

Short communication

Human mast cells release Interleukin-8 and induce neutrophil chemotaxis on contact with activated T cells

Background: Mast cells have recently been shown to control neutrophil recruitment during T-cell mediated cutaneous DTH reaction *in vivo* through TNF- α and MIP-2, the functional murine analogue of human IL-8. Although the nature of signals transmitted from T cells which activate mast cells has not yet been defined, we hypothesized that a direct cross-talk (i.e. heterotypic adhesion) between these two cell populations exists, as has previously been reported.

Aims: The present study was aimed at gaining insight into the functional role of mast cell–T cell contact in expression and release of IL-8, and its effect on neutrophil chemotaxis.

Methods: The IL-8 gene expression was identified by Affymetrix GeneChip arrays, validated by RT-PCR and the protein measured by ELISA. Chemotaxis was evaluated by using a modified Boyden chamber assay.

Results: Mast cells were found to express and release significantly higher concentrations of IL-8 on incubation with membranes obtained from activated, as compared to resting T cells. Supernatants obtained from these activated mast cells induced significant neutrophil chemotaxis that was inhibited by neutralizing mAb to IL-8.

Conclusions: Thus, activated T cells, on heterotypic adhesion to mast cells, deliver the necessary signals for the latter to release cytokines and chemokines necessary for cell migration to sites of antigen challenge, thereby facilitating T-cell mediated inflammatory processes.

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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. A variety of stimuli can activate mast cells to release a diverse array of biologically active products, many of which can mediate potential proinflammatory, anti-inflammatory and/or immunoregulatory effects (1). For example, the synthesis and expression of a plethora of chemokines such as IL-8, RANTES, MCP-1 and eotaxin by human mast cells can influence leukocyte function such as eosinophil migration (2). Observations such as the close physical proximity between mast cells and T cells in inflamed tissues (3) 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 two cell populations (4, 5).

More recently, several studies have shown the involvement of mast cells in the elicitation of T-cell mediated inflammatory responses such as cutaneous DTH and EAE (the animal model of multiple sclerosis) *in vivo* (6, 7). By using W/W^v mast cell deficient mice that were reconstituted with mast cells obtained from TNF^{-/-} mice, it could be

shown that TNF- α and MIP-2 (the functional murine analogue of human IL-8) were essential for appropriate PMN recruitment during T-cell induced cutaneous DTH reactions (6). The induction of MIP-2 and TNF- α were strictly dependent on the presence of mast cells and local activation of memory T cells, which shows that the infiltrating T cells deliver signals that induced both TNF- α and MIP-2 production by mast cells (6). Though the nature of these signals *in vivo* has not yet been defined, it seems reasonable to suggest that a direct cross-talk between these two cell populations exists, since recent *in vitro* studies indicated that mast cells may be triggered to degranulate and release cytokines upon heterotypic adhesion to activated, but not resting, T cells (8, 9).

We have previously reported that direct contact between mast cells and T-lymphocytes caused mast cell degranulation. Mast cells were found to degranulate and release TNF- α in response to direct contact with T cells that had been activated by either PMA or immobilized anti CD3 mAb (8, 9). The present study was aimed at gaining insight into the functional role of mast cell–T cell contact in expression and release of IL-8 and its effect on neutrophil chemotaxis, in the context of mediating T-cell induced inflammatory reactions.

Methods

Cell culture

The HMC-1 cells, a human mast leukemic cell line, and Jurkat T-lymphoma cell cultures were each maintained in RPMI supplemented with 10% FCS, 2 mM L-glutamine, and 1% penicillin-streptomycin-nystatin (Biological Industries, Kibutz Beit Haemek, Israel). Human peripheral blood T lymphocytes were enriched using nonadherence to nylon wool (9). The LAD-2 cells were established from bone marrow aspirates from a patient with mast cell sarcoma/leukemia and maintained as previously described. These mast cells resemble CD34⁺-derived human mast cells, respond to rhSCF and have functional Fc ϵ RI and Fc γ RI receptors. Also, the LAD-2 cells release β -hexosaminidase following Fc ϵ RI or Fc γ RI aggregation (10).

Mast cell activation

Mast cells were activated by co-culturing (at 1 : 1 ratio) with isolated T lymphocytes or Jurkat T cells which were first activated with 50 ng/ml PMA for 60 min at 37°C, followed by extensive washing with PBS. Alternatively, mast cells were incubated with 20 μ g/ml Jurkat T-cell membranes, which were isolated by a method described previously (9).

The IL-8 expression and release

For the analysis of IL-8 mRNA expression, HMC-1 cells were incubated for 3 h with activated or nonactivated T-cell membranes. Total RNA was isolated according to the manufacturer's instructions (QIAGEN, MD, USA). Total RNA was reverse-transcribed using SuperScriptTM II RT (Invitrogen Ltd, Paisley, UK). Amplification of IL-8 was conducted using the sense primer 5'-AGA CATACTCCAAACCTTTCCACC-3', the antisense primer 5'-CTCAGCCCTCTTCAAAAATTCTC.

Supernatants obtained from mast cells incubated overnight with resting or activated T cells or with T-cell membranes, were examined for released IL-8 by a commercial ELISA kit, according to the manufacturer's instructions (R&D Systems Inc., Minneapolis, MN, USA).

Chemotaxis

Human purified PMN cells (98%) were isolated from heparinized venous blood and a 48-well chemotaxis-microchamber (Neuro Probe Inc., Bethesda, MD, USA) was used to determine random migration and chemotaxis (11). Briefly, the tested supernatants were added to the bottom wells. A polycarbonate membrane filter, with 3 μ m pores (Nucleopore Corp., Pleasanton, CA, USA), was placed on top of the wells in the bottom plate. After affixation, PMN cells were added to the upper wells. The assembly was incubated for 60 min in humidified air. The filter was wiped off and stained with May-Grunwald-Giemsa dye. The average number of cells in nine fields was counted under light microscopy with a 20 $\times$ objective and an optical grid at 10 $\times$ magnification. Net chemotaxis was calculated by subtracting the random migration from the chemotactic activity.

Results

We first determined whether mast cell-T cell interactions, which likely occur at sites of inflammation, could

potentiate mast cell production of IL-8. The HMC-1 or the LAD-2 human mast cells were incubated overnight with resting or activated peripheral blood-derived T cells or Jurkat T cells; supernatants were then collected and analyzed for released IL-8. As shown in Fig. 1, co-culturing mast cells with activated, but not resting, T cells resulted in >25-fold increase in IL-8 concentration. The IL-8 levels in resting cells lysates were negligible (100 pg/ml) indicating that this cytokine is not preformed but mainly synthesized *de novo*. The same results were obtained with the LAD-2 cells (not shown; three independent experiments).

To further investigate the effect of heterotypic adhesion on IL-8 expression and release, and to neutralize a possible effect that may be exerted by the activated T cells, we used cell membranes. It has previously been shown that membranes obtained from activated T cells are capable of inducing expression and release of granule-associated mediators such as β -hexosaminidase and MMP-9, and of certain cytokines such as TNF- α and IL-4, following incubation with mast cells (9). In this study, expression of IL-8 was first evaluated by Affymetrix GeneChip arrays which showed a 290-fold increase in mRNA for IL-8 in activated HMC-1 cells and 111-fold increase in LAD-2 cells compared with that of mast cells incubated with membranes of resting T cells. In LAD-2

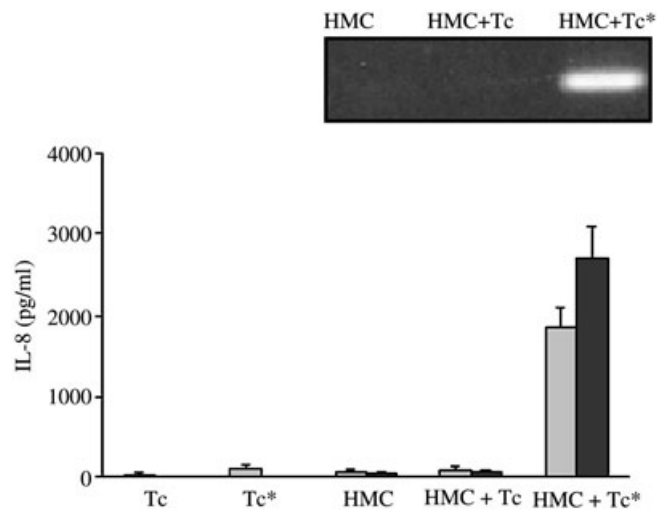


Figure 1. The IL-8 expression and release from mast cells on interaction with T cells. The HMC-1 human mast cells were incubated overnight with resting (Tc) or activated [50 ng/ml PMA; 60 min (Tc*)] T cells (gray bars) or with cell membranes isolated from resting or activated T cells (black bars). Supernatants were collected and analyzed for released IL-8 by ELISA. Data represent mean $\pm$ SD of five independent experiments. In the upper panel, total RNA was extracted from HMC-1 mast cells at the end of a 3 h incubation period with cell membranes obtained from either resting or activated T cells. Expression of IL-8 was analyzed by RT-PCR. Amplified products were subjected to electrophoresis on 1% agarose gels and visualized by ethidium bromide staining.

cells, there was no change in the expression level of the chemokines CXCL1-CXCL7, while in HMC-1 cells there was a 60-fold increase in mRNA for CXCL3. Furthermore, as can be seen in Fig. 1, membranes obtained from activated, but not resting T cells, induced a significant expression of IL-8 mRNA and a pronounced increase (~25-fold) in IL-8 in the supernatants. It should be noted that separation between the two cell populations by a microporous membrane (Transwell; Costar, Cambridge, MA, USA) or supernatants obtained from activated T-cells failed to induce mast cell degranulation or cytokine release (data not shown; three independent experiments). Thus, direct contact between cell surface molecules on mast cells and on activated T-cell membranes is sufficient to transduce the signal in mast cells necessary for IL-8 expression and release, independent of T-cell production of cytokines or other mediators.

To determine whether a functional interaction exists between mast cells and neutrophils, we used a 48 well-chemotactic microchamber migration assay to assess the effect of T cell-induced mast cell IL-8 release on neutrophil chemotaxis (11). Supernatants collected from activated mast cells induced a significant (>4-fold) increase in neutrophil migration compared to HMC-1 cells incubated with medium alone. In contrast, neutrophils showed only a mild specific migration to supernatants collected from mast cells that had been incubated with membranes obtained from resting T cells (Fig. 2).

To determine whether the effects of activated mast cells on neutrophil migration are mediated by IL-8, we added to the supernatants anti IL-8 mAb in concentrations that have previously been shown to exert blocking effects (R&D

Systems Inc.). Indeed, the anti IL-8 mAb blocked the neutrophil migration induced by activated mast cells. We found no significant inhibition of neutrophil migration with the isotype control, which was found to have even some stimulatory effect. Taken together, these results demonstrate that neutrophil migration is induced by IL-8 released from mast cells upon adhesion to activated T cells.

Discussion

Mast cells and T lymphocytes clearly have bidirectional influences on each other, as evidenced during T-cell mediated inflammation (4, 5). Such influences have primarily been attributed to the biological effects of T-cell derived mediators on mast cell function. 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 found to degranulate and release certain cytokines such as TNF- α , MIP-2, IL-4 and IL-6 upon direct contact with activated T cells, a process that may be regulated by LFA-1-ICAM-1 interaction or by lymphotoxin- β receptor activation (8, 12). In this study we have demonstrated that this pathway of activation also results in the expression and release of IL-8, which serves as a potent chemokine in inducing neutrophil migration. The same results were obtained if either HMC-1 (Figs 1 and 2) or LAD-2 (not shown) mast cells were used. The combination of TNF- α and IL-8 is crucial for cell recruitment because TNF- α and IL-8 provide two qualitatively different but synergistic signals. Human mast cells are capable of releasing TNF- α on co-culturing with activated T cells or their membranes (9). The TNF- α levels at 16 h incubation with activated T-cell membranes were 400 pg/ml. There was no significant release of TNF- α on incubation with membranes obtained from resting T cells (9). Mast cell derived TNF- α has been shown to induce adhesion molecule expression required for both lymphocyte and neutrophil attachment to endothelial cells, whereas IL-8 establishes a chemotactic gradient required for diapedesis and directed migration (4). Notwithstanding, this mechanism of mast cell activation may result in the release of several other chemokines/cytokines known to be operative in recruiting inflammatory cells to sites of antigen challenge.

In addition to affecting the elicitation phase (cell recruitment) of DTH, mast cells appear to affect also the induction phase of T cell-induced inflammatory processes by various mechanisms including antigen presentation, and dendritic cell migration (4, 13). Thus, the cascade of events, whereby mast cells are activated by T cells to release certain mediators which are known to be essential for leukocyte extravasation and recruitment to affected sites, points to an important immunoregulatory function of mast cells within the context of T-cell mediated inflammatory processes.

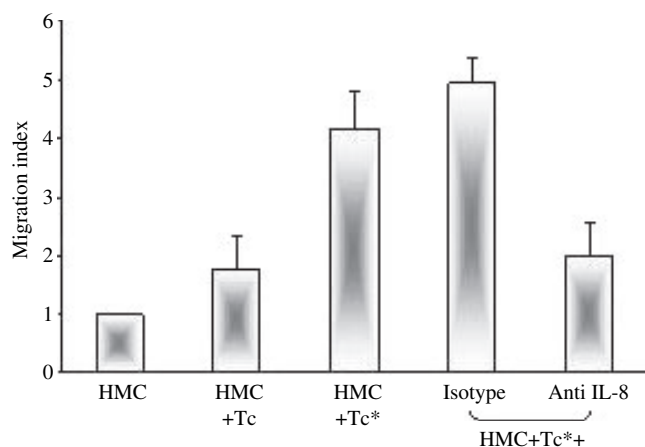


Figure 2. Activated mast cells induce migration of neutrophils. Supernatants were collected from human mast cells (HMC-1) that were either left untreated, or incubated (overnight) with membranes obtained from resting or PMA-activated T cells. Neutrophils were assembled in a migration chamber and migration was measured by counting cells under light microscopy. A migration index of one corresponds to the number of neutrophils that migrated to resting mast cells. Data is mean $\pm$ SE of three independent experiments.

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