Lovastatin Decreases the Receptor-Mediated Degradation of Acetylated and Oxidized LDLs in Human Blood Monocytes During the Early Stage of Differentiation Into Macrophages

N. Hrbőticky, G. Draude, G. Hapfelmeier, R. Lorenz, P.C. Weber

Abstract—3-Hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors are used therapeutically to upregulate the LDL receptor-mediated removal of plasma cholesterol by the liver. Several lines of evidence indicate that these drugs also exert direct effects on the metabolism of native and modified LDL in extrahepatic cells. We studied the effects of lovastatin (LOV) on the degradation of native, acetylated, and oxidized LDL, and on levels of mRNA encoding for the LDL receptor, the class A types I and II macrophage scavenger receptor, and CD36 receptors in human blood monocytes at different stages of their maturation into adherent macrophages. LOV (10 μmol/L) reduced the degradation of acetylated LDL when added to freshly isolated cells cultured for 2 (81 ± 4% of control, P < 0.05) and 5 (76 ± 6%, of control, P < 0.05) days. The degradation of oxidized LDL was also reduced in cells treated with LOV for 2 days after seeding (51 ± 3% of control, P < 0.001) but not in 5-day-old cells. LOV had no significant effect on the degradation of either acetylated or oxidized LDL when added to fully matured macrophages allowed to differentiate under control conditions for 7 days before incubations with 10 μmol/L LOV for an additional 2 days. In contrast, LOV increased the degradation of native LDL in these cells at all 3 stages of cell differentiation. LOV also reduced class A types I and II macrophage scavenger receptor and CD36 mRNA levels in 2- and 5-day-old cells but not in the more mature macrophages. These data suggest that 3-hydroxy-3-methylglutaryl–coenzyme A inhibitors may reduce the expression and function of the class A types I and II macrophage scavenger receptor and CD36 in monocytes, during the early stages of their differentiation into adherent macrophages. These effects, if operative in vivo, may slow down the development of the atherosclerotic plaque and thus contribute to the beneficial effects of these drugs. (Arterioscler Thromb Vasc Biol. 1999;19:1267-1275.)

Key Words: scavenger receptor ▪ LDL receptor ▪ acetylated LDL ▪ oxidized LDL ▪ CD36 ▪ 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitor ▪ monocyte

A key process in the pathogenesis of atherosclerosis is the adhesion and migration of blood monocytes into the vessel wall, followed by their conversion into adherent macrophages and foam cells by the unregulated uptake of modified lipoproteins, through the so-called scavenger receptors. Of the many cell surface proteins discussed as scavenger receptors, the class A types I and II macrophage scavenger receptor (SR-A) and CD36 have been shown to be responsible for the uptake of modified lipoproteins by human blood monocyte-derived macrophages (HMDMs). SR-A exhibits high affinity for both the chemically modified acetylated (acetyl) LDL and oxidized (ox) LDL, the latter of which is formed in vivo and is involved in atherosclerosis. Studies in SR-A–negative mice have recently confirmed the involvement of this receptor in the development of the atherosclerotic plaque and elevated SR-A mRNA have been observed in freshly isolated mononuclear cells of hyperlipidemic patients. The 88-kDa glycoprotein CD36, on the other hand, has been shown to mediate up to 50% of ox LDL metabolism in HMDMs in vitro.

Lovastatin (LOV) is a lipophilic member of the 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors, widely used in the treatment of hypercholesterolemia. Although their main site of action and clearance is the liver, their potential to influence cellular lipid metabolism, growth, and function of peripheral cells is of growing interest. Parallel to their action in the liver, HMG-CoA reductase inhibitors have been shown to suppress cellular cholesterol synthesis and to stimulate the receptor-mediated uptake of LDL in HMDMs. Their effect on the uptake and degradation of modified lipoproteins is less well established. Two earlier studies in HMDM detected no significant effects of HMG-CoA reductase inhibitors on the metabolism of modified LDL. On the other hand, these drugs have been shown to reduce acetyl LDL degradation in mouse peritoneal macrophages and to inhibit SR-A gene expression in phorbol ester–differentiated THP-1 cells. HMG-CoA reductase inhibitors also reduce cholesterol esterification and cholesterol ester deposition in mouse peritoneal macrophages and human monocyte-derived macrophages. Furthermore, these
drugs can reduce the mRNA levels and cell surface expression of CD36 as well as ox LDL binding in the human monocyte U937 cells. 

Studies of human blood monocytes and monocytic cell lines indicate that the cellular expression of both SR-A 25 and CD36 23 is strongly influenced by cell differentiation. The potential of HMG-CoA reductase inhibitors to influence the expression and function of these 2 scavenger receptors may thus be dependent on the stage of cell maturity. Therefore, we investigated the effects LOV on the metabolism of native (nat), acetyl-, and ox LDL, and on the specific mRNA expression for LDL, SR-A, and CD36 receptors in cultured human blood monocytes at 3 different stages of their maturation into adherent macrophages.

Methods

Materials

LOV was a kind gift of Merck Sharp & Dohme. Iodine-125 (carrier free) was purchased from Amersham. Phenol was from Amresco.

Murine leukemia virus reverse transcriptase was from Life Technologies, random hexamers were from Boehringer Mannheim. Taq polymerase and DEAE column were from Applied Biosystems. Ficoll was from Biochrom. Falcon Primaria cell culture dishes were from Becton Dickinson. Cell culture media and ingredients as well as all other chemicals were purchased from Sigma.

Cell Isolation and Culture

Blood was collected from healthy, normolipidemic male volunteers on 10% sodium citrate. Peripheral blood mononuclear cells were isolated by Ficoll density-gradient separation as described. 22 Cells were plated in RPMI 1640 medium containing 4 mmol/L L-glutamine, 200 U/mL penicillin, and 200 μg/mL streptomycin (medium A). Monocytes were allowed to adhere to culture dishes at 37°C in 5% CO2 and 95% air. Nonadherent cells were removed by washing 3 times with medium A at 2 hours after seeding, every subsequent 60 hours, and again before each experiment. Cells were cultivated in medium A also containing 20% autologous serum. LOV or its carrier dimethyl sulfoxide (DMSO; final concentration, 0.1% vol/vol) were added either to freshly isolated cells 2 hours after plating at indicated concentrations for 2 or 5 days, or to matured cells, cultured under control conditions for 7 days before LOV treatment for an additional 2 days. Cell viability, as judged by ethidium bromide/acridin orange staining, was >90% under all conditions.

Isolation, Acetylation, Oxidation, and Labeling of Lipoproteins

LDL (density, 1.019 to 1.063 g/mL) was isolated from plasma of normolipidemic fasting subjects by sequential preparative ultracentrifugation. Acetylation of LDL was performed as in the study by Basu et al. 23 Ox LDL was prepared by incubation of LDL (200 μg/mL) in EDTA-free, O2-saturated PBS containing 5 μmol/L CuSO4 at 37°C for 6 or 20 hours. Oxidation was stopped by the addition of EDTA (final concentration, 0.24 mmol/L) and ox LDL was washed 5 times with PBS containing 0.24 mmol/mL EDTA using Centriflo ultrafiltration cones (CF25 Amicon). Lipoproteins were oxidized by dialysis in 500 volumes of EDTA-free, O2-saturated PBS containing 5 μmol/L CuSO4 at 37°C for 6 or 20 hours. Oxidation was stopped by dialyzing samples in PBS containing 0.48 mmol/L EDTA at 4°C for 2 hours. All lipoproteins were filter-sterilized (0.22 μm) and stored at 4°C. Protein concentrations were determined as in the study by Markwell et al. 26 The extent of oxidation of both unlabeled and radiodinated ox LDL was assessed by means of agarose gel electrophoresis (Ciba Corning Diagnostics). The mean relative mobilities of LDL preparations oxidized for 6 and 20 hours were 2.1±0.1 and 3.6±0.1, respectively. Some preparations of fully oxidized 125I-LDL (20 hours) were centrifuged at 10 000g for 15 minutes to remove large insoluble LDL aggregates. However, these represented only 1% of total radioactivity of these preparations.

125I-Lipoprotein Degradation, Cell Association, and Binding

Degradation and Cell Association

After washing 3 times with medium A, cell monolayers were incubated in 1 mL of medium A with 125I-labeled lipoprotein (10 μg/mL) in 5% CO2 at 37°C for 5 hours, with or without a 25-fold excess of unlabeled lipoprotein. At the end of the incubation period, medium was removed and cells were washed 3 times each with 2 mL of PBS with and without 0.2% BSA. Washed cell pellets were solubilized in 0.2 mol/L NaOH and the protein content was measured. 25 Proteolytic degradation was measured as trichloroacetic acid– and silver nitrate–soluble radioactivity released into the medium. Cell association, representing the sum of bound and internalized lipoprotein at 37°C, was measured as radioactivity contained in the washed and solubilized cell pellets.

Binding

Cells in 1 mL of medium A also containing 25 mmol/L HEPES were incubated with 10 μg/mL 125I-labeled lipoprotein in the presence or absence of a 25-fold excess of unlabeled lipoprotein for 2 hours at 4°C. The cell-associated radioactivity per milligram of cell protein was assessed in washed cell pellets as described above.

Specific degradation, association, and binding were calculated as the difference between total (without unlabeled ligand) and nonspecific (in the presence of 25-fold excess of unlabeled ligand) values.

RNA Isolation

RNA was isolated from adherent cells grown on 10-cm culture dishes as in the study by Danesch et al. 26 Cells were washed with PBS and lysed in 800 μL of 7.5 mol/L guanidine HCl, 25 mmol/L sodium citrate, and 3.5 mmol/L N-lauroylsarcosine, adjusted to pH 5.2 with 1 mol/L acetic acid. The lysed cell mix was passed 5 times through a 0.4-mm-diameter needle to shear chromosomal DNA. RNA was selectively precipitated overnight with 0.5 volumes of 100% ethanol. RNA was dissolved in 500 μL of 50 mmol/L Tris-HCl buffer containing 25 mmol/L NaCl, 5 mmol/L EDTA, 0.1% SDS, and 0.3 mmol/L sodium acetate, pH 5.0, and extracted with water-saturated phenol. The upper phase containing RNA was washed once with diethyl ether to remove residual phenol. RNA was then precipitated overnight with 2.5 volumes of 100% ethanol and dissolved in diethylpoly carbonate (DEPC)-treated water.

Reverse Transcription–Polymerase Chain Reaction (RT-PCR) and HPLC

cDNA was reverse-transcribed from 0.3 μg of total RNA, using murine leukemia virus reverse transcriptase primed with random hexamers. Specific primers were selected to bind to regions with minimal homology, to span at least 1 intron for distinction from genomic DNA and to avoid nonspecific annealing. Primers synthesized according to known cDNA sequences were 5’CCA GGG ACA TGG GAA TGC AA 3’ (forward primer, 20-mer; nucleotides 544 to 563) and 5’CCA GTG GGC CCT CGA TTC CC 3’ (reverse primer, 20-mer; nucleotides 909 to 890) for SR-A types I and II, 5’CAA TGT CTC ACC AAG CTC TG 3’ (forward primer, 20-mer, nucleotides 1125 to 1106) for CD36, 27 5’GAG AAC TGT TAT GGG GCT AT 3’ (forward primer, 20-mer, nucleotides 737 to 756) and 5’TTC AAC TGG AGA GGC AAA GG 3’ (reverse primer, 20-mer, nucleotides 2297 to 2316) and 5’TTC GTC TCG AGG GGT AGC TG 3’ (reverse primer, 20-mer, nucleotides 2554 to 2535) for LDL receptor, 5’GAG AAC TGT TAT GGG GCT AT 3’ (forward primer, 20-mer, nucleotides 737 to 756) and 5’TTC AAC TGG AGA GGC AAA GG 3’ (reverse primer, 20-mer, nucleotides 2297 to 2316) and 5’TTC GTC TCG AGG GGT AGC TG 3’ (reverse primer, 20-mer, nucleotides 2554 to 2535) for LDL receptor.

cDNA was amplified by using Taq polymerase (Sigma) and 15 pmol of each primer in a Cetus thermocycler 480 (Perkin-Elmer) set to the following profile: 95°C melting (5 minutes), 58°C annealing (60 seconds), and 72°C extension (60 seconds) followed by 95°C (30 seconds), 58°C (60 seconds), and 72°C (60 seconds) for 25 cycles,
finished by an extension step at 72°C for 10 minutes. Specific PCR products were obtained for β-actin (540 bp), SR-A types I and II (366 bp), LDL receptor (258 bp), and CD36 (389 bp), respectively. Linearity of amplification was confirmed up to 32 cycles and 1 μg of total RNA in the reverse transcription step.

Specific mRNA levels were quantified by HPLC separation on a nonporous DEAE column (Applied Biosystems) with a 0.3 to 0.6 mol/L NaCl gradient (piston pump 307 and 306 with dynamic mixer 811, 700 μL volume, Gilson-Abimed). Amplificates were detected at 260 nm and quantified by integration of corresponding peak areas (115 Variable Wavelength Detector, System Interface Module 506C, System-Software 712 V1.1, Gilson-Abimed). mRNA amounts were normalized to levels of β-actin mRNA, which served as endogenous standard to compensate for variations in mRNA extraction.

**Fluorescence-Activated Cell Sorting (FACS) Analyses**

Cells were gently scraped from culture dishes, washed twice with PBS containing 1 mmol/L CaCl₂ and MgCl₂, and preincubated with 5% human serum in PBS for 15 minutes on ice to block nonspecific binding to Fc receptors. Cells were then incubated with saturating amounts of mouse monoclonal antibodies against human CD14 (phoeyrthrin-conjugated Mo2-RD1, Coulter Clone), CD36 (FITC-conjugated SMO clone, Camon) or CD11b (FITC-conjugated, Mo-1, Coulter Clone), or the appropriate phoeyrthrin-IgM and FITC-IgM isotype controls, in PBS containing 0.5% BSA for 30 minutes on ice. In some experiments, an unconjugated CD36 antibody (SMO, Camon) was used and cells were stained with FITC-conjugated goat anti-mouse F(ab')₂ Ig fragment (DAKO, Denmark). After staining, all cells were washed twice with FACS buffer (Becton Dickinson), fixed in 2% paraformaldehyde in FACS buffer, and analyzed by FACS (Becton Dickinson). Cell debris and contaminating cells were excluded from the analysis by gating for monocytes in the forward/ sideway scatter. At least 5000 cells were used in each analysis. After correction for nonspecific staining (isotype control), specific mean fluorescence intensity (sMFI) was presented in channels.

**Statistical Analysis**

The results are presented as mean±SEM absolute values or as percentages of control. Statistically significant differences were analyzed by Student’s t test for paired or unpaired data as appropriate. Dose–response effects of LOV were assessed by using linear regression analysis.

**Results**

The effect of length of cell cultivation and LOV on the cellular degradation of modified and nat LDL are depicted in Figure 1. As first shown by Fogelman et al,20 the cellular degradation of nat and modified LDL by HMDMs varied with the length of in vitro cell cultivation. In control cells, the degradation of both 125I-acetyl and 125I-ox LDL increased ∼3-fold between 2 and 5 days after plating, presumably reflecting the differentiation-related increase in the expression of several scavenger receptors in these cells.4,21,31 The degradation of both modified lipoproteins decreased in cells cultivated under control conditions for 9 days, returning to levels seen in 2-day-old cells. Our data on cell-associated radioactivity, representing surface-bound and internalized lipoprotein suggest, that the decrease in lipoprotein degradation in the 9-day-old cells was the consequence of increased cellular accumulation of undegraded lipoprotein (Table 1). The cellular degradation of nat LDL in untreated cells, on the other hand, was the highest after 2 days of culture and decreased to similar levels in 5- and 9-day-old cells (Figure 1).

As expected from its main mechanism of action, LOV (10 μmol/L) increased the cellular degradation of nat LDL. This effect was observed independent of whether LOV was given to freshly seeded cells for 2 and 5 days, or to matured HMDMs cultured for 7 days in normal growth medium before treatment with 10 μmol/L LOV for an additional 2 days (Figure 1c). The levels of mRNA encoding for the LDL receptor also tended to be higher in LOV-treated cells. However, this increase was statistically significant only in cells treated with 10 μmol/L LOV for 5 days after seeding (Figure 2).

Incubations with LOV (10 μmol/L) resulted in a significant decrease in 125I-acetyl LDL degradation when added to freshly isolated peripheral blood mononuclear cells were cultured for 2 and 5 days, or matured macrophages grown in normal culture medium for 7 days were treated with 10 μmol/L LOV or its carrier (DMSO, 0.1% final concentration) for the times indicated. Receptor-specific lipoprotein degradation was determined as described in Methods. Data are mean±SEM values of 4 to 6 separate experiments performed in duplicate. Statistical differences between mean values were determined with the paired t test.

HMDMs cultured for 7 days in normal growth medium before treatment with 10 μmol/L LOV for 5 days after seeding (Figure 2).

Figure 1. Effect of LOV on the degradation of 125I-labeled acetyl (a), ox (b), and nat LDL (c) in HMDMs. Freshly isolated peripheral blood mononuclear cells were cultured for 2 and 5 days, or matured macrophages grown in normal culture medium for 7 days were treated with 10 μmol/L LOV or its carrier (DMSO, 0.1% final concentration) for the times indicated. Receptor-specific lipoprotein degradation was determined as described in Methods. Data are mean±SEM values of 4 to 6 separate experiments performed in duplicate. Statistical differences between mean values were determined with the paired t test.
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TABLE 1. Effect of LOV and Length of Cell Cultivation on the Cell Association of 125I-Labeled Lipoproteins at 37°C

<table>
<thead>
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<th>2 Days</th>
<th>5 Days</th>
<th>7+2 Days</th>
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<tbody>
<tr>
<td>Acetyl LDL Control</td>
<td>251±105</td>
<td>427±88</td>
<td>746±43</td>
</tr>
<tr>
<td>LOV</td>
<td>227±84</td>
<td>382±78</td>
<td>785±75</td>
</tr>
<tr>
<td>Ox LDL Control</td>
<td>634±63</td>
<td>2242±441</td>
<td>1111±146</td>
</tr>
<tr>
<td>LOV</td>
<td>341±48*</td>
<td>2884±748</td>
<td>1230±115</td>
</tr>
<tr>
<td>Nat LDL Control</td>
<td>106±10</td>
<td>60±11</td>
<td>68±18</td>
</tr>
<tr>
<td>LOV</td>
<td>130±15*</td>
<td>80±15</td>
<td>101±29*</td>
</tr>
</tbody>
</table>

Freshly isolated human blood monocytes cultured for 2 or 5 days or matured macrophages grown in normal culture medium for 7 days were treated with 10 μmol/L LOV for the times indicated. Cell associations of 125I-labeled acetyl, ox, and nat LDL were determined as described in “Methods.” Data are mean±SEM percentages of control values from 3 experiments performed in duplicate. The significance of the dose–response effect was assessed by means of linear regression. Differences between means were determined with the paired t test.

The metabolism of ox LDL was studied further in 5-day-old HMDMs. Cells at this stage of differentiation exhibited the highest rate of ox LDL uptake and degradation, whereas the effect of LOV on both parameters was contrary to the downregulation of the SR-A and CD36 scavenger receptors. In a manner similar to its lack of effect on ox LDL uptake and degradation, LOV (10 μmol/L) had no effect on ox LDL binding at 4°C, which was 89.7±8.0% for control, 89.5±7.6% for LOV-treated, and 701±90 and 953±59% for control, respectively (Figure 2). The levels of SR-A mRNA in LOV-treated matured HMDMs, on the other hand, were not significantly different from control cells (89.7±8.9% of control).

In correspondence to the LOV-induced reduction in 125I-acetyl LDL degradation, LOV decreased SR-A mRNA expression, when given to freshly seeded cells for 2 and 5 days, to 74.8±7.9% and 67.9±4.4% of control values, respectively (Figure 2). The levels of SR-A mRNA in LOV-treated matured HMDMs, on the other hand, were not significantly different from control cells (89.7±8.9% of control).

The cellular degradation of ox LDL was also significantly reduced by LOV when given for 2 days to freshly seeded monocytes (Figure 1b). The amount of ox LDL accumulated intracellularly was also significantly reduced in these cells (Table 1). In contrast, both the cellular degradation and accumulation of ox LDL tended to increase in cells receiving 10 μmol/L LOV for 5 days after seeding, although this increase did not achieve statistical significance. As seen for acetyl LDL, LOV had no effect on ox LDL metabolism when given to matured HMDMs cultured for 7 days in normal growth medium before treatment with 10 μmol/L LOV for an additional 2 days. In a manner similar to SR-A mRNA expression, CD36 mRNA levels were significantly reduced in cells incubated with 10 μmol/L LOV for 2 days after seeding (69.2±5.5% of control, Figure 2). CD36 expression tended to be lower in cells treated with LOV for 5 days (78.5±10.5% of control, P=0.08). In contrast, no reduction in CD36 mRNA levels was seen in matured HMDMs treated with LOV for 2 days (94.4±10.5% of control).

The metabolism of ox LDL was studied further in 5-day-old HMDMs. Cells at this stage of differentiation exhibited the highest rate of ox LDL uptake and degradation, whereas the effect of LOV on both parameters was contrary to the downregulation of the SR-A and CD36 scavenger receptors.
In agreement with others,5,6 125I-ox LDL degradation was only partially blocked by a 25-fold excess of unlabeled acetyl LDL (45±13% of total). The combination of unlabeled ox and acetyl LDL had no additive effect on nonspecific 125I-ox LDL degradation (21±5.0% of total), whereas excess nat LDL did not block ox LDL degradation. Because strongly oxidized LDL tends to aggregate and be taken up into cells by receptor-independent mechanisms,33,34 some ox LDL preparations were centrifuged at 10 000 g for 10 minutes to minimize the presence of large ox LDL aggregates. However, in these experiments, as well as in experiments with mildly oxidized LDL (5 μmol/L CuSO4, 6 hours), which may be preferentially taken up by specific ox LDL receptors such as CD36,6 no significant effects of LOV on cellular ox LDL degradation or association could be demonstrated in 5-day-old cells (data not shown). Furthermore, LOV did not influence the degradation and cell accumulation of 125I-ox LDL in the presence of a 25-fold excess of acetyl LDL, used to specifically block the uptake of ox LDL through the SR-A receptor.

Because our data suggested that LOV reduced the cell differentiation–induced increase in the expression of the SR-A and CD36 scavenger receptors, we also examined its effects on the surface expression of CD14 and CD11b, 2 markers of monocyte/macrophage differentiation (Table 2 and Figure 5). A modest increase in the expression of both surface antigens was observed between day 2 and day 5 of culture. Inductions of larger magnitude may have occurred in the earlier stages of in vitro monocyte adhesion and differentiation. On the other hand, the expression of CD14 decreased and that of CD11b remained unchanged in 9-day-old cells. A reduction in the expression of CD14 and other surface antigens has been previously reported in prolonged monocyte culture.35 LOV had no significant effects on the cell surface CD14 and CD11b expression at the cultivation times studied. The process of monocyte differentiation was also evidenced by changes in the morphology of 2-, 5-, and 9-day-old cells, including the typical increase in cell spreading and the appearance of irregularly shaped cells and multinucleated giant cells in the 9-day-old HMDMs (Figure 6). It is noteworthy that the surface expression of CD36 decreased with the length of cultivation. Furthermore, a modest but consistent reduction in CD36 expression was observed in 5- and 7+2-day-old LOV-treated cells.

**Discussion**

In this study, we investigated the effects of the HMG-CoA reductase inhibitor LOV on the receptor-specific degradation of nat LDL, acetyl LDL, and ox LDL in human monocyte-derived macrophages. In agreement with previous studies in human monocyte-derived macrophages,12,13 LOV increased the receptor-specific degradation of nat LDL. This effect was observed when LOV was given to freshly seeded cells during their spontaneous differentiation to adherent macrophages, as well as in matured macrophages allowed to differentiate under control conditions before LOV treatment. This increase in LDL degradation did not, however, always correlate with the receptor-specific degradation of modified lipoproteins in human monocyte-derived macrophages.4,20 This reduction appeared to be the result of a selective action of LOV on SR-A expression, because cell

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**TABLE 2. Effect of LOV and Length of Cell Cultivation on the Cell Surface Expression of CD36, CD11b, and CD14**

<table>
<thead>
<tr>
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<th>2 Days</th>
<th>5 Days</th>
<th>7+2 Days</th>
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<tbody>
<tr>
<td>CD36</td>
<td>349±23</td>
<td>259±60</td>
<td>199±45</td>
</tr>
<tr>
<td>LOV</td>
<td>305±25</td>
<td>221±54*</td>
<td>175±4*</td>
</tr>
<tr>
<td>CD14</td>
<td>356±64</td>
<td>414±35</td>
<td>286±83</td>
</tr>
<tr>
<td>LOV</td>
<td>335±48</td>
<td>454±19</td>
<td>317±75</td>
</tr>
<tr>
<td>CD11b</td>
<td>349±15</td>
<td>408±37</td>
<td>406±33</td>
</tr>
<tr>
<td>LOV</td>
<td>326±23</td>
<td>425±25</td>
<td>389±29</td>
</tr>
</tbody>
</table>

Freshly isolated human blood monocytes cultured for 2 and 5 days or matured human macrophages grown in normal culture medium for 7 days were treated with 10 μmol/L LOV for the times indicated. Cell surface expressions of CD36, CD14, and CD11b were determined as described in “Methods.” Data are mean±SEM values of 4 to 6 experiments and are expressed as units of specific mean fluorescence intensity (sMFI).

*Significant differences between mean values were determined by the paired t test.
differentiation as assessed by the cell surface expression of the differentiation markers CD14 and CD11b was not significantly influenced by this drug. Our findings of decreased function and expression of SR-A after in vitro treatment of cells with HMG-CoA reductase inhibitors are in agreement with similar studies in mouse peritoneal macrophages\textsuperscript{16} and in phorbol ester–differentiated THP-1 macrophages.\textsuperscript{17} In contrast, no differences in ex vivo acetyl LDL degradation

**Figure 5.** Representative FACS analyses of the surface expression of CD36, CD14, and CD11b. Freshly isolated human blood monocytes were cultivated as described in Figure 1. Cell surface expression of CD36, CD14, and CD11b was determined as described in Methods.
was reported in 1 study of 10-day-old monocyte-derived macrophages isolated from patients before and after the onset of an 8-week-long pravastatin therapy. The lower concentration of the drug used and the longer cultivation periods may explain the lack of effect in this study. On the other hand, our results are similar to the only other study on cultured human monocytes, where a 30%, but statistically insignificant, decrease in acetyl LDL degradation after a 2-day incubation with 13 mM mevastatin was observed. In a manner similar to others, we found that 125 I-ox LDL degradation was efficiently inhibited by an excess amount of unlabeled ox LDL (18.8%) but only to 55% by unlabeled acetyl LDL. Thus, in our cells, 45% of the degradation of ox LDL was putatively mediated by receptors other than SR-A. A major candidate in human monocyte-derived macrophages is CD36, as the receptor-specific degradation of ox LDL can be suppressed by 26% to 50% of control by coincubations with monoclonal CD36 antibodies in these cells. In the present studies, LOV decreased the expression of mRNA encoding for both SR-A and CD36 when added to freshly isolated monocytes for 2 and 5 days. However, ox LDL uptake and degradation was only reduced in the 2-day-old cells, and no such reduction was seen in cells treated with LOV for 5 days. The interpretation of studies of ox LDL metabolism are complicated by the heterogeneous nature of in vitro derived ox LDL and indeed of ox LDL isolated from atherosclerotic tissue. Furthermore, the effect of agents such as LOV on ox LDL degradation by macrophages will be the net sum of their effects on SR-A, CD36, and additional cell surface proteins with affinity for ox LDL, all of which most likely participate in the binding and degradation of ox LDL in these cells. The herein observed differences in 2- versus 5-day-old cells suggest that the relative importance of these additional uptake mechanisms in cellular ox LDL metabolism increases with cell differentiation.

The molecular mechanism behind the LOV-induced reduction in the cellular expression of SR-A and CD36 remains to be fully clarified. Additions of the early nonsterol cholesterol precursor mevalonic acid or farnesol have been shown to reverse the LOV-induced reduction in the expression of both receptors. Furthermore, the involvement of prenylated ras proteins in the transcription of SR-A and CD36 has been demonstrated. HMG-CoA reductase inhibitors may thus reduce the transcription of both proteins through their indirect inhibition of protein prenylation. It has been also shown that HMG-CoA reductase inhibitors reduce cellular superoxide radical formation and cellular responses to ox LDL. Because both SR-A and CD36 are induced by oxidative stimuli, the reduction in SR-A and CD36 expression by LOV may also be the indirect consequence of a reduced cellular response to oxidative stimuli. In the present study, LOV suppressed scavenger receptor expression only in the early stages of in vitro macrophage differentiation. A similar dependence of length of cell cultivation has been previously demonstrated in the inhibition of SR-A expression by lipopolysaccharide in these cells. This dependence of cellular response on the stage of macrophage differentiation may also explain the lack of reduction in the surface expression of the adhesion protein CD11b at 2 to 9 days, previously reported in 24-hour-old, LOV-treated HMDMs. It is noteworthy that our acetyl LDL binding studies showed no significant changes in the number of binding sites for acetyl LDL on the cell surface of LOV-treated cells. Bernini et al have also recently shown that HMG-CoA reductase inhibitors reduce the acetyl LDL degradation in mouse peritoneal macrophages but have no effect on acetyl LDL binding sites, as assessed by acetyl LDL binding at 4°C. In a manner similar to Bernini et al, we found acetyl LDL binding to be minimal when compared with degradation, most likely reflecting a highly efficient rate of endocytosis, receptor recycling, and lysosomal degradation by these cells. The simultaneous LOV-related reduction in SR-A expression and acetyl LDL degradation in the presence of the same number of binding sites implies concomitant changes in receptor endocytosis, recycling, or ligand degradation. Fong has recently reported a dissociation between SR-A surface expression and activity. His studies point to a significant posttranslational regulation of SR-A via protein phosphorylation/dephosphorylation mechanisms influencing receptor surface distribution and...
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lignand-receptor internalization. Prenylation of cellular proteins by isoprenoid precursors of cholesterol is involved in a wide spectrum of cellular functions, including receptor-mediated endocytosis. It is thus conceivable that the LOV-induced changes in such posttranslational mechanisms could have contributed to the reduction in acetylated LDL degradation seen in our study.

In conclusion, this study demonstrates that HMGC-CoA reductase inhibitors such as LOV reduce the mRNA expression of the class A type I and II scavenger receptor and CD36 in human monocytes during their in vitro differentiation into adherent mature macrophages. This reduction in mRNA scavenger receptor levels was functionally reflected in a reduction in the cellular uptake and degradation of acetylated LDL and ox-LDL in the early stages of cell differentiation. In vivo, such cells may represent the infiltrating monocytes in the process of migrating through the subendothelial matrix. Thus, as an extension of previous reports, we demonstrate phase-specific effects of HMGC-CoA reductase inhibitors at several levels of handling of modified lipoproteins by monococyte-derived macrophages. These mechanisms, if occurring in vivo, could contribute to slowing down the progression of atherosclerosis documented for this class of drugs.

Acknowledgments

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References

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