Deletion of the NH2-Terminal Residue Converts Monocyte Chemotactic Protein 1 from an Activator of Basophil Mediator Release to an Eosinophil Chemoattractant

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Summary

Chemotactic cytokines of the CC subfamily (CC chemokines) are considered as major mediators of allergic inflammation owing to their actions on basophil and eosinophil leukocytes. The monocyte chemotactic protein (MCP) 1 is a potent inducer of mediator release from basophils but is inactive on eosinophils. To obtain information on the structural determinants of the activities of MCP-1, we have synthesized several NH2-terminally truncated analogues and tested their effects on basophils and eosinophils. Through deletion of the NH2-terminal residue, MCP-1(2-76) was obtained, which was a potent activator of eosinophils, as assessed by chemotaxis, cytosolic free Ca2+ changes, actin polymerization, and the induction of the respiratory burst. In contrast, the activity of MCP-1(2-76) on basophil leukocytes was dramatically decreased (50-fold) compared with that of full-length MCP-1. Deletion of the next residue led to total loss of activity on eosinophil and basophil leukocytes. Analogues with three or four residue deletions, MCP-1(4-76) and MCP-1(5-76), were again active on both cells, whereas all further truncation analogues, MCP-1(6-76) through MCP-1(10-76), were inactive. Thus, a minimal structural modification can change receptor and target cell selectivity of MCP-1. Our observations indicate that the recognition sites of CC chemokine receptors on eosinophils and basophils are similar, although they discriminate between MCP-1 and MCP-1(2-76) and suggest NH2-terminal processing as a potential mechanism for the regulation of CC chemokine activities.

Eosinophil and basophil leukocytes are major effector cells in allergic inflammation. Recent studies have demonstrated that some chemotactic cytokines of the CC subfamily (CC chemokines), in addition to their activity toward monocytes (1-4) and T cell subsets (5-8), can attract and activate eosinophils and basophils (9-15). Monocyte chemoattractant protein (MCP) 1, the best-characterized CC chemokine, is a moderate chemoattractant and a powerful stimulus of histamine and leukotriene release for basophils but does not act on eosinophils (9-11). In contrast, regulated on activation, normal T expressed and secreted (RANTES) and macrophage inflammatory protein (MIP)-1α are strong attractants for both types of cells but only weak inducers of release (12-14). MCP-3 combines the properties of MCP-1 and RANTES and is a powerful stimulus of both release and migration for basophils and eosinophils (15). The differences in the responsiveness of these cells are likely to depend on the patterns of CC chemokine receptor expression (15).

It is well established that the NH2-terminal region is critical for the biological activity of IL-8 (16, 17), and it has been shown that modifications of the corresponding region of MCP-1 profoundly alter the activity of this CC chemokine on mononuclear phagocytes (18, 19). We have now studied the role of the NH2-terminal domain for the activity of MCP-1 on eosinophils and basophils and show here that deletion of the NH2-terminal residue confers to MCP-1 potent activity on eosinophil leukocytes while greatly reducing activity on basophils.

Materials and Methods

Cytokines and Other Agonists. All chemokines and chemokine analogues were prepared on peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA), using the tertiary t-butoxycarbonyl and benzyl protection strategy (19), and C5a was purified from human plasma (20). Recombinant human IL-3 was kindly provided by Sandoz Ltd. (Basel, Switzerland). All MCP-1 truncation analogs were analyzed by reverse-phase HPLC, and each was eluted as a single, uniform peak without detectable closely eluting species. Each analogue was also subjected to electrospray mass spectrometry, and the determined mass corresponded in all cases to the calculated mass within the experimen-
MCP-1(2-76), 8,552.2 ± 1.1 (-0.8); MCP-1(3-76), 8,455.1 ± 0.9 (-0.7); MCP-1(4-76), 8,340.6 ± 1.5 (-0.1); MCP-1(5-76), 8,269.8 ± 0.6 (+0.1); MCP-1(6-76), 8,156.0 ± 0.8 (+0.5); MCP-1(7-76), 8,041.8 ± 0.7 (-0.6); MCP-1(8-76), 7,971.2 ± 0.8 (-0.1); MCP-1(10-76), 7,774.9 ± 0.6 (-0.2).

MCP-1, MCP-3, and RANTES were characterized in former studies (4).

Cells. Leukocytes were isolated from freshly drawn blood of healthy, unselected donors by dextran sedimentation for 90 min. Basophils were partially purified (10–30% pure, with lymphocytes as almost exclusive contaminants) on a discontinuous Percoll gradient (21). Eosinophils (99% pure) were obtained by Percoll density gradient centrifugation followed by negative selection with anti-CD16 mAb–coated magnetic beads (12).

Histamine and Leukotriene C4 (LTC4) Release. A suspension of basophils [200,000 cells/ml] in HACM buffer (20 mM Hepes, pH 7.4, containing 125 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1 mM CaCl2, and 0.25 mg/ml BSA) was prewarmed to 37°C, exposed to 10 ng/ml IL-3 for 10 min, and then stimulated with a chemokine. After 20 min, the reaction was stopped on ice. Histamine and LTC4 were measured in the supernatants (11, 22). Priming with IL-3 enhances responsiveness to agonists and is required for LTC4 generation (11, 20, 22).

Chemotaxis. Chemotaxis of eosinophils was performed essentially as described (12). Briefly, chemokines were added to the lower wells of a 48-well chemotactic chamber (Neuro Probe, Cabin John, MD). Cells were suspended in Gey’s solution supplemented with 20 mM Hepes and 0.25% BSA adjusted to pH 7.4 and placed into the top wells (50,000 cells/well). Migration across a polycarbonate filter (polyvinyl pyrrolidone free, 5-μm pore size; Nucleopore Corp., Pleasanton, CA) was assessed after 50 min at 37°C in 5% CO2. The migrated cells were counted microscopically after fixation and staining on the lower surface of the filter.

Cytosolic-free Calcium [Ca2+]. Changes. Purified eosinophils were loaded with Fura-2/AM (0.3 nmol/106 cells) for 30 min at 37°C, washed, and resuspended in HACM buffer. The cells (106 cells/ml) were stimulated in a stirred cuvette at 37°C and the fluorescence changes monitored (12). In each experiment, maximal and minimal fluorescence was calibrated by addition of 2 μM ionomycin and 3 mM CaCl2, followed by 10 mM MnCl2.

Actin Polymerization. F-actin formation was assessed in eosinophils, according to Howard and Meyer (23). Eosinophils (106 cells/ml in HACM buffer) were prewarmed for 10 min, stimulated with a chemokine, and fixed after 15 s with 3% formaldehyde in PBS (pH 7.4) containing 100 μg/ml lysophosphatidylcholine. After 10 min, the cells were stained for 10 min in the dark with NBD-phallacidin (33 U/ml), and the intracellular fluorescence was monitored by FACScan analysis.

Respiratory Burst. The respiratory burst was measured in eosinophils by lucigenin-dependent chemiluminescence. Samples of 200 μl eosinophil suspension (106 cells/ml) in HACM buffer containing 100 μM lucigenin were placed in microtiter plates thermostated at 37°C, stimulated with a chemokine, and the chemiluminescence was monitored (24).

Results and Discussion

Synthetic analogues of full-length MCP-1 (76 residues), with truncations within the 10-residue NH2-terminal domain preceding the first cysteine, MCP-1(2-76) through

MCP-1(10-76) (19), were tested for activity on basophil leukocytes. As shown in Fig. 1, full-length MCP-1 induced the release of histamine, reflecting granule exocytosis, and the generation and release of LTC4, consistent with previous studies (9–11). For both responses, the concentration of MCP-1 inducing half-maximal release was ~5 nM. Deletion of the NH2-terminal residue, yielding analogue MCP-1(2-76), led to an ~50-fold decrease in potency, and deletion of the following residue led to analogue MCP-1(3-76), which was inactive up to a concentration of 1,000 nM. Some activity was again detected for MCP-1(4-76) and MCP-1(5-76), which were ~300- and 50-fold less potent than full-length MCP-1, respectively, whereas the fur-

![Figure 1](image_url)
ther truncated analogues MCP-1(6-76) through MCP-1(10-76) were inactive. The dramatic loss of potency on basophils resulting from the deletion of the NH2-terminal residue demonstrates that the NH2 terminus is a major requirement for the induction of histamine and leukotriene release. The present results are in general agreement with recent observations on monocytic THP-1 cells, where MCP-1(2-76) was shown to have a 100-fold lower binding affinity than full-length MCP-1.

Because of the striking changes in function on NH2-terminal truncation, it was of interest to test the MCP-1 analogues on eosinophil leukocytes that respond to MCP-3 and RANTES, but not to MCP-1 (12-15). The results were surprising: deletion of the NH2-terminal residue resulted in an MCP-1 analogue with marked activity on eosinophils. As shown in Fig. 2, MCP-1(2-76) is a powerful chemoattractant for human eosinophils with similar efficacy and potency as MCP-3. Additional responses were studied to substantiate the eosinophil-activating properties of MCP-1(2-76). In contrast to full-length MCP-1, the truncated analogue induced actin polymerization, a correlate of the motor response of phagocytes, the respiratory burst reflecting the activation of the NADPH-oxidase, and a transient rise in [Ca2+]i, which is typical for agonists acting through seven-transmembrane, G protein-coupled receptors (25, 26) (Fig. 3). These results show that MCP-1(2-76) elicits in eosinophil leukocytes the characteristic pattern of in vitro leukocyte responses to chemoattractant stimulation.

Full-length MCP-1 and all truncated analogues were also tested on eosinophils for their ability to induce chemotaxis and [Ca2+]i changes. The results with analogues MCP-1(3-76) through MCP-1(10-76) were qualitatively similar on eosinophils and basophils. Analogue MCP-1-(3-76) was inactive, weak but significant responses were obtained with MCP-1(4-76) and MCP-1(5-76), and no activity was observed upon further truncation (Fig. 4). Full-length MCP-1 showed borderline chemotactic activity in some but not all experiments and did not induce [Ca2+]i changes (Fig. 4).

After stimulation, chemokine receptors are characteristically refractory to subsequent stimulation by the same or a
cross-reacting agonist. Previous studies have revealed the presence of two CC chemokine receptors on eosinophils, one binding RANTES and MCP-3, and the other binding RANTES and MIP-1α and, with lower affinity, MCP-3 (13, 15, 26). Desensitization experiments were performed to gain information on the receptors involved in the activation of eosinophils by MCP-1(2-76). As shown in Fig. 5, the response to MCP-1(2-76) was prevented by prior stimulation with MCP-3 or RANTES but not MIP-1α, suggesting that MCP-1(2-76) acts mainly through the receptor that recognizes RANTES and MCP-3 but does not bind MIP-1α. This conclusion is consistent with the observation that MCP-1(2-76) had chemotactic activity comparable to that of MCP-3 and RANTES, and was clearly superior to MIP-1α, which is a weak stimulus of eosinophils (12, 26).

This study demonstrates the critical role of the NH2 terminus for the action of MCP-1 on basophils and eosinophils. MCP-1 is a potent inducer of histamine and leukotriene release in basophils but lacks detectable activity on eosinophils. Upon deletion of the NH2-terminal amino acid, the profile of activity of the chemokine changed dramatically: its potency at inducing mediator release from basophils fell 50-fold, and a new activity emerged, chemotaxis for eosinophils. The \([\text{Ca}^{2+}]_i\] transients induced in eosinophils by MCP-1(2-76) were prevented by prior stimulation with MCP-3 or RANTES, indicating that MCP-1(2-76) acts through the RANTES/MCP-3 receptor (23). Our results suggest that the receptor for MCP-1 on basophils and the receptor for RANTES/MCP-3 on eosinophils must be similar, although they clearly discriminate between MCP-1 and MCP-1(2-76). Similarity is also suggested by the observation that two truncation analogues, MCP-1(4-76) and MCP-1(5-76), were active, whereas the others, MCP-1(3-76) and MCP-1(6-76) through MCP-1(10-76), were inactive on both eosinophils and basophils.

The major effect of the deletion of the NH2-terminal residue of MCP-1 described here suggests processing at the NH2 terminus as a potential mechanism for the regulation of the potency and target cell selectivity of MCP-1 and other CC chemokines. There is no direct information about the processing of MCPs by aminopeptidases, but some MCP analogues with truncated NH2-terminal domains have been identified in cell culture supernatants (2). In this context, eotaxin, a novel chemokine discovered in guinea pigs, is of particular interest. Eotaxin was identified as the main attractant for eosinophils in a model of eosinophilic lung inflammation (27). It has marked sequence similarity with the human MCPs, but its NH2-terminal domain (residues preceding the first cysteine) consists of 7 instead of 10 residues (28). Comparison of the activity of eotaxin on eosinophils, basophils, and monocytes may provide useful information about the determinants for receptor recognition.

Previous studies have demonstrated the critical role of the NH2 terminus for the biological activity of CXC chemokines on neutrophils (16, 17, 29). Several NH2-terminal variants of IL-8 and other CXC chemokines were found in the supernatants of cultured monocytes and tissue cells (25), and it was shown that NH2-terminal processing markedly enhances IL-8 activity (17). However, a change in cellular and receptor selectivity, as described here as the consequence of MCP-1 truncation, has never been observed. Processing by specific aminopeptidases must be considered as a regulatory mechanism for CC chemokine activities in the microenvironment of immune effector cells.

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