Monocyte Chemotactic Protein-2 (MCP-2) Uses CCR1 AND CCR2B as Its Functional Receptors*

(Received for publication, October 23, 1996, and in revised form, March 10, 1997)

Xiaoqi Gong‡, Wanghua Gong‡, Douglas B. Kuhns§, Adit Ben-Baruch‡, O. M. Zack Howard, and Ji Ming Wang¶

From the Intramural Research Support Program and §Clinical Immunology Services, SAIC Frederick and the ¶Laboratory of Molecular Immunoregulation, Division of Basic Sciences, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702.

Monocyte chemotactic protein (MCP)-2 is a member of the C-C chemokine subfamily, which shares more than 60% sequence homology with MCP-1 and MCP-3 and about 30% homology with macrophage inflammatory protein (MIP)-1α, regulated on activation of normal T cell expressed (RANTES), and MIP-1β. Despite this considerable sequence homology to other C-C chemokines, MCP-2 appears to have unique functional properties in comparison with other C-C chemokines such as MCP-1 and MCP-3. Although evidence obtained from studies on leukocytes suggested that MCP-2 may share the receptors with these C-C chemokines, the actual functional receptors for MCP-2 have not yet been identified. In this study, by using radioiodinated MCP-2, we identified high affinity binding sites for MCP-2 on human peripheral blood monocytes. The MCP-2 binding was competed by for MCP-1 and MCP-3, but less well by MIP-1α or RANTES. In experiments using cells transfected with C-C chemokine receptors, 125I-MCP-2 bound to human embryonic kidney 293 cells transfected with CCR1 or CCR2B, known to also bind MIP-1α/RANTES and MCP-1, respectively, but both shared by MCP-3. The binding of 125I-MCP-2 to these receptor-transfected cells was displaced completely by chemokines that bind to these receptors. Both CCR1- and CCR2B-transfected 293 cells showed significant migration in response to MCP-2, in addition to responding to other specific chemokines. These results clearly demonstrate that MCP-2, unlike MCP-1, uses both CCR1 as well as CCR2B as its functional receptors, and this accounts for the unique activities of MCP-2.

Monocyte chemotactic protein (MCP)1-2 is a C-C chemokine co-purified with MCP-1 and MCP-3 from human osteosarcoma cells (1, 2). It shares over 60% amino acid identity with MCP-1 and MCP-3 and has about 30% identity with other C-C chemokines MIP-1α, RANTES, and MIP-1β (1, 2 and reviewed in Ref. 3). MCP-2 is chemotactic for and activates a wide variety of inflammatory cells, and its spectrum of action on leukocytes is similar to that of MCP-3, including monocytes, T lymphocytes, NK cells, basophils, mast cells, and eosinophils (1, 3–9), but differs from MCP-1, which is not active on eosinophils (10). Based on cross-desensitization of calcium mobilization in leukocyte subsets by different chemokines, and the competition by MCP-2 for binding sites of MCP-1 and MIP-1α on monocytes, MCP-2 has been proposed to interact with multiple C-C chemokine receptors, including those used by MCP-1 and MCP-3 (6, 7). However, since leukocytes express a multiplicity of chemokine receptors with promiscuous binding and functional properties (reviewed in Ref. 11), it is difficult to identify the receptors used by a given ligand on these cells. A number of C-C and C-X-C chemokine receptors have been cloned and functionally expressed on non-leukocytic cells. It has been shown that CCR1 is a receptor for MCP-3 and MIP-1α/RANTES (12–14, 26), while CCR2B is shared mainly by MCP-1 and MCP-3 (15, 16, 26). Although CCR2B has been reported not to be functional for MCP-2 (16, 26), the conclusion was derived from the failure of synthetic MCP-2 to induce calcium mobilization in CCR2B transfected HEK 293 cells and the inability of MCP-2 to desensitize MCP-1 induced calcium flux (16, 26). Since MCP-2 was a poor inducer of calcium mobilization in monocytes (6), and its chemotactic activity for monocytes was not Pertussis toxin-sensitive, MCP-2 was proposed to use signaling pathways distinct from other C-C chemokines (6). Despite this, MCP-2 was effective in attenuating the MCP-1-induced monocyte migration, through homologous deactivation, suggesting the utilization of the same MCP-1 receptor domains by MCP-2 (6). However, this cannot be clearly established using native cells, but requires the use of cells transfected to express only a single chemokine receptor. In this study, by determining the binding properties of radiolabeled MCP-2 and performing sensitive chemotaxis assays to assess receptor functions, we demonstrate that CCR1 and CCR2B are both functional receptors for MCP-2.

MATERIALS AND METHODS
Chemokines—Recombinant human MCP-2 and other chemokines, including E. coli-derived MCP3, MCP1, MIP1α, MIP1β, and RANTES were purchased from PeproTech Inc. (Rocky Hill, NJ). Radiodinated MCP-2 was a kind gift from Dr. G. Brown (DuPont NEN). Other radiodinated chemokines were purchased from DuPont NEN. All radiodinated chemokines have a specific activity of 2200 Ci/mmol.

Cells—Human peripheral blood monocytes were isolated from normal donors (National Institutes of Health Clinical Center, Transfusion Department, Bethesda, MD) with an iso-osmotic Percoll (Pharmacia Biotech Inc., Uppsala, Sweden) gradient as described elsewhere (17). Cultured monocytes (kind gifts from Dr. I. Espinosa-Gaetano, NCI) were also used in the study. The monocyte preparations were >90% pure as assessed by morphological criteria.

The CCR1 cDNA clone was isolated as described previously from a natural killer-like cell line YT (12). The CCR2B cDNA was a kind gift provided by Dr. I. F. Charo (San Francisco, CA, Ref. 15). The 293 human embryonic kidney epithelial cell line (HEK 293, a gift from Dr. P. Gray, ICOS Corp., Bothell, WA) was grown in monolayers in Dulbecco’s modified Eagle’s medium (BioWhittaker, Inc., Walkersville, MD) with 10% fetal calf serum (Hyclone Laboratories, Logan, UT) and antibiotics. The HEK 293 cells were either transfected with 5–20 µg of CCR1 cDNA by using DOTAP transfection reagent (Boehringer Mannheim) or with CCR2B cDNA by electroporation (1 × 10⁷ cells, 950 microfarads, 0.2
Stable transfectants were established by adding G418 (800 μg/ml; Life Technologies, Inc.) to maintain selection pressure and were designated CCR1/293 or CCR2B/293 cells.

Binding Assays with Radiolabeled Chemokines—Binding assays were performed by using a single concentration of 125I-labeled chemokines in the presence of increasing concentrations of unlabeled ligands as described previously (12, 19). The binding data were then analyzed with a Macintosh computer program LIGAND (P. Munson, Division of Computer Research and Technology, NIH, Bethesda, MD). The rate of competition for binding by unlabeled chemokines was calculated as follows: % competition for binding = 1 - (counts/min obtained in the presence of unlabeled ligand/cpm obtained in the absence of unlabeled ligand) × 100%.

Chemotaxis Assay—Monocyte migration was evaluated by using a 48-well microchamber technique as described (12, 18, 19). The migration of HEK 293 cells transfected with cDNA clones was also assessed by a 48-well microchamber technique (12). Data were presented as the chemotaxis index and were the representative of at least five experiments performed. The chemotaxis index was calculated by: chemotaxis index = number of cells migrating to chemokines/number of cells migrating to medium.

The significance of the difference between test and control groups was analyzed by using the Student’s t test.

RESULTS AND DISCUSSION

Recombinant human MCP-2 (PeproTech, lot number 85411) was highly effective in inducing human monocyte migration in vitro at an optimal concentration range of 6–12 nM (50–100 ng/ml) (data not shown). This recombinant human MCP-2 did not induce significant calcium mobilization in monocytes at concentrations below 60 nM (500 ng/ml) (not shown) as reported previously (3, 6), even though it did induce calcium flux at concentrations of 6–12 nM in a cultured murine T lymphoma cell line (data not shown). 125I-MCP-2, prepared using the recombinant protein of the same lot number, induced monocytes chemotaxis comparable with that of the unlabeled ligand, indicating that radioiodination did not significantly change the molecular structure of the MCP-2 and its capacity to interact with receptors. It is therefore possible to examine whether human leukocytes express specific binding sites for MCP-2 and whether these binding sites are shared by multiple C-C chemokines as suggested by various investigators (6–9). Fig. 1A shows a typical binding profile of 125I-MCP-2 to monocytes with an estimated Kd value of 2 nM. The binding of 125I-MCP-2 to monocytes was efficiently displaced by MCP-2 itself, and also by MCP-1 and MCP-3 (Fig. 1B), two C-C chemokines with high degree of homology with MCP-2 (1–3). MIP-1α and RANTES, which share about 30% homology with MCP-2, only partially displaced 125I-MCP-2 binding (Fig. 1B). MIP-1β and C-X-C chemokine IL-8 did not compete for 125I-MCP-2 binding sites on monocytes. Our data obtained with monocytes support the suggestion that MCP-2 interacts with receptors shared by multiple C-C chemokines (6–9).

Fig. 1. Binding of radiolabeled MCP-2 to human monocytes. Monocytes were incubated with a fixed concentration of radiolabeled MCP-2 (120 pM) in the presence of increasing concentrations of unlabeled ligand. The radioactivity associated with the cells were analyzed using the LIGAND program and plotted as shown in A. B, displacement of 125I-MCP-2 binding to monocytes by unlabeled chemokines. ●, MCP-2; ○, MCP-1; □, MCP-3; ▲, MIP-1α; △, RANTES.

Fig. 2. Binding of radiolabeled MCP-2 and MIP-1α to HEK 293 cells transfected with CCR1 (CCR1/293 cells). A and B, binding isotherms of 125I-MCP-2 (A) and 125I-MIP-1α (B). C, displacement of MCP-2 binding to CCR1/293 cells by unlabeled chemokines. ●, MCP-2; □, MCP-3; ○, MCP-1; ▲, MIP-1α; △, RANTES; ×, MIP-1β.
To identify receptors for MCP-2, we examined the binding and function of recombinant MCP-2 to cells transfected to express C-C chemokine receptors, including CCR1, which is a common receptor for MCP-3 (12, 26) and MIP-1α/RANTES (13, 14), and CCR2B, which is shared by MCP-1 and MCP-3 (15, 16, 26). The parental HEK 293 cells and cells transfected with vector alone did not bind 125I-MCP-2, nor did they migrate in response to any of the C-C chemokines, including MCP-2 (Ref. 12 and data not shown). HEK 293 cells stably transfected with CCR1 (CCR1/293 cells) showed a high level of specific binding for 125I-MCP-2 (Fig. 2A) and also bound radiolabeled MIP-1α (Fig. 2B) and MCP-3 (Ref. 12). The binding affinity of 125I-MCP-2 for CCR1/293 cells is comparable with 125I-MIP-1α ($K_d = 5 \pm 2$ nM and $4 \pm 0.8$ nM, respectively, Fig. 2, A and B), but lower than 125I-MCP-3 ($K_d = 0.7$ nM, Ref. 12). Cross-competition experiments, as shown in Fig. 2C, indicate that MCP-3, MIP-1α, and RANTES are all effective competitors for 125I-MCP-2 binding to CCR1/293 cells with $IC_{50}$ values of about 5 nM, comparable with MCP-2 itself. MIP-1β had very limited effect. To our surprise, MCP-1, which was a weak competitor for MCP-3, or MIP-1α/RANTES binding to CCR1/293 cells (12, 13), displaced rather effectively the 125I-MCP-2 binding to CCR1/293 cells (Fig. 2C), suggesting MCP-2 may share certain binding domains on CCR1 that react with MCP-1. In fact, MCP-1 at high concentrations did induce signaling in CCR1 (13) and partially displaced MCP-3 binding (12). Unlabeled MCP-2 fully displaced radiolabeled MIP-1α and RANTES binding to CCR1/293 cells (not shown). However, MCP-2 was a less potent competitor for binding of radiolabeled MIP-3 to CCR1/293 cells with an $IC_{50}$ 10 times higher than that of MCP-3 itself (15 nM versus 1.5 nM, not shown), in agreement with the relatively lower binding affinity of MCP-2 to CCR1/293 cells than MCP-3.

Our efforts to obtain reproducible calcium mobilization in chemokine receptor-transfected 293 cells were not successful. We therefore utilized chemotaxis assays as a functional indicator, which has been shown to be a specific, sensitive, and physiologically relevant method of assessing the action of chemokines on their receptors (12, 20, 21). Fig. 3A shows that MCP-2 induced significant migration of CCR1/293 cells in vitro. The MCP-2 concentrations required to elicit optimal cell response ranged from 6 to 12 nM (50–100 ng/ml), comparable with those of MIP-1α and RANTES (12), but higher than effective MCP-3 concentrations (12). A complete cross-attenuation of migration of CCR1/293 cells was achieved by placing equal concentrations of MCP-2, MCP-3, or MIP-1α in upper and/or lower wells of the chemotaxis chamber (not shown).

The capacity of MCP-2 to compete for MCP-1 binding sites and cross-desensitize one another on native leukocytes pointed to the possibility that MCP-2 also interacted with MCP-1 binding sites (6–9). This is supported by the binding data of 125I-MCP-2 to monocytes obtained in this study. Previous study showed no effect of MCP-2 on CCR2B/293 cells based on the inability of MCP-2 to induce calcium flux in these cells and to desensitize the effect of MCP-1 (16, 26). However, in this study, we observed that 125I-MCP-2 did specifically bind to CCR2B/293 cells ($3 \pm 1$ nM, Fig. 4A), although the binding affinity of 125I-MCP-2 to CCR2B/293 cells was relatively lower than that of 125I-MCP-1 ($0.5 \pm 0.1$ nM, Fig. 4B). 125I-MCP-2 binding was fully displaced by both MCP-1 and MCP-3 (Fig. 4C), but only slightly by MIP-1α (Fig. 4C), not by RANTES or MIP-1β (not shown). Unlabeled MCP-2 was able to completely displace the binding of 125I-MCP-3 on CCR2B/293 cells with slightly higher $IC_{50}$ (5 versus 3 nM) than MCP-3 itself. However, unlabeled MCP-2 appeared to be a poor competitor ($IC_{50} > 50$ nM) for 125I-MCP-1 binding to CCR2B/293 cells. This may be explained by a lower affinity of MCP-2 binding to CCR2B/293 cells than MCP-1. Or alternatively, there exists differential occupancy of binding domains on CCR2B by MCP-1 and MCP-2. Studies on the binding pattern of C-X-C chemokines, IL-8, and NAP-2, on IL-8 receptor type B (CXCR2) showed that IL-8 completely displaced NAP-2 binding, whereas IL-8 binding was only partially displaced by NAP-2 (21), even though both ligands are functional for CXCR2. Computer modeling suggested that IL-8 possesses multiple binding domains, only some of these may be shared by NAP-2 (21).

We further examined the function of MCP-2 on CCR2B/293 cells by its capacity to induce directional cell migration. CCR2B/293 cells showed highly reproducible and potent chemotactic responses to MCP-2 as well as to MCP-1 and MCP-3 (Fig. 3B). Furthermore, the chemotactic activity of these three MCPs for CCR2B/293 cells was attenuated by each of the ligands (not shown), in agreement with previously reported results obtained in monocytes (6). However, at least 5-fold more MCP-2 was required to completely attenuate the migration of CCR2B/293 cells to MCP-1, in correlation with the binding competition pattern (see above). Thus our binding and chemotaxis data indicate that CCR2B is also a functional receptor for MCP-2 and the functional domains on CCR2B differentially interact with MCP-2 and MCP-1.

The significance of MCP-2 production in pathophysiological conditions has yet to be defined (3). MCP-2 is constitutively expressed in tumor cells and is inducible by proinflammatory cytokines in mononuclear cells and fibroblasts (1–3). MCP-2, like MCP-3, exhibits a broader spectrum of targeted cells, including cells of dendritic phenotype as we recently reported.
of cell migration is the same. Our current data obtained with CCR2B/293 cells again show that chemotaxis assay provides a powerful approach to identify the ligand-receptor interaction with 293 cells transfected to express a single chemokine receptor, as we have reported previously for both C-C and C-X-C chemokines (12, 20, 21).

Although there is an apparent redundancy in the binding and function of chemokine and receptor family (for reviews, see Refs. 11, 23, and 24), C-C chemokines have been implicated to be important mediators of many pathological conditions such as chronic inflammation, immune diseases, neoplasia, and atherosclerosis (11, 23, 24). Recently, several chemokine receptors have been reported to function as HIV-1 fusion co-factors, and some chemokines were able to interfere with the viral replication (reviewed in Ref. 25). Investigation into the shared and unique functional domains on both chemokine ligands and receptors will prove important in the development of therapeutic approaches to chemokine- and chemokine receptor-mediated pathology.

Acknowledgments—We thank Dr. Joost J. Oppenheim for his critical review of this manuscript and Dr. I. F. Charo (Gladdstone Institute of Cardiovascular Disease, San Francisco, CA) for providing us with the CCR2B cDNA. The radiolabeled MCP-2 was a kind gift from Dr. G. Brown of the DuPont NEN. The technical support from K. Bengali and the secretarial assistance by Ms. T. Covell and Ms. C. Fogel are gratefully appreciated. X. G. was supported in part by a fellowship from The Office of the International Affairs, NCI.

REFERENCES

1. Van Damme, J., Proost, P., Lenaerts, J.-P., and Opdenkogel, K. (1992) J. Exp. Med. 176, 59–65
2. Decock, B., Conings, R., Lenaerts, J.-P., Biliou, A., and Van Damme, J. (1990) Biochim. Biophys. Acta 1019, 117–124
3. Proost, P., and Van Damme, J. (1996) J. Leukocyte Biol. 59, 67–74
4. Taub, D. D., Proost, P., Murphy, W. J., Anver, M., Longo, D. L., Van Damme, J., and Oppenheim, J. J. (1995) J. Clin. Invest. 95, 1370–1376
5. Alleva, P., Bianchi, O., Zhou, D., Van Damme, J., Jilek, P., Sozzani, S., and Mantovani, A. (1994) Eur. J. Immunol. 24, 3233–3236
6. Sozzani, S., Zhou, D., Locati, M., Rieppi, M., Proost, P., Magazin, M., Vita, N., Van Damme, J., and Mantovani, A. (1994) J. Immunol. 152, 3615–3622
7. Ugucioni, M., DiPiazza, M., Luscher, F., Bignall, B., Bagnoli, G., and Charo, I. F. (1996) Eur. J. Immunol. 26, 64–68
8. Weber, M., Ugucioni, M., Ochsenberger, B., Bagnoli, G., Clark-Lewis, I., and Cahn, D. A. (1995) J. Immunol. 154, 4166–4172
9. Alam, R., Fosythe, P., Stafford, S., Heinrich, P., Bravo, J., Proost, P., and Van Damme, J. (1994) J. Immunol. 153, 3155–3159
10. Dahinden, C. A., Geiser, T., Brunner, T., von Thaer, V., Caput, D., Ferrara, P., Muntz, A., and Bagnoli, G. (1994) J. Exp. Med. 178, 753–756
11. Murphy, P. M. (1996) Cytokine Growth Factor Rev. 7, 47–64
12. Franci, C., Wong, L. M., Van Damme, J., Proost, P., and Charo, I. F. (1995) J. Immunol. 154, 6511–6517
13. Colotta, F., Bersani, L., Landazuri, A., Poli, G., and Mantovani, A. (1988) J. Immunol. 140, 5354–5358
14. Falk, W., Goodwin, R. H., Jr., and Leonard, E. J. (1987) J. Immunol. 138, 1599–1608
15. Xu, L., Proost, P., and Charo, I. F. (1995) J. Leukocyte Biol. 57, 259–264
16. Colotta, F., Bersani, L., Landazuri, A., Poli, G., and Mantovani, A. (1988) J. Immunol. 140, 5354–5358
17. Falk, W., Goodwin, R. H., Jr., and Leonard, E. J. (1987) J. Immunol. 138, 1599–1608
18. Xu, L., Proost, P., and Charo, I. F. (1995) J. Leukocyte Biol. 57, 259–264
19. Xu, L., Proost, P., and Charo, I. F. (1995) J. Leukocyte Biol. 57, 259–264
20. Ben-Baruch, A., Bengali, K., Tani, K., Xu, L., Oppenheim, J. J., and Wang, J. M. (1996) J. Biol. Chem. 271, 22123–22128
21. Neste, K., DiGregorio, D., Mak, J. Y., Horuk, R., and Schall, T. J. (1995) Cell 72, 415–425
22. Gao, J.-L., Kuhns, D. B., Tiffany, H. L., McDermot, D., Li, X., Francke, U., and Murphy, P. M. (1993) J. Exp. Med. 177, 1421–1427
23. Charo, J. F., Myers, S. J., Herman, A., Franzi, C., Connolly, A. J., and Coughlin, S. R. (1994) Proc. Natl. Acad. Sci. U. S. A 91, 2752–2756
24. Franci, C., Wong, L. M., Van Damme, J., Proost, P., and Charo, I. F. (1995) J. Immunol. 154, 6511–6517
25. Colotta, F., Bersani, L., Landazuri, A., Poli, G., and Mantovani, A. (1988) J. Immunol. 140, 5354–5358
26. X. G. was supported in part by a fellowship from The Office of the International Affairs, NCI.
Monocyte Chemotactic Protein-2 (MCP-2) Uses CCR1 AND CCR2B as Its Functional Receptors
Xiaoqi Gong, Wanghua Gong, Douglas B. Kuhns, Adit Ben-Baruch, O. M. Zack Howard and Ji Ming Wang

J. Biol. Chem. 1997, 272:11682-11685.
doi: 10.1074/jbc.272.18.11682

Access the most updated version of this article at http://www.jbc.org/content/272/18/11682

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 26 references, 12 of which can be accessed free at http://www.jbc.org/content/272/18/11682.full.html#ref-list-1