Specific Interaction of Oxidized Low-Density Lipoprotein With Macrophage-Derived Foam Cells Isolated From Rabbit Atherosclerotic Lesions

Helga E. de Vries, Bas Buchner, Theo J.C. van Berkel, Johan Kuiper

Abstract—Interaction of oxidized LDL (OxLDL) with macrophage-derived foam cells is one of the key events in the development and progression of atherosclerosis. To study this interaction, macrophage-derived foam cells were isolated from rabbit atherosclerotic lesions and the expression of scavenger receptors for OxLDL was examined. Atherosclerosis was induced in rabbits by denudation of the large arteries, followed by a hypercholesteremic diet. Macrophage-derived foam cells, characterized by immunostaining with an RAM-11 antibody (a macrophage marker), contained a high content of intracellular lipid. Maximal binding of radiolabeled OxLDL to isolated macrophage-derived foam cells (1652±235 ng 125I-OxLDL/mg of cell protein) was 20-fold higher compared with B_max values of monocytes. Levels of association of OxLDL to macrophage-derived foam cells isolated from atherosclerotic lesions 12 weeks after denudation were >3-fold higher compared with the levels expressed by macrophage-derived foam cells isolated after 6 weeks. Association of 125I-OxLDL could be completely blocked by OxLDL, and partially by acetylated LDL and polyinosinic acid, indicating the presence of a specific binding site for OxLDL on macrophage-derived foam cells. The induction of scavenger receptors for OxLDL on macrophage-derived foam cells during the development of atherosclerosis, as described in this study, may facilitate the lipid accumulation in macrophage-derived foam cells, as observed in advanced atherosclerotic lesions. (Arterioscler Thromb Vasc Biol. 1999;19:638-645.)

Key Words: scavenger receptor ■ oxidized lipoproteins ■ macrophage-derived foam cells ■ monocytes

The appearance of lipid-laden macrophages in the vessel wall is one of the characteristic features in the development of atherosclerosis.1 During atherosclerosis, monocytes infiltrate and accumulate in the arterial wall and subsequently transform to foam cells after the uptake of modified lipoproteins, such as oxidatively modified LDL (OxLDL).2–4 Scavenger receptors, which are expressed at the cell surface of macrophages, can mediate the uptake and internalization of modified lipoproteins into the cell.5 So far, 4 different types of receptors for OxLDL have been identified. Two isoforms of a scavenger receptor class A with similar binding properties are characterized, type I and type II (SRAI/SRAII). SRAI/SRAII recognize chemically modified lipoproteins, eg, acetylated LDL, oxLDL, malondialdehyde-conjugates of either LDL or albumin, and polyanions.6–8 Both isoforms have a molecular mass of 220 kd and contain an α-helical and a collagenous-like coiled coil domain. The type I scavenger receptor contains a C-terminal cysteine-rich domain, which is absent in the type II scavenger receptor.3,7 In addition, specific binding of OxLDL to CD36, a 94- to 105-kd protein membrane glycoprotein, is described. CD36 is a member of the class B scavenger receptors and is predominantly expressed on monocytes, platelets, and microvascular endothelial cells.9–11 Recently, a 94- to 97-kd macrophage protein was described that specifically recognizes OxLDL. This protein is identified as macrosialin, the mouse homolog of human CD68.8,12–15 OxLDL is suggested to play a key role in the transformation of monocytes and macrophages into resident foam cells in the atherosclerotic plaque.16,17 OxLDL may also act as a chemotactic agent in the atherosclerotic plaque, which results in an increased infiltration of monocytes into the vessel wall.16–18 The continuous interaction of modified lipoproteins with macrophages in the atherosclerotic plaque may therefore be of importance for the progression of the disease. To study this interaction, we isolated macrophage-derived foam cells directly from in vivo developing atherosclerotic lesions and studied the expression of receptors for OxLDL during the atherosclerotic process. Our study is the first to analyze scavenger receptors on cells isolated from atherosclerotic lesions and we have followed the induction during the development of atherosclerosis. It is concluded that the expression of high levels of scavenger receptors for OxLDL may mediate the extensive lipid accumulation in macrophase-derived foam cells that is observed in advanced lesions.

Received January 5, 1998; revision accepted August 21, 1998.
From the Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Sylvius Laboratories, University of Leiden, PO Box 9503, 2300 RA Leiden, The Netherlands.
Correspondence to Dr J. Kuiper, Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Sylvius Laboratories, Wassenaarseweg 72, 2333 AL Leiden, PO Box 9503, 2300 RA Leiden, The Netherlands. E-mail j.kuiper@lacdr.LeidenUniv.nl
© 1999 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
Methods

Reagents
Polyinosinic acid, collagenase, elastase, and trypsin inhibitor were purchased from Sigma. PCR buffer, dNTPs, MgCl2, SuperScript™ II RT, Taq DNA polymerase, DTT, and BCS were obtained from GibcoBRL Life Technologies. Sodium iodide (125I) (carrier-free) in NaOH was purchased from Amersham Life Science. dX-174 DNA HAE III digest marker was from New England Biolabs. All primers were obtained from Eurogentech. The antibodies RAM-11, HHF-35, and GAMPO were obtained from DAKO. All other chemicals were of analytical grade.

Animals
Female New Zealand White rabbits (3 to 3.5 kg, Broeckman, Someren, The Netherlands) were housed in separate cages. Rabbit chow (LKK-20, Hope Farms) and water were available ad libitum. For denudation of the large arteries, animals were anesthetized by Hypnorm, Janssen Pharmaceutica, Beere, Belgium, 0.3 mL/kg and diazepam 3 mg/kg. A Fogarty embolectomy catheter (3F, Baxter) was inserted via the femoral artery. In the aorta, the balloon was inflated and retracted 3 times along the intimal wall of the aorta, the iliac, and the femoralis. After denudation animals were put on a high cholesterol diet (1% cholesterol in LKK-20).

Immunocytochemistry of Atherosclerotic Arteries
At indicated weeks after denudation, animals were euthanized and atherosclerotic arteries were isolated. Segments of the aorta and iliac containing atherosclerotic lesions were fixated in formaldehyde (37%) and embedded in paraffin. Serial 8- to 10-μm sections were stained for lipid by using oil red O. In addition, sections were immunostained with RAM-11 (a rabbit-specific macrophage marker), and HFF-35 (a monoclonal directed against rabbit muscle actin). As a second antibody, goat anti-mouse peroxidase was used and antibody complexes were visualized by using diaminobenzoic acid.

Isolation Procedure of Macrophage-Derived Foam Cells
At 6, 9, and 12 weeks after denudation, 4, 3, and 3 animals, respectively, were euthanized and lesions were isolated from the atherosclerotic arteries. Lipid-laden macrophages were isolated from the lesions by using an enzymatic method followed by density-gradient centrifugation, essentially according to Rosenfeld et al.19 After isolation, lesions were incubated with an enzyme mixture (1 g of tissue/10 mL of enzyme mixture) containing collagenase (450 U/10 mL), elastase (4.7 U/10 mL), and trypsin inhibitor (10 mg/10 mL) in Hanks’ buffered HEPES buffer (pH 7.4) containing 0.3% bovine serum albumin. Lesions were incubated for 1 hour at 37°C, and subsequently the suspension was filtered by using a nylon sieve (180 μm). Lesions were incubated for 15 minutes at 4°C, 0.25 mL AgNO3 (0.7 mol/L) was added and the suspension was diluted to 180 μmol/L NaOH. Solubilized cells were counted for radioactivity and protein content was measured.

To determine the specific binding of 125I-OxLDL to macrophages, nonspecific binding values were subtracted from the total binding values. Dissociation constant (Kd) and maximal binding (Bmax) were determined from binding studies according to a single-site displacement model by using a computerized nonlinear fitting program (Graphpad Prism, Graphpad Software).

Association and Degradation of 125I-OxLDL by Rabbit Macrophage-Derived Foam Cells and Monocytes
Specificity of the OxLDL binding to macrophage-derived foam cells and monocytes was determined by incubating the cells with 5 μg/mL 125I-OxLDL at 37°C in the presence of various concentrations of competitors, OxLDL, AcLDL, or polyinosinic acid (polyI). After 2 hours of incubation with 3 washes with the same buffer without albumin. Subsequently, the cells were solubilized in 0.1 mol/L NaOH. Solubilized cells were counted for radioactivity and protein content. Degradation of 125I-OxLDL was determined as described by Van Berk et al20 with modifications. Incubation medium (0.5 mL) was washed 3 times with cold PBS/heparin and resuspended in RPMI 1640 containing penicillin/streptomycin (100 μg/mL), 1-glutamine (2 mmol/L), supplemented with 10% BCS. After overnight incubation at 37°C in the incubator (5% CO2, 95% air), nonadherent cells were removed by aspiration followed by washing with RPMI 1640. Monocytes were cultured for various days, positively immunostained with the RAM-11 antibody and were used for the reported studies. In addition, monocytes were also cultured for various days in the absence or presence of rabbit cholesterol-rich serum (1% in RPMI 1640 supplemented with 9% BCS). Cholesterol-rich serum was collected from rabbits 12 weeks after denudation on a hypercholesteremic diet as described above. Serum isolated from these animals contained 17 μg/mL cholesterol.

Lipoprotein Isolation, Modification, and Labeling
LDL (1.024<d<1.055) was isolated from fresh human serum by density-gradient ultracentrifugation according to Redgrave et al.20 LDL was acetylated with acetic anhydride as described by Basu et al.21 LDL was oxidatively modified by incubation of 200 μg/mL of LDL with 10 μmol/L CuSO4 at 37°C. After 2 hours of incubation, the reaction was terminated by administration of 1.01 mmol EDTA (final concentration). The negative charge of acetylated LDL (AcLDL) and OxLDL was routinely checked by agarose gel electrophoresis by using a 1% agarose solution in hipuric acid buffer (Rf=0.52 and 0.53, respectively).

Before oxidative modification of LDL, LDL was iodinated. LDL was radiolabeled with 125I by the ICl method of McFarlane22 as modified by Bilheimer et al.23 The specific activity of radiolabeled OxLDL ranged from 80 to 200 cpm/ng of protein.

Binding of 125I-OxLDL to Rabbit Macrophage-Derived Foam Cells and Monocytes
Cell culture medium was displaced by RPMI 1640 containing 2% BSA for 2 hours. Incubations of cells were performed in RPMI 1640 containing 2% BSA.

Total binding of 125I-OxLDL was measured after incubating cells for 2 hours at 4°C with various amounts of 125I-OxLDL in concentrations ranging from 0.5 to 75 μg/mL. Nonspecific binding was determined after incubating the cells with various amounts of 125I-OxLDL in the presence of a 10-fold excess of unlabelled OxLDL with a minimum of 100 μg/mL. The cells were washed 3 times with a Tris-NaCl buffer (50 mmol/L Tris, 150 mmol/L NaCl, pH 7.4) containing 0.2% BSA, and 5 mmol/L CaCl2 followed by 3 washes with the same buffer without albumin. Subsequently, the cells were solubilized in 0.1 mol/L NaOH. Solubilized cells were counted for radioactivity and protein content was measured.

Association and Degradation of 125I-OxLDL by Rabbit Macrophage-Derived Foam Cells and Monocytes
Specificity of the OxLDL binding to macrophage-derived foam cells and monocytes was determined by incubating the cells with 5 μg/mL 125I-OxLDL at 37°C in the presence of various concentrations of competitors, OxLDL, AcLDL, or polyinosinic acid (polyI). After 2 hours of incubation with ice, and the medium was collected. The cells were washed 3 times with a Tris-NaCl buffer (50 mmol/L Tris, 150 mmol/L NaCl, pH 7.4) containing 0.2% BSA, and 5 mmol/L CaCl2 followed by 3 washes with the same buffer without albumin. Subsequently, the cells were solubilized in 0.1 mol/L NaOH. Solubilized cells were counted for radioactivity and protein content.

Degradation of 125I-OxLDL was determined as described by Van Berk et al20 with modifications. Incubation medium (0.5 mL) was mixed with 0.4 mL 35% trichloroacetic acid. After an incubation for 30 minutes at 4°C, 0.25 mL AgNO3 (0.7 mol/L) was added and the mixture was centrifuged for 10 minutes at 1500g. Of the aqueous phase, 1 mL was counted for radioactivity.

Monocyte Isolation and Culture
Fresh rabbit blood was diluted (3:1) with PBS (150 mmol/L NaCl, 8.5 mmol/L Na2HPO4, 1.5 mmol/L NaH2PO4 pH 7.4) containing 200 U/mL of heparin. Of this solution, 15 mL was brought on 12 mL of Ficoll-Paque density gradient (d=1.077), and centrifuged for 45 minutes at 750g, at room temperature. The mononuclear interphase was collected, washed 3 times with cold PBS/heparin, and resuspended in RPMI 1640 containing penicillin/streptomycin (100 μg/mL), 1-glutamine (2 mmol/L), supplemented with 10% BCS. After overnight incubation at 37°C in the incubator (5% CO2, 95% air), nonadherent cells were removed by aspiration followed by washing with RPMI 1640. Monocytes were cultured for various days, positively immunostained with the RAM-11 antibody and were used for the reported studies. In addition, monocytes were also cultured for various days in the absence or presence of rabbit cholesterol-rich serum (1% in RPMI 1640 supplemented with 9% BCS). Cholesterol-rich serum was collected from rabbits 12 weeks after denudation on a hypercholesteremic diet as described above. Serum isolated from these animals contained 17 μg/mL cholesterol.
Statistical analysis of the receptor binding data was performed by means of Fisher test followed by a 1-way ANOVA and a Student’s Newman–Keuls test.

**RNA Isolation**

All glassware was treated with DEPC to inhibit RNases. Total RNA was extracted from macrophage-derived foam cells according to the method of Chomczynski and Sacchi.\(^3\) After washing the cells twice with PBS, cells were lysed in a denaturing buffer containing 4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, 0.5% lauryl sarcosine, and 0.1 mol/L 2-mercaptoethanol (pH 7.0). RNA was extracted by mixing the cell lysate with an equal volume of phenol, 0.1 volume of sodium acetate (pH 4.0), 0.2 volume of chloroform/isooamy alcohol (49:1). After centrifugation at 10 000\(\times\)g, at 4°C for 20 minutes, the aqueous phase was collected. RNA was precipitated at −20°C for >1 hour in an equal volume of ethanol followed by centrifugation at 10 000\(\times\)g, for 30 minutes at 4°C. The pellet was washed with 70% ice-cold ethanol. RNA was dissolved in 10 mmol/L Tris-Cl, 1 mmol/L EDTA, pH 7.4 buffer and quantified.

**Polymerase Chain Reaction**

Purified total RNA was used as a template for single-stranded cDNA synthesis. The cDNA synthesis mix contained 400 U reverse transcriptase, 4\( \mu \)L first-strand buffer, 200 ng oligo(dT) primers, 5 mmol/L each of dATP, dGTP, dTTP, and dCTP, 0.1 U Taq polymerase, and 5 mmol/L each of dATP, dGTP, dTTP, and dCTP, and 5\( \mu \)L total transcriptase, 4\( \mu \)L PCR buffer. The mixtures were incubated in a Biometra Trio thermal cycler for 38 cycles. Each cycle consisted of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes. Aliquots of PCR reaction mixtures were visualized under UV light.

**Characterization of Macrophage-Derived Foam Cells Isolated From Rabbit Atherosclerotic Lesions**

Macrophage-derived foam cells were isolated from atherosclerotic lesions in the aortic, iliac, and femoral arteries. Isolated cells were characterized by staining with the RAM-11 antibody (Figure 2A) and the cells had a high content of lipid as demonstrated by oil red O staining (Figure 2B). No contaminating cell types were present as determined by immunostaining for muscle actin cell by using HHF-35 (results not shown).

**Receptor-Binding Studies of OxLDL to Rabbit Macrophage-Derived Foam Cells and Monocytes**

A saturation curve of OxLDL binding to macrophage-derived foam cells isolated 12 weeks after denudation is shown in Figure 4A. Specific binding of OxLDL to macrophage-derived foam cells was demonstrated and a \(K_d\) value of 17±4 \(\mu\)g of OxLDL/mL and a \(B_{max}\) value of 1652±235 ng of bound OxLDL per milligram of cell protein was found (Table 1). Maximal binding of radiolabeled OxLDL to macrophage-derived foam cells isolated 6 weeks after denudation was significantly lower (\(P<0.05\)) and \(B_{max}\) values of 716±104 ng of bound OxLDL per milligram of cell protein were found (Table 1).

The maximal binding of OxLDL to monocytes cultured for various days was significantly lower (\(P<0.01\)) compared with \(B_{max}\) values of OxLDL to macrophage-derived foam cells (Table 1). Specific binding curves for the interaction of radiolabeled OxLDL with rabbit monocytes cultured for 1, 5, and 14 days are shown in Figure 4B. Specific binding of OxLDL to monocytes cultured for 1, 5, and 14 days revealed an increasing maximal binding of 98±17, 135±12, and 277±28 ng of \(^{125}\)I-OxLDL/mg of cell protein, respectively, with a constant \(K_d\) (Table 1).

In addition, monocytes were also cultured for 5 and 14 days in the presence of medium supplemented with 1% cholesterol-rich serum (final concentration), which resulted in the formation of monocyte-derived foam cells as demonstrated by oil red O staining. It is noteworthy that no significant differences in the binding parameters of radiolabeled OxLDL to these cells were found compared with monocytes cultured for 5 days in normal serum. Maximal binding of OxLDL to monocytes cultured for 5 days in the

**Results**

**Immunocytochemistry**

Sections of aortic and iliac arteries containing atherosclerotic lesions were analyzed and revealed lesion formation along the lumen of the vessels. Sections were immunostained with the RAM-11 antibody, an antibody specific for rabbit macrophages (Figure 1A). Atherosclerotic lesions contained many RAM-11–positive cells located in the intima of the vessel, which also contained intracellular vesicles. Staining of these sections for lipids using oil red O revealed that cells in the atherosclerotic lesion contained a high content of lipid (Figure 1B). In the media of these atherosclerotic vessels, no lipid-enriched cells were found. Immunostaining of the sections with the antibody HIF-35 directed against rabbit muscle actin showed the presence of smooth muscle cells in the media of the arterial wall (Figure 1C). Furthermore, HIF-35–positive cells were also found in the atherosclerotic lesions, which suggests a migration of smooth muscle cells from the media into the atherosclerotic lesions of the vessel wall.
presence of 1% cholesterol serum revealed a maximal binding ($B_{\text{max}}$) of $135 \pm 15$ ng of $^{125}$I-OxLDL/mg of cell protein with a dissociation constant $K_D$ of $16 \pm 3$ (mean±SEM, n=3). After 14 days of culture in the presence of 1% cholesterol serum, monocytes revealed a $B_{\text{max}}$ of $298 \pm 32$ ng of $^{125}$I-OxLDL/mg of cell protein with a $K_D$ of $14 \pm 4$ (n=3).

**Figure 1.** Immunocytochemical analysis of cryosections of atherosclerotic arteries. Serial sections of fatty streaks in atherosclerotic arteries were immunostained with the RAM-11 antibody, a specific rabbit macrophage marker (A, original magnification ×200). Sections were stained with oil red O for lipid (B, original magnification ×100). Furthermore, sections were immunostained with HHF-35, a monoclonal antibody directed against muscle actin (C, original magnification ×100).

**Association and Degradation of OxLDL to Rabbit Macrophage-Derived Foam Cells and Monocytes**

The specificity of the association to and degradation of $^{125}$I-OxLDL by rabbit macrophage-derived foam cells was determined in competition studies. Macrophage-derived foam cells were isolated at various weeks after denudation and...
incubated with 5 μg/mL 125I-OxLDL in the presence of various concentrations of unlabeled OxLDL, AcLDL or polyI.

Macrophage-derived foam cells isolated 12 weeks after denudation had an association of 125I-OxLDL (5 μg/mL) of 880 ± 661 ng of OxLDL/mg of cell protein after 2 hours. OxLDL could effectively inhibit the association of 125I-OxLDL to macrophage-derived foam cells by 71%, whereas AcLDL was able to block the association by 38%. PolyI could inhibit the association of OxLDL to foam cells by 50% (Figure 5). Degradation of 125I-OxLDL (5 μg/mL) was at a slightly lower level than the level of association, which was previously observed when incubating oxLDL with rat Kupffer cells.35 Macrophage-derived foam cells isolated 6 weeks after denudation revealed an association >3-fold lower compared with macrophage-derived foam cells isolated 12 weeks after denudation. Levels of association and degradation of OxLDL by macrophage-derived foam cells isolated 6, 9, and 12 weeks after denudation are shown in Table 2.

The levels of association and degradation of OxLDL by primary cultures of monocytes were at a significantly lower level (P < 0.01) compared with macrophage-derived foam cells. Monocytes cultured for 1 day displayed an association of 83 ± 7 ng of 125I-OxLDL/mg of cell protein and a degradation of 34 ± 5 ng of 125I-OxLDL/mg of cell protein. It is noteworthy that the levels of association and degradation were 6 times higher in monocytes cultured for 14 days (Table 2). Monocytes cultured for 14 days in the presence of medium supplemented with 1% cholesterol-rich serum showed an increase in cell association and degradation of OxLDL similar to that of monocytes cultured in the absence of cholesterol-rich serum.
Macrophage-derived foam cells isolated from rabbit atherosclerotic lesions possessed a high-affinity binding site for OxLDL, which was coupled to active degradation of the protein. Competition studies indicated that various receptors contribute to the recognition and uptake of OxLDL. First, the presence of the SRAI/AII on isolated macrophage-derived foam cells was established, because the association of OxLDL to foam cells was competed for 38% by AcLDL, indicating that 334 ng of 125I-OxLDL/mg of cell protein associated to SRAI/AII. The presence of SRAI/AII was confirmed by PCR analysis on RNA isolated from these macrophage-derived foam cells. SRAI/AII may recognize a broad group of modified lipoproteins, such as AcLDL and OxLDL, and play an important role in atherosclerosis. A disruption in the SRAI/AII gene in atherosclerotic mice resulted in a reduction of the size of atherosclerotic lesions, indicating their role in atherosclerosis. The strong induction of SRAI/AII expression, as we observed in the macrophages that matured in the plaque, is confirmed by a recent study by Hiltunen et al. who showed that the level of mRNA expression in the entire atherosclerotic plaque was strongly increased (270-fold) at 14 weeks after induction of atherosclerosis.

Because the association of OxLDL to foam cells is not fully competed for by AcLDL, the presence of binding sites for OxLDL other than SRAI/AII is suggested. Specific binding sites for OxLDL are described for monocyte and macrophage cell lines, such as THP-1 and RAW cells, and Kupffer cells.

### TABLE 1. Binding Parameters of 125I-OxLDL to Rabbit Macrophage-Derived Foam Cells and Monocytes

<table>
<thead>
<tr>
<th>Foam Cells</th>
<th>Kd (μg/mL)</th>
<th>Bmax (ng/mg of Cell Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foam cells (12 wk)</td>
<td>17±4</td>
<td>1652±235</td>
</tr>
<tr>
<td>Foam cells (6 wk)</td>
<td>19±3</td>
<td>716±104</td>
</tr>
<tr>
<td>Monocytes (1 d)</td>
<td>16±2</td>
<td>98±17</td>
</tr>
<tr>
<td>Monocytes (5 d)</td>
<td>17±2</td>
<td>135±12</td>
</tr>
<tr>
<td>Monocytes (14 d)</td>
<td>15±2</td>
<td>277±28</td>
</tr>
</tbody>
</table>

Macrophage-derived foam cells were freshly isolated from rabbit atherosclerotic lesions 6 and 12 weeks after denudation. Monocytes were isolated from rabbit blood and cultured for 1, 5, and 14 days. Cells were incubated with increasing concentrations of 125I-OxLDL in the absence of OxLDL. Binding parameters were determined as described in Methods. Data are expressed as mean±SD values of at least 3 different experiments.
The specific binding of OxLDL to macrophage-derived foam cells, as described in this study, may be partly mediated by CD36 or macrosialin. PCR performed on the RNA extracted from isolated macrophage-derived foam cells revealed that mRNA for CD36 as well as for macrosialin was present, which indicates that both proteins may be involved in the specific interaction of OxLDL with macrophage-derived foam cells. Macrosialin is a member of the lamp family and is characterized as a specific OxLDL-binding protein, which also interacts with polyI.35 The role of macrosialin in the uptake and cellular processing of OxLDL, however, remains to be established.17,28,29 The involvement of CD36, a member of the class B scavenger receptors, in the binding and processing of OxLDL by monocytes has been recognized.10,11,36–38 Because the specific binding site for OxLDL on macrophage-derived foam cells isolated from in vivo lesions is not competed for totally by polyI, it is suggested that CD36 is a possible candidate for the specific polyI-insensitive processing of OxLDL by these cells. This part of the oxLDL-binding represents the oxLDL-specific binding (71% of the total oxLDL binding) minus the polyI-sensitive part of the oxLDL binding (50% of the total oxLDL binding), resulting in 21% (185 ng of 125I-oxLDL/mg of cell protein) of the binding, which is most likely mediated by CD36. The binding of oxLDL to CD68 is represented by the polyI-sensitive (50%) minus the AcLDL-compatible part (38%) of the oxLDL-specific binding, which indicates that 12% of the oxLDL binding (106 ng of 125I-oxLDL/mg of cell protein) is most likely mediated by CD68.

The specific interaction of OxLDL with rabbit monocytes was also examined and was compared with that of macrophage-derived foam cells. Monocytes as well as macrophage-derived foam cells used in this study possess specific binding sites for OxLDL. The maximal binding of OxLDL to isolated macrophage-derived foam cells 12 weeks after denudation, however, is 20-fold higher than that of monocytes cultured for 1 day, indicating a strong induction of scavenger receptor expression on macrophage-derived foam cells isolated from atherosclerotic lesions. Maximal binding of OxLDL to rabbit macrophage-derived foam cells is at a 5-fold higher level compared with rabbit peritoneal macrophages.36 The binding parameters of rabbit monocytes for OxLDL obtained in this study are in the same range of those described for human monocyte-derived macrophages with maximal binding values ranging from 200 to 400 ng of 125I-OxLDL/mg of cell protein after various days of culture.38,39

We observed that the processing of OxLDL by macrophage-derived foam cells in the atherosclerotic plaque increases strongly during the development of atherosclerosis. Values for association and degradation were at least 4-fold higher in macrophage-derived foam cells isolated 12 weeks after denudation compared with macrophage-derived foam cells isolated 6 weeks after denudation. These results indicate that during the maturation of macrophage-derived foam cells in the atherosclerotic lesion, an upregulation of OxLDL binding sites on these cells occurs, which leads to increased uptake and degradation of OxLDL. The interaction of OxLDL with macrophage-derived foam cells isolated from the atherosclerotic plaque was >10-fold higher compared with the interaction of OxLDL to monocytes directly after isolation from the blood. Differentiation of rabbit monocytes in culture also resulted in increased levels of association and degradation of OxLDL, but values were still at a much lower level compared with foam cells that had matured in vivo in an atherosclerotic lesion. No significant changes in binding parameters of monocytes were observed in the presence of cholesterol-rich serum, indicating that cholesterol per se does not influence scavenger receptor expression. These results also confirm that the increased expression and production of scavenger receptors per cell on freshly isolated macrophage-derived foam cells is a result of the entry and maturation of the macrophages within the intima. These observations indicate that the macrophage-derived foam cells mature in a different way in the lesion in the vessel wall compared with monocytes in vitro. The expression of specific receptors for OxLDL on macrophage-derived foam cells may be influenced locally by various mediators within the atherosclerotic plaque. The increase in the binding of OxLDL during differentiation may also be the result of an increase in the expression of the scavenger receptor class A type I. An enhanced SRAI/SRAII ratio was observed at the protein level as well as the mRNA levels during the differentiation of human monocytes.40 Also, the SRAI is stated to be of importance in the transformation of macrophages into foam cells. In addition, during their differentiation, monocytes also show alterations in the expression of CD36 and its intracellular transport.11,41 Therefore, enhanced levels of interaction of OxLDL with isolated macrophage-derived foam cells and monocytes may be the result of increased expression of scavenger receptors class A type I and CD36.

In contrast, inflammatory mediators produced by activated macrophages and T lymphocytes in the atherosclerotic plaque may also influence scavenger receptor expression. Various inflammatory mediators, such as cytokines, chemokines, and growth factors, are detected in the atherosclerotic plaque.30,42 Cytokines, such as granulocyte-macrophage colony-stimulating factor, interferon-γ, and tumor necrosis factor are described to influence the expression of scavenger receptor class A I/II and CD36.39,41,43,44 Therefore, increased uptake and processing of OxLDL by foam cells in the course of atherosclerosis may also be influenced by inflammatory mediators. Results described in this study indicate that increased expression of scavenger receptors on macrophage-derived foam cells during the development of the atherosclerotic plaque may facilitate the extensive accumulation of intracellular lipid as detected in the advanced lesions.

Acknowledgment

This work was supported by a grant from The Netherlands Heart Foundation (grant 94.124). The authors would like to thank Dr M.E. Rosenfeld of the University of Washington, Seattle, for his advice for the isolation procedure.

References

4. Freeman M, Ashkenas J, Rees DJG, Kingsley DM, Copeland NG, Jenkins NA, Krieger M. An ancient highly conserved family of cysteine-rich protein domains revealed by cloning type I and type II murine macro-


Specific Interaction of Oxidized Low-Density Lipoprotein With Macrophage-Derived Foam Cells Isolated From Rabbit Atherosclerotic Lesions

Helga E. de Vries, Bas Buchner, Theo J. C. van Berkel and Johan Kuiper

doi: 10.1161/01.ATV.19.3.638

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/3/638