*Proc. Natl. Acad. Sci. USA*
Vol. 87, pp. 5134–5138, July 1990
Cell Biology

# Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells

**(atherosclerosis/oxidized lipoproteins)**

SUSAN D. CUSHING*, JUDITH A. BERLINER†, ANTHONY J. VALENTE‡, MARY C. TERRITO*, MAHAMAD NAVAB*, FARHAD PARHAMI†, ROSS GERRITY‡, COLIN J. SCHWARTZ§, AND ALAN M. FOGELMAN*

Departments of *Medicine and †Pathology, UCLA School of Medicine, Los Angeles, CA 90024-1679; ‡The Cleveland Research Institute, Cleveland, OH 44115; and §Department of Pathology, University of Texas, San Antonio, TX 78284-7750

*Communicated by William N. Valentine, April 23, 1990*

**ABSTRACT** **After exposure to low density lipoprotein (LDL) that had been minimally modified by oxidation (MM-LDL), human endothelial cells (EC) and smooth muscle cells (SMC) cultured separately or together produced 2- to 3-fold more monocyte chemotactic activity than did control cells or cells exposed to freshly isolated LDL. This increase in monocyte chemotactic activity was paralleled by increases in mRNA levels for a monocyte chemotactic protein 1 (MCP-1) that is constitutively produced by the human glioma U-105MG cell line. Antibody that had been prepared against cultured baboon smooth muscle cell chemotactic factor (anti-SMCF) did not inhibit monocyte migration induced by the potent bacterial chemotactic factor f-Met-Leu-Phe. However, anti-SMCF completely inhibited the monocyte chemotactic activity found in the media of U-105MG cells, EC, and SMC before and after exposure to MM-LDL. Moreover, monocyte migration into the subendothelial space of a coculture of EC and SMC that had been exposed to MM-LDL was completely inhibited by anti-SMCF. Anti-SMCF specifically immunoprecipitated 10-kDa and 12.5-kDa proteins from EC. Incorporation of [$^{35}$S]methionine into the immunoprecipitated proteins paralleled the monocyte chemotactic activity found in the medium of MM-LDL stimulated EC and the levels of MCP-1 mRNA found in the EC. We conclude that (*i*) SMCF is in fact MCP-1 and (*ii*) MCP-1 is induced by MM-LDL.**

An important early event in atherogenesis is an increased recruitment of monocytes into the arterial subendothelium (1–4). Previous studies have shown that endothelial cells (EC) (5, 6) and smooth muscle cells (SMC) (7, 8) in culture constitutively produce a chemotactic factor that acts on monocytes but not neutrophils. Graves and colleagues (9) demonstrated that the monocyte chemotactic activity in the supernatants from a number of tumor cell lines was inhibited by an antibody made against baboon smooth muscle cell chemotactic factor (anti-SMCF) (10). Additionally they demonstrated a strong concordance with immunoprecipitation of a protein with an apparent molecular mass of 14.4 kDa. A human glioma cell line, U-105MG, constitutively expresses monocyte chemotactic activity. The protein responsible for this activity has been purified, sequenced, and named monocyte chemotactic protein 1 (MCP-1) (11, 12). Graves and colleagues did not test their antibody against the chemotactic activity secreted by the U-105MG cell line, but based on limited primary sequence data from the baboon smooth muscle chemotactic protein, they concluded that their protein was homologous to MCP-1.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

We have recently demonstrated that low density lipoprotein (LDL) that has been minimally modified (MM-LDL) is indistinguishable from native LDL by the LDL receptor, is not recognized by the scavenger receptor, and induces EC to secrete high levels of monocyte chemotactic activity, whereas native LDL does not (13). We have also shown that MM-LDL induces EC to produce colony-stimulating factors, including monocyte colony-stimulating factor (M-CSF), which has been shown to be a potent monocyte chemoattractant (14).

In this study we provide further evidence that the baboon SMCF is in fact MCP-1. We also demonstrate that MM-LDL induces MCP-1 and that MCP-1 accounts for virtually all of the chemotactic activity produced by EC and SMC in culture before and after exposure to MM-LDL. Furthermore, we show that MM-LDL stimulates monocyte chemotactic activity in cocultures of EC and SMC and that this induced activity is entirely due to MCP-1.

## MATERIALS AND METHODS

**Cell Culture.** Human aortic EC and SMC at passages 6–9 were cultured in Dulbecco's modified Eagle's medium as described (5, 15). The coculture system was maintained in Iscove's modified medium as described (15). The human glioma cell line U-105MG, initiated by J. Ponten and B. Westermark at the University of Uppsala, Uppsala, Sweden (16), was a generous gift from Y. Gillespie (University of Alabama at Birmingham). These cells were cultured in RPMI 1640 medium as described (17). MM-LDL was prepared as described (13) and contained <5 pg of bacterial lipopolysaccharide (LPS) per μg of LDL protein (13). Greater than 1 ng of LPS was required to stimulate chemotactic factor production (data not shown) and no more than 100 pg of LPS was present in any of the experiments described in this manuscript. In contrast to highly oxidized LDL, MM-LDL contained only 2–5 nanomoles of thiobarbituric acid-reactive substances (TBARS) as malondialdehyde equivalents per mg of cholesterol and was recognized by the LDL receptor and not the scavenger receptor. For collection of chemotactic factor produced constitutively or by MM-LDL stimulation, cells were washed in serum-free medium and then incubated at 37°C for 18 hr in the appropriate medium containing 0.1–0.2% heat-inactivated fetal calf serum with or without the addition of MM-LDL. Medium without cells and with or without the addition of MM-LDL was also incubated for 18 hr and used as a control in the chemotaxis assay.

Abbreviations: LDL, low density lipoprotein; MM-LDL, LDL that has been minimally modified by oxidation; MCP-1, monocyte chemotactic protein 1; SMC, smooth muscle cells; EC, endothelial cells; SMCF, smooth muscle cell chemotactic factor; M-CSF, monocyte colony-stimulating factor; fMLP, formyl-Met-Leu-Phe.

**Measurement of Chemotaxis.** Monocytes were obtained by a modification of the Recalde method (18). The ability of the medium to initiate monocyte chemotaxis was tested in a neuroprobe chamber (5). Antibody to baboon SMCF was prepared as described (9). For antibody inhibition studies the conditioned medium was incubated with antibody for 2 hr at 37°C and then centrifuged in a microcentrifuge for 1 min at 8000 × *g* to remove immune complexes before testing for chemotactic activity. Formyl-Met-Leu-Phe (fMLP) at a concentration of 0.1 $\mu$M was used as a reference chemoattractant.

**Metabolic Labeling and Immunoprecipitation.** Human aortic EC in 60-mm dishes were transferred to medium deficient in methionine, 100–250 $\mu$Ci (1 Ci = 37 GBq) of [$^{35}$S]methionine per ml was added, and the cells were incubated for 5 hr. The medium was collected and centrifuged to remove detached cells and debris. Irrelevant antibody (rabbit IgG to human $\beta$-lipoprotein) was added to bind any material that nonspecifically bound IgG; this was then removed by precipitation with protein A-Sepharose. Anti-SMCF was added to the supernatant, the mixture was left overnight at 4°C, and bound molecules were precipitated with protein A-Sepharose. The pellet was washed three times with a solution containing 1% Triton X-100 and 1% bovine serum albumin, and proteins were solubilized in sample buffer containing 2% 2-mercaptoethanol, heated at 100°C for 5 min, and electrophoresed on 12% acrylamide gels. Gels were fixed in 10% methanol/5% acetic acid solution overnight, washed in distilled water, soaked in 1 M sodium salicylate/5% glycerol for 30 min, dried, and exposed to Kodak XAR-5 film.

**Northern Blot Analysis.** Total RNA was isolated from human aortic EC, SMC, and the human glioma U-105MG cell line by the guanidinium isothiocyanate method of Chomczynski and Sacchi (19). The alcohol-precipitated RNA was electrophoresed on formaldehyde/1% agarose gels and transblotted to Biotrans nylon membranes. The blots were hybridized with a $^{32}$P-end-labeled 35-mer oligonucleotide probe. The probe was complementary to nucleotides 257–291 of published cDNA sequence for MCP-1 (20). The sequence of the probe was 5′-CGG-ATG-TTG-GGT-TTG-CTT-GTC-CAG-GTG-GTC-CAT-GG-3′. Hybridization was done overnight at 57°C in a solution containing 0.75 M NaCl, 0.15 M Tris·HCl (pH 8.0), 10 mM EDTA, 5× Denhardt's solution (1× Denhardt's solution: 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrolidone), 0.1% SDS, 0.1% sodium pyrophosphate, 50 $\mu$g of heat-denatured, sheared, salmon sperm DNA per ml, and 2 × $10^6$ cpm of probe per ml. Blots were washed twice with 20 ml of 0.2× SSC (1× SSC: 0.15 M NaCl/15 mM sodium citrate)/0.1% SDS at 57°C for 30 sec, washed twice with 500 ml of the same solution at 57°C for 30 min, dried, and exposed between 16 and 64 hr (within the linear range of the film) to Kodak XAR-5 film with an intensifying screen at −70°C. The autoradiographs were scanned on an LKB Ultrascan laser densitometer and quantitated with the GELSCAN II program for the Apple II computer.

**Monocyte Transmigration Assay.** To determine the effect of MM-LDL on monocyte transmigration into the subendothelial space of the cocultures, these preparations were washed, serum content of the medium was reduced to 2.5%, and the cocultures received 20 $\mu$g/ml of MM-LDL, MM-LDL plus anti-SMCF Fab fragments, or MM-LDL plus irrelevant Fab fragments. These Fab fragments were prepared as described (21). fMLP at a concentration of 0.1 $\mu$M was added to some of the cocultures as a reference chemoattractant. Human monocytes isolated as described above were labeled with the fluorescent compound 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) by incubating at 4°C for 10 min (22). The labeling of monocytes did not affect the monocyte adhesion or transmigration (data not shown). Following a 24-hr treatment of the cocultures with MM-LDL, DiI-labeled monocytes were added to the endothelial side of the preparations and were incubated at 37°C for 60 min. The nonadherent or loosely attached monocytes were washed and removed, and then the preparations were fixed. The number of subendothelial monocytes in each preparation was determined in nine high-power fields under oil immersion.

## RESULTS

As shown in Fig. 1*A*, anti-SMCF completely inhibited the chemotactic activity produced by human aortic EC and SMC in culture but did not inhibit the chemotaxis induced by fMLP. Moreover, the antibody completely inhibited the chemotactic activity in the medium from the glioma cell line U-105MG. Fig. 1*B* shows that MM-LDL itself was not chemotactic (condition C); however, MM-LDL increased the amount of chemotactic activity produced by EC and SMC approximately 2- to 3-fold but did not increase the chemotactic activity produced by the glioma cells. Anti-SMCF completely inhibited the chemotactic activity produced by the MM-LDL-stimulated cells.

Immunoprecipitation of the medium from [$^{35}$S]methionine-labeled, unstimulated EC with anti-SMCF resulted in the presence of several protein bands on acrylamide gels. Two of these (10 kDa and 12.5 kDa) appeared to be specifically recognized by the antibody since they were not precipitated by irrelevant antibody, and their precipitation by anti-SMCF

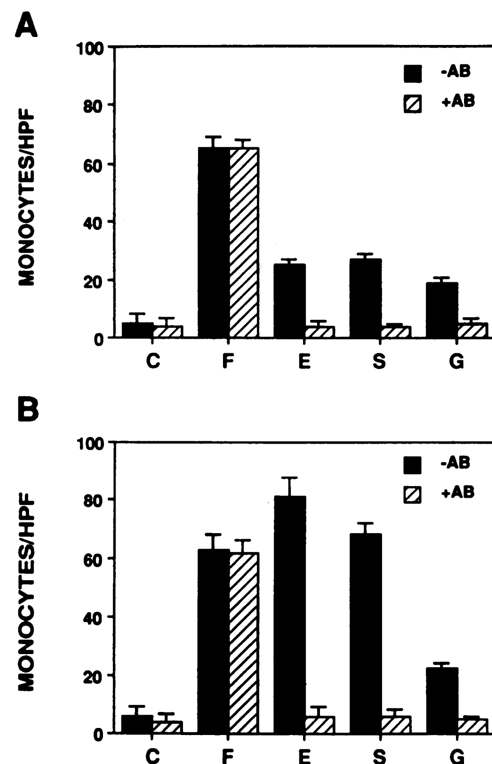


FIG. 1. Effect of anti-SMCF on the monocyte chemotactic activity of human artery wall cells. (*A*) After 18 hr of incubation the media from confluent cultures of human aortic EC (E), human aortic SMC (S), or U-105MG human glioma cells (G) were collected and tested for monocyte chemotactic activity in the absence (−AB) or presence (+AB) of anti-SMCF. Medium from dishes without cells (C) or medium containing the chemotactic factor fMLP (F) were also tested for monocyte chemotactic activity in the presence or absence of antibody. (*B*) The conditions were the same as in *A* except that the media contained 20 $\mu$g of MM-LDL per ml. The values shown are the mean ± SD for quadruplicate determinations. HPF, high-power fields.

was completely inhibited by the addition of highly purified unlabeled SMCF (Fig. 2A). Immunoprecipitation of equal trichloroacetic acid-precipitable cpm taken from the medium of MM-LDL-stimulated cells showed an increase in the incorporation of label into these protein species (Fig. 2B), suggesting an increased biosynthesis and secretion in response to the MM-LDL. The increase was estimated to be 7- to 10-fold as determined by laser densitometric scanning. The increase in monocyte chemotactic activity in the medium from the MM-LDL-treated cells in this experiment was also 7- to 10-fold (data not shown).

Based on the published cDNA sequence for MCP-1 from the U-105MG glioma cells (20, 23), we constructed a 35-mer oligonucleotide probe that was used for Northern blot analysis. Fig. 3A shows that human aortic EC, SMC, and the U-105MG glioma cells all constitutively expressed MCP-1 mRNA (≈0.8 kilobase). The levels of MCP-1 mRNA (but not α-tubulin mRNA) increased in human aortic EC and SMC but not in the U-105MG cells after an 18-hr exposure to 20 μg of MM-LDL per ml. Laser densitometry of the Northern blot autoradiograph (Fig. 3B) showed that the levels of MCP-1 mRNA were increased ≈22-fold in EC cells and 8-fold in SMC.

Fig. 4 shows that MCP-1 message (but not α-tubulin mRNA) was increased by a 16-hr exposure to MM-LDL in a concentration-dependent manner. The absolute increase in MCP-1 message caused by a given concentration of MM-LDL varied with different MM-LDL preparations. However, a concentration-dependent relationship was maintained in all cases, with a plateau in MCP-1 mRNA expression seen with concentrations of MM-LDL between 20 and 30 μg/ml.

The time-dependent induction of MCP-1 mRNA by 20 μg of MM-LDL per ml is shown in Fig. 5. EC and SMC showed large increases in MCP-1 mRNA after a 4-hr exposure to MM-LDL. The laser densitometer scans clearly demonstrate that the time-dependent induction of MCP-1 mRNA was different in the two cell types. EC exhibited a rapid and protracted increase in MCP-1 mRNA, with the highest levels seen after a 20-hr exposure to MM-LDL. However, MCP-1 mRNA expression by MM-LDL-treated SMC reached a peak at 4 hr and declined to nearly constitutive levels after 20 hr.

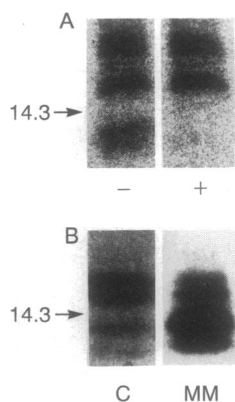


FIG. 2. Immunoprecipitation of EC conditioned medium. (A) Competition with unlabeled SMCF. (B) Comparison of metabolically labeled immunoprecipitated proteins from control (C) and MM-LDL (MM)-treated cells. EC received either no additions [A and control (C) in B] or 10 μg of MM-LDL per ml (MM in B) together with 250 μCi of [$^{35}$S]methionine per ml. The labeled media were collected, preabsorbed with irrelevant antibody, and equal trichloroacetic acid-precipitable cpm were immunoprecipitated with anti-SMCF in the absence (– in A; C and MM in B) or presence (+ in A) of an excess of purified SMCF and analyzed by SDS/PAGE. The arrows indicate the 14.3-kDa size standard.

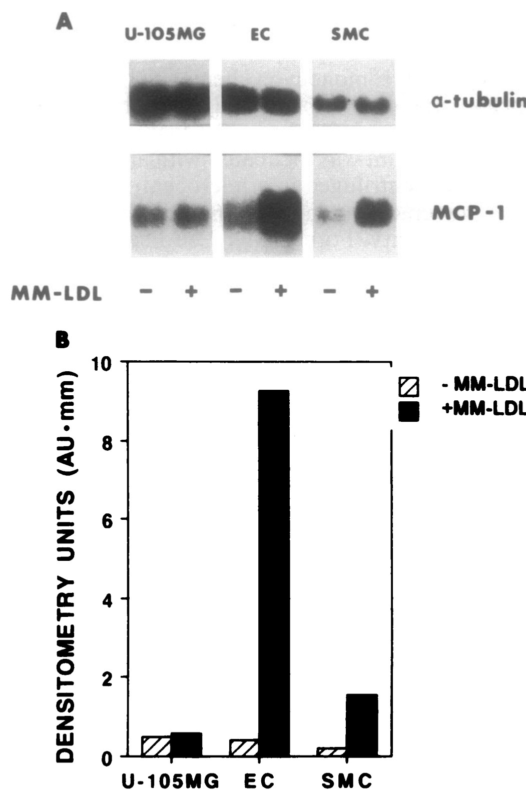


FIG. 3. Expression of MCP-1 mRNA by human cells. Human glioma cells (U-105MG), human EC, and human aortic SMC were incubated with (+) or without (–) 20 μg of MM-LDL per ml for 18 hr. (A) RNA was extracted and Northern blot analysis was performed with MCP-1 and α-tubulin probes. (B) Laser densitometry scans of the Northern blots of MCP-1 mRNA, normalized for α-tubulin. AU, absorbance units.

The ability of MM-LDL to stimulate monocyte chemotactic activity produced by human aortic EC and SMC in coculture separated by a thin layer of collagen is shown in Fig. 6. MM-LDL induced monocyte migration into the subendothelial space of the coculture to the same degree as that induced by fMLP. The increased monocyte migration induced by MM-LDL (but not that induced by fMLP; data not shown) was completely inhibited by anti-SMCF Fab fragments but not by Fab fragments of an irrelevant antibody (Fig. 6). Since it is known that EC produce M-CSF, which can induce monocyte migration (24), and MM-LDL induces M-CSF mRNA (14), the effect of anti-M-CSF Fab fragments on the chemotactic activity was also tested. However, unlike anti-SMCF Fab fragments, anti-M-CSF Fab fragments had no significant effect on monocyte migration into the subendothelial space of the cocultures (data not shown).

## DISCUSSION

A number of monocyte chemotactic factors have been described that may be produced by EC and SMC, including collagen fragments (25), elastin fragments (26), fibronectin fragments (27), transforming growth factor β (28), and 8-kDa to 15-kDa proteins (5, 10, 23).

We have shown here that all of the monocyte chemotactic activity released constitutively into the medium by adult human aortic EC and SMC as well as all of the MM-LDL-induced increase in chemotactic activity was attributable to MCP-1. Anti-SMCF completely inhibited the chemotactic activity produced by either MM-LDL-stimulated or unstimulated EC and SMC as well as the activity released constitutively from the glioma U-105MG cell line, from which

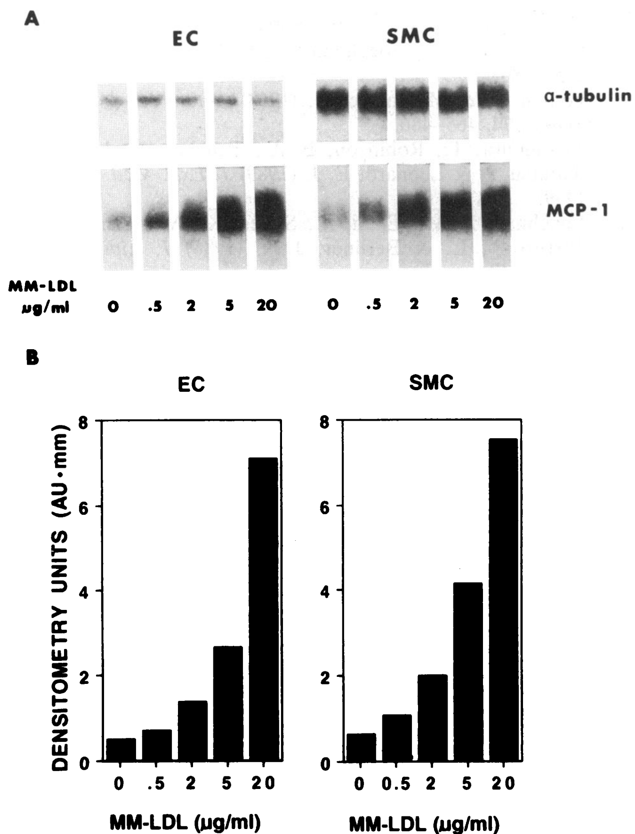


FIG. 4. Dependence of MCP-1 mRNA expression on MM-LDL concentration. Human aortic EC and SMC were incubated for 16 hr with the concentrations of MM-LDL shown on the abscissa. (*A*) RNA was extracted and Northern blot analysis was performed with MCP-1 and α-tubulin probes. (*B*) Laser densitometry scans of the Northern blots of MCP-1 mRNA normalized for α-tubulin. AU, absorbance units.

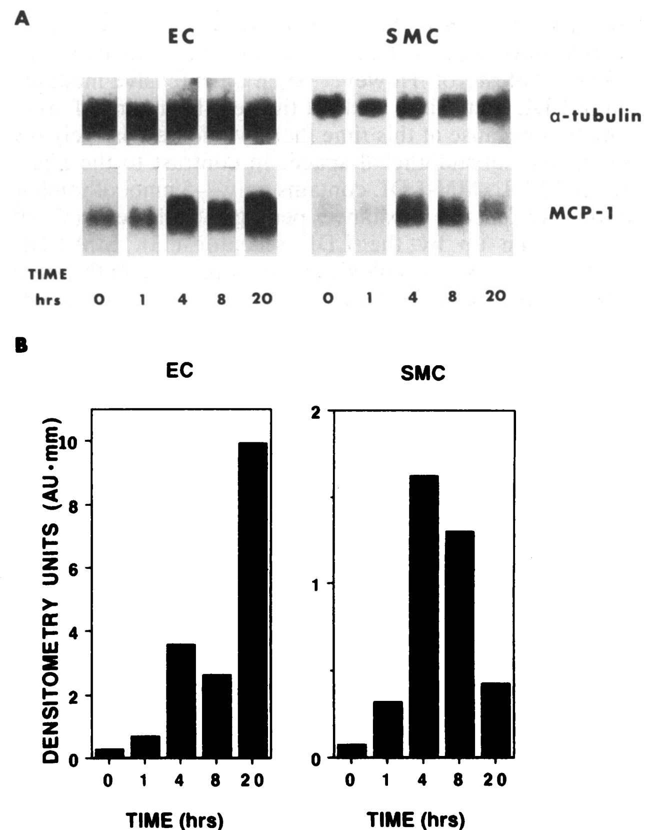


FIG. 5. Time-dependent expression of MCP-1 mRNA by MM-LDL-treated human aortic EC and SMC. EC and SMC were incubated with 20 μg of MM-LDL per ml for the times shown on the abscissa. (*A*) RNA was extracted and Northern blot analysis was performed with MCP-1 and α-tubulin probes. (*B*) Laser densitometry scans of the Northern blots of MCP-1 mRNA normalized for α-tubulin. AU, absorbance units.

MCP-1 was originally isolated. Anti-SMCF also completely inhibited the MM-LDL-induced monocyte migration into the subendothelial space of a coculture of human aortic wall cells. Furthermore, a 35-mer oligonucleotide probe, which was complementary to the published cDNA sequence of MCP-1 (20, 23), specifically hybridized to the same size message in all three cell types and was shown to be dramatically increased in EC and SMC that were treated with MM-LDL. Thus the MM-LDL-stimulated increase in chemotactic activity correlated with the MM-LDL-stimulated increase in MCP-1 message.

Several proteins were immunoprecipitated from EC conditioned medium by anti-SMCF. However, only 10-kDa and 12.5-kDa proteins were increased in stimulated cells and were competitively inhibited by highly purified baboon SMCF. These proteins are similar in size to the monocyte chemotactic proteins isolated from human glioma U-105MG cells (13 kDa and 15 kDa) by Yoshimura and colleagues (17), from the human osteosarcoma MG-63 cell line (9 kDa and 14 kDa), and from baboon SMC (14.4 kDa) by Graves and colleagues (9). Yoshimura and colleagues found the amino acid compositions of the two peptides isolated from the glioma U-105MG cells to be indistinguishable (11). The size differences may be due to cell- and species-specific posttranslational modifications, such as phosphorylation, glycosylation, or degradation. Therefore, we conclude that (*i*) SMCF is in fact MCP-1 and (*ii*) MCP-1 is induced by MM-LDL.

Oxidized lipoproteins have been seen in atherosclerotic plaques (29–33). A number of laboratories have studied LDL that has been modified to the extent that it is no longer recognized by the LDL receptor but is often recognized by the scavenger (acetyl LDL) receptor (34–39). Usually these modifications of LDL have been extensive and the resulting

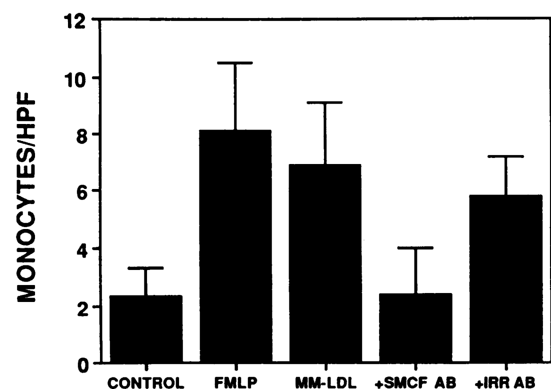


FIG. 6. Monocyte transmigration into the subendothelial space of cocultures of human aortic EC and SMC. The cocultures received either no additions (Control) or received the following: 0.1 μM fMLP; 20 μg of MM-LDL; 20 μg of MM-LDL plus Fab fragments of the antibody to the SMCF (+SMCF AB); 20 μg of MM-LDL plus Fab fragments of an irrelevant antibody (+IRR AB). After 24 hr, human monocytes ($1 \times 10^6$ cells per ml·cm$^2$) that had been fluorescently labeled were added at 37°C for 60 min. Nonadherent leukocytes were removed by washing, the cocultures were fixed, and the number of monocytes that had migrated beneath the endothelial monolayer was determined at high magnification. (×625.) Values are the mean ± SD of number of monocytes in nine high-power fields (HPF). The data shown here are representative of three out of three experiments.

LDL has contained 10–50 nmol of thiobarbituric acid-reactive substances as malondialdehyde equivalents per mg of LDL cholesterol. However, such an extensive modification of LDL seems less likely at the very beginning of lesion formation because at this time there would be relatively few cells in the subendothelial space. In contrast to the highly oxidized LDL, MM-LDL contains only 2–5 nmol of thiobarbituric acid-reactive substances per mg of cholesterol (13, 40) and is taken up by the LDL receptor (13). MM-LDL, therefore, may be physiologically more relevant in the initial stages of atherogenesis than its highly oxidized counterpart. Although MM-LDL itself is not chemotactic (in contrast to highly oxidized LDL; see ref. 6), this study demonstrates that MM-LDL is a potent inducer of MCP-1 in aortic EC and SMC whether cultured independently or together in coculture. Therefore, MM-LDL may be important in the monocyte recruitment into the subendothelium that is seen in the initial stages of atherogenesis.

We thank Drs. Dana Graves, Peter A. Edwards, and Aldons J. Lusis for helpful suggestions, Dr. Susan Imes and Ali Andalibi for help with some of the Northern blots and for reviewing the manuscript, and Laura Almada, Gregory Hough, and Lori Ross for excellent technical assistance. This research was supported by National Institutes of Health Grants HL30568, HL38390, HL26890, RR 865, and ESO 3466 and by the Laubisch Fund. S.D.C. is a Nassi Fellow.

1. Gerrity, R. G., Naito, H. K., Richardson, M. & Schwartz, C. J. (1979) *Am. J. Pathol.* **95**, 775–792.
2. Faggiotto, A., Ross, R. & Harker, L. (1984) *Arteriosclerosis* **4**, 323–340.
3. Jerome, W. G. & Lewis, J. C. (1984) *Am. J. Pathol.* **116**, 56–68.
4. Ross, R. (1986) *N. Engl. J. Med.* **314**, 488–500.
5. Berliner, J. A., Territo, M., Almada, L., Carter, A., Shafonsky, E. & Fogelman, A. (1986) *Arteriosclerosis* **6**, 254–258.
6. Quinn, M. T., Parthasarathy, S., Fong, L. G. & Steinberg, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2995–2998.
7. Jauchem, J. R., Lopez, M., Sprague, E. A. & Schwartz, C. J. (1982) *Exp. Mol. Pathol.* **37**, 166–174.
8. Mazzone, T., Jensen, M. & Chait, A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5094–5097.
9. Graves, D. T., Jiang, Y. L., Williamson, M. J. & Valente, A. J. (1989) *Science* **245**, 1490–1493.
10. Valente, A. J., Graves, D. T., Vialle-Valentin, C. E., Delgado, R. & Schwartz, C. J. (1988) *Biochemistry* **27**, 4162–4168.
11. Yoshimura, T., Robinson, E. A., Tanaka, S., Appella, E. & Leonard, E. J. (1989) *J. Immunol.* **142**, 1956–1962.
12. Robinson, E. A., Yoshimura, T., Leonard, E. J., Tanaka, S., Griffin, P. R., Shabanowitz, J., Hunt, D. F. & Appella, E. (1987) *Proc. Natl. Acad. Sci. USA* **86**, 1850–1854.
13. Berliner, J. A., Territo, M. C., Sevanian, A., Ramin, S., Kim, J. A., Bamshad, B., Esterson, M. & Fogelman, A. M. (1990) *J. Clin. Invest.* **85**, 1260–1266.
14. Rajavashisth, T. B., Andalibi, A., Territo, M. C., Berliner, J. A., Navab, M., Fogelman, A. M. & Lusis, A. J. (1990) *Nature (London)* **344**, 254–257.
15. Navab, M., Hough, G. P., Stevenson, L. W., Drinkwater, D. C., Laks, H. & Fogelman, A. M. (1988) *J. Clin. Invest.* **82**, 1852–1863.
16. Ponten, J. & MacIntyre, E. (1968) *Acta Pathol. Microbiol. Scand.* **74**, 465–486.
17. Yoshimura, T., Robinson, E. A., Tanaka, S., Appella, E., Kuratsu, J. & Leonard, E. J. (1989) *J. Exp. Med.* **169**, 1449–1459.
18. Fogelman, A. M., Elahi, F., Sykes, K., Van Lenten, B. J., Territo, M. C. & Berliner, J. A. (1988) *J. Lipid Res.* **29**, 1243–1247.
19. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
20. Yoshimura, T., Yuhki, N., Moore, S. K., Appella, E., Lerman, M. I. & Leonard, E. J. (1989) *FEBS Lett.* **244**, 487–493.
21. Goding, J. W. (1976) *J. Immunol. Methods* **13**, 215–226.
22. Lo, S. K., Detmers, P. A., Levin, S. M. & Wright, S. D. (1989) *J. Exp. Med.* **169**, 1779–1793.
23. Strieter, R. M., Wiggins, R., Phan, S. H., Wharram, B. L., Showell, H. J., Remick, D. G., Chensue, S. W. & Kunkel, S. L. (1989) *Biochem. Biophys. Res. Commun.* **162**, 694–700.
24. Metcalf, D. (1989) *Nature (London)* **339**, 27–30.
25. Postlethwaite, A. E. & Kang, A. H. (1976) *J. Exp. Med.* **143**, 1299–1307.
26. Senior, R. M., Griffin, G. L. & Mecham, R. P. (1980) *J. Clin. Invest.* **66**, 859–862.
27. Norris, D. A., Clark, R. A. F., Swigart, L. M., Huff, J. C., Weston, W. L. & Howell, S. E. (1982) *J. Immunol.* **129**, 1612–1618.
28. Wahl, S. M., Hunt, D. A., Wakefield, L. M., McCartney-Francis, N., Wahl, L. M., Roberts, A. B. & Sporn, M. B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5788–5792.
29. Morton, R. E., West, G. A. & Hoff, H. F. (1986) *J. Lipid Res.* **27**, 1124–1134.
30. Hoff, H. F. & Gaubatz, J. W. (1982) *Atherosclerosis* **42**, 273–297.
31. Haberland, M. E., Fong, D. & Cheng, L. (1988) *Science* **241**, 215–218.
32. Palinski, W., Rosenfeld, M. E., Yla-Herttuala, S., Gurtner, G. C., Socker, S. S., Butler, S. W., Parthasarathy, S., Carew, T. E., Steinberg, D. & Witztum, J. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1372–1376.
33. Boyd, H. C., Gown, A. M., Wolfbauer, G. & Chait, A. (1989) *Am. J. Pathol.* **135**, 815–825.
34. Morel, D. W., DiCorleto, P. E. & Chisolm, G. M. (1984) *Arteriosclerosis* **4**, 357–364.
35. Steinbrecher, U. P., Parthasarathy, S., Leake, D. S., Witztum, J. L. & Steinberg, D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3883–3887.
36. Heinecke, J. W., Rosen, H. & Chait, A. (1984) *J. Clin. Invest.* **74**, 1890–1894.
37. Cathcart, M. K., Morel, D. W. & Chisolm, G. M. (1985) *J. Leukocyte Biol.* **38**, 341–350.
38. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Witztum, J. L. (1989) *N. Engl. J. Med.* **320**, 915–924.
39. Kosugi, K., Morel, D. W., DiCorleto, P. E. & Chisolm, G. M. (1987) *J. Cell Physiol.* **130**, 311–320.
40. Fox, P. L., Chisolm, G. M. & DiCorleto, P. E. (1987) *J. Biol. Chem.* **262**, 6046–6054.