Suppressor of Cytokine Signaling 1 Interacts with the Macrophage Colony-stimulating Factor Receptor and Negatively Regulates Its Proliferation Signal*

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Macrophage colony-stimulating factor receptor (M-CSF-R) is a tyrosine kinase that regulates proliferation, differentiation, and cell survival during monocytic lineage development. Upon activation, M-CSF-R dimerizes and autophosphorylates on specific tyrosines, creating binding sites for several cytoplasmic SH2-containing signaling molecules that relay and modulate the M-CSF signal. Here we show that M-CSF-R interacts with suppressor of cytokine signaling 1 (Socs1), a negative regulator of various cytokine and growth factor signaling pathways. Using the yeast two-hybrid system, in vitro glutathione S-transferase-M-CSF-R pull-down, and in vivo coimmunoprecipitation experiments, we demonstrated a direct interaction between the SH2 domain of Socs1 and phosphorylated tyrosines 697 or 721 of the M-CSF-R kinase insert region. Moreover, Socs1 is tyrosine-phosphorylated in response to M-CSF. Ectopic expression of Socs1 in FDC-P1/MAC and EML hematopoietic cell lines decreased their growth rates in the presence of limiting concentrations of M-CSF. However, Socs1 expression did not totally suppress long term cell growth in the presence of saturating M-CSF concentrations, in contrast to other cytokines such as stem cell factor and interleukin 3. Taken together, these results suggest that Socs1 is an M-CSF-R-binding partner involved in negative regulation of proliferation signaling and that it differentially affects cytokine receptor signals.

Hematopoiesis is mainly under the control of soluble and membrane-bound factors acting through specific transmembrane receptors (1). These receptors transduce intracellular messages by recruiting and activating a given set of cytoplasmic molecules that initiate specific signaling pathways, which then spread throughout the compartments of the cell, modifying its activity (2). What results from setting off these multiple biochemical signals is a global cellular response: proliferation, differentiation, activation, and/or survival. It is therefore important to determine the identity, stoichiometry, and function of the molecules present in the initial signaling complex formed in concert with the growth factor receptor.

Macrophage colony-stimulating factor receptor (M-CSF); also called CSF-1) is a key regulator of monocytic lineage development. In bone marrow, M-CSF stimulates proliferation and differentiation of committed progenitors, leading to the production of blood monocytes and tissue macrophages (3, 4). Osteopetrotic op/op mutant mice lacking functional M-CSF show impaired monocyte development and deficiency in osteoclasts and macrophages and can be cured by M-CSF injection (5). All the biological effects of M-CSF are mediated by a single receptor, encoded by the protooncogene c-fms, which is expressed on the surface of cells undergoing monocytic development (6, 7). Ectopic expression of the M-CSF receptor (M-CSF-R or Fms) in different hematopoietic cell lines enables M-CSF-dependent proliferation and monocyte/macrophage differentiation (8–10). Fms is a member of class III of the receptor tyrosine kinase family, which includes Kit, Flk3, and the α and β platelet-derived growth factor receptors (11). Ligand binding induces receptor dimerization and trans-autophosphorylation in specific tyrosines of the cytoplasmic domain, creating binding sites for src homology 2 (SH2)-containing proteins (12). Known Fms partners include Src family members (13), Grb2 and Mona adapters (14, 15), the p85 subunit of phosphatidylinositol 3-kinase (16), phospholipase Cγ2 (17), and FMIP (18). Some of these interactions are difficult to detect in vitro, being transient or expressed only at one particular stage of monocyte development, and have been revealed only by means of sensitive techniques such as yeast two-hybrid screen (15, 17, 18).

The suppressor of cytokine signaling 1 (Socs1) protein, also called SSI-1 or JAB, is a member of the Socs family, composed of eight molecular adapters that negatively regulate cytokine signaling pathways (19–24). Analysis of mice with homozygous inactivation of the Socs1 gene confirmed its role as a negative growth signal regulator. Socs1−/− mice died within 21 days of birth, with a complex fatal neonatal disease, due to a defect in the negative regulation of interferon γ signaling (25–27). Socs1 binds to all Janus kinase family members and inhibits their tyrosine kinase activity, which is needed for the activation of

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1 The abbreviation used are: M-CSF, macrophage colony-stimulating factor; M-CSF-R, M-CSF receptor; SH, src homology; Socs1, suppressor of cytokine signaling 1; KI, kinase insert; WT, wild type; GFF, green fluorescent protein; IL, interleukin; CM, conditioned medium; SCF, stem cell factor; BHK, baby hamster kidney; IMDM, Iseove’s modified Dulbecco’s medium; FBS, fetal bovine serum; FACS, fluorescence-activated cell sorting; HA, hemagglutinin; GST, glutathione S-transferase.
signal transducer and activator of transcription (STAT). Because the Janus kinase pathway is also essential for signaling by many cytokine receptors devoid of intrinsic kinase activity, Socs1 may be a general negative regulator of signaling through these receptors.

Socs1 is also a modular multidomain protein that can interact with other signaling proteins. Its N-terminal region contains diproline sites that interact with the SH3-containing proteins Nck, Grb2, the p85 subunit of phosphatidylinositol 3-kinase, Fyn, and Itk kinases. The Socs1 SH2 domain binds to the N-terminal region of the guanine nucleotide exchange factor Vav in a phosphotyrosine-independent manner. The C-terminal Socs box, common to all Socs family members, interacts with B and C elongins (19, 28).

We have previously shown that Socs1 also binds to the Kit and Flt3 receptor tyrosine kinases. The interaction requires Socs1 SH2 domain and phosphorylation of the receptors on as yet unknown tyrosine autophosphorylation sites (19). Although it does not inhibit Kit tyrosine kinase activity, ectopic expression of Socs1 strongly suppresses the proliferative signal of the Kit receptor, suggesting that negative regulation involves not only Janus kinase inhibition but also other mechanisms as yet unknown (19). In the present study, we investigated the interaction between Socs1 and M-CSF-R to 1) identify the receptor site(s) of the interaction as a model for other receptor tyrosine kinases and 2) determine whether Socs1 has the same effect on two closely related receptor tyrosine kinases, Kit and Fms. We show that Socs1 directly associates with tyrosine-phosphorylated M-CSF receptor upon M-CSF binding. In vitro and in vivo experiments demonstrated that this interaction involves the SH2 domain of Socs1 and tyrosines 697 or 721 of the Fms kinase insert (KI) domain. Ectopic expression of Socs1 in two different hematopoietic cell lines inhibited M-CSF-dependent proliferation but only in the presence of limiting concentrations of M-CSF. This result suggests that Socs1 could act as a general negative regulator of receptor tyrosine kinase proliferation signals, but its effects may be quantitatively different depending on the type of receptor.

**EXPERIMENTAL PROCEDURES**

**Yeast Two-hybrid System**—As previously described (17), the LexA-Fms wild-type (WT) or K614A baits contain the entire cytoplasmic domain (amino acids 536–976) of murine Fms. Mutation of lysine 614, located in the ATP binding site, into alanine (K614A) results in a kinase-inactive Fms protein. The YRN974 yeast strain containing an integrated LexA-Operator-GFP cassette. Data are representative of three independent experiments. KD, kinase-dead.

**FIG. 1. Interaction between Fms cytoplasmic domain and Socs1 in the yeast two-hybrid system.** A, schematic representation of the Socs1 protein. P represents a PxxP diproline motif. The region encompassed by the clone 99 is shown. B, flow cytometry analysis of Fms/Socs1 interaction using the yeast strain YRN974 carrying an integrated LexA-Operator-GFP cassette. Data are representative of three independent experiments. KD, kinase-dead.

**FIG. 2. In vitro interaction between Fms KI domain and Socs1 SH2 domain.** GST-Fms KI fusion proteins were produced as phosphoproteins in Epicurian cells, immobilized on glutathione-Sepharose, and incubated with lysates of GP+E-86 cells expressing either WT or mutated (R105K) HA epitope-tagged Socs1. Total cell lysate and bound proteins were run on a 12% polyacrylamide gel, blotted, and probed with anti-HA antibody for the detection of Socs1 protein and with anti-Grb2 antibody as a positive control. A and B, effects of single, double, or triple tyrosine (Y) to phenylalanine (F) mutations in the Fms KI domain. C, effect of arginine (R) 105 to lysine (K) mutation in the phosphotyrosine-binding pocket of Socs1 SH2 domain.
was transformed with two plasmids: pBTM116, encoding LexA-Fms WT or R614A, and pVP16, either empty (as a negative control) or containing the Socs1 partial cDNA referred to here as clone 99, which was isolated in a previous two-hybrid screen using LexA-Kit as a bait (19). Three individual clones were isolated on selective agar plates and expanded in similar selective liquid medium. Exponentially growing cultures were then analyzed for fluorescence intensity using a Becton Dickinson FACScalibur flow cytometer. Plasmids, selective media, and the transformation protocol have been described previously (30).

**Growth Factors, Cell Cultures, and Infections**—The source of M-CSF was conditioned medium of 3T3 insect cells expressing recombinant murine M-CSF from a baculovirus vector (31). X63-interleukin 3 (IL-3) cell-conditioned medium (IL3-CM) was used as a source of IL-3 (3-2). The source of stem cell factor (SCF) was a conditioned medium from BHK/MKL cells (BHK/MKL-CM) (33). M1 cells (34, 35) and their derivatives were maintained in IMDM supplemented with 10% FBS, and either 1% IL3-CM or 1% murine M-CSF from a baculovirus vector (31). Murine Fms (amino acids 668–746) was cloned in pGEX1 (Amersham Pharmacia Biotech). The entire KI region of WT and mutant Fms was expressed as a fusion protein with GST and produced as tyrosine-phosphorylated GST-Fms KI fusion proteins as previously described (15). GST-Fms KI fusion proteins were immobilized on glutathione-Sepharose beads (Amersham Pharmacia Biotech). The beads were washed three times with 1 ml of 1% FBS containing 2 mM Na3VO4 and then stimulated or not with M-CSF (15,000 units/ml) for 1 h at 37 °C. Starved cells were then resuspended in phosphate-buffered saline, 1% FBS containing 2 mM Na3VO4 and then stimulated or not with M-CSF (15,000 units/ml) for different times at 37 °C or for 1 h in ice. Cells were lysed in ice-cold lysis buffer (15) containing a protease inhibitor mixture (Roche Molecular Biochemicals, number 1836170) and phosphatase inhibitor mixture II (Sigma, number P5726). Equalized cell lysates were mixed for 18 h with 5 µl of antibody to Socs1 antiserum and 15 µl of protein G coupled to agarose beads (Amersham Pharmacia Biotech). The beads were washed three times with 1 ml of lysis buffer, and bound proteins were released by boiling for 5 min in Laemmli buffer. Proteins from cell lysates and immunoprecipitates were separated on SDS-polyacrylamide gels, transferred to nitrocellulose membrane, and probed with various antibodies as previously described (15). Antibody binding was visualized using horseradish peroxidase-conjugated secondary antibodies (Sigma) and enhanced chemiluminescence reagent (ECL+, Amersham Pharmacia Biotech).

**Binding Assay Using Phosphorylated Glutathione S-Transferase (GST)-Fms KI Fusion Proteins**—The entire KI region of WT and mutant murine Fms (amino acids 668–746) was cloned in pGEX1 (Amersham Pharmacia Biotech) and produced as tyrosine-phosphorylated GST-KI fusion proteins as previously described (15). For the binding assay, 12.5 µg of immobilized fusion proteins were mixed for 18 h at 4 °C with 750 µg of cell lysates of GP+ or GP-E8+ cells expressing either WT or mutated (R105K) HA epitope-tagged Socs1 (19). The beads were washed three times with 1 ml of lysis buffer, and bound proteins were released by boiling for 5 min in Laemmli buffer. Total cell lysates and bound proteins were then investigated on SDS-polyacrylamide gels, transferred to nitrocellulose membrane, and probed with various antibodies as previously described (15). Antibody binding was visualized using horseradish peroxidase-conjugated secondary antibodies (Sigma) and enhanced chemiluminescence reagent (ECL+, Amersham Pharmacia Biotech).

**RESULTS**

**Interaction of M-CSF-R with Socs1 in the Yeast Two-hybrid System**—In a yeast two-hybrid screen using LexA-Kit as a bait (19), we isolated a VP16-cDNA clone (clone 99) that encompasses the N-terminal and SH2 domain sequences of Socs1 (Fig. 1A). We then investigated the interaction of clone 99 with the LexA-Fms tyrosylphosphatase domain fusion protein, which is able to autophosphorylate in yeast and to interact with specific partners (17). The interaction was analyzed by flow cytometry using the YRN974
yeast strain in which the reporter gene encoded the GFP (29). As shown in Fig. 1B, yeast clones expressing either LexA-Fms wild type (WT) or LexA-Fms kinase-dead (KD) as bait, together with VP16, show only autofluorescence (thin lines). Coexpression of LexA-Fms WT and VP16-clone 99 (Fig. 1B, thick lines) increased the fluorescence intensity (left panel), whereas coexpression of LexA-Fms kinase-dead and VP16-clone 99 had no effect (right panel). These results suggested that one or more M-CSF-R tyrosine phosphorylation sites directly interact with Socs1.

Socs1 SH2 Domain Interacts with M-CSF-R Phosphotyrosine 
697 and 721—To confirm the specific interaction between Socs1 and Fms observed in yeast and to determine which Fms autophosphorylation sites are concerned, we investigated Socs1 interaction with Fms in vitro. GST fusion proteins containing either the WT Fms KI or KI mutated at each different autophosphorylation site (Tyr-697, Tyr-706, Tyr-721) were expressed in the Epicurian TKX1 bacteria strain that enables phosphorylation of tyrosine residues. The phosphorylated fusion proteins immobilized on glutathione-Sepharose were mixed with lysates of GP1E-86 cells expressing HA epitope-tagged Socs1. The presence of Socs1 (and Grb2 as a control) among precipitated proteins was examined by Western blotting using the relevant antibodies (Fig. 2A). Socs1 specifically associated with GST-Fms KI WT but not with GST alone, as was also the case for the positive control Grb2. As expected, mutation of tyrosine 697 abolished Fms interaction with Grb2 adapter (14). Although mutation of all three tyrosines abolished Socs1 interaction, none of the single mutations had an effect on Socs1 binding (Fig. 2A), suggesting that Socs1 is able to bind to two or three Fms phosphotyrosines.

Using the three combinations of double mutants, we found that mutation of both tyrosines 697 and 721 was the only combination that totally abolished Socs1 interaction with Fms (Fig. 2B). However, tyrosines 697 and 721 are binding sites of Grb2 and p85, respectively, both molecules that are able to bind to the diproline motifs in the Socs1 N-terminal region (19). To verify that the interaction observed in vitro between Socs1 and Fms was direct and not mediated by p85 and Grb2, we used a mutant of the phosphotyrosine-binding pocket of the Socs1 SH2 domain (R105K) (19) in the binding assay with GST-Fms KI WT. As shown in Fig. 2C, the R105K mutation totally abrogated Socs1 binding to Fms KI, demonstrating that the interaction is mediated through the Socs1 SH2 domain.

M-CSF-R Interacts with Socs1 and Induces Its Tyrosine Phosphorylation in Vivo—To examine the interaction between M-CSF-R and Socs1 in vivo, we expressed HA-Socs1 in the hematopoietic cell line M1 (34) using the pMiev-HA-Socs1-GFP retrovirus expressing both HA epitope-tagged Socs1 and the GFP protein from a single bicistronic mRNA (19). Because M1 cells do not normally express M-CSF-R (35), WT or triple mutant YTF (Phe-697, Phe-706, Phe-721) Fms was ectopically expressed in these cells by retroviral infections. M1/Fms WT or YTF cells expressing both Fms and Socs1 were either unstimulated or stimulated by M-CSF. Analysis of total cell lysates...
showed that both cell lines expressed similar amounts of Fms and Socs1 (Fig. 3A, bottom panels) and that both receptors were activated by M-CSF, because they induced similar protein tyrosine phosphorylation patterns (Fig. 3A, top panel), except for some bands including the receptor itself, as previously described (36). In addition, Socs1 expression did not modify the overall tyrosine phosphorylation patterns in response to M-CSF (data not shown). After immunoprecipitation with anti-Socs1 antibody, Western blot analysis showed that phosphorylated WT Fms but not YTF Fms coimmunoprecipitated with Socs1 in an M-CSF-dependent manner (Fig. 3B).

Some Fms-binding partners, such as p85 and phospholipase Cγ2, phosphorylate in response to M-CSF-R activation (17). Socs1 tyrosine phosphorylation was then examined after M-CSF stimulation at 37 °C for various times. M-CSF stimulation of M1/Fms WT/Socs1 cells induced very rapid protein tyrosine phosphorylation, which then decreased after 2.5 min (Fig. 3C). As ex-pected, Socs1 expression totally inhibited SCF-dependent proliferation in FDC-P1/MAC cells, but only in the presence of limiting concentrations of M-CSF (Fig. 4B). These results suggest that Socs1 may act as a negative regulator of the M-CSF-R proliferation signal in FDC-P1/MAC cells, but only in the presence of limiting concentrations of M-CSF.

To compare the effect of Socs1 on Kit and Fms signaling in the same cellular background, we expressed Socs1 in EML-Fms cells (38). Because we had previously shown that Socs1 suppresses SCF-dependent proliferation in EML cells (19), Socs1 was retrovirally transduced in EML-Fms cells maintained in the presence of a saturating concentration of M-CSF (1600 units/ml). EML-Fms/Socs1 and control EML-Fms/Miev cells were selected for high GFP expression (Fig. 4A), and Socs1 protein expression was verified by Western blotting (Fig. 5B). Cells were washed free of growth factors and then cultivated in the presence of low (200 units/ml) or high (1600 units/ml) concentrations of M-CSF or in the presence of a saturating concentration of SCF (10% BHK/MKL-CM) (Fig. 5C). As expected, Socs1 expression totally inhibited SCF-dependent proliferation and had no effect on M-CSF-dependent proliferation in the presence of 1600 units/ml M-CSF. However, when limiting concentrations of M-CSF (200 units/ml) were present, Socs1 was then able to block proliferation totally, and more than 90% of the EML-Fms/Socs1 cells died after a week of culture. This result confirmed that, as in FDC-P1/MAC cells, Socs1 negatively regulates the M-CSF-R proliferation signal.

CSF also induced macrophage differentiation in M1-Fms cells, and we did not observe any significant effect of Socs1 expression on this process. The effect of Socs1 on the M-CSF-dependent proliferation signal could not be investigated in M1-Fms cells because of their factor independence and their differentiation in response to M-CSF. As a control, we expressed Socs1 in the FDC-P1/MAC factor-dependent hematopoietic cell line that expresses endogenous M-CSF-R, and it can grow in medium supplemented either with IL-3 or M-CSF (37). Cells were infected by coculture with virus-producing GP+E86 cells expressing either empty pMiev or pMiev-HA-Socs1 retroviral vectors in the presence of saturating concentrations of IL-3 or M-CSF (1000 units/ml) and then sorted for GFP expression by FACS. When cells were infected in the presence of IL-3, we were able to isolate GFP-positive cells only from the control Miev population but not from Socs1-expressing cells. This result was expected, because Socs1 is a strong negative regulator of Janus kinases, which are necessary for the IL-3 signal. Moreover, we have observed in several cell systems that Socs1 overexpression did not allow cells to proliferate and survive in response to IL-3 (data not shown), in accordance with previous reports that IL-3 activation of luciferase reporter genes is suppressed by Socs1 (22, 24). On the contrary, when cells were infected in the presence of M-CSF, we were able to select cells with high GFP expression from both populations, referred to here as FD-MAC/Miev and FD-MAC/Socs1 (Fig. 4A). As with M1-Fms cells, Western blot analysis demonstrated the expression of Socs1 (Fig. 4B), its interaction with phosphorylated Fms, and its tyrosine phosphorylation in response to M-CSF in FD-MAC/Socs1 cells (Fig. 4C).

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The effect of Socs1 on growth rate was then examined using a short term proliferation assay in the presence of various M-CSF concentrations (Fig. 4D). At saturating concentrations of M-CSF (>500 units/ml), there was no significant growth rate effect of Socs1, although FD-MAC/Socs1 cells grew slightly more slowly than FD-MAC/Miev control cells. However, in the presence of limiting M-CSF concentrations (between 30 and 125 units/ml), there was a 50% decrease in the growth of FD-MAC/Socs1 cells. This effect was confirmed in a clonal assay, where FD-MAC/Socs1 cells produced fewer and smaller colonies as compared with the FD-MAC/Miev cells in the presence of 125 units/ml M-CSF (Fig. 4E). These results suggest that Socs1 expression totally inhibited SCF-dependent proliferation in FDC-P1/MAC cells, but only in the presence of limiting concentrations of M-CSF.
only at limiting M-CSF concentrations, suggesting that Socs1 has differential inhibitory effects on Kit and Fms signaling.

DISCUSSION

In the present study, we have characterized the in vitro and in vivo interaction between Socs1 and Fms/M-CSF-R. In vivo interaction between Socs proteins and several receptor tyrosine kinases has been previously reported; Socs1 binds to both Kit and Flt3 (19), Socs2 binds to the insulin-like growth factor receptor (41), and Socs3 binds to the insulin receptor (42). Until now, the receptor autophosphorylation sites involved in these interactions remained to be determined. Here we show that the SH2 domain of Socs1 binds to two phosphotyrosines located in the Fms kinase insert. These two sites are tyrosines 697 and 721, which are the Fms binding site for the Grb2 adapter and the p85 subunit of phosphatidylinositol 3-kinase, respectively. Interestingly, the Socs1 N-terminal region contains diproline motifs that recognize the SH3 domains of Grb2 and p85 (19), suggesting that Socs1 binding may not decrease, but on the contrary extend, the M-CSF-R signaling repertoire. Because these two sites are the first Socs1 binding sites to be described on receptors, it would be interesting to test whether Grb2 and/or p85 sites are also docking sites for Socs1 in other receptor tyrosine kinases. We have also shown that Socs1 was rapidly and transiently tyrosine-phosphorylated in response to M-CSF, as described for other Fms-interacting molecules (17). Socs2 and Socs3 tyrosine phosphorylation has recently been described in response to insulin-like growth factor and IL-2, respectively (43, 44). The precise role and importance of Socs tyrosine phosphorylation in regulating its function is yet unknown. Concerning Socs1, it would be interesting to locate the phosphorylation site(s) and to determine whether tyrosine phosphorylation also occurs in response to other signals and whether it affects such Socs1 functions as Janus kinase inhibition.

The biological relevance of the interaction between Socs1 and M-CSF-R is supported by the fact that Socs1 expression resulted in a decreased M-CSF-dependent proliferation of two different cell lines, including the FDC-P1/MAC cells that express endogenous M-CSF-R and are a physiological model for Fms signaling (37). Socs1 protein is unstable in cells, and Socs1 synthesis is strongly repressed at the translation level (45). Indeed, endogenous Socs1 protein has so far been described only in mouse T lymphocytes after immunoprecipitation (45). For this reason, we expressed Socs1 ectopically to study its functional interactions in vivo. Socs1 promoter expression has been detected in vivo in cells of the monocyte/macrophage lineage (27), suggesting that both M-CSF-R and Socs1 are coexpressed during monocytic development. Interestingly, Socs1

![Figure 5](http://www.jbc.org/)

**FIG. 5.** Socs1 expression in EML-Fms cells abolishes its growth in response to a limiting M-CSF concentration. EML-Fms cells were infected by either empty pMiev or pMiev-HA-Socs1 retroviral vectors in the presence of M-CSF (1600 units/ml) and sorted for GFP expression (A). HA epitope-tagged Socs1 expression was checked by Western blot analysis in EML-Fms/Socs1 cells as compared with control EML-Fms/Miev cells. Equal gel loading was checked by blotting with anti-SHC antibody (B). Cells were washed twice with IMDM and seeded at 5 × 10⁴ cells/ml in 1 ml of IMDM, 20% horse serum containing no growth factor, M-CSF (200 or 1600 units/ml), or SCF (1% of BHK/MKL-CM). The viable cell number was determined every 2 days, and cultures were split and fed. Data represent mean values of two independent experiments.
negatively regulated M-CSF-R proliferation signal only in the presence of nearly physiological limiting concentrations of M-CSF (46).

Altogether, our data suggest that Socs1 may act as a negative regulator of the M-CSF-R proliferation signal; there are different ways in which Socs1 could be involved in Fms signaling. First, M-CSF-R activates the Janus kinase/signal transducer-and-activator of transcription pathway in myeloid cells (47); Socs1 recruitment by M-CSF-R could then specifically inhibit this pathway. Socs1 may also link the receptor to the ubiquitin machinery, as demonstrated for Vav (28), which could occur through the interaction of the Socs box with ubiquitin ligase complexes. The present study also clearly shows that Socs1 does not equally affect proliferation signals of different receptors. Socs1 strongly suppressed IL-3 and SCF receptor signals, whereas its effects on M-CSF-R were less intense. In that respect, Socs1 offers an example of how a signaling molecule can differentially modify receptor signaling and thereby control the balance between self-renewal and commitment to differentiation. Finally, our experiments demonstrated that it is possible to obtain stable expression of Socs1 in IL-3- or SCF-dependent cell lines as long as they are maintained in the presence of high concentrations of M-CSF. Such cells represent valuable models for studying the early effects of Socs1 on other receptor signals after shifting the cells from M-CSF to other growth factor (e.g. IL-3 or SCF)-containing medium. In conclusion, our work provides the first evidence for a connection between M-CSF-R and Socs1, and we are now investigating the role of other members of the Socs family during monocyte development as controlled by M-CSF-R.

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