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The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosialin, the mouse homologue of human CD68

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ABSTRACT **We have previously reported the partial purification of a 94- to 97-kDa plasma membrane protein from mouse peritoneal macrophages that binds oxidatively modified low density lipoprotein (OxLDL) and phosphatidylserine-rich liposomes. We have now identified that protein as macrosialin, a previously cloned macrophage-restricted membrane protein in the lysosomal-associated membrane protein family (mouse homologue of human CD68). Early in the course of purification of the 94- to 97-kDa protein, a new OxLDL-binding band at 190–200 kDa appeared and copurified with the 94- to 97-kDa protein. The HPLC pattern of tryptic peptides from this higher molecular mass ligand-binding band closely matched that derived from the 94- to 97-kDa band. Specifically, the same three macrosialin-derived tryptic peptides (9, 9, and 15 residues) were present in the purified 94- to 97-kDa band and in the 190- to 200-kDa band and antisera raised against peptide sequences in macrosialin recognized both bands. An antiserum against macrosialin precipitated most of the 94- to 97-kDa OxLDL-binding material. We conclude that the binding of OxLDL to mouse macrophage membranes is in part attributable to macrosialin. Our previous studies show that OxLDL competes with oxidized red blood cells and with apoptotic thymocytes for binding to mouse peritoneal macrophages. Whether macrosialin plays a role in recognition of OxLDL and oxidatively damaged cells by intact macrophages remains uncertain.**

Studies in this laboratory have shown that oxidative modification of low density lipoprotein (LDL) leads to enhanced uptake by mouse peritoneal macrophages (1, 2). Subsequent studies showed that oxidative modification of LDL occurs *in vivo* and that antioxidants significantly slow the progression of atherosclerosis in animals (3, 4). Oxidation of LDL can not only account for foam cell formation but can also confer many new biological properties on the LDL particle that make it more atherogenic. Thus, there is intense interest in the nature of the macrophage receptors that interact with oxidized LDL (OxLDL).

Macrophage uptake of OxLDL is in part attributable to the acetyl LDL receptor (1), later cloned by Kodama *et al.* (5). However, as much as 30–70% of the uptake could not be accounted for by the acetyl LDL receptor alone (6, 7). Several macrophage membrane proteins have been shown to bind OxLDL: (*i*) the acetylated LDL receptor (8); (*ii*) the $F_c\gamma$RII-B2 receptor (9); (*iii*) human monocyte antigen CD36 and its mouse homologue (10) and a closely related scavenger receptor, SR-BI (11); and (*iv*) a partially purified and characterized 94- to 97-kDa protein occurring on mouse peritoneal macrophages (MPMs), on rabbit aortic foam cells and carrageenan granulomas (12), and on rat liver Küpffer cells (13). We have now further purified and identified the 94- to 97-kDa OxLDL-binding protein from MPMs and from the RAW 264.7 mouse macrophage cell line as macrosialin (14), the mouse homologue of human CD68 (15). Macrosialin was first fully characterized by Rabinowitz and Gordon (16, 17) as a heavily glycosylated, macrophage-restricted protein of 87–115 kDa, found predominantly in late endosomes but with a low level of expression in the plasma membrane. In the present paper we show by several criteria that the 94- to 97-kDa protein we have proposed as an additional receptor for OxLDL (12) is identical with macrosialin.

MATERIALS AND METHODS

Lipoproteins. Native human LDL (ρ = 1.03–1.063), Cu^{2+}-OxLDL, and acetyl LDL were prepared as described (2, 18). Protein concentrations were obtained by the Lowry method (19) or by the bicinchoninic acid-Lowry protein assay procedure (Pierce).

Cells and Cell Culture. Resident and elicited (thioglycollate broth; Difco) MPMs were prepared as described (12). RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Oxidatively modified human erythrocytes (OxRBCs) were prepared and their binding to macrophages was studied as reported (20).

Preparation of Membrane and Detergent Extracts. Membranes were prepared from RAW 264.7 cells essentially as described (12). The protocol was slightly modified for MPMs and all steps were carried out at 4°C. Approximately 30×10^8 thioglycollate-elicited MPMs from groups of 100 mice were washed in phosphate-buffered saline and resuspended in 240 ml of 20 mM Tris·HCl (pH 8) containing the protease inhibitor mix: 50 units of aprotinin per ml, 5 mM benzamidine, 0.1 mM phenylmethanesulfonyl fluoride, 14.5 μM pepstatin A, 0.1 mM leupeptin, and 1.9 μM D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (Calbiochem). The suspension was stirred at 4°C for 1 hr after which the cells were broken using a Dounce homogenizer (40 strokes with a B pestle). The homogenate was adjusted to 0.15 M NaCl/0.1 mM EDTA and centrifuged at 800 × *g* for 10 min. The supernate was centrifuged for 1 hr at 100,000 × *g* and the pellet was resuspended in buffer A (50 mM Tris·HCl/0.15 M NaCl/0.1 mM EDTA, pH 8) containing protease inhibitor mix and stored at −70°C. Membrane proteins were extracted with 40 mM octyl glucoside

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Abbreviations: lamp, lysosomal-associated membrane protein; LDL, low density lipoprotein; OxLDL, oxidized LDL; OxRBC, oxidatively damaged red blood cell; PS, phosphatidylserine; IEF, isoelectric focusing; MPM, mouse peritoneal macrophage.

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(Sigma) for 1 hr and the insoluble material was sedimented at 100,000 × *g*.

Ligand Blotting. Fractions at various stages of purification were electrophoresed according to Laemmli (21) under nonreducing conditions on 8% polyacrylamide mini gels (NOVEX, San Diego). Proteins were electrotransferred to nitrocellulose (Schleicher & Schuell) and ligand blotting was performed in buffer B [50 mM Tris·HCl (pH 8) containing 50 mM NaCl] using ^{125}I-labeled OxLDL (^{125}I-OxLDL; specific activity, 300–400 cpm/ng) as described (12). Alternatively, ligand binding was detected (22) using sheep anti-(human) apolipoprotein B antiserum (Boehringer Mannheim) followed by rabbit anti-sheep IgG conjugated to alkaline phosphatase (Fisher) and the phosphatase substrates 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Bio-Rad). For blotting with liposomes, phosphatidylserine (PS) liposomes [brain PS, egg phosphatidylcholine, and cholesterol (1:1:1) containing 1% biotinylated dipalmitoyl-L-α-phosphatidylethanolamine (Molecular Probes)] were prepared by extrusion through 0.1-μM polycarbonate membranes (Lipex Biomembranes, Vancouver). Blotting was performed as described (20) and binding was detected using streptavidin conjugated to alkaline phosphatase.

Purification Scheme. Macrophage membrane octyl glucoside extracts were prepared from either thioglycollate-injected mice (500 mice yielding ≈1.5×10^{10} cells = 190 mg of membrane protein) or RAW cells (7×10^9 cells = 130 mg of membrane protein) and fractionated as follows.

Wheat germ agglutinin chromatography. Detergent extracts were applied to a column of wheat germ agglutinin Sepharose 6B (Pharmacia) equilibrated with buffer A containing 40 mM octyl glucoside and the protease inhibitor mix (buffer C). Bound proteins were eluted with buffer C containing 0.5 M *N*-acetylglucosamine (Sigma).

OxLDL affinity chromatography. OxLDL was delipidated (23) and the modified apolipoprotein B, resolubilized in cetyltrimethyl ammonium bromide, was coupled to AvidGel (UniSyn, Tustin, CA). Lectin column fractions were applied to the OxLDL column and the column was first washed with 100 ml of 0.1 M NaCl in buffer D [50 mM Tris·HCl (pH 8) containing 15 mM NaCl, 40 mM octyl glucoside, and protease inhibitor mix]. The bound proteins were eluted with a linear gradient of NaCl (0.1–1.0 M) in buffer D.

Free solution isoelectric focusing (IEF). Fractions from the OxLDL affinity chromatography step were pooled and dialyzed against 5 mM octyl glucoside in deionized water. Focusing medium containing the sample (2 mg) and a final concentration of 2% pH 3–10 ampholines (Pharmacia LKB), 40 mM octyl glucoside, and the protease inhibitor mix in 45 ml of deionized water was focused at 4°C in a Bio-Rad Rotofor preparative IEF cell.

Protein Sequencing. Fractions from the OxLDL chromatographic step (0.5–0.65 mg) or fractions from the IEF step were pooled (8 ml), dialyzed against 0.4 mM octyl glucoside, concentrated to 200 μl by lyophilization, subjected to preparative SDS/PAGE under nonreducing conditions, and transferred to nitrocellulose. The membranes were stained with ponceau S and the bands corresponding to the 94- to 97-kDa and the 190- to 200-kDa OxLDL-binding activities were excised, reduced by incubation with 10 mM tris(carboxyethyl)phosphine hydrochloride in 50 mM ammonium acetate (pH 4.5) for 30 min at room temperature, and subjected to digestion with trypsin and chemical sequence analysis in an Applied Biosystems model 470 protein sequencer as described (24).

Antipeptide Macrosialin Antiserum. Three synthetic peptides were prepared representing different regions of the macrosialin sequence: DS1 from the middle portion ($_{145}$QIQIRILYPIQGGRK$_{159}$), DS3 from the NH_2-terminal region ($_{22}$CPHKKAVTLLPS$_{33}$), and DS4 from the COOH-terminal region ($_{314}$CITRRRQSTYQPL$_{326}$). Cysteine was added to the COOH terminus of DS1 to facilitate coupling to maleimide-activated keyhole limpet hemocyanin (KLH) and separately to bovine serum albumin (BSA) using reagents from the Imject activated immunogen conjugation kit (Pierce). Male Hartley guinea pigs were immunized with three injections of the KLH–peptide conjugate and antisera were screened with the BSA–peptide conjugate as described (25). Titers of antisera were $>>10^5$.

Immunoprecipitation and Western Blotting. Purified fractions (10 μl) were incubated for 16 hr at 4°C with a 1:50 dilution of either pre- or postimmune DS4 antiserum. The immune complexes were adsorbed with a 50-μl suspension of protein A-Sepharose (Boehringer Mannheim) in buffer C for 2 hr at 4°C. The unbound supernate was removed, and the beads were washed three times with buffer C and boiled in 50 μl of 2× SDS sample buffer under nonreducing conditions. Immunoprecipitation with a 6:1 dilution of the ABL93 rat monoclonal antibody to mouse lysosomal-associated membrane protein 2 (lamp-2) (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City) was carried out essentially in a similar manner except that the immune complexes were adsorbed with protein G-Sepharose. Western blotting was performed as described (12), using a $1:10^3$ dilution of the DS4 antiserum containing 0.05% Tween 20.

RESULTS

We set out to further purify the OxLDL-binding membrane protein described by Ottnad *et al.* (12), starting with MPMs and with RAW 264.7 cells. The ligand blotting activities at various stages of purification from RAW cells are shown in Fig. 1. The starting material contained mostly the 94- to 97-kDa OxLDL-binding band (lane 1). After lectin chromatography a ligand binding band at 190–200 kDa became much more prominent (lane 2). The lectin column fractions containing these two ligand-binding activities were pooled and applied to the OxLDL affinity column in 0.15 M NaCl. Under these conditions, CD36 does not bind (12). Fractions eluted with 0.36–0.45 M NaCl were pooled (lane 3) and subjected to further purification by free solution IEF. Again, both ligand-binding activities copurified in the same fractions (pH 2.54–4.75, represented in lane 4). Further studies, described below, showed that these

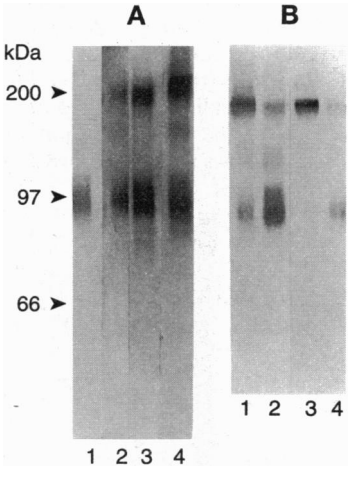

FIG. 1. Ligand blots with OxLDL and PS liposomes of fractions from RAW 264.7 cells. (*A*) Lanes: 1, crude membrane extract; 2, fraction from wheat germ agglutinin column; 3, fraction from delipidated OxLDL column; 4, fraction after IEF. The nitrocellulose strip was incubated with 10 μg of unlabeled OxLDL per ml and bound ligand was detected using anti-apoB antiserum and an alkaline phosphatase-conjugated secondary antibody. (*B*) Aliquots of IEF fractions were blotted with either 10 μg of OxLDL per ml (lanes 1 and 2) or PS liposomes, 0.1 mM total lipid (lanes 3 and 4).

bands represent monomer and dimer, respectively. The results of the purification from elicited MPMs were essentially similar.

PS liposomes, shown earlier to bind to the 94- to 97-kDa OxLDL-binding protein of thioglycollate-elicited mouse macrophages (20), also bound to the dimer in the purified preparations from RAW cells (Fig. 1*B*, lanes 3 and 4).

Fig. 2 shows the SDS/PAGE silver staining profile of fractions during purification. The majority of the proteins in the starting membrane extracts were not retained on the lectin column (lane 1). The eluate from the wheat germ agglutinin column contained a faintly staining protein band at ≈97 kDa (lane 2). After the OxLDL affinity chromatographic step this band was considerably enriched along with the appearance of another prominent band at ≈190–200 kDa (lane 3). Several contaminating proteins were removed after IEF (lane 4).

Purified preparations from elicited MPMs and the RAW cells were fractionated by preparative SDS/PAGE and the electroblotted proteins corresponding to the 94- to 97-kDa and the 190- to 200-kDa OxLDL-binding activities were subjected to internal protein sequence analyses. A tryptic fragment of the MPM 94- to 97-kDa protein eluting at 42.1 min yielded the sequence ILYPIQGGR. A computer homology search using the BLAST program (26) showed that this sequence uniquely matched that of macrosialin residues 150–158 (14). The calculated average mass (MH^+) for this peptide (containing no glycosylation sites) was 1017.12 Da; the observed mass was m/z 1017.1. Fragments with an identical HPLC elution time and mass were also found in the digests of the peritoneal macrophage 190- to 200-kDa band as well as in the 94- to 97-kDa and 190- to 200-kDa bands from RAW cells.

A second tryptic fragment eluting at 63.5 min on the HPLC chromatograms was found to be present in all four of the purified preparations. Because the amount of material in individual fractions was marginal, the fractions were pooled and the following sequence was observed: (E)LQAPLGQ(S)-FX(C)GX(A), where residues in parentheses were determined with <80% confidence and X denotes that no residue could be unambiguously identified in that position. This sequence also matched that of macrosialin residues 238–252 (ELQAPLGQS-FCCGNA).

A third fraction eluting at 36.4 min, again present in all four purified preparations, was found to contain two peptides in an approximately equimolar ratio. One of these (LQAAQLPDK) exactly matched residues 269–277 in macrosialin, whereas the other (EQVLSVSR) exactly corresponded to a fragment of the mouse lamp-2 protein (residues 314–321) (27).

A critical test of whether the OxLDL-binding activity is in fact attributable to macrosialin was carried out using the DS4 antiserum, raised against the COOH-terminal tridecapeptide of macrosialin. Fig. 3*A* shows a representative Western blot of

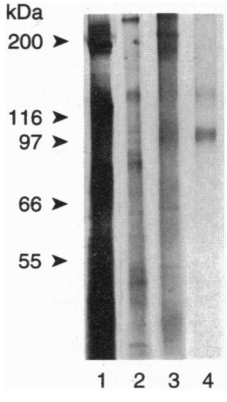

FIG. 2. SDS/PAGE silver staining profile at successive stages of purification from RAW cells. Lanes: 1, unbound fraction from wheat germ agglutinin column; 2, fraction pool from lectin column; 3, fraction from delipidated OxLDL column; 4, fraction after IEF.

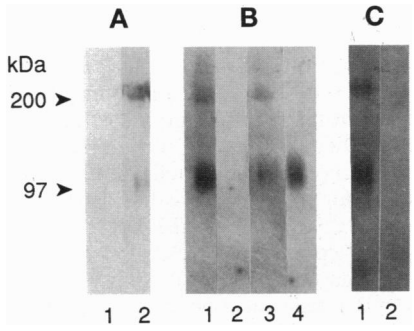

FIG. 3. Immunoadsorption analysis of OxLDL-binding proteins with antibodies to macrosialin and lamp-2. (*A*) Western blotting of wheat germ agglutinin column fraction from MPMs with a 1:1000 dilution of either preimmune serum (lane 1) or DS4 antiserum against macrosialin (lane 2). (*B*) Ligand binding of ^{125}I-OxLDL to supernates (lanes 1 and 3) and to resolubilized immunoprecipitates (lanes 2 and 4) after incubation of OxLDL column fractions from RAW cells with a 1:50 dilution of either preimmune serum (lanes 1 and 2) or DS4 antiserum (lanes 3 and 4). The samples were electrophoresed and the proteins, after transfer to nitrocellulose, were probed with ^{125}I-OxLDL (10 μg/ml) and visualized by autoradiography (−70°C for 2 days). (*C*) Ligand binding of ^{125}I-OxLDL (10 μg/ml) after immunoadsorption of the OxLDL column fraction from RAW cells using a hybridoma supernatant antibody to mouse lamp-2. Lane 1, supernate; lane 2, precipitate. Western blotting confirmed complete removal of the lamp-2 protein from the supernate and its recovery in the precipitate (data not shown).

the purified OxLDL-binding proteins from MPM cells at the lectin column step. Fig. 3*B* shows that immunoprecipitation with this antiserum efficiently removed the bulk of the OxLDL-binding activities at 94–97 kDa and 190–200 kDa (lane 1 vs. lane 3). When the same experiment was done with the lectin fraction from MPMs, all of the OxLDL-binding activity was precipitated (data not shown). The precipitate from the preimmune antiserum incubations did not show any detectable OxLDL-binding activity (lane 2), while the DS4 antiserum precipitate, resolubilized in 4% SDS, showed a strong OxLDL-binding band at 94–97 kDa (the higher SDS concentration dissociates the dimer). In marked contrast, as shown in Fig. 3*C*, a monoclonal antibody against mouse lamp-2 did not remove any of the ligand-binding activity of either the monomer or the dimer (lane 1) relative to a control isotype-matched monoclonal antibody (data not shown). The lamp-2 antibody precipitate did not contain any detectable Ox-LDL ligand-binding activity (Fig. 3*C*, lane 2). Under these conditions the lamp-2 antibody completely precipitated all of the lamp-2 protein (as detected by Western blotting with the same antibody) (data not shown).

Macrosialin is a heavily glycosylated protein (17). To further test the identification of macrosialin as the OxLDL-binding component, detergent extracts of elicited MPMs were treated with *N*-glycanase. This resulted in a significant increase in mobility of the 94- to 97-kDa and the 190- to 200-kDa bands with little change in total ligand-binding activity, and the change in mobilities coincided with that of the Western blotting activities of the two proteins with DS4 antiserum. Antisera DS1 and DS3 (pooled) did not yield positive Western blots with untreated samples but did so after partial deglycosylation (data not shown).

Macrosialin expression is increased in thioglycollate-elicited MPMs. The magnitude of the reported increase was marginal in one study (28) but an order of magnitude in the other (17). Whether the partitioning between surface and internally located macrosialin shifts is not clear. All of our studies to this point had been done with elicited MPMs. To test if the changes on elicitation were accompanied by changes in OxLDL binding, ligand blotting analyses were performed on membrane extracts of resident and elicited MPMs. Fig. 4*A* shows that

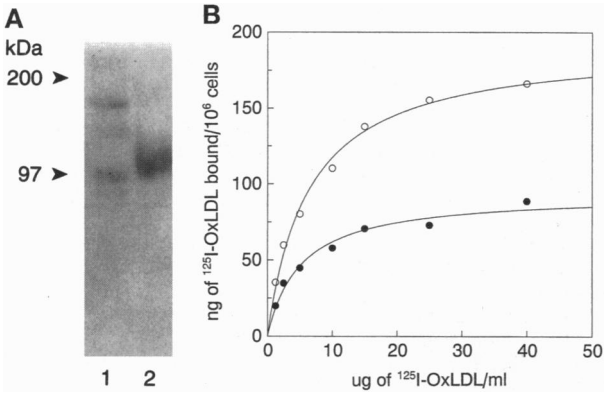

FIG. 4. Increase in OxLDL ligand-binding activity in thioglycollate-elicited peritoneal macrophages. (*A*) Membranes were prepared from resident and thioglycollate-elicited mouse macrophages and subjected to ligand blotting analysis using ^{125}I-OxLDL (10 μg/ml) and autoradiography (−70°C for 2 days). Lane 1, resident macrophages (40 μg); lane 2, elicited macrophages (50 μg). (*B*) Specific binding curves of ^{125}I-OxLDL at 4°C to resident (●) and thioglycollate-elicited (○) macrophages.

elicited MPMs (lane 2) express an OxLDL-binding band that is more diffuse and has a slightly higher apparent kDa than that in resident MPMs (lane 1), compatible with a greater degree of glycosylation, as previously shown by Rabinowitz and Gordon (17). The blot shows that the resident macrophages also contain an intense OxLDL-binding component at ≈180 kDa, presumably representing the dimer, and a fainter ligand-binding band at ≈150 kDa, which is seen only in the final purified preparation of elicited MPM and RAW cells (Fig. 1*A*, lane 4). Western blotting with DS4 antiserum showed a pattern identical to that in the ligand blots. We then tested whether OxLDL and OxRBC binding to elicited MPMs would show a comparable increase. Binding of OxLDL at 4°C increased by 2- to 3-fold (Fig. 4*B*) in contrast to the 17-fold increase in expression of total macrosialin protein reported by Rabinowitz and Gordon (17). To our surprise, binding of OxRBC failed to increase but actually decreased markedly (Table 1).

DISCUSSION

The evidence that the 94- to 97-kDa OxLDL-binding protein of MPMs is identical with macrosialin can be summarized as follows. (*i*) Ligand binding, either with OxLDL or with PS liposomes, detected almost exclusively a single broad band with the appropriate molecular size for macrosialin; this band was identified as macrosialin by Western blotting. (*ii*) Treatment with *N*-glycanase reduced the apparent molecular size of macrosialin, as expected, and the ligand blotting of OxLDL shifted correspondingly. (*iii*) Tryptic digestion of the 94- to 97-kDa band from membranes of both elicited MPMs and the RAW macrophage cell line yielded a set of three tryptic peptides uniquely present in macrosialin. (*iv*) During purification of the 94- to 97-kDa OxLDL-binding protein, a new OxLDL-binding band of 190–200 kDa appeared, suggesting dimerization; tryptic digestion yielded a tryptic peptide pattern apparently identical to that obtained from the 94- to 97-kDa band. (*v*) Antisera raised against synthetic peptides representing macrosialin sequences recognized the 94- to 97-kDa and the 190- to 200-kDa bands. (*vi*) Immunoprecipitation using an antiserum raised against macrosialin removed most or all of the OxLDL-binding proteins from purified preparations and the 94- to 97-kDa OxLDL-binding material was found in the precipitate. (*vii*) An octapeptide sequence unique to mouse lamp-2 (27) was also found in the final preparation. However, immunoprecipitation using an antiserum against lamp-2 removed none of the OxLDL-binding material. (*viii*) Thioglycollate-elicited MPMs, which show a marked increase in expression of macrosialin (ref. 17; Fig. 4*A*), showed a 2-fold increase in specific binding of OxLDL at 4°C. (*ix*) PS liposomes, which compete with OxLDL and OxRBCs for binding to MPMs, bound exclusively to the 94- to 97-kDa and 190- to 200-kDa bands in crude and purified membrane protein preparations.

Table 1. Binding of native RBCs and OxRBCs to resident and thioglycollate-elicited MPMs

	Macrophages binding RBCs, %		RBCs bound per macrophage, no.	
	Resident	Elicited	Resident	Elicited
OxRBCs	91.3	10.5	3.49	0.21
Native RBCs	24.0	33.3	0.41	0.95

RBCs were added to macrophages at a final concentration of 0.1% hematocrit. Resident and elicited macrophages were tested for binding 4 hr after plating. Values represent the percentage of positive macrophages—i.e., a macrophage binding to one or more RBC—and the number of RBCs per macrophage.

Although these data support a role for macrosialin as an OxLDL-binding protein, they do not establish its quantitative contribution to the binding and uptake of OxLDL in intact cells, relative to that of the acetyl LDL receptor (8), CD36 (10), and other candidate OxLDL receptors (9, 11, 12). A recent report (29) shows that binding and uptake of OxLDL by monocyte/macrophages from patients totally lacking CD36 is reduced by about 40%. These studies were carried out with circulating monocytes cultured *in vitro* but not exposed to stimuli that may significantly alter the relative expression of the several OxLDL-binding proteins. Ultimate assessment of the relative importance of the several candidate "OxLDL receptors" may depend upon the use of "knockout" experiments.

The significance of the copurification of lamp-2 along with macrosialin remains to be determined. The two glycoproteins have approximately the same apparent molecular mass and very similar structures so that their copurification during the early steps is not surprising. The binding of OxLDL, however, appears to reflect binding exclusively to macrosialin, not to lamp-2, as shown by immunoprecipitations. Still, we do not rule out the possibility that the macrosialin–lamp-2 association found may reflect a functionally significant association in the intact macrophage.

Macrosialin was originally identified as a macrophage-associated protein with plasma membrane and intracellular location (28). Rabinowitz and Gordon (16, 17) established that the protein was heavily glycosylated, containing N- and O-linked sugars and that thioglycollate elicitation of MPMs increased the amount of core protein and, to an even greater extent, the extent of glycosylation. The cDNA for macrosialin was cloned by Holness *et al.* in 1993 (14) and its structure showed clearly that it belonged to the lamp family. Macrosialin, and its human homologue CD68, cloned by Holness and Simmons (15), have so far been found to be strongly expressed predominantly in macrophages, dendritic cells, and Langerhans cells. The structures of macrosialin and of CD68 place them in the lamp/lgp family of lysosomal glycoproteins but the latter show a much broader tissue distribution than does macrosialin. Moreover, the NH_2-terminal domain of macrosialin and CD68 is an unusual mucin-like domain not found in the lamp family. Holness *et al.* (14) and Fukuda (30) point out that while macrosialin and lamp proteins are found predominantly associated with lysosomes and endosomes, a small percentage is also found on the plasma membrane. Therefore, they considered that this domain might be involved in phagocytosis of foreign organisms and killing of tumor cells.

Does macrosialin play a role in the binding and phagocytosis of OxRBCs? The fact that it binds PS liposomes is suggestive because the binding of OxRBCs and of OxLDL to MPMs is

inhibited by PS liposomes (20, 31), as is the binding of some apoptotic cells (20, 32). However, the preliminary studies reported above do not fit well with that possibility. First, though the level of expression of macrosialin in the RAW 264.7 line of cells is comparable to that in MPMs (as assessed by OxLDL ligand blotting), we could not demonstrate significant binding of OxRBCs to RAW cells (20). Second, elicited MPMs showed no increase in OxRBC binding; in fact, OxRBC binding actually fell significantly (Table 1). Interpretation of these results depends very much on knowing what fraction of the total macrosialin in the elicited MPMs is expressed on the surface of the cell.

Smith and Koch (28) found very little difference in cell surface expression of macrosialin on elicitation. On the other hand, Rabinowitz *et al.* (17, 33) found a huge increase in the total macrosialin of elicited macrophages and found that most of it was present in a late endosomal compartment with only low levels expressed on the plasma membrane (33). The apparent dissociation between binding of OxLDL and binding of OxRBCs could reflect a requirement for participation of additional membrane components for binding of the latter. In any case, these findings cast doubt on a role for macrosialin in the binding of damaged cells but do not completely rule it out. Much will depend on further studies on the distribution and trafficking of macrosialin to and from the plasma membrane.

Note Added in Proof. A recent paper by Rigotti *et al.* (34) demonstrates that SR-BI and CD36, which both bind OxLDL, also binds PS liposomes. Interestingly, however, Ren *et al.* (35) show that cells transfected with the cDNA for CD36 acquire the ability to bind and phagocytose apoptotic neutrophils but the binding in this case does not appear to depend on PS on the outer membrane. Instead the binding involves a vitronectin receptor/CD36 complex mediating binding through an RGDS sequence on thrombospondin (36).

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