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

Sylvie Dejager, Michele Mietus-Snyder, and Robert E. Pitas

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 $^{125}$I-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 $^{125}$I-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.

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KEY WORDS • acetyl LDL receptor • foam cells • macrophages • oxidized LDL • scavenger receptors • smooth muscle cells • modified LDL

In 1979, Goldstein et al.1 reported that macrophages express a receptor (the acetyl low density lipoprotein [LDL]), or scavenger, receptor) that recognizes chemically modified LDL. In contrast to the LDL receptor, acetyl-LDL receptor activity is not regulated by cholesterol within the cells,1 and therefore the uptake of acetyl-LDL leads to lipid accumulation and foam cell formation in macrophages. Malondialdehyde (MDA) modified LDL and oxidized (Ox) LDL also bind to the scavenger receptor.2,3 Whereas acetyl-LDL does not occur in vivo, several lines of evidence suggest that Ox-LDL and MDA-modified LDL do occur in vivo. Ox-LDL and MDA-modified protein have been demonstrated in atherosclerotic lesions by immunocytochemistry4,5 and in extracts of atherosclerotic lesions,4 and autoantibodies directed against Ox-LDL have been detected in the plasma of experimental animals and humans.4 Furthermore, the antioxidant probucol is effective in preventing the development of spontaneous atherosclerotic lesions in genetically hypercholesterolemic Watanabe heritable hyperlipidemic (WHHL) rabbits.5,6 Similarly, butylated hydroxytoluene prevents diet-induced atherosclerosis in New Zealand White rabbits.6 Recently, a probucol analogue that retains its antioxidant activity but has no effect on plasma cholesterol levels was also shown to be antiatherogenic in WHHL rabbits.7 Ox-LDL may, therefore, be an atherogenic physiological ligand for the scavenger receptor.8

Both smooth muscle cells and macrophages are precursors of foam cells found in atherosclerotic lesions. It has been proposed that the uptake of Ox-LDL by
macrophages leads to the generation of foam cells in vivo.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.12,13 We recently demonstrated that rabbit
smooth muscle cells also express the scavenger receptor and that the receptor in these cells is regulated.14,15
Receptor expression is upregulated by platelet secre-
yry products and preincubation of the cells with phor-
bol esters. The phorbol ester-treated rabbit smooth
muscle cells express 10–30% as many receptors as mouse peritoneal macrophages.14 These observations
raise the possibility that lipid accumulation in smooth
muscle cells in vivo might also result from the receptor-
ediated 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 [22]iodide, the
fluorescent probe 1,1'-dioctadecyl-3,3,3',3'-tetrameth-
ylindocarbocyanine perchlorate (DiI), and fucoidan
were obtained as previously described.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.
Monty Krieger, Massachusetts Institute of Technology,
Cambridge, Mass.,16,17 and the vector pSV2neo18 was
obtained from Dr. David Johnson, Gladstone Institute.
The vector pcDNA1 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.19 The human LDLs were
dialyzed against saline-EDTA, sterilized by filtration (0.45
µm).3-20 Oxidation was stopped by cooling and the
thiobarbituric acid-reactive substances (TBARS) were
determined by analysis of the cells solubilized in 0.1N
NaOH by the method of Lowry et al13 using bovine
serum albumin as a standard. All data points are the
average of duplicate determinations. In each experi-
ment, the amount of lipoprotein associated with the
cells at the end of the experiment, which represents
both surface-bound and internalized, nondegraded lip-
protein, was also quantified as described.30 For compe-
tition studies, the 125I-ligand and unlabeled competitors
were premixed before incubation with the cells, either
in DMEM or in DMEM–N2 hydroxyethylpiperazine-N’-2-ethanesulfonic acid containing
10% human lipoprotein-deficient serum for 4°C
experiments.

Cell Culture Experiments

Mouse peritoneal macrophages were obtained from
nonstimulated Swiss Webster mice as described.1 Rab-
bit peritoneal macrophages were obtained from rabbits
4 days after intraperitoneal injection of mineral oil.17
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 µg/ml). The medium
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 µg/ml). The type I and type II
bovine scavenger receptor expression vectors, pXSR7
and pXSR3 (20 µg), respectively, were cotransfected in
CHO cells with pSV2neo (1 µg) using the calcium
phosphate precipitation method as described.28 Colon-
ies resistant to the neomycin analogue G418 (0.4
mg/ml) were selected and screened with DiI-labeled
AcAc-LDL (5 µg/ml for 16 hours) to identify scavenger
receptor-positive colonies. The most highly fluorescent
cells were selected by fluorescence-activated cell sorting
described.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.29
Control CHO cells were stably cotransfected with the
vector pcDNA1 and pSV2neo in the same 20:1 ratio.

Scavenger receptor activity was assessed at 37°C by
measuring cellular degradation of 125I-labeled modified
LDL in 12-well culture dishes. For all lipoprotein
degradation assays, the growth medium was removed from
the cells before the addition of 125I-lipoproteins in
DMEM. The cells were incubated at 37°C for the times
indicated, and the trichloroacetic acid-soluble lipopro-
tein degradation products in the medium were quanti-
fied as described.30 The cellular protein content was
determined by analysis of the cells solubilized in 0.1N
NaOH by the method of Lowry et al13 using bovine
serum albumin as a standard. All data points are the
average of duplicate determinations. In each experi-
ment, the amount of lipoprotein associated with the
cells at the end of the experiment, which represents
both surface-bound and internalized, nondegraded lip-
protein, was also quantified as described.30 For compe-
tition studies, the 125I-ligand and unlabeled competitors
were premixed before incubation with the cells, either
in DMEM for 37°C experiments or in DMEM–N2
hydroxyethylpiperazine-N’-2-ethanesulfonic acid contain-
10% human lipoprotein-deficient serum for 4°C
experiments.
Northern Analysis

A 32P-labeled antisense rabbit RNA probe was generated for Northern hybridization.33 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.34 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.14 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 octylglucoside-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 nondenaturing 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 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.14 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. Premiscuection 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,35,36 the apparent difference in the upregulation of the degradation of these ligands could be due to the accumulation of degraded 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, right panel).
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

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Competition curves for the binding of 125I-modified low density lipoproteins (LDL) to phorbol ester-treated rabbit smooth muscle cells by oxidized (Ox) LDL (○) and acetoacetylated (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.

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Competition curves for the degradation of 125I-modified low density lipoproteins (LDL) in phorbol ester-treated rabbit smooth muscle cells by oxidized (Ox) LDL (○) and acetoacetylated (AcAc) LDL (●). Cells were treated with phorbol myristate acetate as described in the legend to Figure 1. The cells were incubated for 16 hours at 37°C with Dulbecco’s modified Eagle’s medium containing 5 μg/mL of either 125I-AcAc-LDL or 125I-Ox-LDL alone or with increasing concentrations of unlabeled competitors. The amount of lipoprotein degraded was determined as described in “Methods” and is expressed as a percentage of the control value. In the absence of competitor, the amounts of 125I-Ox-LDL and 125I-AcAc-LDL degraded were 4.6 and 8.6 μg/mg cell protein per 16 hours, respectively.

**Figure 4.** Competition curves for the degradation of 125I-modified low density lipoproteins (LDL) in rabbit peritoneal macrophages by oxidized (Ox) LDL and acetoacetylated (AcAc) LDL. The cells were harvested and plated as indicated in “Methods.” The cells were incubated for 5 hours at 37°C with 5 μg/mL of either 125I-AcAc-LDL or 125I-Ox-LDL alone or together with increasing concentrations of unlabeled lipoprotein. The amount of lipoprotein degraded was determined as described in “Methods.” In the control cells in the absence of competitor, 2.4 and 1.1 μg/mg cell protein were degraded for AcAc-LDL and Ox-LDL, respectively.

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 nondialed 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.14

The cross-competition data are similar to those reported by Freeman et al for the binding of Ox-LDL and acetate-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 $^{125}$I-AcAc-LDL or $^{125}$I-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 $^{125}$I-Ox-LDL was competed for to a similar extent by both Ox-LDL and AcAc-LDL. The degradation of $^{125}$I-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 $^{125}$I-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 $^{125}$I-AcAc-LDL or $^{125}$I-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...
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 is 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 degradation of 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 then to horseradish peroxidase (HRP)-conjugated protein A. The ligand blot was incubated sequentially with acetyl low density lipoprotein (acetyl-LDL, 30 µg/mL), an antiserum specific for acetyl-LDL, and then HRP-protein A. The HRP-protein A was detected by incubation with reagents for chemiluminescent detection (see "Methods") before exposure on x-ray film.

![Immunoblot Ligand Blot](image_url)

**FIGURE 8.** Ligand blot and immunoblot of membrane proteins from rabbit peritoneal macrophages and from Chinese hamster ovary (CHO) fibroblasts expressing the type I bovine scavenger receptor. Membrane proteins were solubilized with octylglucoside, applied (nonreduced) to sodium dodecyl sulfate–polyacrylamide (5% acrylamide) slab gels, electrophoresed, and then transferred to nitrocellulose. For both the immunoblot and the ligand blot, 75 µg of membrane proteins from the control CHO cells or the CHO cells expressing the type I receptor were used. For the immunoblot and ligand blot of the rabbit peritoneal macrophage membrane proteins, 500 and 150 µg were used, respectively. The immunoblot was reacted with a mixture of antiseras raised in guinea pigs to specific sequences of the bovine scavenger receptor and then to horseradish peroxidase (HRP)-conjugated protein A. The ligand blot was incubated sequentially with acetyl low density lipoprotein (acetyl-LDL, 30 µg/mL), an antiserum specific for acetyl-LDL, and then HRP-protein A. The HRP-protein A was detected by incubation with reagents for chemiluminescent detection (see "Methods") before exposure on x-ray film.
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 \( 3^2 \text{H-Ox-LDL} \), whereas in other studies, acetyl-LDL did not compete effectively, suggesting the presence of an independent receptor for Ox-LDL. 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 number and type 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 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, 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. The scavenger receptor(s) expressed by rabbit macrophages and smooth muscle cells 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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