Macrophage Inflammatory Protein-1α Activates Basophils and Mast Cells

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Summary

Macrophage inflammatory protein-1 (MIP) is a recently cloned cytokine that causes neutrophilic infiltration and induces an inflammatory response. We studied the effect of MIP-1α on histamine secretion from basophils and mast cells. Leukocytes from allergic and normal subjects were studied. MIP-1α caused dose-dependent release of histamine from basophils of 14 of 20 allergic donors at concentrations of 10^{-9}–10^{-7} M, and the mean release was 13.50 ± 2.9% at the highest concentration. In the same experiments, the mean histamine release by anti-immunoglobulin E and monocyte chemotactic and activating factor (MCAF) (10^{-7} M) was 32 ± 7% and 31 ± 3%, respectively. The cells from only 2 of 10 normal subjects released histamine in response to MIP-1α. Histamine release by MIP-1α was rapid, and almost complete within the first 3 min. MIP-1α-induced degranulation was a calcium-dependent noncytotoxic process. MIP-1α showed chemotactic activity for purified basophils that was comparable to MCAF. Both MIP-1α and MCAF at 10^{-7} M concentration elicited a chemotactic response that was 40% of the maximal response to C5a (1 μg/ml). Murine MIP-1α induced histamine release from mouse peritoneal mast cells in a dose-dependent manner. Thus, we have established that MIP-1α is a novel activator of basophils and mast cells.

Increased expression of various cytokines has been demonstrated in tissue specimens from late phase allergic reactions (1). Cytokines may play a crucial role in the recruitment and activation of inflammatory cells at the site of allergic reactions. Cytokines upregulate adhesion molecules on endothelium and on inflammatory cells such as eosinophils and basophils (2). The role of eosinophils in allergic asthma has been well established. A growing body of evidence suggests that basophils are activated in the late allergic reaction (3). Cytokines that activate basophils include IL-3, GM-CSF, and connective tissue-activating peptide III (CTAPIII)/neutrophil-activating peptide 2[1] (NAP-2) (4, 5). We (6) and others (7) have recently demonstrated that monocyte chemotactic and activating factor (MCAF)/monocyte chemoattractant protein-1 (MCP-1) is one of the most potent histamine releasing factors (HRF). Histamine releasing activity of MCAF is comparable

with that of anti-IgE and FMLP. Basophils from both allergic and nonallergic subjects respond to MCAF. We have also reported that two other members of the 8-kD family of cytokines, namely IL-8 and RANTES inhibit MCAF-induced histamine release from basophils (8). MIP-1α is another member of the 8-kD family of cytokines (9). In this study we show that MIP-1α induces histamine release from basophils and mast cells, and causes chemotaxis of basophils.

Materials and Methods

Reagents. RPMI 1640 was obtained from Gibco Laboratories (Grand Island, NY); human serum albumin, glutamine, Histopaque-1077, Con A, penicillin, streptomycin, FMLP, and C5a were from Sigma Chemical Co. (St. Louis, MO); Hepes was from Research Organics, Inc. (Cleveland, OH); hydroxyethyl starch (HetaStarch) was from American McGaw (Irvine, CA); and human and murine recombinant MIP-1α was from R & D System, Inc. (Minneapolis, MN). Recombinant cytokines were 99% pure by SDS-PAGE and RP-HPLC and were received as a lyophilized material in PBS. A stock-solution of MIP-1α was made at a concentration of 2 × 10^{-6} M in Hepes buffered saline, pH 7.4, containing 0.3% human serum albumin, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Affinity-purified rabbit anti-human IgE serum (1 mg/ml) was from Behring Diagnostics (Somerville, NY). Magnetic beads coated with mAb against CD2 and CD19 were from Dynal, Inc. (Great Neck, NY).

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[1] *Abbreviations used in this paper:* HA, Hepes buffered saline with albumin; HACM, Hepes buffered saline with albumin, $CaCl_2$, and $MgCl_2$; HCM, Hepes buffered saline with $CaCl_2$ and $MgCl_2$; HRF, histamine releasing factor; MCAF, monocyte chemotactic and activating factor; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; and NAP-2, neutrophil-activating peptide 2.

Isolation of PBLs. Donors for this study were selected from a large group of allergic and nonallergic subjects that were screened in our laboratory for histamine release from basophils in response to HRF and anti-IgE. Allergic status was defined by the presence of clinical symptoms, past allergic history, and positive reaction to prick skin testing with a panel of 32 local aeroallergens.

Venous blood from donors was anticoagulated with 10 mM EDTA and sedimented with 1.5% hydroxyethyl starch for 30 min at room temperature (4). The leukocyte-rich buffy coat was collected and washed three times in HA buffer (Hepes buffered saline, pH 7.4, and 0.03% human serum albumin) in a refrigerated centrifuge (4°C) at 300 g. The washed leukocytes were suspended in HACM buffer (Hepes buffered saline, pH 7.4, 0.03% human serum albumin, 2 mM $CaCl_2$, and 1 mM $MgCl_2$). Leukocytes from 1 ml of blood were usually used for one duplicate experiment.

Purification of Basophils. For some experiments, basophils were purified using a discontinuous Percoll gradient (10). Partially separated basophils were further purified by negative selection of basophils using mAb (against CD2 and CD19)-coated magnetic beads (11). The final purity of basophils ranged from 65 to 80%.

Isolation of Mouse Peritoneal Mast Cells. DBA/2 mice were used to obtain peritoneal mast cells. The peritoneum was lavaged with a total of 6 ml of HA buffer. The cells were washed three times with the same buffer and then suspended in HACM. The viability of the cells was checked with trypan blue and was >98%. The mast cell content of the peritoneal lavage fluid was <5%. Approximately $10-20 \times 10^3$ mast cells/tube were used for histamine release assay.

Histamine Release Assay. Aliquots of 50 μl of various dilutions of MIP-1α, MCAF, or anti-IgE antibody were incubated with 50 μl of leukocyte or mast cell suspension for 45 min in a shaking water bath at 37°C. Each experiment was done in duplicate. 400 μl of HA buffer were added to each tube at the end of the incubation. The supernatants were separated from the cells by centrifugation at 600 g for 5 min at 4°C. The histamine content of the supernatants was measured using an automated fluorometric analyzer (4). Spontaneous histamine release was assessed by incubating the cells in HACM buffer alone. The total histamine content of the cells was measured by lysing the cells with 3% perchloric acid. The percentage of histamine release was calculated according to the formula: 100 × (histamine in the supernatant/total histamine in the cells). Spontaneous histamine release from the cells was usually <5%. The values of spontaneous histamine release were subtracted from the calculated histamine release.

Chemotaxis of Basophils. Basophils of 65-80% purity were isolated as above, and suspended in Gey's solution. Chemotaxis was carried out in Boyden microchambers using 5 μm polycarbonate membranes (Nucleopore Corp., Pleasanton, CA). 29 μl of buffer or C5a (1 μg/ml), or various concentrations of MIP-1α were added to the lower chamber. Approximately, 10^5 basophils were placed on the top of the membrane. The incubation was carried out for 1 h at 37°C in a humidified incubator. The membrane was stained with Wright's stain. Basophils from twenty fields of duplicate experiments were identified and counted at a magnification of 1,000 under immersion oil.

Results were expressed as mean ± SEM. Data were analyzed for statistical significance using appropriate tests as indicated in the text.

Results

Leukocytes from thirty subjects were studied for histamine release by MIP-1α. Twenty subjects were allergic and ten were
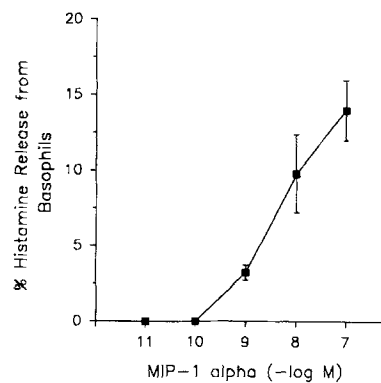

Figure 1. Dose-response curves for MIP-1α-induced histamine release from basophils. Leukocytes from 20 allergic donors were incubated with various concentrations of MIP-1α, and the release of histamine into the supernatant was measured spectrofluorometrically. Results are expressed as percent intracellular release (mean ± SEM) from 14 responders.

healthy subjects. MIP-1α induced histamine release from basophils of 14 of 20 allergic subjects in a dose-dependent manner (Fig. 1). Human recombinant MCAF and anti-IgE were also used in the same experiments. The optimal concentration of anti-IgE for inducing histamine release was 1 μg/ml. The concentration of MCAF was 10^{-7} M. The release of histamine by MIP-1α was compared with MCAF and anti-IgE (Fig. 2). There was no correlation of histamine release between MIP-1α and anti-IgE ($r = -0.07$, $P >0.05$ by Spearman's rank correlation), or between MIP-1α and MCAF ($r = -0.3$, $P >0.05$ by Spearman's rank correlation). Basophils from six allergic subjects did not respond to MIP-1α. Cells from 2 of 10 normal subjects responded to MIP-1α with histamine release (6 and 11% histamine release at 10^{-7} M). In three experiments, basophils from three allergic donors were purified by discontinuous Percoll gradient centrifugation and negative selection using anti-CD2 and -CD19 mAb-coated magnetic beads. The final purity ranged from
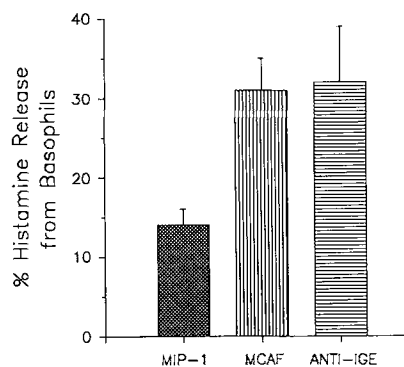

Figure 2. Comparison of histamine release from basophils by MIP-1α, MCAF, and anti-IgE. Leukocytes from allergic donors were incubated with MIP-1α (10^{-7} M), MCAF (10^{-7} M), and anti-IgE (1 μg/ml). Histamine was measured as above and expressed as percent release ($N = 14$ for MIP-1α, and $N = 9$ for both MCAF and anti-IgE).
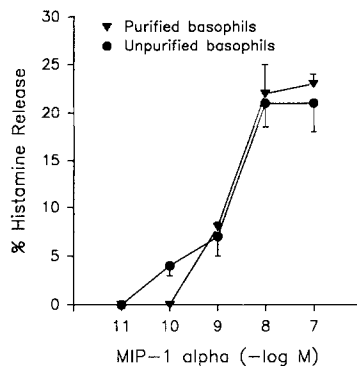
Figure 3. MIP-1α-induced histamine release from partially purified basophils. Basophils from three donors were purified by discontinuous Percoll centrifugation followed by negative selection using anti-CD2 and -CD19 mAb-coated magnetic beads. The final purity ranged from 65 to 80%. The results of one of three similar experiments are shown.

65 to 80%. MIP-1α induced a dose-dependent release of histamine in these experiments (Fig. 3).

The kinetics of histamine release by MIP-1α are shown in Fig. 4. The release was very rapid, and over 80% of the maximal release took place within 30 s. The release was almost complete within the first 3 min. On the other hand, IgE consistently required a lag period of 1–3 min before any noticeable release occurred.

To investigate whether the degranulation of basophils by MIP-1α is a calcium-dependent phenomenon, we conducted the experiments in Hepes-albumin buffer in the presence of 4 mM EDTA containing no calcium or magnesium. In parallel experiments, leukocytes were incubated with MIP-1α in the calcium containing HACM buffer. Anti-IgE was used as a positive control in the experiment. The release of histamine in the absence or presence of calcium and magnesium is shown in Fig. 5. There was no histamine release by MIP-1α in the absence of calcium and magnesium.
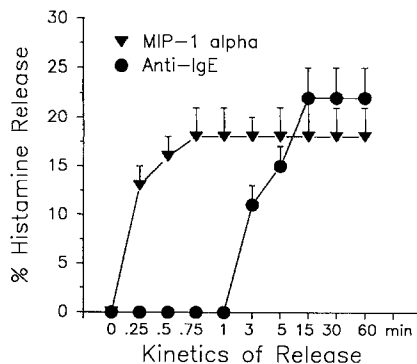

Figure 4. The kinetics of MIP-1α and anti-IgE–induced histamine release from leukocytes. Leukocytes from four different donors were incubated with MIP-1α (10⁻⁷ M) and anti-IgE (10⁻⁴ dilution) for different periods of time as indicated. At the conclusion ice-cold buffer containing 4 mM EDTA was added, and the supernatant was separated by centrifugation at 4°C. Results of four duplicate experiments are shown.
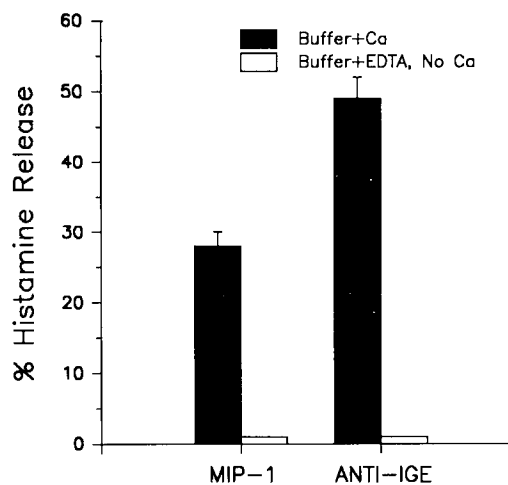

Figure 5. The effect of calcium depletion on MIP-1α–induced histamine release from basophils. Leukocytes were washed in calcium- and magnesium-free buffer containing additionally 4 mM EDTA. The washed leukocytes were then incubated with MIP-1α (10⁻⁷ M) or anti-IgE (10⁻³ dilution) in the same calcium- and magnesium-free EDTA containing buffer as indicated for histamine release. In parallel experiments, leukocytes washed in HA buffer were incubated with the agonists in HACM buffer. Results of four duplicate experiments are shown.

Purified basophils were used to study the effect of MIP-1α on chemotaxis. In the same experiments we investigated the chemotactic activity of MCAF. Both MIP-1α and MCAF induced basophil chemotaxis in a dose-dependent manner. The range of concentration was similar to that required for histamine release (Fig. 6). Both MIP-1α and MCAF elicited ~40% of the maximal chemotactic response that was induced with C5a (1 μg/ml).
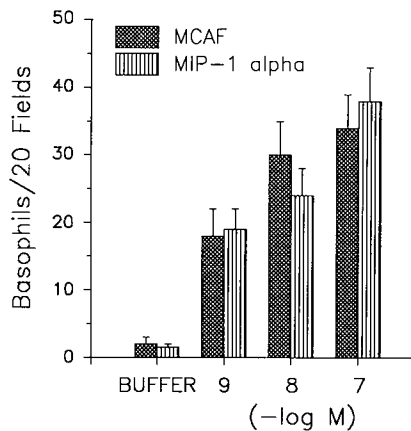

Figure 6. Chemotaxis of purified basophils in response to MIP-1α and MCAF. Basophils from four donors were purified by discontinuous Percoll centrifugation followed by negative selection using anti-CD2 and -CD19 mAb-coated magnetic beads. The final purity ranged from 65 to 80%. Chemotaxis with MIP-1α, MCAF, or C5a was carried out in Boyden microchambers using 5 μm polycarbonate membranes. Results are expressed as basophil count per 20 oil fields. The number of cells migrated to C5a (1 μg/ml) was 90 ± 4/20 oil fields.
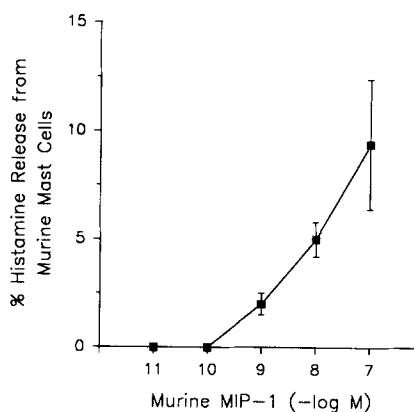
Figure 7. Murine MIP-1α-induced histamine release from murine peritoneal mast cells. Mouse peritoneum was lavaged with HA buffer and the washed cells containing ~5% mast cells were used in the histamine release test. Results of eight duplicate experiments are shown.

The effect of murine MIP-1α was studied on mouse peritoneal mast cells. Peritoneal lavage cells containing ~5% mast cells were obtained from DBA/2 mice. MIP-1α induced a small but significant histamine release from mast cells (Fig. 7). The response of mouse mast cells to MIP-1α was species dependent. Initially we tried peritoneal mast cells from BALB/c species. MIP-1α failed to induce histamine release from BALB/c mast cells. We also studied the effect of murine MIP-1α on human basophils and compared with human MIP-1α. Murine and human MIP-1α induced similar levels of histamine release from human basophils (Fig. 8).

Discussion

MIP-1α is a 8.7-kD protein based on amino acid sequence and cloning data (9, 12), although it migrates as a 13–15-kD protein on SDS-PAGE. MIP-1α belongs to the family of cytokines so-called intercrine -β that have four conserved cysteine residues. The first two cystine residues are adjacent. The
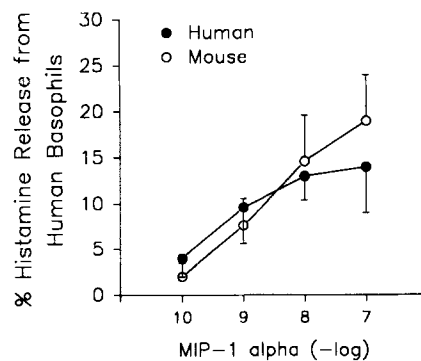

Figure 8. Comparison of histamine releasing activity of human and murine recombinant MIP-1α. Leukocytes from three donors were incubated with various concentrations of human and murine MIP-1α and the released histamine was measured.

other members of this family include MIP-1β, MCAF, hRANTES, hAct-2h, mJE, and mouse T cell activation gene (mTCA) (reviewed in reference 12). Most of these cytokines have ~30–70% sequence homology.

We have demonstrated that MIP-1α has significant histamine releasing activity. MIP-1α causes histamine release from human basophils and mouse mast cells. Its histamine releasing capability is significantly lower than that of anti-IgE and MCAF. However, the true potency of MIP-1α is difficult to determine because it is a doublet in its natural form, and more importantly, it forms 2×10^6 kD multimers as judged by SDS-PAGE (12). The multimers can only be resolved at high ionic strength. Since the histamine release test is a bioassay and is affected by high ionic solutions, the actual potency of MIP-1α cannot be assessed using this assay.

MIP-1α causes histamine release from basophils of some select allergic donors. In contrast, MCAF causes histamine release from basophils of both allergic and normal subjects. Previously, we and others (4, 13) have shown that IL-3 and GM-CSF cause histamine release from select allergic patients. However, a far greater number of donors respond to MIP-1α than to IL-3 and GM-CSF. Basophils from only 3 of the 30 donors released histamine in response to IL-3 and GM-CSF in our laboratory. Although the majority of MIP-1α responders were allergic subjects, there was no correlation of histamine release between MIP-1α and anti-IgE.

Histamine release by MIP-1α in a given responder donor is reproducible. We repeated the histamine release experiment on a group of four people on at least four different occasions (original release experiments, kinetics experiments, calcium-dependency experiments, purified vs. unpurified basophil experiments, and human vs. murine MIP-1 experiments), and the release by a given concentration of MIP-1 was fairly reproducible (± 15%). The reason why basophils from some but not all subjects respond to MIP-1α is unclear. It has been previously shown that the response of basophils from both allergic and normal populations to secretagogues like C5a (14) and anti-IgE (15) is not uniform, and varies from no response to extremely good response. Conroy et al. (15) have introduced the term "releasability" to emphasize the variable intrinsic property of basophils of a given donor to respond to different stimuli. They have identified a group of donors whose basophils do not respond to anti-IgE and Con A despite having a significant number of surface IgE molecules. It has been postulated that nonreleasing basophils lack an early component of the signal transduction pathway (16). It is interesting that IL-3, a cytokine known for its priming effect on basophils, failed to correct this problem.

MIP-1α also induces chemotaxis of basophils. Basophils from donors who gave a positive response in the histamine release assay also responded well in the chemotaxis assay. Many secretagogues for basophils have chemotactic activity. In agreement with the above, we showed that MCAF, a potent secretagogue for basophils, also has chemotactic activity for basophils. MIP-1α is a strong chemokinetic factor for neutrophils (17). We were able to confirm the latter observation in a separate set of control experiments (data not shown).

This is the first report of MIP-1α causing histamine release from basophils and mast cells. Among all the cytokines that we have tested thus far, only a few cause histamine release from basophils, MCAF being the most potent. We also tested human and murine cytokines on human lung and mouse peritoneal mast cells, respectively. All of them, including MCAF, failed to induce histamine release from mast cells (data not shown). Murine MIP-1α is the only cytokine that was found active on mouse peritoneal mast cells. Since there exists considerable heterogeneity among mast cells from different sources, it is possible that MCAF and other cytokines may have activity on mast cells from other anatomical origin, e.g., bronchoalveolar mast cells.

The function of MIP-1α is largely unknown. It causes an intense inflammation when injected in vivo (17). The infiltrating cells are mostly neutrophils, but there is also an increased number of mast cells that occurs within 4 h. MIP-1α has chemokinetic activity for neutrophils. The mecha-

nism of increased mast cell numbers is intriguing and has not been investigated. Our report points to another mechanism of MIP-induced inflammation, namely, the release of mediators from basophils and mast cells.

MIP-1α may play an important role in the pathogenesis of allergic and nonallergic inflammation Cytokines that have histamine releasing activity have been detected in the blister fluid from late phase cutaneous allergic reactions (18), bronchoalveolar lavage fluid (19), and nasal washings (20). Increased synthesis of HRF by mononuclear cells has been reported in asthma (21) and atopic dermatitis with food allergy (22). Also, increased levels of HRF-like activity have been detected in bronchoalveolar lavage fluid from patients with pulmonary fibrosis (23), and in skin chamber fluid from patients with urticaria (24). The individual contribution of MIP-1α to the activity of the crude biological fluid will have to be determined in the future to assess the exact role played by these cytokines in human illnesses.

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