS-2 overexpression experiments performed by Ram and Waxman[85] indicate that SOCS-2 decreases STAT5 activation in response to GH. These data support a concept that SOCS-2 may regulate GH-dependent transcriptional induction of the IGF-I gene, or other STAT5b responsive genes. Although these data suggest an inhibitory role of SOCS-2 on GH action, some initial observations paradoxically indicated that SOCS-2 overexpression enhances the activity of STAT5 reporter genes.[78,83,86] It has been suggested that these dual actions of SOCS-2 may be determined by the levels of expressed SOCS-2 and the possibility that SOCS-2 may attenuate the actions of SOCS family members with more potent inhibitory capabilities.[79,86] Consistent with this concept, the decrease in GH-dependent STAT5 activation that results from SOCS-2 overexpression is not as dramatic as the complete suppression observed when SOCS-1 and SOCS-3 are overexpressed.[85,87] Defining the physiologic role of SOCS-2 has been complicated further by evidence that SOCS-2 interacts with the IGF-I receptor, as well as the GH receptor. Yeast 2-hybrid assays found that SOCS-2 interacts with the intracellular tail of the IGF-I receptor, and biochemical studies revealed that GST-SOCS-2 fusion proteins bind to activated IGF-I receptor.[88] Furthermore, FLAG epitope-tagged hSOCS-2 co-immunoprecipitates with active IGF-I receptor in transiently transfected cell lines.[88] There currently is no evidence that IGF-I is capable of inducing SOCS-2 expression, but these findings imply that SOCS-2, induced by GH or other cytokines, could alter IGF-I as well as GH action.

The phenotype of SOCS-2^{−/−} mice provides considerable evidence that SOCS-2 plays an important in vivo role in GH action, and possibly the actions of IGF-I. SOCS-2^{−/−} mice are viable and, unlike the SOCS-1^{−/−} mice, are healthy from birth to adulthood and show no detectable abnormalities in the hematopoietic system.[89] Male and female SOCS-2^{−/−} mice show a 30% to 40% increase in body weight by 12 weeks of age that involves significant increases in body, tail, and long-bone lengths. The increased body weight in SOCS-2^{−/−} mice is not owing to fat accumulation and many of the internal organs, including the liver, are enlarged in proportion to the increase in body size.[89] Although the original analyses of SOCS-2^{−/−} mice did not examine the intestine, recent preliminary studies show an increase in weight of the small intestine (Heath et al., unpublished observations, 2001). The growth abnormalities in SOCS-2^{−/−} mice have obvious similarities to those reported in GH- and IGF-I–overexpressing transgenic mice,[9,11,90,91] providing strong support for a hypothesis that SOCS-2 modulates GH- or IGF-I–dependent growth. Until recently it was thought that circulating IGF-I was determined largely by the levels of IGF-I expression in the liver and, specifically, hepatocytes. Hepatic levels of IGF-I expression are highly dependent on GH.[81] Unexpectedly, however, SOCS-2^{−/−} mice do not have detect-

able increases in circulating IGF-I levels compared with wild-type mice.[89] SOCS-2 may therefore play a minor role in limiting GH-dependent IGF-I expression in the liver or, because GH induces multiple SOCS family members in the liver,[92] it is possible that they may compensate for any effect of SOCS-2 deficiency on hepatic IGF-I synthesis. The fact that SOCS-2$^{-/-}$ mice exhibit increased growth yet normal circulating IGF-I challenges the view that circulating IGF-I is the major determinant of postnatal body growth, as do observations that postnatal growth is normal in mice with hepatocyte-specific IGF-I gene deletion and 25% normal circulating IGF-I.[81] Increasingly it appears that the levels of local IGF-I expression in nonhepatic tissues may be integral to normal growth.[81] Consistent with this concept, SOCS-2$^{-/-}$ mice show increased expression of IGF-I in a number of nonhepatic tissues. This may lead to organ or body overgrowth and relate to specific or preferential up-regulation of SOCS-2 by GH in these tissues compared with liver. More recently, it has been discovered that the high growth mouse, which also exhibits accelerated post-natal growth,[93] has a mutation resulting in the deletion of the majority of the SOCS-2 coding region.[94] The phenotype of the high-growth mouse is nearly identical to that of the SOCS-2$^{-/-}$ mouse, except that moderately elevated circulating IGF-I levels are detected in high-growth mice in a time- and strain-dependent manner.[93–95]

Distinguishing whether SOCS-2 preferentially regulates either the GH or the IGF-I signaling pathway based on the phenotype of SOCS-2$^{-/-}$ mice is difficult. This is because of the intertwined relationships between GH and IGF-I, low receptor expression in most tissues, and the difficulty in deriving primary cell lines that are GH responsive. However, primary hepatocytes from SOCS-2$^{-/-}$ mice display modest prolongation of STAT5 phosphorylation compared with wild-type hepatocytes, and crossing SOCS-2$^{-/-}$ with STAT5b$^{-/-}$ mice has revealed that STAT5b is critical to the manifestation of the SOCS-2 null growth phenotype.[96] This provides compelling evidence that altered STAT5b activation contributes to the phenotypic consequences of absolute SOCS-2 deficiency.

Our long-term interest in the enterotrophic actions of GH and IGF-I[80] led us to consider the possibility that SOCS-2 may be an important determinant of the functional outcome of GH or IGF-I action on intestinal growth. Similar increases in mass of the small intestinal mucosa and circulating IGF-I levels occur in transgenic mice with widespread expression of GH[9] or IGF-I.[91] However, IGF-I, but not GH transgenics, show sus-

tained increases in crypt cell proliferation through adulthood whereas GH but not IGF-I transgenics show increased sucrase activity.[9,91] In a rat total parenteral nutrition (TPN) model, systemic IGF-I had much more potent actions on crypt cell proliferation than GH, even though GH potently increased circulating IGF-I levels.[97,98] These differences led us to consider the possibility that GH may somehow limit the proliferative actions of IGF-I on the intestine.[80] This is a significant issue because GH is one of few cytokines, if not the only cytokine, used clinically to promote enhanced functional capabilities in patients on long-term TPN owing to short bowel syndrome.[99–101] One concern in the clinical use of GH in these patients is the possibility that sustained GH-dependent increases in crypt cell proliferation could increase susceptibility to colorectal cancer.[80] Our recent studies support the concept that SOCS-2 may serve to limit crypt cell proliferation in response to GH or IGF-I induced by GH as depicted in the hypothetical model in Figure 3. In a rat TPN model, GH selectively induced SOCS-2 but not SOCS-1, SOCS-3, or CIS, and IGF-I did not significantly affect expression of any of the SOCS mRNAs (Miller et al., unpublished data, 2002).[34] This selective effect of GH on SOCS-2 in the small intestine differs from its effects in liver where SOCS-1, -2, -3, and CIS are all induced by GH.[78] It is important to point out that TPN itself does not increase the levels of SOCS-2 expression in the intestine compared with oral feeding (Miller et al., unpublished data, 2002)[34] and we have observed GH-dependent increases in SOCS-2 expression in the intestine of orally fed mice (Miller and Lund, unpublished data, 2002). Thus, it seems likely that SOCS-2 plays a role in GH action on intestines in both TPN-fed and orally fed animals. GH induces SOCS-2 in Caco-2 colon cancer–derived epithelial cell lines, indicating that SOCS-2 is a product of intestinal epithelial cells. Forced overexpression of SOCS-2 in Caco-2 cells reduces spontaneous proliferative activity and the ability of both GH and IGF-I to stimulate Caco-2 cell proliferation (Miller et al., unpublished data, 2002).[102] Together, these findings suggest that SOCS-2 may have a
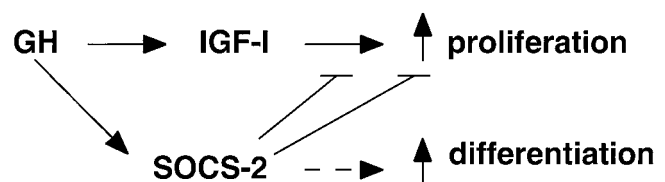
Figure 3. Model predicting the role of SOC-2 in GH-mediated proliferation and differentiation of intestinal epithelium. GH initially induces IGF-I, which stimulates crypt cell proliferation, but subsequent induction of SOCS-2 prevents sustained proliferation. SOCS-2 may impact on differentiation but this has not been examined definitively.

particular role in limiting the magnitude or duration of the proliferative actions of GH and IGF-I on intestinal epithelial cells. Mechanistically, it will be important to establish if the primary role of SOCS-2 is to prevent GH action or to terminate its actions once initiated. Available evidence suggesting that GH-dependent activation of STATs precedes SOCS-2 induction and the established role of SOCS as STAT-induced feedback inhibitors of cytokine action in other systems favor the latter possibility. Ongoing studies are further evaluating SOCS-2 action in additional intestinal epithelial cell lines and in intestines of SOCS-2$^{-/-}$ mice to test if SOCS-2 plays a primary role in regulating proliferation or affects differentiation as well (Figure 3). It is noteworthy that the ability of SOCS-2 to inhibit proliferation of the Caco-2 colon cancer cell line raises the possibility that SOCS-2 could have tumor-suppressive actions in colon cancer. Together with the evidence for a similar role of SOCS-1 in hepatocellular carcinoma, this emphasizes the potential significance of these cytokine suppressors in controlling tumorigenesis within the gastrointestinal tract.

SOCS-2 may be relevant to potential complications of GH therapy in patients with CD. A small, placebo-controlled trial suggested beneficial effects of GH in these patients[103] and experimental data in animal models of intestinal inflammation indicated beneficial actions of GH to stimulate mucosal repair and remission of inflammation.[10,104] However, a potential concern is that GH therapy could exacerbate fibrosis, a complication observed in CD but not UC.[105] This concern derives from observations that increases in locally expressed IGF-I in mesenchymal cells at sites of fibrosis are a common feature of CD and animal models of CD.[105,106] GH could further increase IGF-I expression and fibrosis. In TPN-fed rats, preliminary data show that GH and IGF-I both increase collagen expression, but less potent actions of GH correlate with GH induction of SOCS-2 at sites of collagen synthesis.[107] These preliminary findings in the TPN model provide indirect evidence that SOCS-2 could limit the fibrogenic complications of GH therapy in other situations such as CD. There is in fact in vivo evidence that SOCS-2 limits collagen accumulation in other organs because SOCS-2$^{-/-}$ mice show marked thickening of the dermis owing to increased collagen deposition and collagen accumulation in some ducts and vessels.[89] The possibility that SOCS-2 or other SOCS limit cytokine-induced collagen accumulation warrants further investigation because this is relevant to the incurable complications of fibrosis associated with chronic injury or inflammation of gastrointestinal tissues.

Suppressors of Cytokine Signaling 3

After SOCS-1, SOCS-3 is the most extensively studied member of the SOCS family. SOCS-3 shares several features in common with SOCS-1. This includes a high degree of amino acid homology and induction by a wide range of cytokines including IL-2, IL-3, IL-4, IL-6, IL-10, GH, leptin, prolactin, LIF, EPO, thrombopoietin, IFN-γ, tumor necrosis factor α, and insulin. Similar to SOCS-1, SOCS-3 inhibits the actions of multiple cytokines (Table 1) and is rapidly but transiently induced by cytokines. In addition, SOCS-3 can be immunoprecipitated with JAKs as observed for SOCS-1. However, several studies indicate that in contrast to SOCS-1, SOCS-3 has little[108,109] or no effect[37] on JAK activation in vitro. Because this suggests a different mode of action of SOCS-3 compared with SOCS-1, recent studies assessed the ability of SOCS-3 to bind to potential target phosphopeptide sequences present within JAKs, STATs, and the common gp130 subunit shared by a number of cytokine receptors including the IL-6 and LIF family of cytokines. These studies revealed that SOCS-3 has the highest affinity for the tyrosine 757 of the gp130 receptor and that mutation of the Tyr 757 residue to alanine ablates the ability of SOCS-3 to inhibit gp130 receptor signaling.[110,111] This is intriguing because Tyr 757 is also the site of interaction of SHP-2, a phosphatase that is thought also to be a negative regulator of gp130 signaling.[112–114] In light of the new data showing that SOCS-3 inhibits gp130 signaling by binding to Tyr 757, it is possible that previous reports of negative regulation via Tyr 757 attributed to SHP-2, may in fact be owing to the actions of SOCS-3.

Despite the evidence that SOCS-3 binds to the gp130 subunit and JAKs, the mechanisms by which SOCS-3 suppresses cytokine signaling are not yet fully defined. Evidence that the N-terminal regions of SOCS-3 can be substituted with that of SOCS-1 indicates that at least this region of SOCS-3 may be involved in the binding of JAK, but may not affect its kinase activity.[37] Experimental data examining SOCS-3 suppression of EPO signaling supports such a model in which key residues in SOCS-3 have been defined that are critical for binding the EPO receptor and JAK.[108] However, the actions of SOCS-3 may not be restricted to the termination of signaling but could include signal modification. SOCS-3 is the only SOCS protein known to be phosphorylated[32,115] and Cacalano et al.[116] have discovered that this modification allows SOCS-3 to inhibit STAT activity, but at the same time enhance extracellular-signal–regulated kinase (Erk) activity by recruiting p120 RasGAP, an important me-

diator of Erk activation. A recent study indicated that deletion of the STAT3 binding site in the gp130 receptor leads to impaired induction of SOCS-3 and SOCS-2, and mice with this mutation show gastric ulceration, and sustained SHP-2–mediated Ras/Erk activation.[117] This suggests that SOCS may mediate reciprocal cross-talk between SHP-2/Erk activation and STAT activation. The gastric ulceration phenotype in these mice indicates that normal STAT and SOCS expression or the balance between STAT and SHP-2/Erk activation may be highly relevant to normal mucosal integrity in the stomach.

SOCS-3–deficient mice have been generated and are reported to die in utero between E12 and E16 owing to marked erythrocytosis around the liver and in the abdominal region. Colony assays of hematopoietic progenitor cells from E12–E16 SOCS-3$^{-/-}$ embryos also revealed that IL-3/EPO-stimulated blood forming unit–E and colony forming unit–mix colonies were significantly larger than those derived from wild-type cells. Furthermore, fetal liver erythropoiesis was suppressed completely when SOCS-3 was transgenically overexpressed in embryos.[51] Together these results strongly support initial overexpression studies implicating SOCS-3 as a key regulator of EPO signaling.[108] A more recent study characterized an independently generated SOCS-3$^{-/-}$ mouse and found that these mice die in utero between E11–E13. In contrast to the previous results, these SOCS-3$^{-/-}$ mice do not suffer from erythrocytosis and have no major defects in hematopoiesis or erythropoiesis in a variety of assays.[118] However, these mice do display placental defects at E10.5, which would most likely account for the small size of SOCS-3$^{-/-}$ embryos compared with wild-type embryos and may have contributed to their mortality. Examination of SOCS-3 pre- and postnatal function is hampered by embryonic mortality, but it is hoped that the adoption of other technologies, such as cell-specific, Cre/lox-mediated gene disruption will bypass this impediment.

The importance of SOCS, specifically SOCS-3, to inflammatory processes in the intestine has been brought to light by recent studies in patients with IBD and in animal models of IBD. Inflamed colon samples from patients with UC or CD and from multiple mouse models of spontaneous or inducible T-cell mediated colitis all exhibit elevated levels of SOCS-3.[33,73] The animal models include IL-10$^{-/-}$ mice, mice with macrophage-specific STAT-3 deletion, T-cell receptor α chain–deficient mice, mice with colitis induced by trinitrobenzene-sulfonic acid or sodium dextran sulfate (DSS)-induced colitis, and rats with enterocolitis induced by peptidoglycan

polysaccharides. The DSS colitis model is one of acute mucosal injury and inflammation followed by mucosal repair when DSS is withdrawn. In this model, SOCS-3 mRNA expression is highest after withdrawal of DSS, indicating a potential role in mucosal repair or remission of inflammation.[33,73] SOCS-3 mRNA expression during DSS-induced intestinal inflammation localized largely to the mucosa in hyperplastic epithelial cells and lamina propria cells that may be immune cells or mesenchymal cells or both.[33,73] Particularly intense up-regulation of SOCS-3 occurs at sites of mucosal ulceration.[33] Although 2 studies suggest that SOCS-3 is preferentially up-regulated in IBD and animal models of IBD compared with SOCS-1, SOCS-2, or CIS, it is important to consider the limitations in detection of changes in RNA or protein expression is small subpopulations of cells when analyzing whole tissues. It will be important, therefore, to perform additional analyses of other SOCS family members as well as SOCS-3 at the cellular level. Localization of SOCS-3 to epithelial cells in animal models of IBD[33,73] and to hyperplastic epithelial cells[73] and sites of ulceration[33] raises the intriguing possibility that SOCS-3 may play a role in either mucosal restitution or in limiting the magnitude or duration of crypt hyperproliferation that accompanies mucosal repair. It is difficult at this point to directly test the role of SOCS-3 in intestinal inflammation in vivo without viable adult SOCS-3 knockout or transgenic animals. However, transgenic mice engineered to express low levels of SOCS-1 and -3 proteins containing a point mutation in the kinase inhibitory region do exhibit signs of more severe DSS-induced colitis than wild-type mice, including more severe weight loss, epithelial hyperplasia, goblet cell depletion, and increased STAT-3 activation.[73] Because the level of phosphorylated STAT-3 correlates qualitatively with the severity of colitis across the mouse models,[73] this evidence that SOCS-3 inhibits STAT-3 activation indicates a protective role during intestinal inflammation. Nonetheless, additional studies are required to precisely define the role of SOCS-3 in different models of IBD and in repair after inflammation-induced intestinal injury. Recent adenovirus-mediated delivery of SOCS-3 into the ankle joints of animals with arthritis was shown to drastically reduce disease severity.[119] Adenovirus-mediated SOCS-3 delivery to the intestine therefore holds promise for defining its role in the intestine during experimentally induced inflammation, which in turn would provide insights into whether SOCS-3 may be a useful future target for development of new therapies for IBD.

Cytokine Inducible SH2-Domain Containing Proteins

CIS was discovered in a search for immediate-early cytokine-responsive genes[20] and shares the highest sequence and structural homology with SOCS-2. CIS is induced in response to many hematopoietic cytokines such as IL-1, IL-6, IL-12, IL-13, LIF, G-colony stimulating factor, thrombopoietin, IL-2, IL-3, EPO, but also a number of nonhematopoietic cytokines such as GH, leptin, and prolactin (Table 1). The mRNA induction pattern of CIS is rapid and transient as for SOCS-1 and SOCS-3, except there may be a second or more prolonged phase of CIS induction in some systems.[35] Initial in vitro experiments showed that CIS was capable of inhibiting EPO, IL-2, IL-3, GH, and prolactin signaling to various degrees of effectiveness, but displayed a different mechanism of action compared with the other SOCS studied to date. CIS requires receptor phosphorylation before it can bind to cytokine receptors and its inhibitory effects are JAK independent.[20,120] Overexpression studies have shown that CIS can bind to only one of 2 STAT5 binding sites on the EPO receptor and inhibits only a proportion of STAT5 activity. This had led to a concept that CIS may compete with STAT5 for receptor docking sites.[120,121] This is supported by work examining the role of CIS in GH signaling, which has revealed that CIS inhibition of GH signaling is more complete at lower levels of STAT5b expression.[87] Because of the transient nature of CIS mRNA expression it is an attractive hypothesis that CIS may play a role in the suppression of pulsatile GH-induced STAT5 activity that typifies GH action in men in particular. CIS is capable of binding to the C-terminal portion of the GH receptor that contains STAT5 docking sites[83,85] and can inhibit a proportion of STAT5 activity. The use of the proteasomal degradation blocker, MG132, has shown that CIS inhibition of GH signaling involves a 2-step mechanism: the first is the binding of CIS to STAT5 binding sites, and the second is subsequent targeting of bound receptor complexes for proteasomal-targeted destruction.[87] This 2-step mechanism may be crucial for the time-dependent inhibition of GH signaling that is thought to cycle every 3–4 hours.

As with other SOCS, genetically modified mice were created in an attempt to clarify the physiologic role of CIS. CIS transgenic mice expressing high levels of CIS via the β-actin promoter have been produced and suffer from a complex phenotype. This includes reduced body weights in adult male and female mice, lower levels of the GH-dependent protein MUP, and a failure to lactate owing to defective terminal differentiation of the mammary gland.[122] These phenotypes are reminiscent of mice lacking STAT5α and/or STAT5β.[123–125] CIS transgenic mice also display a depletion in numbers of γδ T cells, natural killer, and natural killer T cells, as well as some inhibition of IL-2 induced by STAT5 activity in T cells. Transgenic expression of CIS in CD4+ T cells also implicates CIS as a regulator of T-cell receptor–mediated T-cell activation.[126] Paradoxically, CIS-deficient animals display no obvious phenotype to date.[51,56] It also is surprising that the CIS transgenic and knockout mice display no abnormality in blood cell production given the predicted importance of CIS in regulating EPO function.[20,120] At present, little is known about CIS in the gastrointestinal tract except that it is expressed (Miller et al., unpublished data, 2002)[34] and may show distinct patterns of regulation in lamina propria vs. peripheral lymphocytes.[74]

Other SOCS Family Members

Relatively little is known about the functions of the other SOCS family members including the signaling systems they regulate. SOCS-4 mRNA is expressed in human colonic mucosa.[32] SOCS-5 mRNA is induced in mouse liver 8–12 hours after injection of IL-6 and inhibits a proportion of IL-6–mediated M1 cell differentiation,[24,37] but a definitive role for this molecule is yet to be discovered. SOCS-6 was recently found to bind to the insulin receptor in an insulin-dependent manner in which it inhibited activation of extracellular-signal–regulated kinase 1/2 and protein kinase B in vivo, as well as IR-directed phosphorylation of insulin receptor substrate-1 in vitro.[127] This indicates a possible role of SOCS-6 in insulin resistance. Recent studies revealed that SOCS-6 binds to insulin receptor substrate-4, a molecule important in insulin receptor signaling and that SOCS-6$^{-/-}$ mice are slightly growth retarded.[128] SOCS-7 (also known as NAP-4) has been found to interact with the SH3 domain of Nck, as well as binding to Ash, phospholipase-Cγ, and the epidermal growth factor receptor in vitro.[129] Whether these observations reflect the physiologic function of this molecule is unknown.

The functions of the SOCS-related proteins that contain a SOCS box but no SH2 domain is obscure. A number of them have been shown to interact with elongin B and C through their SOCS box.[27,28] Extensive analysis of ankyrin repeats-1 transgenic and knockout mice failed to define any hematopoietic role for this protein despite strong expression in the hematopoietic compartment.[130] More recent work has found that Asb-2 is induced rapidly by all-trans retinoic acid, which acts on retinoid receptors or retinoid X receptor binding elements in the ankyrin repeats 2 promoter.[131] Interest-

ingly, chicken WD-40 repeats-1 (also called SwiP-1) is induced by sonic hedgehog in somatic mesoderm explant cultures and developing limb buds.[132] Whether it plays a negative role in sonic hedgehog signaling is yet to be shown, but given the importance of sonic hedgehog in intestinal development, this warrants further study.

Conclusions and Future Implications

Numerous in vitro experiments have highlighted the diverse role that CIS, SOCS-1, -2, and -3 proteins may play in the negative regulation of cytokine signaling. Information is beginning to emerge about SOCS-4, -5, -6, and -7. The generation of genetically modified mice has validated and assigned a number of the SOCS members as key mediators of important physiologic processes (Table 2). A detailed understanding of the biochemical mechanisms of SOCS action is being generated. Further molecular and gene modification studies will help to confirm and extend the current models and hypotheses and define what role SOCS proteins play in specific gastrointestinal functions and diseases. The emerging information indicates that these proteins may be integral to appropriate and coordinated actions of cytokines and growth factors in the gastrointestinal tract and accessory organs. As shown in Figure 4, SOCS appear

likely to play key roles in cytokine or growth factor action on multiple cell types in the gastrointestinal tract and in multiple physiologic and pathophysiologic states. A wealth of evidence suggests that SOCS will impact on cytokine regulation of immune cell function or phenotype and are essential to appropriate termination of inflammation. In this regard, SOCS may be pivotal in dictating whether acute inflammatory states persist to become diseases of chronic inflammation (Figure 4). Emerging evidence suggests a role of SOCS in intestinal mesenchymal cells where inhibition of cytokine/growth factor action by SOCS could help determine the balance between normal repair or excessive cytokine responses leading to fibrosis (Figure 4). In crypt epithelial cells, SOCS may limit hyperproliferative responses associated with renewal, repair, or regeneration of mucosal epithelium. It remains to be established if these roles are particularly important after mucosal damage by proinflammatory cytokines. SOCS also may play a key role in normal crypt homeostasis or as tumor suppressors in the intestine as appears to be the case for SOCS-1 in liver (Figure 4). At present, these putative actions of SOCS in the gastrointestinal tract are based largely on findings in other systems and a number of challenges remain in fully defining the roles of SOCS in the gut and other systems. Their widespread expression in multiple cell types poses

Table 2. Summary of Genetic Modification Studies of SOCS Genes and Their Implications for Function

Protein	Phenotype	Implicated function
CIS		
Knockout	No phenotype	Unknown, redundancy?
Transgenic	Retarded growth, impaired mammary gland development and lactation, reduced T-cell populations	Represses STAT5 activity
SOCS-1		
Knockout	Die prenatally, multiple hematopietic abnormalities, liver necrosis, macrophage organ infiltration	Controls sensitivity to IFN-γ, possible T-cell function/selection
Transgenic		
DN transgenic	Defective thymocyte development and perturbed homeostasis of T cells	Affects sensitivity to IFN-γ as well as other cytokines
Knockin–lacks SOCS box	Low expressors have no overt phenotype, but are more susceptible to DSS-induced colitis	Hyperactivation of STAT3 results in severe colitis, infers SOCS-3 regulates intestinal inflammation responses
	Similar to SOCS-1, but less prolonged signaling disfunction and disease progression	Regulates cytokine signaling, possibly by interacting with E3 ubiquitin ligase
SOCS-2		
Knockout	Excessive growth, some elevated IGF-I mRNA, thicker skin, collagen deposition, lower MUP	Regulation of GH and/or IGF-I pathways
SOCS-3		
Knockout	Embryonic lethal (E11-E13), erythrocytosis in abdominal organs	Controls EPO signaling
	Placental insufficiency	Involved in placental development
Transgenic	Embryonic lethal (E11-E13), erythronemia	Controls EPO signaling
SOCS-6	Mild growth retardation	Unknown
Knockout		
ASB-1		
Knockout	No phenotype	Unknown
Transgenic	No phenotype	Unknown

Multiple actions of SOCS in gastrointestinal tract
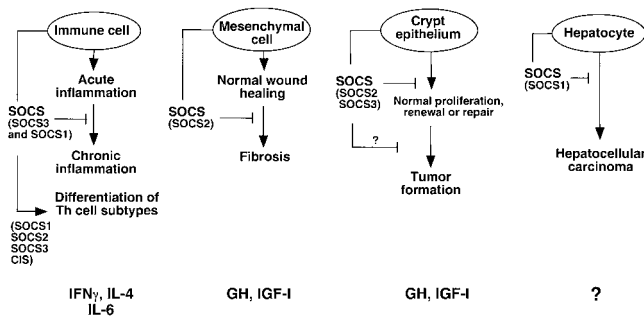
Figure 4. Possible roles of SOCS in normal and diseased gastrointestinal tissue. ⊣, Inhibitory effect; SOCS subtypes in parentheses are those implicated in particular events or cell types based on available evidence in cultured cells or mouse models. Cytokines at the bottom are those for which available evidence suggests that SOCS play a major role in their actions on specific gastrointestinal cell types. It is important to emphasize, however, that it seems likely that SOCS will play a role in the actions of a wider range of cytokines than indicated here.

a challenge in unraveling cell-specific actions. Derivation of mice with cell-specific deletion of particular SOCS, for example, using intestinal epithelial, immune, mesenchymal cell, or hepatocyte-specific promoters, and the Cre/Lox system has particular potential to elucidate the role of individual SOCS proteins in the actions of particular cytokines and growth factors on intestinal growth, inflammation, wound healing, fibrosis, and cancer. Better definition of the cell-specific patterns of expression of each SOCS family member and elucidation of the consequences of expression of multiple SOCS in some cell types or situations vs. predominance of one particular SOCS in others also is desirable. This will require in vivo localization studies in normal and diseased gastrointestinal tissue and creative cross-breeding approaches to derive genetically manipulated mice with cell-specific alterations in multiple SOCS proteins.

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