Monocyte Chemotactic Protein 1 Is a Potent Activator of Human Basophils

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Summary
Cytokines belonging to the RANTES/SIS family are highly induced in a number of pathophysiological processes such as autoimmune disorders, cancers, atherosclerosis, and chronic inflammation. However, apart from their chemotactic activity on monocytes and particular lymphocyte types, the biological activities in the human system of this recently discovered cytokine family are largely unknown. Here we report that one family member, described as monocyte chemotactic protein 1 (MCP-1), strongly activates mature human basophils in a pertussis toxin-sensitive manner. MCP-1 causes a rise in the cytosolic free calcium level in basophils and monocytes, but not in other blood leukocyte types, and triggers basophil degranulation at low concentrations (ED50 = 3–10 nM). Thus, MCP-1 is a cytokine capable of directly inducing histamine release by basophils. Furthermore, MCP-1 promotes the formation of leukotriene C4 by basophils pretreated with interleukin 3 (IL-3), IL-5, or granulocyte/macrophage colony-stimulating factor. MCP-1-induced basophil mediator release may play an important role in allergic inflammation and other pathologies expressing MCP-1.

In contrast to other human cytokines, the members of the bipartite platelet factor 4 superfamily seem to exert a rather restricted profile of biological activities. Members of the "C-X-C branch" (according to the position of the first two cysteines in the conserved motif), also known as neutrophil-activating peptide (NAP)1/IL-8 family, exert proinflammatory activity mainly through their action on neutrophils (e.g., IL-8 and NAP-2), whereas members of the "C-C branch," termed RANTES/SIS family, appear to attract certain mononuclear cells (1–3). RANTES, the macrophage inflammatory protein 1α (MIP-1α) and MIP-1β are chemotactic for distinct lymphocyte populations (3, 4), while MCP-1 has been described as a specific monocyte chemotactic factor (3, 5–7).

Basophils, rare leukocyte types in peripheral blood, are an important source of vasoactive and inflammatory mediators (8), but their participation in disease is largely unknown. They are triggered to release histamine and leukotriene C4 (LTC4) by IgE receptor crosslinking, by chemotactic peptides, i.e., FMLP or C5a, and by cell-derived histamine-releasing factors (9–13). Although certain cytokines, such as the hematopoietic growth factors IL-3, IL-5, GM-CSF, as well as IL-8, can affect basophil mediator release (9–11), none of the known cytokines examined so far consistently induce basophil degranulation by themselves. At least part of the cell-derived histamine-releasing activity (HRA) can be attributed to a fraction of small molecular mass (8–12 kD) cationic peptide(s) (12, 13). In the course of our studies on histamine release induced by cytokines of the platelet factor 4 family, we found that none of the members of the C-X-C branch could account for the HRA of the cationic peptide fraction. In fact, even the most active peptide, IL-8, up to a 100-nM concentration, induces histamine release only in basophils primed with IL-3, GM-CSF, or IL-5 (11). The present study suggests that MCP-1 can account for at least part of the HRA from stimulated mononuclear cells.

Materials and Methods

Reagents. Dextran and Ficoll-Hypaque were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden); Hepes was from Calbiochem-Behring Corp. (La Jolla, CA); BSA, fatty acid free, was from Boehringer Mannheim, Inc. (Mannheim, FRG); EDTA was from Fluka AG (Buchs, Switzerland); and Dynabeads M-450 Pan T and Pan B were from Dynal A.S. (Skoyen, Norway). Pertussis toxin (PT) and its B subunit were from List Biological Laboratories Inc. (Campbell, CA).

Cell Stimulants. Recombinant human (r)MCP-1 (76 amino acids, 8.5 kD; reference 7), expressed in Escherichia coli and purified to 99% as assessed by SDS-PAGE and HPLC, was from PreproTech Inc. (Rocky Hill, NJ). rhIL-3 and rhIL-8 were obtained from Sandoz Ltd. (Basel, Switzerland; and Vienna, Austria). The anaphylatoxins were purified from yeast-activated human serum as described (10, 14). The purified mAb 29C6 directed against the high affinity IgE

1 Abbreviations used in this paper: [Ca2+]i, cytosolic free calcium; HRA, histamine-releasing activity; LTC4, leukotriene C4; MCP-1, monocyte chemotactic protein-1; MIP, macrophage inflammatory protein; NAP, neutrophil-activating peptide; PT, pertussis toxin.
receptor α chain (Kd = 3.2 nM) was provided by Hoffmann-La Roche (Nutley, NJ).

Cell Preparation. PBMC containing 1–6% basophils were isolated from venous blood of unselected human volunteers by Ficoll-Hypaque density centrifugation exactly as described previously (9, 10). For measurement of intracellular calcium levels, basophils, monocytes, and neutrophils were purified from the same leukocyte preparation by discontinuous Percoll gradient centrifugation and negative selection of basophils with immunomagnetic beads as reported previously (9; 14a). Basophils, purified to 80–91%, were contaminated with neutrophils (1–16%), lymphocytes (0–3%), and monocytes (0–3%). Monocytes were purified to ~90% (10% lymphocytes); neutrophil purity was >99%, as assessed by stained cytocentrifuge slides.

Mediator Release Assay. Cells were suspended in HACM buffer (20 mM Hepes, 125 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 0.5 mM glucose, and 0.025% BSA), and experiments were performed in a shaking water bath at 37°C as described (9, 10). The reaction was stopped by placing the tubes in ice-cold water. Histamine and LTC4 release were measured in the cell supernatants as described (9, 10).

Measurement of Cytosolic Free Calcium ([Ca2+]i). After loading the cells with 0.3 nmol fura-2/AM per 106 cells for 30 min at 37°C and washing in HACM buffer, fluorescence of the stirred thermostatted cell suspension was monitored continuously, and the percent fura-2 saturation was calculated as described (15). Resting [Ca2+]i corresponded to 50 ± 2% fura-2 saturation. In some experiments, basophils were preincubated for 90 min at 37°C with or without PT or its β subunit before starting the mediator release experiments of [Ca2+]i measurements.

Results and Discussion

Fig. 1 A shows that rhMCP-1 directly induces histamine release by basophils in a concentration-dependent manner within a range of 3–100 nM. IL-8, under identical experimental conditions, did not trigger basophil degranulation up to a 100-nM concentration, and induced only negligible histamine release at 1 µM. Previously, we reported that IL-3, IL-5, and GM-CSF strongly enhance basophil mediator release in response to diverse agonists (9, 10). Consistent with these studies, IL-3 also enhanced the basophil responsiveness towards MCP-1 (Fig. 1 B). As reported earlier (11), IL-8 induced histamine release in IL-3-primed cells. However, the amount of histamine release induced by MCP-1 was much larger and occurred at lower agonist concentrations (ED50 ~3 nM) when compared with IL-8 (Fig. 1 B). In the absence of hematopoietic growth factors, neither MCP-1 nor IL-8 promoted the generation of detectable amounts of LTC4 (not shown). In the presence of IL-3, however, both agonists induced lipid mediator release (Fig. 1 C). As for histamine release, MCP-1 was a much more potent basophil trigger than IL-8, and induced the formation of large amounts of LTC4 concentration dependently over a range of 1–30 nM. These results demonstrate that basophils are stimulated for mediator release within a similar MCP-1 concentration range as previously shown to activate monocytes (5, 16).

In Fig. 2, the effect of MCP-1 was compared with that of other basophil agonists such as IL-8, the anaphylatoxins C3a and C5a, and anti-IgE receptor antibody (anti-FceR) at near optimal concentrations. The data confirm the results shown in Fig. 1, and demonstrate a qualitatively similar effect of C3a and MCP-1 on basophils. Both agonists triggered histamine release by themselves, but induced LTC4 production only in the presence of IL-3. By contrast, IL-8 and C3a were able to induce mediator release only after pretreatment with IL-3 (11, 14). Identical results were obtained when IL-3 was replaced by IL-5 or GM-CSF (not shown). The data show that MCP-1, at a 30-nM concentration, is a potent basophil agonist, in particular in the presence of IL-3, inducing the release of comparable amounts of mediators as optimal IgE receptor crosslinking. Since mononuclear cell preparations con-
Changes in $[Ca^{2+}]_i$ in response to MCP-1 and IL-8. Basophils (91% purity; 7% neutrophils, 2% lymphocytes), monocytes (90% purity; 10% lymphocytes), and neutrophils (>99% purity) were isolated from the same leukocyte preparation. Cells were loaded with fura-2/AM, washed, and resuspended at a cell density of 0.5 x 10^6/ml (basophils) or 2.0 x 10^6/ml (monocytes, neutrophils). Fluorescence of the cell suspensions was expressed as percent fura-2 saturation. Arrows indicate the time of MCP-1 (30 nM) or IL-8 (30 nM) addition. Similar results were obtained from three other basophil preparations of 80-90% purity and variable degrees of monocyte (0-3%), lymphocyte (2-12%), and neutrophil (1-16%) contamination. Anti-FceR mAb (500 ng/ml) induced a $[Ca^{2+}]_i$ rise of similar magnitude as MCP-1 in all basophil preparations examined, but not in monocytes or neutrophils (not shown). Lymphocytes depleted of monocytes, basophils, and neutrophils did not react with $[Ca^{2+}]_i$ changes towards MCP-1, IL-8, or anti-FcεR (not shown).

The kinetic of the MCP-1-induced histamine and LTC4 release was found to be rapid and identical to that previously reported for C5a (10), further indicating the similarity of action between these two agonists (not shown). Leukocyte chemotactic factors acting through G protein-coupled receptors (i.e., C5a and IL-8) are known to induce a transient rise in $[Ca^{2+}]_i$ in neutrophils as an early event in signal transduction (17, 18). Therefore, we loaded highly purified basophil preparations with fura-2, and monitored fluorescence changes in response to MCP-1 and IL-8, respectively (15). Fig. 3 demonstrates that both MCP-1 and IL-8 promote rapid changes in intracellular calcium concentration ($[Ca^{2+}]_i$) in human basophils. A $[Ca^{2+}]_i$ change of identical magnitude was obtained with the specific basophil agonist anti-FceR (not shown). Interestingly, the $[Ca^{2+}]_i$ changes in response to MCP-1 or IL-8 were similar, indicating that the distinct

Table 1. Effect of PT on Basophil Mediator Release Induced by MCP-1 and Other Agonists

| Mediator release (control) | Percent inhibition of mediator release* |
|---------------------------|----------------------------------------|
|                           | PT-B (5 nM)  | PT (1 nM)  | PT (5 nM)$ |
| **Histamine**             |             |           |             |
| MCP-1                     | 11 ± 1      | -4 ± 6    | 84 ± 14     | 93 ± 12 |
| IL-3 + MCP-1              | 32 ± 5      | -8 ± 9    | 27 ± 16     | 79 ± 9  |
| C5a                       | 23 ± 12     | -12 ± 5   | 58 ± 17     | 90 ± 9  |
| IL-3 + C5a                | 43 ± 19     | 4 ± 4     | 57 ± 34     | 82 ± 17 |
| Anti-FcεR Ab              | 29 ± 17     | 22 ± 9    | 15 ± 8      | 32 ± 27 |
| **LTC4**                  |             |           |             |
| IL-3 + MCP-1              | 9 ± 1       | 3 ± 14    | 73 ± 17     | 90 ± 7  |
| IL-3 + C5a                | 13 ± 3      | -4 ± 8    | 51 ± 35     | 84 ± 18 |
| Anti-FcεR Ab              | 13 ± 6      | 19 ± 10   | 15 ± 5      | 30 ± 24 |

* The toxin effects are expressed as percent inhibition of mediator release according to the formula: 100 x [1 - (release with toxin/release without toxin)]. Mean ± SD of three different experiments performed in duplicates are shown.

† Control values are expressed as percent specific release of total cellular histamine content, or as picograms leukotriene C4 released per nanogram total cellular histamine.

§ PBMC containing basophils were preincubated at 37°C for 90 min without (control) or with 5 nM of the B subunit of Pertussis toxin (PT-B), or with 1 and 5 nM PT, respectively, before stimulating the cells as described in Fig. 2. PT and its B subunit did not induce significant mediator release by themselves at 1-5 nM (not shown).
histamine-releasing capacities of the two agonists are due to differences in signaling events other than calcium mobilization. For comparison, [Ca \(^{2+}\)] changes in response to MCP-1 or IL-8 are also shown for monocytes and neutrophils purified from the same leukocyte preparation. Consistent with published data (16), MCP-1 did not influence [Ca \(^{2+}\)] in neutrophils, in contrast to its prominent effect in monocytes. PBL depleted of monocytes and basophils responded to neither MCP-1 nor to IL-8 (not shown). Collectively, these experiments strongly suggest that MCP-1 activates basophils directly through specific high affinity receptors (19). This conclusion is further supported by cross-desensitization experiments in purified basophils demonstrating that the cells were deactivated towards a second challenge with the same agonist (MCP-1 or IL-8, respectively; not shown), but not if one cytokine was followed by the other (Fig. 3). Similarly, IL-8 preincubation did not inhibit the MCP-1-induced mediator release (not shown), in contrast to its inhibitory activity towards IL-8 stimulation (20).

Since the receptors for C5a, FMLP, and IL-8 are all coupled to PT-sensitive G-protein(s) (17, 18), we examined whether MCP-1-induced basophil activation is inhibited by PT. As a control for G protein–independent toxin effects, the B subunit of PT was included in these experiments. Table 1 illustrates that PT, but not its B subunit, strongly inhibited MCP-1-induced mediator release. As expected, the C5a response was similarly reduced, in contrast to the IgE-dependent stimulation (21). Furthermore, the [Ca \(^{2+}\)] change induced by MCP-1 or C5a was inhibited by >90% by 5 nM PT, whereas the calcium signal induced by anti-FceR was unchanged (not shown). Thus, MCP-1 appears to induce [Ca\(^{2+}\)] changes in a similar way to other chemotactic agonists, presumably by interacting with a specific G protein–coupled receptor.

MCP-1 represents the first identified cytokine capable of directly inducing mediator release by human basophils. In preliminary experiments, we found no histamine release in response to RANTES, MIP-1α, or MIP-1β, indicating that the potent basophil-activating property of MCP-1 may be unique, even among the members of the RANTES/SIS cytokine family. Basophils, as target cells of MCP-1 action, may thus be involved in processes (3, 22, 23) in which MCP-1 is upregulated. This implies the interesting possibility of an additional role of basophils outside allergic disease.

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