Original Article

Distribution of Smooth Muscle Cells and Macrophages Expressing Scavenger Receptor BI/II in Atherosclerosis

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Aim: Scavenger receptors type I and II (SRB I/II) have dual roles in both atherogenic and antiatherogenic functions through interactions with lipoproteins and their expression in macrophages; however, the distribution and density of SRB I/II-positive macrophages and smooth muscle cells (SMCs) as well as their association with lipid metabolism-related proteins in atherosclerotic intima of the human aorta remain unclear.

Methods: Autopsied aortic tissues were double-immunostained with SRB I/II and smooth muscle actin or macrophage-specific antibodies. The density of SRB I/II-positive SMCs and macrophages in intimal lesion was measured. They were also immunostained with antibodies against four apolipoproteins, four phospholipase A2s, and CETP.

Results: SRB I/II was expressed in both macrophages and SMCs distributed in various intimal lesions. The density of SRB I/II-positive SMCs in intimal lesions significantly decreased with the advance of atherosclerosis, whereas the density of SRB I/II-positive macrophages significantly increased with atherosclerotic development. In addition, functional proteins, such as apolipoproteins, secretory phospholipase A2s, and CETP, were distributed in the intimal stroma around SRB I/II-positive cells in all lesion types.

Conclusion: The results indicated that SMCs are involved in lipid metabolism via SRB I/II expression mainly in the early stages of atherosclerosis evolution, and that SRB I/II-positive macrophages are mainly involved in advanced stages.


Key words: Scavenger receptor, Secretory Phospholipase A2, Apolipoprotein, Atherosclerosis, Human aorta

Introduction

High-density lipoprotein (HDL) plays a crucial role in reverse cholesterol transport from the peripheral tissues to the liver and is postulated to have antiatherogenic effects via this function¹). In this process, scavenger receptor type B1 (SRB1) functions as a mediator of cholesterol transfer between HDL particles and cells²). SRB1 also has binding capacity to apolipoprotein B (apoB) containing lipoproteins, including low density lipoprotein (LDL) and very low density lipoprotein (VLDL), and is implicated in the removal of such lipoproteins from the blood circulation³). In addition, SR type BII (SRBII), a variant of SRB1, also has the functions of a selective cellular uptake of cholesterol ester from HDL as well as HDL-dependent cholesterol efflux from cells⁴). From these data on SRB I/II roles in lipid metabolism, SRB I/II
seems to be involved in antiatherogenic functions; however, from in vitro studies using human macrophages and in vivo experiments using mouse models, macrophage SRB1 has been shown to play a dual role in atherogenesis; namely, the development of initial fatty streak lesions is induced by facilitation of the uptake of atherogenic lipoproteins, such as LDL and VLDL, thus inducing foam cell formation, through SRB1 function, and conversely the function in cholesterol efflux to HDL via SRB1 expression protects the arterial wall from an extensive lipid accumulation at a later stage of atherosclerotic evolution. Considering these SRB1/II roles in lipid transfer between the cells and lipoproteins, it is considered that SRB1/II may be a modulator of lipid accumulation in the arterial wall in collaboration with lipoproteins.

Atherosclerosis begins with serum lipids permeating into the subendothelial space, followed by the infiltration of monocyte-derived macrophages into the subendothelial space and the migration of smooth muscle cells (SMCs) into the intima from the media. The macrophages and SMCs become foam cells in the arterial intima, and the increase of these cells and extracellular lipid accumulation in the intima leads to the progression of atherosclerosis. In addition, SMCs and monocyte-derived macrophages in the arterial intima are directly involved in lipid metabolism as well as SRB1/II expression through the production of proteins having a role in cholesterol transfer or lipid deposition, such as apoJ, apoE, cholesteryl ester transfer protein (CETP), and secretory phospholipase A2s (sPLA2s). In spite of a close relationship between intimal cells expressing SRB1/II and lipid-relating proteins, such as apolipoproteins, CETP and sPLA2s, the association of SRB1/II expression with the intracellular and extracellular distribution of these proteins in the human atherosclerotic aorta requires further clarification.

SRB1 is expressed in the endothelial cells, monocyte-derived macrophages and SMCs in the arterial wall. SRB1-II expression is similar to SRB1, but exhibits lower levels than SRB1; however, the sequential change of distribution patterns of SMCs expressing SRB1/II in the whole process of atherosclerotic evolution has not yet been described. From this background, we histopathologically examined the relationship between SRB1/II-expressing cells and the distribution of the four types of apolipoproteins, four types of sPLA2s, and CETP in atherosclerotic aortas. In addition, sequential changes in the density of SRB1/II-positive macrophages and SRB1/II-positive SMCs in the whole process of atherosclerosis development in human aortas were investigated.

**Materials and Methods**

**Tissue Preparation**

Eighty human aortic tissues were obtained from 20 autopsy cases aged 37 to 83 years (15 males and 5 females). The tissues were generous gifts from the patients’ relatives who gave written agreement for usage in this study. All cases had died from cancer, pneumonia, or renal failure, without cardiovascular diseases. The specimens were taken from the thoracic and abdominal aortas and included various macro- scopically evident atherosclerotic lesions as well as non-atherosclerotic portions. They were then fixed in 10% neutral-buffered formalin and embedded in paraffin. From each paraffin block, twenty thin sections (4 μm-thick) were obtained, and two sections were stained with hematoxylin-eosin (HE) and elastic van Giesons (EVG) staining.

**Classification of Lesion Types**

In this study, the intimal lesion types were classified into four categories, types I, II, III, and IV lesions according to the classification of the American Heart Association with minor modification. Adaptive intimal thickening is generally composed of mild SMC proliferation, which contains a small number of macrophages, and the initial lesion contains an increased number of foamy macrophages in comparison to adaptive intimal thickening; however, it is difficult to discriminate adaptive intimal thickening from the initial lesion in one aortic tissue, because they are sometimes mingled in one region. For such a reason, aortic tissue exhibiting an adaptive intimal lesion type or initial lesion is defined as type I lesion. Type II lesion contains an aggregation of foamy cells, which corresponds grossly to a fatty streak. Type III lesion (so-called preatheroma) contains extracellular lipid deposition with scattered lipid-laden cells, which is interpreted as an intermediate lesion between a fatty streak and atheroma. Type IV lesion (atheroma) contains a lipid core and a fibrous cap. In this study, ‘normal’ was designated as an intima with no atherosclerotic lesion and no fibrocellular thickening. The above classification was made by three pathologists (YI, MKM, and TI) through observation of sections with HE and EVG staining as well as immunostaining against α-smooth muscle actin (α-SMA) and human macrophages.

**Immunohistochemistry**

Subserial thin sections were immunostained with rabbit polyclonal antibodies against human sPLA2-IIA, -III, -V and -X using the EnVision + kit (DakoCyto- mation, Carpinteria, CA. USA), goat antibody
against apoA-I, apoB and apoJ (Acris Antibodies GmbH, Hiddenhausen, Germany) using the LSAB method (DakoCytomation), rabbit antibody against SRB1/II (Novus Biologicals Inc., Littleton, CO, USA) and mouse antibody against CETP (Chemicon International, Inc., Temecula, CA, USA) using the CSA-II kit (DakoCytomation), and mouse antibody against apoE (Innogenetics NV, Gent, Belgium) using the EnVision + kit (DakoCytomation) according to the manufacturer's instructions. Immunostaining against α-SMA (clone 1A4; DakoCytomation) and human macrophages (clone HAM56; DakoCytomation) was also performed using the EnVision + kit to identify SMCs and monocytes/macrophages in intimal lesions, respectively. For HAM56 immunostaining, deparaffinized sections were pretreated with 0.1% pronase (DakoCytomation) for 5 min.

The deparaffinized sections were incubated with antibodies against sPLA2s overnight at 4°C, α-SMA and HAM56 for 30 min at room temperature, and apoA-I, apoB, apoE, apoJ, CETP, and SRB1/II for 2 hr at room temperature. After washing, they were reacted with the secondary antibody, and the reaction products were visualized by treating the slides with diaminobenzidine tetrahydrochloride (DAB Liquid System; DakoCytomation). To verify specificity, an additional section from each block was used as a negative control by omitting the primary antibody and replacing it with the corresponding normal mouse, goat or rabbit immunoglobulin.

Double immunostaining with antibodies for α-SMA and SRB1/II as well as HAM56 and SRB1/II was performed on sections from the same paraffin block of each lesion to determine the localization of SRB1/II in intimal cells. In brief, the sections were first immunostained with SRB1/II and visualized by DAB treatment. These sections were then reimmunostained with antibody for α-SMA or HAM56 using EnVision System-AP (DakoCytomation) and visualized by treating the slides with a BCIP/NBT substrate system (DakoCytomation).

The extent of immunopositivity against SRB1/II, CETP, each apolipoprotein, and each sPLA2, in the stroma was arbitrarily graded into four categories using the following scale: −, negative; +, mildly positive; ++, moderately positive; ++++, severely positive. The immunopositivity of SRB1/II in α-SMA or HAM56-positive cells was described as − for a negative result and + for a positive result.

The above semi-quantitative analysis was employed by three pathologists (YI, MKM, and TI) in a blinded manner, and the common grade point evaluated by two or three investigators was adopted.

**Statistical Analyses**

For statistical analyses, sections immunostained with three antibodies for SRB1/II, α-SMA, and HAM-56 as well as double-immunostained sections were used. To assess the density of cells positive for each protein in the intimal lesion, the areas of intimal lesions were measured with an automated image analysis system, Visual Measure 32 (Rise System, Sendai, Japan), and the number of cells positive for each protein in the intimal lesion was counted. The average number of positive cells for each protein per 1 mm² in five sections was defined as the density of positive cells in each lesion type. The fluctuation of the density of SRB1/II-positive cells was statistically examined among the four lesion types, type I, II, III and IV, by one-way ANOVA with Scheffe's F-test. In addition, the fluctuations of the density of SRB1/II-positive SMCs and SRB1/II-positive macrophages were examined by the same method as for SRB1/II-positive cells. Because SRB1/II-positive cells were not recognized in the normal aorta, these analyses were performed for type I, II, III, and IV lesions.

All statistical analyses were carried out using the StatView software package (Abacus Concepts, Berkeley, CA, USA). A p-value less than 0.05 was considered significant.

**Results**

The results of immunohistochemistry are summarized in **Table 1** for the cells and **Table 2** for the stroma. There was no difference in all expression patterns by age, but some differences were demonstrated by the lesion type. In addition, the distribution pattern and location of SRB1/II-positive SMCs and macrophages in each intimal lesion are summarized in **Table 3**.

**Normal Aorta**

In the normal intimal stroma, all apolipoproteins and sPLA2s except for sPLA2-V were sparsely and weakly found, but CETP was not detected. SRB1/II-positive cells were not recognized in the normal intima. Most of the medial SMCs were positive for SRB1/II, CETP, and sPLA2-IIA, -III, -V, and -X. ApoA-I, apoB, and apoE were not recognized in the medial SMCs. ApoA-I, apoB, and CETP were not detected in the normal stroma, but apoE, apoJ, sPLA2-IIA, -III, and -V were detected in the lower third of the media, and sPLA2-X was mildly scattered in all layers of the media. In the media, the distribution pattern of SRB1/II-positive SMCs was similar to the extracellular distribution of CETP, sPLA2-IIA, -III,
## Table 1. Results of immunohistochemistry for aortic wall cells

<table>
<thead>
<tr>
<th>Lesion types</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intima</td>
<td>media</td>
<td>intima</td>
<td>media</td>
</tr>
<tr>
<td>SRB I/II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CETP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>apoA-I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>apoB</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>apoE</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>apoJ</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>sPLA2-IIA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>sPLA2-III</td>
<td>+</td>
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<td>+</td>
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<td>sPLA2-V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>sPLA2-X</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

MΦ, macrophage; SMC, smooth muscle cell; SRB I/II, scavenger receptor B I and B II; CETP, cholesteryl ester transfer protein; sPLA2, secretory phospholipase A2

- , negative; +, positive

## Table 2. Results of immunohistochemistry in the aortic stroma

<table>
<thead>
<tr>
<th>Lesion types</th>
<th>Normal</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intima</td>
<td>media</td>
<td>intima</td>
<td>media</td>
<td>intima</td>
</tr>
<tr>
<td>CETP</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>apoA-I</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>apoB</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>apoE</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>apoJ</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>sPLA2-IIA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>sPLA2-III</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>sPLA2-V</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>sPLA2-X</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

CETP, cholesteryl ester transfer protein; sPLA2, secretory phospholipase A2

- , negative; +, mildly positive; ++, moderately positive; +++, severely positive.

These different grades represent the extent of individual proteins in the comparison between lesions, but not the comparison between the extent of proteins.

## Table 3. Main distribution pattern and location of SRB I/II-positive cells in the intima

<table>
<thead>
<tr>
<th>Lesion types</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pattern</td>
<td>Location</td>
<td>Pattern</td>
<td>Location</td>
</tr>
<tr>
<td>SRB I/II-positive SMCs</td>
<td>scattered</td>
<td>whole area</td>
<td>scattered</td>
<td>frame</td>
</tr>
<tr>
<td>SRB I/II-positive macrophages</td>
<td>scattered</td>
<td>whole area</td>
<td>cluster</td>
<td>central</td>
</tr>
</tbody>
</table>

Main locations of SRB I/II-positive cells indicate the portion in the intimal lesion.
and -V in the media.

**Type I lesion (Fig. 1a–d)**

In the intima, a few macrophages were positive for SRB1/II, but SRB1/II was detected in many SMCs by double immunostaining. These SRB1/II-positive cells were scattered in the thickened intima. All proteins, except for apoA-I, examined in this study were recognized in intimal cells. In the intimal stroma around SRB1/II-positive cells, apoA-I, apoB, apoE, and sPLA2 -X were moderately distributed, and CETP, apoJ, sPLA2-IIA, -III, and -V were mildly found. Medial SMCs just under the intimal lesions showed immunoreactivity for SRB1/II, CETP, apoJ, and sPLA2s, but not for apoA-I, apoB, and apoE. In the medial stroma just under the intimal lesion, all apolipoproteins, sPLA2-III, and -X were moderately distributed, and CETP, sPLA2-IIA, and -V were

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**Fig. 1.** Immunohistochemistry of the aortas with type I lesion (a–d) and type II lesion (e–h). Arrows in a–g indicate the portion of the internal elastic lamina. a–d are the same portion of serial sections of the aorta with type I lesion, and e–g are the same portion of serial sections of the aorta with type II lesion.

- **a.** Scavenger receptors B1/II (SRB1/II) are expressed in the intimal cells and medial smooth muscle cells (SMCs). b. Apolipoprotein (apo) A-I is widely distributed in intimal stroma and mildly found in the media. c. Immunoreactivity for apoE is extensively recognized in the stroma of the intima and media, especially around the internal elastic lamina. d. Type III secretory phospholipase A2 (sPLA2-III) is expressed in intimal and medial cells as well as the stroma of the intima and media. e. SRB1/II expression is detected in intimal cells as well as medial cells in the upper layer of the media. f. Cholesteryl ester transfer protein is expressed in intimal and medial cells and also found in the intimal stroma. g. ApoB is distributed mainly in the intimal stroma and scarcely in foam cells in the intima. h. Double immunostaining using SRB1/II (brown) and smooth muscle actin (blue) antibodies indicates SRB1/II expression in SMCs in the intima.
mildly scattered. CETP, apoB, apoE, and sPLA2-IIA were present in the medial cells or stroma of the upper two-third layers, in where SRB1/II-positive SMCs were abundantly distributed. In addition, apoA-I, apoJ, sPLA2-III, -V, and -X were expressed in the cells and stroma of all of the aortic layers.

**Type II lesion (Fig.1e–h)**

Double immunostaining for SRB1/II and α-SMA demonstrated the distribution of SRB1/II-positive SMCs, including foamy SMCs in the intima around a fatty streak, and these SRB1/II-positive SMCs were scattered. The majority of cells immunopositive for both HAM56 and SRB1/II in the intima were foamy, and tended to aggregate in the central portion of a fatty streak. All proteins, except for apoA-I, were expressed in cells constituting the intimal lesion. In the intimal stroma, CETP and sPLA2-IIA were moderately distributed; apoA-I, apoB, apoE, and apoJ were extensively distributed; and sPLA2-III and -V were uniformly expressed in SMCs. ApoJ and sPLA2-X were found in SMCs distributed in the upper third of the media, but apoA-I, apoB and apoE were not detected in SMCs in the media. In the media stroma, CETP, sPLA2-IIA, -III, and -V were uniformly expressed in SMCs. ApoJ and sPLA2-X were found in SMCs distributed in the upper third of the media, but apoA-I, apoB and apoE were not detected in SMCs in the media. In the media, CETP, sPLA2-IIA, -III, and -V were mildly or moderately scattered, but immunopositivity for all apolipoproteins was intense at the boundary between the intima and media. The distribution pattern of sPLA2-X was a mottled in the medial stroma.

**Type III lesion (Fig.2a–d):**

SRB1/II was detected in many intimal cells, including foamy cells. By double immunohistochemistry, SRB1/II was expressed in both α-SMA-positive cells and macrophages. SRB1/II-positive SMCs were mainly distributed in the surface area of raised lesions, but SRB1/II-positive macrophages were mainly observed in the central portion of the intimal lesion. In the intimal stroma, apoA-I, apoB, apoE, apoJ, sPLA2-V, and -X were extensively distributed, and CETP, sPLA2-IIA, and -III were moderately distributed. All proteins except for apoA-I were expressed in the intimal cells to varying extents.

The medial SMCs just under the intimal lesions showed immunoreactivity for SRB1/II, CETP, sPLA2s, and apoJ, but apoA-I, apoB, and apoE were absent in these cells. In the medial stroma, all proteins, except for SRB1/II, were detected in the upper third layer, and sPLA2-X was expressed more extensively than the other proteins. ApoA-I, apoB, and apoE were more extensively distributed in the upper half layer than the lower half layer of the media, and their distribution patterns were similar to that of SRB1/II.

**Type IV lesion (Fig.2e–j)**

All proteins were recognized in many foam cells around a lipid core. SRB1/II, CETP, apoE, apoJ, and sPLA2s were also expressed in intimal cells observed in the lesion. By double immunohistochemistry, SRB1/II-positive SMCs were mainly scattered in the fibrous cap and shoulder portion of a atheroma lesion. In contrast, SRB1/II-positive macrophages were mainly distributed in the area around the atheroma core. ApoA-I, apoB, apoE, apoJ, sPLA2-III, and -V were widely expressed in the stroma of the intimal lesion, but sPLA2-X was relatively restricted in the subendothelial space. All proteins were observed in a lipid core. The expression patterns of CETP, apolipoproteins, sPLA2-IIA, and -V were similar to that of SRB1/II-positive cells in the intimal lesion.

In the media, SRB1/II, CETP, sPLA2-IIA, -III, and -V were uniformly expressed in SMCs. ApoJ and sPLA2-X were found in SMCs distributed in the upper third of the media, but apoA-I, apoB and apoE were not detected in SMCs in the media. In the media stroma, CETP, sPLA2-IIA, -III, and -V were mildly or moderately scattered, but immunopositivity for all apolipoproteins was intense at the boundary between the intima and media. The distribution pattern of sPLA2-X was a mottled in the medial stroma.

**Statistical Analyses**

The fluctuation of the density of SRB1/II-positive cells in the aortic intima with the progression of atherosclerosis is shown in Fig. 3a. The density of SRB1/II-positive cells tended to significantly decrease (one-way ANOVA: p<0.025) from type I to III lesions (type I vs. type II; p=0.0051, type I vs. type III; p=0.0113, type II vs. type III; p=0.0121 by Scheffe’s F-test), but it was significantly greater in type IV than in type III (p<0.0135).

The density of SMCs was significantly greater in type I than in the other types (p<0.05 by Scheffe’s F-test), and decreased with atherosclerotic development from type I to IV (one-way ANOVA; p=0.0182). The density of SRB1/II-positive SMCs also decreased from type I to III, but the difference was not significant between the values of type I and IV. In addition, the fluctuation pattern of the density of SRB1/II-positive SMCs from type I to III lesions was similar to that of SRB1/II-positive cells, as shown in Fig. 3b. The density of SRB1/II-positive macrophages significantly increased from type I lesion to type IV lesion (p=0.0113; one-way ANOVA) and was significantly greater in type IV lesions than in the other
Fig. 2. Immunohistochemistry of aortas with type III lesion (a−d) and type IV lesion (e−j). a−c are the same portion of serial sections of the aorta with type III lesion, and e−h are the same portion of serial sections of the aorta with type IV lesion. Arrows in a−c and e−h indicate the portion of the internal elastic lamina. Red arrows in e−h indicate the portion of the atheroma core.

a. In the aorta with type III lesion, SRB/ is expressed in many intimal cells and is also found in medial cells in the lower portion of the figure. b. ApoJ is widely expressed in the stroma of the intima and intimal cells. c. Immunoreactivity of sPLA2-X is similar to that of apoJ. d. Double immunostaining using SRB/ (brown) and human macrophage (blue) antibodies indicates SRB/ expression in macrophages (arrows). e. In the aorta with type IV lesion, SRB/Positive cells are distributed in the shoulder portion of an atheroma lesion and in the media. f. Immunohistochemistry using human macrophage antibody indicated macrophage distribution in the shoulder portion of an atheroma lesion, similar to the distribution of SRB/II. g. ApoB is recognized mainly in the atheroma core and intimal stroma. h. Immunoreactivity of sPLA2-V is similar to that of apoB. i. Double-immunostaining using SRB/II (brown) and human macrophage (blue) antibodies indicates SRB/II expression in macrophages (arrows) distributed in the shoulder portion adjacent to an atheroma lesion. j. Double immunostaining using SRB/II (brown) and smooth muscle actin (blue) antibodies indicates SRB/II expression in foamy SMCs in the intima.
lesion types (Fig. 3c); however, the density of macrophages was significantly greater in type III lesion than the other lesion types ($p = 0.027$; one-way ANOVA).

**Discussion**

In the present study, SRB1/II-positive cells were
detected in intimal lesions in all stages of atherosclerosis development. In addition, SRB1/II was expressed in both SMCs and monocytes-macrophages in the intima, and CETP, apolipoproteins, and sPLA2s were expressed in the intimal stroma around SRB1/II-positive cells in all lesion types. Although there were few SRB1/II-positive macrophages in the intimal lesions of types I and II, the density of these cells in the intima increased with the advance of atherosclerosis. In contrast, the density of SRB1/II-positive SMCs in the intimal lesions of types I and II was higher than that of SRB1/II-positive macrophages and decreased with atherosclerosis development. Considering previous reports on the function of these receptors, together with the present results, it is suggested that both SMCs and macrophages commonly play both roles for the removal and/or deposition of lipids in the aortic intima through SRB1/II functions, and that macrophages contribute to lipid deposition in the advanced lesions more than SMCs.

SRB1/II, a receptor for HDL and LDL, mediates the selective uptake of cholesteryl ester from these lipoproteins and the transport of cellular unesterified cholesterol to HDL. Recent studies have shown that SRB1 is expressed only in macrophages in atherosclerotic lesions of the human aorta and coronary arteries. On the other hand, the expression of SRB1 in vascular SMCs has been identified in the human cerebral arteries of normal brains and in brains with Alzheimer's disease and in rat aorta, but not in the SMCs of human aorta and coronary arteries. In the present study, the majority of macrophages positive for SRB1/II in the intima exhibited foamy cells, as seen in previous reports. In addition, SRB1/II was expressed in the medial SMCs mainly distributed in the upper third medial layer of the normal and atherosclerotic aortas as well as in the intimal SMCs of the aortas having adaptive intimal thickening and atherosclerotic lesions, and many SRB1/II-positive SMCs in the intima also exhibited the foamy cell type. These observations suggest that SMCs in a normal aorta and an aorta with an atherosclerotic lesion contribute to the selective uptake or efflux of cholesterol via the function of SRB1/II, the same as macrophages that are distributed in atherosclerotic lesions.

In the present study, CETP was widely distributed in the intimal stroma with various lesions as well as apolipoproteins. ApoA-I circulates in the plasma as a constituent of HDL particles, and apoB as a constituent of LDL and VLDL particles. From such characteristics of apoA-I and apoB, the distribution of apoA-I in the aortic wall is almost the same as that of HDL particles, and the distribution of apoB in the aortic wall is almost the same as the localization of LDL and/or VLDL particles. The colocalization of CETP and apoA-I/B in the intimal lesion, as shown in the present results, therefore indicates that CETP colocalizes with HDL and apoB-containing lipoproteins in the aortic intima. On the other hand, CETP has a role in cholesteryl ester efflux from the extracellular compartments, damaged cells and macrophages, and functions to transfer cholesteryl ester from apoA-I-containing HDL to apoB-containing lipoproteins, thus resulting in a redistribution of cholesteryl ester. In addition, apos A-1, E and J function to remove excess cholesterol and phospholipids from the arterial intima, and apo A-1 and E promote the efflux of cholesterol ester from cells through SRB1 function. In the present study, the distributions of apolipoproteins and CETP in the intima were similar to that of SRB1/II-positive cells. Considering the distribution patterns of CETP, apolipoproteins, and SRB1/II-positive cells in all lesions examined, SRB1/II-positive macrophages and SMCs in the intima contribute to the redistribution of excess cholesterol from the aortic wall to other tissues via the expression of SRB1/II.

The present results demonstrated that the distribution patterns of sPLA2s were similar to those of apoA-I and apoB in the aortic wall. This finding indicates the colocalization of sPLA2s and HDL/LDL particles in atherosclerotic lesions; therefore, HDL and LDL found in the intimal lesions are considered to be modified through the hydrolytic function of sPLA2s for phosphatidylcholine on these lipoproteins. sPLA2-modified HDL is a more efficient substrate for SRB1/II-mediated selective uptake of cholesteryl ester than normal HDL. In addition, sPLA2-modified lipoproteins can easily bind to proteoglycans and promote foam cell formation of macrophages. The similar distribution patterns of sPLA2s to those of apoA-I and apoB in our results suggest that sPLA2s may function in lipid deposition in intimal cells through SRB1 expression and stroma in the entire process of atherosclerotic evolution.

In this study, the density of SRB1/II-positive cells in the intima decreased from type I lesion up to type III lesion, but significantly increased in type IV lesions. This decrease of SRB1/II-positive cells in the intima appears to be caused by a decrease of SRB1/II-positive SMCs, because the density of SRB1/II-positive SMCs in the intima decreased from type I to type III lesion. SMC apoptosis in the intima is generally induced by oxidized LDL, and the density of apoptotic SMCs in the aortic intima increases with atherosclerotic development. A decrease in the density of...
SRBI/II-positive SMCs may be due to an increase of apoptosis or necrosis of SMCs in intimal lesions. On the other hand, the present results demonstrated that the density of SRBI/II-positive macrophages was small in types I and II lesions but abruptly increased in types III and IV lesions. In the intima exhibiting types III and IV lesions, extracellular lipid deposition is observed accompanied with abundant oxidized LDL-positive cells, and oxidized LDL induces SRBI expression of monocyte-derived macrophages. From these previous findings, it is considered that an increase of SRBI/II-positive macrophages may be caused by the induction of degenerated LDL, such as oxidized LDL in the intima. In fact, SRBI/II-positive macrophages were mainly distributed in the central area of intimal lesions with type III and type IV, as shown in Table 3.

The present results indicate that SMCs mainly help to remove excess lipid in early atherosclerotic lesions, such as types I and II lesions, through SRBI/II expression as well as the synthesis of apoE and CETP, whereas macrophages act mainly in advanced lesions, such as types III and IV lesions via SRBI/II function and apoE production; however, the foam cell formation of SMCs and monocyte-macrophages by SRBI/II function further leads to extracellular lipid deposition through their necrosis/apoptosis. It is therefore considered that both SMCs and macrophages are involved in both antiatherogenic and atherogenic roles via complicated interactions between SRBI/II and apolipoproteins, CETP, or sPLA2s. In addition, considering vascular wall relaxation induced by the interaction of HDL and SRBI due to nitric oxide production, an increase of SRBI/II-positive cells in the aortic wall with atherosclerosis evolution may also contribute to aortic wall relaxation in aortas with advanced intimal lesions.

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