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

Pazit Salamon, MSc,[a]* Nitza G. Shoham, PhD,[a]* Ilaria Puxeddu, PhD,[b] Yosef Paitan, PhD,[c] Francesca Levi-Schaffer, PhD,[b] and Yoseph A. Mekori, MD[a] *Kfar Saba, Tel Aviv, and Jerusalem, Israel*

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. (J Allergy Clin Immunol 2008;121:448-55.)

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 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

From [a]the Allergy and Clinical Immunology Laboratory and [c]the Microbiology Laboratory, Meir General Hospital, Kfar Saba, and the Sackler School of Medicine, Tel Aviv University; and [b]the Department of Pharmacology, Faculty of Medicine, the Hebrew University, Jerusalem.
*These authors contributed equally to this work.
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.
Available online October 29, 2007.
Reprint requests: Yoseph A. Mekori, MD, Department of Medicine B, Meir General Hospital, Kfar Saba 44281, Israel. E-mail: ymekori@clalit.org.il.
0091-6749/$34.00

doi:10.1016/j.jaci.2007.08.054

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 E_2 and used as described.[18] The human fetal lung fibroblast line MRC-5 (American Type Culture Collection, Manassas, Va) was cultured in T21/75-cm^2 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% CO_2 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:

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

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

See the Methods section in the Online Repository at www.jacionline.org for more information.

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).