Increased Expression of Lectinlike Oxidized Low Density Lipoprotein Receptor-1 in Initial Atherosclerotic Lesions of Watanabe Heritable Hyperlipidemic Rabbits

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Abstract—A novel lectinlike oxidized low density lipoprotein receptor-1 (LOX-1) was recently identified in bovine aortic endothelial cells. It is strongly suggested to have a potential role in the initiation and development of atherosclerosis. In this study, we have isolated cDNA clones encoding the rabbit homologue of LOX-1 by screening a rabbit placenta cDNA library. In amino acid sequence and domain structure organization, the rabbit LOX-1 is highly conserved with the human counterpart. Transfection of rabbit LOX-1 cDNA to HEK-293 cells confers on them the activity to bind and internalize oxidized low density lipoprotein. Rabbit LOX-1 was identified as a 45-kDa protein by Western blot analysis with a specific monoclonal antibody. Notably, analyses by reverse transcription–polymerase chain reaction and Western blot revealed that LOX-1 was accumulated in 8-week-old Watanabe heritable hyperlipidemic rabbit aortas compared with normal rabbit aortas. Immunostaining confirmed that the augmented expression of LOX-1 was primarily localized within the intima at the earliest stages of atherogenesis. The most prominent staining was in the endothelial cells of lesions. Furthermore, the distinctive staining of LOX-1 was identified in the endothelium of nonlesion areas of Watanabe heritable hyperlipidemic rabbit aortas. Taken together, these findings support the possibility that LOX-1 might be involved in the initiation of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2000;20:1107-1115.)

Key Words: atherosclerosis ■ endothelium ■ oxidized low density lipoproteins ■ lectinlike oxidized low density lipoprotein receptor-1 ■ Watanabe heritable hyperlipidemic rabbits

Endothelial dysfunction is implicated in the pathogenesis of atherosclerosis.1–3 Oxidized LDL (Ox-LDL) is believed to be a key atherogenic component relevant to endothelial injury in vivo.1,4 A number of “scavenger receptors” characterized by binding to Ox-LDL have been identified.5,6 Among them, the lectinlike Ox-LDL receptor-1 (LOX-1) is expressed in the endothelial cells of large arteries. Applying an expression cloning strategy, we originally identified the LOX-1 cDNA from bovine aortic endothelial cells.7 LOX-1 is a type II membrane protein with a C-type lectinlike structure at the C-terminus. In addition to Ox-LDL, LOX-1 binds aged/apoptotic cells, suggesting potential physiological functions.8

LOX-1 gene expression is highly regulated. The inducible expression in cultured endothelial cells by phorbol ester, tumor necrosis factor-α, angiotensin II, and shear stress, as well as the ligand, Ox-LDL, has been reported.7,9–13 Besides these in vitro studies, the expression of LOX-1 was confirmed in normal arteries and atherosclerotic intima in vivo.7,14–16 An enhanced expression in hypertensive rat aorta was also observed.14,15 Integrating inflammatory and fluid mechanical stimuli, the initiation of atherosclerosis develops focally and is accelerated under hypertension. This inducible nature of LOX-1 expression suggests an active role in the complex atherogenic processes.17

The rabbit is an important model in the study of lipoprotein metabolism and atherosclerosis.18 Cloning of rabbit LOX-1 cDNA will facilitate the use of the rabbit for the study of the potential role of LOX-1, in particular, in the pathogenesis of atherosclerosis. Watanabe heritable hyperlipidemic (WHHL) rabbits with defective LDL receptors present spontaneous hyperlipidemia leading to atherosclerosis, and the pathological nature of atherogenesis in WHHL rabbits resembles that in humans.19–21 In this context, we have isolated the cDNA for a rabbit homologue of LOX-1 to study the potential roles of LOX-1 in hyperlipidemia-based atherosclerosis. To investigate the regulation of LOX-1 in vivo during hyperlipidemia and its association with atherogenesis, we characterized the expression of LOX-1 in proatherogenic WHHL rabbit aortas compared with normal Japanese White (JW) rabbit aortas.

In the present study, we used 8-week-old WHHL rabbits to focus on a time frame that possibly precedes the formation of
Transient Expression of Rabbit LOX-1

Rabbit LOX-1 cDNA was subcloned to pME18s expression vector. HEK-293 and Chinese hamster ovary (CHO) cells were transiently transfected with plasmid containing rabbit LOX-1 and empty pME18s vector, respectively, by use of lipofectamine (GIBCO). Then, 48 hours after transfection, the cells were prepared for examination of rabbit LOX-1 expression.

DiI-Ox-LDL Binding and Internalization

The rabbit LOX-1-transfected HEK-293 cells, the vector-transfected HEK-293 cells, and wild-type HEK-293 cells were incubated with DiI-labeled Ox-LDL (3 μg/mL) in DMEM/10% FCS for 3 hours, washed 3 times with culture media, and fixed with 3.7% formaldehyde in PBS. Fluorescence microscopy was performed to detect DiI-Ox-LDL bound and internalized in cells.

Reverse Transcription–Polymerase Chain Reaction

The tissue was homogenized with a Polytron PT1200 homogenizer (Brinkmann) with 1 mL of Trizol solution (GIBCO) per 100 mg of tissue. Total RNA was isolated from rabbit aortic tissues by use of Trizol reagent. The amount of RNA isolated was determined by measuring the absorbance at 260 nm. The integrity of the RNA isolated was confirmed by electrophoresis on a 1.0% agarose gel containing formaldehyde. Then, 1.0 μg of total RNA was reverse-transcribed into cDNA in 50 μL reaction mixtures with the use of 200 U of Superscript II RT (GIBCO) and random hexamer as a primer according to the manufacturer’s protocol. As much as 5% of the reverse-transcribed materials was amplified with LA-Tag DNA-polymerase (Takara) by use of a primer pair specific to rabbit LOX-1 cDNA (sense primer, 5′-caaggctctgaagaatttgg-3′; antisense primer, 5′-tcatcagggctggaagg-3′). The PCR profile was set at 94°C for 40 seconds, 58°C for 1 minute, and 68°C for 1 minute for 30 cycles. Also, primers for rabbit GAPDH (sense primer, 5′-ggcgcgtcagacggtacctg-3′; antisense primer, 5′-tgcgaagttggctgctgcc-3′) were applied for 25 cycles as an internal control for the amount of RNA, RT efficiency, and amplification variability. The amplified transcripts were visualized on 1.5% agarose gels with the use of ethidium bromide. Specific amplification products of the expected size (344 bp for LOX-1 and 465 bp for GAPDH) were observed. Relative intensities of the bands of interest were read by the FLA-2000 (Fujifilm) and analyzed with MacBas V2.5 software (Fujifilm). The ratios of LOX-1 mRNA to GAPDH mRNA were quantified.

Immunoblotting

The tissues were homogenized by use of the Polytron PT1200 homogenizer with 1 mL of lysis buffer (50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, and 1% [vol/vol] Tween 20) overnight to block nonspecific binding sites. It was probed with a monoclonal antibody against LOX-1 (No. 5-2). The membrane was incubated with biotinylated anti-mouse IgG (Vector Laboratories) for 1 hour and washed with PBS containing 0.05% (vol/vol) Tween 20. Visualization of the antigen was performed with a Vectastain Elite ABC Kit (Vector Laboratories) and an Immunostain Kit (Konica).

Generation of Rabbit–LOX-1 Antiserum

An antibody against rabbit LOX-1 (MS-069) was generated. The cDNA fragment corresponding to the extracellular lectinlike domain

Methods

Animals

Twelve 8-week-old male JW rabbits weighing ~1.8 kg were purchased from Japan SLC (Hamamatsu, Japan). Twelve 8-week-old male homozygous WHHL rabbits weighing ~1.6 kg were purchased from Kitayama Labs Ltd (Nagano, Japan). The rabbits had been fed a standard chow diet ad libitum and were killed at the age of 8 weeks. They were euthanized under intravenous pentobarbital (25 mg/kg) injection, and their descending thoracic aortas were immediately collected. Two adjacent intimomedial segments of the thoracic aorta were used for reverse transcription (RT)-polymerase chain reaction (PCR) and Western blotting. A third adjacent segment with ostia at the second intercostal artery level was studied by immunohistochemistry. Meanwhile, preparations of WHHL rabbit aortic tissues by endothelial cell denudation were also made. In brief, the WHHL rabbit aortic segments were divided into 2 pieces by longitudinal section. One piece was scraped 3 times on the luminal surface with a cover slice and washed thoroughly with PBS solution to remove the endothelium. In parallel, the alternative half was set as the control. All animal experiments were conducted in accordance with the Guidelines for Animal Experiments of the National Cardiovascular Center, Japan.

Serum Sampling and Analysis

Blood samples were drawn from rabbits before euthanasia, and serum total cholesterol levels were measured by using enzymatic methods (Cholesterol E-Test, Wako Pure Chemicals).

cDNA Cloning and Sequence Analysis

Rabbit placenta was collected from a preterm (24-day gestation) JW rabbit that was anesthetized with pentobarbital. A rabbit placenta cDNA library was constructed with size-fractionated cDNA (>500 bp) in Agt10. Approximately 5 × 10^6 clones were screened at high-stringency conditions by using the coding region of human LOX-1 cDNA as a probe. The probe was radiolabeled by a random primer DNA labeling kit (Takara) to a specific activity of 10^9 cpm/μg DNA. Southern blotting was performed in 50 mmol/L Tris-HCl (pH 7.5), 1% SDS, 1 mol/L NaCl, and 200 μg/mL yeast tRNA for 12 hours at 65°C. After washes with 2× SSC, with 0.1% SDS and 0.1× SSC, and with 0.1% SDS for 20 minutes each, the filters were exposed to x-ray film (Kodak) at −80°C overnight. Positive clones containing a >1.5-kb insert with the full open reading frame were identified. The inserts were subcloned to pUC18 vector for sequencing and to pME18s vector for gene expression experiments. The nucleotide sequences were determined on both strands by the dideoxyxynucleotide chain–termination method with a DNA sequencer (model 4000L, LI-COR). Nucleotide and amino acid sequences were analyzed and compared with other species sequences by use of Gene Works software (Intelegenetics) on a Macintosh computer.

Preparation Ox-LDL

LDL (relative density 1.019 to 1.063) from fresh human plasma was isolated by sequential ultracentrifugation, as described previously. The oxidative modification of LDL was carried out with 7.5 μmol/L CuSO4 in EDTA-free PBS for 20 hours at 37°C. Oxidation was monitored by measuring the amount of thiobarbituric acid–reactive substances, ~10 nmol malondialdehyde equivalent per milligram protein in Ox-LDL. Agarose-gel electrophoresis showed increased electrophoretic mobility and minimal aggregation of Ox-LDL particles. Labeling of Ox-LDL with 1,1'-dioctadecyl-3,3,3'3'-tetrameth-ylindocarbocyanine perchlorate (DiI, Molecular Probes) was performed as described.

Fatty lesions in large arteries. Hence, this hypercholesterolemic model provides a unique opportunity for exploration of the potential role of LOX-1 in the initiation of atherogenic processes.
of rabbit LOX-1 (amino acids 112 to 278) was amplified by PCR and subcloned into pQE31 vector (Qiagen), sequenced, and expressed in Escherichia coli. The 6×His-tagged fusion protein was purified with Ni-NTA resin (Qiagen) and used as an antigen to immunize guinea pigs. The antiserum was collected 10 days after the third booster injections. Immunoreactivity of the serum was monitored by ELISA. The specificity to rabbit LOX-1 was further confirmed by cell-surface immunobinding to rabbit LOX-1–transfected CHO cells.

**Immunocytochemistry**

Rabbit LOX-1 cDNA was transiently transfected into CHO cells by using lipofectamine as described. At 48 hours after transfection, the cells were immunostained with anti-rabbit LOX-1–transfected LOX-1 antisemur (MS-069) or preimmune serum by the same method as the following immunohistochemistry. The pME18s vector–transfected and untransfected CHO cells were immunostained with the same antibody as the control cells.

**Immunohistochemistry**

The dissected thoracic aortic arteries were kept submerged in a bath of ice-cold PBS and cleaned thoroughly of adventitia. The tissues were then embedded in OCT compound (Miles Laboratories) and snap-frozen in isopentane cooled with dry ice. The samples were sectioned serially at 6-μm thickness. Sections taken at the equivalent site of thoracic aortas were used for comparing LOX-1 expression in JW and WHHL rabbit aortas. The first section was stained with oil red O. The other sections were subjected to immunohistochemical staining. Immunoperoxidase staining was performed on the frozen sections placed on glass slides. In brief, after being fixed with cold acetone, the sections were incubated with 0.1% BSA–Tris-buffered saline containing 10% serum of the second antibody–generated species for 30 minutes and then with primary antibodies overnight at 4°C. To determine LOX-1 expression in normal and WHHL rabbit aortas, we used the guinea pig anti-rabbit LOX-1 antiserum (MS-069). After being washed with 0.1% BSA–Tris-buffered saline, they were incubated with biotinylated goat anti-guinea pig IgG (Vector Laboratories) for 30 minutes and then washed again. Endogenous peroxidase activity was blocked by incubation with methanol containing 0.3% hydrogen peroxide, after which avidin–biotin peroxidase complex (Vectastain ABC Elite Kit, Vector) was added. Antibody binding was visualized with 3-amino-9-ethylcarbazole from Dako, and the sections were counterstained with Mayer’s hematoxylin. The localization and integrity of endothelium were confirmed by immunostaining with anti–von Willebrand factor (vWF) polyclonal antibody (The Binding Site). RAM11 (Dako) was applied for the staining of macrophages. The biotinylated secondary antibodies were from Vector Laboratories.
Immunostaining with type- and class-matched nonimmune IgGs (Funacoshi) served as a negative control for each antibody used in the present study. Oil red O applied for lipid staining was purchased from Wako Pure Chemicals. All immunostaining studies were performed simultaneously with LOX-1 in adjacent sections by the same method.

**Results**

**cDNA Cloning of Rabbit LOX-1**

Four positive clones of rabbit LOX-1 cDNA were obtained by screening $5 \times 10^5$ plaques of the rabbit placenta cDNA library in Agt10 with the human LOX-1 cDNA coding region.
as a probe under conditions of high stringency. The sequences exhibited striking homology to the human and bovine LOX-1 cDNAs. The clone, rG, which contained the entire coding region of rabbit LOX-1, is shown in Figure 1. The sequence spanned 1521 nucleotides and is composed of a 5' untranslated region of 36 nucleotides, an open reading frame of 834 nucleotides, and a 3' untranslated region of 651 nucleotides. The open reading frame encoded a protein containing 278 amino acids with a Mr of 31 625.

Sequence Alignment of Rabbit LOX-1 With Its Counterparts

The cDNAs encoding bovine, human, rat, and mouse LOX-1 have been isolated.7,15,25 In nucleotide and amino acid sequences, the rabbit LOX-1 showed the highest degree of homology to human LOX-1 among all the species identified. The sequence spanned 1521 nucleotides and is composed of a 5' untranslated region of 36 nucleotides, an open reading frame of 834 nucleotides, and a 3' untranslated region of 651 nucleotides. The open reading frame encoded a protein containing 278 amino acids with a Mr of 31 625.

Expression of Rabbit LOX-1

An anti–LOX-1 antibody specifically detected a band with a mass of 45 kDa in Western blot analysis of rabbit LOX-1–transfected cells but not native or empty-vector–transfected cells (Figure 2a). The difference from the calculated molecular weight may be due to glycosylation.7 Under fluorescence microscopy, the LOX-1–transfected HEK-293 cells displayed high activity of internalizing Ox-LDL. In contrast, the wild-type and the mock-transfected HEK-293 cells consistently lacked the activity (Figure 2b). Therefore, the ectopic expression of rabbit LOX-1 in HEK-293 cells conferred the ability to bind and take up Ox-LDL. This result confirmed that rabbit LOX-1 functions as a receptor for Ox-LDL.

Upregulated Expression of LOX-1 in Aortas of WHHL Rabbits

We analyzed the in vivo regulation of LOX-1 expression in WHHL rabbits. The serum total cholesterol concentration of 8-week-old WHHL rabbits was 24.2±1.1 mmol/L, which was markedly higher than that in 8-week-old JW rabbits (1.2±0.2 mmol/L). Extracts of 8-week-old WHHL and JW rabbit aortas were analyzed by RT-PCR and Western blotting for LOX-1 expression. RT-PCR showed specific amplification of the fragment of rabbit LOX-1 cDNA (344 bp). Examined by RT-PCR, the basal level of LOX-1 gene expression in normal JW aortas was minimal, whereas it was much more enhanced in the atherogenic WHHL rabbit aortas. The amount of GAPDH, as an internal control, was not changed (Figure 3). Similarly, Western blotting confirmed the augmented expression of LOX-1 in atherogenic WHHL rabbit aortas compared with noninvolved normal JW rabbit aortas (Figure 4). The apparent size of LOX-1 in aortic tissues was ~45 kDa, consistent with the size in transfected cells. Removing endothelial cells from the intima of WHHL rabbit aortas substantially reduced the signals of LOX-1 in RT-PCR and Western blot analyses.

Localization of LOX-1 in Early Lesions of WHHL Rabbits

The precise localization of LOX-1 expression in aortic arteries of JW and WHHL rabbits was determined by the
ligand, Ox-LDL, and consequently initiating the processes of function as a recognition domain binding to the LOX-1 repeats of cysteines. This is consistent with the predicted was highly conserved among different species, especially the and amino acid sequences. Notably, the lectinlike domain cDNA. It shows high similarity to human LOX-1 in cDNA study, we have cloned and characterized rabbit LOX-1 resemble those of human atherosclerosis.20,21,26 In the present of LOX-1 in hyperlipidemic conditions occurred before was accumulated in early atherosclerotic lesions. The upregulation (Figure 7A and 7C). These results demonstrated that LOX-1 and identifying the LOX-1–positive cells as endothelial cells vWF antibody, confirming the integrity of endothelial cells. In the adjacent sections, both WHHL and JW rabbit aortic endothelial cells were similarly stained with vWF antibody, confirming the integrity of endothelial cells and identifying the LOX-1–positive cells as endothelial cells (Figure 7A and 7C). These results demonstrated that LOX-1 was accumulated in early atherosclerotic lesions. The upregulation of LOX-1 in hyperlipidemic conditions occurred before lesion formation; hence, the enhanced expression of LOX-1 was potentially involved in atherogenesis.

Discussion

The leading risk factor for atherosclerosis is hypercholesterolemia.1 The WHHL rabbit has been used as a model for human atherosclerosis.18 The cellular events that occur during the progression of early vascular lesions in rabbits well resemble those of human atherosclerosis.20,21,26 In the present study, we have cloned and characterized rabbit LOX-1 cDNA. It shows high similarity to human LOX-1 in cDNA and amino acid sequences. Notably, the lectinlike domain was highly conserved among different species, especially the 6 repeats of cysteines. This is consistent with the predicted function as a recognition domain binding to the LOX-1 ligand, Ox-LDL, and consequently initiating the processes of internalization and phagocytosis.15,25 These results indicate that rabbit LOX-1 is a close homologue of human LOX-1 and suggest that analysis of its role in the rabbit model of atherosclerosis may provide significant insight into the prevalent human atherosclerotic diseases.27,28 Interestingly, we found that the structure of the neck domain varies among different species. Rabbit LOX-1 contains only 1 repeating unit, as do human LOX-1 and bovine LOX-1, whereas rat and mouse LOX-1 possess triple repeats in the neck domain.15,25 Each repeat unit consists of 46 amino acid residues that are highly conserved. The neck domain separates the transmembrane domain from the functional lectinlike domain. The triple repeats enable rat and mouse LOX-1 to possess a neck domain that is longer than the rabbit, bovine, and human LOX-1. Although the structure-function relation for the length of the neck domain has not been characterized, the relatively short neck domain in atherosclerosis-susceptible species might be of significance in the interaction with Ox-LDL.

To define the potential role of LOX-1 in relation to early atherogenesis, we took advantage of WHHL rabbits aged 8 weeks, when most of the lesions were in the critical early stages.20,21,26 LOX-1 expression in WHHL rabbit aortas was enhanced compared with that in normal rabbit aortas. The expression was primarily within the intima, as displayed by immunostaining. In the initial atherosclerotic lesions, luminal endothelial cells and infiltrated macrophages were positive for LOX-1 expression. The most intense signals were exhibited by endothelial cells, as confirmed by staining with vWF, a specific endothelial cell marker. In addition, endothelial denudation markedly reduced LOX-1 signals, as examined by RT-PCR and Western blotting. Therefore, in the 8-week-old WHHL rabbit aortas, endothelial cells were the major source of LOX-1. The accumulation of LOX-1 in the proatherogenic aortas under hyperlipidemia directly suggests an important function of LOX-1 in early atherogenesis. More important, the upregulation of LOX-1 was not confined to lesions, it was also exhibited by noninvolved WHHL rabbit aortas. Therefore, the onset of this process seems to be an extremely early event in atherogenesis, even preceding lesion formation. These findings might provide the missing piece of the long-standing puzzle behind endothelial activation/dysfunc-
tion mediated by Ox-LDL in the absence of atherosclerotic lesions.\textsuperscript{3,17,29} The augmentation of LOX-1 in WHHL rabbit aortas could be due to a direct upregulation of the LOX-1 gene by severe hyperlipidemia alone or together with other pathological circumstances. Previous studies have demonstrated that LOX-1 expression can be induced by shear stress, tumor necrosis factor-\(\alpha\), and phorbol 12-myristate,13-acetate in vitro.\textsuperscript{9-11} Furthermore, LOX-1 was upregulated by Ox-LDL in cultured vascular endothelial cells.\textsuperscript{12} Relevant to these findings in vitro, a working hypothesis might be suggested that LOX-1 expression in vivo is enhanced under atherosclerosis-related conditions, such as vessel bifurcation, hyperlipidemia, and even atherosclerosis lesion formation, per se, when intrinsic inflammatory cytokines are involved. Hence, LOX-1 would be involved in initiating and promoting the vicious cycles of atherogenic processes.

The expression of LOX-1 in macrophages has been demonstrated previously.\textsuperscript{11,30,31} The expression of LOX-1 in monocytes seems relatively lower than that in differentiated macrophages\textsuperscript{30} and is also induced by tumor necrosis factor-\(\alpha\), as in endothelial cells.\textsuperscript{9,31} More recently, we have reported the expression of LOX-1 in the advanced atherosclerotic lesions of human carotid arteries.\textsuperscript{16} In the human fibrofatty atheromas, significant staining of LOX-1 was colocalized with the accumulated macrophages and smooth muscle cells. Thus, these findings support the potential roles of LOX-1 in macrophages and macrophage-derived foam cells in atherosclerotic lesions.\textsuperscript{16}

**Figure 6.** Immunohistochemical staining of a representative early atherosclerotic lesion in WHHL rabbit aortas. Photomicrographs A through F are frozen serial sections of a WHHL rabbit thoracic aorta with an early atherosclerotic lesion. The sections were stained with oil red O (A, magnification \(\times 100\); E, magnification \(\times 200\)), anti-macrophage antibody RAM11 (B, magnification \(\times 200\)), preimmune serum (C, magnification \(\times 200\)), antiserum against rabbit LOX-1 (D, magnification \(\times 200\)), and anti-vWF antibody (F, magnification \(\times 200\)). Photomicrograph A was taken at a low magnification to show that the lesion was located at the edge of an intercostal artery ostium. Abundant staining of LOX-1 was found within the intima lesion. Staining with vWF indicated an intact endothelial cell layer. RAM11-stained sections revealed infiltrating macrophages in the lesion. LOX-1 expression by endothelium was most intense at the luminal edge of the lesion, and moderate expression in the subendothelial space was colocalized with intimal macrophages.
In conclusion, we have cloned rabbit LOX-1 and demonstrated increased expression in endothelial cells in the initial atherosclerotic lesions. The upregulation of LOX-1 would be one of the early events in the initiation of atherogenesis and other related diseases. Endothelial dysfunction in the early atherosclerotic lesions was reversible by therapeutic intervention. Inhibition of LOX-1 expression or activity would be a target for such maneuvers.

Figure 7. Immunohistochemical staining of atherosclerosis-free regions of WHHL and JW rabbit aortas. Photomicrographs A and B are serial frozen sections of thoracic aorta of 8-week-old JW rabbit. The sections were stained with anti-vWF antibody (A, magnification ×200) or antiserum against rabbit LOX-1 (B, magnification ×200), respectively. LOX-1 expression was not detectable in the endothelial cells stained with anti-vWF antibody. Photomicrographs C through G are serial frozen sections of thoracic aorta of an 8-week-old WHHL rabbit. The sections were stained with anti-vWF antibody (C, magnification ×200), antiserum against rabbit LOX-1 (D, magnification ×200), preimmune serum (E, magnification ×200), anti-macrophage antibody RAM11 (F, magnification ×200), and oil red O for lipid staining (G, magnification ×200). The negative staining with macrophage and lipid confirmed that the region was atherosclerosis free. The distinctive staining of LOX-1 in the endothelium revealed that it was upregulated in the nonlesion areas of WHHL rabbit aorta.
References

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