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A macrophage receptor for oxidized low density lipoprotein distinct from the receptor for acetyl low density lipoprotein: Partial purification and role in recognition of oxidatively damaged cells

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ABSTRACT **The binding and uptake of oxidatively modified low density lipoprotein (OxLDL) by mouse peritoneal macrophages occurs, in part, via the well characterized acetyl LDL receptor. However, several lines of evidence indicate that as much as 30–70% of the uptake can occur via a distinct receptor that recognizes OxLDL with a higher affinity than it recognizes acetyl LDL. We describe the partial purification and characterization of a 94- to 97-kDa plasma membrane protein from mouse peritoneal macrophages that specifically binds OxLDL. This receptor is shown to be distinct from the acetyl LDL receptor as well as from two other macrophage proteins that also bind OxLDL—the FcγRII receptor and CD36. We suggest that this OxLDL-binding membrane protein participates in uptake of OxLDL by murine macrophages and also represents a receptor responsible for macrophage binding and phagocytosis of oxidatively damaged cells.**

Oxidative modification of lipoproteins appears to play an important role in the atherogenic process (1). Oxidatively modified low density lipoprotein (OxLDL) is taken up by macrophages much more rapidly than is native LDL and almost all that uptake is saturable and specific (2). OxLDL is taken up in part by way of the acetyl LDL (AcLDL) receptor, discovered by Basu *et al.* (3) and later cloned and fully characterized by Kodama *et al.* (4). However, as much as 30–70% of the binding and uptake of OxLDL by mouse peritoneal macrophages is not accounted for by the AcLDL receptor (5, 6), implying the presence of an additional receptor or receptors specific for OxLDL. We recently reported evidence that the same receptor(s) recognizing OxLDL also recognized oxidatively damaged red blood cells (OxRBCs) (7). OxLDL inhibited binding of OxRBCs, but AcLDL did not. It was suggested that this might represent one pathway by which damaged or apoptotic cells might be bound and phagocytosed by macrophages. Such a broad, generalized function would make more understandable the persistence of these receptors through evolution. Abrams *et al.* (8) have reported scavenger receptor-like activity even in *Drosophila* embryo membranes. In an accompanying paper (9), G.R.S. and D.S. demonstrate that OxRBCs and apoptotic thymocytes bind to mouse peritoneal macrophages via a receptor that also binds OxLDL and that phosphatidylserine (PS) liposomes (but not AcLDL) compete for the binding of both OxRBCs and OxLDL.

In the present studies, we describe the partial purification from mouse peritoneal macrophage plasma membranes of a protein that we believe to be that receptor. It is a 94- to 97-kDa protein that binds OxLDL and PS-rich liposomes with high affinity but binds AcLDL with much lower affinity. The same ligand-binding protein was found in membrane fractions from rabbit peritoneal macrophages, foam cells isolated from aortas of cholesterol-fed rabbits, and rabbit subcutaneous carrageenan granulomas. It is shown by several criteria to be distinct from two other OxLDL-binding membrane proteins or murine macrophages—the FcγRII receptor and the mouse homologue of human CD36.

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MATERIALS AND METHODS

Materials. Nitrocellulose membranes were from Bio-Rad. Carrier-free $Na^{125}I$ was from Amersham. *N*-octyl β-D-glucopyranoside and cetyltrimethyl ammonium bromide were from Sigma. Drakeol 6-VR mineral oil was from Penreco (Los Angeles). Purified rat anti-mouse FcγRII monoclonal antibody was from Pharmingen. Monoclonal antibody 2F8 directed against the mouse AcLDL receptor was a generous gift from I. Fraser, D. A. Hughes, and S. Gordon (University of Oxford) (10).

Cells and Tissue. Female Swiss–Webster mice (30 g) were from Simonsen Laboratories (Gilroy, CA). The mice were injected intraperitoneally with 2 ml of 1% thioglycollate broth. On day 3, macrophages were harvested by peritoneal lavage. Rabbits (female New Zealand White; 3 kg) were injected intraperitoneally with 30 ml of mineral oil 3 days before isolation of macrophages. Rabbit granuloma tissue was produced by injecting 15 ml of 1% carrageenan subcutaneously into the abdominal wall of New Zealand White rabbits on a 2% cholesterol diet. After 7 days, the granuloma tissue was harvested according to the procedure of Schwartz *et al.* (11).

RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal calf serum (FCS). THP-1 cells were grown in suspension in RPMI 1640 medium, plated in the presence of phorbol 12-myristate 13-acetate (PMA) (10 ng/ml), and studied 3 days later. Chinese hamster ovary (CHO) cells were maintained in Ham's F-12 medium containing 5% FCS. CHO cells stably transfected with the murine type II AcLDL receptor (a gift from M. Krieger, Massachusetts Institute of Technology) were grown in the same medium with G418 added at 500 μg/ml.

Human skin fibroblasts were cultured in DMEM and scraped off the plate for membrane preparation. Human peritoneal macrophages were obtained from patients with endometriosis during diagnostic laparoscopy. These macrophages have been shown to actively degrade labeled OxLDL (S.P. and A. A. Murphy, unpublished data).

Abbreviations: LDL, low density lipoprotein; OxLDL, oxidatively modified LDL; OxRBC, oxidatively damaged red blood cell; BHT, butylated hydroxytoluene; PS, phosphatidylserine; AcLDL, acetyl LDL; PMA, phorbol 12-myristate 13-acetate.

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Rabbit aortic foam cells were isolated from deendothelialized aortas of cholesterol-fed rabbits (2% cholesterol for 11 weeks) as described by Rosenfeld *et al.* (12).

Membrane Preparation. Cells were suspended in buffer A [50 mM Tris·HCl, pH 8/150 mM NaCl/1 mM EDTA/1 mM phenylmethylsulfonyl fluoride (PMSF)] and homogenized with 20 strokes in a Potter–Elvehjem homogenizer and centrifuged at 800 × *g* for 10 min. The supernate was centrifuged at 10,000 × *g* for 15 min and the resulting supernate was centrifuged at 100,000 × *g* for 120 min to obtain a crude membrane pellet.

To obtain preparations highly enriched in plasma membrane, the cells were suspended in buffer B (5 mM Tris·HCl, pH 8/0.25 M sucrose/1 mM PMSF), homogenized, and centrifuged in isotonic sucrose gradients; the plasma membrane fraction was prepared according to Aronson and Touster (13).

Carrageenan granuloma tissue was disrupted by mincing it and processing it for 1 min with a Polytron (Brinkmann) tissue grinder. The minced tissue was homogenized with a Teflon pestle (20 strokes) and centrifuged as described above to obtain the 100,000 × *g* crude membrane pellet.

Ligand Preparation and Iodination. LDL (density, 1.019–1.063) was isolated by preparative ultracentrifugation from fresh human plasma collected in EDTA (1 mg/ml) (14). In some preparations, 20 μM butylated hydroxytoluene (BHT) was included during isolation. AcLDL was prepared as described by Basu *et al.* (15). OxLDL was obtained by incubating LDL (100 μg/ml) with 10 μM cupric acetate for 18 h at 37°C. Iodination was performed according to Salacinski *et al.* (16). Protein was determined according to Lowry *et al.* (17). Degradation of ^{125}I-labeled LDL (^{125}I-LDL) by mouse peritoneal macrophages was measured as described (5). Binding and uptake of ^{125}I-LDL by macrophages was assessed at 37°C for 1 h in the presence or absence of competitors. Unbound ligand was removed by washing twice with phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin and an additional two times with PBS. Cells were solubilized in 0.2 M NaOH for determination of radioactivity.

Ligand Blotting. Crude membrane proteins were separated by SDS/PAGE under nonreducing conditions. The separated proteins were transferred to a nitrocellulose membrane, which was then incubated with 10 mM Tris·HCl, pH 8/90 mM NaCl/1 mM EDTA containing 5% nonfat dry milk for 1 h. The ^{125}I-ligands (average specific activity, 150–250 cpm/ng) were then added for 1.5 h at room temperature, after which the membrane was washed eight times (each step for 10 min) with buffer containing 1% nonfat dry milk. The strips were dried in air and exposed to Kodak X-Omat AR film for 3–4 days at −70°C. For competition studies the nitrocellulose strips were incubated with the inhibitors for 45 min prior to addition of the radiolabeled ligand.

PS-rich liposomes were prepared as described in the accompanying paper (9) and used in binding competition studies as described above except that the NaCl concentration in the incubation buffer was raised from 90 to 140 mM.

OxLDL Affinity Chromatography. OxLDL was delipidated by the method of Bligh and Dyer (18). The modified apolipoprotein B was resolubilized in cetyltrimethyl ammonium bromide (25 mg/ml). The solubilized protein was again precipitated with acetone and washed several times with water. The delipidated OxLDL, which could be resolubilized in water, was coupled to AH-Sepharose with an efficiency of 80%. The macrophage membrane proteins, solubilized in octyl glucoside (5 mg), were applied and the column was initially washed with 50 mM Tris·HCl/15 mM NaCl/40 mM octyl glucoside, pH 8.0 (column buffer) and then eluted with NaCl, either in two steps (first with 250 mM NaCl and then with 1 M NaCl) or by using an increasing linear gradient of NaCl. In later studies, AvidGel (UniSyn, Tustin, CA) was substituted for Sepharose and a step-gradient elution was performed from 15 to 250 mM NaCl.

Antibody Against the Mouse Homologue of CD36. Endemann *et al.* (19), using expression cloning, have identified the mouse homologue of CD36 as an OxLDL-binding protein. Based on the registered sequence of this gene (GenBank data base, accession no. L23108) and that of the human CD36 (20), we chose a highly conserved sequence (representing amino acid residues 169–244 of the mouse CD36). The fragment was amplified from murine CD36 cDNA (a generous gift of Gerda Endemann, Scios Inc., Mountain View, CA) by PCR. The amplified fragment was inserted in-frame into a pGEX-2T vector (Pharmacia) and expressed as a fusion protein with glutathione *S*-transferase. The fusion protein was isolated with glutathione-Sepharose 4B beads (Sigma) and used to generate an antiserum in guinea pigs. The antiserum was used for Western blotting and was detected with an alkaline phosphatase-conjugated goat anti-guinea pig IgG (Sigma).

RESULTS

The binding of ^{125}I-OxLDL to membrane proteins from mouse peritoneal macrophages is shown in Fig. 1. OxLDL showed predominant binding to a protein of 94–97 kDa (lane A). Native LDL, prepared carefully in the presence of BHT, showed no binding (lane B). On several occasions, however, "native" LDL that had not been carefully protected against oxidative damage did bind to the 94- to 97-kDa band. The binding of OxLDL to these membrane proteins was completely inhibited by the addition of excess unlabeled oxidized LDL (lane C). Delipidated ^{125}I-OxLDL also bound to the 94- to 97-kDa band, indicating that the protein moiety of the OxLDL is recognized by the receptor (data not shown).

As shown in Fig. 2 (lane A), ^{125}I-AcLDL showed ligand binding to a band at ≈210 kDa, presumably representing the mouse homologue of the AcLDL receptor described by Kodama *et al.* (4), but it also bound to the 94- to 97-kDa protein. The latter binding was abolished when excess unlabeled OxLDL was present during the blotting (lane B), whereas the binding of AcLDL to the 210-kDa band resisted competition by unlabeled OxLDL. Studies in Krieger's laboratory (21) have shown that OxLDL does bind to the AcLDL receptor on intact cell membranes but with a lower affinity than does AcLDL. The K_d for OxLDL binding to the 94- to 97-kDa ligand-binding band was 5.5 μg/ml. Only when very high concentrations of the ligand were used did we see definite binding of ^{125}I-OxLDL to a 140- or 210-kDa band.

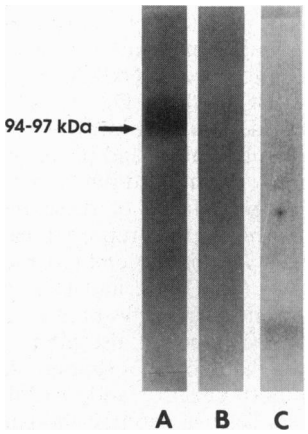

FIG. 1. Ligand blots of membrane proteins from mouse peritoneal macrophages. Mouse peritoneal macrophage membrane proteins (100 μg) were run on a SDS/9% polyacrylamide gel under nonreducing conditions. After transfer to nitrocellulose the strips were incubated with 10 μg of ^{125}I-OxLDL per ml (lane A), 10 μg of ^{125}I-LDL per ml that had been isolated in the presence of 20 μM BHT (lane B), and 10 μg of ^{125}I-OxLDL per ml in the presence of 300 μg of unlabeled OxLDL per ml (lane C).

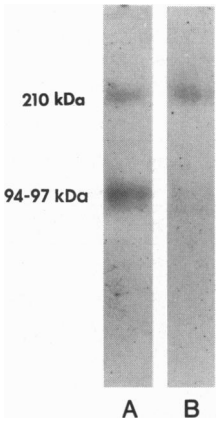

FIG. 2. Binding of ^{125}I-AcLDL to mouse peritoneal macrophage membrane proteins in the absence and presence of excess unlabeled OxLDL. Solubilized mouse peritoneal macrophage membrane proteins (100 μg) were separated on a SDS/3–15% polyacrylamide gel under nonreducing conditions. After transfer to nitrocellulose, the strips were incubated with 10 μg (230 cpm/ng) of ^{125}I-AcLDL per ml in the absence (lane A) or in the presence (lane B) of 550 μg of unlabeled OxLDL per ml.

It has been shown that binding of OxLDL to mouse peritoneal macrophages is inhibited by PS-rich liposomes (22). We therefore tested the ability of PS liposomes to compete for binding of ^{125}I-OxLDL in a ligand blot. PS liposomes (0.25 mM total lipid) very strongly reduced binding of OxLDL (2.5 μg/ml) (data not shown).

We next isolated fractions highly enriched in plasma membrane. Fractions enriched in 5′-nucleotidase, a marker enzyme for plasma membrane, were enriched also in the 94- to 97-kDa OxLDL-binding proteins (Fig. 3). No OxLDL-binding band was detected in the membrane fraction freed of plasma membranes (results not shown).

Partial purification of the OxLDL-binding protein was achieved by preparative gel electrophoresis and affinity chromatography. When solubilized mouse macrophage membrane proteins were applied to an OxLDL-Sepharose affinity column, the OxLDL-binding protein was adsorbed and could then be eluted with 250 mM NaCl containing 40 mM octyl glucoside. The eluted fractions, when separated by SDS/PAGE, showed only a single band, at 94–97 kDa, that bound ^{125}I-OxLDL. However, Coomassie staining showed multiple bands. Better purification was obtained by using an AvidGel OxLDL affinity column and stepwise or continuous gradient elution. Fractions eluted between 150 and 250 mM NaCl showed OxLDL-binding bands at 94–97 kDa and at ≈220 kDa, presumably representing the OxLDL-binding protein and the AcLDL receptor, respectively. As shown in Fig. 4, affinity chromatography also resolved the OxLDL receptor from the mouse homologue of CD36, which was cloned as an OxLDL-binding protein by Endemann *et al.* (19).

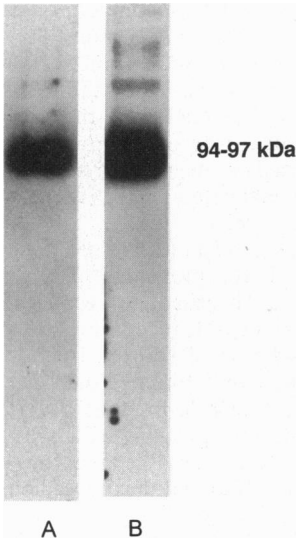

FIG. 3. Ligand blotting of ^{125}I-OxLDL to membrane preparations from mouse peritoneal macrophages. Lanes: A, 100 μg of crude macrophage membrane protein; B, 100 μg of purified plasma membrane protein. Samples were separated on a SDS/9% polyacrylamide gel and transferred to nitrocellulose membranes.

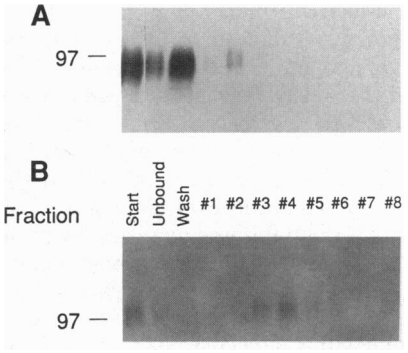

FIG. 4. Resolution of CD36 and 94- to 97-kDa protein by OxLDL affinity chromatography. Affinity chromatography was performed as described starting with 7 mg of the 100,000 × *g* membrane fraction in 10 ml of column buffer. After loading, the column was washed with 50 ml of buffer and then eluted with a linear gradient of sodium chloride (0.015–1.0 M) in column buffer. Ten 5-ml fractions in increments of 60 mM were collected and aliquots of either 6 μg (lane 1) or 3–4 μg (lanes 2–11) were electrophoresed, transferred to nitrocellulose, and probed for either CD36 Western blotting activity (*A*) or ^{125}I-OxLDL ligand blotting activity (*B*).

As shown in Fig. 5, rabbit peritoneal macrophages, foam cells isolated from the aortas of cholesterol-fed rabbits, and macrophages from rabbit carrageenan granulomas also expressed a 94- to 97-kDa OxLDL-binding protein. The protein was particularly strongly expressed in the rabbit aortic foam cells (lane C). A faint band was also seen in membranes from

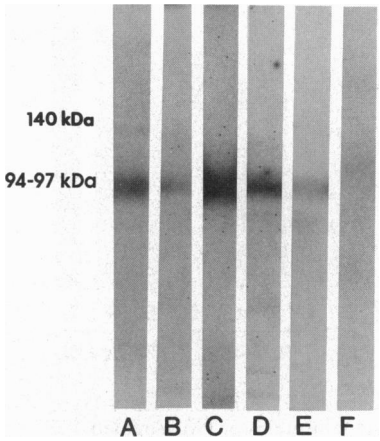

FIG. 5. Binding of ^{125}I-OxLDL to membrane proteins from various cell types. Lanes: A, mouse peritoneal macrophages; B, RAW 246.7 cells; C, rabbit aortic foam cells; D, rabbit carrageenan granuloma cells; E, rabbit peritoneal macrophages; F, human fibroblasts. Either 100 μg (lane A) or 200 μg (lanes B–F) of crude membrane proteins was separated on a SDS/8% polyacrylamide gel under nonreducing conditions and transferred to nitrocellulose membranes. The nitrocellulose membranes were incubated with 10 μg of ^{125}I-OxLDL per ml (240 cpm/ng) for 1.5 h, washed, and autoradiographed.

a macrophage-like cell line, RAW 264.7 (lane B). Human fibroblast membranes showed no OxLDL binding.

Treatment of human-derived THP-1 cells with PMA induces expression of the AcLDL receptor (23, 24). As shown in Fig. 6, treatment of THP-1 cells with PMA for 3 days did indeed induce a significant increase in the binding of ^{125}I-AcLDL to the 220-kDa band, as expected (lanes E and F). Untreated THP-1 cells showed a low level of binding of ^{125}I-OxLDL to a band at ≈120 kDa and this was increased by prior incubation with PMA (lanes B and C). There was no binding in the 94- to 97-kDa region. Human peritoneal macrophages obtained from patients with endometriosis also expressed an OxLDL-binding protein with a molecular mass of ≈120 kDa (lane D). These cells also exhibited an AcLDL-binding protein at 220 kDa (lane G).

Sambrano *et al.* (7) found that the binding of OxRBCs to mouse peritoneal macrophages was inhibited by OxLDL but not by AcLDL. To test further the possibility that the AcLDL receptor might be involved in the binding of OxRBCs, we looked for binding to CHO cells stably transfected with the cDNA for the mouse AcLDL receptor type II. No binding of OxRBCs was observed (data not shown).

In further tests of the possible involvement of the AcLDL receptor, we used a rat monoclonal antibody that recognizes the mouse AcLDL receptor (10). As shown in Table 1, it inhibited 37°C binding of AcLDL and OxLDL to the transfected CHO cells. As expected, this antibody was also effective in inhibiting uptake of AcLDL by macrophages (65% of total uptake). However, it had no effect on the binding of OxRBCs to macrophages (Table 1).

DISCUSSION

These studies identify a 94- to 97-kDa membrane protein in mouse peritoneal macrophages that represents a receptor for OxLDL—and possibly for OxRBCs (7, 9)—distinct from the AcLDL receptor. A ligand-binding band for OxLDL was also found in human monocyte-like THP-1 cells and in human peritoneal macrophages but in these human cells it had a larger molecular mass (≈120 kDa). Expression of this membrane protein was enhanced by treatment of the cells with PMA, a known inducer of the expression of the AcLDL receptor in those cells (23, 24).

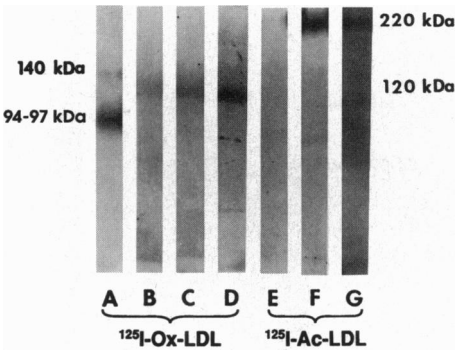

FIG. 6. Binding of ^{125}I-OxLDL (lanes A–D) or ^{125}I-AcLDL (lanes E–G) to membrane proteins of PMA-treated THP-1 cells and membrane proteins of human peritoneal macrophages. Lanes: A, 100 μg of mouse peritoneal macrophage membrane proteins; B and E, 445 μg of THP-1 membrane proteins from untreated cells; C and F, 445 μg of THP-1 membrane proteins from PMA-treated cells; D and G, 240 μg of membrane protein from solubilized human peritoneal macrophages. Proteins were separated on a SDS/5–15% polyacrylamide gel under nonreducing conditions and transferred to nitrocellulose membrane. Nitrocellulose strips were incubated with 10 μg of ^{125}I-OxLDL per ml (250 cpm/ng) (lanes A–D) or with 10 μg of ^{125}I-AcLDL per ml (250 cpm/ng) (lanes E–G).

Table 1. Effects of a monoclonal antibody (mAb) against the AcLDL receptor (R) on binding and uptake of AcLDL and OxLDL by CHO cells expressing the AcLDL receptor and on binding of OxRBCs to mouse peritoneal macrophages

Competitor added	% inhibition: Binding and uptake by CHO cells, ^{125}I-AcLDL	% inhibition: Binding and uptake by CHO cells, ^{125}I-OxLDL	% inhibition: Binding of OxRBCs to macrophages
mAb against AcLDL R	65	58	1
Unlabeled OxLDL	12	99	90
Unlabeled AcLDL	92	89	2

Binding/uptake studies were performed at 37°C for 1 h in DMEM. ^{125}I-AcLDL and ^{125}I-OxLDL were added at 10 μg/ml (final concentration) and OxRBC was at 0.1% hematocrit. Unlabeled OxLDL and AcLDL were at 150 μg/ml and the monoclonal antibody (2F8) was at 80 μg/ml (final concentrations). Values represent percentage inhibition of binding calculated from μg of ^{125}I-ligand per mg of cell protein or percentage of macrophages binding 1 or more OxRBC.

Several lines of evidence distinguish this receptor from the AcLDL receptor. First, it is much smaller (97 kDa vs. 220 kDa). Second, the affinity of the 94- to 97-kDa protein for OxLDL is greater than its affinity for AcLDL; in contrast, the AcLDL receptor cloned by Kodama and coworkers (21) and transfected into CHO cells showed a higher affinity for AcLDL than for OxLDL. Third, reducing conditions did not affect the apparent molecular size of the 94- to 97-kDa protein or its capacity to bind OxLDL; in contrast, reducing conditions result in disruption of the AcLDL receptor and sharply reduce its ability to bind AcLDL (21). Fourth, as shown in an accompanying paper (9), whereas OxRBCs bind strongly to mouse peritoneal macrophages, they did not bind to CHO cells overexpressing the mouse AcLDL receptor. Fifth, neither AcLDL nor a monoclonal antibody directed against the AcLDL receptor inhibited binding of RBCs to macrophages, whereas OxLDL was a very effective competitor. Finally, PS liposomes competed for OxLDL binding to the 94- to 97-kDa protein but they did not bind to the 210-kDa band or to the AcLDL receptor expressed in CHO cells (25).

Because the ligand-binding domain of the AcLDL receptor is unusual, interpretation of competition studies should be made cautiously. As Krieger *et al.* point out (26), the array of ligands that bind to the receptor is much broader than that seen with most other receptors. Thus, the failure of AcLDL to compete for binding of OxRBCs to the macrophage does not necessarily rule out a role for it. For example, the negative result might be based on the need for a cooperating membrane protein in order to make the AcLDL receptor competent in the binding of damaged cells.

The work of Stanton *et al.* (27) raised the possibility that the macrophage FcγRII receptor might play a role in uptake of OxLDL; however, their subsequent results (19) showed that an antibody against the FcγRII receptor was without effect on the uptake and degradation of OxLDL by mouse macrophages. We have confirmed that finding (data not shown), casting doubt on an important role for the FcγRII receptor. In addition, the binding and phagocytosis of OxRBCs has been shown not to involve the FcγRII receptor (7).

Endemann *et al.* (19) cloned the mouse homologue of human CD36 (21) as an OxLDL-binding protein by expression cloning from a mouse peritoneal macrophage library. Functional tests for a role of this homologue in the internalization and degradation of OxLDL by mouse macrophages were not reported. However, binding of OxLDL to human THP-1 cells was inhibited by OKM5, a monoclonal antibody against human CD36 (19). In collaboration with others, we have studied CHO cells stably transfected

with CD36 (R. H. Lipsky, G.S., Y. Tang, and D.S., unpublished results). The results confirmed that ^{125}I-OxLDL binds to cells expressing CD36. However, there was no measurable internalization and degradation. Using an antibody against the mouse homologue of CD36, we show (Fig. 4) that it can be resolved from the 94- to 97-kDa OxLDL-binding protein by affinity chromatography. Again, CD36 might act cooperatively with the 94- to 97-kDa protein or another macrophage membrane protein in the uptake of OxLDL (and/or Ox-RBCs) and so a role for CD36 in OxLDL uptake is by no means ruled out.

Data supporting the presence of distinct AcLDL and OxLDL receptors in rat liver have been reported by van Berkel *et al.* (28). When they injected both labeled AcLDL and labeled OxLDL intravenously into rats, the Kupffer cells took up OxLDL much more rapidly than AcLDL, whereas the reverse was true for the sinusoidal endothelial cells. Recently, using ligand blotting, De Rijke and van Berkel (29) have demonstrated the presence of a 95-kDa OxLDL-binding protein in rat Kupffer cells.

How do we account for the persistence through evolution of the "scavenger receptors"? Abrams *et al.* (8) have shown that scavenger receptors can be found all the way back to *Drosophila*, so they probably have some very fundamental biological function. Sambrano *et al.* (7) have proposed that this function is to recognize damaged or apoptotic cells so that they can be taken up rapidly. One possibility is that oxidized lipid–protein complexes in damaged cell membranes may be structurally homologous to oxidized lipid–protein complexes in OxLDL and therefore taken up by the same receptors (30). There is a large body of literature addressing the issue of how macrophages recognize and remove aged or damaged cells (reviewed in ref. 31). Possibilities that have been suggested include a lectin receptor (32), a vitronectin/RGD receptor that may bind to the damaged RBCs via thrombospondin (33), and, finally, a receptor that recognizes clusters of PS residues on the external leaflet of the plasma membrane (22, 33–35). The results of the present studies suggest that a receptor with specificity for OxLDL, but not AcLDL, is involved in recognition of damaged RBCs (and, more broadly, damaged cells in general). The 94- to 97-kDa protein of the mouse macrophage meets this criterion. The accompanying paper (9) provides additional evidence that the 94- to 97-kDa OxLDL-binding protein is involved in recognition of damaged RBCs and that PS clusters play a role in that recognition.

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