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 formation 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. Uptake of Ox-LDL in macrophages by receptor-mediated endocytosis appears to be involved in cellular accumulation of cholesteryl ester and subsequent foam cell transformation. So far, several different cell-surface receptors, such as scavenger receptors class A (SR-A), CD36, SR-BI, CD68, and lectin-like receptor for Ox-LDL (LOX-1), have been identified to support cellular uptake of Ox-LDL; however, additional molecules may also be involved in the endocytosis of Ox-LDL. Recently, by expression cloning from a cDNA library of phorbol 12-myristate 13-acetate–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). 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, polyinosinic 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. 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
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′-ACTCAGCAGCAGGCAATGGCACC-3′ and 5′-GGTATTGGCTCCCAAACG-3′) and GAPDH (5′-CTCTGTCACCAGGGCTGCTTTT-3′ and 5′-CATGAGGTCCACCACCCTGTT-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 peroxidase 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 kit, Vector Labs) were added. Staining with the antibodies was visualized with 3,3′-diaminobenzidine tetrahydrochloride (Vector Labs) and then counterstained with Mayer’s hematoxylin (Wako). Untransfected COS-7 cells were immunostained with the same antibodies to serve as negative controls.

Single-Labeled Immunohistochemistry
Frozen sections were fixed for 2 minutes in cold acetone, and then immunohistochemical staining with the rabbit anti-human SR-PSOX polyclonal antibodies was performed as described above. Immunohistochemical analyses of adjacent sections with cell-type-specific antibodies were also carried out by use of anti-human CD68 monoclonal antibody (DAKO) and anti-human smooth muscle α-actin monoclonal antibody (DAKO). Staining with nonimmune rabbit IgG (Zymed) served as a negative control. For detection of lipids accumulated in foam cells, oil-red O staining was also performed.

Double-Labeled Immunohistochemistry
For double-labeled immunohistochemistry, after sections were fixed with cold acetone, they were first incubated with the anti-human SR-PSOX polyclonal antibody and then incubated with biotinylated goat anti-rabbit IgG (DAKO), which was followed by incubation with an avidin-biotin peroxidase conjugate and then 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

Figure 1. RT-PCR detection of SR-PSOX mRNA in human atherosclerotic plaques. Total cellular RNA (250 ng) isolated from 2 normal aortas, 8 atherosclerotic plaques, and 2 human monocyte-derived macrophages were reverse-transcribed. Transcribed cDNA fragments were used for PCR with specific sets of primers for human SR-PSOX (top) and GAPDH (bottom). PCR products were then subjected to electrophoresis through 1% agarose gels and visualized by ethidium bromide staining. Lanes are as follows: 1 and 2, normal aortas; 3 through 10, atherosclerotic plaques; and 11 and 12, human monocyte-derived macrophages.
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 atherectomy. 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

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

*Figure 2.* Single-labeled immunocytochemistry of human SR-PSOX-transfected COS-7 cells (A) and untransfected COS-7 cells (B) with anti-human SR-PSOX polyclonal antibody. At 48 hours after the transfection, cells were fixed with cold acetone and then immunostained with antibodies by avidin-biotin complex peroxidase method as described in Methods. Original magnification ×200.

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

*Figure 3.* Immunohistochemical staining of human carotid atherosclerotic lesions. A through E, Single-labeled immunohistochemistry of human carotid end-arterectomy specimens. Serial fresh frozen sections were fixed with cold acetone and stained with anti-human SR-PSOX polyclonal antibody (original magnification ×100 [A] and ×1000 [B]), anti-human CD68 monoclonal antibody (original magnification ×100 [C]), anti-human smooth muscle α-actin monoclonal antibody (original magnification ×100 [D]), and nonimmune rabbit IgG (original magnification ×100 [E]) as described in Methods. F, Oil red O staining of the adjacent sections for detection of lipids accumulated in foam cells in the atherosclerotic plaques (original magnification ×100). G, Double-labeled immunohistochemistry of human carotid atherosclerotic lesions with anti-human SR-PSOX and anti-human CD68 antibodies (original magnification ×1000). Anti-SR-PSOX antibody was visualized by avidin-biotin complex peroxidase technique and appeared brown on the slides, whereas the cell-specific antibody was visualized by alkaline phosphatase method and appeared red.
anti-CD68 antibodies showed that these SR-PSOX-positive cells were mostly macrophages (Figure 4B), as is the case with carotid endarterectomy specimens. Oil red O staining also showed that SR-PSOX-positive cells, in fact, accumulate lipids (Figure 4C).

Discussion

Several lines of evidence have shown that Ox-LDLs may play crucial roles in the pathogenesis of atherosclerosis. Ox-LDL and its lipid constituents have been shown to transcriptionally induce endothelial genes relevant to atherogenesis and receptor-mediated endocytosis of Ox-LDLs appears to play key roles in cholesteryl ester accumulation and the subsequent foam cell transformation of macrophages or vascular smooth muscle cells. Multiple different molecules, including SR-A, CD36, SR-BI, CD68, and LOX-1, 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 as well as CD36. 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. Studies with SR-A 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, bacteria, advanced glycation end product, and β-amyloid protein, 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. 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/Bonzo. 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 and CD36 in macrophages, although these cytokines can induce LOX-1 expression. 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 but not SR-A. 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.
Acknowledgments

This work has been supported, in part, by a grant from the Center of Excellence (No. 12CE2006); grants-in-aid (Nos. 11838008, 11694266, 11307018, and 32644) from the Minister of Education, Science, Sports, and Culture of Japan; grant RFTP97L00803 from the Japan Society for the Promotion of Science; and grants from the Takeda Science Foundation and the Novartis Foundation for Gerontological Research.

References

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 and Toru Kita

doi: 10.1161/hq1001.096652
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/21/11/1796

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at: http://atvb.ahajournals.org/subscriptions/