Distinct Signaling Pathways for MCP-1-dependent Integrin Activation and Chemotaxis*

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Transmigration of monocytes to the subendothelial space is the initial step of atherosclerotic plaque formation and inflammation. Integrin activation and chemotaxis are important functions involved in monocyte transmigration. To delineate the signaling cascades leading to integrin activation and chemotaxis by monocyte chemoattractant protein-1 (MCP-1), we have investigated the roles of MAPK and Rho GTPases in THP-1 cells, a monocytic cell line. MCP-1 stimulated β1 integrin-dependent, but not β2 integrin-dependent cell adhesion in a time-dependent manner. MCP-1-mediated cell adhesion was inhibited by a MEK inhibitor but not by a p38-MAPK inhibitor. In contrast, MCP-1-mediated chemotaxis was inhibited by the p38-MAPK inhibitor but not by the MEK inhibitor. The inhibitor of Rho GTPase, C3 exoenzyme, and a Rho kinase inhibitor abrogated MCP-1-dependent chemotaxis but not integrin-dependent cell adhesion. Further, C3 exoenzyme and the Rho kinase inhibitor blocked MCP-1-dependent p38-MAPK activation. These data indicate that ERK is responsible for integrin activation, that p38-MAPK and Rho are responsible for chemotaxis mediated by MCP-1, and that Rho and the Rho kinase are upstream of p38-MAPK in MCP-1-mediated signaling. This study demonstrates that two distinct MAPKs regulate two dependent signaling cascades leading to integrin activation and chemotaxis induced by MCP-1 in THP-1 cells.

Several lines of evidence indicate that monocyte chemoattractant protein-1 (MCP-1) is involved in the pathogenesis of atherosclerosis by promoting directed migration of inflammatory cells, such as monocytes and T lymphocytes (1, 2). During the progression of atherosclerosis, there is an accumulation of low-density lipoprotein within macrophages present in the intimal layer. Deposition of lipids within these cells leads to the formation and eventual enlargement of atherosclerotic lesions. Boring et al. (3) noted an overall decrease in atherosclerotic lesion size in mice deficient for the MCP-1 receptor, CCR2, when they are crossed with ApoE knockout mice. Gu et al. (4) also found decreased atherosclerotic lesions in MCP-1-deficient mice when they are crossed with the low-density lipoprotein receptor knockout mice. These studies have demonstrated that MCP-1 and CCR2 play a crucial role in the initiation of atherosclerosis by recruiting monocytes to the vessel wall.

According to the multistep theory, monocytes roll on the endothelial cells, interact with E-selectin, adhere to the endothelial cells by firm adhesion to ICAM-1 and VCAM-1, and then migrate into the subendothelium (5). Rolling of monocytes on endothelial cells is dependent on the binding of E-selectin and sialyl Lewis X, and adhesion to the endothelium is dependent on the interaction of integrins on monocytes and adhesion molecules on the endothelial cells, such as VCAM-1 and ICAM-1. Integrins consist of several subtypes, and each subtype is specific for its ligand. For example, α4β1 integrin, very late antigen-4, binds to VCAM-1, and β2 integrins bind to ICAM-1. Fibronectin, one of the extracellular matrix proteins, is also known to bind to β1 integrins, mainly to α5β1 integrin. In these serial events of the multistep theory, MCP-1 can play a key role in monocyte recruitment by both integrin activation and by promoting migration to the vessel wall. However, the signal transduction pathways leading to integrin activation and chemotaxis have not been fully elucidated.

We recently demonstrated that the βγ subunit of heterotrimeric G protein, Gi, plays a key role in MCP-1-induced chemotaxis (6). In that study, we reported that activation of ERK was not involved in chemotaxis by MCP-1. MAPK family members, ERK, JNK, and p38-MAPK, have been implicated in events necessary for proliferation, differentiation, apoptosis, and certain kinds of stress responses (7). These MAPKs are activated by specific cascades responsible for certain stimuli and eventually induce a variety of cell responses. Recently, several groups have reported on the involvement of MAPK and Rho in chemotaxis (8, 9). Most of the studies on signal transduction of chemotaxis and cell adhesion have been conducted in adherent cells. However, in adherent cells it would be difficult to separate chemotaxis and integrin activation, because these two functions are closely connected. Therefore, the aim of our study was to examine whether integrin activation and chemotaxis can be separated by studying the signaling cascades leading to integrin activation and chemotaxis mediated by MCP-1 in human monocytic THP-1 cells and to elucidate the role of MAPK and Rho in these biological functions.

EXPERIMENTAL PROCEDURES
Reagents—RPMI medium was obtained from Nissui Pharmaceuticals Co. Ltd. (Tokyo, Japan). Fetal calf serum was purchased from Grand Cayman (British West Indies). L-Glutamine and penicillin/strepto-
tomycin were obtained from Bio Whittaker (Walkersville, MD). Recombinant human MCP-1 was obtained from PeproTech EC Ltd. (London, England). Recombinant human soluble VCAM-1, ICAM-1, and E-selectin were from Genzyme/Techne (Minneapolis, MN). Fibronectin, BSA, arginine-glycine-aspartate-serine (RGDS) peptides, and arginine-glycine-glutamate-serine peptides were from Sigma. Anti-human α4 (very late antigen-4) antibody was from Upstate Biotechnology (Lake Placid, NY). PD98059 and SB203580 were from Calbiochem. C3 exoenzyme was a generous gift from Dr. S. Narumiya (Kyoto University). A Rho kinase inhibitor, Y-27632, was a generous gift from Welfide Corporation (Osaka, Japan).

Cell Lines—The monocytic cell line THP-1 was a generous gift from Dr. K. Nishida (Daiichi Pharmaceuticals Co. Ltd., Tokyo, Japan) and was cultured in RPMI supplemented with L-glutamine and penicillin/streptomycin plus 10% fetal calf serum in an atmosphere of 95% air and 5% CO₂ at 37 °C.

Cell Adhesion Assay—Polystyrene 96-well flat-bottomed microtiter plates (Costar 3595, Corning Incorporated, Corning, NY) were coated with 25 μl of soluble VCAM-1 (2.5 μg/ml), soluble ICAM-1 (2.5 μg/ml), soluble E-selectin (2.5 μg/ml), or fibronectin (10 μg/ml) for 1 h at room temperature. After incubation, wells were blocked by incubation with 225 μl of 10 mg/ml heat-denatured BSA for 30 min at room temperature. Control wells were filled with 10 mg/ml heat-denatured BSA. 100 μl of THP-1 cells suspended at a concentration of 10⁶/ml in 0.1% BSA-RPMI were incubated for the indicated times in a CO₂ incubator at 37 °C in the presence or absence of MCP-1. After incubation nonadherent cells were removed by centrifugation (top side down) at 48 × g for 5 min (11). Attached cells were fixed with 5% glutaraldehyde for 30 min at room temperature. Cells were washed three times with water, and 100 μl of 0.1% crystal violet in 200 mM MES (pH 6.0) was added to each well and incubated at room temperature for 20 min. Excess dye was removed by washing with water three times, and the bound dye was solubilized with 100 μl of 10% acetic acid (12, 13). The absorbance of each well at 595 nm was then measured using a multiscan enzyme-linked immunosolvent assay reader (SPECTRA classic; TECAN). Each sample was assayed in triplicate. The absorbance was linear to the cell number up to an OD of 1.9 (data not shown). For example, 0.05 of OD represents adhesion of about 2,000 cells, and 0.5 of OD represents adhesion of about 25,000 cells.

Chemotaxis Assay—The migration of THP-1 cells was determined using a modification of the method of Campbell et al. (14). Briefly, THP-1 cells were resuspended in 0.1% BSA-RPMI. After adjusting the cell density to 1 × 10⁶ cells/ml, 100,000 cells in 100 μl were added to the top chamber of a 24-transwell apparatus (6.5-mm diameter, 5-μm pore...
MCP-1 Increased Adhesion of THP-1 Cells to VCAM-1 and Fibronectin—To determine the regulation of integrin avidity by MCP-1, we studied adhesion of THP-1 cells to purified adhesion molecules. Cell adhesion to soluble E-selectin, soluble ICAM-1, and soluble VCAM-1 was determined in the presence or absence of 10 nM MCP-1 under static conditions. MCP-1 increased adhesion of THP-1 cells to VCAM-1 by more than 2-fold but not to E-selectin or ICAM-1 (Fig. 1), indicating increased avidity of the β1 integrin by MCP-1. In the absence of MCP-1, more THP-1 cells adhered to VCAM-1 than control. In time course experiments, we also examined cell adhesion to fibronectin as a ligand for α5β1 integrin and found that MCP-1 increased cell adhesion to both VCAM-1 and fibronectin by more than 3-fold in a time-dependent manner (Fig. 2). MCP-1-dependent adhesion to VCAM-1 was also increased in a dose-dependent manner, reaching a plateau at 1 nM MCP-1 (Fig. 3). Adhesion to fibronectin was also increased by MCP-1 stimulation in a dose-dependent manner (data not shown). To show that this MCP-1-mediated adhesion is dependent on e4β1 and α5β1 integrins, we preincubated the cells with anti-α4 antibody and the RGDS peptide. Preincubation of THP-1 cells with anti-α4 antibody inhibited MCP-1-dependent and -independent cell adhesion to VCAM-1 by about 80% but not with control IgG. Preincubation with the RGDS peptide, but not with the RGES peptide, inhibited MCP-1-dependent and -independent cell adhesion to fibronectin (Fig. 4). These data indicate that cell adhesion in our assay depends on the interaction between integrins and their ligands and that MCP-1 increased the avidity of both e4β1 and α5β1 integrins on THP-1 cells.

Inhibition of ERK but Not p38-MAPK Abrogated MCP-1-induced Adhesion—To determine whether MAPK activation is involved in MCP-1-mediated integrin activation, we next pretreated the cells with MEK- or p38-MAPK-specific inhibitors and examined the effect of ERK and p38-MAPK on MCP-1-dependent and -independent cell adhesion to VCAM-1 and fibronectin. As shown in Fig. 6, MEK inhibition (PD98059) blocks MCP-1-dependent adhesion to VCAM-1 and fibronectin in a dose-dependent manner. MCP-1 increased adhesion of THP-1 cells to VCAM-1 by more than 2-fold but not to E-selectin or ICAM-1 (Fig. 1), indicating increased avidity of the β1 integrin by MCP-1. In the absence of MCP-1, more THP-1 cells adhered to VCAM-1 than control. In time course experiments, we also examined cell adhesion to fibronectin as a ligand for α5β1 integrin and found that MCP-1 increased cell adhesion to both VCAM-1 and fibronectin by more than 3-fold in a time-dependent manner (Fig. 2). MCP-1-dependent adhesion to VCAM-1 was also increased in a dose-dependent manner, reaching a plateau at 1 nM MCP-1 (Fig. 3). Adhesion to fibronectin was also increased by MCP-1 stimulation in a dose-dependent manner (data not shown). To show that this MCP-1-mediated adhesion is dependent on e4β1 and α5β1 integrins, we preincubated the cells with anti-α4 antibody and the RGDS peptide. Preincubation of THP-1 cells with anti-α4 antibody inhibited MCP-1-dependent and -independent cell adhesion to VCAM-1 by about 80% but not with control IgG. Preincubation with the RGDS peptide, but not with the RGES peptide, inhibited MCP-1-dependent and -independent cell adhesion to fibronectin (Fig. 4). These data indicate that cell adhesion in our assay depends on the interaction between integrins and their ligands and that MCP-1 increased the avidity of both e4β1 and α5β1 integrins on THP-1 cells.

Inhibition of ERK but Not p38-MAPK Abrogated MCP-1-induced Adhesion—To determine whether MAPK activation is involved in MCP-1-mediated integrin activation, we next pretreated the cells with MEK- or p38-MAPK-specific inhibitors and examined the effect of ERK and p38-MAPK on MCP-1-de-
CO2 at 37 °C. After incubation, cells were subjected to chemotaxis indicated concentrations for 1 h in an atmosphere of 95% air and 5% CO2 at 37 °C. After incubation, cells were subjected to chemotaxis assays as described under “Experimental Procedures.” The concentrations of MCP-1 in the lower chamber were 0 (closed columns) and 1 nM (open columns). Data represent the mean ± S.D. of duplicate measurements. Results are representative of six separate experiments.

Next, to examine the role of Rho GTPase and the Rho kinase, we pretreated the cells with C3 exoenzyme and Y-27632 on MCP-1-induced chemotaxis of THP-1 cells. However, pretreatment of the cells with C3 exoenzyme and Y-27632 abrogated MCP-1-induced phosphorylation of p38-MAPK but not of ERK (Fig. 8).

DISCUSSION

In this study we have elucidated the role of MAPK and Rho GTPase in MCP-1-mediated cell adhesion and chemotaxis. We show that MCP-1 induced activation of the integrins α4β1 and α5β1 in THP-1 cells and that the integrin activation is dependent on ERK activation. In contrast, MCP-1-dependent chemotaxis was dependent on activation of Rho and p38-MAPK. Thus, as depicted in Fig. 10 two important biological functions mediated by MCP-1 utilize two distinct MAPK-dependent signaling pathways.

In this study we used sensitive cell adhesion assays to demonstrate important functions of MCP-1. Although Weber et al. (16) demonstrated that binding of monocytes to VCAM-1 was reduced at 15 min under stimulation with MCP-1 in a similar assay, we found greater than a 2-fold increase in cell adhesion to this molecule at 10 to 20 min. In preliminary experiments,
we have found a basal increase in cell adhesion after labeling the cells with fluorescent dye and washing the cells. We speculate that this is because of some stress on the cells. Further, a basal increase in cell adhesion after spinning down and washing the cells might be because of increased MAPK activation during these procedures. In support of this hypothesis, MacKenna et al. (17) reported that in cardiac fibroblasts ERK and JNK are activated by mechanical stretch. Therefore, it would be important to avoid stress on the cells as much as possible in this experiment. Work is now in progress to determine the effect of mechanical stress on MAPK activation and cell adhesion.

We found that in the monocytic cell line β1 integrins but not β2 integrins are activated by MCP-1. However, Weber et al. (18) have reported that MCP-1 induces a prolonged increase in the binding of monocytes to ICAM-1 in a static adhesion assay. This difference may be because of a difference in the way to remove nonadherent cells. We removed nonadherent cells by centrifugation, but they did it by plate washer. So we speculate that binding of β2 integrin and ICAM-1 is not strong enough to overcome the centrifugation force. Further, Chan et al. (19) have reported that activation of β1 integrin by chemokines might be much stronger than that of β2 integrin and that β1 integrin/VCAM-1 interaction activates β2 integrin-mediated cell adhesion in human T cells. Therefore, in *in vitro* situations, activation of β1 integrins might be stronger and more important in the early phase of cell migration.

FIG. 8. Activation of p38-MAPK, but not ERK by MCP-1, is blocked by C3 exoenzyme and Y-27632. THP-1 cells were pre-incubated with or without 15 μg/ml of C3 exoenzyme for 24 h (A) or 10 μM Y-27632 for 1 h (B) in an atmosphere of 95% air and 5% CO₂ at 37 °C. After the incubation, cells were stimulated with 10 nM MCP-1 for 1 min and subjected to the MAPK assay as described under “Experimental Procedures.” Results are representative of three (A) and two (B) independent experiments.

FIG. 9. MCP-1-dependent Ca²⁺ flux is not affected by PD98059 or SB203580. THP-1 cells were pre-incubated with 50 μM PD98059 (B) or 10 μM SB203580 (C) for 1 h in an atmosphere of 95% air and 5% CO₂ at 37 °C. In A, cells were pre-incubated with 0.2% Me₂SO. After the incubation, cells were stimulated with 10 nM MCP-1, and Ca²⁺ flux was measured as described under “Experimental Procedures.”

FIG. 10. A model proposed to explain MCP-1-mediated chemotaxis and integrin activation. MCP-1-mediated chemotaxis is mediated through Rho, Rho kinase, and p38-MAPK, and MCP-1-mediated activation of β1 integrin is dependent on ERK. These two distinct signaling cascades are supposed to be required for transmigration of monocytes.

In the cell adhesion assay, we could not abrogate MCP-1-dependent adhesion to fibronectin by the RGDS peptide, even though we have used sufficient concentrations of the peptide.
We speculate that the inhibitory effect of this peptide on the interaction between fibronectin and integrins is not so strong (20). Because RGDS-independent adhesion of fibronectin has been reported (21), it is also possible that RGDS-independent cell adhesion is induced by MCP-1.

In this study we showed that in THP-1 cells ERK is responsible for integrin activation by MCP-1 but not p38-MAPK or Rho. Laudanna et al. (22), however, reported that Rho is also involved in integrin activation by interleukin-8 in neutrophils and lymphocytes. The reasons for these differences are not clear, but signaling through integrin activation in response to chemokines might be cell type-specific. It is also possible that different types of integrins in leukocytes might be activated in response to each chemokine.

Recently, several reports have shown that p38-MAPK is involved in chemotaxis induced by serum, lysophosphatidylcholine, and chemokines in leukocytes and smooth muscle cells (8, 9). Our study has also shown that p38-MAPK is involved in chemotaxis induced by MCP-1 in THP-1 cells. In contrast, Yen et al. (23) showed that ERK is responsible for MCP-1-mediated chemotaxis. Knall et al. (24), on the other hand, have shown that ERK or p38-MAPK is not involved in interleukin-8-mediated chemotaxis. The reason for these differences is not clear, but in the system of Yen et al. (23) the activation of integrin might have been required for monocyte chemotaxis. Rho family GTPases have also been shown to be involved in cell migration (25). Thus our data are consistent with the others that p38-MAPK and Rho are involved in chemotaxis. However, the relationship between Rho and MAPK is quite complicated. For example, Zhang et al. (26) have shown that p38-MAPK is downstream of Rho in interleukin-1-mediated signaling. In contrast, Hippenstiel et al. (27) have reported that LPS-induced activation of p38-MAPK is not affected by Clostridium difficile toxin B-10463, a specific inhibitor of Rho. In terms of ERK and Rho, some reports have claimed that Rho is upstream of ERK (28–30), whereas others have demonstrated that Rho and ERK are activated independently (31). However, few studies have been conducted to examine whether ERK and p38-MAPK are differentially affected by Rho. In this paper, we clearly show that MCP-1 phosphorylates ERK and p38-MAPK in THP-1 cells and that Rho is upstream of p38-MAPK but not of ERK in MCP-1-mediated signal transduction. We have also shown that the Rho kinase (32) is between Rho and p38-MAPK in THP-1 cells and that Rho is upstream of p38-MAPK but not of ERK in MCP-1-mediated signal transduction. We have also clearly shown that the Rho kinase (32) is between Rho and p38-MAPK and is a key molecule for chemotaxis. However, downstream targets of p38-MAPK leading to chemotaxis still remain to be determined. Although we found that MCP-1 also activated JNK (data not shown), the role of JNK activation in MCP-1-mediated signaling was not determined in this study.

In summary, we have provided clear evidence that two distinct signaling cascades are present to mediate MCP-1-induced activation of β1 integrins and chemotaxis in THP-1 cells. These two distinct signaling cascades would be important for transmigration of monocytes through endothelial cells. The most intriguing aspect of this study is that we could separate two important biological functions of leukocytes, integrin activation and chemotaxis, by different assays and found that two distinct signaling cascades mediate these two functions. In adherent cells, however, segregation of integrin activation and chemotaxis would be very difficult to assess. As depicted in Fig. 10, identification of signaling molecules located at the bifurcation to ERK and p38-MAPK would be important to delineate the signaling cascades through CCR2. Further, it would be intriguing to determine the signaling cascades in a condition closer to in vivo situations.

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REFERENCES

1. Libby, P. (2000) J. Intern. Med. 247, 349–358
2. Reape, T. J., and Groot, P. H. (1999) Atherosclerosis 147, 213–225
3. Boring, L., Gosling, J., Cleary, M., and Charo, I. F. (1996) Nature 384, 897–899
4. Gu, L., Okada, Y., Clinton, S. K., Gerard, C., Sukhova, G. K., Libby, P., and Rollins, B. J. (1998) Mol. Cell 2, 275–281
5. Butcher, E. C. (1991) Cell 67, 1033–1036
6. Arai, H., Teu, C.-L., and Charo, I. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 14495–14499
7. Lopez-Illasara, M. (1996) Biochem. Pharmacol. 56, 269–277
8. Ayala, J. M., Goyal, S., Liverton, N. J., Claremon, D. A., O’Keefe, S. J., and Hanlon, W. A. (2000) J. Leukocyte Biol. 67, 869–875
9. Jing, Q., Xin, S. M., Zhang, W. B., Wang, P., Qin, Y. W., and Pei, G. (2000) Circ. Res. 87, 52–59
10. Humphries, M. J., Olden, K., and Yamada, K. M. (1986) Science 233, 467–470
11. Taooka, Y., Chen, J., Vednock, T., and Sheppard, D. (1999) J. Cell Biol. 145, 413–420
12. Makarem, R., Newham, P., Askari, J. A., Green, L. J., Clements, J., Edwards, J. M., Humphries, M. J., and Mould, A. P. (1994) J. Biol. Chem. 269, 4005–4011
13. Hama, Z., Kutsun, K. L., Herrera-Velit, P., Nandhan, D., and Reiner, N. E. (1999) J. Biol. Chem. 274, 1050–1057
14. Campbell, J. J., Qin, S., Bacon, K. B., Mackay, C. R., and Butcher, E. C. (1996) J. Biol. Chem. 271, 1063–1073
15. Myers, S. J., Wong, L. M., and Charo, I. F. (1995) J. Biol. Chem. 270, 5786–5792
16. Weber, C., Alon, R., Moser, B., and Springer, T. A. (1996) J. Cell Biol. 134, 413–420
17. MacKenna, D. A., Dolfi, F., Vuori, K., and Ruoslahti, E. (1998) J. Clin. Invest. 101, 301–310
18. Weber, K. S., Klickstein, L. B., and Weber, C. (1999) Mol. Cell Biol. 19, 861–873
19. Chan, J. R., Hyduk, S. J., and Cybulsky, M. I. (2000) J. Immunol. 164, 746–753
20. Gepstein, L., Aigner, S., Hubbe, M., Yagita, H., and Altevogt, P. (1995) J. Cell Biol. 131, 1881–1891
21. Silletti, S., Mei, F., Sheppard, D., and Montgomery, A. M. P. (2000) J. Cell Biol. 149, 1485–1501
22. Laudanna, C., Campbell, J. J., and Butcher, E. C. (1996) Science 271, 981–985
23. Yen, H., Zhang, Y., Penfold, S., and Rollins, B. J. (1997) J. Leukoc. Biol. 61, 489–492
24. Knall, C., Worthen, G. S., and Johnson, G. L. (1997) Proc. Natl. Acad. Sci. U. S. A.
25. Allen, W. E., Zicha, D., and Jones, G. E. (1998) J. Cell Biol. 141, 1147–1157
26. Zhang, S., Han, J., Sells, M. A., Chernoff, J., Knaus, U. G., Ulevitch, R. J., and Bokoch, G. M. (1995) J. Biol. Chem. 270, 23934–23936
27. Hippenstiel, S., Soeth, S., Kellis, B., Fuhrmann, O., Seybold, J., Krull, M., Michel-Streiber, C., Goeberl, M., Ludwig, S., and Suttrop, N. (2000) Blood 95, 3044–3051
28. Numaguchi, K., Eguchi, S., Yamakawa, T., Motley, E. D., and Inagami, T. (1999) Circ. Res. 85, 5–11
29. Costello, P. S., Walters, A. E., Mee, P. J., Turner, M., Reynolds, L. F., Prisco, A., Sarner, N., Zamoyska, R., and Tybulewicz, V. L. (1999) Proc. Natl. Acad. Sci. U. S. A.
30. Costello, P. S., Walters, A. E., Mee, P. J., Turner, M., Reynolds, L. F., Prisco, A., Sarner, N., Zamoyska, R., and Tybulewicz, V. L. (1999) Proc. Natl. Acad. Sci. U. S. A.
31. Costello, P. S., Walters, A. E., Mee, P. J., Turner, M., Reynolds, L. F., Prisco, A., Sarner, N., Zamoyska, R., and Tybulewicz, V. L. (1999) Proc. Natl. Acad. Sci. U. S. A.
32. Matsui, T., Amano, M., Yamamoto, T., Chihara, K., Nakafuku, M., Ito, M., Nakano, T., Okawa, K., Yamatsuka, A., and Kaibuchi, K. (1996) EMBO J. 15, 2208–2216
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