Molecular Cloning of a Novel Scavenger Receptor for Oxidized Low Density Lipoprotein, SR-PSOX, on Macrophages*

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Receptor-mediated endocytosis of oxidized low density lipoprotein (OxLDL) by macrophages has been implicated in foam cell transformation in the process of atherogenesis. Although several scavenger receptor molecules, including class A scavenger receptors and CD36, have been identified as OxLDL receptors on macrophages, additional molecules on macrophages may also be involved in the recognition of OxLDL. From a cDNA library of phorbol 12-myristate 13-acetate-stimulated THP-1 cells, we isolated a cDNA encoding a novel protein designated SR-PSOX (scavenger receptor that binds phosphatidylserine and oxidized lipoprotein), which acts as a receptor for OxLDL. SR-PSOX was a type I membrane protein consisting of 254 amino acids, expression of which was shown on human and murine macrophages with a molecular mass of 30 kDa. SR-PSOX could specifically bind with high affinity, internalize, and degrade OxLDL. The recognition of OxLDL was blocked by polyinosinic acid and dextran sulfate but not by acetylated low density lipoprotein. Taken together, SR-PSOX is a novel class of molecule belonging to the scavenger receptor family, which may play important roles in pathophysiology including atherogenesis.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF275260, AF277001, and AF277000 for human SR-PSOX, murine SR-PSOX, and porcine SR-PSOX, respectively.

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The abbreviations used are: OxLDL, oxidized low density lipoprotein; SR-A, class A scavenger receptors; PMA, phorbol 12-myristate 13-acetate; HMDM, human monocyte-derived macrophages; PS, phosphatidylserine; AcLDL, acetyl LDL; Dil, 1,1′-dioctadecyl-3,3′,3′′-tetramethylindocarbocyanin perchlorate; poly I, polyinosinic acid; PCR, polymerase chain reaction; LDL, low density lipoprotein; SR, scavenger receptor; CHO, Chinese hamster ovary; kb, kilobase; EST, expressed sequence tag.
The amino acid sequences of human, murine, and porcine SR-PSOX were shown to proteolytically degrade 125I-OxLDL, which was completely inhibited by OxLDL but not significantly by AcLDL or native LDL (Fig. 3A). Thus, SR-PSOX was shown to be an OxLDL-specific receptor.

Scatchard analysis indicated that COS-hSR-PSOX cells specifically bound 125I-OxLDL with an approximate dissociation constant (Kd) of 5.3 µg/ml (Fig. 3B), which is comparable with that of other scavenger receptors (3, 4, 9). COS-hSR-PSOX cells were shown to proteolytically degrade 125I-OxLDL, which was blocked by excess amounts of unlabeled OxLDL but not by unlabeled AcLDL or native LDL (Fig. 3C). The amounts of bound and degraded 125I-OxLDL in COS-hSR-PSOX cells appeared to be comparable with those in CHO cells stably transfected with SR-A (CHO-SR-A cells; data not shown). In addition, the AcLDL used in the present study was shown to similarly inhibit uptake of DiI-OxLDL in CHO-SR-A (data not shown).
shown) as described previously (17). These findings indicate that SR-PSOX is a specific receptor for OxLDL, whereas neither AcLDL nor native LDL is a ligand for SR-PSOX with high affinity.

SR-PSOX Is a 30-kDa Glycoprotein Expressed on Macrophages—To analyze SR-PSOX at the protein level, we developed polyclonal antibodies directed against human and murine SR-PSOX. Immunoblotting with these anti-human and anti-murine SR-PSOX antibodies revealed that SR-PSOX expressed in COS-hSR-PSOX cells, PMA-stimulated THP-1 cells, HMDM, COS-mSR-PSOX cells, and murine thioglycollate-elicited peritoneal macrophages. The molecular masses of both human and murine SR-PSOX expressed in the cDNA-transfected COS-7 cells were 27 and 30 kDa, whereas we observed only 30-kDa forms in human and mouse macrophages (Fig. 4). The expression level of human SR-PSOX on HMDM pre-cultured for 10 days, which are differentiated macrophages, is higher than that on HMDM pre-cultured for only 2 h, which can be considered to be premature macrophages. Immunoblotting with antibodies against other amino acid residues of human and murine SR-PSOX showed similar findings (data not shown).

Northern blot analysis also showed that PMA-stimulated THP-1 cells and the 2 h-cultured HMDM produced only 2.5-kb mRNA for SR-PSOX, and the 10 day-cultured HMDM produced higher amounts of mRNA for SR-PSOX with both 1.8 and 2.5 kb (Fig. 5, A and B). Thus, Western blot analysis and Northern blot analysis suggest that expression of SR-PSOX was induced in monocytes during differentiation into macrophages. To explore SR-PSOX expression in vivo, we carried out Northern blot analysis with mRNA isolated from various human organs. As shown in Fig. 5C, SR-PSOX was expressed mainly as 2.5-kb mRNA in liver, lung, peripheral blood leukocytes, prostate, heart, kidney, and pancreas and mainly as 1.8-kb mRNA in spleen, thymus, and testis. SR-PSOX was shown to be expressed in various organs except for brain, skeletal muscle, or colon.

**DISCUSSION**

We have successfully isolated a cDNA clone encoding a novel scavenger receptor on macrophages designated SR-PSOX, which specifically bind to OxLDL. We also identified murine and porcine homologues of SR-PSOX, which specifically bind and internalize OxLDL, suggesting SR-PSOX plays a significant role in not only humans but also other mammals. Uptake of OxLDL by macrophages accumulated in the arterial intima...
HMDM cultured for 2–10 days in tissue culture plates (A). RNA isolated from THP-1 cells with or without PMA stimulation (B), human multiple organs (C) were ascertained by hybridization with human elongation factor 1α (EF1α) (A) and with β-actin (B).

Fig. 5. Northern blot analysis of human SR-PSOX. Total cellular RNA isolated from THP-1 cells with or without PMA stimulation, THP-1 cells, peripheral blood leukocytes, spleen, thymus, prostate, testis, heart, lung, liver, skeletal muscle, kidney, pancreas, porcine fetal liver (C), and mouse skeletal muscle RNA were used for Northern blot analysis in Fig. 5. 32P-labeled human SR-PSOX cDNA as a probe. The quantities of analyzed RNA were ascertained by hybridization with human elongation factor 1α (EF1α) and with β-actin. The Northern blot analysis in Fig. 5 showed that SR-PSOX was expressed as 1.8- and 2.5-kb mRNA with different proportions among various organs. Almost 100 EST clones encoding human SR-PSOX showed essentially the same nucleotide sequence in the coding region, suggesting that SR-PSOX could recognize OxLDL but not AcLDL effectively (Figs. 1–3). Such ligand specificity of SR-PSOX is similar to that of LOX-1 (17), although it is different from that of SR-A, which binds AcLDL, as well as OxLDL. Scavenger receptor family molecules have been shown to have a variety of ligands (5), and it also remains to be determined whether SR-PSOX can act as a receptor for ligands other than OxLDL of scavenger receptor family molecules including PS on aged and apoptotic cells. Further studies will elucidate the roles of SR-PSOX in various physiological settings including atherogenesis.

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