

Oncostatin M causes eotaxin-1 release from airway smooth muscle: Synergy with IL-4 and IL-13

Débora S. Faffe, MD, PhD,^{a,b} Lesley Flynt, BSc,^a Matthew Mellema, DVM,^a Paul E. Moore, MD,^c Eric S. Silverman, MD,^a Venkat Subramaniam, BSc,^a Matthew R. Jones, PhD,^a Joseph P. Mizgerd, ScD,^a Timothy Whitehead, MSc,^a Amy Imrich, BSc,^a Reynold A. Panettieri, Jr, MD,^d and Stephanie A. Shore, PhD^a
Boston, Mass, Rio de Janeiro, Brazil, Nashville, Tenn, and Philadelphia, Pa

Background: Eotaxin is implicated in asthmatic eosinophilia. Oncostatin M (OSM) causes eotaxin release from fibroblasts. **Objective:** We sought to examine the effects and mechanism of action of OSM and other IL-6 family cytokines on eotaxin release from human airway smooth muscle cells.

Methods: Eotaxin 1 release was measured by means of ELISA. Western blotting was used to examine mitogen-activated protein kinase and signal transducer and activator of transcription 3 (STAT-3) phosphorylation. Eotaxin promoter activity was analyzed in cells transfected with wild-type STAT-3, a mutant form of STAT-3 that cannot be phosphorylated, and a constitutively active form of STAT-3. The mRNA and protein expression of IL-4R α , the signaling receptor for IL-4 and IL-13, was evaluated by means of real-time PCR and flow cytometry, respectively.

Results: OSM increased eotaxin 1 release and augmented IL-4- or IL-13-induced eotaxin release, whereas other IL-6 family cytokines did not. OSM caused a greater increase in STAT-3 phosphorylation and STAT-3-mediated gene transcription than other IL-6 family cytokines. OSM increased eotaxin promoter activity and augmented IL-13- and IL-4-induced increases in promoter activity. The constitutively active form of STAT-3 increased eotaxin promoter activity, whereas the mutant form of STAT-3 that cannot be phosphorylated significantly reduced eotaxin promoter activity induced by OSM or IL-4 plus OSM. OSM increased IL-4R α mRNA and protein levels.

Conclusions: OSM induces eotaxin 1 expression in human airway smooth muscle cells by a mechanism involving STAT-3. OSM synergizes with IL-13 and IL-4 to increase eotaxin 1

expression, possibly as a result of effects on IL-4R α expression. (*J Allergy Clin Immunol* 2005;115:514-20.)

Key words: Signal transducer and activator of transcription 3, extracellular signal-regulated kinase, c-Jun N-terminal kinase, IL-4R α , monocyte chemoattractant protein 1, vascular endothelial growth factor

The C-C chemokine eotaxin is an eosinophil chemoattractant factor that has been implicated in asthma. Antigen-sensitized and antigen-challenged eotaxin-deficient mice exhibit a marked decrease in eosinophil recruitment to the airways compared with wild-type mice.¹ Furthermore, eotaxin is upregulated in the bronchoalveolar lavage fluid and airway tissue of asthmatic patients.^{2,3} Expression of eotaxin mRNA and protein is also upregulated after allergen challenge in asthmatic patients.⁴

It is likely that airway smooth muscle contributes to the eotaxin release observed in patients with asthma. Hamid et al⁵ reported eosinophils in and around airway smooth muscle in lungs from asthmatic subjects, and immunohistochemical analysis demonstrated substantial expression of eotaxin in smooth muscle, as well as in airway epithelial cells.⁶ Furthermore, human airway smooth muscle (HASM) cells in culture produce eotaxin constitutively, and eotaxin expression is upregulated by the inflammatory cytokines TNF- α and IL-1 β and the T_H2 cytokines IL-4 and IL-13.⁶⁻⁹

The IL-6 family cytokine oncostatin M (OSM) causes a dose-dependent increase in eotaxin release from fibroblasts.¹⁰ Moreover, nasal inoculation of mice with an adenoviral vector encoding murine OSM increases pulmonary expression of eotaxin mRNA and causes marked eosinophilic inflammation.

In addition to OSM, the IL-6 family of cytokines includes IL-6 itself, leukemia inhibitory factor, IL-11, cardiotropin 1, and ciliary neutrophilic factor (CNTF), as well as other cytokines. Members of this family activate receptors that signal through activation of signal transducer and activator of transcription 3 (STAT-3), extracellular signal-regulated kinase (ERK), and, in some cell types, c-Jun N-terminal kinase (JNK).¹⁰⁻¹⁵ Receptors for IL-6 family cytokines are present on HASM cells, and ligation of some of these receptors, particularly the OSM

From ^athe Physiology Program, Harvard School of Public Health, Boston;

^bthe Laboratory of Respiratory Physiology, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; ^cthe Department of Pediatrics, Vanderbilt University, Nashville; and ^dthe Pulmonary and Critical Care Division, Department of Medicine, University of Pennsylvania, Philadelphia.

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Reprint requests: Stephanie Shore, PhD, Physiology Program, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115. E-mail: sshore@hsph.harvard.edu.

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Abbreviations used

CNTF:	Ciliary neurotrophic factor
ERK:	Extracellular signal-regulated kinase
GAPDH:	Reduced glyceraldehyde-phosphate dehydrogenase
HASM:	Human airway smooth muscle
JAK:	Janus kinase
JNK:	c-Jun N-terminal kinase
MCP-1:	Monocyte chemoattractant protein 1
OSM:	Oncostatin M
OSMR:	OSM receptor
STAT:	Signal transducer and activator of transcription
STAT-3-C:	Constitutively active STAT-3
STAT-3F:	Nonphosphorylatable form of STAT-3
STAT-3-WT:	Wild-type STAT-3
VEGF:	Vascular endothelial growth factor

receptor (OSMR), has been shown to induce STAT-3 and ERK phosphorylation in these cells.^{11,16}

We hypothesized that OSM and other IL-6 family members induce eotaxin expression in HASM cells, as occurs in murine fibroblasts,¹⁰ and that ERK, JNK, and STAT-3 modulate these events. Because IL-4 and IL-13 have been shown to induce eotaxin release from HASM cells^{8,9,17,18} and because these cytokines have been implicated in the pathogenesis of asthma, we also examined the effects of OSM in combination with IL-4 or IL-13 on eotaxin release. Our results indicate that OSM, but not other IL-6 cytokines, increases eotaxin release from HASM cells. OSM also augments the ability of IL-4 and IL-13 to cause eotaxin release, possibly because it increases expression of IL-4R α , the common signaling receptor for IL-4 and IL-13. Consistent with this hypothesis, OSM also augments the ability of IL-4 and IL-13 to cause vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein 1 (MCP-1) release. Our results also indicate a role for STAT-3 in OSM-induced eotaxin promoter activity. This is the first report of a role for STAT-3 in eotaxin expression in any cell type.

METHODS

Cell culture

HASM cells were obtained from lung transplant donor tracheas in accordance with procedures approved by the University of Pennsylvania Committee on Studies Involving Human Beings and cultured as previously described.^{9,19-21} Except as noted, cells were confluent, serum deprived, and hormone supplemented for 24 to 48 hours before use and used in passages 4 through 7.

ELISA

HASM cells in 24-well plates were treated with cytokines (R&D Systems, Minneapolis, Minn) for 24 hours. The supernatant was removed, and eotaxin 1, VEGF, and MCP-1 were analyzed by means of ELISA (R&D Systems). In experiments involving mitogen-activated protein kinase inhibitors, cells were treated with inhibitor 2 hours before cytokines. To assess the role of p38, JNK, and ERK

in response to OSM, we used SB-203580, SP-600125, or U0126 (Calbiochem, San Diego, Calif), respectively, in concentrations previously shown to be specifically effective.²⁰⁻²⁴ Cells from 2 to 5 donors were used in each situation.

Transfection of HASM cells

Cells from 2 to 3 donors were transfected with 1 μ g of an eotaxin promoter–firefly luciferase reporter construct¹⁷ and 1 μ g of a renilla luciferase control construct. For experiments involving STAT-3 expression constructs, the cells were transfected with the eotaxin promoter reporter construct (1 μ g) along with 1 μ g of either a wild-type STAT-3 (STAT-3-WT) or a mutant form of STAT-3 that cannot be janus kinase (JAK) phosphorylated (STAT-3F).²⁵ These were a gift of Dr Toshio Hirano (Osaka University, Japan). For measurement of STAT-3–mediated gene transcription, cells were transfected with 1 μ g of a STAT-3–luciferase promoter–reporter plasmid along with 1 μ g of a control β -gal expression construct. This promoter–reporter plasmid was generated by replacing the SV40 promoter region of the pGL3 control vector (Promega, Madison, Wis) with 4 tandem STAT-3–binding M67 sequences upstream of a minimal herpes simplex virus thymidine kinase promoter excised from p4xM67-tk-Luc.²⁶ We also examined the effect of a constitutively active form of STAT-3 (STAT-3-C) on M67 activity and on eotaxin promoter activity. STAT-3-C was generated by means of substitution of 2 cysteine residues in the C-terminal loop of the SH2 domain of STAT-3, producing a molecule that dimerizes spontaneously, binds to DNA, and activates transcription.²⁷ p4xM67-tk-Luc and STAT-3-C were provided by Dr James E. Darnell (Rockefeller University, New York, NY). Constructs were transfected into HASM cells with Eugene 6 (Roche, Indianapolis, Ind), according to the manufacturer's protocol. Twenty-four hours after transfection, the cells were either left untreated or were treated with cytokines (OSM at 20 ng/mL with or without IL-4 at 3 ng/mL) for 24 hours. Luciferase and β -gal were assayed as previously described.^{17,28}

Western blotting

Confluent cells were treated with 20 ng/mL cytokines for up to 4 hours and then lysed, and solubilized proteins (30 μ g per lane) were separated by means of SDS-PAGE and transferred to a BioTrace polyvinylidene difluoride membrane, as previously described.¹¹ The membranes were blocked and then probed with rabbit anti-phosphoTyr705-STAT-3, anti-phospho-p44/p42, anti-phospho-JNK, or anti-phospho-p38 (Cell Signaling, Inc, Woburn, Mass) overnight at 4°C. Primary antibodies were detected with goat anti-rabbit horseradish peroxidase–conjugated antibodies and visualized by means of enhanced chemiluminescence (Cell Signaling, Inc).

RNA extraction and real-time PCR

HASM cells were treated with OSM (2–20 ng/mL for 6 hours) or left untreated. In experiments involving U0126, cells were treated with the compound for 2 hours before OSM treatment. Total RNA was extracted with TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, Calif) in accordance with the manufacturer's instructions. Reverse transcription was performed with 2 μ g of total RNA, 1 μ g of random hexamers (Invitrogen, Life Technologies), 50 nmoles of deoxyribonucleoside triphosphate, 25 U of rRNasin RNase inhibitor (Promega), 200 U of M-MLV reverse transcription (Promega) in a 25- μ L total volume consisting of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, and 10 mM dithiothreitol at 37°C for 60 minutes. Real-time PCR was performed with an iCycler iQ Real Time Detection System and iQ SYBR Green Supermix in accordance with the manufacturer's instructions (BioRad, Hercules, Calif). The primer set 5'-GAG AGC AGC AGG GAT GAC TT-3' (forward) and

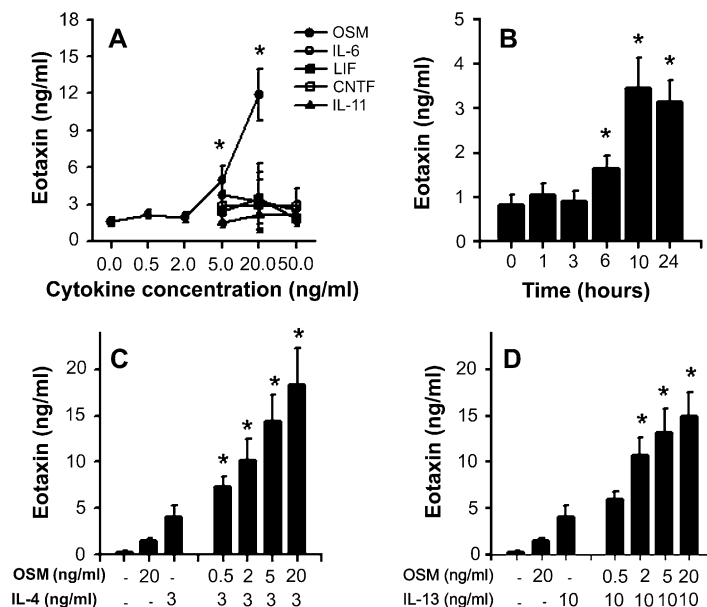


FIG 1. **A**, Dose-response effect of 24 hours of treatment with IL-6 family cytokines (0-50 ng/mL). * $P < .05$ compared with no treatment. **B**, Time course of effect of OSM (20 ng/mL). * $P < .05$ compared with time 0. **C** and **D**, Effect of OSM plus IL-4 or IL-13 for 24 hours. * $P < .01$ compared with the sum of IL-4 or IL-13 alone plus OSM alone. Results are presented as means $\pm$ SE from 4 to 10 wells from 2 to 4 donors.

5'-TCCACCGCATGTACAACTC-3' (reverse) was used to amplify a 435-bp fragment of the human *IL4RA* mRNA gene. The product was cloned into a TOPO-TA vector (Qiagen, Valencia, Calif) and used to construct a standard curve. The primer set 5'-GAGTC-CACTGGCGTCTTCA-3' (forward) and 5'-TTCAGCTCAGG-GATGACCTT-3' (reverse) was used to amplify and subsequently clone a fragment of reduced glyceraldehyde-phosphate dehydrogenase (*GAPDH*) for use as a positive control. Changes in *IL4RA* mRNA transcript copy number were assessed relative to changes in *GAPDH* mRNA transcript copy number.

Flow cytometry

Near-confluent cells (approximately 70%) were stimulated for 24 hours with 20 ng/mL OSM. Cells were removed by using cell dissociation buffer (Sigma, St Louis, Mo) and washed twice in ice-cold serum-free media. Cells (10^5) were resuspended in 200 μ L of monoclonal anti-human IL-4R antibody (2.5 μ g/mL, R&D Systems) or mouse IgG2a control (2.5 μ g/mL) for 30 minutes at 4°C, washed twice, and resuspended in 200 μ L (10 μ g/mL) of Alexa Fluor 488 F(ab)2 goat anti-mouse antibody (Molecular Probes, Inc, Eugene, Ore). After 30 minutes in the dark at 4°C, cells were washed and resuspended in 0.5 mL of ice-cold serum free media. IL-4R antibody binding was quantitated on a Coulter ELITE flow cytometer (Coulter Corp, Miami, Fla) by using an argon laser (488 nm). Propidium iodide allowed separation of live cells. Change in mean fluorescence intensity ($\times 1024$ scale factor) from IgG control to IL-4R antibody was measured in 3 donors (≥ 2000 live cells). Samples were normalized for cell size (forward scatter).

RESULTS

OSM caused a concentration-dependent (Fig 1, A) and time-dependent (Fig 1, B) increase in eotaxin release from HASM cells. In contrast, there was no significant effect of

leukemia inhibitory factor, IL-6, IL-11, or CNTF (Fig 1, A). There was also marked synergism between OSM and IL-13 or IL-4 (Fig 1, C and D).

OSM caused ERK and JNK phosphorylation, whereas the other cytokines did not (Fig 2). There was no significant phosphorylation of the p38 mitogen-activated protein kinase by any of the cytokines (data not shown). With the exception of CNTF, all the IL-6 family cytokines increased STAT-3 phosphorylation compared with untreated cells, but OSM was more potent than the other cytokines (Fig 2). There were no significant effects of any of the cytokines on unphosphorylated levels of STAT-3, ERK, or JNK (data not shown). To quantitate differences among the IL-6 family cytokines in their ability to induce STAT-3-dependent gene transcription, we transfected HASM cells with a STAT-3 reporter construct (M67). OSM increased M67 promoter activity to $199\% \pm 51\%$ of untreated cells ($P < .05$), whereas the other cytokines had no effect (data not shown).

The ERK kinase-, p-38-, and JNK-specific inhibitors U0126, SB-203580, and SP-600125, respectively, had no effect on basal eotaxin release or on eotaxin release induced by OSM (5 or 20 ng/mL, data not shown). U0126 significantly decreased IL-4-induced eotaxin release, as previously reported (Fig 3).⁹ In contrast, neither SB-203580 nor SP-600125 had any effect on eotaxin release induced by IL-4 alone (data not shown). U0126 also inhibited eotaxin release in cells treated with OSM plus IL-4 (Fig 3), whereas SB-203580 and SP-600125 were without effect (data not shown). Nevertheless, there was still significant synergy between OSM and IL-4, even in U0126-treated cells. Similar results were obtained with IL-13 (data not shown).

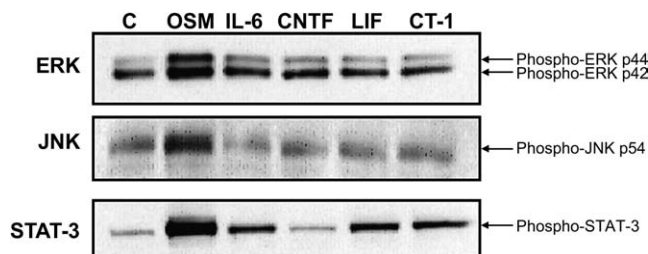


FIG 2. Representative Western blots showing immunoreactivity for phospho-ERK, phospho-JNK, and phospho-STAT-3 in untreated control cells (C, control) and cells treated with OSM, leukemia inhibitory factor (LIF), IL-6, cardiotropin 1 (CT-1), or CNTF, each at 20 ng/mL for 15 minutes. Similar results were obtained in cells from 3 donors.

IL-4, IL-13, and OSM each caused a small but significant increase ($P < .01$) in eotaxin promoter activity (Fig 4, A). Addition of OSM also caused a significant increase ($P < .01$) in IL-4- or IL-13-induced promoter activity compared with cells treated with IL-4 or IL-13 alone. Western blotting confirmed a robust increase in STAT-3 immunoreactivity in cells transfected with expression constructs for either STAT-3-WT or a mutant form of STAT-3 (STAT-3F) that cannot be JAK phosphorylated (data not shown).²⁵ OSM caused an approximate 2-fold increase in eotaxin promoter activity in cells transfected with STAT-3-WT (Fig 4, B). Indeed, the increase was greater than in cells in which STAT-3 was not overexpressed (Fig 4, A). In contrast, OSM-induced eotaxin promoter activity was significantly reduced in cells transfected with STAT-3F (Fig 4, B). OSM plus IL-4 also resulted in significantly greater eotaxin promoter activity in cells transfected with STAT-3-WT than in cells transfected with STAT-3F. Nevertheless, OSM plus IL-4 still increased eotaxin promoter activity in these cells, presumably through IL-4-induced STAT-6 activation, as described by others.²⁹

To further evaluate the potential for STAT-3 to influence eotaxin expression, we transfected cells with either wild-type or constitutively active STAT-3. In cells transfected with STAT-3-C, M67 promoter activity averaged $216\% \pm 35\%$ of activity in cells transfected with STAT-3-WT ($P < .01$), confirming STAT-3-C activity. Eotaxin promoter activity was also increased in cells transfected with STAT-3-C ($173\% \pm 26\%$ of activity in cells transfected with STAT-3-WT, $P < .01$).

To examine the possibility that OSM synergizes with IL-4 or IL-13 by increasing expression of their common signaling receptor, we measured IL-4R α mRNA and protein expression. OSM caused a concentration-related increase in ratio of *IL4RA* mRNA/*GAPDH* mRNA transcript copy number compared with that seen in control cells ($170\% \pm 38\%$, $243\% \pm 55\%$, and $310\% \pm 98\%$ of untreated cells after treatment with 2, 5, and 20 ng/mL OSM, respectively; $P < .05$ for 5 and 20 ng/mL OSM). Treatment with U0126 (10 μ M) had no effect on OSM-induced increases in *IL4RA* mRNA (data not shown). OSM (20 ng/mL) also increased IL-4R α protein expression compared with that seen in untreated cells. The change in green fluorescence/cell size ratio from IL-4R α

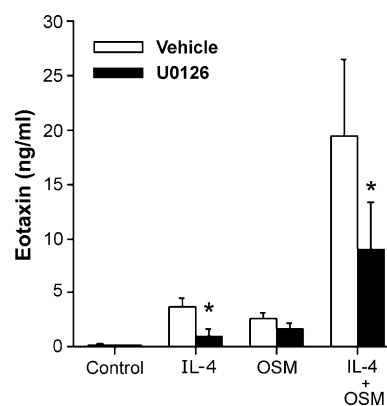


FIG 3. Cells were treated with vehicle (0.01% dimethyl sulfoxide) or with the ERK kinase inhibitor U0126 (10 μ M) for 2 hours before addition of OSM (20 ng/mL) or IL-4 (3 ng/mL) alone or in combination. Results are presented as means $\pm$ SE of data from 6 to 8 wells from 3 donors. * $P < .05$ compared with vehicle.

antibody to IgG control antibody averaged 51.8 ± 5.6 for control cells and 111.0 ± 21.1 for OSM-treated cells ($P < .05$).

To evaluate other interactions between OSM and IL-4 or IL-13, we examined the effects of OSM on IL-13- and IL-4-induced VEGF and MCP-1 release. OSM increased VEGF and MCP-1 release in HASM cells (Fig 5), as reported for other cell types.³⁰⁻³⁴ IL-4 and IL-13 each caused a small but significant increase in VEGF and MCP-1 release in control cells, and the effects of IL-4 and IL-13 on VEGF and MCP-1 release were significantly increased in cells treated with OSM (Fig 5).

DISCUSSION

Our data indicate that OSM causes eotaxin release from HASM cells, whereas other IL-6 family cytokines do not (Fig 1, A). Indeed, the levels of eotaxin released suggest that HASM cells might be a major source of eotaxin in the airways. Similarly, OSM, but not other IL-6 cytokines, increases eotaxin release from murine lung fibroblasts.¹⁰ Moreover, intranasal inoculation of mice with an adenoviral vector expressing OSM results in increased eotaxin

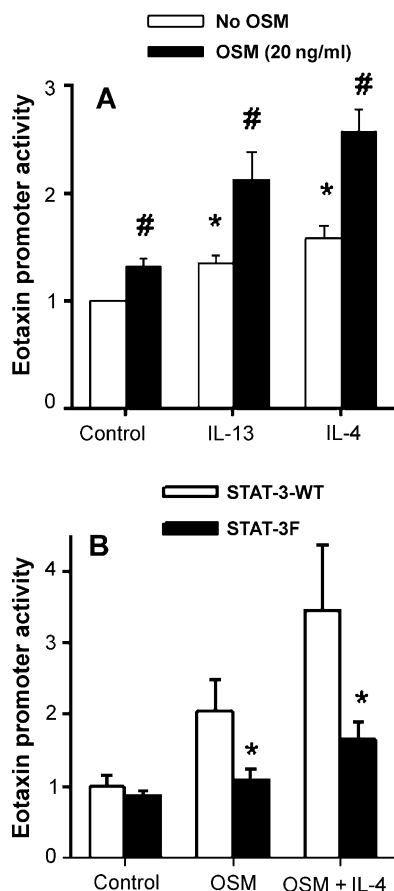


FIG 4. Eotaxin promoter activity. **A**, Activity in cells treated with IL-13 (10 ng/mL), IL-4 (3 ng/mL), OSM (20 ng/mL), or OSM plus IL-13 or IL-4 each for 24 hours. * $P < .01$ compared with control. # $P < .01$ compared with the same treatment without OSM. **B**, Activity in cells transfected with STAT-3-WT or STAT-3F. Results are normalized for luciferase expression in untreated STAT-3-WT. * $P < .05$ compared with STAT-3-WT with same treatment. Results are presented as means $\pm$ SE of data from 8 to 14 cell wells from 2 to 3 donors.

expression in the lung, as well as a marked recruitment of eosinophils.

Members of the IL-6 cytokine family share a common receptor subunit, glycoprotein 130.³⁵ OSM binding to the OSMR results in the formation of an OSMR–glycoprotein 130 heterodimer. Glycoprotein 130 and OSMR are constitutively associated with JAKs. After receptor binding, JAKs become phosphorylated and, in turn, tyrosine phosphorylate glycoprotein 130 and OSMR. STAT-3 binding to phosphorylated glycoprotein 130 or OSMR¹⁴ leads to JAK phosphorylation of STAT-3. Other members of the IL-6 family also use glycoprotein 130 in combination with other receptors for signaling, but only OSM activates OSMR. We observed STAT-3 phosphorylation in response to all IL-6 family cytokines examined except CNTF, although the effect was much greater for OSM than for the other cytokines (Fig 2). Thus the increased ability of OSM to cause STAT-3 phosphorylation is likely related to

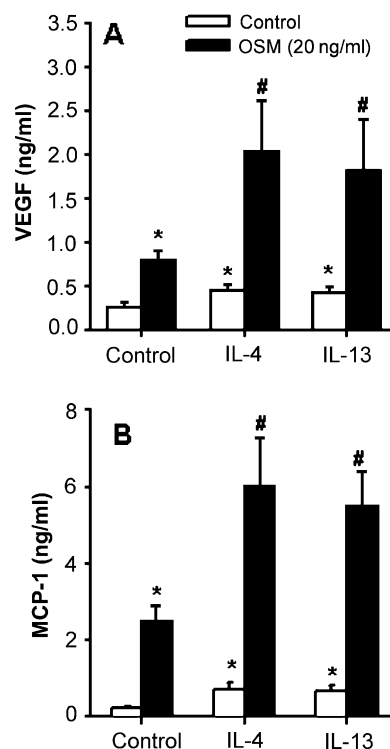


FIG 5. MCP-1 and VEGF release from HASM cells. Cells were treated with OSM (20 ng/mL), IL-4 (3 ng/mL), or IL-13 (10 ng/mL) alone or in combination. Results are presented as means $\pm$ SE of data from 5 donors studied in duplicate. * $P < .05$ compared with untreated cells. # $P < .05$ compared with cells treated with OSM alone. Similar results were obtained with 5 ng/mL OSM.

its unique ability to activate the OSMR. We also observed activation of a STAT-3–dependent promoter only in response to OSM. The observation that other IL-6 family cytokines are capable of inducing STAT-3 phosphorylation, albeit to a lesser extent than OSM (Fig 2), but that only OSM caused activation of a STAT-3–dependent promoter reporter suggests that OSM, but not other IL-6 family cytokines, not only phosphorylates STAT-3 but might also lead to other events that increase nuclear translocation, DNA binding, or transcriptional activation of STAT-3.

The effect of OSM on eotaxin is likely mediated at the transcriptional level because OSM also increased eotaxin promoter activity (Fig 4, A). Furthermore, our data indicate that this effect is dependent on STAT-3 activation (Fig 4, B) because overexpression of a transcriptionally inactive form of STAT-3 significantly reduced OSM-induced eotaxin promoter activity compared with STAT-3-WT (Fig 4, B). Indeed, our data showed increased eotaxin promoter activity in cells transfected with a constitutively active form of STAT-3, indicating that activation of STAT-3 is sufficient for eotaxin promoter activity. Thus it is likely that the lack of effect of other IL-6 family cytokines on eotaxin release (Fig 1, A) is related to their inability to induce STAT-3–dependent gene transcription. To our knowledge, this is the first report of a role for

STAT-3 in eotaxin expression in any cell type. Langdon et al¹⁰ also reported OSM-induced eotaxin release from fibroblasts and reported activation of STAT-3 by OSM but did not examine the role of STAT-3 on eotaxin release in these cells. The mechanistic basis for the effects of STAT-3 remains to be established.

OSM, but not other IL-6 family cytokines, also caused activation of ERK and JNK, which is consistent with reports in other cell types.^{10,12-15} However, differences in ERK and JNK activation by OSM compared with other IL-6 family cytokines do not appear to contribute to differences in their ability to induce eotaxin because neither an ERK kinase inhibitor nor a JNK inhibitor had any effect on OSM-induced eotaxin release. In contrast, ERK does play a role in OSM-induced eotaxin release in fibroblasts.¹⁰

We observed a marked synergy between OSM and IL-13 or IL-4 in the induction of eotaxin (Fig 1, C and D). IL-4 and IL-13 have also been shown to synergize with TNF- α or IL-1 β in the induction of eotaxin expression both in HASM cells^{8,9} and in other cell types,^{29,36} but this is the first report of synergy between OSM and T_H2 cytokines in any cell or tissue. Both IL-4 and IL-13 signal through IL-4R α .³⁷ Although it is possible that other factors are involved, our data are consistent with the hypothesis that the synergy between OSM and these T_H2 cytokines might be the result of OSM-induced increases in IL-4R α expression because OSM increased IL-4R α mRNA and protein expression. In contrast, the ability of IL-1 β to synergize with IL-4 or IL-13 occurs in the absence of changes in IL-4R α expression.⁸ Other members of the IL-6 family of cytokines have been shown to increase expression of IL-4R α in other cell types.^{38,39} However, to our knowledge, this is the first report of effects of OSM on IL-4R α expression in any cell type. These data indicate that OSM might influence the full spectrum of IL-4- or IL-13-dependent allergic responses. Indeed, our results demonstrate that OSM also augmented the ability of IL-13 and IL-4 to induce VEGF and MCP-1 release (Fig 5).

The ability of OSM to enhance IL-4- and IL-13-induced eotaxin release from HASM cells (Fig 1, C and D) is at least partly dependent on STAT-3 because eotaxin promoter activity induced by OSM plus IL-4 was attenuated in cells transfected with a transcriptionally inactive form of STAT-3 compared with cells transfected with STAT-3-WT (Fig 4, B). We also observed an attenuation of eotaxin release induced by IL-4 and OSM or IL-13 and OSM in cells in which ERK activation was inhibited with U0126 (Fig 3). However, U0126 also inhibited eotaxin release in cells treated with IL-13 or IL-4 alone, which is consistent with the observation that IL-13 and IL-4 also activate ERK.^{8,37} Thus it is likely that the effect of U0126 is the result of inhibition of events mediated downstream of IL-13 or IL-4 activation rather than the result of inhibition of effects of OSM.

There are currently no data regarding the expression of OSM in the airways of asthmatic patients. OSM is not expressed in HASM cells, even though IL-6 and IL-11 are expressed.^{11,40} However, other cells in the lungs and

airways, including macrophages, T lymphocytes, neutrophils, eosinophils, and dendritic cells, have been shown to produce OSM.⁴¹⁻⁴⁷ Furthermore, coculture of alveolar macrophages with airway epithelial cells results in a marked increase in OSM expression in response to ambient particles compared with either cell type on its own.⁴⁸ Hence it is possible that OSM might be expressed in asthma, a disease characterized by airway infiltration with inflammatory cells, and that OSM contributes to the increased eotaxin expression and the eosinophilia of asthma, especially given its capacity to synergize with IL-13 and IL-4. The observation that the OSMR is expressed in abundance on airway smooth muscle of asthmatic patients⁴⁹ increases that likelihood.

In summary, our results indicate that OSM induces eotaxin expression in HASM cells by a mechanism involving STAT-3. This is the first report of a role for STAT-3 in the expression of eotaxin in any cell type. OSM also enhances IL-4- and IL-13-induced eotaxin, VEGF, and MCP-1 release from HASM cells, likely as a result of changes in expression of IL-4R α . Given the importance of IL-4 and IL-13 for many other aspects of allergic asthma, our results indicate that OSM has the capacity to play an important role in amplifying the pathophysiologic manifestations of asthma.

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