

Human mast cells release oncostatin M on contact with activated T cells: Possible biologic relevance

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Background: We have recently demonstrated that mast cells can be activated by heterotypic adhesion to activated T cells.

Objective: We sought to perform gene expression profiling on human mast cells activated by either IgE cross-linking or by T cells and to characterize one of the cytokines, oncostatin M (OSM).

Methods: Gene expression profiling was done by means of microarray analysis, OSM expression was validated by means of RT-PCR, and the product was measured by means of ELISA in both the LAD 2 human mast cell line and in cord blood–derived human mast cells. Immunocytochemistry was used to localize OSM in human mast cells, and its biologic activity was verified by its effect on the proliferation of human lung fibroblasts.

Results: OSM was expressed and released specifically on T cell–induced mast cell activation but not on IgE cross-linking. OSM was localized to the cytoplasm, and its expression was inhibited by dexamethasone and mitogen-activated protein kinase inhibitors. OSM was also found to be biologically active in inducing lung fibroblast proliferation that was partially but significantly inhibited by anti-OSM mAb. *In vivo* mast cells were found to express OSM in both biopsy specimens and bronchoalveolar lavage fluid from patients with sarcoidosis.

Conclusion: The production of OSM by human mast cells might represent one link between T cell–induced mast cell activation and the development of a spectrum of structural changes in T cell–mediated inflammatory processes in which mast cells have been found to be involved.

Clinical implications: Mast cells might serve as a target for treating T cell–mediated fibrotic processes. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: *Oncostatin M, mast cells, T cells*

Abbreviations used

BAL:	Bronchoalveolar lavage
CBMC:	Cord blood mast cell
ERK:	Extracellular signal-regulated kinase
GAPDH:	Glyceraldehyde 3-phosphate dehydrogenase
JNK:	Jun N-terminal kinase
MAPK:	Mitogen-activated protein kinase
OSM:	Oncostatin M
PMA:	Phorbol 12-myristate 13-acetate
QR-PCR:	Quantitative real-time PCR
SCF:	Stem cell factor
Tc-m:	Nonactivated Jurkat T-cell membrane
Tc*-m:	Activated Jurkat T-cell membrane

Most commonly known for their role in the elicitation of IgE-mediated allergic inflammation, mast cells have been implicated in a range of other nonallergic inflammatory processes. Observations such as the close physical proximity between mast cells and T cells in inflamed tissues and the capability of the former to release a wide range of immunomodulatory mediators and to express surface molecules important in costimulation in both adaptive and innate immunity have led investigators to propose a functional relationship between these 2 cell populations.¹⁻⁴ Indeed, morphologic studies have documented an increase in the local density of mast cells and their activation during T cell–mediated inflammatory processes, as observed in cutaneous delayed-type hypersensitivity, graft-versus-host reactions, sarcoidosis, Crohn's disease, rheumatoid arthritis, and fibrosis.^{1,4-7}

We have previously reported on the effects of direct contact between mast cells and T lymphocytes on mast cell activation and mediator release. Mast cells were found to degranulate in response to direct contact with activated T cells or their membranes and to produce cytokines, such as TNF- α and IL-8.^{5,8} Moreover, studies with murine mast cells and phorbol 12-myristate 13-acetate (PMA)– or anti-CD3–activated T cells attributed the T cell–induced mast cell activation to interactions of surface molecules, such as intercellular adhesion molecule 1 and lymphotoxin- β receptor, with their respective ligands.^{9,10} Thus direct

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Supported in part by a research grant from the Israel Science Foundation, founded by the Israel Academy of Sciences and Humanities, and by the Frederick Reiss chair in dermatology, Tel Aviv University.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication December 7, 2006; revised August 22, 2007; accepted for publication August 30, 2007.

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doi:10.1016/j.jaci.2007.08.054

contact between surface molecules on mast cells and on activated T cells was found to provide the stimulatory signal in mast cells necessary for degranulation and cytokine release independent of T-cell intracellular function and in the absence of demonstrable soluble mediators.

Oncostatin M (OSM) is a multifunctional cytokine that belongs to the IL-6 subfamily and produced mainly by activated T cells, neutrophils, monocytes, and macrophages.¹¹ More recently, it has also been demonstrated that OSM is produced by dendritic cells.¹² However, and in contrast to other members of the IL-6 family, there is no evidence to suggest that OSM is produced by resident cells, such as mast cells or smooth muscle cells. Because of its pleiotropic nature, there is a vast array of biologic activities exhibited by OSM. These activities include inhibition of tumor cell growth, induction of neurotrophic peptides, stimulation of fibroblast proliferation and collagen production, regulation of cholesterol metabolism, and induction of cartilage destruction.^{11,13-16}

Recent studies have shown that OSM might have a role in T cell-mediated inflammatory processes in which mast cells have also been found to be involved, including rheumatoid arthritis, multiple sclerosis, and pulmonary fibrosis.^{7,14} We thus hypothesized that cell-to-cell contact with activated T cells might result in OSM release from mast cells. As will be shown, OSM is specifically expressed on T cell-induced mast cell activation but not on IgE cross-linking. Also, heterotypic adhesion to activated, but not resting, T cells resulted in OSM release from mast cells that was found to be biologically active in inducing human lung fibroblast proliferation. *In vivo*, OSM was expressed in human lung mast cells from patients with sarcoidosis. To the best of our knowledge, this is the first report of OSM in human mast cells. Based on the previously reported biologic effects of OSM, our results suggest that production of OSM might represent one link between T cell-induced mast cell activation and the development of a spectrum of structural changes in T cell-mediated inflammatory processes in which mast cells have been found to be involved.

METHODS

Cells

Reagents for cell culture were purchased from Biological Industries (Beit Haemek, Israel). Human peripheral blood T lymphocytes were isolated from healthy donors, and the Jurkat human T-cell line was maintained as previously described.⁸ The human LAD 2 mast cells were maintained as described elsewhere.¹⁷ The primary cultured human cord blood mast cells (CBMCs) were derived from human cord blood mononuclear cells in the presence of stem cell factor (SCF), IL-6, and prostaglandin E₂ and used as described.¹⁸ The human fetal lung fibroblast line MRC-5 (American Type Culture Collection, Manassas, Va) was cultured in T21/75-cm² flasks in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin and used for assays between the second and seventh passage. In all experiments the fibroblasts were incubated overnight with serum-free Dulbecco's modified Eagle's medium before stimulation.

Mast cell activation

Freshly isolated human peripheral blood T lymphocytes or Jurkat T cells (1×10^6 /mL) were first activated with 50 ng/mL PMA (60 minutes at 37°C), followed by extensive washing, to study the effects of coculture with T cells. When indicated, T cells were fixed with 1% paraformaldehyde for 20 minutes (which preserves membrane integrity but prevents soluble factor secretion) and then washed.¹⁹ Mast cells (1×10^6 /mL) were then cocultured for various time periods with resting/activated T cells (at 1:1 ratio). Alternatively, mast cells were incubated with 20 µg/mL activated (Tc*-m) or nonactivated (Tc-m) Jurkat T-cell membranes, which were isolated by using a method described previously.⁸ For IgE-mediated activation, cells were sensitized overnight with 100 ng/mL human myeloma IgE-biotin (Calbiochem; Merck KGaA, Darmstadt, Germany) and then stimulated with 100 ng/mL streptavidin (Jackson ImmunoResearch Laboratories, West Grove, Pa).¹⁷

Fibroblast proliferation assay

Subconfluent human lung fibroblasts (5×10^3 /0.2 mL) were incubated (24 hours at 37°C in a 5% CO₂ environment) with supernatants obtained from either Tc-m- or Tc*-m-activated CBMCs. In some experiments 1 µg/mL neutralizing anti-OSM mAb was added to the culture; 1 ng/mL recombinant human OSM served as a positive control (R&D Systems, Minneapolis, Minn). Tritiated thymidine (1 µCi per well; NEN Life Science Products, Inc, Boston, Mass) was added as a final 24-hour pulse, and samples were processed as previously described.²⁰ Inhibition of proliferation was calculated as follows:

$$\left[1 - \frac{(\text{sup. of CBMC} + \text{Tc}^*\text{-m} + \text{Ab} - \text{sup. of CBMC alone})}{(\text{sup. of CBMC} + \text{Tc}^*\text{-m} - \text{sup. of CBMC alone})} \right] \times 100.$$

Human cytokine assays

For the semiquantitative detection of multiple cytokines, supernatants obtained from mast cells incubated with either fixed T cells or T-cell membranes were incubated with the RayBio human cytokine antibody array III (RayBiotech, Inc, Norcross, Ga), as per the manufacturer's instructions. OSM release in supernatants of activated mast cells, as indicated above, was determined by using a commercial ELISA kit per the manufacturer's instructions (DuoSet, R&D Systems).

Microarray analysis and PCRs

See the [Methods](#) section in this article's Online Repository at www.jacionline.org for more information. FcεRI- or T-cell membrane-mediated activation of LAD 2 mast cells was verified before microarray analysis or PCR. In all experiments, a significant degranulation (>28%), as measured by β-hexosaminidase release, was observed in the respective groups.

Immunocytochemistry and histochemistry

See the [Methods](#) section in the Online Repository at www.jacionline.org for more information.

Immunofluorescence

OSM was detected in CBMCs (1×10^5) incubated with Tc-m or Tc*-m (4 hours at 37°C in a 5% CO₂ environment), as described in the [Methods](#) section in the Online Repository at www.jacionline.org.

Statistical analysis

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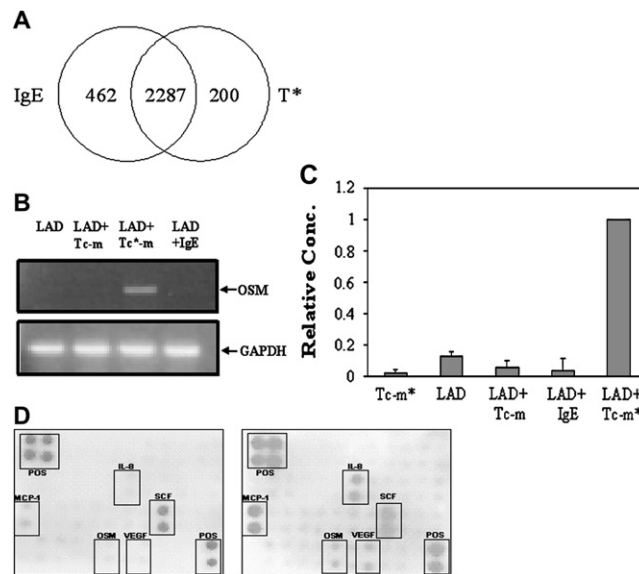


FIG 1. Gene and cytokine expression profiles in resting mast cells or in mast cells activated by either IgE cross-linking or activated T-cell membranes (Tc*-m). **A**, Numbers of upregulated genes in IgE- and T cell-activated mast cells. **B**, RT-PCR analysis of OSM gene expression in LAD 2 cells treated as above. **C**, OSM expression in activated LAD 2 mast cells, as measured by means of QR-PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as an internal control for constitutive gene expression (mean $\pm$ SD, $n = 3$). **D**, Cytokine antibody array of supernatants of mast cells incubated with fixed activated (*right panel*) or resting (*left panel*) T cells.

RESULTS

Gene expression by human mast cells after activation by T cells

A critically important outcome of the interaction of activated T cells with mast cells would be the upregulation of families of genes the products of which facilitate the ability of mast cells to interact with T cells and in turn would contribute to the initiation or propagation of an inflammatory response. We thus examined the global gene expression program engaged by the interaction of the LAD 2 human mast cells with either resting or activated T cells compared with the gene profile induced by IgE-mediated activation. By using microarray technology, we selected genes for further study that were upregulated at least 2-fold in mast cells exposed to activated T-cell membranes versus IgE cross-linking. We identified 462 genes that were specifically upregulated at 3 hours of IgE-mediated activation but not in mast cells activated by T-cell membranes (Fig 1, A). The intersection of T cell- and IgE-activated mast cells revealed a common set of 2287 regulated genes, whereas activation by T cells specifically resulted in the upregulation of 200 genes. The latter were selected for further analysis by grouping as to function (see Table E1 in this article's Online Repository at www.jacionline.org for the list of these genes).

The cytokine/chemokine group accounted for 5% of all selected genes. In this group the most strongly induced genes were IL-8 (23-fold induction), the chemokine ligand CXCL-12 (SDF-1; 56-fold induction), colony-stimulating factor 1 (3-fold induction), amphiregulin (478-fold

induction), activin A (48-fold induction), and OSM (11-fold induction).

OSM is regulated on T cell-induced mast cell activation

Among the transcripts, the OSM gene was of particular interest because of strong induction in LAD 2 human mast cells, as well as its direct association with T cell-mediated inflammatory processes in which mast cells have been found to be involved. Furthermore, the expression of OSM has not heretofore been reported in human mast cells, and we therefore focused on the characterization of this cytokine. RT-PCR and quantitative real-time PCR (QR-PCR) have validated these findings. As shown in Fig 1, B, OSM gene expression was detected in mast cells incubated with activated T-cell membranes. The QR-PCR analysis (Fig 1, C) revealed a 10- to 20-fold increase in OSM gene expression on direct contact with activated T-cell membranes compared with IgE- cross-linking, resting LAD cells, or LAD cells incubated with resting T-cell membranes. Only a miniscule OSM gene expression was found in activated T-cell membranes alone, indicating that mast cells are the only significant source of OSM in this system.

Released protein products that resulted from the heterotypic adhesion-induced mast cell activation have been analyzed by using a cytokine antibody array. As can be seen in Fig 1, D (right panel), coculturing mast cells with fixed activated T cells resulted in the release of OSM, vascular endothelial growth factor, and IL-8. In contrast, these cytokines were not detected in the supernatants of mast cells incubated with fixed resting T cells (left panel). Monocyte chemoattractant protein 1 and SCF were found

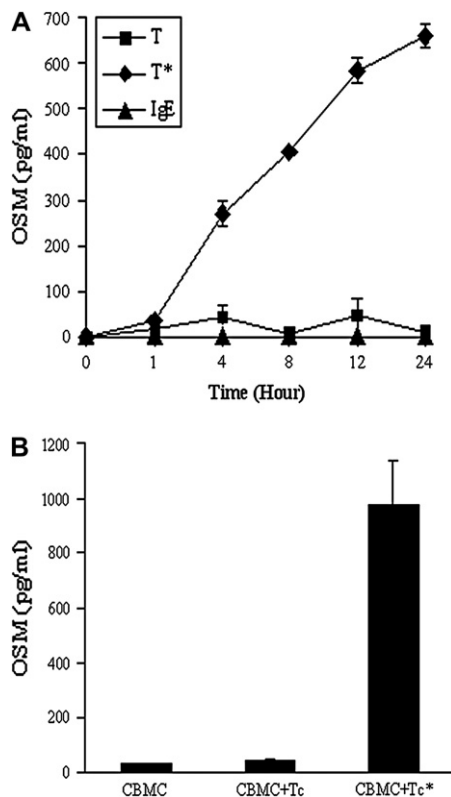


FIG 2. A, Kinetics of OSM release on incubation of LAD 2 cells with T-cell membranes. **B**, OSM release from CBMCs incubated with Tc-m or Tc*-m for 8 hours. Results are shown as means $\pm$ SES of 3 independent experiments done in duplicates.

in both groups, with the latter added as a growth factor to the culture medium. Because similar results were obtained with either peripheral blood T lymphocytes or Jurkat T cells, subsequent experiments were performed with Jurkat T cells. As has been previously reported by us,⁹ using a microporous membrane (Transwell; Costar, Cambridge, Mass) to separate between the 2 cell populations or supernatants from activated T cells did not induce β -hexosaminidase release or OSM from LAD 2 mast cells (data not shown).

In the next set of experiments, we analyzed the kinetics of OSM release induced by activated T cells. Thus LAD 2 mast cells were cocultured with fixed resting or PMA-activated T cells or with their membranes for an incubation period that lasted 24 hours. Sample supernatants were collected at several time points for measurement of OSM release by means of ELISA (Fig 2). As shown in Fig 2, A, OSM was first detected at 4 hours and peaked at 12 to 24 hours. Cultures in which LAD 2 mast cells were incubated with nonactivated T cells or with IgE plus antigen did not show any significant release of OSM in the supernatants (Fig 2, A). T-cell membranes alone did not release any significant amount of OSM (<20 pg/mL at 24 hours, data not shown).

Experiments were also performed with human mature CBMCs. Similar to our findings with the LAD 2 cells, a

marked increase in β -hexosaminidase release (>4 fold, data not shown) was observed, as well as a pronounced augmentation in OSM release in response to direct contact of these primary mast cell cultures with activated T-cell membranes (Fig 2, B).

Intracytoplasmic localization of OSM

By using immunoperoxidase staining, we examined the intracellular localization of OSM in human mast cells. Immunoreactive OSM was expressed in the cytoplasm of T cell-stimulated LAD 2 cells (Fig 3, A, right panel) but not in unstimulated mast cells (Fig 3, A, left panel). As can also be seen in Fig 3 and as has previously been reported, activated, but not resting, mast cells formed aggregates that were found to be heterotypic in nature.⁹ The same results were obtained when CBMCs were incubated for 4 hours with T-cell membranes, as demonstrated by means of immunofluorescent staining (Fig 3, B). Confocal microscopy identified OSM to be expressed in the cytoplasm of CBMCs; however, specific granular localization could not be confirmed by means of double staining with both anti-mast cell tryptase and anti-OSM mAb (not shown).

Regulation of OSM production in mast cells

Corticosteroids have been found to be potent modulators of cytokine production by various cells, including lymphocytes, eosinophils, and mast cells. Furthermore, dexamethasone has been specifically found to inhibit LPS-induced OSM production by PMN cells.²¹ We therefore tested the effect of dexamethasone on inducible OSM production by human mast cells. As shown in Fig 4, A, pretreatment of LAD 2 cells with dexamethasone resulted in a significant concentration-dependent inhibition of OSM production induced by activated T-cell membranes (inhibition of 37%, 50%, and 78% with dexamethasone concentrations of 1, 10, and 100 μ M/L, respectively).

OSM has been found in different cells to be induced by various stimuli, including cytokines such as TNF- α , GM-CSF, LPS, and prostaglandins.^{21,22} We thus investigated a possible stimulatory effect of either TNF- α or LPS on human mast cells. Neither TNF- α at 100 U/mL nor LPS at 100 ng/mL had any effect on OSM release by LAD 2 cells. The possibility of an autocrine effect of mast cell-derived TNF- α or prostaglandins was also ruled out by the lack of effect of blocking anti-TNF- α mAb or indomethacin (0.5–5 μ M/L) on OSM release (data not shown).

It has recently been reported that T cell-induced mast cell activation is associated with phosphorylation of the mitogen-activated protein kinases (MAPKs) extracellular signal-regulated kinase (ERK) and p38.²³ On the basis of this observation, we have investigated the possible involvement of MAPKs in T cell-induced OSM production in the LAD 2 human mast cells. Specific inhibitors to MAPK kinase, Jun N-terminal kinase (JNK), and p38 (PD 98059, curcumin, and SB 202190, respectively) have been introduced to the cell cultures 30 minutes before the addition of T-cell membranes. OSM release was measured at the end of the 18-hour incubation period. As shown in Fig 4, B, each of these 3 compounds partly

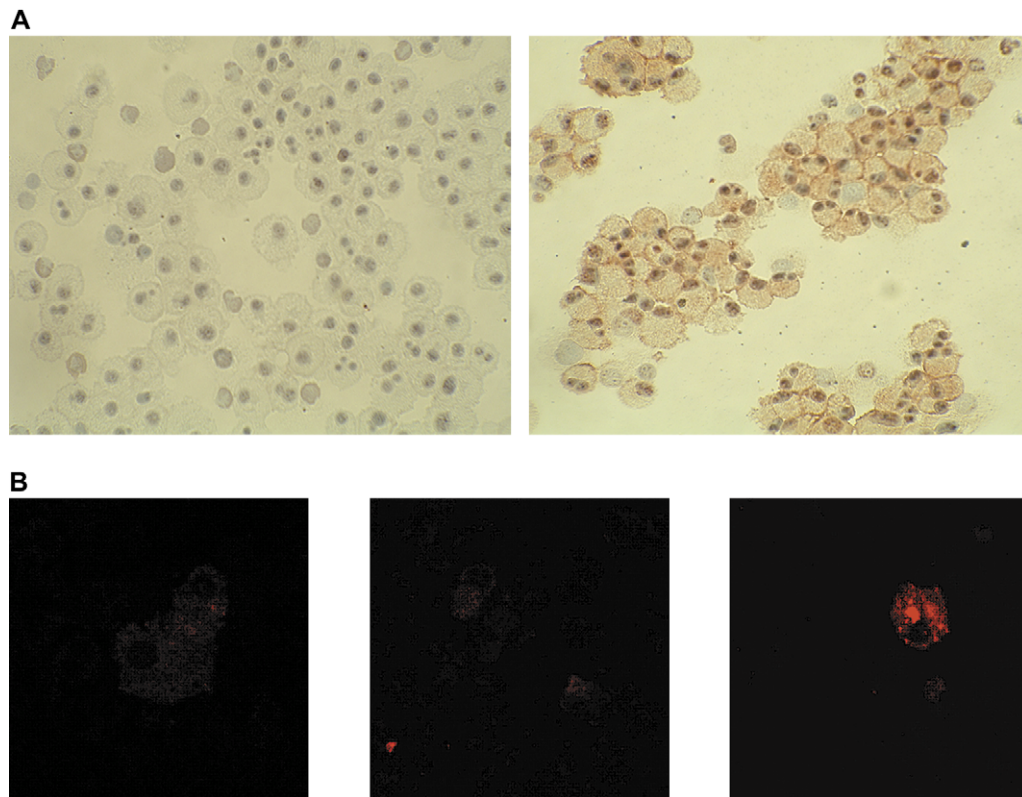


FIG 3. Intracellular localization of OSM. **A**, Immunocytochemical staining of resting (*left panel*) or T cell-activated (*right panel*) LAD 2 cells with anti-OSM mAb (brown; magnification $\times 40$). **B**, Immunofluorescent staining of CBMCs. Left to right: Naive CBMCs, CBMCs incubated with Tc-m, and CBMCs incubated with Tc*-m.

inhibited the T cell–induced OSM release. When added together, they almost completely abrogated OSM release, thus indicating the involvement of the MAPK signaling pathway in T cell–induced mast cell activation and OSM release.

Effect of mast cell–derived OSM on the proliferation of lung fibroblasts

OSM has been found to exert profibrotic properties by inducing fibroblast proliferation and collagen synthesis.²⁰ Because mast cells have been found to be involved in fibrotic processes, such as pulmonary fibrosis, we have analyzed whether this effect can be mediated by mast cell–derived OSM. The human fetal lung fibroblast line MRC-5 has previously been found to express OSM receptors and to proliferate in response to recombinant human OSM.²⁰ When these fibroblasts were incubated with supernatants obtained from CBMCs, which had been stimulated for 8 hours with membranes of activated T cells, a significant mitotic activity was found, as assessed on the basis of tritiated thymidine incorporation. As shown in Fig 5, a small and insignificant increase (23%) of fibroblast proliferation was found when fibroblasts were exposed to supernatants obtained from CBMCs incubated with membranes of resting T cells compared with supernatants of mast cells cultured alone. In contrast, supernatants of activated mast cells (containing 800 pg/mL OSM)

induced a 58% and 45% increase of tritiated thymidine incorporation compared with supernatants obtained from either CBMCs alone or CBMCs incubated with membranes of resting T cells, respectively. As can also be seen in Fig 5, 1 ng/mL recombinant human OSM induced a 69% increase of mitotic activity compared with supernatants of resting CBMCs. The addition of neutralizing anti-OSM mAb led to a significant inhibition (52%) of fibroblast proliferation, although not a complete one, indicating that other mediators released from mast cells (on contact with activated T cells) might act in concert with OSM to induce fibroblast proliferation. These findings were consistent with those obtained by means of direct cell counting (not shown).

OSM expression in human lung mast cells of patients with sarcoidosis

Mast cell hyperplasia occurs ubiquitously in diverse interstitial lung diseases, including idiopathic pulmonary fibrosis, systemic sclerosis, and sarcoidosis.^{7,24} Furthermore, a pathogenetic link between mast cells and fibroblast activation in the sarcoid lung has been suggested. Therefore lung tissues and bronchoalveolar lavage (BAL) fluid from patients with pulmonary sarcoidosis or control patients were examined by using immunostaining with antibodies to OSM and mast cell tryptase or c-kit to determine whether OSM is expressed in the lung mast

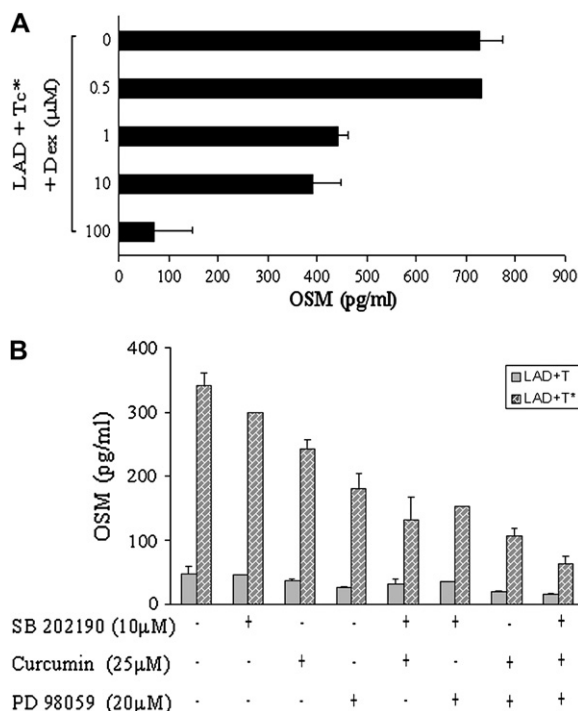


FIG 4. Regulation of OSM production. **A**, Effect of dexamethasone on OSM release by LAD 2 cells incubated with either Tc-m or Tc*-m. Data points are means $\pm$ SES of 2 independent experiments done in duplicates. **B**, Effect of MAPK inhibitors. Data points are means $\pm$ SES of 3 independent experiments done in duplicates.

cells in sarcoidosis. In normal lung tissues, we found that mast cells are positive for tryptase or c-kit; however, these cells stained negative for OSM (not shown). As can be seen in Fig 6, A, mast cells in BAL fluid from a patient with pulmonary sarcoidosis express both tryptase (brown) and OSM (black). LAD 2 mast cells, which were activated with T-cell membranes, were also stained with tryptase and OSM and served as a positive control (Fig 6, B). In both panels there is one cell representative of several that were positively stained in these specimens. In lung biopsy specimens obtained from patients with pulmonary sarcoidosis, we could find both T cells and mast cells within the sarcoid granuloma (Fig 6, C), with the latter positive for OSM (Fig 6, D), thus indicating the presence of OSM in mast cells in this T cell-mediated disease.

DISCUSSION

Mast cells and T lymphocytes clearly have bidirectional influences on each other, as evidenced during T cell-mediated inflammation and parasitic infections.^{1,2} Such influences have primarily been attributed to the biologic effects of T cell-derived soluble mediators on mast cell function.² However, recent investigations of mast cell/T-cell interactions have revealed a novel intercellular communication exclusively involving the binding of cell-surface molecules. Mast cells have been shown to degranulate and produce several cytokines and

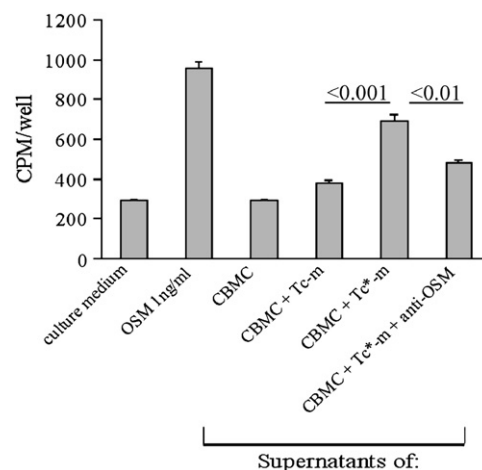


FIG 5. Mast cell-derived OSM induces human lung fibroblast proliferation. Human lung fibroblasts were incubated with supernatants obtained from either resting or T cell-activated CBMCs. Proliferation was measured by means of tritiated thymidine incorporation. Data are expressed as means $\pm$ SES of 2 independent experiments.

chemokines on direct contact with activated T cells, a process that might be regulated by leukocyte function-associated antigen 1/intercellular adhesion molecule 1 interaction or by lymphotoxin- β receptor activation.^{8,10} Considering that such T cell-dependent mast cell activation might result specifically in the expression of certain genes, in the present study we used a cDNA microarray to screen for differentially expressed genes that are relevant to T cell-mediated inflammatory processes. Of the cytokine genes that were analyzed, 6 were specifically expressed at significant levels only in mast cells that were activated by heterotypic adhesion to T cells and not after IgE cross-linking. Under these conditions, OSM was found to be strongly induced in both the LAD 2 and the primary cultured CBMCs only on T cell-induced activation (Fig 1). Other cytokine/chemokine genes, such as IL-8, activin A, and amphiregulin, were indeed induced only on T cell-mediated activation in the LAD 2 cells; however, they were also found to be upregulated on IgE cross-linking in CBMCs.^{25,26} This probably reflects the differences in level of differentiation between these 2 mast cell populations. The fact that OSM mRNA and protein were induced after adhesion to activated T cells but were almost undetectable in unstimulated mast cells suggests that OSM is newly synthesized and released on stimulation in the context of T cell-mediated immune response.

To our knowledge, the results presented herein are the first evidence of OSM regulation in mast cells by means of direct contact with another leukocyte population. Such contact with T lymphocytes correlated with induction of degranulation; release of TNF- α , IL-8, and matrix metalloproteinase 9; and expression of the cytokines IL-4 and IL-6.^{5,8}

Mechanisms mediating T-cell effects on mast cell activation after cell-to-cell contact remain to be elucidated, although it has been reported that activated lymphocytes

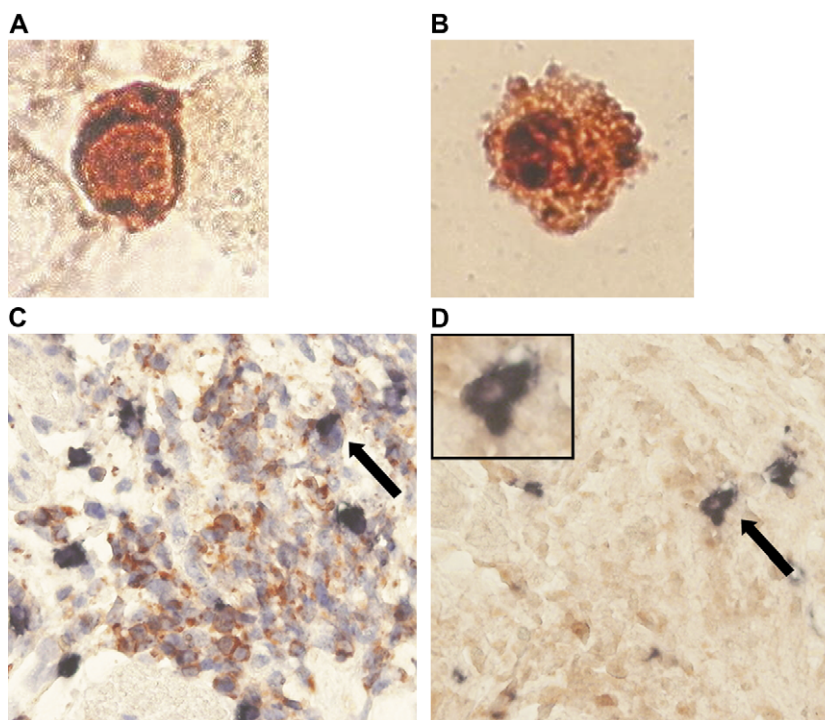


FIG 6. Pulmonary mast cells from patients with sarcoidosis express OSM. Cytopsin preparations of BAL fluid (**A**) or Tc*-m-activated LAD 2 mast cells (**B**) were double stained with anti-mast cell tryptase (brown) and anti-OSM (black). This is representative of 3 patients in whom mast cells were positively stained for OSM. **C** and **D**, Paraffin-embedded cross-sections of lung biopsy specimens from a patient with pulmonary sarcoidosis double stained with anti-c-kit (black) and anti-CD45RO mAb (brown; **Fig 6, C**) or anti-OSM mAb (brown; **Fig 6, D, inset**). One representative mast cell is shown by an arrow. This is representative of 3 patients in whom mast cells were positively stained for OSM.

might affect mast cells at the genome level.²⁷ We have recently shown that direct contact of human mast cells with T-lymphocyte membranes resulted in the phosphorylation of 2 members of the MAPK family (ie, ERK and p38).²³ The MAPKs are known to play an important role in mediator release in human mast cells after IgE cross-linking or on activation by inducers, such as SCF and TNF- α .^{28,29} In accordance with these findings, we were able to show that T cell-induced OSM release is regulated by MAPKs because it was abrogated on the addition of specific inhibitors to p38, JNK, and ERK (**Fig 4**). Furthermore, dexamethasone inhibited, in a dose-dependent manner, the production of OSM by T cell-activated mast cells (**Fig 4**). One mechanism by which glucocorticoids exert their anti-inflammatory effects is the induction of MAPK phosphatase 1. This enzyme dephosphorylates and inactivates all members of the MAPK family, including JNK, ERK 1 and 2, and p38,³⁰ thus explaining the inhibitory effect of dexamethasone on OSM release.

OSM has been implicated in T cell-mediated inflammatory diseases in which mast cells were found to be involved, including rheumatoid arthritis, multiple sclerosis, and inflammatory bowel diseases, and in fibrotic processes, such as scleroderma.⁷ Relevant to the pathogenesis of fibrosis, OSM has been found to stimulate proliferation of fibroblasts and production of collagen and

glycosaminoglycans through finely regulated JAK/signal transducer and activator of transcription and MAPK signaling pathways and expression of own receptor chains.³¹ Indeed, we could show that supernatants obtained from T cell-activated CBMCs induced human lung fibroblast proliferation, an effect that was partially blocked by anti-OSM mAb (**Fig 5**). *In vivo* increased OSM expression has been found in scleroderma, particularly in patients with pulmonary fibrosis.⁷

Pulmonary sarcoidosis is a T cell-mediated disease that is associated with fibrosis and in which mast cells have been implicated.^{24,32} We therefore hypothesized that mast cell-derived OSM might contribute to the pathogenesis underlying this disease. By using double immunostaining with antibodies against c-kit or tryptase in addition to anti-OSM mAb, we found OSM expression in tissue mast cells located within sarcoid granulomas in the lung or in the BAL fluid of patients with pulmonary sarcoidosis (**Fig 6**). Mast cells frequently accumulate at the site of lung fibrosis of widely differing causes.¹ Given that OSM is released by mast cells on activation by heterotypic adhesion to activated T cells, is present in the lungs of patients with sarcoidosis, and induces proliferation of lung fibroblasts, it is possible that mast cell-derived OSM might promote the proliferation of fibroblasts in the lung and skin and thus contribute to fibrosis. In addition to OSM, other mediators

released by mast cells, including TNF- α , TGF- β , platelet-derived growth factor, basic fibroblast growth factor, amphiregulin, and tryptase, have also been demonstrated to exert fibrogenic effects by causing fibroblast proliferation, increased collagen synthesis, or both.^{1,2} It can be envisioned that these mediators released simultaneously on mast cell activation might act synergistically to exert their physiologic or pathophysiologic effects. Interestingly, recent reports have suggested a role for OSM in airway remodeling because of its effects on airway smooth muscle.³³⁻³⁵

In summary, we demonstrated for the first time that both OSM mRNA and protein are induced in human mast cells by means of heterotypic adhesion to activated T cells. Mast cell–derived OSM can induce the proliferation of lung fibroblasts, and its presence was demonstrated in the lungs of patients with sarcoidosis. These results suggest that human mast cells might contribute to T cell–mediated fibrotic inflammatory processes by means of local release of OSM after direct activation by T cells.

We thank Drs Evgeny Edelshtein and Alexander Guber for providing the lung tissue specimens and analyzing the results.

REFERENCES

- Mekori YA. The mastocyte: the “other” inflammatory cell in immunopathogenesis. *J Allergy Clin Immunol* 2004;114:52-7.
- Galli SJ, Kalesnikoff J, Grimbaldston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as “tunable” effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005;23:749-86.
- Marshall JS, Jawdat DM. Mast cells in innate immunity. *J Allergy Clin Immunol* 2004;114:21-7.
- Salamon P, Shoham NG, Gavrieli R, Wolach B, Mekori YA. Human mast cells release Interleukin-8 and induce neutrophil chemotaxis on contact with activated T cells. *Allergy* 2005;60:1316-9.
- Maruotti N, Crivellato E, Cantatore FP, Vacca A, Ribatti D. Mast cells in rheumatoid arthritis. *Clin Rheumatol* 2006;26:1-4.
- Atamas SP, White B. Cytokine regulation of pulmonary fibrosis in scleroderma. *Cytokine Growth Factor Rev* 2003;14:537-50.
- Baram D, Vaday GG, Salamon P, Drucker I, Hershkovich R, Mekori YA. Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF- α . *J Immunol* 2001;167:4008-16.
- Nakae S, Suto H, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cells enhance T cell activation: importance of mast cell-derived TNF. *Proc Natl Acad Sci U S A* 2005;102:6467-72.
- Inamura N, Mekori YA, Bhattacharyya SP, Bianchini PJ, Metcalfe DD. Induction and enhancement of Fc(ϵ)RI-dependent mast cell degranulation following coculture with activated T cells: dependency on ICAM-1- and leukocyte function–associated antigen (LFA)-1-mediated heterotypic aggregation. *J Immunol* 1998;160:4026-33.
- Stopfer P, Mannel DN, Hehlhans T. Lymphotoxin- β receptor activation by T cells induces cytokine release from mouse bone marrow-derived mast cells. *J Immunol* 2004;172:7459-65.
- Tanaka M, Miyajima A. Oncostatin M, a multifunctional cytokine. *Rev Physiol Biochem Pharmacol* 2003;149:39-52.
- Suda T, Chida K, Todate A, Ide K, Asada K, Nakamura Y, et al. Oncostatin M production by human dendritic cells in response to bacterial products. *Cytokine* 2002;17:335-40.
- Koshy PJ, Lundy CJ, Rowan AD, Porter S, Edwards DR, Hogan A, et al. The modulation of matrix metalloproteinase and ADAM gene expression in human chondrocytes by interleukin-1 and oncostatin M: a time-course study using real-time quantitative reverse transcription-polymerase chain reaction. *Arthritis Rheum* 2002;46:961-7.
- Pelletier JP, Martel-Pelletier J. Oncostatin M: foe or friend? *Arthritis Rheum* 2003;48:3301-3.
- Hui W, Rowan AD, Richards CD, Cawston TE. Oncostatin M in combination with tumor necrosis factor alpha induces cartilage damage and matrix metalloproteinase expression in vitro and in vivo. *Arthritis Rheum* 2003;48:3404-18.
- Barksby HE, Hui W, Wappler I, Peters HH, Milner JM, Richards CD, et al. Interleukin-1 in combination with oncostatin M up-regulates multiple genes in chondrocytes: implications for cartilage destruction and repair. *Arthritis Rheum* 2006;54:540-50.
- Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA, et al. Characterization of a novel stem cell factor responsive human mast cell line LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia. *Leuk Res* 2003;27:677-82.
- Piliponsky AM, Gleich GJ, Nagler A, Bar I, Levi-Schaffer F. Non-IgE-dependent activation of human lung- and cord blood-derived mast cells is induced by eosinophil major basic protein and modulated by the membrane form of stem cell factor. *Blood* 2003;101:1898-904.
- Wittmann M, Alter M, Stunkel T, Kapp A, Werfel T. Cell-to-cell contact between activated CD4+ T lymphocytes and unprimed monocytes interferes with a TH1 response. *J Allergy Clin Immunol* 2004;114:965-73.
- Scaffidi AK, Mutsaers SE, Moodley YP, McNulty RJ, Laurent GJ, Thompson PJ, et al. Oncostatin M stimulates proliferation, induces collagen production and inhibits apoptosis of human lung fibroblasts. *Br J Pharmacol* 2002;136:793-801.
- Grenier A, Dehoux M, Boutten A, Arce-Vicioso M, Durand G, Gougerot-Pocidalo MA, et al. Oncostatin M production and regulation by human polymorphonuclear neutrophils. *Blood* 1999;93:1413-21.
- Repovic P, Benveniste EN. Prostaglandin E2 is a novel inducer of oncostatin-M expression in macrophages and microglia. *J Neurosci* 2002;22:5334-43.
- Brill A, Baram D, Sela U, Salamon P, Mekori YA, Hershkovich R. Induction of mast cell interactions with blood vessel wall components by direct contact with intact T cells or T cell membranes in vitro. *Clin Exp Allergy* 2004;34:1725-31.
- Edwards ST, Cruz AC, Donnelly S, Dazin PF, Schulman ES, Jones KD, et al. c-Kit immunophenotyping and metalloproteinase expression profiles of mast cells in interstitial lung diseases. *J Pathol* 2005;206:279-90.
- Cho SH, Yao Z, Wang SW, Alban RF, Barbers RG, French SW, et al. Regulation of activin A expression in mast cells and asthma: its effect on the proliferation of human airway smooth muscle cells. *J Immunol* 2003;170:4045-52.
- Wang SW, Oh CK, Cho SH, Hu G, Martin R, Demissie-Sanders S, et al. Amphiregulin expression in human mast cells and its effect on the primary human lung fibroblasts. *J Allergy Clin Immunol* 2005;115:287-94.
- Oh CK, Metcalfe DD. Activated lymphocytes induce promoter activity of the TCA3 gene in mast cells following cell-to-cell contact. *Biochem Biophys Res Commun* 1996;221:510-4.
- Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. *J Allergy Clin Immunol* 2006;117:1214-25.
- Wong CK, Tsang CM, Ip WK, Lam CW. Molecular mechanisms for the release of chemokines from human leukemic mast cell line (HMC)-1 cells activated by SCF and TNF- α : roles of ERK, p38 MAPK, and NF- κ B. *Allergy* 2006;61:289-97.
- Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med* 2005;353:1711-23.
- Ihn H, Tamaki K. Oncostatin M stimulates the growth of dermal fibroblasts via a mitogen-activated protein kinase-dependent pathway. *J Immunol* 2000;165:2149-55.
- Amano H, Kurosawa M, Ishikawa O, Chihara J, Miyachi Y. Mast cells in the cutaneous lesions of sarcoidosis: their subtypes and the relationship to systemic manifestations. *J Dermatol Sci* 2000;24:60-6.
- O'Hara KA, Kedda MA, Thompson PJ, Knight DA. Oncostatin M: an interleukin-6-like cytokine relevant to airway remodelling and the pathogenesis of asthma. *Clin Exp Allergy* 2003;33:1026-32.
- Faffe DS, Flynt L, Mellema M, Moore PE, Silverman ES, Subramaniam V, et al. Oncostatin M causes eotaxin-1 release from airway smooth muscle: synergy with IL-4 and IL-13. *J Allergy Clin Immunol* 2005;115:514-20.
- Faffe DS, Flynt L, Mellema M, Whitehead TR, Bourgeois K, Panettieri RA Jr, et al. Oncostatin M causes VEGF release from human airway smooth muscle: synergy with IL-1 β . *Am J Physiol Lung Cell Mol Physiol* 2005;288:L1040-8.

METHODS

Microarray analysis

LAD 2 cells were activated by either T-cell membranes or IgE cross-linking, as described above. Total RNA was isolated 3 hours after stimulation (and validation by β -hexosaminidase release) by using the RNeasy Mini Kit (Qiagen, Inc, Hilden, Germany). The cDNA probes were generated and hybridized to the Affymetrix GeneChip human genome U133 (HG-U133), according to the manufacturer's protocol (Affymetrix, Santa Clara, Calif; http://www.affymetrix.com/support/technical/datasheets/human_datsheet.pdf). Global scaling was done by all probes and analyzed with Affymetrix GCOS 1.2. A detection call, which indicates whether a transcript is reliably detected (present) or not detected (absent), was generated for pairwise comparison. The signal log ratio was calculated by comparing each probe pair on the 2 arrays under comparison, and this number is computed by using a 2-step Tukey biweight method, taking a mean of the log ratios of probe pair intensities across the 2 arrays. Fold change was obtained by reversing the log scale by $2^{\text{signal log ratio}}$ (see also Wang et al²⁶).

Semiquantitative RT-PCR and QR-PCR

LAD 2 cells were incubated for 4 hours with Tc-m or Tc*-m. Total RNA was isolated by using the RNeasy Mini Kit (Qiagen). RNA samples (5 μ g) were reverse transcribed into cDNA by using 20 U of SuperScript II (Invitrogen, Carlsbad, Calif) and amplified by using OSM-specific sets of primers. The linear ranges of PCR amplification were determined for each transcript, and amplification was performed within those linear ranges. PCR samples were subjected to electrophoresis on 1% agarose gel. GAPDH was used as an internal control.⁸ The oligonucleotide primers for OSM were synthesized by Invitrogen as follows: OSM sense, 5'-CATCGAGGACTTGGAGAAGC; OSM antisense, 5'-TCTCATCCACAGAGCACCTG.

For quantification of gene expression, equal amounts of cDNA generated as indicated above was amplified by using the same sets of primers of OSM and GAPDH. QR-PCR was performed in duplicates on a Corbett Rotor-Gene RG3000 (Corbett Life Science, Sydney, Australia) by using ABsolute Blue QPCR SYBR Green Mix (ABgene House, Epsom, United Kingdom). Specific targets were amplified by using the following program: stage 1, 95°C for 15 minutes; stage 2, 40 cycles of 97°C for 10 seconds, 58°C for 20 seconds, and 72°C for 15 seconds; stage 3, melt curve of 72°C to 99°C. For quantitative analysis, standard curves for OSM and GAPDH were created with cDNA obtained from LAD 2 cells incubated with Tc*-m and were used to quantify the concentration of each gene, according to its standard curve. Then the expression of the OSM gene was normalized in each sample with the internal reference

gene (GAPDH) and relatively normalized by using the Two Standard Curves method with Rotor-Gene 6000 series software 1.7 (Build 40; Corbett Research, Morrelake, Australia). Specificity for all reactions was verified by means of melting curve analysis and agarose gel detection of single product.

Immunocytochemistry and histochemistry

Immunocytochemistry. Cytospin preparations of BAL fluid from patients with pulmonary sarcoidosis (after signing informed consent) or Tc-m/Tc*-m-activated LAD 2 cells (5×10^4) were fixed in methanol (Bio Lab Ltd, Jerusalem, Israel). Endogenous peroxidase activity was quenched in 1% H₂O₂, and permeabilization was done with 0.2% Triton X-100 (Sigma-Aldrich, St Louis, Mo).

Immunohistochemistry. Paraffin-embedded sections of lung biopsy specimens from patients with sarcoidosis were deparaffinized, immersed in EDTA (pH 8) solution, and heated in a microwave oven.

The double-staining procedure for both methods was done with the Histostain-DS Kit (Zymed LAB-SA Detection System; Zymed laboratories, Invitrogen Immunodetection, San Francisco, Calif), according to the manufacturer's instructions. The following primary antibodies were used: mouse anti-human mast cell tryptase (Chemicon International, Inc, Temecula, Calif), mouse anti-human OSM (R&D Systems), rabbit anti-human c-kit, CD117 (DAKO, Glostrup, Denmark), and mouse anti-human CD45R0 (DAKO). Some sections were counterstained with Mayer's haematoxylin.

Irrelevant isotype-matched antibodies were used to exclude nonspecific staining. No staining was observed in these procedures.

Immunofluorescence

Cytospin preparations of CBMCs (1×10^5) incubated with Tc-m or Tc*-m (4 hours at 37°C in a 5% CO₂ environment) were fixed in 2% buffered paraformaldehyde, permeabilized with 0.2% Triton X-100, blocked in 1% BSA, and incubated with mouse anti-human OSM antibody (R&D Systems) or with an irrelevant isotype-matched IgG antibody. Binding was detected with Cy5 goat anti-mouse fluorescent secondary antibody (Jackson ImmunoResearch Laboratories). Cells were examined by using a 410-laser scanning fluorescence microscope attached to the Axiovert 135 M inverted microscope with a C-apochromat water immersion lens (Carl Zeiss, Thornwood, NY).

Statistical analysis

Results are expressed as means $\pm$ SE. Statistical analysis was performed by using the Student paired *t* test. A *P* value of less than .05 was considered statistically significant. In the fibroblast proliferation assay, a parametric ANOVA, followed by the Tukey Kramer multiple comparisons test, was used to compare the effects. A *P* value of less than .05 was considered statistically significant.

TABLE E1. Genes specifically upregulated after Tc-m* activation compared with IgE cross-linking†

Gene	Protein description	Signal LAD + IgE	Detection LAD + IgE	Signal LAD + Tc*-m	Detection LAD + Tc*-m	Fold induction
Transcription						
<i>LAMC1</i>	Laminin, γ 1 precursor	72.6	A	283	P	4.9
<i>BRF2</i>	Butyrate response factor 2	17.1	A	76	P	3.5
<i>ZFP36L2</i>	Butyrate response factor 2	28.8	A	51.6	P	2.5
<i>ZFP36</i>	Zinc finger protein 36, C3H type, homolog (mouse)	79.5	A	531.7	P	5.7
<i>ATF3</i>	Activating transcription factor 3 long isoform	26.5	A	101.3	P	2.6
<i>KIAA0685</i>	KIAA0685 gene product	46.8	A	78.1	P	2.3
<i>PER1</i>	Period 1	3.6	A	391.3	P	64
<i>MEF2D</i>	MADS box transcription enhancer factor 2, polypeptide D	13.8	A	99.8	P	9.85
<i>C11ORF9</i>	Chromosome 11 open reading frame 9	20	A	67.1	P	2.5
<i>FOXO3A</i>	Forkhead box O3A	25.8	A	133.2	P	5.7
<i>IRF4</i>	Interferon regulatory factor 4	10.3	A	181.5	P	13.9
<i>SERPINB2</i>	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	10.2	A	462.3	P	45
<i>KIAA0997</i>	KIAA0997 protein	9.8	A	39	P	3.7
<i>BCL2A1</i>	BCL2-related protein A1	13.5	A	61.4	P	3.7
<i>PAX2</i>	Paired box gene 2	26.2	A	87.9	P	3.7
<i>MAF</i>	v-maf musculoaponeurotic fibrosarcoma (avian)oncogene homolog	49.1	A	566.8	P	11.3
<i>GTF2A1</i>	TFIIA α , p55	20	A	91.3	P	4.3
<i>KIAA0189</i>	KIAA0189 gene product	38.4	A	236.3	P	6.5
<i>EGR4</i>	Early growth response 4	39.3	A	177.7	P	3
<i>EGR4</i>	Early growth response 4	18.8	A	215	P	13
<i>CHD3</i>	Chromodomain helicase DNA binding protein 3	51.6	A	225.7	P	5.3
<i>NCOR2</i>	Nuclear receptor corepressor 2	4.8	A	64.6	P	11.3
<i>FOS</i>	v-fos FBJ murine osteosarcoma viral oncogene homolog	30.3	A	469	P	13.9
<i>BTEB2</i>	Transcription factor BTEB2	17.4	A	39	P	2.14
<i>MAF</i>	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	9.4	A	354.6	P	42
<i>B3GALT2</i>	B3GALT2	0.4	A	32.7	P	68.6
<i>ING1</i>	Inhibitor of growth family, member 1	55.9	A	117.7	P	2
<i>FKHRLIP2</i>	Fork head-like protein	1.6	A	75.2	P	18.4
<i>KIAA0397</i>	KIAA0397 protein	38.5	A	113.3	P	2.8
<i>KIAA0934</i>	KIAA0934 protein	155.4	A	307.4	P	2.5
<i>TCF8</i>	Transcription factor 8	27.6	A	81.8	P	3.5
<i>KIAA1199</i>	KIAA1199 protein	5.2	A	41.2	P	9
<i>KIAA0346</i>	KIAA0346 protein	26.9	A	1666.5	P	32
<i>FBII</i>	HIV-1 inducer of short transcripts binding protein	22.7	A	34.3	P	2
<i>KIAA0810</i>	KIAA0810 protein	30.2	A	74.5	P	2.8
<i>ZNF277P</i>	Zinc finger protein 277 pseudogene	5.5	A	45.1	P	11.3
<i>B3GALT2</i>	UDP-Gal:betaGlcNAc β 1,3-galactosyltransferase, polypeptide 2	4.3	A	77.5	P	9
<i>NUP188</i>	Nucleoporin 188 kd	50.8	A	99.6	P	2
<i>FLJ11137</i>	Hypothetical protein FLJ11137	19.4	A	127.8	P	3.5
<i>NGAP</i>	Ras GTPase activating protein-like	15.3	A	169.7	P	10.6
<i>AF5Q31</i>	ALL1 fused gene from 5q31	62.8	A	210	P	3.3
<i>GALNT4</i>	Polypeptide N-acetylgalactosaminyltransferase 4	9.1	A	87.8	P	5.7
<i>FLJ20307</i>	Hypothetical protein FLJ20307	6	A	28	P	3.3
<i>ABCB2</i>	ATP-binding cassette, subfamily B (MDRTAP), member 2	63.3	A	130	P	2.8

TABLE E1. (Continued)

Gene	Protein description	Signal LAD + IgE	Detection LAD + IgE	Signal LAD + Tc*-m	Detection LAD + Tc*-m	Fold induction
Signaling						
<i>MACS</i>	Myristoylated alanine-rich protein kinase C substrate	12.4	A	259.2	P	17
<i>MACS</i>	Myristoylated alanine-rich C-kinase substrate	5.4	A	136.5	P	22.6
<i>SLC6A8</i>	Solute carrier family 6 (neurotransmitter transporter, creatine), member 8	57.2	A	759.6	P	8
<i>SERPINE1</i>	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	39.9	A	119.3	P	3
<i>PKIA</i>	Protein kinase, cAMP-dependent, catalytic, inhibitor α	37.2	A	373.2	P	9
<i>RRAD</i>	Ras-related associated with diabetes	5.5	A	270.5	P	24.3
<i>RRAD</i>	Ras-related associated with diabetes	44	A	503.1	P	12
<i>PDGFA</i>	Platelet-derived growth factor α polypeptide (PDGFA)	15.4	A	76.3	P	3.7
<i>PNOC</i>	Prepronociceptin	15.2	A	273.3	P	13
<i>RASA3</i>	RAS p21 protein activator 3	12.7	A	193.7	P	10.6
<i>NRG1</i>	Neuregulin 1 isoform HRG-beta2	5.9	A	1134.9	P	181
<i>SPINK1</i>	Serine protease inhibitor, Kazal type 1	17.2	A	272.7	P	13.9
<i>NRG1</i>	Neuregulin 1 isoform SMDF	1.2	A	831.2	P	338
<i>BAIAP2</i>	BAI1-associated protein 2, isoform 2	24	A	63.4	P	2.6
<i>NRG1</i>	Neuregulin 1 isoform ndf43	6.1	A	807.3	P	64
<i>NRG1</i>	Neuregulin 1 isoform ndf43	5.9	A	267	P	22.6
<i>TC21</i>	Oncogene TC21	0.6	A	169.9	P	194
<i>TRIO</i>	Triple functional domain (PTPRF interacting)	15.4	A	210.6	P	9
<i>TRIO</i>	Trio isoform	265.2	A	2050.4	P	9.85
<i>RGS</i>	Regulator of G protein signaling	28.1	A	101.5	P	4
<i>LAMA5</i>	Laminin subunit α -5	96.9	A	335.1	P	3.3
<i>SLC6A8</i>	GABA noradrenaline transporter	90	A	780.4	P	8
<i>SBF1</i>	SET binding factor 1	51.4	A	105.2	P	2
<i>TC21</i>	Oncogene TC21	1.5	A	160.9	P	104
<i>TC21</i>	Oncogene TC21	5.5	A	263.7	P	45
<i>ARHE</i>	Ras homolog gene family, member E	38.6	A	1387.7	P	37
<i>GAL</i>	Galanin	9.2	A	111.7	P	9.85
<i>RGS12</i>	Regulator of G-protein signalling 12	3.2	A	49.8	P	15
<i>SLC6A10</i>	Creatine transporter (SLC6A10)	48.5	A	459.4	P	12
<i>GLRA3</i>	Glycine receptor, α 3	13	A	30.9	P	2.6
<i>DRM</i>	DRM	0.8	A	24.6	P	22.6
<i>FLJ10628</i>	Hypothetical protein FLJ10628	48.6	A	294.9	P	4.6
<i>FLJ11360</i>	Hypothetical protein FLJ11360	29.6	A	69.3	P	2
Protein transport						
<i>STX1A</i>	Syntaxin 1A (brain)	57.2	A	485.9	P	6.1
<i>AP3D1</i>	Adaptor-related protein complex 3, delta 1 subunit	50.1	A	164.6	P	3
<i>EHD1</i>	EH domain containing 1	32.6	A	283	P	11.3
<i>MDR1</i>	P-glycoprotein	15.4	A	99.1	P	4.6
<i>MDR1</i>	P-glycoprotein	3	A	72.6	P	12
<i>KPNB1</i>	Karyopherin β 1	48.1	A	157.9	P	2.6
Enzyme						
<i>PYGB</i>	Phosphorylase, glycogen; brain	80.3	A	214.9	P	2.8
<i>ADCY9</i>	Adenylate cyclase 9	58.6	A	178.6	P	2.3
<i>MMP19</i>	Matrix metalloproteinase RASI-1	1.7	A	90.8	P	15
<i>MYOM2</i>	Myomesin 2	30.7	A	279.8	P	5.3
<i>KIAA0750</i>	KIAA0750 gene product	30.7	A	70.4	P	2.8
<i>PTP4A3</i>	Protein tyrosine phosphatase type IVA, member 3	17.9	A	101.8	P	4.6

TABLE E1. (Continued)

Gene	Protein description	Signal LAD + IgE	Detection LAD + IgE	Signal LAD + Tc*-m	Detection LAD + Tc*-m	Fold induction
<i>CCNT1</i>	Cyclin T1	35.1	A	90.8	P	2.3
<i>SLC7A11</i>	Solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	13.5	A	97.4	P	5.7
<i>PCTK1</i>	PCTAIRE protein kinase 1	68.1	A	211.7	P	2.8
<i>SYNJ2</i>	Synaptojanin 2	13.3	A	62.6	P	5.3
<i>PDE4A</i>	3,5-cyclic AMP phosphodiesterase HSPDE4A7A	30.1	A	106.8	P	2.6
<i>AKT2</i>	Rac protein kinase-beta	60.4	A	149.4	P	2.6
<i>PDE4A</i>	Phosphodiesterase	14.9	A	85.5	P	5.7
<i>CREBBP</i>	CREB-binding protein	6.3	A	56.6	P	6.1
<i>PDE4A</i>	Cyclic AMP-specific phosphodiesterase HSPDE4A10	2.7	A	30.2	P	4.3
<i>KIAA1096</i>	KIAA1096 protein	25.4	A	158.9	P	5.7
<i>KIAA0871</i>	KIAA0871 protein	22.6	A	48.3	P	2.14
<i>SLC7A11</i>	Solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	1.9	A	142.2	P	32
<i>HSA275986</i>	Transcription factor (SMIF gene)	20.6	A	121	P	5.7
<i>CCNK</i>	Cyclin K	10.7	A	66.8	P	4.3
<i>CAPN10</i>	Calpain 10, isoform a	48.1	A	150.6	P	2.8
<i>FLJ20707</i>	Hypothetical protein FLJ20707	20.1	A	107.9	P	4.3
<i>DOCK6</i>	Dedicator of cytokinesis 6	59.9	A	133.3	P	2
DNA binding						
<i>NR4A1</i>	Nuclear receptor subfamily 4, group A, member 1	86.6	A	2396.5	P	21
<i>FOSB</i>	FBJ murine osteosarcoma viral oncogene homolog B	49.1	A	473.6	P	10.6
<i>SATB1</i>	Special AT-rich sequence binding protein 1	26.8	A	112.2	P	6.1
<i>LIG3</i>	Ligase III, DNA, ATP-dependent, isoform α	116.7	A	163.4	P	2
<i>NR4A2</i>	Nuclear receptor subfamily 4, group A, member 2	42.3	A	454.3	P	8
<i>ATF5</i>	Activating transcription factor 5	31.4	A	40.4	P	2.3
<i>DNASE1L2</i>	Deoxyribonuclease I-like 2	25.2	A	70.5	P	2.14
<i>MYCN</i>	v-myc Avian myelocytomatosis viral related oncogene, neuroblastoma derived	12.2	A	152.8	P	11.3
<i>NR4A1</i>	TR3 β	11.5	A	104.2	P	9.85
<i>HMG1Y</i>	High-mobility group protein-R	69.9	A	1138.3	P	9
<i>NAK1</i>	DNA-binding protein	7.7	A	126.4	P	30
<i>MYCN</i>	N-MYC	8.1	A	52.8	P	7
<i>SSBP</i>	Single-stranded DNA-binding protein	28.9	A	148.3	P	3.3
<i>DRIL2</i>	Dead ringer-like 2	58.6	A	235.4	P	4.6
RNA binding						
<i>KIAA0682</i>	KIAA0682 gene product	22.6	A	147.2	P	5.3
<i>LUCA-15</i>	LUCA-15 protein	7.8	A	78.3	P	7
<i>D6S81E</i>	HLA-B-associated transcript-1	10.8	A	77.8	P	5.3
<i>KIAA0185</i>	KIAA0185 protein	156.7	A	261.9	P	2.14
<i>SAM68</i>	GAP-associated tyrosine phosphoprotein p62 (Sam68)	19.7	A	43.4	P	2
Cytoskeleton						
<i>KIAA0992</i>	Palladin (KIAA0992)	16.7	A	137.1	P	7.5
<i>KIAA0992</i>	Palladin	20.5	A	91.6	P	4.3
<i>SMTN</i>	Smoothelin	58.7	A	517.4	P	7
<i>SMTN</i>	Smoothelin large isoform L2	60.4	A	539.7	P	5.7
<i>ARC</i>	ARC	2.2	A	1459.7	P	549
<i>PXN</i>	Paxillin β	25.6	A	403.7	P	16
<i>MYO10</i>	Myosin X	1.3	A	88.4	P	30

TABLE E1. (Continued)

Gene	Protein description	Signal LAD + IgE	Detection LAD + IgE	Signal LAD + Tc*-m	Detection LAD + Tc*-m	Fold induction
Cytokines and chemokines						
<i>IL8</i>	IL-8 C-terminal variant	24	A	464.5	P	23
<i>IL8</i>	IL-8	5.9	A	227.8	P	15
<i>INHBA</i>	Inhibin β A subunit precursor	9	A	140.7	P	9
<i>AREG</i>	Amphiregulin (schwannoma-derived growth factor)	6.5	A	2387.4	P	478
<i>hIRH</i>	Intercrine- α	1.8	A	71.9	P	56
<i>INHBA</i>	Inhibin, β A (activin A, activin AB α polypeptide)	2.7	A	141.7	P	48
<i>TNFSF11</i>	Osteoprotegerin ligand	1.2	A	17	P	8.6
<i>TRAIL-R4</i>	TRAIL-R4-B	37	A	87.2	P	2.3
<i>CSF1</i>	Colony-stimulating factor 1	57.4	A	152.7	P	3
<i>CSF1</i>	Macrophage-colony stimulating factor γ precursor	60.6	A	151.5	P	3
<i>OSM</i>	Oncostatin M	22.4	A	366.2	P	11
Cell surface						
<i>CRK</i>	v-crk Avian sarcoma virus CT10 oncogene homolog isoform a	69.3	A	147	P	2.3
<i>ITGAV</i>	Integrin, α V (vitronectin receptor, α polypeptide, antigen CD51)	30.7	A	280.3	P	7.5
<i>IL1R1</i>	IL-1 receptor, type I	5.7	A	90.6	P	8.6
<i>GABBR1</i>	Gamma-aminobutyric acid (GABA) B receptor 1, isoform a precursor	108.9	A	290.5	P	2.5
<i>PLXNB3</i>	Plexin B3	21.6	A	107.4	P	4.9
<i>CBLB</i>	Cas-Br-M (murine) ectropic retroviral transforming sequence	2.7	A	24.8	P	13
<i>MC5R</i>	Melanocortin 5 receptor	23.4	A	172.61	P	8.6
<i>NRP1</i>	Soluble neuropilin-1	1.7	A	55.4	P	22.6
<i>NRP1</i>	Neuropilin 1	26.5	A	183	P	5.3
<i>GPR3</i>	G protein-coupled receptor 3	4	A	186.8	P	37
<i>SEMA6A</i>	Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin)	0.5	A	17.3	P	13
<i>CD44</i>	Human soluble CD44 (CD44)	25.1	A	158.4	P	6.1
<i>MFI2</i>	Melanoma-associated antigen p97 precursor	54.3	A	318.4	P	4.6
Cell cycle						
<i>TCF8</i>	Transcription factor 8	46.7	A	758.8	P	11.3
<i>MNB</i>	MNB	24.3	A	87.1	P	3
<i>TMEPAI</i>	Transmembrane, prostate androgen-induced RNA	18.3	A	480.7	P	21
<i>FLJ20538</i>	BANP homolog, SMAR1 homolog	105.1	A	217.1	P	2.14

MADS, MCM1-agamous-deficiens = serum response factor; *ATP*, adenosine triphosphate; *SMDF*, sensory and motor neuron derived factor; *AMP*, adenosine monophosphate; *GAP*, GTPase-activating protein.

†The signal value "A" stands for "absent," meaning that the transcript was not detected; the signal value "P" stands for "present," meaning that the transcript was detected. Signal log ratio was calculated by means of comparison analysis between the expression profiles after Tc-m* activation compared with IgE cross-linking. The fold induction was obtained by reversing the log scale by $2^{\text{signal log ratio}}$ (see the Online Repository Methods section).