Oxidized Low Density Lipoproteins Bind to the Scavenger Receptor Expressed by Rabbit Smooth Muscle Cells and Macrophages

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We have previously demonstrated that the acetylated low density lipoprotein (LDL), or scavenger, receptor expressed by rabbit smooth muscle cells (SMCs) is regulated. Phorbol ester treatment of the cells increased the number of scavenger receptors expressed and the metabolism of acetoacetylated (AcAc) LDL. The current studies examined the interaction of oxidized (Ox) LDL with the rabbit scavenger receptor. The internalization and degradation of both Ox-LDL and AcAc-LDL were increased to a similar extent by phorbol ester treatment of the SMCs. In cross-competition experiments, both Ox-LDL and AcAc-LDL competed equally for the degradation of 125I-Ox-LDL, suggesting that there is no independent receptor for Ox-LDL on these cells. In contrast, only AcAc-LDL competed totally for the degradation of 125I-AcAc-LDL. Similar results were obtained in cross-competition experiments with rabbit macrophages. To determine whether these data were consistent with the binding of both ligands to a single receptor, competition studies were conducted in Chinese hamster ovary fibroblasts transfected with the bovine scavenger receptor. After transfection, the metabolism of both AcAc-LDL and Ox-LDL was increased, in agreement with the previous data from other investigators, and cross-competition studies yielded essentially identical results to those obtained in the SMCs and macrophages. Northern blot analysis with an antisense rabbit scavenger receptor probe detected the same mRNA species in total RNA from rabbit macrophages and SMCs and showed that scavenger receptor mRNA increased dramatically after phorbol ester treatment of SMCs. The probe also detected bovine scavenger receptor mRNA. A mixture of bovine scavenger receptor antipeptide antibodies detected a scavenger receptor of -260 kD among membrane proteins of both SMCs and macrophages. This 260-kD protein bound acetyl-LDL in ligand blots. The data demonstrate that the scavenger receptor expressed by rabbit SMCs and macrophages binds both Ox-LDL and chemically modified LDL and that the rabbit receptor is related to the bovine scavenger receptor. (Arteriosclerosis and Thrombosis 1993;13:371-378)

KEY WORDS • acetyl LDL receptor • foam cells • macrophages • oxidized LDL • scavenger receptors • smooth muscle cells • modified LDL
macrophages leads to the generation of foam cells in vivo.\textsuperscript{1,10,11} We and others have shown that the acetyl-LDL receptor is, in fact, expressed by macrophage-derived foam cells freshly isolated from atherosclerotic lesions.\textsuperscript{12,13} We recently demonstrated that rabbit smooth muscle cells also express the scavenger receptor and that the receptor in these cells is regulated.\textsuperscript{14,15} Receptor expression is upregulated by platelet secretary products and preincubation of the cells with phorbol esters. The phorbol ester-treated rabbit smooth muscle cells express 10–30% as many receptors as mouse peritoneal macrophages.\textsuperscript{14} These observations raise the possibility that lipid accumulation in smooth muscle cells in vivo might also result from the receptor-mediated endocytosis of Ox-LDL. The current studies were undertaken to determine whether both chemically modified LDL and Ox-LDL bind to the scavenger receptor expressed by smooth muscle cells and whether the metabolism of Ox-LDL is enhanced by phorbol ester treatment.

**Methods**

**Materials**

Fetal bovine serum (FBS), Dulbecco’s modified Eagle’s medium (DMEM), Dulbecco’s phosphate-buffered saline, penicillin, streptomycin, sodium \([\text{NaCl}]\) iodide, the fluorescent probe 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate (Dil), and fucoidan were obtained as previously described.\textsuperscript{15} Phorbol 12-myristate 13-acetate (PMA) and the neomycin analogue G418 were purchased from Sigma Chemical Co., St. Louis, Mo.; lactalbumin hydrolysate was purchased from GIBCO, Baltimore, Md.; and mineral oil was obtained from Squibb, Princeton, N.J. The type I and type II bovine scavenger receptor expression vectors pXSR7 and pXSR3 were a generous gift from Dr. David Johnson, Gladstone Institute. Monty Krieger, Massachusetts Institute of Technology, San Diego, Calif. and the vector pcDNAI was purchased from Invitrogen, San Diego, Calif.

**Lipoproteins**

Human LDLs (\(d=1.02–1.05\) g/mL) were obtained from plasma (1 mg/mL EDTA) by sequential density gradient ultracentrifugation.\textsuperscript{19} The human LDLs were dialyzed against saline-EDTA (0.15 M NaCl and 0.01% EDTA) and then sterilized by filtration through a 0.45-\(\mu\)m filter. Before oxidation, LDLs were extensively dialyzed against Dulbecco's phosphate-buffered saline to remove all traces of EDTA. The LDLs (250 \(\mu\)g protein per milliliter) were oxidized in a cell-free system by incubation for 24 hours at 37°C in a medium containing copper (CuCl\(_2\) 10 \(\mu\)M in Ham’s F-10 medium).\textsuperscript{20} Oxidation was stopped by cooling and the addition of EDTA (0.1 mM). The Ox-LDLs were then dialyzed in saline-EDTA, sterilized by filtration (0.45 \(\mu\)m), and stored at 4°C. As a measure of oxidation, thiobarbituric acid–reactive substances (TBARS) were determined\textsuperscript{21} and expressed as MDA equivalents per milligram protein.

The LDLs were labeled with the fluorescent probe Dil\textsuperscript{22,23} and then acetylated or acetoacetylated as described.\textsuperscript{24,25} Acetyl-LDL and acetoacetylated (AcAc) LDL are both ligands for the scavenger receptor.\textsuperscript{23–25} Lipoproteins were iodinated by the method of Bilheimer et al.\textsuperscript{26} When iodinated lipoproteins were required, the LDLs were first iodinated and then acetylated, acetoacetylated, or oxidized. The specific activity of the iodinated lipoproteins ranged from 150 to 250 cpm/ng.

**Cell Culture Experiments**

Mouse peritoneal macrophages were obtained from nonstimulated Swiss Webster mice as described.\textsuperscript{1} Rabbit peritoneal macrophages were obtained from rabbits 4 days after intraperitoneal injection of mineral oil.\textsuperscript{27} New Zealand White rabbit smooth muscle cell lines SMC2 and SMC3 were provided by Drs. Lisa Minor and George Rothblat, Medical College of Pennsylvania, Philadelphia, Pa. Cells were grown in DMEM containing 10% heat-inactivated FBS, penicillin (100 units/mL), and streptomycin (100 \(\mu\)g/mL). The medium was changed 48 hours before an experiment.

Wild-type Chinese hamster ovary (CHO) fibroblasts were maintained in 50% DMEM–50% Ham’s F-12 medium containing 10% FBS, penicillin (100 units/mL), and streptomycin (100 \(\mu\)g/mL). The type I and type II bovine scavenger receptor expression vectors, pXSR7 and pXSR3 (20 \(\mu\)g), respectively, were cotransfected in CHO cells with pSV2neo (1 \(\mu\)g) using the calcium phosphate precipitation method as described.\textsuperscript{28} Colonies resistant to the neomycin analogue G418 (0.4 mg/mL) were selected and screened with Dil-labeled AcAc-LDL (5 \(\mu\)g/mL for 16 hours) to identify scavenger receptor-positive colonies. The most highly fluorescent cells were selected by fluorescence-activated cell sorting as described.\textsuperscript{15} Stock cultures of CHO cells expressing the type I or type II bovine scavenger receptor were maintained in a medium that selects against cells' spontaneously losing scavenger receptor expression.\textsuperscript{29} Control CHO cells were stably cotransfected with the vector pcDNAI and pSV2neo in the same 20:1 ratio.

Scavenger receptor activity was assessed at 37°C by measuring cellular degradation of \(^{125}\text{I}-\)labeled modified LDL in 12-well culture dishes. For all lipidprotein degradation assays, the growth medium was removed from the cells before the addition of \(^{125}\text{I}-\)lipoproteins in DMEM. The cells were incubated at 37°C for the times indicated, and the trichloroacetic acid–soluble lipoprotein degradation products in the medium were quantified as described.\textsuperscript{30} The cellular protein content was determined by analysis of the cells solubilized in 0.1N NaOH by the method of Lowry et al.\textsuperscript{31} using bovine serum albumin as a standard. All data points are the average of duplicate determinations. In each experiment, the amount of lipoprotein associated with the cells at the end of the experiment, which represents both surface-bound and internalized, nondegraded lipoprotein, was also quantified as described.\textsuperscript{30} For competition studies, the \(^{125}\text{I}-\)ligand and unlabeled competitors were premixed before incubation with the cells, either in DMEM or in DMEM–N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid containing 10% human lipoprotein-deficient serum for 4°C experiments.\textsuperscript{22,32}
Northern Analysis

A 32P-labeled antisense rabbit RNA probe was generated for Northern hybridization. The probe was prepared as follows. Twenty-five micrograms of an antisense oligonucleotide primer, corresponding to bases 1,007-1,039 of the bovine scavenger receptor sequence linked to a 29-bp T7 promoter sequence, was annealed to 5 μg of total RNA from rabbit peritoneal macrophages, and cDNA was synthesized using reverse transcriptase. Enzymatic amplification of this cDNA was accomplished by polymerase chain reaction using as primers the antisense oligonucleotide mentioned earlier and a second oligonucleotide corresponding to bases 760-780 (sense) of the bovine scavenger receptor cDNA. The 32P-labeled probe was generated from this amplified product using reverse transcriptase.

Total RNA was isolated from cultured cells by guanidinium thiocyanate extraction and purified by centrifugation in cesium chloride. Total RNA samples (15 μg each) were electrophoresed using a 1% agarose gel containing 2.2 M formaldehyde, transferred to a nylon membrane, and fixed by both ultraviolet irradiation and vacuum baking. The filter was prehybridized at 60°C for 2 hours and then hybridized with the antisense RNA probe overnight at 60°C.

Immunoblot and Ligand Blot Analysis

All procedures for antibody preparation and immunoblot and ligand blot analysis have been described. Peptides corresponding to amino acids 17-27, 104-119, and 204-217 of the bovine scavenger receptor were synthesized, and antibodies were raised in guinea pigs. Total octylglycoside-solubilized membrane proteins from rabbit macrophages, control CHO cells, and CHO cells transfected with the type I bovine scavenger receptor were separated by electrophoresis under nonreducing conditions. The proteins were then electrophoretically transferred to nitrocellulose and processed for immunoblotting or ligand blotting. Immunoblotting was performed with a mixture of polyclonal antipeptide antibodies. Ligand blotting was performed with acetylated LDL as the ligand, and the bound acetyl-LDL was detected with anti-acetyl-LDL as described. The specificity of the antibodies for the scavenger receptor is demonstrated in Figure 8.

Results

Previous data have demonstrated that phorbol ester treatment of rabbit smooth muscle cells results in an increase in the binding, internalization, and degradation of AcAc-LDL. The current studies were undertaken to determine whether the metabolism of Ox-LDL was also upregulated and whether AcAc-LDL and Ox-LDL bind to the same receptor. Preincubation of the smooth muscle cells with phorbol esters resulted in a sevenfold increase in the degradation of Ox-LDL and a 12-fold increase in the degradation of AcAc-LDL (average of nine experiments, data not shown). Because Ox-LDLs have been shown to be resistant to degradation in other cell types, the apparent difference in the upregulation of the degradation of these ligands could be due to the accumulation of undegraded Ox-LDL within the cells. For this reason, the amount of lipoprotein that remained cell associated (bound and internalized) after incubation of the phorbol ester–treated cells with 125I-AcAc-LDL and 125I-Ox-LDL at 37°C was also determined. On average, the cellular association of Ox-LDL was three to four times higher than that of AcAc-LDL (data not shown). When the total amount of lipoprotein metabolized by the cells (total of the lipoprotein bound, internalized, and degraded) was compared, it became apparent that phorbol ester treatment upregulated the metabolism of both lipoproteins to a similar extent, as shown in a representative experiment (Figure 1). Consistent with the binding of Ox-LDL to the scavenger receptor, fucoidan effectively competed for the binding and degradation of Ox-LDL (90% competition), whereas native LDL did not (data not shown).

The data suggest either that phorbol esters upregulate two receptors to a similar extent, one for AcAc-LDL and one for Ox-LDL, or that there is one receptor that binds both modified lipoproteins. To address the question of the type and number of receptors expressed by the phorbol ester–treated rabbit smooth muscle cells, cross-competition experiments were performed at both 4°C and 37°C. In four separate competition assays performed at 4°C, the binding of 125I-Ox-LDL was inhibited by both ligands, but always to an equal or greater extent by AcAc-LDL than by Ox-LDL (Figure 2, left panel). On the other hand, the binding of 125I-AcAc-LDL was competed for only by AcAc-LDL (Figure 2,
modified low density lipoproteins (LDL) to phorbol ester-treated rabbit smooth muscle cells by oxidized (Ox) LDL (○) and acetooacetylated (AcAc) LDL (●). Cells were grown and treated with phorbol myristate acetate as described in the legend to Figure 1. The cells were precooled for 10 minutes on ice and then incubated with cold 10% lipoprotein-deficient serum in Dulbecco’s modified Eagle’s medium containing 5 μg/mL of 125I-AcAc-LDL or 125I-Ox-LDL alone or with increasing concentrations of unlabeled competitors for 2 hours at 4°C. In the absence of competitors the amounts of 125I-Ox-LDL and 125I-AcAc-LDL bound were 60 and 70 ng/mg cell protein, respectively.

right panel). At 37°C, both AcAc-LDL and Ox-LDL competed equally for the degradation of 125I-Ox-LDL (Figure 3, left panel), whereas only AcAc-LDL competed totally for the degradation of 125I-AcAc-LDL (Figure 3, right panel). In a series of nine separate experiments, the degradation of 125I-Ox-LDL was competed for essentially to the same extent by both AcAc-LDL and Ox-LDL, even though the total amount competed in individual experiments varied from 70% to 90%. The degradation of 125I-AcAc-LDL was partially inhibited by a 100-fold excess of unlabeled Ox-LDL (range 25-60%, mean 50%), whereas a 100-fold excess of AcAc-LDL competed essentially completely (more than 90%). We compared the competition by Ox-LDL before (TBARS of 40 nM MDA equivalents per milligram protein, range 30-44) and after (TBARS of 5 nM MDA equivalents per milligram) the last saline-EDTA dialysis and did not observe any significant difference in competition. However, at the highest protein concentration (250–500 μg/mL), the nondialyzed Ox-LDL was more cytotoxic, and cells were detached from the tissue culture dish.

Cross-competition experiments were performed with macrophages to determine whether the pattern of competition would be the same as in the smooth muscle cells. A representative experiment is shown in Figure 4. In rabbit peritoneal macrophages, both AcAc-LDL and Ox-LDL were equally good competitors for the degradation of 125I-Ox-LDL (n=5, range 70–96%), whereas only AcAc-LDL was an effective competitor for the degradation of 125I-AcAc-LDL (75–95%). In mouse peritoneal macrophages, similar results were obtained (data not shown). We compared the level of scavenger receptor activity in the upregulated smooth muscle cells to the activity of the receptor expressed by mouse peritoneal macrophages. In five experiments, the specific degradation of Ox-LDL in the PMA-treated smooth muscle cells was 22–38% (mean 30%) of the level of degradation by mouse peritoneal macrophages (data not shown), consistent with previous Scatchard analyses of the binding of AcAc-LDL, which demonstrate that phorbol ester–treated smooth muscle cells express 10–30% as many scavenger receptors as mouse peritoneal macrophages.

The cross-competition data are similar to those reported by Freeman et al.29 for the binding of Ox-LDL and acetyl-LDL to the bovine scavenger receptor. To determine whether we could reproduce their results under our experimental conditions, CHO cells were transfected with either the type I or II bovine scavenger receptor expression vector, and the effect on the metabolism of AcAc-LDL and Ox-LDL was examined. Type I receptor expression increased the metabolism of both Ox-LDL and AcAc-LDL (Figure 5). Similar results were obtained with the type II receptor (data not shown). Cross-competition experiments in the trans-
FIGURE 5. Bar graphs showing the effect of expression of the type I bovine scavenger receptor in Chinese hamster ovary fibroblasts on the metabolism of acetoacetylated (AcAc) low density lipoproteins (LDL) and oxidized (Ox) LDL. The cells were grown for 3 days and then incubated for 16 hours at 37°C with either [125I]-AcAc-LDL or [125I]-Ox-LDL (5 μg/mL). The lipoprotein degraded (cross-hatched portion of the bar) and the amount of lipoprotein associated with the cells at the end of the experiment, representing both surface-bound and internalized, nondegraded lipoprotein and shown in the solid portion of the bar, were determined as described in “Methods.” Nonspecific degradation and cell association (i.e., the amount obtained in the presence of a 100-fold excess of unlabeled AcAc-LDL or Ox-LDL) have been subtracted from the data. Nonspecific degradation of AcAc-LDL and Ox-LDL to control and transfected cells was ~15% while nonspecific cell association was ~20%.

Transfected CHO cells (Figure 6) gave results similar to those for the rabbit smooth muscle cells and macrophages. The degradation of [125I]-Ox-LDL was competed for to a similar extent by both Ox-LDL and AcAc-LDL. The degradation of [125I]-AcAc-LDL was inhibited partially by unlabeled Ox-LDL (mean 50%, range from 32% to 60% in five experiments), but completely by AcAc-LDL. The data are consistent with the binding of both ligands to the same receptor.

FIGURE 6. Competition curves for the degradation of [125I]-modified low density lipoproteins (LDL) in Chinese hamster ovary fibroblasts expressing the type I bovine scavenger receptor by oxidized (Ox) LDL and acetoacetylated (AcAc) LDL. The cells were incubated for 16 hours at 37°C in medium containing 5 μg/mL of either [125I]-AcAc-LDL or [125I]-Ox-LDL alone or with increasing concentrations of unlabeled lipoprotein. The amount of lipoprotein degraded was determined as described in “Methods.” In control cells in the absence of competitor, 0.92 and 0.65 μg/mg cell protein of AcAc-LDL and Ox-LDL were degraded, respectively.

The similarity of the results of the competition studies with the two ligands, Ox-LDL and AcAc-LDL, in the rabbit smooth muscle cells and macrophages suggest that both share a similar scavenger receptor. The data further imply that the receptor may be related to the bovine scavenger receptor. To explore these possibilities, Northern blot analysis of total RNA from these cells was performed (Figure 7). The probe detected mRNA for the bovine scavenger receptor in transfected CHO cells but not in control cells. The same antisense RNA probe hybridized to three mRNA species (~3.6 kb, 2.6 kb, and 1.7 kb) in the blots of total RNA from the phorbol ester–treated smooth muscle cells and the rabbit macrophages. These same three bands were detected in control nonstimulated smooth muscle cells after longer exposure. The probe was protected in RNase protection assays of total RNA from the phorbol ester–treated smooth muscle cells and macrophages (data not shown).

Immunoblot experiments were performed to determine whether the rabbit macrophage scavenger receptor was recognized by antibodies raised to peptide sequences of the bovine scavenger receptor. These antibodies recognized the bovine scavenger receptor expressed in transfected CHO cells but detected no receptor in nontransfected control CHO cells (Figure 8). In immunoblots of membrane proteins from rabbit macrophages, the antibodies recognized a 260-kD protein, consistent with the size expected for a trimeric scavenger receptor. In ligand blots the 260-kD rabbit scavenger receptor specifically bound acetyl-LDL. As a negative control, the ligand blots were processed with all reagents except acetyl-LDL, and no binding proteins were detected. As a positive control, the binding of acetyl-LDL to the trimeric 220-kD bovine scavenger receptor is also shown. The lower-molecular-weight binding proteins in the total membrane proteins from

FIGURE 7. Northern blot showing the expression of scavenger receptor mRNA by rabbit smooth muscle cells (SMC) and macrophages. Total RNA from Chinese hamster ovary (CHO) fibroblasts stably transfected with the type I bovine scavenger receptor, CHO control cells, rabbit SMCs, rabbit SMCs treated with phorbol myristate acetate (PMA), or rabbit peritoneal macrophages were used. One 2.2-kb species of bovine mRNA was detected, whereas three distinct mRNAs were seen with the induced rabbit SMCs and rabbit peritoneal macrophages.
the macrophages and the transfected CHO cells are most likely nonspecific interactions because binding proteins at these lower molecular weights are also detected in membrane proteins from the control CHO cells that do not express the scavenger receptor.

Discussion

Previous studies have demonstrated that rabbit fibroblasts and smooth muscle cells express a scavenger receptor with properties similar to the acetyl-LDL receptor. Receptor activity was regulated and the degradation of AcAc-LDL was increased in these cell types when they were treated with phorbol esters. In this study, we have demonstrated that phorbol ester treatment of rabbit smooth muscle cells increases the metabolism of Ox-LDL and AcAc-LDL to a similar extent, suggesting the upregulation of a single receptor that binds both AcAc-LDL and Ox-LDL. This hypothesis is supported by the observation that only Ox-LDL and AcAc-LDL competed equally for the degradation of 125I–Ox-LDL. In contrast, unlabelled Ox-LDL did not compete completely for the degradation of 125I–AcAc-LDL. In cross-competition experiments using rabbit and mouse peritoneal macrophages, essentially identical results were obtained. This “nonreciprocal” cross-competition had previously been reported by Freeman et al for the bovine scavenger receptors expressed in CHO cells. We confirmed their results in CHO cells transfected with type I or type II bovine scavenger receptor expression vectors and obtained competition curves similar to those obtained with rabbit smooth muscle cells and macrophages. Taken together, these data indicate that both Ox-LDL and AcAc-LDL bind to the same receptor and suggest that there is one receptor that is upregulated by phorbol ester treatment.

Receptor activity is increased by treatment of the smooth muscle cells with phorbol esters and mezerein, a nonphorbol activator of protein kinase C, suggesting that receptor activation is protein kinase–C mediated. It appears that human smooth muscle cells can also express the scavenger receptor and that receptor activity is regulated. We have previously demonstrated that scavenger receptor protein mass is increased by phorbol ester treatment of smooth muscle cells. The current data indicate that this increase in scavenger receptor synthesis is the consequence of an increase in scavenger receptor mRNA. Whether the increase in receptor mRNA results from an increase in synthesis or increased stability on phorbol ester induction remains to be determined.

Several lines of evidence indicate that the same scavenger receptor is expressed by the smooth muscle cells and macrophages. First, the rabbit smooth muscle cell and macrophage scavenger receptors cross-react with polyclonal antipeptide antibodies to sequences of the bovine scavenger receptor, and both scavenger receptors are essentially of the same molecular weight. In addition, the macrophage and smooth muscle cell scavenger receptors both interact with Ox-LDL and AcAc-LDL, yielding identical cross-competition patterns. Finally, both cell types have the same scavenger receptor mRNA species.

It appears that the rabbit scavenger receptor is structurally related to the bovine scavenger receptors that have been cloned. This hypothesis is supported by the fact that antipeptide antibodies raised to sequences of the bovine scavenger receptor cross-react with the rabbit macrophage and smooth muscle cell scavenger receptor. In addition, an antisense RNA probe that recognized the mRNA for the bovine scavenger receptor expressed in transfected CHO cells also recognized mRNA for the rabbit macrophage and smooth muscle scavenger receptor. The significance of the three rabbit scavenger receptor mRNA species is unknown. Two of the mRNAs could correspond to the message for the type I and type II receptors. The third mRNA may be an unprocessed mRNA or could represent a third variant of the scavenger receptor. The difference in the apparent molecular weight of the bovine scavenger receptor (~220 kD) and the rabbit scavenger receptor (~260 kD) in the immunoblots may be due to differences in the primary sequence of the receptors or to differences in glycosylation.

In agreement with the published properties of the bovine scavenger receptor, both Ox-LDL and AcAc-LDL bind to the scavenger receptor expressed by rabbit
and mouse peritoneal macrophages and smooth muscle cells, suggesting that there is no independent receptor for Ox-LDL in these cells. The previously reported data for mouse peritoneal macrophages have varied more. In some reports, Ox-LDL and acetyl-LDL competed equally for the degradation of 3H-Ox-LDL,

3,38 whereas in other studies, acetyl-LDL did not compete effectively, suggesting the presence of an independent receptor for Ox-LDL.36,39 The reason for these differences in the data is unknown; however, they could result from differences in the degree of oxidation of the LDL used, variation in the preparations of mouse peritoneal macrophages, or both. The question of the type and number of receptors for modified LDL will be clarified when antibodies are available that block the binding of both AcAc-LDL and Ox-LDL to the type I and II scavenger receptors.

It has been suggested that Ox-LDL,10 is a physiological ligand for the scavenger receptor that contributes to the formation of macrophage-derived foam cells in atherosclerotic lesions. Although there may be scavenger receptors specific for Ox-LDL,35,36,39,40 it is clear that the type I and II bovine scavenger receptors can bind acetyl-LDL or AcAc-LDL as well as Ox-LDL and that bovine scavenger receptor–mediated uptake of Ox-LDL by cells in culture can lead to lipid accumulation.29 The scavenger receptor(s) expressed by rabbit smooth muscle cells and macrophages also binds Ox-LDL and, in fact, is related to the bovine type I and II scavenger receptors. Taken together, the data suggest that the uptake of Ox-LDL by scavenger receptors expressed by rabbit macrophages and smooth muscle cells could contribute to lipid accumulation by cells and to foam cell formation.

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