Oncostatin M-Induced and Constitutive Activation of the JAK2/STAT5/CIS Pathway Suppresses CCL1, but Not CCL7 and CCL8, Chemokine Expression[1]

Christoph Hintzen,* Claude Haan,† Jan P. Tuckermann,‡ Peter C. Heinrich,§ and Heike M. Hermanns[2]*

The recruitment of leukocytes to injured tissue is crucial for the initiation of inflammatory responses as well as for immune surveillance to fight tumor progression. In this study, we show that oncostatin M, a member of the IL-6-type cytokine family and potent proinflammatory cytokine stimulates the expression of the chemokines CCL1, CCL7, and CCL8 in primary human dermal fibroblasts at a faster kinetic than IL-1β or TNF-α. The production of CCL1 and CCL8 is important for migration of monocytes, while specific Abs against CCL1 additionally inhibit the migration of T lymphocytes. We identify the mitogen-activated protein kinases ERK1/2 and p38 as crucial factors for the enhanced expression of CCL1 and CCL8. Depletion of the ERK1/2 target genes c-Jun or c-Fos strongly decrease CCL1 and CCL8 expression, while p38 MAPK prolongs the half-life of CCL1, CCL7, and CCL8 mRNA through inhibition of tristetraprolin. None of the STAT transcription factors STAT1, STAT3, or STAT5 stimulate transcription of CCL1 or CCL8. However, we identify a negative regulatory function of activated STAT5 for the gene expression of CCL1. Importantly, not STAT5 itself, but its target gene cytokine inducible SH2-domain containing protein is required for the STAT5 inhibitory effect on CCL1 expression. Finally, we show that constitutive activation of STAT5 through a mutated form of JAK2 (JAK2 V617F) occurring in patients with myeloproliferative disorders similarly suppresses CCL1 expression. Taken together, we identify novel important inflammatory target genes of OSM which are independent of STAT signaling per se, but depend on MAPK activation and are partly repressed through STAT5-dependent expression of cytokine inducible SH2-domain containing protein. *The Journal of Immunology,* **2008, 181: 7341–7349.**

The appropriate immune response relies on the interaction of various cell types orchestrated by direct cell contact or soluble factors. As an initial step, recruitment of leukocytes to sites of tissue damage or invaded pathogens occurs. This process is mainly controlled by members of the chemokine superfamily, in particular by the inducible "inflammatory" chemokines (1–3). This subfamily comprises the majority of the so far known 50 chemokines in humans and is distinguished from the constitutively expressed "homeostatic" chemokines. Apart from their function, chemokines can be sorted according to their structure into four groups, designated C, CX_3C, CXC, and CC depending on the number and spacing of conserved cysteines (1–3). The family of monocyte-chemoattractant proteins (CCL2/MCP-1, CCL7/MCP-3, CCL8/MCP-2, and CCL13/MCP-4) has mainly proinflammatory activities and exerts its biologic effects through binding to the G-protein coupled receptors CCR1 and CCR2, which are present on the cell surface of a variety of cell types (1, 3). CCL1/I-309 has been originally identified as a gene expressed in activated T cell lines (4) and specifically binds to CCR8, which is expressed on the cell surface of polarized Th2 cells and regulatory T cells as well as macrophages (5–8).

CCL1 as well as the MCP family members CCL7 and CCL8 are considered to play an important role in the recruitment of monocytes and T lymphocytes to sites of inflammation (9–11). Their enhanced expression during inflammatory processes is stimulated by different cytokines in various cell types, i.e., by IL-1, TNF-α, and IFN-γ in human airway smooth-muscle cells (12), by IL-1 and IFN-γ in both fibroblasts and epithelial cells (13) and by endogenous IL-1 in monocytes (14).

In this study, we describe the regulation of CCL1, CCL7, and CCL8 by the IL-6-type cytokine oncostatin M (OSM)[3] in primary human dermal fibroblasts. OSM is a known proinflammatory cytokine, which is secreted by activated monocytes, neutrophils, and T lymphocytes (15). The human cytokine can signal through two receptor complexes: the type I receptor complex consisting of gp130, the common receptor subunit of all IL-6-type cytokines, and the LIF receptor or the type II receptor complex composed of gp130 and the OSM receptor β subunit (16). In contrast, murine OSM only signals through the type II receptor complex (17). Both, human and murine OSM, are potent activators of the JAK/STAT- and MAPK-pathways. The canonical JAK/STAT-pathway results in the phosphorylation of the STAT transcription factors STAT1,

*Rudolf Virchow Center, Deutsche Forschungsgemeinschaft Research Center for Experimental Biomedicine, University of Würzburg, Würzburg, Germany; †Life Science Research Unit, University of Luxembourg, Luxembourg; ‡Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany; and §Department of Biochemistry, University Hospital Rheinisch-Westfälische Technische Hochschule Aachen, Aachen, Germany

Received for publication April 21, 2008. Accepted for publication September 22, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the Deutsche Forschungsgemeinschaft (SFB 542; TP B6 and Forschungszentrum FZ82 to H.M.H. and Tu220/3 to J.T.).

[2] Address correspondence and reprint requests to Dr. Heike M. Hermanns, Rudolf Virchow Center, Deutsche Forschungsgemeinschaft Research Center for Experimental Biomedicine, University of Würzburg, Versbacher Str. 9, Würzburg, Germany. E-mail address: heike.hermanns@virchow.uni-wuerzburg.de

[3] Abbreviations used in this paper: OSM, oncostatin M; HDF, human dermal fibroblast; MEF, mouse embryonic fibroblast; PEI, polyethylenimine; siRNA, small interfering RNA; TTP, tristetraprolin; CIS, cytokine inducible SH2-domain containing protein.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

Downloaded from www.jimmunol.org on June 12, 2010

STAT3, and STAT5, their dimerization and subsequent translocation into the nucleus where they bind to STAT-specific DNA-binding elements (18, 19). In many cell types, OSM stimulation has also been reported to activate the MAPK ERK1/2 and the stress-activated protein kinases p38 and JNK (20–22). These kinases have been implicated in the regulation of OSM-mediated chemokine expression in previous studies, i.e., eotaxin expression in fibroblasts, stromal-derived factor-1 in mesenchymal stem cells and CCL21 in microvascular endothelial cells (23–25).

To further elucidate the role of OSM within an inflammatory context, the intracellular mechanisms leading to the release of CCL1, CCL7, and CCL8 in human dermal fibroblasts (HDFs) were investigated. OSM-induced transcription of CCL1, CCL7, and CCL8 was dependent on activation of ERK1 and ERK2 and their downstream targets c-Jun and c-Fos, forming the transcription factor complex AP-1. The chemokine mRNAs were stabilized by p38 MAPK-dependent inhibition of tristetraprolin and their secretion stimulated the migration of monocytes and T lymphocytes. Furthermore, we identified a novel negative regulatory mechanism for the induction of CCL1, which is dependent on STAT5-induced cytokine inducible SH2-domain containing protein (CIS) expression. This negative regulatory loop also appears to function in acute myeloid leukemia cells, which express a constitutively active form of JAK2.

Materials and Methods

Cell culture and cytokines

Primary HDFs and murine embryonic fibroblasts (MEFs) were maintained in DMEM (Invitrogen). STAT5a/b heterozygous mice were intercrossed; single E14.5 embryos were collected and genotyped as described (26). MEFs were isolated from these embryos and immortalized as described (27). Primary monocytes, primary T lymphocytes, and HEL cells were grown in RPMI 1640 medium (Invitrogen). All media were supplemented with 10% FCS or 1% human serum (PAA) for primary blood cells. Cells were grown at 37°C in a water-saturated atmosphere containing 5% CO_2. HDFs were generated as described previously (28). Human PBMCs were isolated from fresh blood or from buffy coats. Monocytes and T cells were isolated with a Dynal Monocyte Negative Isolation Kit or a Dynal T Cell Negative Isolation Kit, respectively, as described by the manufacturer (Invitrogen). Recombinant human OSM, TNF-α, and IL-1β were obtained from Cell Concepts and murine OSM from R&D Systems. Human LIF was purchased from Sigma-Aldrich. rIL-6 and soluble IL-6R were prepared as described (29).

Cell lysis and Western blotting

HDF and MEF cells were stimulated for the indicated times with 20 ng/ml human or murine OSM. Immediately after stimulation, cells were lysed in Triton lysis buffer as described previously (30). All steps of cell lysis were performed at 4°C using ice-cold buffers. Proteins were separated by SDS-PAGE in 10% gels, followed by electroblotting onto a polyvinylidene difluoride membrane (PALL). Western blot analysis was conducted using the indicated Abs and the ECL kit (GE Healthcare) according to the manufacturer's instructions. Before reprobing, blots were stripped in 2% SDS and 100 mM 2-ME in 62.5 mM Tris-HCl (pH 6.7) for 20 min at 70°C. The pharmacological inhibitors U0126 (Promega), SB202190, SP600125, AG490, and JAK inhibitor 1 (Merck AG) were applied 30 min prior stimulation with cytokines.

Antibodies

The phosphospecific polyclonal Abs against STAT1(Tyr^{701}), STAT3(Tyr^{705}), STAT5(Tyr^{694}), ERK1/2(Thr^{202}/Tyr^{204}), MK2(Thr^{222}), and JAK2 ($Tyr^{1007/1008}$), as well as STAT1, STAT3, MK2, JAK2, ERK1/2, c-Fos, c-Jun, and α/β-tubulin Abs were purchased from Cell Signaling Technology. The polyclonal Abs against STAT5b (C-17) and p38 (C-20) were obtained from Santa Cruz Biotechnology and the antiserum against active-p38 from Promega. The HRP-conjugated secondary Abs were purchased from DakoCytomation.

RT-PCR

Total RNA was isolated from HDFs and MEFs stimulated for the indicated times with 20 ng/ml human or murine OSM, respectively, using RNeasy columns (Qiagen) according to the manufacturer's instructions. RT and PCR were performed with 1 μg of total cell RNA using the OneStep RT-PCR kit (Qiagen). Detection of specific mRNA for SOCS1, SOCS3, CIS, CCL1, CCL7, CCL8, and TCA-3 was achieved by using primers designed to amplify at least one exon (across one intron/exon border to exclude contamination of cDNA with genomic DNA). GAPDH was used as an internal standard (31). Amplification was conducted with 35 cycles of 40 s denaturation at 94°C, 30 s annealing at 53–58°C and 30 s extension at 72°C. The amplification was terminated with an extension step for 10 min at 72°C after the last cycle. PCR products were separated on 1% agarose gels and stained with ethidium bromide.

RNase protection assays

Total RNA was isolated as described above and protection assays were carried out using the mCK-5c and hCK8 RiboQuant MultiProbe Template set from BD Biosciences. Autoradiographies were quantified using a Typhoon 9400 (GE Healthcare).

ELISA

Subconfluent cultures (70–80%) were stimulated for the indicated time periods and with the indicated cytokines in normal supplemented medium. Supernatants were collected and analyzed by ELISA. Human CCL1 and CCL8 as well as murine TCA-3 ELISA kits were purchased from R&D Systems and used according to the manufacturer's protocol.

Transfection of murine embryonic fibroblasts

MEFs were transfected using polyethylenimine (PEI). All polyplexes consisting of DNA and PEI were prepared in a sterile isotonic glucose solution at pH 7.4. The preparation occurred in two steps: 8 μg DNA, resuspended in a total volume of 304 μl isotonic glucose solution, were incubated for 10 min at room temperature. Then, 96 μl PEI solution (2 mg/ml) were added and after thorough mixing incubated for 20 min at room temperature. The transfection mixture was added to 1×10^5 MEFs in 2.6 ml OptiMEM/10% FCS (Invitrogen). After 4 h at 37°C the transfection medium was removed and replaced by DMEM/10% FCS. Forty-four hours later cells were stimulated and lysed as described above.

Modified Boyden chamber assay

Primary monocytes and T-lymphocytes were incubated for 2 h after isolation in RPMI 1640 containing 1% human serum and maintained in DMEM without serum throughout the assay. Transwell inserts (6.5 mm diameter, 8 μm pore size; Corning Costar) were coated with human fibronectin. Into each transwell, 1×10^5 monocytes or T-lymphocytes/100 μl were transferred. The transwell was inserted into a 24-well plate containing 800 μl supernatant of OSM-stimulated HDFs or IL-1β-stimulated HEL cells and monocytes, respectively. Cell migration to the bottom chamber was assessed 4 h later. To block migration, specific Abs (R&D Systems) were added to the supernatant in the bottom chamber. Every migration assay was performed at least three times in triplicate.

Small interfering RNA (siRNA) transfection

HDFs were seeded in 6-well plates and allowed to grow to 70% confluency for 24 h. Transient transfections were performed with DharmaFECT1 transfection reagent (Perbio Europe) according to the manufacturer's protocol. In brief, 75 nM siRNA (OnTargetPlus SMARTpools; Perbio Europe) or nonsilencing off-target siRNA (Qiagen) and DharmaFECT1 transfection reagent were each diluted first in reduced serum medium (OptiMEM; Invitrogen) and then mixed. The mixtures were allowed to incubate for 30 min at room temperature and then added in drops to each culture dish containing medium without FCS. Two hours later, complete DMEM medium was added. Forty-eight hours after transfection the cells could be stimulated and further investigated.

Statistical analysis

All data are expressed as mean $\pm$ SD for at least three separate experiments (Student's t test). A probability $p < 0.05$ was considered significantly different. All analyses were performed using the statistical software GraphPad Prism (GraphPad Prism for Windows, Version 3.00, GraphPad Software).

Results

Oncostatin M stimulates production of CCL1, CCL7, and CCL8 by primary human dermal fibroblasts

To elucidate the effect of OSM on the expression of CCL1, CCL7, and CCL8 in HDFs, their mRNA levels were determined by RNase

Downloaded from www.jimmunol.org on June 12, 2010

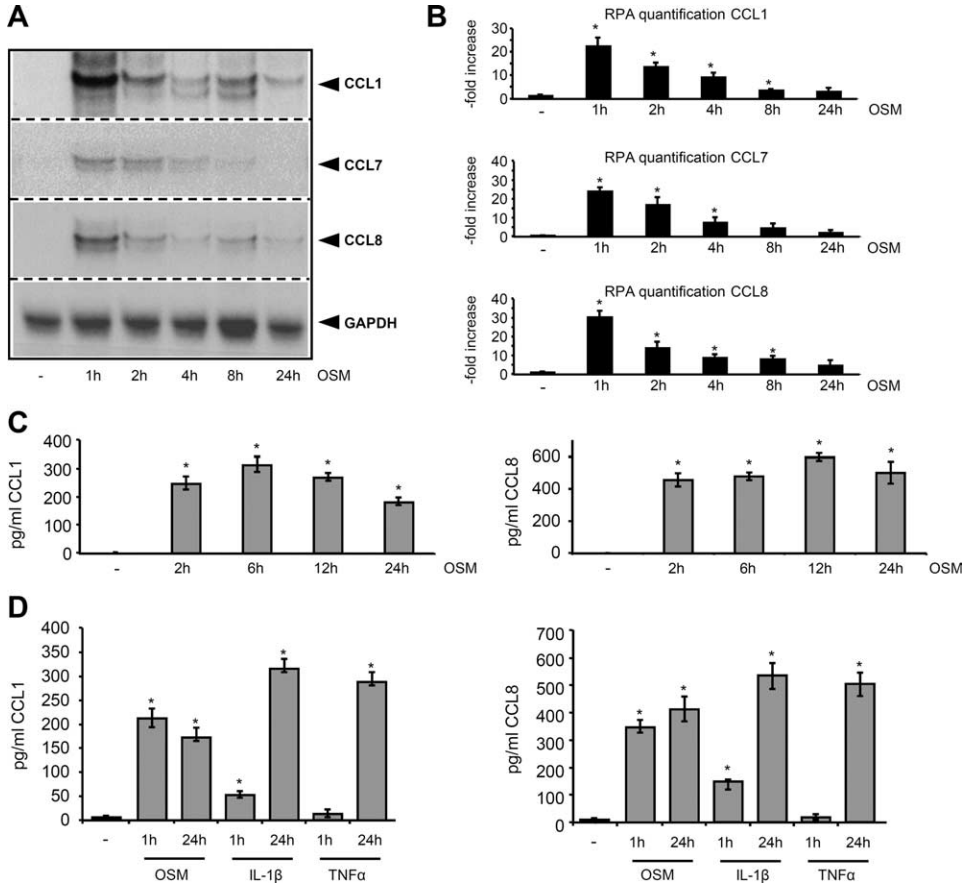

FIGURE 1. OSM-induced expression of CCL1, CCL7, and CCL8. *A*, The mRNA levels of CCL1, CCL7, and CCL8 in HDFs treated with 20 ng/ml OSM are determined by RNase protection assays and one representative of three independent experiments is shown. *B*, RNase protection assay results were quantified by phosphoimager, the relative mRNA levels were normalized to those of GAPDH and expressed as fold induction relative to unstimulated cells. The values shown are mean ± SD ($n = 3$). *, $p < 0.05$ vs control. *C*, Protein amounts of CCL1 and CCL8 in culture supernatants of OSM-treated HDFs (20 ng/ml) were determined by ELISA. Data are expressed as mean ± SD ($n = 4$). *, $p < 0.05$ vs control. *D*, Protein amounts of CCL1 and CCL8 in culture supernatants of OSM- (20 ng/ml), IL-1β- (10 ng/ml) or TNF-α- (10 ng/ml) treated HDFs (1 and 24 h) were determined by ELISA. Data are expressed as mean ± SD ($n = 3$). *, $p < 0.05$ vs control.

protection assays. Human OSM (20 ng/ml) stimulated the transcription of CCL1, CCL7, and CCL8 transcription time dependently with a peak at 60 min. Significant quantities of all mRNAs were still detectable 4 h after stimulation (Fig. 1, *A* and *B*). ELISAs, conducted for CCL1 and CCL8 to quantify protein levels in HDF supernatants, confirmed the secretion of up to 300 pg/ml CCL1 and up to 600 pg/ml CCL8 (Fig. 1*C*). Once produced, the proteins were stable for at least 12 h in cell supernatants. Furthermore, analysis of the required OSM dose demonstrated that enhanced transcription of the chemokines starts at ~1 ng/ml and reached a plateau at 10 ng/ml (Supplementary Figure S1*A*).[4] To evaluate the ability of other inflammatory cytokines to induce CCL1, CCL7, and CCL8, HDFs were stimulated for 4 h with 100 ng/ml LIF, 20 ng/ml IL-6 (in combination with 0.5 μg/ml soluble IL-6R), 10 ng/ml TNF-α, or 10 ng/ml IL-1β. TNF-α and IL-1β up-regulated CCL1, CCL7, and CCL8 mRNA levels to a similar extent as OSM (Supplementary Figure S1, *B–D*). However, for IL-1β- and TNF-α-treated HDFs, a remarkable delay in protein secretion was detectable; only OSM induced maximal chemokine secretion already 1 h after cytokine stimulation. After 24-h stimulation though, all three cytokines stimulated substantial release of chemokines (Fig. 1*D*). In contrast to the proinflammatory cytokines, IL-6 stimulation induced only marginal increases in CCL7 and CCL8 mRNA levels and LIF completely failed to initiate mRNA expression of the analyzed chemokines (Supplementary Figure S1, *B*). In summary, OSM is a potent inducer of CCL1, CCL7, and CCL8 expression comparable to IL-1β and TNF-α, however, maximal chemokine secretion is achieved at earlier time points.

[4] The online version of this article contains supplementary material.

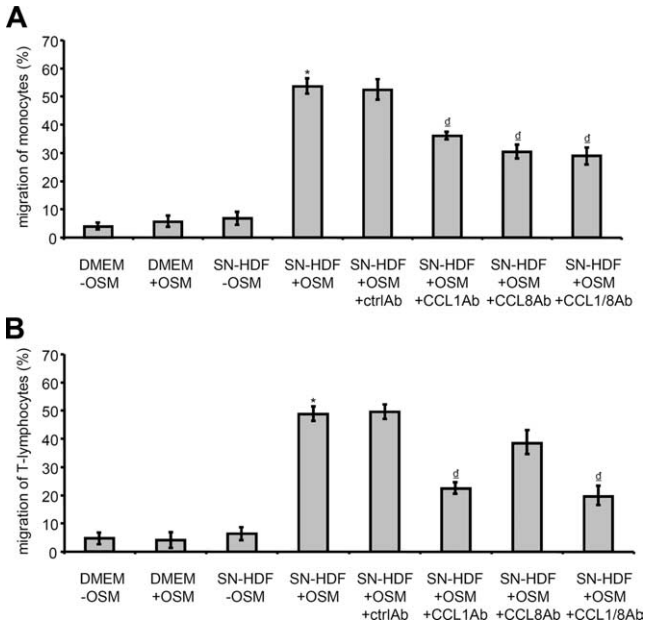

FIGURE 2. OSM-induced CCL1 and CCL8 expression mediates migration of primary monocytes and T lymphocytes. *A* and *B*, Culture supernatants of OSM-treated HDFs (20 ng/ml, 3 h) were used to analyze the potential of secreted chemokines to induce migration in a modified Boyden chamber assay as described in *Materials and Methods*. The numbers of migrated monocytes or T lymphocytes were determined and compared with controls (untreated DMEM). Values shown are mean ± SD ($n = 3$). *, $p < 0.05$ vs control; d, $p < 0.05$ vs Ab control.

Downloaded from www.jimmunol.org on June 12, 2010

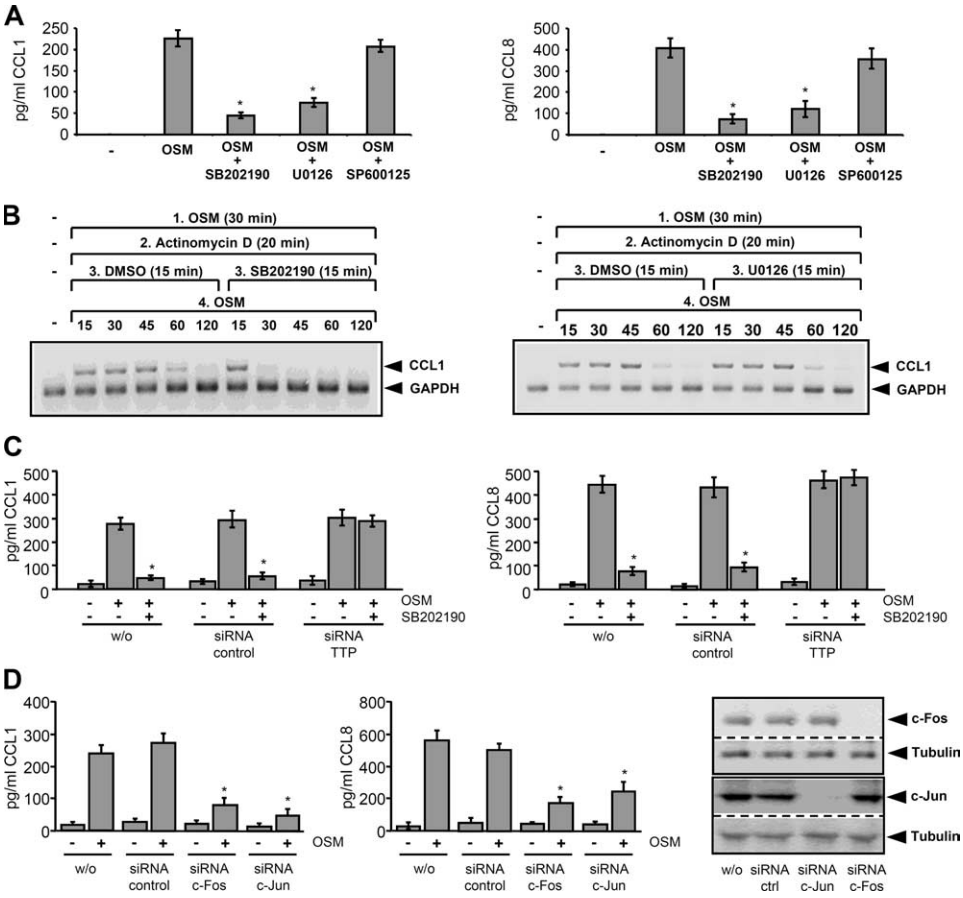

FIGURE 3. OSM-induced CCL1 and CCL8 expression is dependent on MAPK signaling. *A*, HDFs were pretreated with 10 μM SB202190, 10 μM U0126, or 10 μM SP600125 for 30 min, exposed to 20 ng/ml OSM for 3 h and secreted protein amounts of CCL1 or CCL8 were measured by ELISA from culture supernatants. Data are expressed as mean ± SD ($n = 5$). ∗, $p < 0.05$ vs control. *B*, HDFs were stimulated with 20 ng/ml OSM for 30 min. Subsequently, cells were washed and cultivated for additional 20 min in OSM-free medium containing actinomycin D (4 μM) to block transcription. Afterward, 10 μM SB202190 or 10 μM U0126 were added to the medium before the cells were finally stimulated a second time with OSM for the indicated time periods. CCL1 mRNA and GAPDH mRNA levels were analyzed by RT-PCR. One representative of three independent experiments is shown. *C*, HDFs were transfected with TTP siRNA as described in *Materials and Methods*. Forty-eight hours after transfection, cells were pretreated with 10 μM SB202190 for 20 min and subsequently stimulated with 20 ng/ml OSM for 3 h. The amounts of CCL1 or CCL8 in culture supernatants were measured by ELISA. Data are expressed as mean ± SD ($n = 3$). ∗, $p < 0.05$ vs control. *D*, HDFs were transfected with control, c-Jun, or c-Fos siRNA, respectively. The amounts of CCL1 or CCL8 in culture supernatants were measured by ELISA. Data are expressed as mean ± SD ($n = 4$). ∗, $p < 0.05$ vs control. To analyze knock-down efficiency whole cellular lysates were subjected to Western blot analysis using specific Abs against c-Fos and c-Jun. Tubulin expression served as loading control.

OSM-induced CCL1 and CCL8 expression leads to migration of monocytes and T lymphocytes

As both CCL1 and CCL8 have been implicated in the recruitment of monocytes and T lymphocytes, we wondered whether the amounts of CCL1 and CCL8 induced by OSM are sufficient to stimulate leukocyte migration. HDFs were stimulated with 20 ng/ml OSM for 3 h and the chemokine-containing supernatant was added to a modified Boyden chamber assay. Direct OSM treatment or supernatant from untreated HDFs had no influence on the migration of the leukocytes (Fig. 2, *A* and *B*, *second and third column*). In contrast, the supernatant of OSM-stimulated HDFs induced the migration of ~50% of the seeded monocytes or T lymphocytes to the bottom reservoir of the transwell (*fourth column*). To evaluate the contribution of CCL1 and CCL8 to migration, we added neutralizing Abs to both chemokines, alone or in combination. As expected, neutralization of CCL1 reduced the migration of monocytes (Fig. 2*A*, *sixth column*) and T lymphocytes (Fig. 2*B*, *sixth column*) while the blockade of CCL8 significantly reduced migration of monocytes (Fig. 2*A*, *seventh column*), but only had a minor inhibitory effect on T lymphocytes (Fig. 2*B*, *seventh column*). Simultaneous addition of both Abs revealed no additive effect (Fig. 2, *A* and *B*, *eighth column*).

In conclusion, OSM induced the migration of leukocytes via HDF-released factors mediated in part by CCL1 and CCL8.

OSM-induced ERK1/2 activation is involved in transcription of CCL1, CCL7, and CCL8 while p38 MAPK stabilizes the mRNA through a tristetraprolin (TTP)-inhibiting mechanism

Next, we investigated which signal transduction pathways participate in CCL1, CCL7, and CCL8 transcription. Established pharmacological inhibitors of the three MAPK families ERK, p38, and JNK served as tools to assess their roles in OSM-mediated CCL1 and CCL8 induction. Preincubation of HDFs with the p38 MAPK inhibitor SB202190 or the ERK1/2 inhibitor U0126, but not the JNK inhibitor SP600125, led to a significant reduction in the protein levels of CCL1 and CCL8 detected in HDF supernatants 3 h after OSM stimulation (Fig. 3*A*). Analysis of mRNA levels by RNase protection assays proved to be consistent with the results of protein assays (data not shown). Effectiveness of all inhibitors was

Downloaded from www.jimmunol.org on June 12, 2010

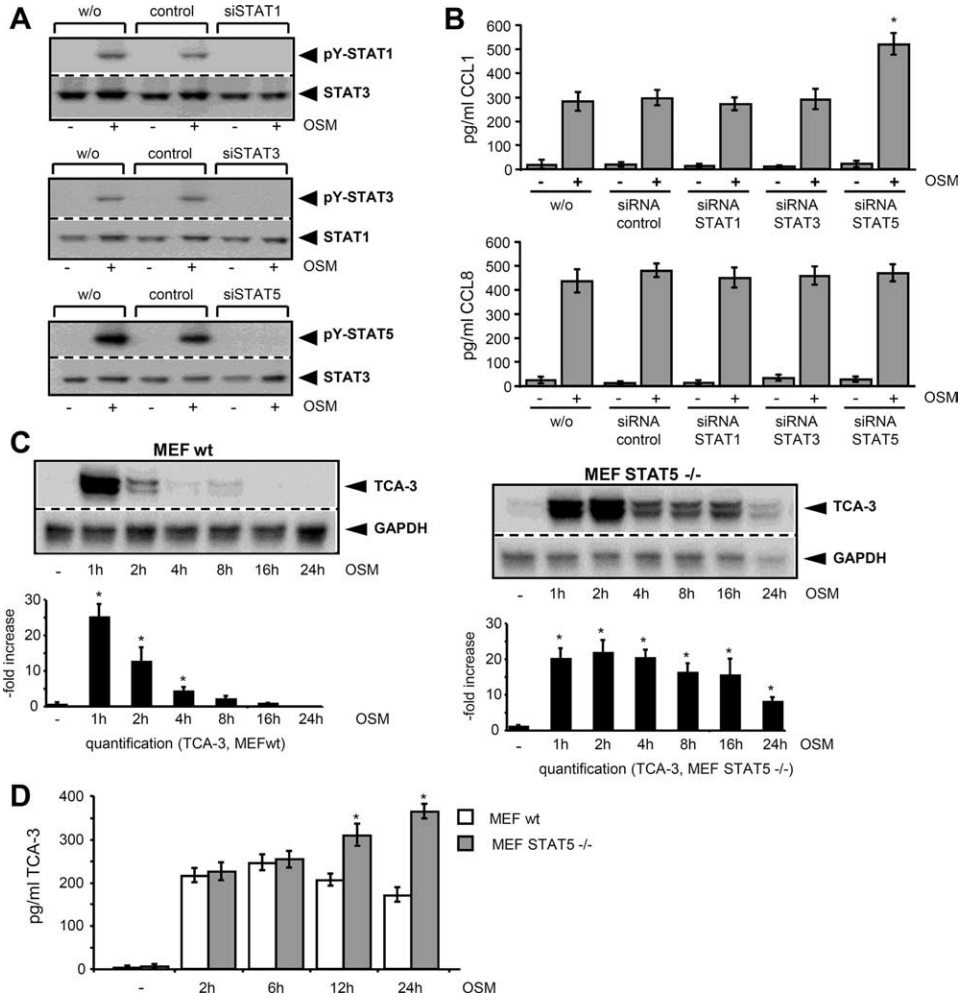

FIGURE 4. CCL1 and TCA-3 expression are negatively regulated by STAT5 activation. *A*, Western blot detection of whole cellular extracts of siRNA transfected HDFs, treated with OSM (20 ng/ml, 3 h), using specific antisera against phospho-STAT1, -STAT3, or -STAT5. Subsequently the blots were stripped and reprobed with antisera recognizing proteins irrespectively of their activation. *B*, Amounts of CCL1 or CCL8 in culture supernatants of transfected HDFs were measured by ELISA. Data are expressed as mean ± SD ($n = 5$). *, $p < 0.05$ vs control. *C*, MEF cells were treated with 20 ng/ml murine OSM for the indicated time periods. The mRNA levels of TCA-3 were determined by RNase protection assays and a representative experiment of three independent experiments is shown. RNase protection assay results were quantified and the relative levels of TCA-3 following OSM stimulation were normalized to those of GAPDH and compared with unstimulated cells. The values shown are mean ± SD ($n = 3$). *, $p < 0.05$ vs control. *D*, ELISA to detect protein amounts of TCA-3 in MEF cells treated with murine OSM (20 ng/ml) for the indicated time periods.

demonstrated by Western blot analysis of target proteins (Supplementary Figure S2*A*).

To corroborate whether p38 and ERK1/2 were directly involved in initiation of transcription or stabilization of mRNA, we compared the time-dependent loss of CCL1, CCL7, and CCL8 mRNA in the presence or absence of OSM and the respective pharmacological inhibitors SB202190 or UO126 (Fig. 3*B* and Supplementary Fig. S2*B*). Chemokine expression in HDFs was induced by stimulation with OSM for 30 min. Afterward, OSM was removed and actinomycin D was added to the medium to prevent ongoing mRNA synthesis. After 20-min incubation with actinomycin D, OSM was added to restart activation of signal transduction in the absence or presence of the MAPK inhibitors SB202190 or UO126. Whereas CCL1 mRNA induced by 30 min OSM treatment was degraded only after 60 min of additional OSM treatment, the presence of the p38 inhibitor SB202190 in the supernatant prevented stabilization of the mRNA levels and led to rapid degradation already after additional 15 min incubation (Fig. 3*B*, *left panel*). The MEK1/2 inhibitor UO126, however, had no destabilizing effect on CCL1 mRNA (Fig. 3*B*, *right panel*). Equivalent results were obtained for CCL7 and CCL8 mRNA (Supplementary Fig. S2*B*). Control experiments demonstrated that OSM was not able to induce chemokine transcription in the presence of actinomycin D (Supplementary Fig. S2*B*, *right*).

Stabilization of AU-rich mRNAs can be mediated by p38 MAPK-dependent phosphorylation and subsequent degradation of the mRNA destabilizing protein TTP. Therefore, one could envisage a similar mechanism for OSM-activated p38 MAPK. Consequently, chemokine mRNA should be stabilized in TTP knockdown cells irrespective of treatment with the p38 inhibitor SB202190. Indeed, mRNA levels of CCL1, CCL7, and CCL8 showed a prolonged half-life in TTP siRNA-treated HDFs despite inhibition of p38 by SB202190 (Supplementary Fig. S2*C*). Consistently, the strong reduction of OSM-induced CCL1 and CCL8 secretion in the presence of SB202190 was abrogated in cells pretreated with TTP siRNA (Fig. 3*C*, compare *sixth column* with *ninth column*).

Because inhibition of ERK activation had no influence on chemokine stability, we investigated a direct involvement in transcription by depleting the mRNAs of two prominent ERK1/2 immediate early target genes, c-Jun and c-Fos (Fig. 3*D*, *right panel*). Indeed, lack of c-Jun or c-Fos strongly reduced the capacity of OSM to induce transcription and secretion of CCL1 (Fig. 3*D*, *left panel*) and CCL8 (Fig. 3*D*, *middle panel*).

OSM-induced production of CCL1, but not CCL8, is negatively regulated by STAT5

To investigate whether the OSM-stimulated CCL1 and CCL8 expression requires the JAK/STAT signaling cascade as well, HDFs were transfected with specific siRNAs against STAT1, STAT3, and STAT5, respectively. Although the analysis of whole cellular extracts indicated a complete knock-down of activated STAT factors (Fig. 4*A*), neither STAT1 nor STAT3 absence affected CCL1 or CCL8 production (Fig. 4*B*). Knock-down of STAT5, however, resulted in a strong increase of CCL1 protein secretion (Fig. 4*B*, *upper panel*).

Downloaded from www.jimmunol.org on June 12, 2010

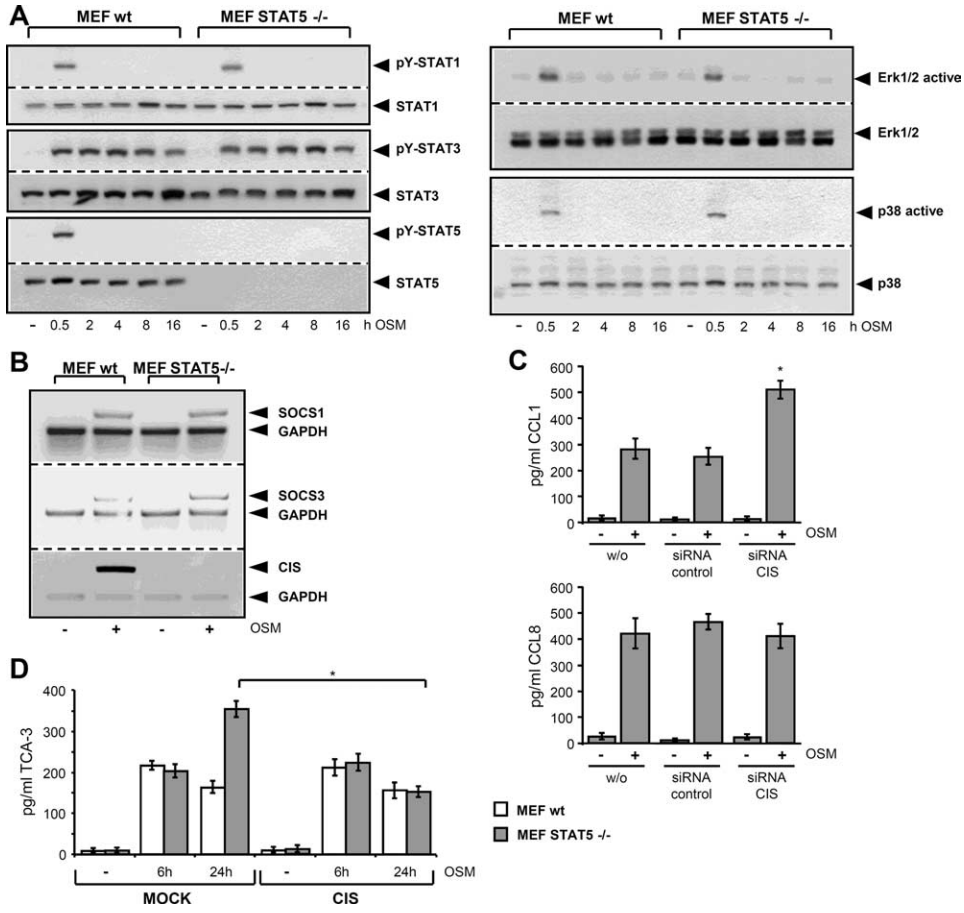

FIGURE 5. The STAT5-dependent negative regulation of CCL1 expression is mediated by CIS. *A*, Western blot detection of whole cellular extracts of MEFs, treated with OSM (20 ng/ml for the indicated time periods), using Abs specific for phosphorylated STAT1, STAT3, STAT5, ERK1/2, and p38. The blots were stripped and reprobed with antisera recognizing the proteins irrespectively of their activation. *B*, RT-PCR to detect SOCS1, SOCS3, and CIS mRNA levels in MEFs stimulated with 20 ng/ml murine OSM for 1 h. One representative of three independent experiments is shown. *C*, HDFs were transfected with CIS siRNA and then stimulated with 20 ng/ml human OSM for 3 h. The protein amounts of CCL1 or CCL8 in culture supernatants were measured by ELISA. Data are expressed as mean ± SD ($n = 4$). *, $p < 0.05$ vs control. *D*, MEF cells were transiently transfected with expression vectors for muCIS or control vector and stimulated with mOSM as indicated. The protein amounts of TCA-3 in culture supernatants were measured by ELISA. Data are expressed as mean ± SD ($n = 3$). *, $p < 0.05$ vs MOCK-transfected control.

Previous studies have shown that STAT5 can be directly activated by JAK2 after cytokine stimulation (30, 32). To corroborate our results, we treated HDFs with AG490, an inhibitor with higher affinity to JAK2 than to other JAK family members. In fact, significantly increased CCL1 production is also observed after the administration of AG490 (Supplementary Fig. S3, *left panel*). However, the inhibitor does not exert any effect on the OSM-induced CCL8 expression (*right panel*).

TCA-3 represents the murine homologue to human CCL1. We therefore assessed the TCA-3 mRNA and protein levels in wild-type and STAT5$^{-/-}$ MEF cells after OSM stimulation. A clear though transient induction of TCA-3 mRNA was observed in wild-type cells (Fig. 4*C*, *left*), whereas significantly elevated TCA-3 mRNA levels were detectable in STAT5-deficient MEFs up to 24 h after OSM stimulation (Fig. 4*C*, *right*). These elevated levels of TCA-3 mRNA in STAT5-deficient MEFs resulted in clearly elevated protein levels of TCA-3 in the supernatants after 24 h of stimulation (Fig. 4*D*).

To further investigate which factor(s) might be responsible for the negative regulation of TCA-3 in wild-type MEFs, STAT5 itself, a STAT5-inducible gene or secondary players, the activation patterns of prominent members of the OSM-inducible pathways were analyzed (Fig. 5*A*). We observed very similar phosphorylation kinetics for STAT1, STAT3, ERK1/2, and p38 in wild-type compared to STAT5$^{-/-}$ MEFs after stimulation with OSM over the indicated periods of time. As expected, the STAT5 activation was not detectable in STAT5$^{-/-}$ cells (*third panel, left*). The analysis of common feedback inhibitors of IL-6-type cytokine signaling, SOCS1 and SOCS3, shows that both proteins are inducible by OSM in wild-type and STAT5-deficient cells. Induction of the STAT5-dependent feedback inhibitor CIS, however, is not observed in STAT5$^{-/-}$ MEFs (Fig. 5*B*, *third panel*). Based on these results, we studied the role of CIS on OSM-induced CCL1 expression. We transfected HDFs with CIS siRNA and subsequently stimulated with OSM (Supplementary Fig. S4*A*). The secreted amount of CCL1 was significantly increased in CIS siRNA-transfected cells 3 h post OSM stimulation (Fig. 5*C*, *upper panel*), whereas the OSM-induced CCL8 expression is not affected by the knock-down of CIS (Fig. 5*C*, *lower panel*). We conclude that the elevated levels of CCL1 in the absence of STAT5a/b are due to the failure of OSM to induce CIS expression. Consequently, overexpression of CIS reduced the elevated mRNA and protein expression of TCA-3 in STAT5-deficient MEFs to levels produced by wild-type MEFs (Fig. 5*D*, Supplementary Fig. S4, *B* and *C*). Neither STAT5 nor CIS knock-down affected the IL-1β or TNF-α-mediated expression of CCL1 (Supplementary Fig. S4*D*).

Constitutive activation of the JAK2/STAT5 pathway in acute myeloid leukemia cells suppresses CCL1 expression

The STAT transcription factors, particularly STAT3 and STAT5, are known to be constitutively activated in a number of tumor cells. Recently, a mutation in human JAK2 (JAK2 V617F) has been identified in patients with myeloproliferative disorders (33). This mutation in the pseudokinase domain of JAK2 results in the constitutive activation of the enzyme and consequently in the activation of STAT5 and ERK1/2 (Fig. 6*A*, *lane 1*). Because we identified a negative regulatory function of STAT5/CIS in suppressing the expression of CCL1, we predicted that this chemokine production is also constitutively suppressed in acute myeloid leukemia cells harboring the JAK2 V617F mutation (HEL cells). Indeed, these cells constitutively express CIS (Fig. 6*B*, *lane 1*) and, in contrast to primary monocytes from healthy donors, stimulation

Downloaded from www.jimmunol.org on June 12, 2010

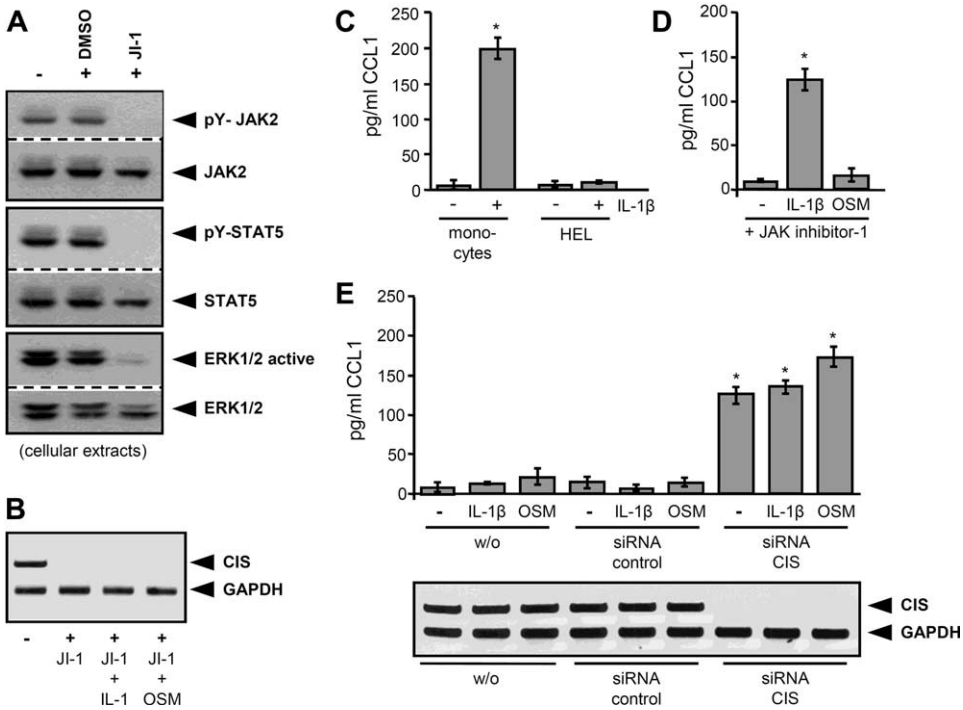

FIGURE 6. The JAK2 V617F mutation leads to CCL1 suppression. *A*, HELs were left untreated or incubated with 1 μM JAK inhibitor-1 (JI-1) or the solvent DMSO for 12 h. Lysates were prepared and subjected to Western blot analysis using Abs specific for phosphorylated JAK2, STAT5, and Erk1/2. The blots were stripped and reprobed with antisera recognizing the proteins irrespectively of their activation. *B*, HELs were preincubated with 1 μM JAK inhibitor-1 (JI-1) for 12 h and then stimulated with 10 ng/ml IL-1β or 20 ng/ml OSM for 1 h. RNA was isolated and RT-PCR used to detect CIS mRNA. *C*, Primary monocytes and HEL cells were stimulated with 10 ng/ml IL-1β for 3 h. The protein amounts of CCL1 in culture supernatants were measured by ELISA. Data are expressed as mean $\pm$ SD ($n = 4$). $*$, $p < 0.05$ vs control. *D*, HELs were preincubated with 1 μM JI-1 for 12 h and then stimulated 10 ng/ml IL-1β or 20 ng/ml OSM for 3 h. The protein amounts of CCL1 in culture supernatants were measured by ELISA. Data are expressed as mean $\pm$ SD ($n = 3$). $*$, $p < 0.05$ vs control. *E*, HELs were transfected with control or CIS siRNA and stimulated with 10 ng/ml IL-1β or 20 ng/ml OSM for 3 h. The protein amounts of CCL1 in culture supernatants were measured by ELISA. Data are expressed as mean $\pm$ SD ($n = 3$). $*$, $p < 0.05$ vs control. To analyze knock-down efficiency RT-PCR was used to detect CIS mRNA levels.

with IL-1β or OSM could not induce secretion of CCL1 in HEL cells (Fig. 6*C*, f*ourth column*; Fig. 6*E*, *second* and *third column*).

To abrogate the constitutive activation of JAK2, HEL cells were incubated for 12 h with the JAK inhibitor-1, a general inhibitor of all Janus kinases. This treatment abrogated the constitutive activation of JAK2, STAT5, and ERK1/2 (Fig. 6*A*, *lane 3*). Furthermore, constitutive expression of CIS was inhibited (Fig. 6*B*, *lane 2*). Because OSM itself, however, relies on the activation of JAK1 and JAK2 to initiate its own signaling cascade, treatment of HEL cells with JI-1 precludes stimulation with OSM. Thus, OSM which is usually a strong inducer of CIS (Fig. 5*B*) could no longer stimulate CIS expression (Fig. 6*B*, *lane 4*) or CCL1 expression (Fig. 6*D*, *third column*). IL-1β, however, signals via IRAK and IKK and therefore independent of Janus kinases. Hence, this cytokine is still actively working in the presence of JI-1 and was additionally used for the experiments. Interestingly, in the absence of the constitutive active JAK2/STAT5/CIS pathway, IL-1β stimulation of HEL cells strongly induced CCL1 expression (Fig. 6*D*, *second column*).

To directly prove the involvement of CIS in the suppression of CCL1 expression in HEL cells we abrogated the constitutive CIS expression through siRNA knock-down (Fig. 6*E*, *lower panel*). Exclusive knock-down of CIS resulted in constitutive secretion of CCL1 (Fig. 6*E*, *upper panel*, *seventh column*). Because we could show that c-Jun and c-Fos are important positive regulators of CCL1 expression (Fig. 3*D*, *left panel*), the constitutive activation of the ERK1/2 pathway in HEL cells explains the constitutive expression of CCL1. This constitutive expression could be slightly increased by additional stimulation with OSM (Fig. 6*E*, *ninth column*), but not by IL-1β (*eighth column*). Because IL-1β itself does not induce CIS expression knock-down of CIS in JAK inhibitor-1 treated HEL cells had no further stimulatory effect on IL-1β-induced CCL1 (Supplementary Fig. S5, *eighth column*). However, it is important to note that the constitutively expressed CIS (due to the JAK2 V617F mutation) can inhibit IL-1β-induced CCL1 expression in trans in HEL cells.

Discussion

The results presented in this study demonstrate for the first time that human OSM is able to induce CCL1, CCL7, and CCL8 expression, both at mRNA and protein levels in HDFs to levels comparable to those found for the hitherto strongest stimulators IL-1β and TNF-α. Compared with these well-known proinflammatory cytokines OSM acts significantly faster on HDFs leading to maximal secretion of the chemokines already after 1 h of stimulation. The migration assays performed for monocytes and T lymphocytes underline the physiological relevance and efficacy of the OSM-mediated CCL1 and CCL8 induction. Former publications demonstrated that OSM-induced eotaxin secretion is important in the eosinophilic inflammatory process (23) and an increased production of CCL21 in lymphatic endothelial cells promoted migration of dendritic cells into lymph nodes (25).

Interestingly, the close relative of OSM, LIF, cannot induce CCL1 and/or CCL8, indicating that OSM-induced chemokine expression of CCL1, CCL7, and CCL8 is likely to be mediated via the OSMR/gp130 receptor complex (type II) and not by the LIFR/gp130 receptor complex (type I), also used by LIF.

Downloaded from www.jimmunol.org on June 12, 2010

Previous reports indicated that OSM uses the JAK/STAT- or the MAPK-pathway to induce the chemokines CCL2, eotaxin, or stromal-derived factor-1 (23, 25, 34, 35). In this study, we show for the first time the involvement of OSM-induced signaling pathways in CCL1, CCL7, and CCL8 transcription by the use of siRNA interference as well as well established pharmacological inhibitors (SB202190, U0126, and SP600125). These studies demonstrated that JNK is dispensable for the expression of CCL1 or CCL8, whereas p38 and ERK1/2 are crucial for the expression of these chemokines. Closer investigation of the underlying molecular mechanism demonstrated that p38 MAPK is involved in mRNA stabilization of CCL1, CCL7, and CCL8 through a TTP-destabilizing activity. TTP is a well-known mRNA-destabilizing protein mediating mRNA degradation of many proinflammatory mediators through binding to AU-rich elements (36, 37).

ERK1/2, however, appeared to be involved directly in the enhanced transcription through AP-1 transcription factors because depletion of c-Jun or c-Fos strongly reduced expression of CCL1 and CCL8. A similar result has recently been described for CCL2 (35). Of note, the activation of ERK1/2 alone appears not to be sufficient for stable mRNA levels of these chemokines, because IL-6 is also a potent activator of this MAPK in dermal fibroblasts as shown previously (21), however, does not stimulate substantial transcription of the chemokines. None of the siRNAs used to down-regulate STAT1, STAT3, or STAT5 affected the expression of CCL8. This finding suggests a STAT-independent transcription of CCL8 in response to OSM and further supports the observed importance of the MAPK.

STAT1 and STAT3 have no influence on the expression of CCL1, whereas OSM-activated STAT5 appears to have a repressive effect on the secretion of CCL1 as demonstrated by: 1) increased expression after transfection of HDFs with siRNA against STAT5, 2) prolonged transcription and enhanced secretion of the CCL-1 homologue in murine STAT5-deficient MEFs (TCA-3), and 3) pretreatment of HDFs with the JAK2 inhibitor AG490 before OSM-stimulation which significantly enhanced the expression of CCL1. Previous work has highlighted the importance of JAK2 for the STAT5 tyrosine phosphorylation (30, 32).

Interestingly, activated STAT3 has been attributed to a comparable role in tumor cells, where it represses the expression of inflammatory cytokines and chemokines and therefore constrains the immune system in fighting the tumor (38). Because activated STAT5 is found in a large variety of tumors, particularly various leukemias, it is tempting to speculate that activated STAT5 could act in a similar fashion. Indeed, we demonstrate that IL-1β can stimulate expression of CCL1 in a human acute myeloid leukemia cell line that expresses a constitutively active variant of JAK2 (V617F) only if the JAK/STAT-pathway is inhibited by the JAK inhibitor-1.

The enhanced expression of CCL1 in the absence of STAT5 is not due to aberrant phosphorylation of STAT3 or STAT1 by OSM as described for STAT5a/b liver specific mutant mice in response to growth hormone (39). Also no perturbances of p38 and ERK1/2 expression and activity relevant for the induction of CCL1 and CCL8 have been observed in STAT5$^{-/-}$ MEFs. However, the comparison of the feedback inhibitors SOCS1, SOCS3, and CIS within the OSM-induced pathway showed a lack of induction of CIS in STAT5$^{-/-}$ MEF cells, a classical STAT5 target gene and a feedback inhibitor of STAT5 (40, 41). We could functionally prove that the missing expression of CIS is responsible for the elevated CCL1 expression, because deficiency of CIS led to an increase of CCL1 secretion and overexpression of CIS reduced elevated CCL1 amounts in STAT5-deficient cells back to wild-type levels. Similarly, knock-down of the constitutive CIS expression in the acute myeloid leukemia cell line resulted in constitutive CCL1 secretion. In the classical feedback process, CIS binds to phosphorylated tyrosine residues within the cytoplasmic domain of STAT5-activating receptors and blocks further activation of STAT5 (41). In line with this, CIS-transgenic mice are virtually identical to STAT5a/b knock-out mice (42). This classical feedback mechanism, however, cannot serve as explanation for the observed negative effect of STAT5 because we would have expected to see a decrease of CCL1 expression upon knock-down of CIS. Therefore, another molecular mechanism has to be involved. CIS has also been described to act as a scavenger protein guiding phosphorylated proteins, e.g., receptors, to degradation via the ubiquitin/proteasome-dependent pathway (43, 44).

Our findings that all investigated pathways besides STAT5, i.e., STAT1, STAT3, ERK1/2, and p38, are unaffected in STAT5-deficient MEFs treated up to 16 h with OSM argues against an involvement of CIS in degradation of gp130, the oncostatin M receptor or membrane-proximal signaling events. Remarkably, CIS does not only suppress CCL1 activation in response to cytokines which normally induce expression of CIS like OSM, i.e., it does not only act in *cis*-, but also inhibits CCL1 expression mediated through cytokines like IL-1β which themselves do not induce CIS expression, i.e., it acts also in trans. This result argues for a CCL1-promoter proximal activity of CIS. Additional experiments are therefore required to elucidate the molecular mechanism(s) how CIS is involved in the regulation of CCL1 expression.

Taken together, we identified novel important inflammatory target genes of OSM which are independent of STAT signaling per se, but depend on MAPK activation and are partly repressed through cytokine-induced or constitutive expression of CIS.

Acknowledgments

We are grateful to Hans F. Merk and Yvonne Marquardt (Dermatology, University Hospital RWTH Aachen, Germany) for providing the primary dermal fibroblasts, Lothar Hennighausen (Laboratory of Genetics and Physiology, National Institutes of Health, Bethesda, MD) for providing J.T. STAT5a/b knock-out mice and Akihiko Yoshimura (Medical Institute of Bioregulation, Kyushu University, Japan) for the expression vector for murine CIS.

Disclosures

The authors have no financial conflict of interest.

References

1. Baggiolini, M. 2001. Chemokines in pathology and medicine. *J. Intern. Med.* 250: 91–104.
2. Gerard, C., and B. J. Rollins. 2001. Chemokines and disease. *Nat. Immunol.* 2: 108–115.
3. Mackay, C. R. 2001. Chemokines: immunology's high impact factors. *Nat. Immunol.* 2: 95–101.
4. Miller, M. D., S. D. Wilson, M. E. Dorf, H. N. Seuanez, S. J. O'Brien, and M. S. Krangel. 1990. Sequence and chromosomal location of the I-309 gene: relationship to genes encoding a family of inflammatory cytokines. *J. Immunol.* 145: 2737–2744.
5. Cantor, J., and K. Haskins. 2007. Recruitment and activation of macrophages by pathogenic CD4 T cells in type 1 diabetes: evidence for involvement of CCR8 and CCL1. *J. Immunol.* 179: 5760–5767.
6. Goya, I., J. Gutierrez, R. Varona, L. Kremer, A. Zaballos, and G. Marquez. 1998. Identification of CCR8 as the specific receptor for the human β-chemokine I-309: cloning and molecular characterization of murine CCR8 as the receptor for TCA-3. *J. Immunol.* 160: 1975–1981.
7. Roos, R. S., M. Loetscher, D. F. Legler, I. Clark-Lewis, M. Baggiolini, and B. Moser. 1997. Identification of CCR8, the receptor for the human CC chemokine I-309. *J. Biol. Chem.* 272: 17251–17254.
8. Tiffany, H. L., L. L. Lautens, J. L. Gao, J. Pease, M. Locati, C. Combadiere, W. Modi, T. I. Bonner, and P. M. Murphy. 1997. Identification of CCR8: a human monocyte and thymus receptor for the CC chemokine I-309. *J. Exp. Med.* 186: 165–170.
9. Loetscher, P., M. Seitz, I. Clark-Lewis, M. Baggiolini, and B. Moser. 1994. Monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3 are major attractants for human CD4$^+$ and CD8$^+$ T lymphocytes. *FASEB J.* 8: 1055–1060.
10. Miller, M. D., and M. S. Krangel. 1992. The human cytokine I-309 is a monocyte chemoattractant. *Proc. Natl. Acad. Sci. USA* 89: 2950–2954.

Downloaded from www.jimmunol.org on June 12, 2010

11. Schaerli, P., L. Ebert, K. Willimann, A. Blaser, R. S. Roos, P. Loetscher, and B. Moser. 2004. A skin-selective homing mechanism for human immune surveillance T cells. *J. Exp. Med.* 199: 1265–1275.
12. Pype, J. L., L. J. Dupont, P. Menten, E. Van Coillie, G. Opdenakker, J. Van Damme, K. F. Chung, M. G. Demedts, and G. M. Verleden. 1999. Expression of monocyte chemotactic protein (MCP)-1, MCP-2, and MCP-3 by human airway smooth-muscle cells: modulation by corticosteroids and T-helper 2 cytokines. *Am. J. Respir. Cell Mol. Biol.* 21: 528–536.
13. Struyf, S., E. Van Collie, L. Paemen, W. Put, J. P. Lenaerts, P. Proost, G. Opdenakker, and J. Van Damme. 1998. Synergistic induction of MCP-1 and -2 by IL-1β and interferons in fibroblasts and epithelial cells. *J. Leukocyte Biol.* 63: 364–372.
14. Selvan, R. S., L. J. Zhou, and M. S. Krangel. 1997. Regulation of I-309 gene expression in human monocytes by endogenous interleukin-1. *Eur. J. Immunol.* 27: 687–694.
15. Tanaka, M., and A. Miyajima. 2003. Oncostatin M, a multifunctional cytokine. *Rev. Physiol. Biochem. Pharmacol.* 149: 39–52.
16. Mosley, B., C. De Imus, D. Friend, N. Boiani, B. Thoma, L. S. Park, and D. Cosman. 1996. Dual oncostatin M (OSM) receptors: cloning and characterization of an alternative signaling subunit conferring OSM-specific receptor activation. *J. Biol. Chem.* 271: 32635–32643.
17. Lindberg, R. A., T. S. Juan, A. A. Welcher, Y. Sun, R. Cupples, B. Guthrie, and F. A. Fletcher. 1998. Cloning and characterization of a specific receptor for mouse oncostatin M. *Mol. Cell. Biol.* 18: 3357–3367.
18. Heinrich, P. C., I. Behrmann, G. Müller-Newen, F. Schaper, and L. Graeve. 1998. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem. J.* 334: 297–314.
19. Taga, T. 1997. The signal transducer gp130 is shared by interleukin-6 family of haematopoietic and neurotrophic cytokines. *Ann. Med.* 29: 63–72.
20. Amaral, M. C., S. Miles, G. Kumar, and A. E. Nel. 1993. Oncostatin-M stimulates tyrosine protein phosphorylation in parallel with the activation of p42MAPK/ERK-2 in Kaposi's cells: evidence that this pathway is important in Kaposi cell growth. *J. Clin. Invest.* 92: 848–857.
21. Böing, I., C. Stross, S. Radtke, B. E. Lippok, P. C. Heinrich, and H. M. Hermanns. 2006. Oncostatin M-induced activation of stress-activated MAP kinases depends on tyrosine 861 in the OSM receptor and requires Jak1 but not Src kinases. *Cell. Signal.* 18: 50–61.
22. Wang, Y., O. Robledo, E. Kinzie, F. Blanchard, C. Richards, A. Miyajima, and H. Baumann. 2000. Receptor subunit-specific action of oncostatin M in hepatic cells and its modulation by leukemia inhibitory factor. *J. Biol. Chem.* 275: 25273–25285.
23. Langdon, C., C. Kerr, L. Tong, and C. D. Richards. 2003. Oncostatin M regulates eotaxin expression in fibroblasts and eosinophilic inflammation in C57BL/6 mice. *J. Immunol.* 170: 548–555.
24. Lee, M. J., H. Y. Song, M. R. Kim, S. M. Sung, J. S. Jung, and J. H. Kim. 2007. Oncostatin M stimulates expression of stromal-derived factor-1 in human mesenchymal stem cells. *Int. J. Biochem. Cell Biol.* 39: 650–659.
25. Sugaya, M., L. Fang, A. R. Cardones, T. Kakinuma, S. H. Jaber, A. Blauvelt, and S. T. Hwang. 2006. Oncostatin M enhances CCL21 expression by microvascular endothelial cells and increases the efficiency of dendritic cell trafficking to lymph nodes. *J. Immunol.* 177: 7665–7672.
26. Cui, Y., G. Riedlinger, K. Miyoshi, W. Tang, C. Li, C. X. Deng, G. W. Robinson, and L. Hennighausen. 2004. Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation. *Mol. Cell. Biol.* 24: 8037–8047.
27. Todaro, G. J., and H. Green. 1963. Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *J. Cell Biol.* 17: 299–313.
28. Dreuw, A., S. Radtke, S. Pflanz, B. E. Lippok, P. C. Heinrich, and H. M. Hermanns. 2004. Characterization of the signaling capacities of the novel gp130-like cytokine receptor. *J. Biol. Chem.* 279: 36112–36120.
29. Weiergräber, O., U. Hemmann, A. Küster, G. Müller-Newen, J. Schneider, S. Rose-John, P. Kurschat, J. P. Brakenhoff, M. H. Hart, S. Stabel, and P.C. Heinrich. 1995. Soluble human interleukin-6 receptor: expression in insect cells, purification and characterization. *Eur. J. Biochem.* 234: 661–669.
30. Hintzen, C., C. Evers, B. E. Lippok, R. Volkmer, P. C. Heinrich, S. Radtke, and H. M. Hermanns. 2008. Box 2 region of the oncostatin M receptor determines specificity for recruitment of Janus kinases and STAT5 activation. *J. Biol. Chem.* 283: 19465–19477.
31. Stevenson, N. J., S. Haan, A. E. McClurg, M. J. McGrattan, M. A. Armstrong, P. C. Heinrich, and J. A. Johnston. 2004. The chemoattractants, IL-8 and formylmethionyl-leucyl-phenylalanine, regulate granulocyte colony-stimulating factor signaling by inducing suppressor of cytokine signaling-1 expression. *J. Immunol.* 173: 3243–3249.
32. Fujitani, Y., M. Hibi, T. Fukada, M. Takahashi-Tezuka, H. Yoshida, T. Yamaguchi, K. Sugiyama, Y. Yamanaka, K. Nakajima, and T. Hirano. 1997. An alternative pathway for STAT activation that is mediated by the direct interaction between JAK and STAT. *Oncogene* 14: 751–761.
33. Levine, R. L., A. Pardanani, A. Tefferi, and D. G. Gilliland. 2007. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. *Nat. Rev. Cancer* 7: 673–683.
34. Langdon, C., J. Leith, F. Smith, and C. D. Richards. 1997. Oncostatin M stimulates monocyte chemoattractant protein-1- and interleukin-1-induced matrix metalloproteinase-1 production by human synovial fibroblasts in vitro. *Arthritis Rheum.* 40: 2139–2146.
35. Lin, S. K., S. H. Kok, F. T. Yeh, M. Y. Kuo, C. C. Lin, C. C. Wang, S. R. Goldring, and C. Y. Hong. 2004. MEK/ERK and signal transducer and activator of transcription signaling pathways modulate oncostatin M-stimulated CCL2 expression in human osteoblasts through a common transcription factor. *Arthritis Rheum.* 50: 785–793.
36. Blackshear, P. J. 2002. Tristetraprolin and other CCCH tandem zinc-finger proteins in the regulation of mRNA turnover. *Biochem. Soc. Trans.* 30: 945–952.
37. Sandler, H., and G. Stoecklin. 2008. Control of mRNA decay by phosphorylation of tristetraprolin. *Biochem. Soc. Trans.* 36: 491–496.
38. Kortylewski, M., M. Kujawski, T. Wang, S. Wei, S. Zhang, S. Pilon-Thomas, G. Niu, H. Kay, J. Mule, W. G. Kerr, et al. 2005. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. *Nat. Med.* 11: 1314–1321.
39. Cui, Y., A. Hosui, R. Sun, K. Shen, O. Gavrilova, W. Chen, M. C. Cam, B. Gao, G. W. Robinson, and L. Hennighausen. 2007. Loss of signal transducer and activator of transcription 5 leads to hepatosteatosis and impaired liver regeneration. *Hepatology* 46: 504–513.
40. Ram, P. A., and D. J. Waxman. 1999. SOCS/CIS protein inhibition of growth hormone-stimulated STAT5 signaling by multiple mechanisms. *J. Biol. Chem.* 274: 35553–35561.
41. Yoshimura, A., T. Ohkubo, T. Kiguchi, N. A. Jenkins, D. J. Gilbert, N. G. Copeland, T. Hara, and A. Miyajima. 1995. A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *EMBO J.* 14: 2816–2826.
42. Matsumoto, A., Y. Seki, M. Kubo, S. Ohtsuka, A. Suzuki, I. Hayashi, K. Tsuji, T. Nakahata, M. Okabe, S. Yamada, and A. Yoshimura. 1999. Suppression of STAT5 functions in liver, mammary glands, and T cells in cytokine-inducible SH2-containing protein 1 transgenic mice. *Mol. Cell. Biol.* 19: 6396–6407.
43. Verdier, F., S. Chretien, O. Muller, P. Varlet, A. Yoshimura, S. Gisselbrecht, C. Lacombe, and P. Mayeux. 1998. Proteasomes regulate erythropoietin receptor and signal transducer and activator of transcription 5 (STAT5) activation: possible involvement of the ubiquitinated Cis protein. *J. Biol. Chem.* 273: 28185–28190.
44. Zhang, J. G., A. Farley, S. E. Nicholson, T. A. Willson, L. M. Zugaro, R. J. Simpson, R. L. Moritz, D. Cary, R. Richardson, G. Hausmann, et al. 1999. The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. *Proc. Natl. Acad. Sci. USA* 96: 2071–2076.

Downloaded from www.jimmunol.org on June 12, 2010