RANTES and MCP-3 Antagonists Bind Multiple Chemokine Receptors*

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Antagonists of multiple chemokines could be more effective than inhibitors of specific chemokines for controlling cell migration and inflammation. To attempt to identify such antagonists we characterized a number of truncated analogs of regulated on activation normal T cell expressed protein (RANTES), monocyte chemotactant protein (MCP)-3, and MCP-1. On the basis of their ability to compete for binding of their parent chemokines, three analogs were selected for cross-reactivity studies: RANTES (9–68), MCP-3 (10–76), and MCP-1 (9–76). These analogs bound to THP-1 monocytic cells with dissociation constants that were within 4–6-fold of their native counterparts, but they did not promote detectable chemotaxis of THP-1 cells or enzyme release from purified human monocytes. The RANTES (9–68) analog competed for the binding and inhibited the activities of all three chemokines. In contrast, native RANTES was specific for RANTES binding sites. However, truncation of either MCP-1 or MCP-3 did not change their respective binding specificity. MCP-3 and MCP-3 (10–76) competed for binding of all three labeled chemokines. MCP-1 (9–76) competed strongly for binding of labeled MCP-1, but only weakly for the other two labeled ligands and inhibited the activities induced by MCP-1 and MCP-3 but not RANTES. Although RANTES (9–68) and MCP-3 (10–76) inhibited all three chemokines, the RANTES analog was significantly more potent than RANTES-induced activity. The results indicate that NH₂-terminal residues partly determine the receptor specificity of RANTES, and deletions within this region permit binding to multiple chemokine receptors. The findings suggest the feasibility of design of high affinity multi-specific CC chemokine antagonists.

Inflammation pathology involves the migration of various types of blood cells into affected tissue sites (1). The chemokines are a family of protein mediators with potent chemotactic activity for granulocytes, monocytes and lymphocytes (2–4). The human chemokines number about 20 and fall into two classes according to the relative positions of the first two cysteines. For the CXC chemokines, the first two cysteines are separated by one residue; and for the CC chemokines, the first two cysteines are adjacent. Chemokines share significant sequence similarity (24–76% identity), including conservation of the four cysteines that are involved in two disulfide bridges (reviewed in Refs. 2 and 5). Furthermore, the structural fold of the polypeptide of the CC chemokines, MIP-1α (6) and RANTES (7), and the CXC chemokine, interleukin-8 (8), are very similar, suggesting that chemokines share similarity at the level of their three-dimensional structures (5). Functionally the CC class acts on several types of leukocytes including monocytes, basophils, eosinophils, and lymphocytes but do not stimulate neutrophils. In contrast, neutrophil activity is a distinguishing feature of members of the CXC class (2).

Multiple receptors mediate the complex and overlapping functional activities of CC chemokines. Three receptors have been identified and their polypeptide sequences deduced from cDNA clones. These are: chemokine receptor (CKR)-1, which binds MIP-1α, RANTES, and MCP-3 (9–13); CKR-2, which binds MCP-1 and MCP-3 (14, 15); and CKR-3, which has been shown to bind RANTES, MIP-1α, and MIP-1β (12). In addition a fourth receptor, termed K5-5, which binds MCP-1, MIP-1α, and RANTES, has been identified (16). For all these receptors, either mRNA or receptor protein is expressed by peripheral blood monocytes. However despite this sequence information, most of our understanding of the function and ligand specificity of the receptors has come from experiments with cells isolated from blood or cell lines that are representative of a particular lineage (17–23). These experiments suggest that additional functional receptors are also involved and that cross-reactive binding of several CC chemokines is a frequently observed feature of the receptors.

The aim of the present study is to generate and characterize receptor antagonists for CC chemokines. With defined antagonists we can test whether cellular infiltration and inflammatory disease progression is retarded in vivo and evaluate the possibility that antagonists could have therapeutic value. Previously we characterized a series of analogs of MCP-1 and found that some truncated forms were potent antagonists for MCP-1 in vitro (23). Other studies indicated that MCP-1 forms with deletions in the NH₂-terminal region also had some antagonistic properties (24). Our results (23) also demonstrated that different regions within the NH₂-terminal domain are important for binding and activity. Based on this study, we...
hypothesized that modification of MCP-1 or other chemokines that bind to several different receptors may lead to antagonists that block multiple CC chemokine receptors. In this study we have compared truncated analogs of three potent CC chemokines, namely RANTES, MCP-3, and MCP-1, which have differing cellular and receptor specificity, but all stimulate monocytes and the THP-1 monocytic cell line (17, 19, 23, 25, 26). The results indicate that MCP-3 and RANTES analogs could form the basis for antagonists with broad anti-inflammatory activity.

MATERIALS AND METHODS

Synthetic Proteins—Synthetic chemokines and their analogs were synthesized using tBoc chemistry on an Applied Biosystems (Foster City, CA) 430A peptide synthesizer, folded by air oxidation, and purified using at least two steps of reverse phase HPLC as described (27). The molecular mass of each was determined by electrospray mass spectrometry (27, 28) on a API-3 triple quadrupole mass spectrometer (SCIEX Thornhill, ON) with an HPLC interface for sample delivery.

Cell Preparations—Peripheral blood monocytes were isolated from buffy coats supplied by the Central Laboratory of the Swiss Red Cross. Centrifugation on Ficoll-Paque was followed by centrifugation on a Percoll gradient (29). The final cell preparations consisted of 90–95% monocytes and 5–10% lymphocytes. The human monocytic cell line THP-1 (ATCC, Rockville, MD) was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum.

Chemotaxis Assay—Cell migration was evaluated by using Boyden microchambers (Neuroprobe, Cabin John, MD) as described for THP-1 cells (23) and monocytes (17), respectively. All determinations were performed in triplicate. The percentage of cell migration was calculated by dividing the mean number of migrating cells by the mean number of cells migrating in medium alone.

N-Acetyl-β-D-glucosaminidase Release Assay—The release of N-acetyl-β-D-glucosaminidase in response to stimulation of cytokalasin B-pretreated monocytes was determined as described previously (17). For measuring antagonistic activity, the procedure was slightly modified. Briefly, the monocytes (1.2 × 10⁶) that had been treated for 2 min with cytokalasin B (2.7 μg/ml) were exposed to the antagonist for 2 min and then stimulated with the appropriate agonist for 3 min. The final volume of the incubation mixture was 0.4 ml.

Changes in Intracellular Free Ca²⁺—(Ca²⁺⁻⁴)—Monocytes were loaded with Fura-2 (0.4 mM) Fura-2-acetoxyethyl ester (10⁻⁴ M) and then washed and stimulated, and the (Ca²⁺⁻⁴) changes were recorded as described previously (17). Antagonistic activity was assayed by sequential addition of antagonist and then agonist at a 1 min interval.

Iodination of Chemokines—MCP-1 and MCP-3 were labeled with monooxidated Bolton-Hunter reagent (specific activity, 2,200 Ci/mmol⁻¹; DuPont NEN) according to the method supplied. Briefly, 10 μg of MCP-1 was reacted with 1 mCi of ¹²⁵I-Bolton-Hunter reagent at 4 °C in sodium borate buffer (0.1 mM, pH 8.5) for 20 min. Labeled MCP-1 was separated from unreacted Bolton-Hunter reagent by Sephadex G-25 chromatography. The specific activity of the ¹²⁵I-labeled MCP-1 and MCP-3 was 130 and 150 Ci/mmol⁻¹, respectively. RANTES could not be labeled by the Bolton-Hunter method and therefore was labeled using lactoperoxidase. One mCi (3.7 MBq) of Na¹²⁵I (ICN Biomedicals, Irvine, CA) and 1 μg of lactoperoxidase (80–150 units/mg⁻¹, Sigma) were added to 5 μg of RANTES in 50 μl of 0.5% sodium acetate, pH 6.5, at room temperature for 3 min. Saturated tyrosine (150 μl) was added to stop the reaction, and the proteins were separated from the label by Sephadex G-25 chromatography. The specific activity of ¹²⁵I-labeled RANTES was 260 Ci/mmol⁻¹. The labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis to establish that label was associated only with molecules of the apparent Mᵣ of these proteins. They were also analyzed for functional activity to check that their biological potency was retained following labeling.

Competition Binding—Receptor binding of analogs was assayed by competition with ¹²⁵I-labeled chemokines. First, the maximal binding of the labeled preparation was determined by measuring binding at saturating concentrations (30). The bindability of the labeled proteins was about 20%. For binding assays, cells (5 × 10⁵) were incubated with 4 μM of ¹²⁵I-labeled chemokine and increasing concentrations of unlabeled competitor (10⁻⁴ to 10⁻⁵ M) in 200 μl of RPMI medium, containing Hepes (25 mM), bovine serum albumin (10 mg/ml⁻¹), and sodium azide (0.1%). The incubations were carried out for 30 min at 4 °C, except for RANTES, which was incubated at room temperature due to its low solubility at 4 °C. Cell-associated radioactivity was directly separated by spinning the cells through a 2:3 mixture of diacetylphthalate and dibutylphthalate, and the cpm was determined. The cpm that specifically bound to cells was calculated by subtracting the nonspecific cpm (the cpm bound in the presence of 100-fold molar excess of the corresponding unlabeled chemokine) from the total cpm that was bound to the cells. Dissociation constants (Kᵣ values) were determined by Scatchard analysis. A second low affinity class of binding sites (Kᵣ > 2,000 nM) were apparent for some ligands; however, the specificity and significance of these is uncertain, and they were not included in the analysis.

RESULTS

Truncated Analogs of RANTES, MCP-3, and MCP-1—To characterize the antagonistic activity and specificity of NH₂-terminally modified CC chemokine analogs, we synthesized several NH₂-terminal variants of RANTES, MCP-3, and MCP-1. These included: RANTES (6–68), (7–68), (8–68), (9–68), and (10–68); MCP-3 (2–76), (8–76), (9–76), (10–76), and (11–76), and eleven truncated MCP-1 analogs that have been described previously (23). The NH₂-terminal sequences of these analogs are shown in Fig. 1.

The yield of purified folded protein was in the 10–100 mg range, depending on the amount of peptide-resin synthesized (0.05–0.3 mmol corresponding to 0.5–2.0 g of unpurified pep-
### Antagonists that Block Multiple Chemokines

**Fig. 2. Chemoattractant activity of chemokine analogs.** Shown is the migration of THP-1 cells toward the indicated concentrations of RANTES (9–68) (■), MCP-3 (9–76) (●), and MCP-1 (9–76) (▲). Control migration is that obtained toward medium alone. The values shown represent the mean ± S.D. of triplicate determinations from one of two separate experiments.

### Functional Activity of the Shortened Analogs—Based on Preliminary Studies of their Competition for Native Ligand, RANTES (9–68), MCP-3 (10–76), and MCP-1 (9–76) were selected for further examination of their agonist and antagonist activities. In contrast to the full-length native forms, RANTES (9–68), MCP-3 (10–76) (Fig. 2), and MCP-1 (9–76) (23) lacked detectable chemoattractant activity for human THP-1 mononuclear cells. In addition, for human blood-derived monocytes, neither chemotaxis nor N-acetyl-β-D-glucosaminidase release was detected up to 1 μM (not shown). RANTES (6–68), (7–68), (8–68), and (10–68) as well as MCP-3 (8–76), (9–76), and (11–76) also lacked activity (not shown). Thus, residues within the 1–5 region of RANTES and 1–7 region of MCP-3 are essential for their functional activities. Previously we had shown that residues within the 1–6 region of MCP-1 were critical for its chemoattractant activity and receptor activation (23), and analysis of the MCP-3 analogs showed that as with MCP-1 (2–76), MCP-3 (2–76) greatly reduced activity, indicating that the NH2-terminal residue of both MCP-1 and MCP-3 is important for function (data not shown).

**Inhibition of Chemokine Activities—RANTES (9–68), MCP-3 (10–76), and MCP-1 (9–76) were tested for their antagonist activities. Both inhibited the activity induced by the corresponding native forms. As shown in Fig. 3A, RANTES (9–68) fully blocked the chemoattractant activity of RANTES for THP-1 cells, and 41 nM was required to inhibit the agonist activity of 10 nM native RANTES by 50% (IC50 = 41 nM).** Similar results were obtained for migration of peripheral blood monocytes (not shown). The ability of RANTES (9–68) to inhibit chemokine-induced exocytosis from human blood monocytes was assessed by measuring the release of N-acetyl-β-D-glucosaminidase (Fig. 4A). A 10-fold lower concentration (IC50 = 4 nM) of RANTES (9–68) was required to inhibit RANTES-induced enzyme release than was needed for similar inhibition of chemotaxis (Table I).

**Receptor sharing and cross-binding is known to occur amongst members of the CC family of chemokines.** Because the truncated antagonists have lost a part of their structure that is important for receptor activation, it is reasonable to suggest that their receptor selectivity may be altered. To test the specificity of the three truncated antagonists, their ability to inhibit the activities induced by RANTES, MCP-3, and MCP-1 was examined. The RANTES (9–68) analog completely inhibited both MCP-3- and MCP-1-induced chemotaxis (Fig. 3A) and N-acetyl-β-D-glucosaminidase release (Fig. 4A). The respective IC50 values were 200 nM and 820 nM for chemotaxis and 170 nM and 220 nM for release activity (Table I). Thus...
RANTES (9–68) inhibited all the chemokines, but it had the highest potency for RANTES.

MCP-3 (10–76) was found to inhibit MCP-3-induced monocyte N-acetyl-β-D-glucosaminidase release with an IC₅₀ of 37 nM and also chemotaxtactant activity IC₅₀ 470 nM (Fig. 3B). The RANTES analog competed for binding of labeled MCP-3 (Fig. 5). MCP-3 (10–76) blocked enzyme release from monocytes (Fig. 4B). The IC₅₀ values for both RANTES- and MCP-3-induced activities with similar effectiveness to its inhibition of MCP-3 elicited function. Furthermore, MCP-3 (10–76) also inhibited MCP-1- and MCP-1-induced activities with similar effectiveness to its inhibition of MCP-3 elicited function. Moreover, MCP-3 (10–76) blocked enzyme release from monocytes (Fig. 4B). The IC₅₀ values for both RANTES- and MCP-3-induced activities were around 10-fold lower than for chemotaxis of either MCP-1 receptors, MCP-3 (9–76) had about a 15-fold more potent than MCP-3 (10–76) in all the inhibition assays (not shown).

As shown in Fig. 3C and Table I, MCP-1 (9–76) inhibited MCP-1-induced chemotaxtactant activity (IC₅₀ = 72 nM) and was also a potent inhibitor of MCP-3 activity (IC₅₀ = 51 nM). MCP-1 (9–76) did not inhibit RANTES- and MCP-3-induced N-acetyl-β-D-glucosaminidase release activity, but it did not block RANTES-elicted release, at least over the concentration range tested (Fig. 4C). Thus, the ability of MCP-1 (9–76) to inhibit migration and release was similar.

Binding Competition Analysis—Competition binding studies were performed to determine if the observed antagonist properties correlated with their interaction with chemokine binding sites. Because we wished to correlate inhibition of function with binding interactions, we used monocytes cells for both studies, rather than cells engineered to express single receptors. Thus we have measured chemokine binding sites rather than receptor molecules. Despite the presence of multiple receptors on these cells, the binding data always fitted a one site model, suggesting that either the receptors involved have similar affinities or the interaction with one receptor predominates.

The RANTES (9–68) analog competed for binding of labeled RANTES as shown in Fig. 5A. From this data the dissociation constant (Kₐ) was calculated to be 19 nM. Furthermore, RANTES (9–68) also displaced both labeled MCP-3 (Kₐ = 57 nM) and MCP-1 (Kₐ = 120 nM) with moderate efficiency (Fig. 5, A–C, and Table I). Although, as expected, native RANTES competed strongly for RANTES binding (Fig. 5A). The competition for labeled MCP-3 (Fig. 5B) and MCP-1 (Fig. 5C) was very weak and insufficient to derive a Kₐ value. Thus, truncation of RANTES resulted in a markedly increased affinity for both MCP-1 or MCP-3 binding sites. MCP-3 (10–76) competed for binding of MCP-3 with a Kₐ of 57 nM (Fig. 5E and Table I) and also competed for binding of both RANTES (Kₐ = 50 nM) and MCP-1 (Kₐ = 69 nM) (Fig. 5F and I, and Table I). The competition for the MCP-3 (10–76) analog for labeled MCP-3 was only 2-fold weaker than that of full-length MCP-3. For RANTES receptors, MCP-3 and MCP-3 (10–76) had about the same affinity. On the other hand, for MCP-1 receptors, MCP-3 (Kₐ = 13 nM) had about a 5-fold higher affinity than MCP-3 (10–76) (Kₐ = 69 nM). The results indicate that MCP-3 (10–76) had similar affinity for the binding sites of all three chemokines. Similar results were obtained with MCP-3 (9–76) (not shown).

Consistent with previous observations (23), MCP-1 (9–76) competed for binding of labeled MCP-1 with a Kₐ of 9 nM (Fig. 5I and Table I). It also competed for binding of labeled MCP-3, although in this case the Kₐ (128 nM) was considerably higher (Fig. 5H and Table I). Thus, MCP-1 (9–76) exhibited only weak binding (Kₐ = 340 nM) to RANTES receptors (Fig. 5G and Table I). Thus, MCP-1 (9–76) had high affinity for MCP-1 binding sites but only low affinity for MCP-3 or RANTES binding sites. When MCP-1 and MCP-1 (9–76) were compared, they were similar in affinity for both MCP-3 and RANTES binding sites, but for MCP-1 binding sites, MCP-1 (9–76) was around 4-fold lower (Fig. 5, G–I, and Table I).

The cross-reactivity of RANTES (9–68) antagonist was unexpected and prompted us to evaluate other truncated
RANTES analogs for their specificity. A series of five RANTES analogs was tested for inhibition of binding of labeled RANTES and MCP-1 (Fig. 6). There was a general correlation in the ability of the RANTES analogs to displace the two labeled chemokines, although for the (7–68), (8–68) and (9–68) analogs the \( K_d \) was around 10-fold higher for MCP-1 than for RANTES (Fig. 6). The order of the efficiency of displacement (low to high \( K_d \)) was: RANTES (9–68) > (8–68) > (7–68) = (10–68) > (6–68). Only RANTES (8–68) and (9–68) completely inhibited the binding of labeled MCP-1, with the 9–68 analog having a 5-fold higher binding affinity. Similar results were obtained when competition with labeled MCP-3 was tested. Thus, the results indicate that amongst the truncated RANTES analogs tested, RANTES (9–68) had the highest affinity for MCP-1, MCP-3, and RANTES binding sites.

Calcium Desensitization—The truncated analogs RANTES (9–68), MCP-3 (10–76), and MCP-1 (9–76) were tested for their ability to inhibit the transient rise of \( [Ca^{2+}]_i \) induced in monocytes by native RANTES, MCP-3, and MCP-1. As shown in Fig. 7, in all three cases the truncated analogs inhibited the \( [Ca^{2+}]_i \) rise induced by the native chemokines. The most potent effect was observed with RANTES (9–68), which at 30 nM totally prevented the \( [Ca^{2+}]_i \) changes induced by 10 nM RANTES. For inhibition of the responses to MCP-3 and MCP-1, markedly higher concentrations of the corresponding truncated analogs were required. At high concentration (1.000 nM), all truncated analogs also attenuated or even prevented the \( [Ca^{2+}]_i \) changes induced by either of the three chemokines (Fig. 8). Interestingly, RANTES (9–68) markedly decreased the \( [Ca^{2+}]_i \) induced by MCP-3 and MCP-1. In contrast, desensitization experiments indicate that native RANTES does not interact with the receptors that mediate MCP-1 responses in monocytes (17).

**DISCUSSION**

Antagonists for three CC chemokines, RANTES, MCP-3, and MCP-1, were identified and further characterized to determine their specificity as inhibitors of chemokine functions and correlate this with their selectivity for receptor binding sites. These antagonists, RANTES (9–68), MCP-3 (10–76), and MCP-1 (9–76) were selected because of their high affinity for binding sites on monocyteic cells.

The affinity of full-length RANTES for MCP-3 and MCP-1 binding sites was marginal at the highest concentration tested. However, the binding affinity of RANTES (9–68) to sites for both was considerably higher. MCP-3 differed in that it bound to cellular sites for all three ligands with comparable affinity. Furthermore, the MCP-3 antagonist also bound to these sites, but it did not have high affinity for any of the chemokine binding sites, including MCP-3. MCP-1 had high affinity for MCP-1 sites but competed only very weakly for RANTES or MCP-3 binding sites. However, truncation of MCP-1, unlike RANTES, did not reveal a significant change in affinity for MCP-3 and RANTES sites.

The ability to inhibit function correlated with the binding data. The truncated analogs of RANTES or MCP-3 blocked the activities of all three chemokines. The inhibitory potency of RANTES (9–68) for MCP-3 and MCP-1 activity was lower than for native RANTES. The MCP-3 antagonist had the same broad specificity as the parent agonist, but overall it did not have high potency. Subsequent experiments indicated that MCP-3 (9–76) was 2–3-fold more potent than MCP-3 (10–76) in its inhibition of function. Thus, as was the case for MCP-1 (23), the MCP-3 (9–76) analog was the optimal truncated antagonist (not shown). When the inhibitory activities of RANTES (9–68) and MCP-3 (10–76) are compared, the RANTES analog was more potent for RANTES, the MCP-3 analog was more potent for MCP-1, and both inhibited MCP-3. On average, for all three chemokines, the RANTES (9–68) was the most effective antagonist.

The results for binding and inhibition of function were consistent with the data for desensitization of calcium induction. The truncated analogs attenuated or blocked the calcium response to all three ligands with the RANTES (9–68) being the most effective. This suggests a probable link between the equilibrium binding, the inhibition of intracellular signaling events, and the inhibition of the functional responses.

RANTES (9–68) was a particularly potent inhibitor of monocyte release activity with only 4 nM required to inhibit 30 nM RANTES by 50%. In contrast, 19 nM (9–68) was required to displace 5 nM of labeled RANTES from THP-1 cells in the receptor binding studies. The reasons for this difference are not known. One possible explanation is that the binding studies were done with THP-1 cells, whereas the release activity was measured on human peripheral blood monocytes, and therefore different receptors may be involved. However, against this is the observation that the inhibition of RANTES-induced migration of both THP-1 cells and monocytes was similar (not shown). THP-1 cells did not show detectable exocytosis in response to chemokines so the release activities could not be compared. In addition, functionally important but low abundance receptors may not be detected in the binding studies.

These findings clearly indicate that the RANTES analog is binding to receptor(s) that are not accessible to native RANTES. Truncation of RANTES has resulted in decreased binding selectivity, which could be due to loss of residues that normally prevent it from binding to other receptors, for example, by a steric hindrance mechanism. For the series of RANTES analogs with between 5 and 9 residues deleted, the affinity for RANTES, MCP-1, and MCP-3 binding sites correlated, suggesting that the determinants of receptor specificity are located within residues 1–6. However, for MCP-1 there was no difference in the selectivity of the full-length or the truncated analog, suggesting that the determinants of its receptor specificity are located elsewhere in the protein. The selectivity and affinity of the antagonists can probably be modified by alterations to residues outside the NH$_2$-terminal motif. Nevertheless, the results clearly demonstrate that the binding selec-
tivity of RANTES can be modified to generate multiple chemokine antagonists.

The observation that RANTES (9–68) has 50-fold higher affinity than RANTES (10–68) demonstrates that residue 9 contributes to the receptor binding of the RANTES antagonist. However, residues within the (10–68) domain also contribute to binding, because the (10–68) analog did bind, but the RANTES (1–9) peptide did not. Analogs that had residues 6–8 of RANTES intact had decreased binding. One possibility is that flexibility of residues 6–8 region results in loss of binding. For all three chemokines 1 or 2 residues NH$_2$-terminal to the first cysteine were required for optimal binding and antagonist activity. In a previous investigation of MCP-1 analogs, we concluded that residues 1–6 were critical for receptor activation and function and residues 7–10 were important for receptor binding. The current findings for RANTES and MCP-3 are consistent with the model for receptor interactions proposed for MCP-1 (23).

Our findings can be interpreted in terms of interactions with known receptor proteins. The RANTES response could be due to interactions with three known RANTES receptors, CKR-1, CKR-3, or K5–5. The truncated RANTES analog is likely to be also interacting with the CKR-2 protein, which has been shown to bind MCP-1 (14) and MCP-3 (15) but not RANTES (14). MCP-3 also binds to CKR-1 (12, 13), which is well known to be a receptor for RANTES (9–11). Thus the observations with both MCP-3 and the MCP-3 antagonist could be explained by their interaction with these two receptors. MCP-1 and MCP-1 (9–76) responses are likely to be due to interactions with the CKR-2 receptor. The weak binding to RANTES binding sites may be due to low level cross-reactivity with CKR-2 or binding to K5–5. It is possible that not all the receptors on monocytes are represented on THP-1 cells, and therefore some receptor interactions were not included in our binding studies. Conversely, these antagonists may also interact with receptors that are only found on other cell types.

An argument could be made that a general chemokine antagonist would be the most effective anti-inflammatory molecule. Inflammation involves the infiltration of multiple cell types that is likely to occur through the interaction of different chemokines with distinct functional receptors. Blocking the infiltration of multiple effector cells could be highly effective in breaking the inflammatory cycle. Further experiments are required to test this idea. It remains to be determined which other chemokines are inhibited and to which receptor proteins they bind. Our aim was to correlate binding with inhibition of chemokine-induced function, therefore we used chemokine-responsive cells for the binding studies rather than cell lines engineered to over-express single receptors. However, to determine which receptors the antagonists bind, experiments with individual receptors are required. It will be important to know the effects of the antagonists on other cells such as basophils.
eosinophils, and T lymphocytes. The chemokine antagonists described here are potent enough to demonstrate their effects in disease models and thus establish a precedent for the usefulness of chemokine receptor antagonists as anti-inflammatory agents.

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REFERENCES
1. Furie, M. B., and Randolph, G. J. (1995) Am. J. Pathol. 146, 1287–1301
2. Baggiolini, M., Dewald, B., and Moser, B. (1994) Advances Immunol. 55, 97–179
3. Schall, T. J., and Bacon, K. B. (1994) Curr. Opin. Immunol. 6, 865–873
4. Kelvin, D. J., Michiel, D. F., Johnston, J. A., Lloyd, A. R., Sprenger, H., Oppenheim J. J., and Wang, J.-M. (1993) J. Leukocyte Biol. 54, 604–612
5. Lodì, P. J., Garrett, D. S., Kuszewski, J., Tsang, M. L.-S., Weatherbee, J. A., Leonard, W. J., Gronenborn, A. M., and Clore, G. M. (1994) Science 263, 1762–1767
6. Skelton, N. J., Aspiras, F., Ogez, J., and Schall, T. J. (1995) Biochemistry 34, 5329–5342
7. Clore, G. M., Appella, E., Yamada, M., Matsushima, K., and Gronenborn, A. M. (1990) Biochemistry 29, 1689–1696
8. Clark-Lewis, I., Kim, K.-S., Rajarathnam, K., Gong, J.-H., Dewald, B., Moser, B. M., Baggiolini, M., and Sykes, B. D. (1995) J. Leukocyte Biol. 57, 703–711
9. Gao, J. L., Kuhns, D. B., Tiffany, H. L., McDermott, D., Li, X., Francke, U., and Murphy, P. M. (1993) J. Exp. Med. 177, 1421–1427
10. Nedić, K., DiGregorio, D., Mak, J., Y., Horuk, R., and Schall, T. J. (1993) Cell 72, 415–425
11. Nomura, H., Nielsen, B. W., and Matsushima, K. (1993) Int. Immunol. 5, 1239–1249
12. Combadiere, C., Ahuja, S. K., and Murphy, P. M. (1995) J. Biol. Chem. 270, 16491–16494
13. Ben-Baruch, A., Xu, L., Young, P. R., Bengail, K., Oppenheim, J. J., and Wang, J. M. (1995) J. Biol. Chem. 270, 22123–22128
14. Chao, I. F., Myers, S. J., Herman, A., Franci, C., Connolly, A. J., and Coughlin, S. R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2752–2756
15. Franci, C., Wong, L. M., Van Damme, J., Proost, P., and Chao, I. F. (1995) J. Immunol. 154, 6511–6517
16. Power, C. A., Meyer, A., Nemeth, K., Bacon, K. B., Hoogewerf, A. J., Proudfoot, A. E. I., and Wells, T. N. C. (1995) J. Biol. Chem. 270, 19495–19500
17. Ugucioni, M., D’Apuzzo, M., Loetscher, M., Dewald, B., and Baggiolini, M. (1995) Eur. J. Immunol. 25, 68–68
18. Sozzani, S., Molino, M., Locati, M., Luini, W., Cerletti, C., Vecchi, A., and Mantovani, A. (1993) J. Immunol. 150, 1544–1553
19. Wang, J. M., McVicar, D. W., Oppenheim, J. J., and Kelvin, D. J. (1993) J. Exp. Med. 177, 699–705
20. Dahinden, C. A., Geiser, T., Brunner, T., von Tscharner, V., Caput, D., Ferrara, P., Minty, A., and Baggiolini, M. (1994) J. Exp. Med. 179, 751–756
21. Van Riper, G., Siciliano, S., Fischer, P. A., Meurer, R., Springer, M. S., and Rosen, H. (1993) J. Exp. Med. 177, 851–856
22. Baggiolini, M., and Dahinden, C. A. (1994) Immunol. Today 15, 127–133
23. Gong, J.-H., and Clark-Lewis, I. (1995) J. Exp. Med. 181, 631–640
24. Zhang, Y. J., Rutledge, B. J., and Rollins, B. J. (1994) J. Biol. Chem. 269, 15918–15924
25. Van Damme, J., Proost, P., Lenaerts, J.-P., and Opdenakker, G. (1992) J. Exp. Med. 176, 59–65
26. Schall, T. J., Bacon, K., Toy, K. J., and Goeddel, D. V. (1990) Nature 347, 669–671
27. Clark-Lewis, I., Moser, B., Walz, A., Baggiolini, M., Scott, G. J., and Aebersold, R. (1991) Biochemistry 30, 3128–3125
28. Clark-Lewis, I., Dewald, B., Loetscher, M., Moser, B., and Baggiolini, M. (1994) J. Biol. Chem. 269, 16075–16081
29. Colotta, F., Peri, G., Villa, A., and Mantovani, A. (1984) J. Immunol. 132, 930–944
30. Calvo, J. C., Radieleni J. P., and Charrueau, E. H. (1983) Biochem. J. 215, 259–264
31. Schall, T. J., Ongsttra, J., Dyer, B. J., Jorgenisen, J., Clayboriger, C., Davis, M. M., and Krenskey, A. M. (1988) J. Immunol. 141, 1018–1025
32. Opdenakker, G., Froyen, G., Fiten, P., Proost, P., and Van Damme, J. (1993) Biochem. Biophys. Res. Commun. 191, 535–542
33. Yoshimura, T., Yuki, N., Moore, S. K., Appella, E., Lerman, M. I., and Leonard, E. J. (1989) FEMS Let. 244, 487–493
34. Furutani, Y., Nomura, H., Notake, M., Oyamada, Y., Fukui, T., Yamada, M., Larsen, C. G., Oppenheim, J. J., and Matsushima, K. (1989) Biochem. Biophys. Res. Commun. 159, 249–255
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