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Inhibition of IL-6 and IL-10 Signaling and Stat Activation by Inflammatory and Stress Pathways

Simi T. Ahmed* and Lionel B. Ivashkiv²*†

The development and resolution of an inflammatory process are regulated by a complex interplay among cytokines that have pro- and anti-inflammatory effects. Effective and sustained action of a proinflammatory cytokine depends on synergy with other inflammatory cytokines and antagonism of opposing cytokines that are often highly expressed at inflammatory sites. We analyzed the effects of the inflammatory and stress agents, IL-1, TNF-α, LPS, sorbitol, and H₂O₂, on signaling by IL-6 and IL-10, pleiotropic cytokines that activate the Jak-Stat signaling pathway and have both pro- and anti-inflammatory actions. IL-1, TNF-α, and LPS blocked the activation of Stat DNA binding and tyrosine phosphorylation by IL-6 and IL-10, but not by IFN-γ, in primary macrophages. Inhibition of Stat activation correlated with inhibition of expression of IL-6-inducible genes. The inhibition was rapid and independent of de novo gene induction and occurred when the expression of suppressor of cytokine synthesis-3 was blocked. Inhibition of IL-6 signaling was mediated by the p38 subfamily of stress-activated protein kinases. Jak1 was inhibited at the level of tyrosine phosphorylation, indicating that inhibition occurred at least in part upstream of Stats in the Jak-Stat pathway. Experiments using Stat3 mutated at serine 727 and using truncated IL-6Rs suggested that the target of inhibition is contained within the membrane-proximal region of the cytoplasmic domain of the gp130 subunit of the IL-6 receptor and is different from the SH2 domain-containing protein-tyrosine phosphatase/suppressor of cytokine synthesis-3 docking site. These results identify a new level at which IL-1 and TNF-α modulate signaling by pleiotropic cytokines such as IL-6 and IL-10 and provide a molecular basis for the previously described antagonism of certain IL-6 actions by IL-1.


Interleukin-6 is a multifunctional cytokine that plays an important role in immune and inflammatory responses (reviewed in Ref. 1). IL-6 works by regulating the expression of immune/inflammatory genes and regulating cell proliferation, differentiation, and survival. Since IL-6 expression is elevated in inflammatory diseases and is induced by inflammatory stimuli, such as IL-1 and TNF-α, IL-6 has been considered a proinflammatory cytokine (1–3). Many of its proinflammatory and immune properties are secondary to potent effects on driving B cell Ab production, promoting T cell function, and promoting the expression of chemokines and adhesion molecules on endothelial cells (1, 4, 5). In contrast to its effects on lymphocytes and endothelial cells, IL-6 and the related cytokine IL-11 that signals through the same receptor subunit (see below) have suppressive effects on macrophages (6, 7), astrocytes (8), and fibroblasts (9) and suppress the expression of IL-12, IFN-γ, TNF-α, adhesion molecules, and proteases both in vitro and in vivo (6–12). In IL-6 knockout mice, there is decreased IL-10 production and increased IL-12 production from macrophages compared with those in normal mice (12). One mechanism that underlies the anti-inflammatory effects of IL-6 and IL-11 appears to be inhibition of NF-κB (13). IL-6 also induces the expression of multiple factors with anti-inflammatory properties, including IL-1R antagonist, soluble TNF receptors, IL-10, acute phase reactants, glucocorticoids, protease inhibitors (such as tissue inhibitor of metalloproteinase-1), and suppressors of cytokine signaling (SOCS) proteins (12, 14–20). Consistent with these anti-inflammatory effects, IL-6 has been shown to attenuate inflammatory lung disease (21) and to play a chondroprotective role in zymosan-induced arthritis (22), and IL-11 is an effective anti-inflammatory agent in collagen-induced arthritis (23) and psoriasis (24). These observations suggest that induction of IL-6 and IL-11 expression during inflammation, similar to induction of IL-10, may contribute to a negative feedback loop. The overall roles of IL-6 and IL-11 in a particular inflammatory process are determined by the balance between their pro- and anti-inflammatory actions on different cell types.

IL-6 is one member of a family of related cytokines (IL-6, IL-11, oncostatin M (OsM), leukemia-inhibitory factor (LIF), and c-diostrobin) that bind to receptors that consist of cytokine-specific α-chains and a shared gp130 receptor subunit that functions in signal transduction (1). The α subunits determine the specificity of binding of these cytokines to their receptors and in the case of LIF and OsM also contribute to signaling. The α subunits specific for IL-6 and IL-11 play no known role in signal transduction, and thus signals generated by IL-6 and IL-11 are similar, since in both cases they are mediated by gp130 homodimers. Some of the differences in biological activity of IL-6 and related cytokines are probably explained by cell type-specific expression of receptor α subunits. Dimerization of gp130 after binding of IL-6 results in activation of the receptor-associated protein tyrosine kinases Jak1, Jak2, and

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3 Abbreviations used in this paper: SOCS, suppressor of cytokine synthesis; OsM, oncostatin M; LIF, leukemia-inhibitory factor; SAPK, stress-activated protein kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular stimulus-regulated kinase; IP, immunoprecipitation; IRF, IFN-regulated factor; PIAS, protein that interacts with activated Stat; CA, constitutively active; NT3, neurotropin-3; hSIE high-affinity, SIS-inducible element; MKK, MAPK kinase; MAPKAP, MAPK-activated protein.
inhibition of NF-κB transcription factors (25). In many cells, including macrophages and other inflammatory cells, IL-6 activates predominantly Stat3, although Stat1 can be activated by high doses of cytokine in certain cell types. Stat3 has different, and even opposite, functions in different cells depending upon cell type and activation status, can induce either proliferation or growth arrest, and may have both pro- and anti-apoptotic properties. In the immune system, Stat3 promotes T cell survival and function (26) and B cell Ab production (27). In contrast, in myeloid cells, deletion of Stat3 results in hyperactivation of macrophages, dramatic increases in inflammatory cytokine production, and inflammatory bowel disease, thus indicating a role for Stat3 in down-regulation of macrophage activation (28).

IL-1 and TNF are major inflammatory cytokines that activate the expression of adhesion molecules, chemokines, and cytokines (2, 3). IL-1 and TNF activate several signaling pathways, leading to the downstream activation of NF-κB transcription factors and activation of c-Jun N-terminal kinases (JNKs) and the p38 kinases (29), collectively termed stress-activated protein kinases (SAPKs), that constitute two subfamilies of mitogen-activated protein kinases (MAPKs) (30). SAPKs are also activated by cellular stressors, including reactive oxygen intermediates, osmolar shock, and UV radiation, some of which are present during inflammation. SAPKs phosphorylate and activate transcription factors, including AP-1, and have been strongly implicated in mediating IL-1 and TNF inflammatory effects (29, 30). IL-1 and TNF are expressed at most inflammatory sites, where they regulate the expression of other cytokines and interact with other immune/inflammatory cytokines in a cytokine network (31). Interestingly, both synergistic and antagonistic interactions of IL-1 and TNF with cytokines of the IL-6 family have been described. IL-1 and IL-6 work together in the induction of type I acute phase protein genes (14), metalloproteases (32), and HIV expression (33). In contrast, IL-6, IL-11, and Osm antagonize IL-1-, TNF-, and LPS-induced expression of tissue inhibitor of metalloproteinase-3 (9), adhesion molecules (8), TNF-α, IL-8, and GM-CSF (6, 10, 24) and inhibit IL-1-dependent cartilage degradation (34), possibly by a mechanism that involves inhibition of NF-κB (13). Going in the other direction, IL-1 and TNF block IL-6 induction of type II acute phase response genes such as thiostatin and fibrinogen (14, 35) and inhibit IL-6-induced proliferation of thymocytes (36). The mechanisms underlying the antagonism of the actions of IL-6 by IL-1 or TNF are not known.

We have previously described a novel and rapid mechanism of inhibition of IL-6 signaling that was mediated by the extracellular stimulus-regulated kinase (ERK) subfamily of MAPKs (37, 38). Within minutes of activation, ERKs were found to inhibit IL-6-mediated activation of Stat3 via a mechanism that probably involved modification of existing signaling components in the cell. Importantly, this inhibition was found to occur upstream of Stat3 in the Jak-Stat cascade, such that Stat3 did not become tyrosine phosphorylated. Given that ERKs and SAPKs can work together by phosphorylating similar motifs on common or different substrates (29), we investigated whether SAPKs, which are activated by inflammatory cytokines and stress factors, modulate IL-6 signaling and Stat activation. Herein, we describe inhibition of IL-6-induced Stat activation by inflammatory cytokines and stress-inducing agents and demonstrate that inhibition of IL-6 signaling can be accomplished by the p38 subgroup of the SAPKs. This inhibitory pathway is activated by stimuli different from those that activate ERK-dependent inhibition of IL-6 and appears to have a different target of inhibition. These results suggest an important role for MAPK pathways in inhibition and modulation of signaling by cytokines that use the Jak-Stat signaling pathway.

Materials and Methods

Cell isolation and tissue culture

Monocytes were obtained from PBMC immediately after isolation or after 2 days of culture using a kit to deplete nonmonocytic cells (Miltenyi Bio- tec, Auburn, CA) or by positive selection using anti-CD14 magnetic beads as recommended by the manufacturer (Miltenyi Biotec) and were >97% pure as verified using flow cytometry as previously described (39). Monocytes were used fresh or after 2 days of culture under adherent conditions under which differentiation into macrophages was initiated, as assessed by increased cell size and granularity. Similar results were obtained regardless of the method of monocyte purification or culture. MM6 human myeloid cells (40) and U266 human myeloma cells were cultured in RPMI, and 293T cells were cultured in DMEM supplemented with 10% FBS. When agents that were dissolved in DMSO were used (PD98059, SB203580), DMSO was added to control cells to keep concentrations of DMSO (0.1 or 0.2%) equal in all wells.

EMSAs

Cell extracts were prepared as previously described (41). Extracts corresponding to 3.3 × 10⁶ cells (∼12 µg of protein) were incubated for 15 min at room temperature with 0.5 µg of [γ-32P]ATP and 2 µg of poly(dI-dC) (Pharmacia, Piscataway, NJ), as previously described (41), and complexes were resolved on nondenaturing 4.5% polyacrylamide gels.

Immunoblotting, immunoprecipitation (IP), and kinase assays

Cell lysates or immunoprecipitates (see below) were fractionated on 7.5% SDS-polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and incubated with phospho-specific ( Tyr185/186) Stat3 Ab, phospho-specific (Thr180/Tyr182) p38 Ab, phospho-specific (Thr202/Tyr204) ERK1/2 Ab (New England Biolabs, Beverly, MA), monoclonal Stat3 and ERK1/2 Abs (Transduction Laboratories, Lexington, KY), anti-phosphotyrosine Ab (4G10, Upstate Biotechnology, Lake Placid, NY), FLAG Ab (M2), and p38, Jak1, and JNK1 Abs (Santa Cruz Biotechnologies, Santa Cruz, CA). For immunoprecipitations, extracts corresponding to 10–20 × 10⁶ cells were adjusted to a 0.5-ml volume in IP buffer (38) and incubated with 4 µg of FLAG, Stat3, Jak1, or JNK1 Abs (Santa Cruz Biotechnologies). Immunoprecipitates were collected using protein G-and protein A-agarose beads and washed three times with IP buffer, and once with PBS. For kinase assays, 25% of the IPs were saved for immunoblot analysis, and the remaining 75% were washed and resuspended in 50 µl of kinase buffer. JNK kinase activity was assayed by incubation at room temperature for 30 min with 10 µCi of [γ-32P]ATP and 5 µg of GST-Jun substrate.

Nuclear extract preparation

A nuclear minipreparation procedure (42) was used with modifications. MM6 cells (∼8 × 10⁶) were washed in HBSS, resuspended, and incubated in 160 µl of buffer A (10 mM HEPES, 10 mM KCl, 1.5 mM MgCl₂, 1 mM DTT, 10 µg/ml L-soybean trypsin inhibitor, 0.2%). The nuclear fraction was pelleted by centrifugation and lysed in 10 mM HEPES, 10 mM KCl, 1.5 mM MgCl₂, 10 µg/ml L-soybean trypsin inhibitor, 10 mM KCl, and 100 mM NaCl in 0.02% Triton X-100 containing 1 mM DTT and 50 mM PMSF, and centrifuged to remove the supernatant. For EMSAs, 25% of the IPs were saved for immunoblot analysis, and the remaining 75% were washed and resuspended in 50 µl of kinase buffer. JNK kinase activity was assayed by incubation at room temperature for 30 min with 10 µCi of [γ-32P]ATP and 5 µg of GST-Jun substrate.

Analysis of mRNA levels

Total cellular RNA was isolated using TRizol (Life Technologies, Gaithersburg, MD) according to the instructions of the manufacturer. For RT-PCR, RNA was treated with RNase-free DNase, and cDNA was obtained using Moloney murine leukemia virus reverse transcriptase (Life Technologies). Each cDNA (2.5%) was subjected to 22–25 cycles of PCR using conditions that result in a single specific amplification product of the correct size, as previously described (39, 43). 30-s denaturation at 94°C, 1-min annealing at 55°C, and 30-s extension at 72°C in a GeneAmp 9600 thermal cycler (Perkin-Elmer, Norwalk, CT). The DNTPs were used at 100 µM, and 1 µCi of [α-32P]dATP was added to each reaction. No amplification products were obtained when reverse transcriptase was omitted, indicating the absence of contaminating genomic DNA. Amplification was empirically determined to be in the linear range. Oligonucleotide primers used are as follows: GAPDH, GTG AAG GTC GGA GTC AAC and TGG AAT GCA TCG GAA; GGA TCT GGA AAC and TGG AAT GCA TCG GAA; SOCS3, CCC GCC GGC ACC TTT CTG and AGG GCC CGG CTC AAC ACC; IFN-regulated factor-1 (IRF-1), ATG AGA CCC TGG CTA GAG and AAG CAT CCG GTA CAC TCG; Stat1, GAA...
GTG CTG AGT TGG CAG and GCT TTC AAT CCA AAG CCA GAA; and protein that interacts with activated Stat (PIAS3), GCC CAC CAG CCT TGC ATC and GCT CGG CCC ATT CTT GGT. PGE2 receptor primers were provided by C. Beadling and K. Smith (44).

**Transient transfections and reporter gene assays**

293T cells were transfected in duplicate in 100-mm dishes, using the calcium phosphate coprecipitation technique with expression plasmids encoding constitutively active (CA-)MAPK kinase 3 (MKK3) and CA-MKK6 (provided by R. Davis (45)), a CA-MEK1 encoding plasmid (containing the S218E and S222D mutations and an amino-terminal deletion of residues 30–49), or a control empty vector. Cells were cotransfected with plasmids encoding carboxyl-terminal FLAG-tagged Stat3 (46), and β-galactosidase, and the total amount of DNA added per transfection was 20 μg. Six hours post-transfection, cells were washed and cultured with fresh medium for another 18 h, split onto replicate 60-mm tissue culture dishes, allowed to incubate for an additional 24 h, and stimulated with cytokines. Transfection efficiency was monitored by assaying for β-galactosidase activity. In reporter gene experiments, a plasmid encoding 4x-IRF-γ-activated sequence-luciferase (46) was used, and luciferase activity was normalized for β-galactosidase activity encoded by a cotransfected internal control plasmid. Each transfection experiment was performed three to five times.

**Results**

IL-1, TNF-α, LPS, and stress agents inhibit activation of Stats by IL-6 and IL-10, but not by IFN-γ, in primary macrophages and myeloid and B cell lines

The effects of inflammatory cytokines and LPS on IL-6 signaling and Stat activation were determined using primary monocytes/macrophages. IL-6 treatment of monocytes resulted in the rapid induction of DNA-protein complexes that bound the hSIE oligonucleotide (Fig. 1A, top panel, lane 2), which, as previously reported (41, 47), contained Stat1 and Stat3, based on supershift assays with specific Abs (data not shown). A 20-min pretreatment with TNF-α, IL-1, or LPS inhibited Stat DNA binding (lanes 3–5). Tyrosine phosphorylation of Stat1 and Stat3 at a conserved tyrosine residue is necessary for dimerization and DNA binding (25). IL-6-induced tyrosine phosphorylation of both Stat1 and Stat3 was inhibited by TNF-α, IL-1, and LPS (Fig. 1A, second and fourth panels) in a manner that correlated with inhibition of DNA binding (Fig. 1A, top panel). Immunoblotting of the same extracts showed comparable levels of Stat1 and Stat3 proteins in all lanes (Fig. 1A, panels 3 and 5), demonstrating that the observed inhibition of DNA binding and tyrosine phosphorylation was not secondary to reduced Stat protein levels. Since IL-6 may have both pro- and anti-inflammatory effects on macrophages, the effects of inflammatory stimuli on signaling by IL-10, a potent inhibitor of macrophage function (48), were determined. As previously reported (49), IL-10 activated predominantly Stat3 in macrophages (Fig. 1B and data not shown), and similar to IL-6, IL-10 activation of Stat3 was inhibited by TNF-α, IL-1, and LPS at both DNA binding and tyrosine phosphorylation levels (Fig. 1B). In contrast, activation of Stat1 by the proinflammatory cytokine IFN-γ was not inhibited (Fig. 1C). These results are distinct from those reported by Stoiber et al. (50), who showed that long periods of preincubation with LPS (4–48 h) suppressed IFN-γ signaling. These results, therefore, suggest that at early time points after addition to cells, inflammatory cytokines preferentially block Jak-Stat signaling by cytokines that have anti-inflammatory properties. Since Stat3 clearly subserves an anti-inflammatory function in myeloid cells (28), inhibition of Stat3 activation suggests that IL-1, TNF-α, and LPS block anti-inflammatory signals generated by IL-6 and IL-10.

To assess whether inhibition of Jak-Stat signaling by inflammatory factors occurs in other cell types and to identify cell lines that would be useful for further mechanistic studies, the effects of inflammatory and stress factors on Stat activation were assessed in MM6 myeloid cells, U266 myeloma cells, 293T cells, primary fibroblasts, and HepG2 cells. Because not all these cells express significant levels of IL-1Rs, and different inflammatory cytokines and stress factors activate SAPKs in a cell type-specific manner (30), the stress factors sorbitol (hyperosmolar shock), UV radiation, anisomycin, and H2O2 (oxidative stress) were also used.

IL-6 activated primarily Stat3 in MM6 cells (Fig. 2A, top panel, lane 2, and data not shown), and a 20-min pretreatment with IL-1, H2O2, UV light, or sorbitol served to inhibit DNA binding by Stat3 (Fig. 2A, lanes 3, 5, 6, and 8), whereas anisomycin had no such effect. IL-1 did not inhibit IFN-γ-induced Stat1 activation or IL-4-induced Stat6 activation in MM6 cells, demonstrating specificity of inhibition (data not shown). A similar pattern of inhibition of DNA binding was detected with U266 cells (Fig. 2B). Inhibition of Stat activation was also observed in 293T cells (with UV, IL-1, sorbitol, and H2O2) and primary fibroblasts (only IL-1 tested; data not shown). In HepG2 cells, which are a major target of IL-6 action, IL-1 strongly inhibited Stat3 (Fig. 2C). Pretreatment of MM6 and U266 cells with stress agents inhibited accumulation of tyrosine-phosphorylated Stat3 (Fig. 2D) in a manner that correlated with inhibition of DNA binding (Fig. 2, A and B). Our results with myeloid cells agree with those of Bode et al. (51), although our observation with HepG2 cells is in apparent contrast with their results, where they describe weak or no inhibition of IL-6 signaling by, respectively, TNF-α and LPS. This difference may be secondary to inherent differences between IL-1 and LPS/TNF or due to

**FIGURE 1.** Inflammatory cytokines suppress Stat activation by IL-6 and IL-10, but not by IFN-γ, in primary macrophages. Purified macrophages obtained using magnetic beads, as described in Materials and Methods, after 2 days of culture were treated for 20 min with TNF-α (100 ng/ml), IL-1β (100 ng/ml), or LPS (1 μg/ml), followed by a 15-min stimulation with IL-6 (50 ng/ml; A), IL-10 (100 ng/ml; B), or IFN-γ (16 ng/ml; C); all cytokines were used at saturating concentrations. Cell extracts were used in EMSAs with the hSIE Stat-binding oligonucleotide as previously described (41), and the same extracts were analyzed using immunoblotting with Abs against tyrosine-phosphorylated Stat1 or Stat3, followed by probing the same filter with Abs against Stat1 or Stat3. A. Two different gels were run to separately analyze Stat1 and Stat3. Similar results were produced using monocytes/macrophages obtained using several different purification methods and culture systems, as described in Materials and Methods.
inefficient signaling by the latter agents in HepG2 cells. The authors, in fact, indicate that LPS may not be signaling in their system (51). The cell lines tested did not have detectable Stat activation in response to IL-10, and thus the effects of stress factors on IL-10 signaling could not be assessed. Overall, these results indicate that inflammatory and stress factors that activate SAPKs are capable of inhibiting IL-6-triggered Stat activation in several primary cell types and cell lines.

Given that nuclear translocation of Stats typically depends upon previous tyrosine phosphorylation (25), one may predict that inhibition of tyrosine phosphorylation would block nuclear translocation of Stat3. Nuclear extracts were prepared from MM6 cells, and Stat3 translocation was analyzed using immunoblotting. Treatment of cells with increasing doses of IL-6 (2.5–50 ng/ml) resulted in proportionately higher levels of Stat3 protein in the nucleus (Fig. 3, top panel, lanes 2–6). Pretreatment with IL-1 for 20 min resulted in a marked reduction in nuclear Stat3 (lane 7), with a parallel decrease in tyrosine-phosphorylated Stat3 as well (Fig. 3, bottom panel). These results show that after IL-1 treatment, Stat3 is prevented from accumulating in the nucleus, where it activates transcription.

Stress agents inhibit expression of IL-6-inducible genes

To assess the functional consequences of inhibition of IL-6 activation of Stats, semiquantitative RT-PCR was used as previously described (39, 43) to determine the effect of inflammatory/stress agents on steady state mRNA levels of IL-6-inducible genes. IL-6 activation of PIAS1, PIAS3 (52), and PGE2 receptor (44) genes was suppressed by IL-1 (Fig. 4A). More than 30 genes were tested, and several patterns of gene regulation were observed, including activation of genes by both IL-1 and IL-6 and inhibition of IL-1 by IL-6 (data not shown). Thus, IL-1 did not cause a generic reduction of IL-6-dependent gene regulation. In U266 cells, IL-6-induced expression of SOCS3, IRF-1, Stat1, and PIAS3 was suppressed by a 20-min pretreatment with sorbitol or H2O2 (Fig. 4B). Transcription of genes encoding Stat1, IRF-1, and SOCS3 is Stat dependent (19, 5230 INHIBITION OF IL-6-TRIGGERED Stat3 ACTIVATION BY p38

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**FIGURE 2.** Inhibition of IL-6 Stat activation by multiple factors that activate stress kinases. A, MM6 cells were treated for 20 min with IL-1 (50 ng/ml), anisomycin (10 μg/ml), H2O2 (1.1 mM), UV light (50 J/m2), or sorbitol (400 mM), followed by IL-6 (50 ng/ml) stimulation (12 min). Whole-cell extracts were assayed for binding to the hSIE oligonucleotide using EMSA (top), and the same extracts were analyzed by immunoblotting (IB) with specific Stat3 antiserum (bottom). B, U266 myeloma cells were used. C, HepG2 cells were used. D, Stat3 immunoprecipitates (MM6) or whole-cell extracts (U266) were analyzed by immunoblotting with Abs against tyrosine-phosphorylated Stat3 (top panel), and the same filter was probed with anti-Stat3 (bottom panel). pY, Phosphotyrosine.

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**FIGURE 3.** IL-1 prevents nuclear translocation of Stat3. MM6 cells were treated with increasing doses of IL-6, and nuclear extracts were assayed for both tyrosine-phosphorylated and total Stat3 protein levels by immunoblotting.

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**FIGURE 4.** Inflammatory/stress agents suppress the expression of IL-6-inducible genes. A, Monocytes were cultured for 2 days and were treated with IL-1 (100 ng/ml) for 20 min and with IL-6 (50 ng/ml) for 3 h. B, U266 cells were treated with sorbitol (400 mM) or H2O2 (1.1 mM) for 20 min before IL-6 treatment (50 ng/ml) for 1.5 h. Semiquantitative RT-PCR was used as previously described (39, 43) to measure mRNA levels.
25), and thus inhibition of Stat activation probably contributes to inhibition. Interestingly, sorbitol and H2O2 did not induce SOCS3 expression (Fig. 4B), suggesting that inhibition of signaling in B cells was not mediated by SOCS3 (see below). GAPDH levels were comparable in all lanes, demonstrating that inhibition was not secondary to nonspecific effects of the stress agents on transcription. Thus, inhibition of IL-6 signaling by IL-1 and stress agents bears physiologic consequences with regard to gene expression.

**Inhibition of IL-6 signaling by IL-1 is independent of de novo gene expression**

Several reports indicate that cytokine signaling can be inhibited by the induction of inhibitory molecules such as SOCS (18–20, 53, 54). Inflammatory cytokines such as TNF induce SOCS3 expression, probably mediated by the p38 pathway, and induction of SOCS3 correlated with inhibition of Stat3 activation in response to IL-6 (51). More recently, association between SOCS3 and gp130 (the signaling subunit of the IL-6R) has been shown to suppress IL-6 signaling (55, 56). We therefore investigated whether the inhibition of IL-6 signaling seen in our system was dependent on de novo gene induction, especially that of SOCS3. Preincubation of primary macrophages with actinomycin D (an inhibitor of RNA synthesis) had no effect on IL-1-mediated inhibition of IL-6 signaling when IL-1 was added either 20 min or 1 h before IL-6 (Fig. 5, A and B). In addition, although IL-1 induced SOCS3 expression at both time points (Fig. 5C, lanes 2 and 4), actinomycin D essentially blocked completely this induction (lanes 3 and 5). Studies using cycloheximide, a protein synthesis inhibitor, did not yield informative data, as cycloheximide treatment alone significantly blocked IL-6 signaling (data not shown). These results indicate that inhibition of Stat3 activation by IL-6 in our system occurs via a rapid and inducible pathway that does not rely on the expression of new genes.

**Role for p38 in inhibition of IL-6 signaling**

Different inflammatory/stress agents activate distinct MAPKs in different cell types (30), and we investigated possible roles played by different MAPKs in inhibition of IL-6 signaling. Activation of ERKs, JNKs, and p38 in MM6 cells by inflammatory/stress factors was determined using both phosphotyrosine/threonine immunoblotting and kinase assays, and the results are summarized in Table I. In addition, in HepG2 cells, IL-1 and other stress agents activated both ERKs and p38 (data not shown). As indicated in Table I, none of the three agents tested activated ERK1/2 over background levels, and only UV and sorbitol activated JNK. The p38 kinase, however, was activated by all agents that also inhibited Stat3 DNA binding, namely, IL-1, H2O2, UV, and sorbitol. Interestingly, anisomycin was the only stress agent that did not activate p38 (or the other kinases) in our system, which correlates with its inability to inhibit Stat3 DNA binding (Fig. 2A and Table I). These results suggested that p38 may play a role in the suppression of Stat3 activation.

To investigate whether p38 is important in mediating inhibition of IL-6 signaling, SB203580, a specific inhibitor of p38 activation and kinase activity (45), was used. When MM6 cells were incubated with SB203580 before IL-1 treatment, inhibition of Stat3 DNA binding by IL-1 was completely reversed (Fig. 6A, top panel, lanes 4 and 5). Reversal of this inhibition correlated with inhibition of p38 activation (Fig. 6A, middle panel), suggesting a role for p38 in this process. We have previously shown that PMA treatment blocked Stat3 activation in a fashion that was dependent on the MEK-ERK pathway, which is distinct from the p38 activation pathway, and was reversed by the MEK inhibitor, PD98059 (37, 38). We determined whether ERKs and p38 could play distinct roles in inhibition by different factors. As expected, PMA treatment of MM6 cells resulted in inhibition of Stat3 that was reversed by PD98059 (Fig. 6B, lanes 3 and 4) and not by SB203580 (lane 5). In contrast, IL-1-mediated inhibition was reversed by SB203580 (lanes 6 and 8), but not by PD98059 (lane 7). This differential sensitivity to kinase inhibitors suggests that different MAPKs can inhibit IL-6 signaling, and that inhibition can be achieved through the activation of distinct signaling pathways.

The role of the p38 stress kinase pathway in inhibition of IL-6 signaling was directly tested using the expression of CA-MKK3 and CA-MKK6 kinases, which are immediately upstream of p38 and activate p38 by phosphorylation (30). This approach has been used extensively, and expression of CA-MKK3 and CA-MKK6 does not result in cross-activation of JNKs or ERKs (45). 293T cells were transfected with either a control empty vector or the same vector encoding the CA-MKK3 and CA-MKK6 kinases and with wild-type Stat3 containing a carboxyl-terminal FLAG tag. CA-MKK3 and CA-MKK6 dramatically suppressed tyrosine phosphorylation of Stat3 in response to IL-6 (Fig. 7, top panel).

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** Inhibition of IL-6 signaling by IL-1 is independent of de novo gene expression. A, Macrophages purified after 2 days of culture were treated with actinomycin D (Act. D; 5 μg/ml) for 15 min before treatment with IL-1 (50 ng/ml) for 20 min, followed by a 15-min stimulation with IL-6 (50 ng/ml). B, The same assay as that in A was conducted, except that IL-1 was added 1 h before adding IL-6. C, Macrophages in wells parallel to those used in A and B were treated with IL-1 for either 60 or 20 min. Act. D was added 15 min before adding IL-1. Semiquantitative RT-PCR was used to measure mRNA levels.

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*a*, in the direction of the arrow; *−*, against the direction of the arrow.

*Assayed by immunoblotting with phosphotyrosine/threonine-specific Abs.

*Assayed by kinase assays using the GST-Jun substrate.*
Inhibition of IL-6 activation of Stat3 by IL-1 is reversed by the p38 kinase inhibitor SB203580. MM6 cells were incubated with IL-1 (50 ng/ml) for 20 min followed by a 12-min stimulation with IL-6. Cells were preincubated with SB203580 (10 μM) or PD98059 (20 μM) for 45 min. Cell extracts were assayed for binding to the hSIE oligonucleotide using EMSA (upper panels). The same extracts were analyzed by immunoblotting (IB) with Abs against activated p38, total p38, or Stat3.

Inhibition of IL-6-TRIGGERED Stat3 ACTIVATION BY p38

Activation of p38 did not result in inhibition of Jak1 or Jak2 when these kinases were expressed in transfected cells, suggesting that Jaks are not direct targets of p38 and are not inhibited when they are not associated with a cytokine receptor (data not shown). To further define the upstream target(s) that is inhibited by the MKK3/6–p38 pathway, we determined which of these mechanisms, if any, were operative in our system. The inhibitory effect of CA-MEK1 or CA-MKK3 and CA-MKK6 was confirmed (Fig. 7, second panel). Activation of p38 by CA-MKK3 and CA-MKK6 was confirmed (Fig. 7, third panel, lanes 3 and 4), and activation of ERKs or JNKs was not detected (Fig. 7, bottom panel, and data not shown). These results demonstrate that selective activation of the MKK3/6–p38 pathway is sufficient for inhibiting IL-6-triggered Stat3 activation.

To further strengthen the role of the MKK3/6–p38 pathway in regulating Stat3 activation and to assess the functional consequences of this process on the regulation of transcription by Stat3, reporter gene assays were performed using a 4× IRF-γ-activated sequence-luciferase reporter construct that contains four Stat3 binding sites upstream of the thymidine kinase promoter and is dependent on Stats for cytokine-activated transcription (46). Cells were cotransfected with either a control plasmid, CA-MKK3- and CA-MKK6-encoding plasmids, or a CA-MEK1-encoding plasmid (which specifically activates the ERKs). IL-6 treatment resulted in the induction of reporter gene activity, which was strongly inhibited by the CA-MKK3 and CA-MKK6 kinases (Fig. 8). A representative experiment of three performed is shown. Similar to our previous results in HepG2 cells (37), CA-MEK1 also inhibited gene transcription, although inhibition was not as strong as that seen with MKKs (Fig. 8). Weaker inhibition of IL-6-induced reporter gene activity by CA-MEK1 in 293T cells (Fig. 8) relative to HepG2 cells (37) was consistently observed, and the basis for this is not currently understood. When transfected cells were treated with IL-6 together with soluble IL-6Rα (which results in signaling through gp130 homodimers), activation over background was also strong, as expected. As with IL-6, CA-MKK3 and CA-MKK6 effectively blocked this induction, but inhibition mediated by CA-MEK1 was minimal. In contrast, comparable inhibition by CA-MKK3, CA-MKK6, and CA-MEK1 was seen when LIF, which signals through gp130 and a LIF-specific α-chain, was used. This is consistent with previously described inhibition of LIF signaling by ERKs (57) and confirms that CA-MEK1 is indeed active in our system. The inhibitory effect of CA-MEK1 or CA-MKK3 and CA-MKK6 was not detected when IFN-γ was used, suggesting that these kinases do not nonspecifically block all Jak-Stat signaling, consistent with the lack of inhibition of IFN-γ Stat1 activation (Fig. 1C). These results clearly demonstrate that the MKK3/6 → p38 pathway is capable of inhibiting Stat3-dependent reporter gene activity. They also suggest that this effect may be distinct from inhibition by the MEK→ERK pathway.

Inhibition of IL-6 signaling by MKKs/p38 occurs upstream of Stat3 activation

We have previously shown that inhibition of IL-6 signaling by the ERKs occurs upstream of Stat3 activation (37). Alternatively, others have shown that MAPKs can directly modify Stat3 by phosphorylating it on serine 727 (58–61). We therefore set out to determine which of these mechanisms, if any, were operative in our system. First, we wished to investigate whether Stat3 itself may be a target for inhibition via phosphorylation on serine 727 by the p38 pathway. This was addressed directly using a Stat3 S727A mutant that abolishes serine phosphorylation at this site (58). CA-MKK3 and CA-MKK6 inhibited tyrosine phosphorylation of Stat3 S727A after IL-6 treatment (Fig. 9A, top panel). These results indicate that phosphorylation of Stat3 on serine 727 cannot explain the inhibitory effects seen in our experiments.

To determine whether inhibition occurred upstream of Stat3 in the IL-6 signaling pathway, the levels and activation of the Jak1 kinase, which is the principal Jak required for IL-6 action (62, 63), were analyzed. Treatment of U266 cells (in which IL-6 strongly activates the Jak-Stat pathway, therefore allowing activation of Jak1 to be detected) with sorbitol or H2O2 significantly inhibited Jak1 phosphorylation (Fig. 9B). Reprobing the same filter for Jak1 protein demonstrated comparable Jak1 levels in all lanes. These results indicate that p38-mediated inhibition of IL-6 signaling occurred at least in part upstream of Stats.

Activation of p38 did not result in inhibition of Jak1 or Jak2 when these kinases were expressed in transfected cells, suggesting that Jaks are not direct targets of p38 and are not inhibited when they are not associated with a cytokine receptor (data not shown). To further define the upstream target(s) that is inhibited by the MKK3/
6→p38 pathway, we studied signaling through a truncated fusion receptor that consists of the extracellular domain of TrkC (binds neurotropin-3 (NT3)) and the membrane-proximal 113 aa of the gp130 cytoplasmic domain (of 273 aa present in the wild-type gp130 cytoplasmic domain) to which a Stat3 docking site (GGYMPQ) is fused (64). This receptor signals and activates Stat3 when addition of NT3 leads to dimerization (64). This receptor lacks the Y759 SH2 domain-containing protein-tyrosine phosphatase/SOCS3 docking site (55, 56, 64), therefore allowing inhibition to be studied in the absence of potential inhibitory interactions between gp130 and such molecules. 293T cells were transiently transfected with vector alone, CA-MKK3 and CA-MKK6, or CA-MEK1, and FLAG-tagged Stat3. NT3-induced signaling through the truncated receptor (TrkC-gp130-Ty5), as assayed by Stat3 DNA binding and tyrosine phosphorylation, was inhibited by CA-MKK3 and CA-MKK6 (Fig. 9C, first and second panels, lane 4 vs 2) in a fashion similar to the inhibition seen when signaling was triggered via the wild-type IL-6 receptor (Fig. 7). These results indicate that the target of the MKK3/6→p38 inhibitory pathway is different from Y759, which is the target of SOCS3-mediated inhibition. Interestingly, CA-MEK1 was unable to inhibit Stat3 activation in this system (Fig. 9C), even though it strongly activated the ERKs (data not shown), consistent with results reported by Terstegen et al. showing that ERK-mediated inhibition of gp130 targets Y759 (65).

Discussion

We have investigated the molecular basis for interactions between IL-1, an inflammatory cytokine, and IL-6, a pleiotropic cytokine. IL-1 and IL-6 regulate each other’s expression and are often expressed together at sites of inflammation (2, 3). IL-1 inhibited IL-6-mediated Jak-Stat signaling by a rapidly inducible mechanism, and inhibition of IL-6-induced Stat3 activation was mediated by p38, a kinase that is activated by multiple inflammatory/stress factors and has been strongly implicated in driving inflammation (29, 30). Previously described inducible mechanisms of inhibition of Jak-Stat signaling involve synthesis of inhibitory proteins such as SOCS (18–20, 54), potentially target the Stat molecule itself on a conserved carboxyl-terminal serine (serine 727 in Stat3) (58, 60, 66), or use the ERK subfamily of MAPKs (37) or PKCα or PKCδ isoforms (67). Instead, the mechanism described herein is rapid, independent of de novo expression of inhibitory molecules such as SOCS, independent of modification of Stat3 on serine 727, and occurs at least in part upstream of Stat3 in the IL-6 signaling pathway. p38 is activated by stimuli distinct from those that activate ERKs (30) and appears to target the membrane-proximal region of gp130, which is different from the molecular target of inhibition mediated by ERKs (37, 65) (Figs. 8 and 9). Thus, these findings identify a novel mechanism of inhibition of cytokine Jak-Stat signaling and have important implications for regulation of the balance between progression and deactivation of inflammation (see below).

Several lines of evidence support a role for the MKK3/6→p38 pathway in inhibition of IL-6-triggered Stat3 activation. These include a strong correlation between activation of the p38 kinase by stress agents (IL-1, sorbitol, UV, and H2O2) and inhibition of IL-6 activation of Stat3 (Table I), and reversal of inhibition by the p38 kinase inhibitor SB203580 (Fig. 6 and Table I). Inflammatory and stress agents did not activate ERKs in the cell lines that were used (except for HepG2 cells), and inhibition of Stat3 was not reversed.
by the MEK/ERK inhibitor PD98059. Most convincingly, overexpression of constitutively active MKK3 and MKK6 kinases, which are immediately upstream of p38, served to block Stat3 activation in response to IL-6, resulting in inhibition of DNA binding, tyrosine phosphorylation, and Stat-dependent reporter gene activity (Figs. 7 and 8). The constitutively active MKKs did not activate either the ERKs or the JNKs in our system, confirming that activation of p38 alone was sufficient to inhibit Stat3 activation. It remains possible that the most downstream effector molecule in the MKK3/6→p38 pathway is not p38 but a substrate, possibly a kinase such as MAPK-activated protein (MAPKAP) kinase-2, and we have not excluded that JNKs may play a role in inhibition of IL-6 when cells are exposed to stimuli that activate both p38 and JNKs.

Recent reports show that inflammatory agents such as TNF-α and LPS activate SOCS3 expression, probably via the p38 pathway (51), and SOCS3 docks onto gp130 and inhibits signaling by IL-6 (55, 56). These results have led to the suggestion that TNF and LPS inhibit IL-6 signaling by a SOCS3-dependent mechanism, although to date the evidence for this is correlational. The mechanism of inhibition described in this report is different, in that inhibition is independent of de novo induction of genes, such as SOCS (Fig. 5), occurs in B cells when SOCS3 is not induced (Figs. 2B and 4B), and occurs when the SOCS3 docking site is deleted from gp130 (Fig. 9). Thus, inhibition of IL-6 can occur by two different p38-dependent pathways (see Fig. 10).

Since levels of SOCS3 can remain elevated 4–12 h after addition of LPS to macrophages (50), one may therefore postulate that at early time points after addition of IL-1 or LPS, both the direct p38-dependent and the indirect p38- and SOCS3-dependent pathways may function in inhibition of Stats, whereas at later time points the SOCS3 pathway may predominate. The delayed, possibly SOCS3-dependent, pathway also inhibits IFN-γ signaling (50) and thus is less specific in terms of cytokines that are inhibited than the direct p38-dependent inhibitory pathway described herein. In addition, the relative importance of these pathways may be cell type specific, depending on the intensity and duration of MAPK and SOCS induction. Our data indicate that at early time points, the SOCS3-independent inhibitory pathway is necessary for inhibition of IL-6 signaling to occur.

When cells are treated with high concentrations of sorbitol, stress kinases can contribute to Stat activation (68, 69). Sorbitol (600 mM) was required for effective Stat activation, which was independent of gp130 or Jaks, but seemed to depend upon cell shrinkage (68, 69). We did not detect Stat activation when 400 mM sorbitol was used, possibly secondary to low levels of Stat activation at this concentration, as previously reported (68, 69). Our results are consistent with the experience from a large number of laboratories that physiologic activators of stress kinases, such as IL-1 and TNF, do not typically activate Stat tyrosine phosphorylation or DNA binding (70).

An important issue is the identity of the molecular target(s) of p38-mediated inhibition. Previous work on the interactions between MAPK and Jak-Stat pathways has focused on MAPK-dependent phosphorylation of a conserved carboxyl-terminal serine residue in the Stat proteins themselves (46, 58–61, 66, 71–79). There is general agreement that phosphorylation of Stat1 and Stat3 on serine 727 enhances the transcriptional potency of tyrosine-phosphorylated Stat dimers (46, 61, 74, 76, 78, 79), and one study suggests that DNA binding is enhanced as well (72). However, several studies have suggested that serine phosphorylation of Stats actually suppresses tyrosine phosphorylation and DNA binding (58, 60, 66, 80), although only one of these studies tested this directly using a mutated Stat (58). We have not excluded that serine phosphorylation of Stats may contribute to modulating Stat activity in our system, but several lines of evidence suggest that the predominant site of inhibition occurs upstream of Stats. Inhibition of Jak1 (Fig. 9), the Jak most important for IL-6 signaling (62, 63), indicates that inflammatory/stress stimuli inhibited IL-6 signaling at least in part upstream of Stat activation. Additional support for inhibition upstream of Stats includes the following. 1) Inhibition correlated with the receptor, and not with the Stat, that was activated. Thus, Stat1 and Stat3 activation was blocked when IL-6 or IL-10 was used, but not when IFN-γ or IFNα was used (Fig. 1) (37). 2) Inhibition of Stat3 mutated at serine 727 (Fig. 9); it is unlikely that inhibition of Stat3-S727A can be explained on the basis of phosphorylation of other serine residues in Stat3, since phosphopeptide mapping experiments have shown that serine 727 is the predominant site of serine phosphorylation (46, 58, 72, 74). 3) Inhibition of IL-6 activation of ERKs (which are downstream of Jak5, but independent of Stats) occurred in primary fibroblasts, where, in contrast to hemopoietic cells, IL-6 activation of ERKs was detectable (L. Ivashkiv, unpublished observations).

The two most likely targets of inhibition upstream of Stat3 in the IL-6 signaling pathway are the Jaks or the IL-6R. Although Jak2 can be phosphorylated on serine/threonine residues and inhibited by PKCδ (67), Jaks were not inhibited by p38 in cotransfection experiments (S. Ahmed, unpublished observations). Additional evidence suggesting that Jak1 is not the direct target for inhibition is the lack of inhibition of signaling by IFN-γ and IL-4 receptors that use Jak1 (Fig. 1 and S. Ahmed, unpublished observations). However, this conclusion is subject to the caveat that since IFN-γ is a much stronger activator of Jak1 than IL-6 in the cells that were used (38) (L. Ivashkiv, unpublished observations), the specificity of inhibition observed in Fig. 1 may represent a quantitative, rather than a qualitative, difference. The results obtained using the truncated gp130 fusion receptor (Fig. 9) suggest that the target for p38-mediated inhibition lies in the membrane-proximal 113 aa of

**FIGURE 10.** A model for different roles of p38 in the modulation of cytokine Jak-Stat signaling. The cumulative effect of both positive and negative signaling mediated by p38 probably dictates the outcome of its effect on Stat activation. The negative sign denotes negative regulation of IL-6 signaling via two independent p38-mediated pathways: a direct pathway and one that is SOCS3 dependent. The direct pathway is necessary for inhibition to occur at early time points after addition of IL-1, TNF, or LPS. The SOCS3 pathway may be the predominant pathway at later time points, when it may inhibit IFN-γ signaling as well (not shown). The positive signs denote p38-mediated activation of Stat1 and Stat3 transcriptional activities via serine phosphorylation.
the gp130 cytoplasmic tail, which contains one consensus phosphorylation site for MAPKs, and a serine-rich region (1, 64). Mutation of the MAPK site resulted in an inactive signaling receptor (S. Ahmed, unpublished observations), probably because this site overlaps Jak-receptor interaction sites, and the effects of mutations in the serine-rich region will be tested in future experiments. Another key feature of the truncated receptor is that it lacks the Y759 SH2 domain-containing protein-tyrosine phosphatase/SOCS3 docking site (55, 56, 64), lending further support for the SOCS-independent nature of the inhibition seen in our system. Interestingly, the MEK–ERK pathway failed to inhibit signaling through the truncated gp130 fusion receptor, demonstrating a difference between the p38- and ERK-mediated pathways of inhibition. This is consistent with the observation that ERK-mediated inhibition of IL-6 signaling is dependent on the SOCS3-gp130 interaction (65) via the Y759 docking site. Therefore, different cytoplasmic sequences of the IL-6R complex may be targets for different MAPK pathways.

The opposing actions of IL-1 and MAPK pathways on the IL-6R (inhibit signaling) and on Stat1 and Stat3 (activate transcriptional potency of Stats that are tyrosine phosphorylated and dimerized) may appear paradoxical, but it is becoming increasingly clear that many cytokines and growth factors simultaneously activate multiple signals that may act synergistically or oppose each other (Fig. 10). Regulation of the balance between positive and negative signals also provides an opportunity for cells to fine-tune signals and often determines the ultimate action of cytokines (27, 81). As such, p38 may have both a positive and a negative regulatory role in IL-6 signaling. Inhibition upstream of Stats would result in a lower nuclear concentration of Stat dimers, but these Stats would be serine phosphorylated and transcriptionally active (Fig. 10). Under these conditions, there would be continuing high level expression of promoters that compete effectively for Stats when nuclear Stats are present in limiting amounts, but extinction of expression of genes whose promoters no longer bind Stats. This represents a plausible model to explain the complex effects of IL-1 on IL-6 signaling, in which some IL-6-inducible genes are superactivated, but others are suppressed (14, 32, 33, 35). In contrast to the situation with IL-6, IFN-γ signaling was not blocked by inflammatory cytokines when these cytokines were added 20 min before adding IFN-γ (Fig. 1), but Stat1 is serine phosphorylated and transcriptionally activated by p38 (77, 79). This is predicted to result in increased expression of IFN-γ-inducible genes, as previously reported (50, 71).

Blocking of the anti-inflammatory actions of IL-6 and IL-10 may be important to allow an inflammatory reaction to proceed in the face of expression of the counter-regulatory factors that are often highly expressed at sites of inflammation (2, 31). Interestingly, IL-6 activated expression of three genes, PIA1, PIA3, and the PGE2 receptor, that probably subserve inhibitory or anti-inflammatory functions (44, 52), and IL-1 suppressed IL-6 induction of these genes (Fig. 4A). However, consistent with the complex interplay between IL-1 and IL-6, several patterns of gene regulation were seen, including activation of genes by both IL-1 and IL-6, inhibition of IL-6 by IL-1, and inhibition of IL-1 by IL-6 (consistent with previous reports (14, 32, 33, 35)). To date, synergistic activation of genes (as reported in other systems (14, 32, 33)) has not been observed, but it is something we anticipate demonstrating in future work examining larger arrays of genes. Stress inhibition of IL-6 signaling also suppressed the expression of Stat-dependent IRF-1, Stat1, SOCS1, and PIA5 genes in U266 cells (Fig. 4B) and of a Stat-dependent reporter gene (Fig. 7) and inhibited the proliferation of U266 cells, which are dependent on IL-6 for growth and survival (82) (L. B. Ivashkiv, unpublished observed). These results therefore indicate that inhibition of Stat3 activation by inflammatory/stress factors has important functional consequences for the regulation of cell physiology. In conjunction with our previous results (37), we have now demonstrated that both the ERK and p38 pathways are capable of inhibiting Jak-Stat signaling by differing mechanisms. A large number of receptors important in immune function, including FcRs, complement receptors, Ag receptors, costimulatory molecules, and inflammatory cytokines, activate MAPKs, and FcRs (83), complement receptors (84), and the TCR (43) have been shown to inhibit cytokine Jak-Stat signaling. Thus, modulation of Jak-Stat signaling by MAPKs may play an important role in regulation of the immune cell phenotype.

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References


