Patterns of Vascular Cell Adhesion Molecule-1 and Intercellular Adhesion Molecule-1 Expression in Rabbit and Mouse Atherosclerotic Lesions and at Sites Predisposed to Lesion Formation

Kaeko Iiyama, Leena Hajra, Motoi Iiyama, Hongmei Li, Maria DiChiara, Benjamin D. Medoff, Myron I. Cybulsky

Abstract—The recruitment of mononuclear leukocytes and formation of intimal macrophage-rich lesions at specific sites of the arterial tree are key events in atherogenesis. Inducible endothelial cell adhesion molecules may participate in this process. In aortas of normal chow-fed wild-type mice and rabbits, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), but not E-selectin, were expressed by endothelial cells in regions predisposed to atherosclerotic lesion formation. En face confocal microscopy of the mouse ascending aorta and proximal arch demonstrated that VCAM-1 expression was increased on the endothelial cell surface in lesion-prone areas. ICAM-1 expression extended into areas protected from lesion formation. Hypercholesterolemia induced atherosclerotic lesion formation in rabbits, LDL receptor and apolipoprotein E knockout mice, and Northern blot analysis demonstrated increased steady-state mRNA levels of VCAM-1 and ICAM-1, but not of E-selectin. Immunohistochemical staining revealed that VCAM-1 and ICAM-1 were expressed predominantly by endothelium in early lesions and by intimal cells in more advanced lesions. In early and advanced lesions, staining was most intense in endothelial cells at and adjacent to lesion borders. ICAM-1 staining extended into the uninvolved aorta. These expression patterns were highly reproducible in both species. The only difference was that VCAM-1 expression in endothelium over the central portions of lesions was found frequently in rabbits and rarely in mice. The expression of VCAM-1 by arterial endothelium in normal animals may represent a pathogenic mechanism or a phenotypic marker of predisposition to atherogenesis. (Circ Res. 1999;85:199-207.)

Key Words: atherosclerosis ■ endothelium ■ vascular cell adhesion molecule-1 ■ intercellular adhesion molecule-1 ■ expression pattern

Atherosclerosis is a disease process in which blood leukocytes and medial smooth muscle cells migrate into the arterial intima and are organized to form lesions with characteristic morphological features.1,2 Adhesion molecules expressed by endothelium and intimal cells may have pathogenic functions during this process. They may contribute to lesion initiation by participating in the recruitment of blood monocytes and lymphocytes to the intima in the earliest stages of atherogenesis and to lesion expansion at the periphery of lesions.3 Adhesion molecule expression within lesions may influence the organization of cells and promote cytokine/growth factor production and migration of medial smooth muscle cells into the intima, as well as influence cell replication.1

In a variety of species ranging from pigeons to humans, leukocyte accumulation and atherosclerotic lesion formation occur reproducibly at specific sites in the arterial tree, such as the lesser curvature of the aortic arch or adjacent to arterial branches.4–6 Leukocyte composition in lesions is highly regulated, consisting of monocytes and lymphocytes, but not polymorphonuclear leukocytes. These features suggest that local events contribute to leukocyte recruitment during atherogenesis. Potential mechanisms include hemodynamic factors, production of chemokines specific for mononuclear leukocytes, and distinct patterns of local adhesion molecule expression.

Our focus was on the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin, because their expression on vascular endothelium and other cell types is regulated by induction of transcription7 and they may be potential targets for therapy. Other endothelial cell adhesion molecules

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may also be relevant to atherogenesis. For example, P-selectin expression on the endothelial surface can be upregulated rapidly by translocation from cytoplasmic granules. In contrast, ICAM-2 is expressed constitutively by most endothelial cells and, therefore, is not likely to contribute to the topographic pattern of lesion formation.

Our goal was to evaluate and compare inducible adhesion molecule expression in normal and hypercholesterolemic mice and rabbits. This may provide valuable clues to similarities or differences in mechanisms of lesion formation. Atherosclerotic lesion formation has been studied extensively in rabbits, and mice have been used in many recent studies. Through genetic manipulation, mice provide a unique opportunity to assess the roles of adhesion molecules in atherosclerotic lesion formation. Expression patterns of adhesion molecules will provide insights into potential functions at specific stages of lesion formation and will aid in the interpretation of data from genetically altered animals.

Two knockout models, apolipoprotein E (ApoE<sup>−/−</sup>) and LDL receptor (LDLR<sup>−/−</sup>) mice, have been particularly popular for studies of atherogenesis.<sup>8</sup>–<sup>10</sup> Both develop marked hypercholesterolemia and lesions throughout the aorta. Lesions in ApoE<sup>−/−</sup> and LDLR<sup>−/−</sup> mice have morphological features closely resembling human atherosclerosis,<sup>11</sup>–<sup>13</sup> which suggests that similar pathogenic mechanisms may be involved. LDLR<sup>−/−</sup> mice fed a normal chow diet have only a 2-fold elevation in plasma cholesterol and do not develop lesions.<sup>10</sup> When fed a 1.25% cholesterol diet (including 7.5% cocoa butter, 7.5% casein, and 0.5% cholic acid), these mice develop marked hypercholesterolemia and extensive lesions throughout the aorta.<sup>15</sup> ApoE<sup>−/−</sup> mice develop hypercholesterolemia and atherosclerotic lesions when fed a normal chow mouse diet; however, if they are fed a Western-type diet (0.15% cholesterol, 21% fat) lesions develop more rapidly.<sup>8</sup>,<sup>11</sup> Each model has advantages and disadvantages. An appealing feature of LDLR<sup>−/−</sup> mice is that the atherogenic process is initiated with the start of cholesterol feeding, whereas the onset of this process is more difficult to pinpoint in the ApoE<sup>−/−</sup> model.

**Materials and Methods**

**Animals and Diets**

Animals were maintained in accordance with guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication No. [NIH] FS-23) and the Canadian Council on Animal Care. Male and female LDLR<sup>−/−</sup> mice (mixed 129-C57BL/6 strain) and ApoE<sup>−/−</sup> mice (crossed 6 times into the C57BL/6 strain) were purchased from The Jackson Laboratory (Bar Harbor, ME). In LDLR<sup>−/−</sup> mice, hypercholesterolemia was induced by feeding a 1.25% cholesterol diet containing 0.5% cholic acid<sup>16</sup> for 4, 5, 8, 10, 12, 20, or 24 weeks (4 to 6 mice per group). Control groups of LDLR<sup>−/−</sup> mice and ApoE<sup>−/−</sup> mice received standard mouse laboratory chow. For in vivo experiments, C57BL/6 and LDLR<sup>−/−</sup> mice (crossed 10 times into the C57BL/6 strain) were purchased from The Jackson Laboratory. At 2 to 4 months of age, they were fed standard chow or a cholesterol-free AIN-76A semipurified diet containing high fat (40% of energy intake) and 1.25% cholesterol (diet D12108, Research Diets, Inc).<sup>14</sup>

Rabbits were assigned to 1 of 2 dietary groups: standard rabbit chow (Purina) or a diet with 0.3% cholesterol and 9% partially hydrogenated coconut oil (Research Diets Inc). Food and water were provided ad libitum. Groups of rabbits were maintained on standard chow for at least 2 weeks and on cholesterol chow for 3, 6, or 9 weeks (5 rabbits per group). Watanabe heritable hyperlipidemic rabbits 10 weeks to 1 year of age were purchased from the NIH (Bethesda, MD) and were fed standard chow.

**Systemic Activation of Endothelium**

Mice received an intraperitoneal injection of 100 μg Escherichia coli 055:B5 endotoxin (lipopolysaccharide [LPS], Sigma). Rabbits were injected intravenously with 50 μg of LPS. Animals were euthanized 4 hours after LPS injection.

**Immunohistochemistry on Aortic Cross Sections**

The arterial tree of mice anesthetized with ethyl ether was perfused at 100 mm Hg via the left ventricle with 20 mL PBS (pH 7.0), followed by 25 mL 2% paraformaldehyde (4°C). Unfixed aortas were harvested from rabbits euthanized by intravenous sodium pentobarbital overdose. Aortas were removed into ice-chilled PBS, and adipose tissue was removed in situ. Segments of aorta were immersed in OCT compound, snap frozen in liquid nitrogen-cooled 2-methylbutane, and stored at −80°C.

Serial aortic cross sections (5 to 6 μm) were cut on a cryostat and placed on tissue section adhesive–coated slides (Vectabond, Vector Laboratories). Rabbit sections were air dried, whereas mouse sections were incubated at 45°C overnight to firmly adhere tissue to the slide. Slides were fixed for 5 minutes in acetone at −20°C and incubated with primary antibodies for 1 hour at 22°C (rabbit) or overnight at 4°C (mouse). Subsequent steps included the following: biotinylated polyclonal secondary antibodies, blocking endogenous peroxidase activity with 0.3% hydrogen peroxide in PBS (20 minutes), avidin-biotin-peroxidase complexes (ABC Elite kit, Vector Laboratories), 3-amino-9-ethylcarbazole, Gill’s hematoxylin counterstain, and glycerol gelatin mount (Sigma).

**Negative controls** were nonimmune rat IgG and E1/C15, a mouse IgG<sub>1</sub>-specific IgG<sub>1</sub> monoclonal antibody that did not bind to rabbit tissues. Primary antibodies included the following: monoclonal rat anti-mouse VCAM-1 (M/K-2.7, IgG1, American Type Culture Collection), ICAM-1 (YN1/1.7.4, IgG2b, American Type Culture Collection), and CD31-platelet/endothelial cell adhesion molecule-1 (IgG2a, PharMingen Inc), and mouse anti-rabbit VCAM-1 (Rb1/9, IgG1), ICAM-1 (Rb2/3, IgG1), E-selectin (14G2, IgG1, Hoffman-La Roche Inc), and macrophages (RAM 11, IgG1, Dako). Rabbit endothelial cells were identified with cross-reacting antibodies to human von Willebrand factor (polyclonal goat, Atlantic Antibodies) or human CD31 (JC/70A, mouse IgG, monoclonal antibody, Dako). Negative controls were nonimmune rat IgG and E1/C15, a mouse IgG<sub>1</sub> monoclonal antibody that did not bind to rabbit tissues.

**Northern Blotting**

Aortas were pooled from 4 mice per group, and total RNA was obtained by homogenization in guanidinium thiocyanate and cesium chloride ultracentrifugation.<sup>15</sup> RNA was isolated by oligo(dT) cellulose chromatography.<sup>15</sup> Rabbit aortas were divided into segments. Total cellular RNA was extracted with an acid-guanidinium thiocyanate-phenol-chloroform mixture<sup>16</sup> from the portions of the ascending aorta and portions of arch and descending thoracic aorta. Immunohistochemistry was performed on segments containing the brachiocephalic and fourth pair of intercostal artery ostia, and oil red O staining<sup>17</sup> on abdominal aortas and thoracic segments with the fifth pair of intercostal artery ostia.

RNA was electrophoresed through formaldehyde-containing 1% agarose gels (45 V, overnight), capillary transferred to nylon membranes (Bio-Trans+®, ICN), and hybridized overnight at 65°C with<sup>15</sup> [α-<sup>32</sup>P]dCTP-labeled cDNA probes (random sequence decanucleotide primer kit, Amersham Corp). Washed membranes were exposed at −80°C to XAR-5 film (Kodak) with enhancing screens (Dupont), and autoradiographs were analyzed by densitometry.
Rabbit VCAM-1, ICAM-1, and E-selectin cDNAs were constructed by hybridizing an LPS-activated rabbit endothelial cell cDNA library, and rabbit MCP-1 cDNA was produced by reverse transcriptase–polymerase chain reaction. Murine VCAM-1 cDNA was obtained from Biogen Inc and β-actin from Ambion Inc. Probes were generated by restriction enzyme digestion and purified by agarose gel electrophoresis and glass beads.15

Mapping of Sites Predisposed and Resistant to Atherosclerotic Lesion Formation in Proximal Aortas of Mice

The arterial tree of halothane-anesthetized mice was perfused with PBS, pH 7.4 (5 minutes), and then 2% paraformaldehyde (20 minutes) at 100 mm Hg via the left ventricle. The ascending aorta and arch were harvested, and adipose tissue was dissected while immersed in cold PBS. Regions predisposed and resistant to atherosclerotic lesion forma-

En Face Analysis of Endothelial Cell Surface VCAM-1 Expression in Normal Mice

Immunostaining for endothelial cell surface VCAM-1 was carried out in 2- to 4-month-old C57BL/6 and LDLR−/− mice fed standard chow. After perfusion fixation and dissection, the ascending aorta

Figure 1. Northern analysis of adhesion molecule expression in aortas of normal and hypercholesterolemic mice and rabbits. a, mRNA (4 μg/lane) was pooled from 4 aortas of wild-type C57BL/6 (BL/6+ +), LDLR−/−, and ApoE−/− mice fed standard chow or cholesterol diet for 12 weeks (w). The age at euthanization is indicated. En face oil red O (ORO)–stained aortas from LDLR−/− mice fed standard chow (chow) or a 12-week cholesterol diet (chol.) demonstrate the distribution of atherosclerotic lesions (dark regions). Bar=1 mm. b, Total RNA (10 μg/lane) from the thoracic aorta of individual rabbits fed a standard chow (control) or 0.3% cholesterol diet for 9 weeks. Segments of ORO-stained thoracic and abdominal aorta are above and below ethidium bromide (EtBr)–stained ribosomal RNA after transfer to a nylon membrane. Location of 28S and 18S ribosomal RNA is indicated. E-SEL indicates E-selectin.
and proximal arch segment were incubated with 20% donkey serum (15 minutes) and then overnight on a rotator with M/K-2.7 hybridoma supernatant (4°C). The secondary antibody was Cy-3–labeled donkey anti-rat IgG (1:200 dilution, 30 minutes, 22°C). Aortas were washed 3 times with PBS between incubations, stained with green nucleic acid stain (1:100 dilution, 30 minutes, Sytox, Molecular Probes), opened as above, and mounted on slides using mounting medium (Vectashield, Vector Laboratories). The distal arch served as the negative control and was incubated with nonimmune rat IgG (10 μg/mL) instead of M/K-2.7. Images of the endothelial cell monolayer were obtained using a Bio-Rad MRC-600 confocal microscope equipped with a krypton/argon laser and a 60× 1.4–numerical aperture objective (Nikon). For each mouse, images were obtained from regions with high and low probabilities (HPs and LPs, respectively) for lesion development (3 or 4 images per region) and from the negative control using the same confocal settings. Fluorescence in the Cy-3 channel (excitation, 568 nm; emission, 585 nm) was quantified using the confocal software frequency histogram function. For each mouse, the negative control was used to establish a pixel intensity that eliminated 99% of the background signal. Background fluorescence was then subtracted by applying this threshold to all HP and LP images, and the percentage pixels with remaining signal and the average signal intensity were determined for each image. The data showed a normal distribution, and statistical differences were evaluated with an unpaired t test. Detection of only cell surface VCAM-1 was verified by immunostaining with a goat polyclonal antibody to β-catenin (Santa Cruz Biotechnology). β-Catenin is a cytoplasmic protein and was not detected unless cells were permeabilized before staining with 0.2% Triton X-100.

**Results**

**Aortic VCAM-1 and ICAM-1 Expression Is Upregulated by Hypercholesterolemia**

Northern blot analysis was used to quantify adhesion molecule expression in aortas of normal or hypercholesterolemic rabbits and mice (Figure 1). VCAM-1 and ICAM-1 steady-state mRNA was increased 2- to 3-fold (normalized to β-actin) in LDLR<sup>−/−</sup> mice fed a cholesterol diet for 12 weeks and in 34-week-old ApoE<sup>−/−</sup> mice fed a chow diet (Figure 1a and Table 1). Similar increases in VCAM-1 and ICAM-1 expression (2.7- and 10-fold, respectively, normalized to ethidium bromide–stained 28S...
rRNA) were observed in rabbits fed a cholesterol diet for 9 weeks (Figure 1b and Table 2). In rabbits, the magnitude of VCAM-1, but not ICAM-1, expression correlated \((r=0.99)\) with the extent of surface area involved by oil red O–stained lesions in segments of descending thoracic and abdominal aorta from individual rabbits (Table 2). In mice, 2 VCAM-1 transcripts were detected (Figure 1a). The upper band (below 28S rRNA) is the 7 Ig–like domain form, and the lower (below 18S rRNA) band is an alternatively spliced 3 Ig–like form that is unique to rodents.\(^{19,20}\) Two VCAM-1 mRNA transcripts were also detected in rabbit aortas (Figure 1b). The upper band may be an alternatively polyadenylated or spliced form of VCAM-1. We identified an 8 Ig–like domain form of VCAM-1 in LPS-activated rabbit endothelium (data not shown). The additional Ig-like domain was unique to rabbits, was located between the 7th and transmembrane domains, and was homologous to nonfunctional domains.

E-selectin expression was not detected in aortas of mice (not shown) and rabbits (Figure 1b), yet E-selectin mRNA was readily detected in lungs of LPS-treated rabbits and mice, which indicates that the sensitivity of Northern blotting did not account for the absence of detectable transcripts in the aorta.

VCAM-1 and ICAM-1 Are Expressed Predominantly by Endothelial Cells in Early Atherosclerotic Lesions and by Intimal Cells in More Advanced Lesions

In early lesions composed predominantly of macrophage foam cells, expression of VCAM-1 and ICAM-1, but not of E-selectin, was detected by immunohistochemistry predominantly in endothelium. In more advanced lesions, expression was most abundant in intimal cells (Figure 2), which consist mostly of macrophages with variable numbers of smooth muscle cells. Smooth muscle cells in the intima and in culture can express VCAM-1.\(^{21,22}\) Expression of VCAM-1, but not of ICAM-1, was frequently detected in medial smooth muscle cells adjacent to lesions.

Endothelial Cell VCAM-1 Expression Is Restricted to Lesions, Whereas ICAM-1 Expression Extends Into the Uninvolved Aorta

In hypercholesterolemic mice and rabbits, VCAM-1 expression by endothelial cells was restricted to intimal lesions (Figures 2 and 3). Expression by endothelium was most intense at edges (borders) of lesions and extended only several cells beyond lesions. In endothelium over central portions of lesions, expression of VCAM-1 was variable and different between mice and
rabbits. In mice, the majority of these endothelial cells did not express VCAM-1, particularly in more advanced lesions, whereas in rabbits, VCAM-1 was detected frequently in early and advanced lesions (Figure 3). When LDLR<sup>−/−</sup> cholesterol-fed mice were injected with LPS, VCAM-1 (and ICAM-1) expression was found in all endothelial cells over lesions (Figure 4), indicating that these cells have the potential to express adhesion molecules when appropriately activated.

Endothelial cell expression of ICAM-1 was upregulated at edges and over lesions, but unlike VCAM-1, it extended much farther into the uninverted aorta (Figure 3). In regions such as the aortic arch, ICAM-1 staining was detected in virtually all endothelial cells, even when lesions involved only a fraction of the lumen circumference.

In Normal Animals, Endothelial Cells at Sites Predisposed to Lesion Formation Express VCAM-1 and ICAM-1

Initially, we evaluated adhesion molecule expression in random cross sections of the descending thoracic aorta from normal rabbits and C57BL/6 mice. VCAM-1 was expressed in occasional endothelial and medial smooth muscle cells, endothelial ICAM-1 expression was more abundant, and E-selectin was not detected. (All endothelial cells expressed these molecules after LPS treatment.) The descending thoracic aorta provides abundant tissue for analysis, but only small areas adjacent to intercostal artery ostia are predisposed to atherosclerotic lesion formation. Cross sections of the aortic arch were studied to determine whether a different adhesion molecule expression pattern occurs in the lesser curvature, a site predisposed to lesion formation. In mice and rabbits fed standard chow, expression of VCAM-1 was observed in endothelial cells of the lesser curvature and over intimal cushions of the brachiocephalic artery (Figure 5). ICAM-1 expression colocalized with VCAM-1 but was more diffuse. In many mice, ICAM-1 staining was nearly circumferential. Significant differences were not seen between C57BL/6 and standard chow-fed LDLR<sup>−/−</sup> mice (<6 months of age). VCAM-1 and ICAM-1 expression was also observed in the thoracic and abdominal aortas of rabbits and mice near ostia of intercostal, mesenteric, and renal arteries (not shown).

Endothelial cell surface expression of VCAM-1 in normocholesterolemic mice was quantified in regions with different probabilities for developing lesions. HP and LP regions were mapped in the ascending aorta and proximal arch of LDLR<sup>−/−</sup> mice (Figure 6). VCAM-1 expression on the endothelial cell surface was quantified by immunostaining and en face confocal microscopy in standard diet-fed LDLR<sup>+/+</sup> and mice.
LDLR<sup>−/−</sup> mice (C57BL/6 background, ≥10 generations). In every mouse, VCAM-1 expression was detected in HP regions, and staining appeared more intense than in LP regions (Figure 7). Quantitative analysis of the percentage area covered by specific VCAM-1 signal, as well as the average intensity of the signal, revealed significant differences between HP and LP regions (Table 3). VCAM-1 staining was over the entire endothelial cell surface in HP regions, and in some cells, staining was concentrated at intercellular junctions (Figure 7). Previously, VCAM-1 was observed at endothelial cell junctions in the rabbit carotid artery.  

Discussion

Immunostaining of normal aortas clearly demonstrated endothelial cell VCAM-1 expression in regions predisposed to atherosclerotic lesion formation. These observations are consistent with our Northern blot data, which showed readily detectable levels of VCAM-1 steady-state mRNA in normal mouse and rabbit aortas. Recently, Nakashima et al<sup>24</sup> observed that endothelial VCAM-1 expression was upregulated by hypercholesterolemia found in ApoE<sup>−/−</sup> mice before lesion formation but did not detect significant expression in lesion-prone sites of normal C57BL/6 mice. The immunostaining approaches used in our study may have been more sensitive and may account for our ability to detect VCAM-1 expression in aortas of normal animals. Our observations of ICAM-1 expression in HP regions of normal animals and lower levels in LP regions are consistent with those of Nakashima et al.<sup>24</sup>

In normocholesterolemic animals, expression of VCAM-1 and ICAM-1 in endothelial cells at sites predisposed to lesion formation may be related to complex hemodynamics in these regions. In vitro, introduction of shear stress can activate
various endothelial cell signal transduction pathways and influence the expression of adhesion molecules, and different shear stress profiles can induce unique repertoires of endothelial cell gene expression. Similar phenomena may occur at HP sites in vivo. Also, hemodynamics may increase local permeability or transport of lipoproteins by endothelium at HP sites and promote lipoprotein retention in the intima. Local oxidation of lipoproteins trapped in the intima may generate soluble factors that induce endothelial adhesion molecule expression.

The expression of VCAM-1 and ICAM-1 by aortic endothelium in normal animals may result in occasional recruitment of monocytes into the intima. Intimal monocytes/macrophages have been reported at lesion-predisposed sites of normal rabbits. These intimal cells may contribute to the accumulation and were highly reproducible and similar in rabbits and LDLR mice fed cholesterol-containing diets. Expression was most pronounced in endothelial cells at edges (shoulders) of both large and small lesions and extended several cells beyond the edge. VCAM-1 expression was essentially restricted to lesions, whereas ICAM-1 expression extended into the uninvolved aorta. These data suggest that similar mechanisms may influence adhesion molecule expression in atherosclerotic lesions of mice and rabbits, but the regulation of VCAM-1 expression is controlled more precisely than ICAM-1 by lesion-derived factors. Although not presented, virtually identical VCAM-1 and ICAM-1 immunohistochemical staining patterns were obtained from ApoE−/− mice and Watanabe heritable hyperlipidemic rabbits fed standard laboratory chow. This indicates that hypercholesterolemia, and not other dietary factors, was responsible for upregulated VCAM-1 and ICAM-1 expression in lesions.

We found a difference between mice and rabbits regarding the extent of VCAM-1 expression by endothelium overlying central regions of lesions. In both species, VCAM-1 was expressed at edges of lesions, but in rabbits, expression was relatively abundant over the central regions of early and large foam cell lesions, although there was variability between lesions. In LDLR−/− mice, endothelial cells over the central portions of lesions generally did not express VCAM-1 but retained the potential, as was demonstrated by treating mice with LPS (Figure 4). The mechanism for this species difference in VCAM-1 expression is not obvious. One could speculate that a minor difference in the promoters of the mouse and rabbit VCAM1 genes could result in slightly different regulation of expression. Alternatively, the environment in the central regions of mouse and rabbit lesions may be different, eg, the degree of oxidative stress or production of a different growth factor(s)/cytokine(s). In advanced human atherosclerotic lesions, lumen endothelial VCAM-1 expression was associated with subendothelial monocyte accumulation and was not abundant.

We did not detect E-selectin expression in mouse or rabbit atherosclerotic lesions. These data are consistent with other observations in rabbits, but they differ from reports of E-selectin expression in human lesions. Possible explanations are that human lesions were more advanced, some patients had associated conditions that induced E-selectin expression, and the antibody to human E-selectin used in some of these studies (BBA 1) was subsequently found to cross-react with P-selectin.

### Table 3. VCAM-1 Endothelial Cell Surface Expression in HP and LP Regions of the Proximal Mouse Aorta

<table>
<thead>
<tr>
<th>LDLR Genotype*</th>
<th>Percentage of Pixels With Signal</th>
<th>Average Pixel Intensity</th>
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<tr>
<td></td>
<td>HP†</td>
<td>LP†</td>
</tr>
<tr>
<td>+/+</td>
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<td>3.14±2.05</td>
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<td>2.85±1.20</td>
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<td>0.59±0.44</td>
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</tr>
<tr>
<td>−/−</td>
<td>9.83±4.20</td>
<td>1.98±2.96</td>
</tr>
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</table>

*All mice were in the C57BL/6 background, fed standard chow, and used at 2 to 4 months of age.
†Mean±SD obtained from 3 or 4 images from each HP and LP region.
A comparison of endothelial cell VCAM-1 and ICAM-1 expression in lesions and lesion-predisposed sites suggests different regulatory mechanisms. In normal animals, expression was scattered throughout lesion-predisposed sites and probably was influenced by local hemodynamic forces. However, even in early lesions, expression was localized to lesion borders, and in mice, VCAM-1 expression was absent from the center. Therefore, intrinsic properties of lesions, rather than hemodynamics, are likely the dominant factors responsible for this expression pattern. Potential mechanisms include oxidative stress, NF-κB activation, and altered nitric oxide production.

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