Expression of SR-PSOX, a Novel Cell-Surface Scavenger Receptor for Phosphatidylserine and Oxidized LDL in Human Atherosclerotic Lesions

Manabu Minami, Noriaki Kume, Takeshi Shimaoka, Hiroharu Kataoka, Kazutaka Hayashida, Yoshinori Akiyama, Izumi Nagata, Kenji Ando, Masakiyo Nobuyoshi, Michiya Hanyuu, Masashi Komeda, Shin Yonehara, Toru Kita

Abstract—Receptor-mediated endocytosis of oxidized low density lipoprotein (Ox-LDL) by macrophages and the subsequent foam cell transformation in the arterial intima are key events in early atherogenesis. Recently, we have identified a novel macrophage cell-surface receptor for Ox-LDL by expression cloning from a cDNA library of phorbol 12-myristate 13-acetate–stimulated THP-1 cells, designated as the scavenger receptor for phosphatidylserine and oxidized lipoprotein (SR-PSOX). Here, we examined SR-PSOX expression in human atherosclerotic lesions. Total cellular RNA and fresh frozen sections were prepared from human carotid endarterectomy specimens (from 21 patients) and directional coronary atherectomy specimens (from 11 patients). Fragments of human aortas of 2 patients without visible atherosclerotic lesions served as negative controls. Quantitative reverse transcription–polymerase chain reaction demonstrated that SR-PSOX mRNA expression was prominent in atherosclerotic lesions but undetectable in normal aortas. Immunohistochemistry showed that SR-PSOX was predominantly expressed by lipid-laden macrophages in the intima of atherosclerotic plaques in carotid endarterectomy and directional coronary atherectomy specimens, although its expression was not detectable in normal arterial wall. Double-labeled immunohistochemistry confirmed that SR-PSOX is expressed by intimal macrophages. Taken together, SR-PSOX may be involved in Ox-LDL uptake and subsequent foam cell transformation in macrophages in vivo and thus may play important roles in human atherosclerotic lesion formation. (Arterioscler Thromb Vasc Biol. 2001;21:1796-1800.)

Key Words: atherosclerosis ■ immunohistochemistry ■ lipoproteins ■ macrophages ■ receptors

Accumulation of lipid-laden foam cells derived from a monocyte-macrophage lineage in the arterial intima appears to be a key event in early atherogenesis. Several lines of evidence suggest that oxidized LDLs (Ox-LDLs) may play crucial roles in the pathogenesis of atherosclerosis.1,2 Uptake of Ox-LDL in macrophages by receptor-mediated endocytosis appears to be involved in cellular accumulation of cholesterol ester and subsequent foam cell transformation. So far, several different cell-surface receptors, such as scavenger receptors class A (SR-A),3-5 CD36,6 SR-BI,7 CD68,8 and lectin-like receptor for Ox-LDL (LOX-1),9 have been identified to support cellular uptake of Ox-LDL. Recently, by expression cloning from a cDNA library of phorbol 12-myristate 13 acetate (PMA)-stimulated THP-1 cells, we have identified a novel cell-surface receptor for Ox-LDL, which has been designated the scavenger receptor for phosphatidylserine and oxidized lipoprotein (SR-PSOX).10

Human SR-PSOX is a 30-kDa type I membrane protein consisting of 254 amino acids, which does not share any structural homology with other Ox-LDL receptors. SR-PSOX can bind and internalize Ox-LDL but not a significant amount of acetylated or native LDL. Internalized Ox-LDL, in cells expressing SR-PSOX, was subjected to lysosomal degradation. SR-PSOX also recognizes phosphatidylserine, polyninosinic acid, and dextran sulfate but not polycytidylic acid or chondroitin sulfate. In addition to PMA-stimulated THP-1 cells, expression of SR-PSOX has also been shown on human monocyte-derived macrophages and murine thioglycollate-elicited peritoneal macrophages.10 These data demonstrate that SR-PSOX is a novel class of molecule that belongs to the scavenger receptor family; however, the relation of this novel receptor to atherogenesis has not yet been clarified.

In the present study, therefore, we have explored the expression of SR-PSOX in atherosclerotic lesions of human aortas.
carotid and coronary arteries. We provide evidence that SR-PSOX is abundantly expressed by lipid-laden macrophages in the intima of atherosclerotic plaques, although its expression was not detectable in normal arterial walls.

**Methods**

**Tissue Samples**
Fresh frozen sections (6 μm) were prepared from human carotid endarterectomy specimens from 21 patients who had transient ischemic attacks or minor completed strokes before their operations, and sections were also prepared from human directional coronary atherectomy specimens from 11 patients who underwent elective percutaneous coronary interventions because of angina pectoris or asymptomatic myocardial ischemia. Fragments of human aortas without visible atherosclerotic lesions were obtained from 2 patients who underwent cardiovascular surgery. These tissue samples were embedded in Tissue-Tek OCT compound (Miles Laboratories Inc), frozen in liquid nitrogen, and stored at −80°C until use. Total cellular RNA was isolated from 8 carotid endarterectomy specimens and 2 aortic tissue samples by Trizol reagent (GIBCO-BRL) after homogenization as previously described.11

**Reverse Transcription–PCR Analysis**
Equal amounts of total cellular RNA (250 ng) was reverse-transcribed with oligo(dT) primer by use of AMV Reverse Transcriptase (Takara). Transcribed cDNAs were used for polymerase chain reaction (PCR) with specific primers for human SR-PSOX (5′-ACTCAGCCAAGCAATGCAAC-3′ and 5′-GTTTTCAGGTCAGGGTCCACC-AGGGTGCTTTT-3′ and 5′-CATAAGGTACCACCACCCAGTGG-3′) with Ex Taq DNA polymerase (Takara). PCR products were then subjected to electrophoresis through 1% agarose gels and ethidium bromide staining.

**Immunocytochemistry of COS-7 Cells Transfected With Human SR-PSOX**
COS-7 cells were cultured onto Laboratory-Tek II chamber slides (Nalge Nunc) and transfected with the mammalian expression vector containing the full length of human SR-PSOX cDNA by use of LipofectAMINE Plus (GIBCO-BRL). At 48 hours after the transfection, cells were fixed for 2 minutes in cold acetone and then stained with 2 different rabbit anti-human SR-PSOX polyclonal antibodies, which we have previously generated,10 by the avidin-biotin complex method. In brief, cells were incubated with these anti-human SR-PSOX polyclonal antibodies, followed by incubation with a biotinylated goat anti-rabbit IgG (DAKO). Endogenous peroxidase activity was blocked with methanol containing 0.3% hydrogen peroxide, after which avidin-biotin peroxidase complexes (ABC Elite Kit, Vector Labs) were added. Staining with the antibodies was visualized with 3,3′-diaminobenzidine tetrahydrochloride (Vector Labs). Sections were subsequently incubated with the cell-specific antibodies; this incubation was followed by incubation with alkaline phosphatase–conjugated anti-mouse IgG (PharMingen) and visualized with fast red alkaline phosphatase substrate solution (Vector Labs).

**Results**

**Upregulated Expression of SR-PSOX mRNA in Human Atherosclerotic Plaques**
To examine the levels of SR-PSOX mRNA expression in human atherosclerotic plaques, total cellular RNA was isolated from carotid endarterectomy specimens and normal aortic tissue samples, and then reverse transcription (RT)-PCR analyses were performed. As shown in Figure 1, SR-PSOX mRNA expression was prominent in human atherosclerotic plaques. In contrast, in the unaffected human aortas, SR-PSOX mRNA was not detectable. Amounts of GAPDH mRNA, which served as an internal control, were not significantly different. Total RNA isolated from human peripheral monocyte-derived macrophages showed RT-PCR products with the same molecular size.

**Immunoreactivity of Anti-Human SR-PSOX Polyclonal Antibodies**
To explore the expression of SR-PSOX in atherosclerotic lesions by immunohistochemistry, we generated 2 different rabbit polyclonal antibodies against human SR-PSOX, which were raised by immunization with conjugates of carrier protein and synthetic peptides corresponding to extracellular (181 to 200) and intracellular (235 to 254) amino acid residues.10 Both of these polyclonal antibodies were equally bound to human SR-PSOX expressed on the cell surface of COS-7 cells, which had been transfected with human SR-PSOX cDNA but did not react with untransfected COS-7 cells. Figure 2 shows the immunocytochemistry of COS-7 cells expressing human SR-PSOX by use of 1 of the 2 polyclonal antibodies.

**Increased Expression of SR-PSOX by Intimal Macrophages in Human Carotid Atherosclerotic Lesions**
Immunohistochemical staining of human carotid endarterectomy specimens with the anti-SR-PSOX antibodies showed that SR-PSOX was abundantly expressed in the intima of
atherosclerotic plaques (Figure 3A). At higher magnification, SR-PSOX was found to be expressed by macrophage-like cells in the intima (Figure 3B). In fact, immunostaining with these 2 different anti-human SR-PSOX antibodies showed the same results. In addition, staining of the serial sections by cell-type-specific antibodies indicated that SR-PSOX-positive cells were mostly CD68-positive macrophages (Figure 3C) but not α-actin-positive smooth muscle cells (Figure 3D). Oil red O staining of the adjacent sections showed lipid deposition in these SR-PSOX-positive macrophages in the intima of atherosclerotic lesions (Figure 3F). Furthermore, double-labeled immunohistochemistry with use of the anti-SR-PSOX and anti-CD68 antibodies confirmed that SR-PSOX was expressed by intimal macrophages (Figure 3G). In contrast, SR-PSOX expression was not detectable in normal arterial wall (data not shown).

SR-PSOX Expression in Human Coronary Atherosclerotic Lesions

In addition to carotid atherosclerotic lesions, we have examined the expression of SR-PSOX in human coronary atherosclerotic lesions obtained by directional coronary atherec- tomy. As shown in Figure 4A, SR-PSOX was focally expressed by cells accumulated in the intima of atherosclerotic plaques. Immunostaining of the adjacent sections with
attachment end product, 26,27 and receptor-mediated endocytosis of Ox-LDLs also significantly induce endothelial genes relevant to atherogenesis, 12,13 and receptor-mediated endocytosis of Ox-LDL and its lipid constituents have been shown to transcriptionally induce other scavenger receptors (SR-A) 3 and CD36 (CD36), 6 SR-BI, 7 CD68, 8 and LOX-1, 9 have been identified as cell-surface receptors for Ox-LDL. Previous studies have shown that macrophages accumulated in the intima of human atherosclerotic plaques can highly express SR-A 14–17 as well as CD36. 18 Expression of LOX-1, which was initially identified as an endothelial scavenger receptor, has also been demonstrated in human atherosclerotic lesions not only on vascular endothelial cells but also on intimal macrophages and smooth muscle cells. 11 Studies with CD36 knockout mice, so far, have shown that these molecules may play significant roles, at least in part, in atherosclerotic lesion formation of hypercholesterolemic mice in vivo. In addition, these scavenger receptor family molecules, in general, have a variety of biological ligands, including apoptotic cells, 21–23 bacteria, 24,25 advanced glycation end product, 26,27 and β-amyloid protein, 28 suggesting that these molecules may also play important roles in the pathogenesis of various diseases.

SR-PSOX is a novel class of cell-surface receptors for Ox-LDL, isolated from a cDNA library of PMA-stimulated THP-1 cells. Although SR-PSOX does not share any structural homology with other scavenger receptor families, it can bind and internalize Ox-LDL with high affinity. In addition to PMA-stimulated THP-1 cells, expression of SR-PSOX has been demonstrated on human monocyte-derived macrophages and murine thioglycollate-elicited peritoneal macrophages in vitro. 10 As shown in the present study, SR-PSOX is abundantly expressed by lipid-laden macrophages accumulated in the intima of human atherosclerotic lesions but not by endothelial cells or smooth muscle cells. Therefore, SR-PSOX may be involved in Ox-LDL uptake and subsequent foam cell transformation in macrophages and thus may play important roles in atherosclerotic lesion formation. In addition, SR-PSOX appears identical to CXCL16, a novel membrane-anchored chemokine directed to activated T lymphocytes, which express its counterreceptor CXCR6/Bonzon. 29,30 Therefore, SR-PSOX might also act as a chemokine for certain subsets of T lymphocytes accumulated with macrophages in atherosclerotic lesions.

Previous studies have indicated that the regulation of scavenger receptor expression varies among different molecules. For example, tumor necrosis factor-α and transforming growth factor-β inhibit the expression of SR-A 31,32 and CD36 33 in macrophages, although these cytokines can induce LOX-1 expression. 34,35 Peroxisome proliferator-activated receptors are transcriptional factors considered as important regulators in lipid and glucose metabolism as well as monocyte-macrophage differentiation. Peroxisome proliferator-activated receptor γ ligands can upregulate CD36 expression 36 but not SR-A. 37 It remains unclear whether SR-PSOX expression can be regulated by these proinflammatory stimuli or nuclear receptors. As shown in other Ox-LDL receptors, SR-PSOX might also be expressed in other cell types, including vascular smooth muscle cells, under certain pathological conditions; however, the present study shows that macrophages are the only cell type that can express SR-PSOX in human atherosclerotic lesions.

In summary, our present study provides the first evidence that SR-PSOX is abundantly expressed in lipid-laden macrophages accumulated in human atherosclerotic lesions. Further studies related to the regulatory mechanisms of SR-PSOX expression in macrophages and the pathophysiological consequences of Ox-LDL uptake through this novel receptor may provide new insights into the pathogenesis of atherosclerosis.
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References


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