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MAST CELL CHEMOTACTIC ACTIVITY OF RANTES

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Summary: RANTES is a cytokine produced by activated T-lymphocytes that has been shown to exert chemotactic activity for memory-type CD4 T-lymphocytes and eosinophils. In this study, RANTES caused directional migration of human mast cells. When compared to other potential chemoattractants of the same cells, RANTES was found to be more potent than fibronectin and the c-kit receptor ligand, on a molar basis. This cytokine may be a common mechanism in allergic reactions which culminate in the selective migration of memory CD4 T-lymphocytes, eosinophils and mast cells at the tissue site. Asthma and allergic rhinitis may represent possible clinical examples.

Mast cell accumulation occurs in many inflammatory and immune reactions, and local activation of these cells may contribute to functional derangement and tissue damage (1-3).

The factors which stimulate human mast cell migration have been poorly investigated. Previous reports have implicated IL-3 (4), the c-kit ligand (SCF) (5), TGF-β_1 (6), matrix substances such as laminin and fibronectin (7,8), or tumor-derived factors (9), mostly using animal cells and cell lines. In this report, we evaluated the ability of human recombinant RANTES in promoting chemotaxis of human pulmonary mast cells versus the other potential chemoattractants.

RANTES is a member of the "C-C" branch of the recently discovered intercrine/chemokine superfamily of chemotactic peptides that possess a rather restricted target cell specificity (10,11). On the basis of previous reports (11-14), this cytokine is produced by activated T-cells and preferentially induces

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Abbreviations: IL-3, Interleukin-3; SCF, Stem Cell Factor; TGF-β_1, Transforming Growth Factor-beta$_1$; HPF, High Power Field.

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the migration of CD4 T-lymphocytes of the memory phenotype and eosinophils.

Materials and Methods

Mast cell isolation: Pulmonary tissue was removed from 15 patients with lung cancer at the time of surgical operation. After resection, macroscopically normal tissue samples were taken as far away as possible from the malignancy. Cells were dispersed by enzymatic digestion (15), layered onto a 60-80% Percoll discontinuous gradient, and centrifuged at 500g for 20 minutes at 4°C. Cells at the bottom of the gradient and between the 70/80% interface were 65-72% pure mast cells. Contaminating cells were lymphocytes and monocytes and these were removed by negative selection, using magnetic beads. To do that, cells were incubated with the following mouse monoclonal antibodies to the surface markers of T-cells, B-cells and monocytes for 45 minutes at 4°C: anti-Leu-5b (anti-CD2), anti-Leu-16 (anti-CD20), anti-Leu-M3 (anti-CD14) (Becton Dickinson, Milano, I). Cells were then washed and incubated with goat anti-mouse IgG-coated magnetic beads (Advanced Magnetics Inc., Cambridge, MA) for 30 minutes at 4°C. The immunomagnetic rosetted cells were removed from suspension by a magnetic field. Purified cells (92 to 98 % mast cells) were counted in 0.1% toluidine blue. Viability was greater than 90% by trypan blue exclusion.

Chemotaxis: Mast cell migration was evaluated using a modification (16) of a 48-well microchemotaxis assay (17). The chemotactic factors tested were: recombinant human RANTES, recombinant human IL-3, recombinant human SCF, recombinant human TGF-β_1 (all from R&D Systems Europe, Oxon, UK), and human plasma fibronectin (Life Technologies, Renfoe, Scotland). Cell suspensions and chemotactic factors were diluted in GIBCO serum-free medium supplemented with 1% bovine serum albumin (pH 7.4). Chemotactic factors or assay buffer alone (35 µL) were placed in the lower compartment of the 48-well microchemotaxis chamber (Neuroprobe, Cabin John, MD). This was covered with 2 filters: a lower 5-µm cellulose nitrate filter and an upper 8-µm polycarbonate filter. Fifty µL of the cell suspensions (5 x 10^5 mast cells/mL) were placed in the upper compartments. The chemotactic chamber was incubated for 3 hours at 37°C. Then, the lower cellulose nitrate filters were fixed in 2-propanol, stained in acid hematoxylin, cleared and mounted on microscope slides. Chemotaxis was quantitated by counting the number of mast cells that had traversed the upper polycarbonate filter and were attached to the surface of the lower cellulose nitrate filter. Five fields were evaluated on each of duplicate filters at x400 magnification (HPF), and data from each experiment were expressed as the mean number of cells per HPF.

Neutralizing experiments: A neutralizing polyclonal antibody against RANTES and preimmune serum were obtained from R&D Systems Europe.

Results and Discussion

When exposed to concentrations of RANTES ranging from 10^{-12} to 10^{-7}M, human pulmonary mast cells migrated as reported in

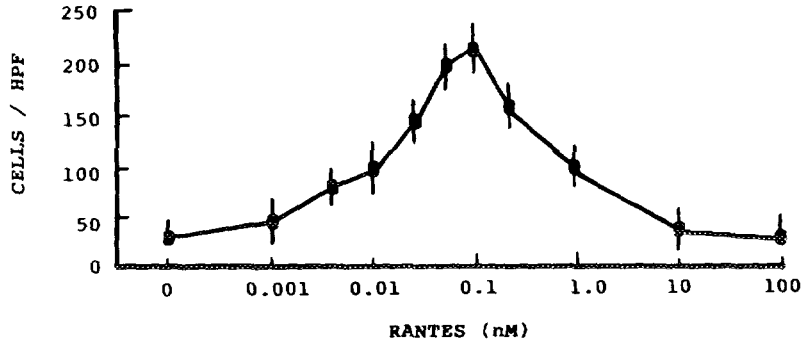

Figure 1. Chemotaxis of human pulmonary mast cells to RANTES. The assay was carried out with the indicated concentrations of RANTES or the assay buffer alone. Data of the concentration-response curve are presented as the mean ± SEM (n=15) of migrated cells per HPF (x400).

Figure 1. Most of the chemotactic activity was observed with concentrations from 10^{-11} to 10^{-9}M, and the optimal chemotactic concentration was approximately 0.1 nM. Based on previous reports on the eosinophil chemotactic activity of RANTES (13,14), this indicates that RANTES is 10- to 100-fold more potent as mast cell chemoattractant. Comparison with the lymphocyte chemotactic activity observed by others (12) is not possible on a molar basis.

The mast cell migratory response to RANTES was due to directional rather than random cell movement, because it required a positive gradient of the chemoattractant (16,17) (Figure 2).

We also determined the specificity of the chemotactic response. RANTES was therefore preincubated with saturating concentrations of a polyclonal antibody to human RANTES, or with preimmune serum as control, and retested for chemotactic activity. As shown in Figure 3, mast cell chemotaxis to the optimal concentration of RANTES was almost completely inhibited by the specific antibody, whereas preimmune serum had no effect, indicating that the response was solely due to RANTES and not to any contaminating chemotactic substance in RANTES preparation.

Finally, we compared the activity of RANTES to that of the other previously reported mast cell chemoattractants (4-8), and migration assay was repeated using predetermined concentrations of RANTES, fibronectin, SCF and TGF-β_1 that caused equivalent mast cell movement. As demonstrated in Figure 4, RANTES was 2,000-fold more potent than fibronectin and 5-fold more potent

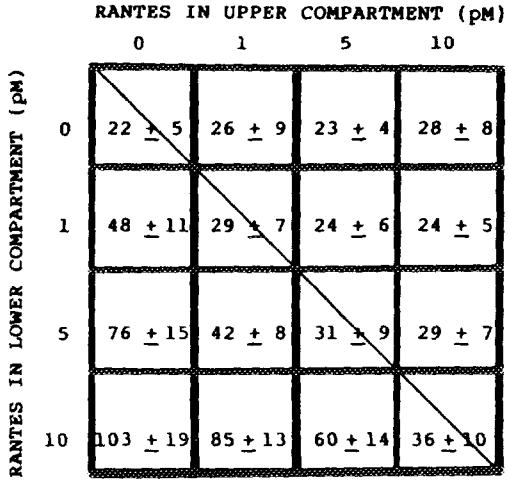

Figure 2. Checkerboard analysis of mast cell movement, as determined in a 48-well microchemotaxis assay by varying the concentrations of RANTES added simultaneously to the upper and lower compartments. The numbers represent mean ± SEM (n=6) of migrated mast cells per HPF (x400). Squares along the diagonal represent equal concentrations of RANTES above and below the filters and measure random (chemokinetic) motion. Squares below the diagonal represent a positive gradient of RANTES and measure directed migration (chemotaxis) of the cells. Squares above this line represent reversed gradients of the factor.

than SCF, on a molar basis. However, it was 200-fold less active than TGF-β_1, which therefore represents the most potent mast cell chemoattractant so far tested.

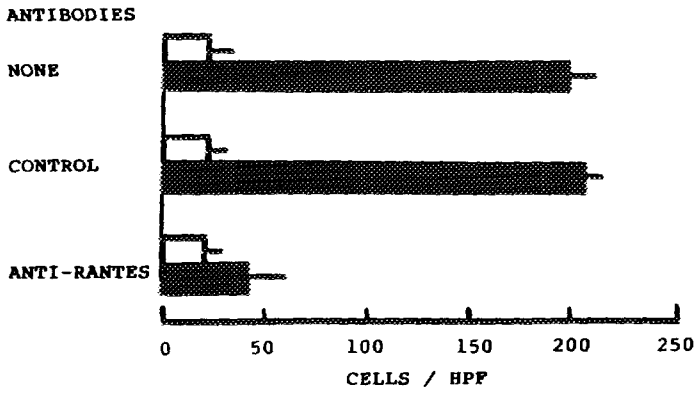

Figure 3. Neutralizing effect of anti-RANTES on mast cell chemotaxis. Samples containing either 0.1 nM RANTES (closed squares) or buffer alone (open squares) were pretreated with a polyclonal anti-human RANTES (25 µg per mL) or preimmune serum for 1 hour at 37°C and assayed for mast cell chemotactic activity. Numbers represent mean ± SEM (n=15) of migrated cells per HPF (x400).

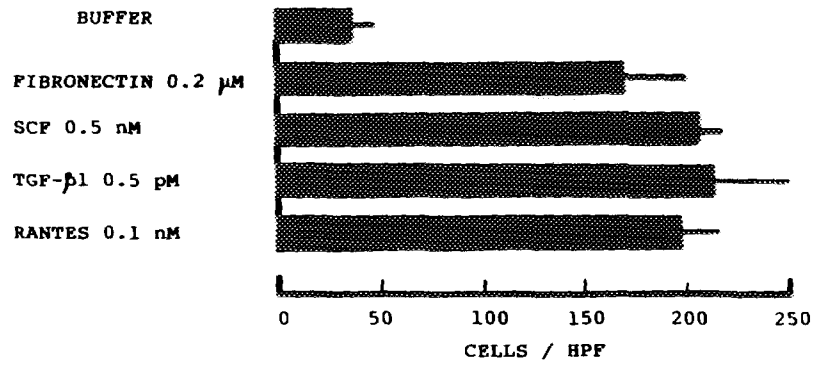

Figure 4. Comparison of the chemotactic activity of recombinant human RANTES and other mast cell chemoattractants. Mast cell migration was tested at the indicated concentrations of chemoattractants or with the assay buffer alone. Data are presented as mean ± SEM (n=6) of migrated cells per HPF (x400).

We also assayed two additional factors which have been previously shown to exert mast cell chemotactic activity (4,7, 8): recombinant human IL-3 (10^{-12} to 10^{-6}M) and laminin (10^{-9} to 10^{-5}M). None of them induced migration of human pulmonary mast cells, in keeping with the evidence that pulmonary mast cells express receptors for fibronectin but not for laminin (18) and that human mast cells do not possess IL-3 receptors (19,20).

It is worth noting that among the mast cell chemoattractants tested here only RANTES is also known to exert chemotactic activity for both eosinophils and memory T-lymphocytes.

Intraepithelial accumulation of mast cells, along with the mucosal infiltration of eosinophils and memory CD4 T-cells, characterizes the inflammatory process in allergic diseases (1-3, 21,22). This process seems to be at least in part orchestrated by some product of activated T-lymphocytes (3,22). The cell source and the unique target cell specificity of RANTES, together with the recent evidence of increased release of this cytokine in allergic patients (23), make it candidate for being that T-cell derived product.

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