

HMG-CoA Reductase Inhibitors Decrease CD11b Expression and CD11b-Dependent Adhesion of Monocytes to Endothelium and Reduce Increased Adhesiveness of Monocytes Isolated From Patients With Hypercholesterolemia

CHRISTIAN WEBER, MD, WOLFGANG ERL, PhD, KIM S. C. WEBER, MBBS,*
PETER C. WEBER, MD

Munich, Germany and Boston, Massachusetts

Objectives. This study sought to determine whether inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase affect CD11b expression and adhesiveness of monocytes *in vitro* and after treatment of patients with hypercholesterolemia.

Background. HMG-CoA reductase inhibitors improve survival of patients with coronary heart disease (CHD) and prevent CHD in hypercholesterolemic men. Because these drugs have been shown to modulate monocyte functions, they may act by reducing monocyte adhesion to endothelium, which is crucial in atherogenesis.

Methods. Isolated human blood monocytes were subjected to flow cytometric detection of CD11b and adhesion assays on fixed human endothelial cells after treatment with lovastatin *in vitro* or *ex vivo* before and after treatment of hypercholesterolemic patients with HMG-CoA reductase inhibitors.

Results. The integrin heterodimer CD11b/CD18 expressed on monocytes interacts with intercellular adhesion molecule-1 on endothelium and is involved in monocyte adhesion to endothelium. Treatment of monocytes with lovastatin *in vitro* slightly and dose dependently reduced surface expression of CD11b on mono-

cytes. Moreover, lovastatin inhibited CD11b-dependent adhesiveness to fixed endothelium of unstimulated monocytes or monocytes stimulated with monocyte chemotactic protein 1. Coincubation with mevalonate, but not with low density lipoprotein (LDL), reversed the effects of lovastatin, suggesting that early cholesterol precursors, but not cholesterol, are crucial for adhesiveness of CD11b. In hypercholesterolemic patients, adhesion of isolated monocytes to endothelium *ex vivo* was dramatically increased over values in healthy control subjects. Treatment of these patients with the HMG-CoA reductase inhibitors lovastatin or simvastatin (20 to 40 mg/day) for 6 weeks slightly decreased total and LDL cholesterol plasma levels and monocyte CD11b surface expression but resulted in a significant reduction of monocyte adhesion to endothelium ($p < 0.01$, $n = 7$).

Conclusions. The reduction of CD11b expression and inhibition of CD11b-dependent monocyte adhesion to endothelium may crucially contribute to the clinical benefit of HMG-CoA reductase inhibitors in CHD, independent of cholesterol-lowering effects.

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Inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase are used in the treatment of hypercholesterolemia. They improve the survival of patients with coronary heart disease (CHD) and prevent CHD in hypercholesterolemic men (1,2). However, the clinical benefits of HMG-CoA reductase inhibitors such as simvastatin and pravastatin are not strictly correlated with their lipid-lowering effects (1,3). The action of HMG-CoA reductase inhibitors on cellular chole-

sterol biosynthesis in the liver, blood cells and vasculature may affect membrane integrity and regulation of membrane-related processes (4) that are associated with changes in numerous cellular functions. Cholesterol precursors—i.e., farnesyl or geranyl pyrophosphates—have been implicated in protein-protein interactions and protein anchoring to lipid membranes (5,6), essentially contributing to receptor-stimulated Ca^{2+} increase, superoxide anion formation, low density lipoprotein (LDL) oxidation, eicosanoid synthesis and differentiation in monocytic cells (7-10). The functional consequences of these effects may be relevant for CHD and atherosclerosis, but they remain to be elucidated in detail.

Monocyte recruitment into the vascular wall is crucial for the initiation and progression of atherosclerotic lesions (11). Interaction of the monocytic surface receptor CD11b/CD18, a β_2 integrin heterodimer, with intercellular adhesion molecule-1 (ICAM-1) has been involved in the adhesion to and migration across endothelium of monocytes, particularly when stimulated (12-14). Hence, we studied the effects of HMG-

From the Institut für Prophylaxe der Kreislaufkrankheiten, Ludwig-Maximilians-Universität, Munich, Germany; and *Brigham and Women's Hospital, Boston, Massachusetts. This study was funded in part by Grants We 681/7-1 and We 1913/1 from the Deutsche Forschungsgemeinschaft, Bonn, Germany. It was presented in part at the 17th Congress of the European Society of Cardiology, Amsterdam, The Netherlands, August 1995 and published in abstract form (Eur Heart J 1995;16:273).

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Address for correspondence: Dr. Christian Weber, Institut für Prophylaxe der Kreislaufkrankheiten, Ludwig-Maximilians-Universität, Pettenkoferstrasse 9, D-80336 Munich, Germany.

Abbreviations and Acronyms

CHD	= coronary heart disease
GTP	= guanosine triphosphate
HDL	= high density lipoprotein
HMG-CoA	= 3-hydroxy-3-methyl-glutaryl coenzyme A
HUVECs	= human umbilical vein endothelial cells
ICAM-1	= intercellular adhesion molecule-1
LDL	= low density lipoprotein
mAb	= monoclonal antibody
MCP-1	= monocyte chemoattractant protein-1
PBMCs	= peripheral blood mononuclear cells

CoA reductase inhibitors on monocyte CD11b surface expression and adhesion to endothelium *in vitro*, as well as on increased adhesiveness of monocytes (15) isolated from hypercholesterolemic patients before and after cholesterol-lowering treatment. Our study used a recently established *ex vivo* assay with fixed human endothelial cells (16).

Methods

Monocyte isolation. Peripheral blood mononuclear cells (PBMCs) were isolated from 40 ml of anticoagulated blood collected from healthy donors by using Ficoll density-gradient separation. To separate monocytes from lymphocytes, PBMCs were allowed to adhere to plastic for 2 h and subsequently washed. For treatment with lovastatin, mevalonate (in dimethylsulfoxide), LDL or 0.1% dimethylsulfoxide (control) at indicated concentrations, monocyte-enriched fractions were cultured in 6-well plates (Corning) in Mono Mac 6 medium (10) with 10% fetal calf serum or autologous serum in 5% CO₂ at 37°C for 24 h. LDL was prepared as described (14). For flow cytometry and adhesion assays, adherent monocytes were washed and gently removed with a cell scraper, yielding purities of ~60%, as judged by CD14 immunofluorescence. Under all conditions, cell viability was ≥95%.

In addition, 20 to 40 ml of blood was collected from consenting patients with isolated hypercholesterolemia before and 6 weeks after initiation of treatment with lovastatin (Mevinacor, Merck Sharp & Dohme, 20 to 40 mg/day, n = 3) or simvastatin (Zocor, Dieckmann, 20 mg/day, n = 4) and from seven age-matched healthy control donors. Treatment with lovastatin or simvastatin showed similar effectiveness in lowering plasma lipid levels, as well as in reducing monocyte adhesion. PBMCs were prepared by Ficoll density-gradient centrifugation, and monocytes were isolated from PBMCs by magnetic cell separation (Miltenyi) with Fe-dextran conjugated CD14 monoclonal antibody (mAb), yielding purities ≥85%. The study was approved by the ethical committee of the University of Munich.

Immunofluorescence. Cells were treated with saturating amounts of phycoerythrin conjugated monocyte antigen 2 (Mo2) mAb and fluorescein isothiocyanate-conjugated IgM or phycoerythrin-IgM isotype control and fluorescein

isothiocyanate-monocyte antigen 1 (Mo1) mAb (Coulter) in phosphate-buffered saline solution with 0.5% bovine serum albumin for 30 min on ice (17). To avoid binding to Fc receptors, cells were preincubated with 5% human serum for 15 min on ice. Cells were washed and analyzed with 10,000 cells/sample in a flow cytometer (Becton Dickinson). Mo1 mAb recognizes CD11b, Mo2 mAb recognizes a CD14 epitope expressed on mature monocytes. After correction for unspecific binding, specific mean fluorescence intensity was given in channels on a log₁₀ scale. Cell culture or treatment of PBMCs with lovastatin for 24 h did not affect surface expression of monocyte-specific CD14 (data not shown).

Endothelial cell culture and adhesion assay. Human umbilical vein endothelial cells (HUVECs) were grown in low serum endothelial cell growth medium (PromoCell), as described (18). Purity was assessed by morphology and Factor VIII expression. HUVECs derived from one preparation (passage 2) were grown to confluence in 48-well plates, fixed with 2% paraformaldehyde and stored desiccated and protected from light, as described (16). This procedure resulted in little retraction of HUVECs from their tight junctions and no significant change in surface expression of ICAM-1 or other receptors (e.g., platelet endothelial cell adhesion molecule-1), as assessed by immunofluorescence, and minimized interassay variations that were found when using living HUVECs, particularly in longitudinal adhesion studies. Isolated monocytes were resuspended (10⁵/ml) in medium 199 with 10 mmol/liter HEPES buffer (M199). HUVECs were washed with M199 before addition of monocytes and incubated at 37°C, 5% CO₂, 90% humidity for 60 min (16). The cell suspension was withdrawn, HUVECs were washed twice with M199, and inverted plates were centrifuged (50g, 5 min). Cells were then treated with formalin/ethanol and stained with Coomassie/Giemsa solution. Adherent monocytes were counted in 20 separate areas with the use of light microscopy (×400) and expressed as percent of cells added. Monocytes were pre-treated with CD11b (mAb 44) or isotype control mAb (Sero-tec, all 10 μg/ml) for 15 min, and for *in vitro* assays, cells were stimulated with monocyte chemotactic protein 1 (MCP-1, 10 ng/ml) for 10 min, and allowed to adhere for 30 min. Inhibition of adhesion with CD11b mAb was obtained with or without preincubation of monocytes with gamma-globulin to block Fc receptors. A F(ab')₂ fragment of the mAb was also effective (data not shown).

Cholesterol and triglyceride determination. Plasma concentrations of total, LDL and high density lipoprotein (HDL) cholesterol and triglycerides were determined by standard enzymatic methods (Boehringer Mannheim test kit). All other reagents were from Sigma Chemical Co.

Statistical analysis. All data were expressed as mean value ± SD and were analyzed by analysis of variance combined with a Scheffé F test as a multiple comparison procedure and by *t* test where appropriate. Comparison was made between control subjects and patients with hypercholesterolemia, and between patients before and after treatment. Values of *p* < 0.05 were considered significant.

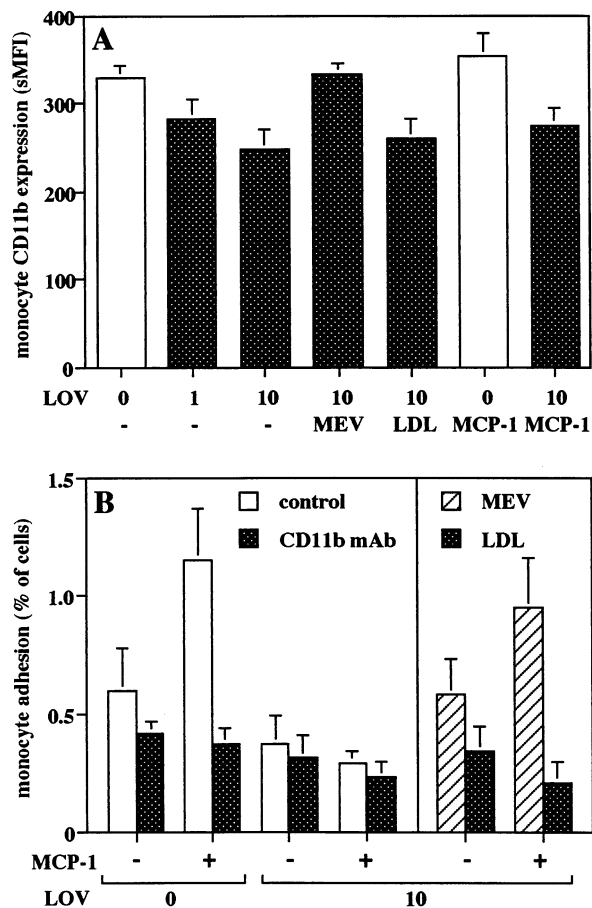


Figure 1. Effect of lovastatin (LOV) on monocyte CD11b expression (A) and adhesion to endothelium (B). Monocytes were treated with or without lovastatin (1 or 10 $\mu\text{mol/liter}$), mevalonate (MEV, 500 $\mu\text{mol/liter}$) or LDL (50 $\mu\text{g/ml}$) for 24 h and carefully harvested, pretreated with CD11b mAb (10 $\mu\text{g/ml}$) for 20 min on ice or prestimulated with MCP-1 (10 ng/ml) for 10 min, as indicated. **A**, Cells were stained with mAb to CD14, CD11b or isotype control and analyzed by flow cytometry. After gating for CD14-positive cells, specific mean fluorescence intensity (sMFI) for CD11b was recorded. Data expressed in channels are mean value \pm SD of three independent experiments. **B**, After coincubation with fixed endothelium for 30 min at 37°C, the number of adherent monocytes was determined. Data expressed as percent of cells added are mean \pm SD of four independent experiments performed in triplicate.

Results

Lovastatin affects monocyte CD11b expression and adhesion to endothelium. We studied the effect of lovastatin on monocyte CD11b surface expression in vitro. After gating for CD14-positive monocytes, untreated cells showed marked CD11b expression, which remained constant for 24 h of cell culture. Treatment with lovastatin dose-dependently reduced CD11b expression (Fig. 1A) from 328 ± 17 to 247 ± 28 channels at 10 $\mu\text{mol/liter}$ ($p < 0.05$, $n = 3$). The effect was not detectable at 2 h and less pronounced at 12 h of incubation (287 ± 18 channels). Co-incubation with mevalonate (500 $\mu\text{mol/liter}$) but not LDL (50 $\mu\text{g/ml}$) reversed the reduction of CD11b expression by lovastatin (Fig. 1A), suggesting that

isoprenoid intermediates but not cholesterol are crucial for expression of CD11b. Chemokines such as MCP-1 are known to upregulate CD11b/CD18 expression on leukocytes (12). In our assay, MCP-1 minimally increased monocyte CD11b expression, but treatment with lovastatin still decreased CD11b expression on MCP-1-stimulated monocytes (Fig. 1A, $p < 0.05$ vs. untreated cells, $n = 3$).

Because the adhesiveness of the ICAM-1 receptor CD11b/CD18 has been shown to require its activation independent of changes in surface expression (12), we studied effects of lovastatin on CD11b-dependent adhesion to fixed HUVECs expressing ICAM-1 but not vascular adhesion molecule-1 (VCAM-1) or E-selectin (14,18). MCP-1 markedly stimulated adhesion of monocytes to fixed HUVECs (Fig. 1B) from $0.60 \pm 0.18\%$ to $1.15 \pm 0.22\%$ ($p < 0.01$, $n = 4$). Preincubation of monocytes with mAb to CD11b reduced adhesion of unstimulated monocytes and completely inhibited MCP-1-stimulated increases in adhesion to fixed HUVECs (Fig. 1B), whereas isotype control mAb had no effect (data not shown). This finding confirms the involvement of CD11b/CD18 and shows that its activation mediates the increase in monocyte adhesiveness. Pretreatment of monocytes for 24 h with lovastatin (10 $\mu\text{mol/liter}$) reduced adhesion of unstimulated monocytes to HUVECs and markedly inhibited adhesion of MCP-1-stimulated monocytes ($0.29 \pm 0.05\%$, $p < 0.01$, $n = 4$) to HUVECs (Fig. 1B). Combination with CD11b mAb showed no additional inhibition, whereas coincubation with mevalonate (500 $\mu\text{mol/liter}$) but not with LDL (50 $\mu\text{g/ml}$) reversed the effect of lovastatin (Fig. 1B). Hence, lovastatin may interfere with activation of CD11b/CD18 adhesiveness by chemokines in monocytes as a result of decreased availability of isoprenoid intermediates.

Effect of lovastatin or simvastatin on monocytes from hypercholesterolemic patients. We next studied whether HMG-CoA reductase inhibitors are also effective in monocytes from patients with hypercholesterolemia. Total, LDL or HDL cholesterol and triglyceride plasma levels in control donors and in patients before and after treatment are shown in Figure 2A. Consistent with previous results (15), we found that the adhesion to endothelium ex vivo of monocytes from patients with isolated hypercholesterolemia was markedly greater (Fig. 2B) than that of monocytes from healthy control subjects with normal plasma cholesterol levels ($p < 0.001$, $n = 7$). The adhesion of monocytes from control subjects ranged from 0.43% to 1.01% (mean \pm SD $0.65 \pm 0.18\%$), whereas adhesion of monocytes from patients was greatly enhanced (range 2.4% to 9.8%, mean $5.3 \pm 2.3\%$). Preincubation with a CD11b mAb inhibited the adhesion of monocytes isolated from patients before treatment to $2.6 \pm 1.2\%$, indicating the involvement of CD11b/CD18 in increased monocyte binding to fixed HUVECs. Treatment of patients with lovastatin (20 to 40 mg/day, $n = 3$) or simvastatin (20 mg/day, $n = 4$) for 6 weeks reduced plasma levels of total and LDL cholesterol while increasing that of HDL cholesterol (Fig. 2A). The adhesiveness of monocytes isolated from patients was dramatically reduced after 6 weeks of treatment (range 0.66% to 2.9%,

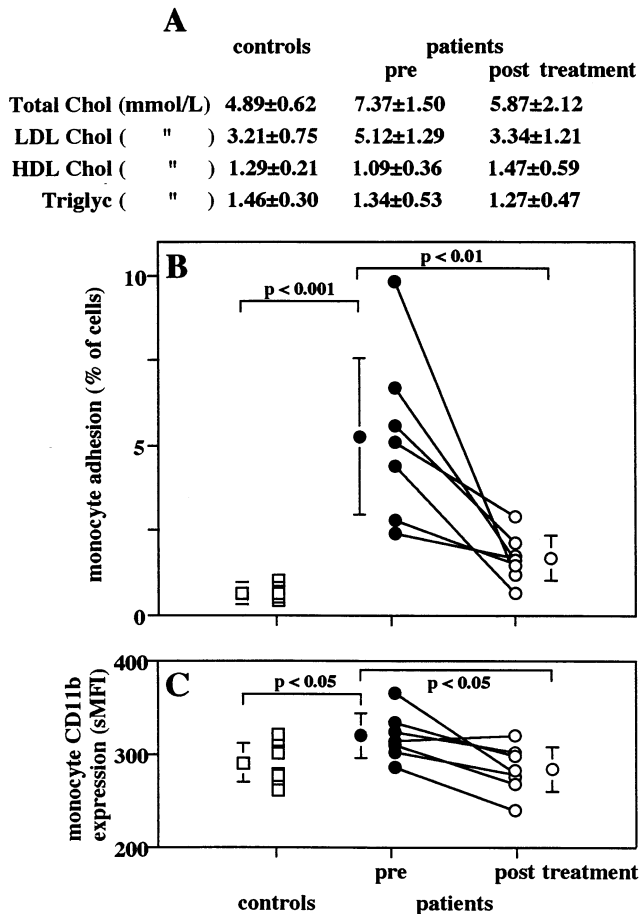


Figure 2. Plasma lipid levels (A), adhesion to endothelium (B) and CD11b expression of isolated monocytes (C) in control donors (controls) and patients with hypercholesterolemia (patients) before (pre) or after (post) treatment with lovastatin (20 to 40 mg/day) or simvastatin (20 mg/day) for 6 weeks. A, Plasma cholesterol (Chol) and triglyceride (Triglyc) levels were determined by standard enzymatic assays. B and C, Monocytes were isolated by magnetic cell separation. After coincubation with fixed endothelium for 60 min at 37°C, the number of adherent monocytes was determined. Experiments were performed in triplicate, and data are expressed as percent of cells added (B). Monocytes were also stained for CD14, CD11b or isotype control, analyzed by flow cytometry and, after gating for CD14-positive cells, specific mean fluorescence intensity (sMFI) for CD11b was recorded and expressed in channels (C). Shown are individual values and mean value \pm SD of control subjects (n = 7, open squares) and patients (n = 7) before (closed circles) or after (open circles) treatment. L = liter.

mean $1.67 \pm 0.65\%$; $p < 0.01$, n = 7) (Fig. 2B). The changes in cholesterol plasma levels were not significantly correlated with those in monocyte adhesiveness (data not shown).

To elucidate possible mechanisms for changes in monocyte adhesiveness, we studied monocyte CD11b expression ex vivo. After gating for CD14-positive monocytes, CD11b expression on monocytes isolated from hypercholesterolemic patients before treatment ranged from 286 to 366 channels (specific mean fluorescence intensity) (mean 319 ± 24 , n = 7), compared with 261 to 322 channels (mean 290 ± 21) in control

donors (Fig. 2C). Treatment with lovastatin or simvastatin for 6 weeks slightly reduced monocyte CD11b expression to 283 ± 24 channels ($p < 0.05$, n = 7). The regulation of CD11b surface expression has been dissociated from its adhesive function (19,20). Therefore, the pronounced decrease in adhesion after treatment with lovastatin or simvastatin may be independent of the slight reduction in CD11b expression.

Discussion

Monocyte accumulation after adhesion to endothelium and extravasation plays a crucial role in the formation of atherosclerosis, to perpetuation of a chronic inflammatory reaction and lipid deposition into monocyte-derived foam cells (11). These cells further contribute to the instability and disruption of atherosclerotic plaques by secretion of proteolytic enzymes that degrade extracellular matrix constituents, and may thus trigger clinical manifestations of atherosclerosis (21). We show that inhibitors of HMG-CoA reductase decrease CD11b-dependent adhesion of unstimulated and MCP-1-stimulated monocytes to human endothelium in vitro. Treatment with HMG-CoA reductase inhibitors is also effective in reducing the increased adhesiveness to fixed endothelium of monocytes isolated from hypercholesterolemic patients ex vivo. The reduction in adhesiveness after HMG-CoA reductase inhibition was more marked than changes in CD11b expression. This suggested that the decrease in monocyte adhesion was due to a reduction in adhesiveness of CD11b/CD18, which can occur independently of its surface expression. The adhesiveness of CD11b/CD18 may rather be mediated by a small subpopulation of molecules undergoing conformational changes in response to stimulation (22). Moreover, the changes in adhesiveness of monocytes ex vivo were poorly correlated with the reduction of total cholesterol and LDL cholesterol plasma levels. The reversibility with mevalonate but not with LDL in vitro also demonstrated that the reduction of CD11b expression and CD11b-dependent adhesion of monocytes was due to decreased availability of isoprenoid cholesterol precursors, but not of cholesterol. Furthermore, increased cholesterol uptake is assumed to compensate for a reduction in cholesterol synthesis during cell culture (9).

Potential mechanisms of action. Isoprenoid molecules posttranslationally modify various proteins and mediate protein-membrane anchoring and protein-protein interactions. Some of the isoprenylated proteins identified are small guanosine triphosphate (GTP)-binding proteins of the ras family, such as rac and rho, as well as the gamma subunits of heterotrimeric G proteins (6). Chemoattractant receptors are coupled to GTP-binding protein heterotrimers, and integrin-dependent binding of leukocytes to endothelium can involve a G-protein regulated activation event (23). Lovastatin has been shown to inhibit Ca^{2+} influx stimulated by G-protein-coupled receptors and localization of ras-like proteins into the membrane of monocytic cells (7). Expression of a constitutively active form of the GTPase R-ras results in activation of β_1 and β_3 integrin avidity, and inactivation of the GTPase rho

suggests its participation in signaling by way of chemoattractant receptors to trigger activation of CD11b/CD18 (24,25). Hence, HMG-CoA reductase inhibitors may impair activation of CD11b/CD18 involving small GTPases or stimulation of G-protein-heterotrimer coupled receptors by MCP-1 or other autocrine chemokines. Alternatively, these drugs may act by interfering with the transport, dimerization or anchoring of CD11b/CD18 in the plasma membrane to maintain its expression on the cell surface. HMG-CoA reductase inhibitors may affect GTP-binding protein-membrane or protein-protein interactions by preventing their prenylation or farnesylation, thus impairing activation and expression of CD11b/CD18.

Increased adhesion of monocytes to endothelium *ex vivo* was recently shown (15) in patients with hypercholesterolemia; however, inhibitory effects of HMG-CoA reductase inhibitors could not be demonstrated. In contrast, the use of fixed endothelium from one preparation minimized interassay variations, particularly in longitudinal adhesion studies (16), and may have thus enabled us to detect inhibitory effects of HMG-CoA reductase inhibitors on monocyte adhesion. The increased adhesion of monocytes from hypercholesterolemic patients has been attributed (15,26) to abnormal monocyte function with respect to eicosanoid metabolism and superoxide anion generation. Hence, HMG-CoA reductase inhibitors may act by inhibiting superoxide anion formation and subsequent LDL oxidation by an NADPH oxidase in activated monocytes that requires assembly of various cytosolic components including small GTP-binding proteins (6,8). Consistently, intake of the antioxidant vitamin C has been demonstrated to prevent the increased CD11b-dependent adhesiveness of monocytes isolated from smokers (16), which may involve similar oxidative pathways.

Limitations of the study. A certain limitation of this study is the small number of patients. However, a small sample size does not preclude the demonstration of potential mechanisms underlying the action of HMG-CoA reductase inhibitors, since the effect of these drugs on monocyte adhesion was highly significant. A second aspect involves the *in vitro* studies that required culture of monocyte preparations over 24 h for treatment with lovastatin. Because CD11b expression and the range of monocyte adhesion remained almost unchanged during this period, and because the effects of lovastatin were comparable to those seen in freshly isolated monocytes, this assay allows valuable insights into mechanisms responsible for the effects of HMG-CoA reductase inhibitors. Thirdly, the isolation procedure may result in a partial activation of monocytes. Thus, the results of this study may reflect the adhesive properties of monocytes that may be activated to some degree.

Conclusions. The reductions in relative risk of CHD after simvastatin reported in the Scandinavian Simvastatin Survival Study (4S) trial (1) did not significantly differ between quartiles for any lipid variable. This finding may be due to effects on crucial mechanisms involved in atherogenesis other than cholesterol lowering. Moreover, the beneficial efficacy of pravastatin in the Regression Growth Evaluation Statin Study

(REGRESS) study (3) is reduced by smoking, which increases oxidative stress and monocyte adhesiveness (16). In conclusion, the reduction of monocyte CD11b expression and CD11b-dependent adhesiveness to endothelium by HMG-CoA reductase inhibitors provides a novel mechanism of action of these compounds and may explain their beneficial effects in the prevention and treatment of CHD (1,2). Our results highlight actions of HMG-CoA reductase inhibitors that are not related to their cholesterol-lowering effects, but may rather be due to interference with cellular isoprenoid metabolism and prenylation-dependent processes.

References

1. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344:1383-9.
2. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 1995;333:1301-7.
3. Jukema JW, Bruschke AVG, van Boven AJ, et al. Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels. The regression growth evaluation statin study (REGRESS). *Circulation* 1995;91:2528-40.
4. Yeagle PL. Lipid regulation of cell membrane structure and function. *FASEB J* 1989;3:1833-42.
5. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990;343:425-30.
6. Glomset JA, Gelb MH, Farnsworth CC. Prenyl proteins in eukaryotic cells: a new type of membrane anchor. *Trends Biochem Sci* 1990;15:139-42.
7. Haag H, Grünberg B, Weber C, Vauti F, Aepfelbacher M, Siess W. Lovastatin inhibits receptor-stimulated Ca^{2+} -influx of retinoic acid differentiated U937 and HL-60 cells. *Cell Signal* 1994;7:735-42.
8. Giron LM, Davignon J, Naruszewicz M. Simvastatin inhibits the oxidation of low-density lipoproteins by activated human monocyte-derived macrophages. *Biochim Biophys Acta* 1993;1165:335-8.
9. Hrboticky N, Tang L, Zimmer B, Weber PC. Lovastatin increases arachidonic acid levels and stimulates thromboxane synthesis in human liver and monocytic cell lines. *J Clin Invest* 1994;93:195-203.
10. Weber C, Erl W, Weber PC. Lovastatin induces differentiation of Mono Mac 6 cells. *Cell Biochem Funct* 1995;13:273-7.
11. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-9.
12. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;76:301-14.
13. Meerschaert J, Furie MB. The adhesion molecules used by monocytes for migration across endothelium include CD11a/CD18, CD11b/CD18, and VLA-4 on monocytes and ICAM-1, VCAM-1, and other ligands on endothelium. *J Immunol* 1995;154:4099-112.
14. Weber C, Erl W, Weber PC. Enhancement of monocyte adhesion to endothelial cells by oxidatively modified low density lipoprotein is mediated by activation of CD11b. *Biochem Biophys Res Commun* 1995;206:621-8.
15. Jongkind JF, Verkerk A, Hoogerbrugge N. Monocytes from patients with combined hypercholesterolemia-hypertriglyceridemia and isolated hypercholesterolemia show increased adhesion to endothelial cells *in vitro*: II: influence of intrinsic and extrinsic monocyte binding. *Metabolism* 1995;44:374-8.
16. Weber C, Erl W, Weber K, Weber PC. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. *Circulation* 1996;93:1488-92.
17. Erl W, Weber C, Wardemann C, Weber PC. Adhesion properties of Mono Mac 6, a monocytic cell line with characteristics of mature human monocytes. *Atherosclerosis* 1995;113:99-107.
18. Weber C, Erl W, Pietsch A, Ströbel M, Ziegler-Heitbrock HWL, Weber PC. Antioxidants inhibit monocyte adhesion by suppressing nuclear factor- κ B mobilization and induction of vascular cell adhesion molecule-1 in endothe-

- lial cells stimulated to generate radicals. *Arterioscler Thromb* 1994;14:1665-73.
19. Buyon JP, Abramson SB, Philips MR, et al. Dissociation between increased surface expression of Gp165/95 and homotypic neutrophil aggregation. *J Immunol* 1988;140:3156-60.
 20. Vedder NB, Harlan JM. Increased surface expression of CD11b/CD18 (Mac-1) is not required for stimulated neutrophil adherence to cultured endothelium. *J Clin Invest* 1988;81:676-82.
 21. Galis ZS, Sukhova GK, Kranzhofer R, Clark S, Libby P. Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. *Proc Natl Acad Sci U S A* 1995;92:402-6.
 22. Diamond MS, Springer TA. A subpopulation of Mac-1 (CD11b/CD18) molecules mediates neutrophil adhesion to ICAM-1 and fibrinogen. *J Cell Biol* 1993;120:545-56.
 23. Bargatze RF, Butcher EC. Rapid G protein-regulated activation event involved in lymphocyte binding to high endothelial venules. *J Exp Med* 1993;178:367-72.
 24. Laudanna C, Campbell JJ, Butcher EC. Role of rho in chemoattractant-activated leukocyte adhesion through integrins. *Science* 1996;271:981-3.
 25. Zhang Z, Vuori K, Wang H-G, Reed JC, Ruoslahti E. Integrin activation by R-ras. *Cell* 1996;85:61-9.
 26. Stragliotto E, Camera M, Postiglione A, Sirtori M, DiMinno G, Tremoli E. Functionally abnormal monocytes in hypercholesterolemia. *Arterioscler Thromb* 1993;13:944-50.