RANTES and Macrophage Inflammatory Protein 1α Induce the Migration and Activation of Normal Human Eosinophil Granulocytes

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Summary

The cellular infiltrates of certain inflammatory processes found in parasitic infection or in allergic diseases consist predominantly of eosinophilic granulocytes, often in association with activated T cells. This suggests the existence of chemotactic agonists specific for eosinophils and lymphocyte subsets devoid of neutrophil-activating properties. We therefore examined four members of the intercrine/chemokine superfamily of cytokines (monocyte chemotactic peptide 1 [MCP-1], RANTES, macrophage inflammatory protein 1α [MIP-1α], and MIP-1β), which do not activate neutrophils, for their ability to affect different eosinophil effector functions. RANTES strongly attracted normal human eosinophils by a chemotactic rather than a chemokinetic mechanism with a similar efficacy as the most potent chemotactic myeloid cell agonist, C5a. MIP-1α also induced eosinophil migration, however, with lower efficacy. RANTES and MIP-1α induced eosinophil cationic protein release in cytochalasin B–treated eosinophils, but did not promote leukotriene C_4 formation by eosinophils, even after preincubation with interleukin 3 (IL-3), in contrast to other chemotactic agonists such as C5a and formyl-methionyl-leucyl-phenylalanine (FMLP). RANTES, but not MIP-1α, induced a biphasic chemiluminescence response, however, of lower magnitude than C5a. RANTES and MIP-1α both promoted identical transient changes in intracellular free calcium concentration ($[Ca^{2+}]_i$), with kinetics similar to those induced by chemotactic peptides known to interact with G protein–coupled receptors. No cross-desensitization towards other peptide agonists (e.g., C5a, IL-8, FMLP) was observed, suggesting the presence of specific receptors. Despite its weaker eosinophil-activating properties, MIP-1α was at least 10 times more potent on a molar basis than RANTES at inducing $[Ca^{2+}]_i$ changes. Interestingly, RANTES deactivated the MIP-1α-induced $[Ca^{2+}]_i$ changes, while the RANTES response was preserved after MIP-1α stimulation. MCP-1, a potent monocyte chemoattractant and basophil agonist, as well as MIP-1β, a peptide with pronounced homology to MIP-1α, did not activate the eosinophil functions tested. Our results indicate that RANTES and MIP-1α are crucial mediators of inflammatory processes in which eosinophils predominate.

Eosinophil granulocytes (eosinophils), similar to other leukocytes, can leave the circulation and accumulate at inflammatory sites. Chemoattractants produced at the site of inflammation have been implicated in the induction of leukocyte migration into the inflamed tissue and the subsequent activation of these effector cells. However, the cellular composition of the leukocyte infiltrate (monocytes, lymphocytes, basophils, eosinophils, and neutrophils) is distinct in inflammatory reactions of different etiologies, suggesting the existence of chemoattractants specific for either one or only a few leukocyte types. It is still unclear how such a "specificity" of a certain type of an inflammatory response is determined, since the chemotactic factors discovered some time ago, such as C5a, FMLP, and platelet-activating factor (PAF)[1], attract and activate all myeloid cell types. For example, no specific eosinophil chemotactic agonist devoid of neutrophil-activating properties has yet been identified, with the exception of CD4 binding proteins recently reported to attract eosinophils (1). Nevertheless, even in the absence of target cell–specific

[1] *Abbreviations used in this paper:* $[Ca^{2+}]_i$, intracellular free calcium concentration; ECP, eosinophil cationic protein; LTC_4, leukotriene C_4; MCP, monocyte chemotactic peptide; MIP, macrophage inflammatory protein; NAP, neutrophil-activating peptide; PAF, platelet-activating factor; RANTES, regulated upon activation in normal T cells expressed and secreted.

chemotaxins, the effector functions of a given leukocyte type may also be controlled by the presence of "modulatory" cytokines, particularly hematopoietic growth factors. For instance, IL-3 and IL-5 enhance the different cellular responses of eosinophils and basophils, but not neutrophils, towards diverse chemotactic agonists (2–6).

The discovery of a large group of homologous cytokines that belong to the platelet factor 4/intercrine/chemokine superfamily has led to the identification of chemotactic peptides with a rather restricted target cell specificity (reviewed in references 7–10). Most members of the C-X-C branch (according to the position of the first two cysteines in the conserved motif), such as IL-8/neutrophil-activating peptide 1 (NAP-1), NAP-2, gro/macrophage inflammatory protein 2 (MIP-2) peptides, and ENA-78, are relatively specific neutrophil attractants (7, 8, 11–14), with the exception of IL-8, which may also have some lymphocyte chemotactic activity (15) and weakly attracts and/or activates IL-3- or IL-5-primed eosinophils and basophils (3, 6, 16). By contrast, the members of the human C-C branch of chemokines, such as RANTES, MIP-1α, MIP-1β, and monocyte chemotactic peptide 1 (MCP-1), do not seem to activate neutrophils but rather are chemotactic for different mononuclear cell types. MCP-1 is a potent monocyte chemoattractant (8–10, 17), while others, such as RANTES and MIP-1α, preferentially induce the migration of lymphocyte subsets (10, 18).

Helminthic infections and allergic as well as certain autoimmune diseases are associated with an eosinophilic infiltration of the affected tissue. Furthermore, eosinophils and lymphocytes tend to appear in the same type of inflammatory lesions in the absence of a marked neutrophilic infiltration (19, 20), suggesting the existence of (a) common agonist(s) for lymphocytes and eosinophils devoid of neutrophil-activating properties. The recent discovery of potent basophil-activating properties of MCP-1 (21, 22) further suggests that some family members of the C-C branch of chemokines may activate distinct granulocytic effector cell types such as eosinophils. For these reasons, we investigated the effect of MCP-1, MIP-1α, MIP-1β, and RANTES on different effector functions of normal human eosinophils purified to homogeneity.

Materials and Methods

Peptides. Natural human C5a was purified to homogeneity from yeast-activated human serum (5). Recombinant human (rhu)MCP-1 was obtained from Prepro Tech. Inc. (Rocky Hill, NJ).

Recombinant RANTES, huMIP-1α, and huMIP-1β were produced in *Escherichia coli* by linking cDNAs encoding the mature, secreted forms of the molecules (devoid of the mammalian signal sequence) to the bacterial STII promoter in an expression plasmid.

Recombinant *E. coli* expressing human RANTES were harvested by centrifugation, the extracellular medium was discarded, and the cell pellet was stored frozen at −70°C. Cell pastes were thawed and dispersed in 50 mM glycine, 250 mM NaCl, pH 3.0, using an Ultra Turrax homgenizer (Tekmar Corp., Cincinnati, OH). The cells were then mechanically disrupted in a homogenizer (1104; Microfluidics, Newton, MA) operating at 23,000 psi cooled to room temperature. Cell debris were removed by centrifugation, the supernatant adjusted to pH 6.0, and loaded onto an S-Sepharose fast flow column (Pharmacia, Uppsala, Sweden) equilibrated in 20 mM sodium citrate, pH 6.0. The bound material was eluted with a linear gradient of 0–1.0 M NaCl. Reverse-phase (RP)-HPLC analysis of eluted fractions (using an 8-μm, 4,000-Å resin [Polymer Labs, Amherst, MA] and a linear gradient from 10 to 60% acetonitrile in 0.1% TFA, at 50°C) showed that the peak RANTES levels occurred at ~0.7 M NaCl. The S-Sepharose pool was conditioned by addition of solid ammonium sulfate to 1.8 M, and loaded onto a Phenyl Toyopearl column (Tosa-Haas, Inc., Philadelphia, PA) equilibrated in 100 mM sodium phosphate, pH 6.0. Bound protein was eluted with a linear gradient (1.5–1.0 M) ammonium sulfate in 100 mM sodium phosphate, pH 6.0. RANTES-containing fractions were selected for pooling by RP-HPLC, and ammonium sulfate was removed by diafiltration versus 10 mM sodium citrate, 450 mM NaCl, pH 5.0, across a 3-kD cutoff membrane. huMIP-1α was purified from *E. coli* fermentation medium after first removing the cells by low-speed centrifugation. The pH of the supernatant was then adjusted to ~3 with phosphoric acid, and the resulting precipitate was removed by a second low-speed spin. After adjustment to pH 5, the huMIP-1α contained in the clear supernatant was captured on a bare silica column and eluted with 15% ethanol/1 M NaCl. The silica pool was diafiltered into 10 mM Tris buffer at pH 7 and loaded onto a DEAE-Sepharose column, from which huMIP-1α was eluted with 0.3 M NaCl in Tris buffer. The product was further purified by C4 RP-HPLC (Waters radial compression cartridge) using a linear gradient of acetonitrile. Solvent was removed by capture and elution from DEAE-Sepharose as described above, and the purified product was formulated for administration by diafiltration into an isotonic citrate/sodium chloride buffer.

huMIP-1β was purified from recombinant *E. coli* by dispersing cell paste in phosphate buffer at neutral pH, followed by homogenization at ~20,000 psi. The homogenate was adjusted to pH 3 and centrifuged briefly to pellet cell debris. The supernatant was decanted and adjusted to pH 6 with sodium hydroxide, initiating the precipitation of huMIP-1β. After centrifugation, the supernatant was discarded and the pellet dissolved with ~1 M NaCl/20 mM acetic acid, pH 3. This solution was then applied to a column of phenyl Toyopearl equilibrated in the same buffer. After analysis by RP-HPLC, selected fractions of the column flow-through were pooled and diafiltered across a 5-kD cellulose membrane versus 20 mM acetic acid, pH 3.2.

Purity as judged by HPLC was >99% for all peptides and the pyrogen content was <1 endotoxin unit (EU)/mg for RANTES and MIP-1α and <5 EU/mg for MIP-1β as determined by limulus amoebocyte lysate test. All peptides were stored in Hepes buffer containing 1 mg/ml BSA in small aliquots at −70°C and added to the cells at 1:1,000 to 1:100 (vol/vol) ratio.

Purification of Eosinophils. Eosinophils were purified from dextran-sedimented leukocytes (6% dextran T70 [Pharmacia] in 0.9% NaCl) of EDTA anticoagulated blood (10 mM EDTA; Fluka AG, Buchs, Switzerland) of normal human volunteers exactly as described (23) using a combination of discontinuous Percoll gradient centrifugation and negative selection with anti-CD16-coated immunomagnetic microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany), except that the Percoll density used was higher (1.090g/ml) in order to further enrich the eosinophils and to save anti-CD16 beads. The resulting eosinophil purity was >99.5% as determined by microscopic examination of Giemsa-stained cytospin preparations. All rare contaminating cells were neutrophils. After isolation, cells were resuspended in HACM buffer (20 mM Hepes; Calbiochem-Behring Corp., La Jolla, CA; 125 mM NaCl, 5 mM KCl, 0.5 mM glucose, 0.025% BSA, fatty acid free, low endo-

toxin; Boehringer Mannheim GmbH, Mannheim, Germany; 1 mM $CaCl_2$ and 1 mM $MgCl_2$) in all experiments at the concentrations indicated, except in chemotaxis assays, for which a HBSS containing Ca^{2+} and Mg^{2+} supplemented with 0.05% BSA was used.

Respiratory Burst of Normal Human Eosinophils. Oxygen radical production of eosinophils was assessed by measuring the H_2O_2/peroxidase-dependent chemiluminescence of luminol exactly as described (24) using 5×10^5 pure eosinophils in 500 μl HACM buffer containing 10 U/ml horseradish peroxidase and 10 μM luminol (both from Sigma Chemical Co., St. Louis, MO), with the exception that NaN_3 (for inhibition of cell-derived myeloperoxidase) was omitted since no granule release occurred under the experimental conditions. Chemiluminescence was continuously monitored in a six-channel chemiluminometer (Biolumat LB505; Berthold Laboratory, Berlin, Germany) at time intervals of 10 s.

Eosinophil Cationic Protein (ECP) Release and Leukotriene C_4 (LTC_4) Generation. Cytochalasin B (5 min at 5 μg/ml after a warm-up period of 10 min at 37°C; Sigma Chemical Co.)-treated eosinophils (3×10^5 cells/ml) in HACM buffer were exposed to the peptides for 20 min. Then, the cells were placed on ice, and ECP was released into the supernatants obtained after centrifugation (10 min at 4°C and 500 *g*) was measured by RIA (Pharmacia). Total ECP content was determined from cell lysates obtained after three freeze/thaw cycles. Leukotriene generation by eosinophils pretreated with and without IL-3 (30 ng/ml) was determined as described previously (4, 25).

Changes of Intracellular Calcium Concentration ($[Ca^{2+}]_i$). Purified eosinophils were loaded with 0.3 nmol fura-2/AM (Fluka AG, Buchs, Switzerland) per 10^6 cells in HACM buffer for 30 min at 37°C. After a short centrifugation (5 min, 150 *g*, room temperature) the freshly loaded cells were resuspended in prewarmed HACM buffer at a concentration of $0.5–2 \times 10^6$ cells/ml. Fura-2 fluorescence changes (excitation wavelength, 340 nm; emission wavelength, >490 nm) of the cell suspensions in response to cell agonists were continuously monitored at 0.25-s intervals and analyzed as described (14, 22). Each measurement was standardized by adding ionomycin (5 μM final concentration; Sigma Chemical Co.) leading to 100% fura-2 saturation and subsequent quenching of the fluorescence with $MnCl_2$ (1 mM final concentration).

Measurement of In Vitro Eosinophil Chemotaxis. Eosinophil chemotaxis assays were performed as described (26). Briefly, eosinophils (10^6/ml) were placed in the top wells of a 48-well chemotactic chamber (Neuroprobe, Cabin John, MD), which were separated from the bottom wells containing buffer or peptides at the concentrations indicated by a polycarbonate filter with 5-μm pores (Nucleopore, Pleasanton, CA). For testing chemokinetic responses of eosinophils to RANTES, the cytokine was added to the bottom, the top, or on both sides of the filter. The chambers were disassembled after a 1-h incubation at 37°C, in 5% CO_2, a time point previously established to be optimal for eosinophil migration (26). The eosinophils that migrated across the filter and adhered to the bottom side of the filter were stained with Giemsa (Merck, Darmstadt, Germany) and counted in a randomly chosen area of 1 mm^2 corresponding to each well by an Optomax V image analyzer (AiTektron, Meerbusch, Germany). The number of eosinophils obtained was multiplied by eight to get the total number of migrated eosinophils for each well (well diameter, 3.2 mm). The number of migrated nonadherent (drop-off) eosinophils was determined in two experiments. For each concentration, the number of drop-off cells was found to be proportionate to the number of eosinophils adherent to the filter and to constitute <20% of the total migrated cell number. Since the adherent eosinophils were representative of the total migrated eosinophil population, all of the results from the chemotaxis experiments were expressed as the mean number of migrated adherent eosinophils (in percent of the total input). For each tested attractant, the mean chemotactic efficacy (number of eosinophils migrated at the optimal chemotactic concentration) and mean migration index (number of cells that migrated at the optimal chemotactic concentration divided by the number of cells that migrated to buffer control) were determined. All experiments were performed in duplicates or triplicates and repeated at least five times with eosinophil preparations from different donors. Statistical analysis was performed using the student's *t* test.

Results

Chemotactic Activity of RANTES and Related Peptides. Among the β-intercrines/chemokines tested, RANTES and, to a much lesser extent, MIP-1α induced the migration of eosinophils above the level of random migration in a concentration-dependent manner (Fig. 1). In five experiments with different eosinophil preparations, RANTES had a mean chemotactic efficacy of 40.9 ± 6% (SEM) and a mean migration index of 5.7 ± 0.7 (SEM) at 10^{-7} M, whereas MIP-1α, at its optimal 10^{-8}-M concentration, attracted fewer eosinophils (mean efficacy, 15.5 ± 1.9%; mean migration index, 2.4 ± 0.3; $p < 0.05$ versus buffer control). C5a, a potent chemotactic agonist for all myeloid cells, including eosinophils, was used as a positive control and attracted at its optimal 10^{-8}-M concentration 43.7 ± 6.7% (SEM) eosinophils (mean migration index, 6.1 ± 0.5). Eosinophil migration in response to MCP-1 and MIP-1β did not significantly differ

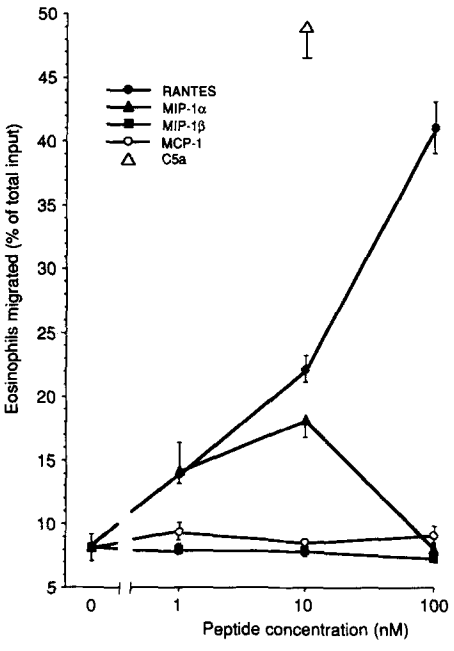

Figure 1. Migration of pure human eosinophils in response to chemokines. Buffer controls (O) or the peptides at the concentrations indicated (*abscissa*) were placed in the bottom wells of the chemotaxis chamber. C5a (10 nM) was included for comparison. Migration (*ordinate*) is expressed as the percentage of the total number of cells added to the chamber. Mean values ± SD of one experiment performed in triplicates out of five are shown.

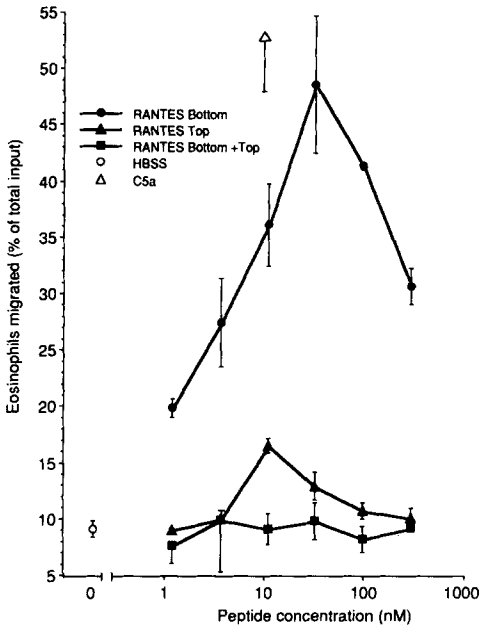

Figure 2. Chemotaxis vs. chemokinesis of eosinophils in response to RANTES. RANTES was placed in the bottom (*circles*), in the top (*triangles*), or on both sides (*squares*) of the filters at the concentrations indicated. Otherwise, experimental conditions and presentation of the data are as in Fig. 1.

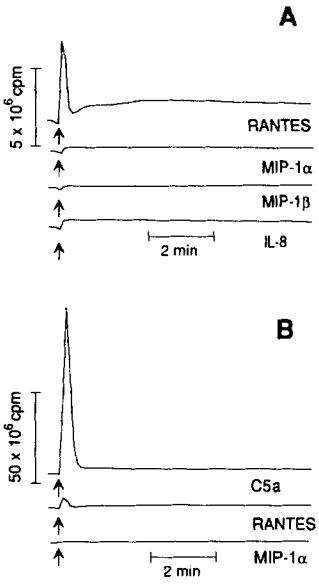

Figure 3. Oxygen radical–induced chemiluminescence in pure normal eosinophils in response to peptide agonists. 0.5×10^6 cells in 500-μl aliquots were warmed up for 10 min at 37°C before adding the peptides indicated at 10^{-7} M. Oxygen radical production was assessed by the H_2O_2/peroxidase–induced chemiluminescence of luminol as described in Materials and Methods. Representative recordings of chemiluminescence (in cpm) over time (10-s intervals) are shown. Arrows indicate the time of peptide addition. Identical kinetics were obtained in six different experiments. Chemiluminescence of resting eosinophils was $234 \pm 61 \times 10^3$ cpm. Peak chemiluminescence induced by RANTES was $5{,}083 \pm 863 \times 10^3$ cpm and C5a $88{,}450 \pm 2{,}192 \times 10^3$ cpm (mean ± SD) in six experiments. (*A*) Chemiluminescence induced by chemokines. The recordings after MCP-1 addition were identical to that of MIP-1β (not shown). (*B*) Comparison to the response induced by C5a (100 nM). Note the difference in scale between *A* and *B*.

from that of the buffer control (mean migration indexes of 1.4 ± 0.2 and 1.2 ± 0.2, respectively). Fig. 2 demonstrates that the eosinophil migration induced by RANTES was due to a chemotactic rather than a chemokinetic mechanism since the response was negligible when the peptide was placed either in the top well only or on both sides of the filter.

Oxygen Radical–induced Chemiluminescence Response of Human Eosinophils. RANTES, in contrast to MIP-1α, MIP-1β, and MCP-1, induced a biphasic chemiluminescence response in human eosinophils at 10^{-7} M (Fig. 3 *A*). The IL-8 response was negligible, in agreement with previous studies using eosinophils form hypereosinophilic donors (27). However, when compared with the response to C5a, which is the most potent chemotactic agonist for the induction of the respiratory burst by eosinophils (27), RANTES induced the production of clearly smaller amounts of oxygen radicals (Fig. 3 *B*).

Eosinophil Degranulation and LTC_4 Generation. A number of previous studies have shown that the chemotactic factor agonists examined so far promote granule release by neutrophils and eosinophils (12, 14, 27), provided that the cells have been pretreated with cytochalasin B. Consistent with their chemotactic activity on eosinophils, RANTES as well as MIP-1α induced the release of ECP at 10^{-8}–10^{-7} M concentrations, in contrast to MIP-1β, MCP-1, and IL-8, which were ineffective (Fig. 4). At 10^{-7} M, RANTES promoted a more pronounced degranulation response than MIP-1α. However, maximal effects induced by MIP-1α were reached at lower ($\geq 10^{-8}$ M) concentrations. When compared with C5a and FMLP (27), RANTES and MIP-1α induced the release of considerably less ECP (Fig. 4).

Our previous studies showed that the chemotactic agonists FMLP and C5a induce the generation of LTC4 by IL-3-pretreated eosinophils. FMLP, but not C5a, also leads to LTC_4 synthesis by freshly isolated normal human eosinophils, albeit in smaller amounts than by IL-3-primed eosinophils (4). By contrast, neither RANTES nor MIP-1α were capable of promoting the generation of detectable amounts of LTC_4 even by eosinophils preincubated for 90 min with 30 ng/ml IL-3 (<30 pg $LTC_4/10^6$ eosinophils formed in response to RANTES or MIP-1α at 10^{-7} M with or without preincubation with 30 ng/ml IL-3 during 90 min in experiments performed with cells from five different donors in which C5a and FMLP were included as positive controls).

Changes of $[Ca^{2+}]_i$. Chemotactic myeloid cell agonists are known to rapidly induce a transient change in $[Ca^{2+}]_i$ as an early event of receptor-mediated signal transduction (7, 11, 12, 14, 22, 27). Similarly to other chemotactic peptide

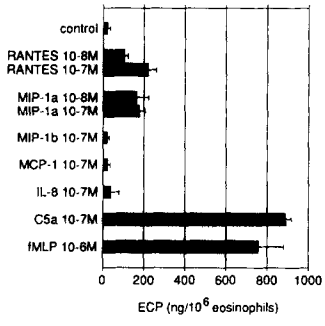

Figure 4. ECP release by human eosinophils. Pure eosinophils (300,000 cells/ml) were treated with cytochalasin B (5 μg/ml) for 5 min before adding the peptides at the concentrations indicated for 20 min. ECP released into the supernatant (mean ± SD of three experiments) is shown. ECP release induced by C5a corresponds to 65% of the total cellular ECP content.

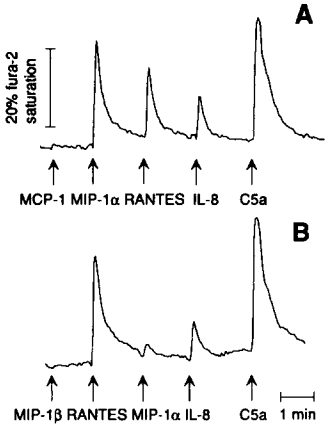

Figure 5. Changes of $[Ca^{2+}]_i$ in eosinophils induced by peptide agonists. Fura-2-loaded cells were suspended at 2 × 10^6/ml and continuously monitored for fluorescence changes. Peptide agonists were added at the time points indicated by arrows in 1:200 dilutions (vol/vol), resulting in final concentrations of 50 nM. $[Ca^{2+}]_i$ changes in response to the sequential addition of MCP-1, MIP-1α, RANTES, IL-8, and C5a (*A*), and of MIP-1β, RANTES, MIP-1α, IL-8, and C5a (*B*). Identical results were obtained with cells of five different blood donors, with the exception that the response to IL-8 was in some experiments even smaller than shown here. The order of addition of C5a, IL-8, and RANTES or MIP-1a did not alter the $[Ca^{2+}]_i$ changes induced by each agonist (not shown).

agonists such as C5a, RANTES and MIP-1α induced rapid and transient $[Ca^{2+}]_i$ changes. MCP-1 and MIP-1β, however, were ineffective (Fig. 5). Despite their marked difference in chemotactic efficacy and despite the inability of MIP-1α to induce a respiratory burst, as opposed to RANTES, the extent and kinetics of the $[Ca^{2+}]_i$ changes were virtually indistinguishable at 100 and 50 nM for both peptides (Figs. 5 and 6). Dose-response studies even revealed that MIP-1α was at least 10 times more effective on a molar basis as compared with RANTES (ED_{50} for maximal $[Ca^{2+}]_i$ increase for RANTES at 3–10 nM, ED_{50} for MIP-1α at 0.3–1 nM) for inducing transient $[Ca^{2+}]_i$ changes (Fig. 6).

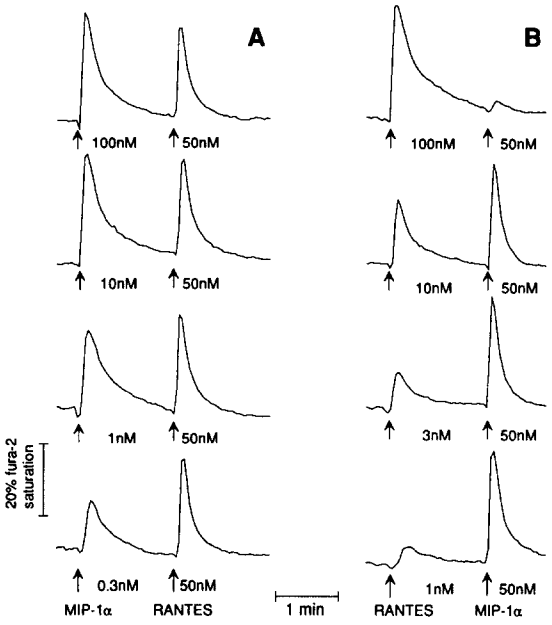

Figure 6. Cross-densensitization of $[Ca^{2+}]_i$ changes induced by RANTES and MIP-1α in eosinophils. Experimental conditions are as in Fig. 5. (*A*) Eosinophils were exposed to decreasing concentrations (100, 10, 1, 0.3 nM, respectively) of MIP-1α followed by a constant quantity of RANTES (50 nM final concentration) 90 s later. (*B*) RANTES (100, 10, 3, 1 nM, respectively) was followed by MIP-1α addition (50 nM).

No information is yet available on the expression and specificity of receptors for MIP-1α and RANTES on human leukocytes. When cells have been exposed to a certain cell agonist capable of inducing $[Ca^{2+}]_i$ responses at sufficiently high concentrations, a second $[Ca^{2+}]_i$ change is abolished if the same peptide or a different agonist interacting with a shared receptor is used as a second trigger (11, 12, 14, 22, 28). This phenomenon has been referred to as receptor-specific cell desensitization. By contrast, we found negligible mutual influence of sequential $[Ca^{2+}]_i$ responses for a variety of diverse agonists interacting with separate receptors in different effector cell types. (22, our unpublished observations). Therefore, to examine whether RANTES and MIP-1α activate eosinophils through specific receptors, $[Ca^{2+}]_i$ changes were monitored in eosinophils sequentially exposed to different agonists in various combinations. Exposure of eosinophils to MIP-1α and/or RANTES affected neither the $[Ca^{2+}]_i$ response to IL-8 nor C5a (Fig. 5) and vice versa (data not shown). Fig. 5 also demonstrates the poor effectiveness of IL-8 for inducing $[Ca^{2+}]_i$ changes, consistent with its predominant neutrophil-activating properties and previous studies performed with hypereosinophilic cells (3, 27). Furthermore, exposure of eosinophils to RANTES or MIP-1α did not affect the $[Ca^{2+}]_i$ changes induced by two other unrelated chemotactic agonists, FMLP and PAF (data not shown). Sequential addition of the two members of the C-C subfamily of chemokines, RANTES and MIP-1α, however, resulted in alterations of the second signal (Figs. 5 and 6). Most surprising was the observation that RANTES at 50 and 100 nM almost totally abolished the $[Ca^{2+}]_i$ changes induced by MIP-1α despite its lower potency in promoting $[Ca^{2+}]_i$ changes by itself (as compared with MIP-1α), while preincubation with MIP-1α even at 100 nM hardly affected the eosinophil response to RANTES (Figs. 5 and 6). Fig. 6 also shows the concentration dependency of these deactivation phenomena.

Discussion

The members of the intercrine/chemokine family of peptides are small molecular mass cationic cytokines (7–10 kD) whose major biological function lies in their chemotactic activity towards different leukocyte effector cells (7–10). For the members of the C-X-C or IL-8 subfamily, much progress has been made regarding their biological functions and characterization of specific receptors, in contrast to the members of the C-C subfamily for which information is still scarce (10).

So far, the best examined cytokine of the C-C family is MCP-1, a potent attractant and activator of human monocytes (8–10, 17). RANTES has recently been shown to be a chemotactic factor of lymphocytes of Th memory subsets (18). This study demonstrates that RANTES, and more weakly MIP-1α, in contrast to MCP-1 and MIP-1β, are also chemotactic for eosinophils. RANTES was found to be a particularly efficacious chemoattractant for eosinophils, attracting almost as many eosinophils at optimal concentrations as the most potent known chemotaxin, C5a. In comparison with

C5a or FMPL (4, 27), however, RANTES was a weaker (oxygen radical production, ECP release) or an ineffective (LTC_4 synthesis) activator of human eosinophil effector function. None of the C-C members examined (RANTES, MIP-1α, MCP-1, MIP-1β) were, however, capable of activating neutrophils, at least at concentrations up to 100 nM (10, 22, our unpublished observations). Thus, RANTES and MIP-1α appear to be the first recognized peptide agonists chemotactic for eosinophils but not for neutrophils.

Calcium measurements indicated that RANTES and MIP-1α activate human eosinophils through specific receptors. The kinetics of $[Ca^{2+}]_i$ changes in response to RANTES and MIP-1α in eosinophils are similar to those induced by other humoral and cell-derived chemotactic agonists, such as C5a and IL-8 in neutrophils, suggesting that these cytokines interact with G protein–coupled receptors. Although cross-desensitization experiments are only a preliminary and indirect way of demonstrating the specificity of novel receptors, previous studies in other myeloid cell types with all agonists performed so far (C5a, C3a, FMLP, PAF, IL-8, NAP-2) have revealed an excellent predictive value of this experimental approach (11, 12, 28). In fact, in all myeloid cells, sequential challenge with 100 nM of any of these agonists results in complete desensitization towards a second challenge with the same agonist (our unpublished observations). Thus, the inability of MIP-1α and RANTES to affect $[Ca^{2+}]_i$ change towards other peptide agonists indicates that eosinophils express novel yet to be defined receptors specific for these cytokines. The mutual influence of RANTES and MIP-1α on each other's $[Ca^{2+}]_i$ response is more difficult to interpret. The data shown in Fig. 6 would be consistent with the existence of two types of receptors recognizing RANTES and MIP-1α, respectively, one being specific for RANTES and the other recognizing MIP-1α with high and RANTES with lower affinity. This interpretation is also consistent with the presented data on eosinophil function: RANTES, as opposed to MIP-1α, has the capacity to activate the respiratory burst of eosinophils and has a higher efficacy in promoting eosinophil chemotaxis and degranulation at maximally effective concentrations. MIP-1α, however, on a molar basis is considerably more potent than RANTES in inducing $[Ca^{2+}]_i$ changes and ECP release. The capacity of MIP-1α to induce $[Ca^{2+}]_i$ changes at low concentrations together with its relatively poor eosinophil activating properties may indicate that MIP-1α could be a specific agonist for another, yet to be determined, cellular response in eosinophils, such as gene expression or cell survival.

MCP-1 was unable to activate eosinophils in all the cell functions tested and did not induce $[Ca^{2+}]_i$ changes, suggesting that eosinophils do not express MCP-1 receptors and that MCP-1 cannot interact with MIP-1α or RANTES receptors, respectively, on eosinophils. Apart from being a monocyte chemoattractant, MCP-1 has recently been shown to represent the most potent cell-derived basophil agonist (21, 22). Our studies performed so far revealed that basophils and eosinophils are closely related effector cell types with regard to the profile of cytokines and cell agonists regulating the function of these myeloid cell types (4–6). Thus, MCP-1 appears to be the first basophil trigger incapable of activating eosinophils. On the other hand, in addition to the effects on eosinophils as reported here, RANTES was also found to be a potent chemotactic factor for basophils without strongly activating other basophil effector function (our unpublished observations). It therefore appears that the main function of RANTES lies in its ability to attract particular sets of leukocytes, namely Th cell subsets, eosinophils, and basophils, without affecting neutrophil functions. Despite the fact that MIP-1β is more homologous to MIP-1α than MIP-1α to RANTES (10), MIP-1β did not activate eosinophils nor induce $[Ca^{2+}]_i$ changes, and did not desensitize the MIP-1α or RANTES response. This observation is particularly surprising if one postulates the existence of one receptor type recognizing both MIP-1α and RANTES.

The effects of RANTES and MIP-1α on eosinophil function may explain, at least in part, why eosinophilic cellular infiltrates largely devoid of neutrophils can be observed in different diseases. Furthermore, eosinophilic inflammation is often associated by concomitant infiltration with activated T helper cells, an association that can be explained by the chemotactic effect of RANTES and/or MIP-1α on these two cell types (29, 30). RANTES and MIP-1α may thus be particularly important mediators of immediate-type hypersensitivity diseases, such as asthma, by promoting the selective attraction and/or activation of eosinophils, basophils, and Th cells that are found to dominate in allergic inflammatory sites.

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