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The Chemoattractants, IL-8 and Formyl-Methionyl-Leucyl-Phenylalanine, Regulate Granulocyte Colony-Stimulating Factor Signaling by Inducing Suppressor of Cytokine Signaling-1 Expression

Nigel J. Stevenson,²* Serge Haan,²* Angela E. McClurg,* Michael J. McGrattan,* Marilyn A. Armstrong,* Peter C. Heinrich,† and James A. Johnston³*

Suppressors of cytokine signaling (SOCS) are encoded by immediate early genes known to inhibit cytokine responses in a classical feedback loop. SOCS gene expression has been shown to be induced by many cytokines, growth factors, and innate immune stimuli, such as LPS. In this paper, we report that the chemoattractants, IL-8 and fMLP, up-regulate SOCS1 mRNA in human myeloid cells, primary human neutrophils, PBMCs, and dendritic cells. fMLP rapidly up-regulates SOCS1, whereas the induction of SOCS1 upon IL-8 treatment is delayed. IL-8 and fMLP did not signal via Jak/STATs in primary human macrophages, thus implicating the induction of SOCS by other intracellular pathways. As chemoattractant-induced SOCS1 expression in neutrophils may play an important role in regulating the subsequent response to growth promoting cytokines like G-CSF, we investigated the effect of chemoattractant-induced SOCS1 on cytokine signal transduction. We show that pretreatment of primary human neutrophils with fMLP or IL-8 blocks G-CSF-mediated STAT3 activation. This study provides evidence for cross-talk between chemoattractant and cytokine signal transduction pathways involving SOCS proteins, suggesting that these chemoattractants may desensitize neutrophils to G-CSF via rapid induction of SOCS1 expression. The Journal of Immunology, 2004, 173: 3243–3249.

Cytokines and chemokines regulate immune function by controlling growth, proliferation, differentiation, and migration of leukocytes. Many cytokines act via pathways such as the Jak/STAT and MAPK pathways (1, 2). Chemokines are a group of small (8–10 kDa) structurally related cytokines that induce migration of various immune cells via interactions with the seven transmembrane G protein-coupled receptors (GPCRs)³. They can activate protein kinases such as ERK, JNK, and AKT to induce cell growth (3–7). Other chemoattractants, like fMLP, a formylated tripeptide released by bacteria at the site of infection, also signal via GPCRs and thereby induce migration of phagocytic cells like neutrophils, macrophages, and monocytes (8). Neutrophil chemoattractants such as IL-8 and fMLP regulate cell migration and extravasation, and thus attract cells toward inflammatory sites. Both fMLP and IL-8 attract neutrophils to the site of infection and can also cause the release of neutrophil granules, oxygen burst, and stimulate the adherence of neutrophils to endothelial cells via adhesion molecules such as Mac-1 (9–11). As with IL-8, fMLP induces migration, adherence, and degranulation, as well as the production of toxic oxygen metabolites in neutrophils (12, 13). Neutrophil growth factors such as G-CSF induce the proliferation and differentiation of myeloid progenitor cells to the neutrophil lineage. Signaling through G-CSF is regulated at least partially by suppressors of cytokine signaling (SOCS) proteins (14).

The SOCS proteins have been identified as inhibitors of cytokine signaling and shown to act in a classical feedback loop. The prototype members of this family, cytokine-inducible Src homology 2-containing protein (CIS) and SOCS1, were cloned as cytokine-inducible immediate early genes that could inhibit the activation of STAT factors and block biological responses to several cytokines (15–18). To date, SOCS are known to be induced by α helical cytokines, such as IL-6, IL-2, and G-CSF, by innate immune stimulatory factors, such as LPS, and also by inflammatory cytokines, such as TNF and IL-1 (19–21). They control signaling by interacting with membrane proximal signaling intermediates such as Jak kinases and tyrosine-phosphorylated residues on the cytoplasmic portion of the receptors. When a cell responds to a chemoattractant, it rapidly migrates along a chemotactic gradient and can desensitize the cell to other signals. Chemotactic cytokines or chemoattractants are known to induce down-regulation of many signaling pathways, particularly signaling via other GPCRs by a process referred to as nonspecific desensitization (22, 23). This thought to be brought about by phosphorylation of serine/threonine residues on the cytoplasmic tail of GPCRs. However, whether chemoattractant desensitization could also affect other cytokine signaling pathways has not been explored. To date, it has been suggested that SOCS3 may be up-regulated in response to stromal cell-derived factor (SDF)-1α, via the Jak/STAT pathway (24), however, the importance of this is not clear, and whether other

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*Abbreviations used in this paper: GPCR, G protein-coupled receptor; CIS, cytokine-inducible Src homology 2-containing protein; PMN, polymorphonuclear neutrophils; SDF, stromal cell-derived factor; SOCS, suppressors of cytokine signaling.
host-derived chemoattractants like IL-8 or bacterial-derived chemoattractants regulate SOCS expression has not been explored.

In this paper, we show that SOCS1 expression can be regulated by the chemoattractants, IL-8 and fMLP, in myeloid cell lines and freshly isolated human neutrophils. We show that SOCS1 is a potent inhibitor of G-CSF signaling, and report in this study that pretreatment of primary neutrophils with fMLP or IL-8 impairs subsequent G-CSF signaling. Therefore, chemoattractant-induced SOCS proteins may act by blocking the G-CSF pathway and thus neutrophil differentiation.

Materials and Methods

Cell line culture conditions

U937 and HL60 cells were obtained from American Type Culture Collection (Manassas, VA) and were grown in RPMI 1640 supplemented with 10% FCS, 2 mM l-glutamine, 1 mM sodium pyruvate, 50 U/ml penicillin, and 50 μg/ml streptomycin. Cells were cultured at 37°C in 5% CO2. For stimulation, cells were treated with 9 ng/ml fMLP (Fluka, Buchs, Switzerland), 50 ng/ml IL-8 or 20 ng/ml IL-6 (PeproTech, Rocky Hill, NJ), 30 ng/ml G-CSF (Rhone-Poulenc Rorer, Collegeville, PA), or 1 μg/ml LPS (Sigma-Aldrich, St. Louis, MO).

RT-PCR analysis of SOCS expression in human polymorphonuclear neutrophils (PMN), U937, and HL60 cells

Blood samples obtained from healthy donors who had given informed consent were layered onto Ficoll-Hypaque, and PMNs were isolated by centrifugation and hypotonic lysis of erythrocytes. Cells were resuspended in serum-free medium for 2 h (PMN) or 5 h (U937 and HL60 cell lines), and then stimulated with 50 ng/ml IL-8, 9 ng/ml G-CSF, or 1 μg/ml LPS for the times indicated. Total RNA was isolated from PMNs (5 × 10⁶ cells) using Trizol and cultured U937 or HL60 cells (1 × 10⁶ cells/cell RT-PCR) following the RNA STAT-60 manufacturer’s protocol (Biogenesys, Poole, UK). RT-PCR was performed with 1 μg of total cell RNA using a OneStep RT-PCR kit (Qiagen, Hilden, Germany). PCR amplification was performed using primer pairs specific for human CIS (upstream primer, 5’-GATGCTGTTGATAGCCCA-3’; downstream primer, 5’-AGAGGCTTCGACTGCCTCTT-3’), SOCS1 (upstream primer, 5’-GAGAGCTTCGACTGCCTCTT-3’; downstream primer, 5’-AGTTAAGAGGTTCCAGAGTCA-3’), SOCS2 (upstream primer, 5’-ATGAGGCAGAAGGTGAGAGA-3’; downstream primer, 5’-ATGGTTGAGGGCATCCTGATT-3’), SOCS3 (upstream primer, 5’-TCTCAAGACCTTCGATCCGCAA-3’; downstream primer, 5’-TTCTGAGTTCCG-3’), SOCS4 (upstream primer, 5’-CTTAGATATTCCTGTTGGGC-3’; downstream primer, 5’-ATGAGCCTTGAATGCTT-3’), SOCS5 (upstream primer, 5’-TACAGAGAAGCATGACTGAGCG-3’; downstream primer, 5’-ACAGAAGAGAGTGGCCTCC-3’), SOCS6 (upstream primer, 5’-TCTCCATGTGCCTTCCCA-3’; downstream primer, 5’-GAGGCTTCGACTGCCTCTT-3’), SOCS7 (upstream primer, 5’-GAGTGGGGCTGTCCTT-3’; downstream primer, 5’-TTACTCTTGGAGGATGCTC-3’), SOCS8 (upstream primer, 5’-AGGCTGGCATTGGCGCT-3’; downstream primer, 5’-AAAGGCTGCTG-3’), and human GAPDH as a control (upstream primer, 5’-TGAGTACAAGTCAGAGTGAG-3’; downstream primer, 5’-TTACTCTTGGAGGATGCTC-3’). The predicted products for CIS, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, SOCS7, and GAPDH were 445, 562, 500, 554, 346, 810, 465, 565, and 244 bp, respectively. The PCR products were separated on a 2% agarose gel and visualized by ethidium bromide staining.

Isolation and growth of primary human cells

To study SOCS protein induction after IL-8, fMLP, and G-CSF treatment, neutrophils (5 × 10⁵ cells/RT-PCR) were extracted from donor blood (as above), or PBMCs were removed from the plasma/Ficoll interface upon Ficoll separation. Dendritic cells were also cultured from monocytes of the PBMC layer. PBMCs (2 × 10⁶ cells/ml) were incubated in a tissue culture flask for 90 min at 37°C in 5% CO2 to allow monocyte adherence, before being grown in fresh 10% FCS RPMI 1640. The cells were differentiated over 7 days into immature dendritic cells using GM-CSF (50 ng/ml) and IL-4 (1000 U/ml) on day 1 and 4. The immature status of the dendritic cells was confirmed using FACS analysis of their low CD14 expression and high CD1a expression. Contamination from T and B lymphocytes was ruled out by analyzing CD3 and CD19 expression of the cell population. Primary human macrophages were prepared as previously described (25).

Immunoblot analysis of protein induction

The dendritic cells were rested on day 7 for 2 h in 0% FCS RPMI 1640, or freshly extracted neutrophils or PBMCs were rested in 0% FCS RPMI 1640 for 2 h, before stimulation with 9 ng/ml G-CSF, 50 ng/ml IL-8, or 30 ng/ml G-CSF for the times indicated. Cells were lysed in 75 μl of lysis buffer (50 mM Tris-HCL, pH 8, 150 mM NaCl, 1% Brij 97, 0.1 mM EDTA), supplemented with aproton (5 μg/ml), leupeptin (5 μg/ml), and Na3VO4 (1 mM). Protein quantification was conducted according to the manufacturer’s protocol (Bio-Rad, Hercules, CA). Total lysates (50 μg) were resolved by SDS-PAGE and transferred to Immobilon membrane (Stratagene, La Jolla, CA). SOCS1 was detected using a SOCS1-Ab (a kind gift from Dr. D. Hiltner, Walter and Eliza Hall Institute for Medical Research and Cooperative Research Center for Cellular Growth Factors, Parkville, Victoria, Australia), and was visualized using goat anti-mouse HRP-conjugate Ab (Amershams Biosciences, Piscataway, NJ) and ECL (Amershams Biosciences).

To monitor the activation of STAT1, STAT3, and STAT5 in U937 cells, 1 × 10⁶ cells were stimulated with 30 ng/ml G-CSF, 20 ng/ml IL-6, 9 ng/ml fMLP, or 50 ng/ml IL-8 (unless otherwise noted) for the times indicated, and all were lysed with 1 ml of lysis buffer supplemented with aproton (5 μg/ml) leupeptin (5 μg/ml), and Na3VO4 (1 mM). The lysates were then subjected to Western blot analysis using phosho-specific Abs for STAT1, STAT3, and STAT5 (Cell Signaling Technology, Beverly, MA). The bands were then reprobed with Abs directed against STAT1 (E23) or STAT3 (C20) (Santa Cruz Biotechnologies, Santa Cruz, CA) or STAT5 (Fusion Antibodies, Belfast, Northern Ireland).

Primary human macrophages were stimulated with 30 ng/ml G-CSF, 20 ng/ml IL-6, 9 ng/ml FMLP, or 50 ng/ml IL-8 for the times indicated, and subjected to Western blot analysis as described above.

LPS endotoxin removal via polymyxin B

To remove the possibility that LPS may be a contaminant of fMLP, it was passed through polymyxin B (endotoxin removal) columns (20344; Pierce, Rockford, IL). To remove the polymyxin B columns were initially regenerated using 1% sodium deoxycholate (British Drug Houses, Poole, U.K.), followed by three washes with pyrogen-free water. The fMLP solution was passed through the polymyxin B column and collected. The lipid A region of any contaminating LPS bound the polymyxin B polypeptide and removed it from the collected fMLP.

Results

fMLP and IL-8 induce SOCS1 mRNA in the monocye cell lines HL60 and U937

SOCS genes are known to be induced by a variety of cytokines, including the IL-2 and IL-6 family, growth hormone, GM-CSF, proinflammatory cytokines including IL-1 and TNF-α, growth factors such as epidermal growth factor, and bacterially derived LPS, but whether neutrophil chemoattractants can induce their expression has not yet been examined.

To investigate the possible regulation of SOCS in response to chemoattractants, we first analyzed mRNA expression of all SOCS family members in myelomonocytic cell lines after treatment with IL-8 or fMLP. Fig. 1 shows that both SOCS1 and SOCS3 mRNA are up-regulated in HL60 cells upon treatment with IL-8 or IMLP. Interestingly, the kinetics of mRNA expression were found to be different. SOCS1 induction after fMLP treatment occurred at ~30 min and decreased to low levels within 2 h, whereas IL-8-induced SOCS1 mRNA was not observed until 3 h after stimulation, but persisted up to 7 h. Similarly, SOCS3 mRNA was rapidly up-regulated in HL60 cells 15 min after fMLP stimulation, whereas the IL-8-induced increase of SOCS3 mRNA was observed only after 1–2 h. Similar results were obtained with the human monocyte precursor cell line U937 (data not shown). In four repeated experiments, apart from SOCS1 and SOCS3, none of the other SOCS family members were regulated in either HL60 (Fig. 1) or U937 cells (data not shown) after treatment with IMLP or IL-8.
fMLP and IL-8 induce SOCS1 mRNA and protein expression in primary human cells

To check whether fMLP and IL-8 were also able to induce SOCS1 mRNA in primary human cells, neutrophils (PMN) were isolated from normal healthy donors using Ficoll gradient separation techniques, stimulated with IL-8 or fMLP, and analyzed by RT-PCR. Fig. 2A shows that both fMLP and IL-8 induce SOCS1 mRNA in primary neutrophils. The kinetics of the induction were comparable to that observed in HL60 and U937 cells, with fMLP rapidly up-regulating SOCS1 mRNA and IL-8 showing a delayed induction of SOCS1 mRNA.

In the case of SOCS3, the up-regulation of mRNA upon treatment with fMLP or IL-8 could not be clearly shown in primary neutrophils as high basal expression of SOCS3 mRNA was frequently observed in these cells (data not shown). As in U937 and HL60 cells, none of the other SOCS family members were regulated in PMN by fMLP or IL-8 (data not shown).

Next, we investigated whether the observed induction of SOCS1 mRNA was reflected at the protein level. Primary human neutrophils were treated with fMLP or IL-8, and total cell lysates were subjected to Western blot analysis using SOCS1 Ab. As shown in Fig. 2B, expression of SOCS1 was observed after stimulation with either fMLP or IL-8. Furthermore, we observed rapid induction of SOCS1 protein upon fMLP treatment, with SOCS1 protein expression being detected as early as 1.5 h following fMLP, and remaining expressed at 2 h. IL-8-induced SOCS1 protein expression was observed as early as 3 h following treatment, and remained expressed at 4 h. Human PBMCs also respond to IL-8 and fMLP, and we therefore wished to ascertain whether SOCS1 protein was expressed in these cells in response to chemotactic stimuli. PBMCs were isolated from healthy donors and treated with either fMLP or IL-8 for the times indicated, before SOCS1 expression was analyzed by Western blot. We again observed rapid up-regulation of SOCS1 levels in response to fMLP, which were sustained until 2 h (Fig. 2C, upper panel). IL-8 also induced SOCS1 protein expression in PBMCs; however, as in PMN, this occurred later, between 3 and 5 h (Fig. 2C, lower panel). This indicates that SOCS1 expression in response to chemotactic stimulation was not limited to PMN.

We also wondered whether dendritic cells that respond to these stimuli could also up-regulate SOCS1 expression following chemotaxtractant stimulation. Therefore, we generated dendritic cells from monocytes and stimulated again with either fMLP or IL-8. Induced SOCS1 protein expression was observed in these cells with kinetics identical with that identified in PMN and PBMC (Fig. 2D). These data clearly prove that SOCS1 protein and mRNA expression are rapidly regulated in response to chemotactic stimuli in many primary human leukocytes.

LPS is not responsible for the rapid up-regulation of SOCS1 mRNA upon fMLP stimulation

Previous reports have demonstrated that LPS can induce SOCS3 and SOCS1 mRNA in macrophages (20, 26–28). The fMLP used in our work was guaranteed to be >99% pure (Fluka, and Riedel, Seelze, Germany); therefore, it was possible that there could be a maximum of 1% LPS (0.09 ng/ml) endotoxin contamination of the fMLP we used. To analyze the maximal amount of SOCS1 mRNA that could originate from LPS contamination of fMLP, HL60 cells...
were treated with increasing concentrations of LPS, and SOCS1 mRNA induction was compared with an fMLP control using RT-PCR. Fig. 3A shows that a maximal possible contamination of <0.09 ng/ml LPS leads to a barely detectable up-regulation of SOCS1 mRNA, whereas the fMLP control potently induces SOCS1 mRNA. To further exclude the possibility of LPS contamination, fMLP was passed through polymyxin B columns to remove all traces of LPS. The fMLP was subsequently used to stimulate HL60 cells for 15, 30, and 60 min. Fig. 3B clearly demonstrates that endotoxin-free fMLP could still potently induce SOCS1 mRNA expression, and that this did not result from LPS contamination.

**The rapid induction of SOCS1 upon fMLP stimulation is not mediated via the Jak/STAT pathway**

The best known pathway leading to the induction of SOCS expression is the Jak/STAT pathway. Upon cytokine stimulation, activation of STATs leads to the induction of SOCS proteins, which then negatively regulate signaling in a classical feedback loop. Indeed, the activation of STAT factors by chemokine and other GPCRs was reported by a number of groups (29–32). More recently, Wu and coworkers (33) reported the fMLP-induced activation of STAT3 upon stimulation of HEK 293 cells overexpressing a transfected fMLP receptor and Gsα. Moreover, SOCS3 has been reported to be induced by SDF-1α, through the G protein-coupled CXCR4 receptor via the Jak/STAT pathway. Thus, we investigated whether the observed induction of SOCS1 involved the activation of STAT factors. First, U937 cells were stimulated with fMLP or IL-8 for various times and the activation of STAT1, STAT3, and STAT5 was monitored (Fig. 4A). As positive controls, the cells were treated with G-CSF or IL-6. As illustrated in Fig. 4A, neither fMLP nor IL-8 lead to the activation of STAT1, STAT3, or STAT5, whereas G-CSF and IL-6 induced prominent STAT phosphorylation. We next checked whether we could detect dose-dependent STAT3 phosphorylation upon fMLP stimulation of U937 cells. Fig. 4B shows that although G-CSF dose-dependently controls activated STAT3, fMLP was not able to induce STAT3 phosphorylation even with high doses. To verify these observations, we next investigated STAT1 and STAT3 activation by fMLP, IL-6, G-CSF, and IL-8 in primary human macrophages (Fig. 4, C and D). Again, in contrast to IL-6 and G-CSF controls, we did not observe STAT3 or STAT1 phosphorylation upon fMLP or IL-8 stimulation in these cells (Fig. 4, C or D). Interestingly, fMLP led to a strong activation of p42/p44 MAPK whereas IL-6, G-CSF, and IL-8 showed a less pronounced p42/p44 phosphorylation. Furthermore, although IL-6 dose-dependently induced STAT3 and STAT1 phosphorylation, fMLP did not. These data strongly suggest that STAT activation is not involved in fMLP-mediated up-regulation of SOCS1, and that the reported STAT3 activation in fMLP receptor-transfected HEK 293 cells (33) may not be physiologically relevant. Furthermore, we did not detect any activation of STATs up to 1 h after IL-8 stimulation, confirming that neither IL-8 nor fMLP activated STATs.

**The chemoattractants fMLP and IL-8 strongly block G-CSF-mediated signal transduction in primary human neutrophils**

As SOCS proteins are known to be potent inhibitors of cytokine signaling, we next investigated whether pretreatment of neutrophils with fMLP or IL-8 would affect subsequent cytokine responses. We have recently shown that both SOCS1 and SOCS3 are induced by G-CSF in primary human neutrophils and negatively regulate G-CSF receptor-mediated signaling (14). Thus, we pretreated primary human neutrophils with either fMLP or IL-8, and subsequently monitored G-CSF-mediated STAT3 phosphorylation. As shown in Fig. 5A, unstimulated and fMLP- or IL-8-treated neutrophils did not show phosphorylation of STAT3 (lanes 1–3), whereas G-CSF alone led to prominent STAT3 activation (lane 4). Pretreatment of cells with fMLP for 1.5 h or IL-8 for 4 h totally abolished the G-CSF-mediated phosphorylation of STAT3. Fig. 5B again demonstrates that SOCS1 protein was observed upon fMLP treatment for 90 min and IL-8 for 4 h. These experiments were performed three times in neutrophils from three different donors and we consistently observed SOCS1 expression and a block in G-CSF signaling after challenge with the chemoattractant stimulus. Interestingly, after the removal of fMLP, STAT3 phosphorylation in response to G-CSF did not recover, suggesting that the G-CSF signaling was blocked for a prolonged period (data not shown). We also analyzed neutrophil G-CSF receptor expression, and found that neither fMLP nor IL-8 pretreatment significantly altered the receptor levels (data not shown). This provides clear evidence for SOCS-mediated cross-talk between chemoattractant and cytokine signaling pathways.

**Discussion**

The findings presented in this study demonstrate that SOCS expression can be regulated by chemoattractants such as IL-8 and fMLP, in primary human peripheral blood neutrophils, dendritic cells, and in PBMCs. The data further shows that upon induction of SOCS1 by chemoattractants, it can act as a potent inhibitor of G-CSF signaling, as pretreatment of neutrophils with chemoattractants induces a marked block in G-CSF signal transduction. This suggests that chemoattractants can modulate cross-talk between pathways by inducing potent inhibition of cytokine signaling through the expression of SOCS proteins.

Inflammatory cells migrate to the sites of infection or tissue damage in response to the production of chemokines at the site of inflammation. These chemokines attract their targets by interacting with GPCRs expressed on the cell surface (22). Chemokine receptors are known to respond rapidly to chemoattractants by migrating along a chemotactic gradient and during this process, induce cross-desensitization of other chemotactic receptors (23, 24). For instance, IL-8 is known, at concentrations higher than 100 nmol, to induce desensitization of chemotaxis in a process termed homologous desensitization, thus blocking responsiveness to further IL-8 treatment. This is thought to be triggered by phosphorylation of the receptor in the cytoplasmic domain and subsequent binding of
arrestin to the cytoplasmic domain. Arrestin binding serves to uncouple the GPCRs from the G proteins and functionally link the receptors to other signaling pathways, including MAPK cascades, and to mediate the internalization of clathrin-coated pits (34). However, heterologous cross-desensitization can also occur and results in fMLP blocking further IL-8 responses (23). Cross-desensitization has been observed between the GPCRs of opioids and chemokine receptors (35), however, to date, chemoattractants and chemokines have not been shown to induce the desensitization of other cytokine pathways that signal through receptors other than GPCRs, such as the G-CSF receptor.

However, SOCS proteins, particularly SOCS1 and SOCS3, are known to potently inhibit signaling via cytokines. SOCS1 is thought to bind to Jak-kinases, particularly Jak1 and Jak2, and strongly inhibit their activation (36). This inhibition of Jak-kinase activity leads to a complete block in signaling through cytokines, such as IL-6 and G-CSF. However, it has recently been shown that other cytokines such as TNF-α, IL-1, and bacterial substances, such as LPS, can induce marked expression of SOCS1 and SOCS3 (20). This expression of SOCS1 and SOCS3 leads to marked inhibition and represents a pathway leading to possible cross-talk between different signaling pathways.

G-CSF is a major regulator of granulocyte hemopoiesis and stimulates proliferation, differentiation, and survival of committed myeloid progenitors, and is commonly used in the treatment of neutropenia following chemotherapy (37, 38). Interaction with its cell surface receptor specifically activates the Janus kinases, Jak1 and Jak2, and the transcription factors, STAT1, STAT3, and STAT5. STAT3 activation is mediated by the G-CSF receptor, and treated with 9 ng/ml fMLP, 20 ng/ml IL-6, 30 ng/ml G-CSF, or 50 ng/ml IL-8 for the times indicated, and the activation of STAT1, STAT3, and p42/p44 MAPK was determined by Western blot analysis.
STAT5 (39). STAT3 activation has been implicated in G-CSF-mediated differentiation (40, 41), whereas STAT5 seems to be involved in G-CSF-mediated proliferation and survival (42). The SOCS family of proteins has been shown to negatively regulate G-CSF signal transduction in a negative feedback loop. It is interesting that the rapid response to cytokines such as G-CSF induces expression of many SOCS proteins, including SOCS1 and SOCS3 (14). However, the data presented in this study suggests that chemotactic cytokines, such as IL-8 and fMLP, only induce SOCS1 expression. Because SOCS1 is known to be the most potent inhibitor of cytokine signaling, our findings illustrate that GPCRs, such as the fMLP receptor and the IL-8Rs, have the ability to induce SOCS1 expression and induce a marked inhibition of G-CSF-signaling, representing a new pathway for cross-talk between GPCRs and cytokine receptors.

Both SOCS1 and SOCS3 have a strong inhibitory effect on many cytokine pathways such as G-CSF, IL-6, and IL-2 (14, 16–19). Therefore, the sharp and transient rise in SOCS1 expression when a neutrophil detects fMLP may result in blunt cytokine responses. This cytokine pathway inhibition may direct the cell from proliferation, growth, and differentiation to a more migratory and phagocytotic phenotype, to allow rapid movement toward the site of bacterial infection. The delay in the IL-8-induced SOCS1 induction may thus allow neutrophils to proliferate, differentiate, and migrate to the site of infection, before the pathways regulating these processes are shut down. Therefore, SOCS1 induction may prevent other signals and help to direct the mature neutrophils to the inflammatory site.

A number of reports have suggested that the fMLP and IL-8Rs may signal via different G proteins perhaps explaining why the induction of SOCS1α occurs more rapidly in response to fMLP. fMLP can signal via G12/13, a pertussis toxin-insensitive pathway (43), while IL-8 responds via CXCR1 and -2, which reportedly signal through G1, for chemotaxis and Gαq for gene induction (44–46).

Soriano et al. (24) have suggested that SOCS3 is induced by SDF-1α in IM-9 cells via Jak/STAT, and others have suggested, using overexpression studies, that fMLP signals via the Jak/STAT pathway (33). However, our results clearly demonstrate that neither fMLP nor IL-8 signal through the Jak/STAT pathway, indicating a completely different mode of induction. It would be important to determine whether other chemotactic factors such as MIP1α, RANTES or MCP1 also have a similar effect on SOCS expression. It is interesting that chemokines induce SOCS1 expression, because initially it was thought that SOCS genes were only induced by the Jak/STAT pathway. However, the findings that TNF and LPS can induce SOCS expression suggest that other signaling pathways, including the NF-κB pathway and perhaps the MAPK pathways, may play a significant role in SOCS expression. Recently, two groups have determined, using SOCS1 knockout mice, that LPS-induced SOCS1 rapidly and transiently inhibits further LPS signaling. This suggests that SOCS1 may play a role inhibiting TLR signaling, as well as cytokine signaling pathways. Although some reports have suggested the tyrosine kinases of the Tec family may be involved in TLR signaling, how SOCS1 induces LPS tolerance is unclear (47).

In conclusion, the data show that IL-8 and fMLP both induce SOCS1 in myeloid cells, and provides evidence for cross-talk mechanisms by which chemotaxtrants shut down cytokine pathways and enable the cell to prioritize its responses by switching off G-CSF signaling during migration to a chemotactic stimuli. Our findings strongly suggest that SOCS proteins induced by chemokines may play an important role in the regulation of cytokine signals through cross-talk, and it will be interesting to determine whether this is a conserved mechanism used by other chemokines.

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References


