Anti–Monocyte Chemoattractant Protein-1/Monocyte Chemotactic and Activating Factor Antibody Inhibits Neointimal Hyperplasia in Injured Rat Carotid Arteries

Yutaka Furukawa, Akira Matsumori, Naohiro Ohashi, Tetsuo Shioi, Koh Ono, Akihisa Harada, Kouji Matsushima, Shigetake Sasayama

Abstract—Monocyte chemoattractant protein-1 (MCP-1)/monocyte chemotactic and activating factor (MCAF) has been suggested to promote atherogenesis. The effects of in vivo neutralization of MCP-1 in a rat model were examined in an effort to clarify the role of MCP-1 in the development of neointimal hyperplasia. Competitive polymerase chain reaction analysis revealed maximum MCP-1 mRNA expression at 4 hours after carotid arterial injury. Increased immunoreactivities of MCP-1 were also detected at 2 and 8 hours after injury. Either anti–MCP-1 antibody or nonimmunized goat IgG (10 mg/kg) was then administered every 12 hours to rats that had undergone carotid arterial injury. Treatment with 3 consecutive doses of anti–MCP-1 antibody within 24 hours (experiment 1) and every 12 hours for 5 days (experiment 2) significantly inhibited neointimal hyperplasia at day 14, resulting in a 27.8% reduction of the mean intima/media ratio ($P<0.05$) in experiment 1 and a 43.6% reduction ($P<0.01$) in experiment 2. This effect was still apparent at day 56 (55.6% inhibition; $P<0.05$). The number of vascular smooth muscle cells in the neointima at day 4 was significantly reduced by anti–MCP-1 treatment, demonstrating the important role of MCP-1 in early neointimal lesion formation. However, recombinant MCP-1 did not stimulate chemotaxis of vascular smooth muscle cells in an in vitro migration assay. These results suggest that MCP-1 promotes neointimal hyperplasia in early neointimal lesion formation and that neutralization of MCP-1 before, and immediately after, arterial injury may be effective in preventing restenosis after angioplasty. Further studies are needed to clarify the mechanism underlying the promotion of neointimal hyperplasia by MCP-1. (Circ Res. 1999;84:306-314.)

Key Words: monocyte chemoattractant protein-1/monocyte chemotactic and activating factor ■ angioplasty ■ restenosis ■ macrophage ■ smooth muscle cell

Late restenosis after balloon angioplasty remains the most limiting factor with regard to the long-term effectiveness of the procedure, and it is partially attributed to neointimal hyperplasia.1,2 Despite some success of various treatments3-5 in reducing neointimal hyperplasia in animal models, no sufficiently effective therapy has been confirmed in clinical trials. In the development of neointima, several cell types, such as monocytes/macrophages, vascular smooth muscle cells (VSMCs), and T lymphocytes, play diverse roles. Growth factors and cytokines mediate the interactions among these cells.6,7 One of these soluble factors, monocyte chemoattractant protein-1 (MCP-1)/monocyte chemotactic and activating factor (MCAF), has been reported to be expressed early in the injured arterial wall.8 MCP-1 is a potent chemotactic factor of monocytes9,10 and is produced by activated endothelial cells11 and VSMCs.12 It has also been detected in human atheromatous plaques using immunohistochemical staining and in situ hybridization.13 Monocytes/macrophages recruited into injured arterial walls become foam cells, the focal accumulation of which may play a pivotal role in atherogenesis.6,14 Including in restenosis. A recent clinical study found that activation of blood monocytes before angioplasty promotes late lumen loss after percutaneous transluminal coronary angioplasty.15 Thus, MCP-1 may have a promoting role in neointimal hyperplasia. However, two opposite effects have been reported with respect to the mitogenic activity of MCP-1 on cultured VSMC proliferation.16,17 This suggests diverse effects of MCP-1 during the development of neointimal hyperplasia.

The purpose of this study was to clarify which action, facilitating or inhibitory, of MCP-1 is more prominent in vivo. First, the time course of the expression of MCP-1 in injured artery was studied by competitive reverse transcriptase–polymerase chain reaction (PCR) and tissue ELISA. On the basis of these results, the effects of anti–MCP-1 treatment in a rat carotid arterial injury model were investigated.
Materials and Methods

Anti–MCP-1 Antibody (Ab) and Other Reagents

Anti–MCP-1 polyclonal Ab (anti-rat MCP-1 goat IgG) and control (nonimmunized) goat IgG were generously provided by Toray Basic Research Laboratory (Kanagawa, Japan). The neutralizing activity of this purified polyclonal anti-rat MCP-1 Ab was confirmed by monocyte chemotaxis assay.16 This Ab shows no cross-reactivity against recombinant rat RANTES and detected a single band in the same fraction as recombinant rat MCP-1 on Western blot analysis, when concanavalin A–stimulated spleen cell culture supernatant was fractionated by heparin-HPLC.18

Recombinant rat MCP-1 that contained <0.1 ng endotoxin/mg of rat MCP-1 protein (data from the manufacturer) was obtained from DIACLONE Research. Recombinant human platelet-derived growth factor (PDGF) was purchased from Gibco/BRL.

Animal Model Preparation

Male Sprague-Dawley rats 11 to 12 weeks old were obtained from Shizuoka Agricultural Cooperation Association. The animals were housed in plastic and stainless-steel cages, with controlled 12-hour light/12-hour dark cycles and access to food and water as desired. They were anesthetized with sodium pentobarbital (50 mg/kg IP). The endothelium of the left common carotid artery was denuded by passage of an inflated 2F Fogarty embolectomy catheter (Baxter Health Care) with a modification of the method of Clowes et al.19

RNA Preparation and cDNA Synthesis

Carotid arteries were harvested at 1, 2, 4, 8, 24, 72, or 120 hours after balloon injury (n = 3 for each time period). Right common carotid arteries were used as controls. Total RNA was prepared from the arteries by the guanidinium thiocyanate/phenol/chloroform/soybean alcohol isolation method.20 One microgram of total RNA template was subjected to first-strand cDNA synthesis with dNTP (Perkin-Elmer Corp) and Moloney murine leukemia virus reverse transcriptase (Gibco/BRL) under supplier-recommended conditions.

Competitive PCR

To estimate MCP-1 mRNA expression quantitatively, competitive PCR analysis was performed as previously described.21 Gene-specific oligonucleotide primers and mimic PCR primers for the MCP-1 and GAPDH genes were purchased from Oligos Etc, Inc. A sense primer (A) and an antisense primer (B) for each were synthesized using the published cDNA sequences for MCP-122 and GAPDH23 as follows.

Gene-Specific Primers

MCP-1 (A): 5'-CCGAACTCCGACACTCTACGAGCCAGAT-3'  
MCP-1 (B): 5'-CGTCAATCTTGGTGAGGGGCATTAAG-3'  
GAPDH (A): 5'-TGAGTGCCCAGCTGAAAGCATTGGCC-3'  
GAPDH (B): 5'-CATGATGCATGGTCACTTACACCC-3'

Mimic PCR Primers

Mimic MCP-1 (A): 5'-AACCTCTCACTGAGGCCAGATCTTCCGAATGGAAATCTCCGGC-3'  
Mimic MCP-1 (B): 5'-AGTTAGGTTGGGCAATATTCTGCTAGGGAACAAAGCTTACCT-3'  
GAPDH (A): 5'-TGAGTGCCCAGCTGAAAGCATTGGCC-3'  
GAPDH (B): 5'-CATGATGCATGGTCACTTACACCC-3'

Enzyme-Linked Immunosorbent Assay

Carotid arteries were harvested at 2, 8, 24, or 120 hours after injury (n = 3 for each time period). Right common carotid arteries were used as controls. The total length of each common carotid artery was homogenized in 1 mL of PBS containing 0.05% NaN₃, with an ultrasonic processor, ASTRASON (Misonix Inc), and then centrifuged. MCP-1 in the supernatant was quantified using an ELISA kit (Biosource International). All measurements were performed in duplicate. The values were corrected by protein concentrations measured by a modification of Lowry's method.24

Experiment 1

Animals were randomly assigned to an anti–MCP-1 treatment group or a control group. Rats in the anti–MCP-1 treatment group (n = 5) received 10 mg/kg anti–MCP-1 Ab, via the tail vein, 30 minutes before arterial injury and 12 and 24 hours after the first injection. Control rats (n = 5) were given 10 mg/kg nonimmunized goat IgG.

Experiment 2

Rats in the anti–MCP-1 treatment group (n = 6) and control group (n = 6) received 10 mg/kg anti–MCP-1 Ab or nonimmunized goat IgG, respectively, via the tail vein, every 12 hours for 5 days starting at 30 minutes before injury. This second series of experiments was performed to test the value of a longer treatment with anti–MCP-1 Ab.

Experiment 3

To determine the persistence of the inhibitory effects of anti–MCP-1 Ab on neointimal hyperplasia, the arteries were examined 56 days after carotid arterial injury, when cell proliferation in the injured arterial walls returns to the basal level.19 Rats in the anti–MCP-1 treatment group (n = 5) and the control group (n = 5) received 5 doses of 10 mg/kg anti–MCP-1 Ab or nonimmunized goat IgG, respectively, via the tail vein, every 12 hours starting at 30 minutes before injury. This third series of experiments was performed to test whether the inhibitory effect was limited to a delay of lesion progression.

Light-Microscopic Examination and Morphometry of Neointima

Fourteen days after injury in experiments 1 and 2, and 56 days after injury in experiment 3, the rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and received 200 μL of 2% Evans blue dye in PBS injected into the tail vein. They then received heparin (100 U/rat) intravenously, and after perfusion with saline, the left common carotid arteries were perfusion fixed with 10% neutral buffered formalin as described previously.25 The carotid arteries were removed and fixed further. Central portions of the blue-stained areas were embedded in paraffin. Five cross sections of each artery situated 2 mm and 1 mm proximal to the center, at the center, and 1 mm and 2 mm distal to the center, were stained with elastic van Gieson stain. Intimal and medial areas were blindly measured with a

\[ V_C = \frac{V_T (V_C + V_T)}{V_C} \times \frac{C_C}{C_T} \]

\[ V_T / V_C \times C_C / C_T \]

\[ C_T \]

\[ C_T \]
staining of macrophages, the carotid arteries were removed 14 days after injury and embedded in OCT compound tissue medium (Miles Inc). Short axial 4-μm cryostat sections were cut from proximal, middle, and distal segments for each sample and fixed for 10 minutes in acetone at 4°C. To label macrophages, ED1 Ab (BMA Biomedicals Ltd)26 was used as primary Ab. Labeling of VSMCs or proliferating cells was performed using the 20 or 5 formalin-fixed, deparaffinated sections of carotid arteries obtained at day 4 or 48 hours, respectively. As primary Ab, mouse monoclonal Ab against muscle actin (HFF35, ENZO Diagnostics Inc) or mouse monoclonal Ab against proliferating cell nuclear antigen (PCNA; PC10, YLEM Srl) was used. The sections were incubated with the following: primary Ab at a dilution of 1:50 overnight at 4°C, biotinylated secondary Ab (rabbit anti-mouse IgG; DAKO) at 1:500 concentration of 50 ng/mL in some of the experiments. Six filters were used, respectively. To test whether MCP-1 can augment the chemotactic activity of VSMCs when PDGF-BB was used as a chemoattractant, MCP-1 was added to the upper chamber at a final concentration of 20 ng/mL. Migration of rat aortic VSMCs was assayed with a 48-well modified Boyden-chamber apparatus (Neuro Probe Inc).28,29 The wells were covered with a polyvinylpyrrolidone-free filter with 8-μm pores (Nuclepore Corp), coated with 2.7 μg/well Matrigel (Collaborative Research),28 or 100 μg/mL type I collagen (Sigma). Cultured VSMCs were trypsinized and suspended at a concentration of 5.0×10^3 cells/mL in serum-free DMEM with streptomycin and penicillin. Fifty microliters of the cell suspension was added to each upper chamber. DMEM containing recombinant rat MCP-1 at the final concentration of 20, 50, or 100 ng/mL was added to the lower chamber in a volume of 50 μL. As negative and positive controls, DMEM without MCP-1 and DMEM with recombinant human PDGF-BB (Gibco/BRL), with a final concentration of 20 ng/mL were used, respectively. To test whether MCP-1 can augment the chemotactic activity of VSMCs when PDGF-BB was used as a chemoattractant, MCP-1 was added to the upper chamber at a final concentration of 50 ng/mL in some of the experiments. Six filters were used for each treatment. After incubation in a humidified atmosphere (5% CO2/95% air) at 37°C, cells on the upper membrane surface were removed, and those on the lower membrane surface were fixed in methanol and stained with Diff-Quik staining solution (International Reagents Corp). The number of cells per four 200× high-power fields was counted under a microscope, and the mean number of cells represented migration activity.

### Statistical Analysis

Values for relative MCP-1 gene–product levels and MCP-1 immunoreactivity are expressed as mean±SEM. Values for intimal areas, medial areas, intima/media ratio, numbers of cells, percentage ED1-positive cells, percentage proliferating cells, and migration activity are expressed as mean±SD. Values for intimal areas and intima/media ratio in experiment 3 were compared using the Mann-Whitney U test, since they were nonparametrically distributed. Values for migration activity were compared using ANOVA. Other values were compared using the 2-tailed unpaired Student t test.

### Results

**Time Course of MCP-1 Gene Expression in Injured Rat Carotid Arteries**

Complimentation of sample cDNA with serially diluted mimic cDNA showed a close correlation between the amount of...
mimic cDNA and target cDNA/mimic cDNA ratio for each reaction (Figure 1A). Induction of MCP-1 mRNA was demonstrable as early as 1 hour, and peak expression occurred at 4 hours after arterial injury (Figure 1B). MCP-1 mRNA level decreased at 8 hours, although relatively low but upregulated MCP-1 gene expression was still present 120 hours after injury.

**Enhanced MCP-1 Immunoreactivity in the Injured Arterial Wall**

Immunoreactivity of MCP-1 in the injured arteries increased as early as 2 hours after injury (129.0±6.8 pg/mg protein; mean±SEM), and the upregulation was still detectable at 8 hours (118.5±18.3) and 24 hours (82.5±14.6). At 120 hours, MCP-1 immunoreactivity had returned to the level of noninjured arteries. In the noninjured arterial tissues, no significant changes were observed throughout the observation period (Figure 2).

**Effects of Anti–MCP-1 Treatment on Neointimal Hyperplasia**

Significant neointimal hyperplasia was observed in injured arteries 14 days after injury. Intravenous administration of 3 doses of anti–MCP-1 Ab significantly inhibited neointimal hyperplasia, compared with the control group (Table 1, Figure 3A). The mean intima/media ratio was reduced to 0.679±0.174 (mean±SD), in contrast to 0.941±0.062 in the control group (P<0.05), representing a 27.8% inhibition in the anti–MCP-1 treatment group. Administration of anti–MCP-1 Ab every 12 hours for 5 days (10 doses) resulted in 43.6% inhibition (Table 1, Figures 3B and 4). The mean intima/media ratio was 0.465±0.163 in the anti–MCP-1–treated group versus 0.825±0.093 in the control group (P<0.01). In experiment 3, this inhibitory effect was still present 56 days after injury, and the mean intima/media ratio was reduced by 55.6% in the anti–MCP-1 treatment group (P<0.05; Table 1, Figures 3C and 4).

**Population of Macrophages in the Neointima**

The number of nuclei and ED1-positive cells in the neointima of 3 sections was counted for each sample, and the percentage of ED1-positive cells was calculated. The number of ED1-positive cells in the neointima tended to be lower in the anti–MCP-1 treatment group (80±26 in the anti–MCP-1 treatment group versus 115±27 in the control group; P=0.054). The number of nucleated cells also tended to be lower (1646±273 in the anti–MCP-1 treatment group versus 2074±419 in the control group; P=0.071). As a result, the percentages of ED1-positive cells relative to the nucleated cells in the neointima were comparable in both groups (4.81±1.03% in the anti–MCP-1 treatment group versus 5.50±0.65% in the control group; P=0.228, Table 2).

**Effect of Anti–MCP-1 Ab on the Number of VSMCs in Early Neointimal Lesions**

In this rat model, VSMCs appear in the intima 4 days after injury.19 The number of intimal VSMCs was thus compared between the anti–MCP-1 treatment group and the control group at day 4, representing migration activity and intimal

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**TABLE 1. Mean Intimal and Medial Areas of Rat Carotid Arteries 14 Days After Balloon Injury**

<table>
<thead>
<tr>
<th>Experiment/Treatment</th>
<th>n</th>
<th>Mean Intimal Area, mm²</th>
<th>Mean Medial Area, mm²</th>
<th>Mean Intima/Media Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonimmunized IgG</td>
<td>5</td>
<td>0.117±0.022</td>
<td>0.124±0.017</td>
<td>0.941±0.062</td>
</tr>
<tr>
<td>Anti–MCP-1 Ab</td>
<td>5</td>
<td>0.070±0.024*</td>
<td>0.102±0.015</td>
<td>0.679±0.174*</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonimmunized IgG</td>
<td>6</td>
<td>0.825±0.017</td>
<td>0.104±0.017</td>
<td>0.825±0.093</td>
</tr>
<tr>
<td>Anti–MCP-1 Ab</td>
<td>6</td>
<td>0.052±0.021*</td>
<td>0.112±0.006</td>
<td>0.465±0.163†</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonimmunized IgG</td>
<td>5</td>
<td>0.126±0.076</td>
<td>0.103±0.018</td>
<td>1.146±0.549</td>
</tr>
<tr>
<td>Anti–MCP-1 Ab</td>
<td>5</td>
<td>0.055±0.018</td>
<td>0.109±0.020</td>
<td>0.509±0.170*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

*P<0.05 vs nonimmunized IgG; †P<0.01 vs nonimmunized IgG.
proliferation of VSMCs in the early neointimal lesion. Table 3 shows that the number of VSMCs in the intima was decreased by treatment with anti–MCP-1 Ab ($P<0.05$).

**Effect of Anti–MCP-1 Ab on Initial Medial Proliferation After Mechanical Injury**

Forty-eight hours after injury, immunohistochemical staining with anti-PCNA Ab showed no differences, between the anti–MCP-1 treatment group and the control group, in the population of proliferating cells in the media (Table 4). Percentage PCNA-positive cells was $35.8\pm2.9\%$ in the anti–MCP-1 treatment group, and that in the control group was $34.4\pm7.2\%$.

**Effects of MCP-1 on VSMC Migration In Vitro**

Recombinant rat MCP-1 failed to stimulate chemotactic activity of VSMCs into coated Matrigel, even at high concentrations, which have been observed to exert a significant effect in a monocyte chemotaxis assay. MCP-1 also failed to increase PDGF-BB–stimulated VSMC migration activity when added directly into the upper chamber (Figure 5). Similar results were obtained in the assays using type I collagen as a coating material (data not shown).

**Discussion**

The present study shows that anti–MCP-1 treatment before and soon after arterial injury reduced neointimal hyperplasia, an effect that persisted during the chronic stage. Rats that had undergone carotid arterial injury were treated with anti–MCP-1 Ab for different lengths of time. Treatment for 5 days (10 doses) seemed more effective in limiting the neointimal lesions than treatment limited to 3 doses, although there was not a notable difference in the inhibitory effects between the 2 treatment durations. This suggests that anti–MCP-1 treatment exerts beneficial effects mostly by neutralizing MCP-1 produced soon after arterial injury. We demonstrated in experiment 3 that the effects of early anti–MCP-1 treatment did not just delay the development of neointimal lesions, but also influenced the severity of neointimal hyperplasia in the chronic stage. As a possible mechanism of the inhibitory effect of anti–MCP-1 treatment, it is noteworthy that the number of VSMCs in the early neointimal lesions at day 4 decreased significantly. In addition, the accumulation of macrophages tended to decrease with anti–MCP-1 treatment. These observations suggest an important role of MCP-1 in the development of accelerated atherosclerosis.

In the pathogenesis of atherosclerosis, various vascular and inflammatory cells, including monocytes/macrophages and VSMCs, play important interacting roles. Soluble factors and adhesion molecules regulate the pathophysiological activities of these cells. As MCP-1 is one of the soluble factors that participate in atherogenesis, the pathophysiological roles of MCP-1 have been studied in cultured cells. Expression of MCP-1 is regulated by various stimuli such as growth factors and those from adhesion molecules.

In this study, MCP-1 mRNA and protein were rapidly induced by mechanical arterial injury, with a time course comparable to that in previous experiments performed in a rabbit model. Because MCP-1 is a potent chemoattractant of
monocytes.\textsuperscript{9,10} This induction of MCP-1 may contribute to early inflammatory responses in the injured arterial walls and local accumulation of monocytes/macrophages into the neointima. Recently, Boring et al.\textsuperscript{38} reported that additional depletion of CCR2 gene, a receptor for MCP-1, to apolipoprotein E (ApoE) gene markedly attenuated atherosclerotic lesions in ApoE-deficient mice by inhibiting macrophage accumulation; these results strongly support the important role of MCP-1 in atherosclerotic lesion formation, particularly macrophage-rich lesions. In fact, in our experiments, the number of macrophages accumulated in the neointima tended to be decreased by anti–MCP-1 Ab treatment, although the population of macrophages is smaller in the rat than in other models of arteriosclerosis.\textsuperscript{39,40} This may partially explain the inhibitory effects of anti–MCP-1 Ab on neointimal hyperplasia in the rat arterial injury model.

In addition to its well-known chemotactic activity on monocytes/macrophages, other properties of MCP-1 have

\begin{table}
\centering
\begin{tabular}{lllll}
\hline
Treatment         & n   & Number of Nucleated Cells & ED1-Positive Cells & n & \%  \\
\hline
Nonimmunized IgG  & 5   & 2074±419                   & \text{115±27}       & 5.50±0.65   \\
Anti–MCP-1 Ab     & 6   & 1646±273                   & \text{80±26}        & 4.81±1.03   \\
\hline
\end{tabular}
\caption{Populations of ED1-Positive Cells in the Neointima}
\end{table}

TABLE 2. Populations of ED1-Positive Cells in the Neointima

Nucleated cells and ED1-positive cells in the intima per 3 cross sections were counted for each sample at a magnification of ×400. Values are expressed as mean±SD.
been described that may stimulate atherosclerotic lesion formation. Some investigators have reported that MCP-1 stimulates chemotaxis of T lymphocytes and VSMCs. Ikeda et al. reported that MCP-1 increased the expression of intercellular adhesion molecule-1 on rat aortic VSMCs. Another study has shown that MCP-1 has direct mitogenic effects on the proliferative response of cultured rat VSMCs, although controversy remains. Thus, several reports have shown biological effects of MCP-1 on VSMCs, despite the absence of a clear demonstration of receptors for MCP-1. Recent observations suggest the existence of chemokine receptors for MCP-1 on the surface of VSMCs. Schecter et al. reported that human VSMCs may express a distinct receptor for MCP-1 from CCR2, which is expressed on monocytes and T lymphocytes, by binding assay and reverse transcriptase–PCR, and another group demonstrated the expression of CCR2 mRNA in unstimulated cultured human VSMCs. There is a discrepancy between these 2 reports with respect to the type of VSMC receptor. Nevertheless, they suggest that MCP-1 may modulate the function of VSMCs and that neutralization of MCP-1 may inhibit neointimal lesion formation not only by reducing the accumulation of macrophages, but also by blocking the biological effects of MCP-1 on VSMCs. Since the intimal population of macrophages is much greater in humans than in this rat model, the clinical effects of anti-MCP-1 treatment cannot be immediately predicted. However, together with the reduction of macrophage-rich atherosclerotic lesions by CCR2 gene depletion in a recent study, the inhibitory effects of anti-MCP-1 treatment in this rat model suggest that such treatment may reduce the formation of neointimal lesions after balloon angioplasty, lesions that contain more VSMCs and fewer macrophages than primary atherosclerotic plaques.

Previous studies have suggested that migration and intimal proliferation are more critical steps in the development of intimal hyperplasia than first-round medial VSMC proliferation.

### Table 3. Effect of Anti–MCP-1 Ab on the Number of Smooth Muscle Cells in the Intima at Day 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of VSMCs in the Intima</th>
<th>Number of VSMCs in the Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonimmunized IgG</td>
<td>6 410±140</td>
<td>7868±255</td>
</tr>
<tr>
<td>Anti–MCP-1 Ab</td>
<td>6 227±134*</td>
<td>7812±510</td>
</tr>
</tbody>
</table>

Smooth muscle cells in the intima per 20 cross sections were counted in each sample at a magnification of ×400. Values are expressed as mean±SD. *P<0.05 vs nonimmunized IgG.

### Table 4. Effect of Anti–MCP-1 Ab on the Initial Proliferative Activity in the Media

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Nucleated Cells</th>
<th>Number of PCNA(+) Cells</th>
<th>% Proliferating Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonimmunized IgG</td>
<td>4 1159±64</td>
<td>401±105</td>
<td>34.4±7.2</td>
</tr>
<tr>
<td>Anti–MCP-1 Ab</td>
<td>5 1193±181</td>
<td>425±59</td>
<td>35.8±2.9</td>
</tr>
</tbody>
</table>

Nucleated cells and PCNA-positive cells in the media per 5 cross sections were counted in each sample at a magnification of ×400. Values are expressed as mean±SD.

Figure 5. Effects of recombinant rat MCP-1 and PDGF-BB on the migration of cultured VSMCs. A, Recombinant rat MCP-1 was added at a concentration of 20, 50, or 100 ng/mL in the lower chamber of the modified Boyden apparatus. PDGF-BB was used as a positive control chemoattractant for VSMCs in this assay. B, MCP-1 was added at a concentration of 50 ng/mL in the upper chamber, and 20 ng/mL of PDGF-BB was added in the lower chamber as a chemoattractant to test whether MCP-1 can augment the chemotactic activity of VSMCs in the presence of PDGF-BB. Values are expressed as mean±SD. *P<0.01 (ANOVA).
intimal VSMCs on day 4 between anti–MCP-1 treatment group and control group, but no difference in the number of proliferating VSMCs at 48 hour, suggesting that MCP-1 stimulates migration or intimal proliferation of VSMCs in vivo. The effects of recombinant MCP-1 on migration of cultured VSMCs were then examined. In the migration assay, MCP-1 did not directly act as a chemotaxant for VSMCs, nor did it stimulate migration activity of VSMCs into Matrigel or collagen I. Our results differ from those of previous reports with regard to the effects of MCP-1 on VSMC migration.43,44 Differences in the species of experimental cells used, or in the preparation of the cells, may explain this discrepancy. It is also possible that the effects of MCP-1 on VSMC migration in vivo are different from those in a cell culture. Nevertheless, the results of the migration assay suggest other effects of MCP-1 on VSMCs causing an early decrease in the number of VSMCs in the intima. Since there was no difference in the number of proliferating medial VSMCs between the anti–MCP-1 treatment group and the control group, we considered the possibility that anti–MCP-1 treatment did not affect first-round medial VSMC proliferation. However, the possibility that anti–MCP-1 Ab inhibited second-round proliferation of VSMCs in the intima cannot be excluded, since the proliferative activity of VSMCs in the injured arterial walls is biphasic,49 and >70% of intimal VSMCs are proliferating at day 4.19 Thus, a decrease in the number of VSMCs in the neointima by anti–MCP-1 treatment at day 4 could result from inhibition of second-wave VSMC proliferation in the intima. Studies using cultured VSMCs revealed variable proliferative responses of VSMCs to MCP-1 depending on the status of the cells.16,17 It is, therefore, possible that differences exist in between the proliferative responses of intimal VSMCs versus medial VSMCs. Another possible biological effect of MCP-1 on VSMCs, which may enhance the second wave of proliferative response, consists of stimulation and prolongation of procoagulant activity. MCP-1 produced by VSMCs and macrophages may induce procoagulant activity and contribute to mural thrombus formation via tissue-factor induction in atherosclerotic lesions.47 Thrombi that are formed after arterial injury contain chemotaxants and mitogens for VSMCs and seem to function as a matrix for the migration and proliferation of VSMCs.50 Therefore, anti–MCP-1 treatment may attenuate neointimal hyperplasia by reducing procoagulant activity in the injured arterial walls.

In conclusion, this study demonstrated that the expression of MCP-1 was induced early in a rat model of carotid arterial injury. Anti–MCP-1 treatment resulted in a significant attenuation of neointimal hyperplasia in studies of 2 different treatment periods, and the inhibitory effect persisted long after injury. As a possible mechanism of this inhibitory effect, neutralization of MCP-1 may affect not only accumulation of macrophages but also an early increase of VSMCs in the intima. These results suggest that MCP-1 acts as an early promoting factor in neointimal hyperplasia after mechanical injury and that anti–MCP-1 treatment before and soon after angioplasty may inhibit postprocedural intimal hyperplasia. Further investigations are needed to clarify the diverse biological effects of MCP-1 on both leukocytes and vascular cells in neointimal lesion formation and to measure the efficacy of anti–MCP-1 treatment in the prevention of restenosis after balloon angioplasty.

Acknowledgments

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References


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