Direct Evidence for a Crucial Role of the Arterial Wall in Control of Atherosclerosis Susceptibility

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Background—Inbred mouse strains C57BL/6J (B6) and C3H/HeJ (C3H) exhibit marked differences in atherosclerosis susceptibility. We sought to determine whether the difference in atherosclerosis susceptibility resides at the level of arterial walls.

Methods and Results—Thoracic aortic segments from 8-week-old female B6 and C3H apolipoprotein E–deficient mice were transplanted into the infrarenal aorta of 10-week-old female F1 mice. After transplantation, recipients were maintained on a chow diet for 16 weeks. The donor aortic segments of B6 mice developed significantly larger atherosclerotic lesions than those of C3H (44 983 ± 11 702 versus 5600 ± 4885 μm² per section; P=0.011). Expression of vascular cell adhesion molecule (VCAM)-1 by endothelial cells was examined both in vitro and in vivo. B6 mice expressed significantly more VCAM-1 than their C3H counterparts. Sequence analysis of VCAM-1 cDNA revealed a nucleotide difference in the coding region that resulted in substitution of an amino acid in the protein product.

Conclusions—These data provide direct proof that factors operating in the vessel wall, particularly endothelial cells, can serve as atherosclerosis modifiers and suggest a possibility for the contribution of VCAM-1 to atherosclerosis susceptibility. (Circulation. 2006;114:2382-2389.)

Key Words: atherosclerosis ■ endothelium ■ grafting ■ transplantation

Atherosclerosis is a complex and chronic inflammatory disease of the large and medium arteries resulting from interactions among lipids, blood cells, and arterial wall cells. Hyperlipidemia results in deposition of apoprotein (apo) B–containing lipoproteins such as low-density lipoprotein (LDL) in the subendothelium of the arterial wall. The accumulated LDL undergoes oxidative modification by arterial wall cells to become oxidized LDL (OxLDL). OxLDL or its components then stimulate arterial wall cells to express monocyte chemotactic protein-1 (MCP-1), macrophage colony-stimulating factor (M-CSF), vascular cell adhesion molecule-1 (VCAM-1), and other proinflammatory molecules that promote monocyte transmigration into the subendothelium and differentiation into macrophages.1 Subsequently, the macrophages take up OxLDL to become foam cells, the hallmark of atherosclerosis.

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The availability of numerous mouse strains that differ in susceptibility to atherosclerosis provides an experimental method for identifying the role of various cellular components in atherogenesis. C57BL/6 (B6) and C3H/HeJ (C3H) mice are 2 commonly used inbred strains that differ strikingly in the development of atherosclerosis when fed an atherogenic diet2 or when deficient in apoE (apoE−/−).3 Previous studies have revealed various differences between B6 and C3H in atherogenic processes involving vascular wall cells. Indeed, B6 mice exhibit a significant increase in apoB-containing lipoproteins in the arterial wall compared with C3H mice when fed an atherogenic diet or when deficient in apoE.4 In response to OxLDL, endothelial cells and smooth muscle cells from B6 mice express significantly more MCP-1, M-CSF, and VCAM-1 than cells from C3H mice.5,6 Moreover, endothelial responses to OxLDL cosegregate with the size of atherosclerotic lesions, as observed in a set of recombinant inbred strains derived from strains B6 and C3H.3 Direct evidence for the contribution of arterial walls to atherosclerosis susceptibility is still missing, however.

Aorta transplantation in the mouse provides a direct means for studying the role of the vascular wall in a biological process in vivo. This technique was initially described by Koulack et al,7 who transplanted a segment of thoracic aorta into the infrarenal aorta of a recipient mouse. Although the aorta transplantation procedure is used primarily to study

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long-term graft rejection, it also is used to investigate the progression or regression of atherosclerotic lesions in apoE/--; mice.8,9 Grafting from parental to F1 strains avoids allogeneic rejection responses; thus, in the present study, we performed aorta transplantation to determine atherosclerotic lesion formation of aortic grafts from B6 and C3H mice in their F1 hybrids. Previously, we observed a 7-fold difference in VCAM-1 mRNA levels at the baseline in endothelial cells of B6 and C3H mice.3 Because VCAM-1 is an adhesion molecule that functions to recruit monocytes, the present study also tested the hypothesis that VCAM-1 contributes to the variation in atherosclerosis susceptibility of the 2 strains.

Methods

Mice

Female 8-week-old B6.apoE/--; and C3H.apoE/--; mice were used as aorta donors; 10-week-old female F1 mice between the 2 strains served as recipients. Female recipients were selected because it is much easier to perform aortic interposition grafts as a result of the absence of gonadal vessels over the site of surgery that are prone to bleeding when mobilized. Female B6.apoE/--; mice at the N10 backcross, constructed from B6;129.apoE/--; mice,10 were purchased from The Jackson Laboratory (Bar Harbor, Maine), and C3H.apoE/--; mice were generated in our laboratory by initially crossing B6.apoE/--; mice with C3H/HeJ mice, followed by 12 sequential backcrossings with C3H/HeJ mice. F1 mice were generated by crossing female B6.apoE/--; mice with male C3H.apoE/--; mice. All procedures were carried out in accordance with current National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee.

Aorta Transplantation Procedure

The aorta transplantation procedure in the mice was performed as described by Reis et al. Briefly, both donors and recipients were anesthetized with ketamine/xylazine, 60 to 80/5 to 10 mg/kg, by intramuscular injection. For donor operation, once appropriate sedation was achieved, the anterior chest wall, lungs, and esophagus were removed under sterile conditions. The aorta was flushed thoroughly with phosphate-buffered saline containing 5 U/mL heparin before the left ventricle of the heart. Descending thoracic aorta was dissected out from surrounding tissues, transected, and kept in ice-cold saline before use. Aortic segments (~5 mm in length) from 1 donor mouse were serially transplanted into 3 recipients. For recipient operation, a midline abdominal incision was made, and the visceras were retracted outside the abdominal cavity with a moist cotton swab. Then the aorta and vena cava were exposed and separated from each other, and 2 microvascular clamps were placed on the infrarenal abdominal aorta. The aorta between the clamps was transected with microvascular scissors, and the prepared aortic graft was anastomosed end to end with the recipient aorta with a total of 6 to 8 sutures for each anastomosis. After completion of the anastomosis, the distal clamp was removed, followed by the proximal clamp. The intestines were returned to the abdominal cavity, the abdominal musculature and the skin were closed in 2 layers, and 1 mL saline was injected into the peritoneal cavity. After the procedure, mice were maintained on a regular rodent chow diet for 16 weeks.

Tissue Preparation and Lesion Quantification

Sixteen weeks after the vascular transplantation procedure, recipient mice (5 to 9 mice in each group) were euthanized by cervical dislocation after isoflurane anesthesia. The aorta was perfused with 4% paraformaldehyde via the left ventricle of the heart. The donor aortic graft, together with adjacent recipient aorta, was dissected out and embedded in optical coherence tomography compound. In a separate group, the infrarenal aorta of 30-week-old female B6.apoE/--; (n=4) and C3H.apoE/--; (n=6) mice without undergoing aorta transplantation was harvested to assess atherosclerotic lesions. Serial 10-μm-thick cryosections were collected and mounted on poly-L-lysine–coated slides. Sections were stained with oil red O and hematoxylin and counterstained with fast green, and the lesion areas were counted by light microscopy.

Immunohistochemical Analysis

The presence of macrophages, T lymphocytes, dendritic cells, and endothelial cells in aortic grafts was determined by staining with antibodies to mouse MOMA-2 (Accurate Chemicals, Westbury, NY), CD3, or CD11c (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) or to the von Willebrand factor (Sigma-Aldrich, St Louis, Mo) as previously described. Briefly, frozen sections were fixed with acetone, followed by incubation with the primary antibodies. After a thorough wash, the sections were incubated with biotinylated secondary antibodies (Vector Laboratories, Burlingame, Calif). The reactions were visualized with peroxidase chromogen kits (Vector Laboratories).

Plasma Lipid Analysis

Mice were fasted overnight before being killed, and blood was collected by retro-orbital venous plexus puncture with the animals under isoflurane anesthesia. Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were determined with the Thermo DMA (Louisville, Colo) cholesterol and triglyceride kits, which were adapted for a microplate assay. Briefly, 6 μL plasma samples, lipid standards, and controls were loaded in a 96-well plate and then mixed with 150 μL cholesterol or triglyceride reagents. After a 5-minute incubation at 37°C, the absorbance at 500 nm was read on a Molecular Devices (Menlo Park, Calif) plate reader. HDL cholesterol levels were determined after precipitation of very-low-density lipoprotein and LDL cholesterol fractions with a precipitating reagent provided by the company.

Lipoprotein Isolation and Modification

LDL (density, 1.019 to 1.069 g/mL) was isolated from the serum of healthy human donors by density-gradient ultracentrifugation as previously described by Havel et al,13 dialyzed in phosphate-buffered saline containing 0.3 mmol/L ethylenediaminetetraacetic acid (EDTA), filtered through 0.22-μm filters, and stored at 4°C. Lipoprotein concentrations were expressed as protein content. Ox-LDL was prepared by incubating LDL at a concentration of 5 mg/mL with 7 μmol/L FeSO4.5 Detection of VCAM-1 on Endothelial Cells

Endothelial cells were isolated from the thoracic aorta of female wild-type B6 and C3H mice by an established explantation technique. Briefly, the aortic segments were placed on Matrigel and incubated in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 90 μg/mL heparin, 60 μg/mL endothelial cell growth supplements, and 100U/mL fungizone. The vessel segments were removed once cell outgrowth was observed. The cells were passaged with Dispase and then plated into gelatin-coated culture dishes. The subsequent passages were performed with 0.25% trypsin-EDTA. At passage 4, cells were plated in 96-well tissue culture plates and allowed to grow to confluence. Confluent cells were incubated overnight in Dulbecco’s modified Eagle’s medium containing 1% fetal bovine serum before being treated with medium containing 100 μg/mL Fe++-modified LDL and 2 μg/mL lipopolysaccharide (LPS). After 4 hours of incubation, the medium was removed, and the cells were fixed with 1% paraformaldehyde in phosphate-buffered saline. VCAM-1 on endothelial cells was determined by enzyme-linked immunosorbent assay (ELISA). Briefly, endothelial cells were washed and incubated with 100 μL rat anti-mouse VCAM-1 antibody (1:250 dilution, BD Pharmingen, San Diego, Calif) per well for 45 minutes at 37°C. Cell monolayers were then washed and incubated with a biotinylated rabbit anti-rat IgG (1:200 dilution) for 45 minutes. After washing, cells were incubated with avidin-alkaline phosphatase for 45 min-


Western Blot Analysis for VCAM-1

Expression of VCAM-1 in the aortic wall of 8-week-old apoE<sup>−/−</sup> mice fed a chow diet was determined by Western blot analysis. Aorta protein was prepared as we previously described.<sup>3,4</sup> Briefly, the aorta was washed thoroughly with phosphate-buffered saline containing 5 U/mL heparin and 1 mmol/L EDTA, cleaned of periadventitial fat and connective tissues, and snap-frozen in liquid nitrogen. The frozen aorta was mechanically broken up; dispersed in lysis buffer containing 10 mmol/L Tris, pH 8, 1 mmol/L EDTA, 2.5% sodium dodecyl sulfate, and 5% mercaptoethanol; and centrifuged at 500 g for 10 minutes at 4°C. The supernatant was collected and used for detection of VCAM-1. Aorta protein (10 µg) was separated by electrophoresis on 4% to 12% Tris polyacrylamide gels and electrophoretically transferred onto polyvinyl difluoride membranes (Invitrogen, Carlsbad, Calif). The membranes were incubated with a primary antibody against mouse VCAM-1 (BD Pharmingen) overnight and then incubated for 0.5 hour with an alkaline phosphatase–conjugated secondary antibody. Signals were detected by the chemiluminescence method (Invitrogen).

Measurements of Soluble VCAM-1

Mice were fasted overnight before blood was collected through retro-orbital veins under isoflurane anesthesia. An ELISA kit for soluble mouse VCAM-1 assay was purchased from R&D Systems (Minneapolis, Minn), and the assays were performed by following the manufacturer’s instructions.

Sequence Analysis of VCAM-1

Total RNA was isolated from endothelial cells of B6 and C3H mice, and cDNA was prepared by use of the Superscript reverse transcription polymerase chain reaction kit (Invitrogen). The cDNA of VCAM-1 was analyzed by sequencing in both directions. The polymerase chain reaction primers used for sequencing were as follows: 5'-GAC CTTCAGCCAGAAGACGA-3'/5'-CCAGATCTTCGATCC-3', 5'-AGTCGCTTCTGTG-3'/5'-GCTGAACTGTTGCTG-3', 5'-ATTGCTTGTGACG-3'/5'-GAGATTTTGCTTC-3', 5'-GGATGGCAG-3'/5'-GAGAGGAACAT-3', and 5'-GGATAAT-3'/5'-GTGGTCGACAT-3'.

Statistical Analysis

All values are expressed as mean±SEM, with n indicating the number of mice. Student <i>t</i> test was used to determine the statistical differences between the 2 strains in all measurements. Differences were considered statistically significant at <i>P</i>&lt;0.05.

The authors had full access to the data and take responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

The Arterial Wall Being a Source of Difference in Atherosclerosis Susceptibility

To determine whether the arterial wall was a source of the difference between the B6 and C3H mouse strains in atherosclerosis susceptibility, a segment of donor thoracic aorta from female B6.apoE<sup>−/−</sup> and C3H.apoE<sup>−/−</sup> mice was transplanted into divided infrarenal aorta of female F1 mice. On a regular rodent chow diet, F1 recipient mice developed moderate hyperlipidemia (Figure 1). In general, F1 mice transplanted with B6 grafts had comparable total cholesterol, HDL cholesterol, and triglyceride levels relative to those transplanted with C3H grafts. Sixteen weeks after transplantation, lesion formation in donor aortic grafts was examined by light microscopy. As shown in Figure 2, donor aortic segments from either strain developed atherosclerotic lesions in the F1 recipients, as determined by oil red O staining. The average lesion area per section was 44 983±11 702 µm<sup>2</sup> per section for each B6 donor aorta (n=9) and 5600±4885 µm<sup>2</sup> per section for each C3H donor aorta (n=5). The difference in lesion sizes between the 2 different grafts was statistically significant (<i>P</i>=0.011). Atherosclerotic lesions in the infrarenal aorta of age-matched B6.apoE<sup>−/−</sup> and C3H.apoE<sup>−/−</sup> mice without aorta transplantation also were assessed when mice were fed the chow diet. B6.apoE<sup>−/−</sup> mice (n=4) developed significantly larger atherosclerotic lesions than C3H.apoE<sup>−/−</sup> mice (n=6) (28 000±7874 versus 667±422 µm<sup>2</sup> per section; <i>P</i>=0.040). Compared with the aortic grafts, the aorta of B6.apoE<sup>−/−</sup> mice without transplantation developed significantly smaller atherosclerotic lesions (<i>P</i>=0.031).

Immunohistochemical analysis revealed the presence of numerous macrophage foam cells in the lesions of both B6 and C3H grafts (Figure 3). Scattered T lymphocytes and dendritic cells were observed in the lesions of B6 grafts. In both grafts, a thin layer of endothelial cells lining the inner surface of the vessel was detected.

Differential VCAM-1 Expression on Endothelial Cells

Expression of VCAM-1 on endothelial cells derived from wild-type B6 and C3H mice after treatment with medium, OxLDL, and LPS for 4 hours was analyzed by ELISA (Figure 4). The baseline VCAM-1 level was significantly higher in B6 than in C3H cells (optical density, 0.77±0.11 versus 0.13±0.01; <i>P</i>=0.021; n=3 for each strain). Moreover, both OxLDL and LPS significantly increased VCAM-1 expression on endothelial cells of B6 mice (<i>P</i>&lt;0.05). In contrast,
VCAM-1 on C3H endothelial cells was not significantly induced by OxLDL or LPS ($P > 0.05$).

Differential VCAM-1 Expression in Aortic Walls

To determine whether differences in VCAM-1 expression could be detected in vivo, protein was prepared from the aorta of 8-week-old B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice on a chow diet and analyzed by Western blot analysis. As shown in Figure 5, VCAM-1 protein had 2 different forms: the full-length long form and the truncated form. The long form has a molecular weight of 110 kDa; the short form has a molecular weight of 47 kDa.15 The expression of VCAM-1 in aortic walls was lower in C3H than in B6 mice for both of the forms, but the reduction was more dramatic for the truncated form in C3H.

Soluble VCAM-1 Levels in Plasma

Soluble VCAM-1 levels in plasma of 8-week-old B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice on the chow diet were measured by ELISA (Figure 6). Plasma VCAM-1 levels were significantly higher in B6.apoE$^{-/-}$ mice than in C3H.apoE$^{-/-}$ mice (671±42 versus 451±17 ng/mL; $P=0.0018$; $n=6$ for each strain).

Sequence Variation Between B6 and C3H

VCAM-1 cDNA

Nucleotide sequences in both promoter and coding regions of the VCAM1 gene were analyzed for B6 and C3H mice. A single base-pair change (A2077G) was identified within the coding region at 2077 bp from A of the start codon (ATG), which results in amino acid substitution (Figure 7A). The

Figure 2. A, Representative light photomicrographs of atherosclerotic lesions in aortic grafts from female B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice. Sections were stained with oil red O and hematoxylin. B, Quantitative comparisons of mean areas of atherosclerotic lesions in aortic grafts from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice (left) and in the infrarenal aorta of the 2 strains without undergoing aorta transplantation (right). All mice were fed a regular chow diet. Values are mean±SE for 4 to 9 mice. *$P < 0.05$ vs C3H; †$P < 0.05$ versus B6 aorta.

Figure 3. Immunohistochemical analysis to determine the presence of macrophages, T-lymphocytes, dendritic cells, and endothelial cells in aortic grafts from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice. Sections were stained with antibodies to mouse macrophage antigen MOMA-2, CD3, or CD11c or to the von Willebrand factor.
Amino acid change occurred at residue 693, where B6 has an asparagine and C3H has an aspartic acid. Within the promoter region of the VCAM1 gene, a nucleotide change at −243 bp upstream of the start code ATG was located (C-243A) (Figure 7B).

Discussion

In the present study, we performed aorta transplantation to determine whether the arterial wall was a source of the difference between B6 and C3H mice in atherosclerosis susceptibility. One major finding was that aortic grafts from atherosclerosis-susceptible B6.apoE−/− mice developed significantly larger atherosclerotic lesions than those from atherosclerosis-resistant C3H.apoE−/− mice in identical F1 mice. Previously, we observed that OxLDL induces marked expression of proinflammatory genes such as MCP-1, M-CSF, and VCAM-1 in endothelial cells and vascular smooth muscle cells from B6 mice, whereas cells from C3H mice show small or no induction of these genes.5,6 In recombinant inbred strains derived from B6 and C3H strains, we found that endothelial responses to OxLDL with respect to the induction of proinflammatory genes cosegregate with the size of atherosclerotic lesions.3 Because MCP-1, M-CSF and VCAM-1 are associated with monocyte recruitment to the arterial wall and differentiation into macrophages, it is plausible that the arterial wall contributes to the difference between B6 and C3H mice in atherosclerosis susceptibility. Thus, in this study, we determined the role of arterial walls in the control of atherosclerosis susceptibility by transplanting aortic grafts from B6.apoE−/− and C3H.apoE−/− mice into the infrarenal aorta of F1 mice. The present finding that donor aortas from B6.apoE−/− mice developed significantly larger lesions than those from C3H.apoE−/− mice provides the first direct proof that factors operating in the vessel wall can serve as atherosclerosis modifiers.

We took advantage of the apoE−/− mouse model to investigate atherosclerosis development of transplanted aortic grafts in a hyperlipidemic environment. ApoE−/− mice represent a mouse model in which spontaneous hyperlipidemia and atherosclerosis occur even on a chow diet.10,16 Moreover, these mice develop all phases of atherosclerotic lesions seen in humans in large and medial arteries in a time-dependent manner.17 In contrast, the diet-induced mouse model of atherosclerosis develops only small fatty streak lesions that are largely limited to the aortic root, even after being fed the Paigen diet for 14 weeks to 9 months.18 In this study, donor aortic grafts were obtained from 8-week-old apoE−/− mice. At this stage, both strains are known to have no detectable atherosclerotic lesions in the aorta.3,17 Thus, the difference between the donor aortas in lesion formation was not associated with differences in preexisting lesions. F1 hybrids between B6.apoE−/− and C3H.apoE−/− mice were used as recipients for aortic grafts, thus avoiding allogeneic rejection as a contributing factor for lesion formation. Studies of male grafts to female recipients would be confounded by rejection across minor histocompatibility antigens encoded by genes on the Y chromosome. In addition, the present results may not be extrapolated to male mice because they were not studied.

There are a 100-fold difference in the size of atherosclerotic lesions at the aortic root3 and a 40-fold difference in the infrarenal aorta (Figure 3) between B6.apoE−/− and C3H.apoE−/− mice on the chow diet. In the present study, however, we observed only a >10-fold difference in the lesion size of transplanted aortas from the 2 donors. One explanation for the discrepant result is that systemic

Figure 4. Induction of VCAM-1 protein in aortic endothelial cells from wild-type B6 and C3H mice after incubation with medium only (control), OxLDL, or LPS for 4 hours. Induction of VCAM-1 protein was measured by ELISA and expressed as optical density. Values are mean±SE of 3 individual mice. *P<0.05 vs C3H with the same treatment; +P<0.05 vs control.

Figure 6. Plasma concentrations of soluble VCAM-1 (ng/mL) in B6.apoE−/− and C3H.apoE−/− mice on chow diet. Values are mean±SEM for 6 mice. *P<0.05 vs C3H.apoE−/− mice.

Figure 5. Western blot analysis of VCAM-1 in descending thoracic aortic walls of 8-week-old B6.apoE−/− and C3H.apoE−/− mice on a regular chow diet. Protein (10 μg) from the aorta was electrophoresed on Tris-polyacrylamide gels, transferred to nitrocellulose membranes, and probed with antibody for the protein. Each lane represents an individual mouse.
factors also contribute to the difference in atherosclerosis susceptibility of the 2 strains.

Transplanted aortas developed much larger atherosclerotic lesions compared with those that were not transplanted, suggesting that the transplantation procedure had resulted in aggravated lesion formation. Recently, we have compared the 2 strains in lesion formation after arterial injury and found that B6.apoE/H-H11002/H11002 mice are more responsive to arterial injury than C3H with regard to lesion sizes.11 Thus, the difference between aortic grafts of the 2 strains in lesion formation in the F1 recipients could be partially attributable to a differential response to injury that occurred during the transplantation procedure.

In the present study, we observed a marked difference between B6 and C3H mice in VCAM-1 expression by endothelial cells both at baseline and after stimulation with OxLDL and LPS. The endothelial cells of B6 mice expressed significantly more VCAM-1 protein than the cells of C3H mice. Moreover, we demonstrated a dramatic reduction in VCAM-1 expression in the aortic wall of C3H mice compared with B6 mice in vivo. In mice, VCAM-1 exists in 2 forms: the long form and the truncated form. Moy et al19 first cloned the truncated form of mouse VCAM-1 cDNA resulting from alternative splicing of the VCAML gene and characterized the truncated VCAM-1 as a fully functional adhesion molecule. Shortly thereafter,
Kumar et al. cloned and characterized the mouse Vcam1 gene, mapped the gene to chromosome 3, and further characterized the truncated VCAM-1. Both long and truncated forms of VCAM-1 are found to be inducible in multiple tissues with stimulation of endotoxin. We previously have observed that both forms of VCAM-1 mRNA are induced by OxLDL in endothelial cells of B6 but not C3H mice. In this study, we found that both forms of VCAM-1 were reduced in C3H, but the reduction was more dramatic for the truncated form. VCAM-1 is a member of the immunoglobulin superfamily known to be expressed by endothelial cells for recruitment of leukocytes during inflammation. VCAM-1 interacts with the integrin α4β1 (very-late-acting antigen 4), which is constitutively expressed on the surface of leukocytes. Blockade of the VCAM-1/very-late-acting antigen 4 pathway reduces monocyte adhesion and infiltration and disruption of VCAM-1 domain 4 reduces monocyte migration and inhibits atherosclerotic lesion formation in apoE−/− and LDL receptor−deficient mice. A lower expression of this proinflammatory molecule by endothelial cells is expected to lead to fewer monocytes being recruited to the subendothelial space where they differentiate, take up lipids, and form foam cells. Thus, the difference in VCAM-1 expression by endothelial cells is likely to contribute to the difference between B6 and C3H mice in atherosclerosis susceptibility.

Interestingly, we observed a significant reduction in plasma soluble VCAM-1 levels in C3H mice. This lower plasma level of soluble VCAM-1 appears to reflect the lower expression of VCAM-1 in the arterial wall. Indeed, although the origins of circulating adhesion molecules have not been clarified, these soluble forms are most likely derived from shedding or proteolytic cleavage from endothelial cells on which these molecules are originally expressed.

Although the expression level of VCAM-1 is likely to contribute to the variation in atherosclerosis susceptibility, its structural differences also may contribute. Sequence analysis of B6 and C3H VCAM-1 cDNA revealed 1 amino acid difference between the 2 strains at residue 693, with B6 having asparagine and C3H having aspartic acid. We also identified a nucleotide substitution in the promoter region of the VCAM1 gene, which was likely to contribute to the variation in VCAM-1 expression. The amino acid substitution could potentially influence the function of VCAM-1.

The demonstration that the difference in atherosclerosis susceptibility between mouse strains B6 and C3H are due, at least in part, to genetic differences in the arterial wall provides the first direct proof that genetic factors in atherosclerosis act at the level of vessel walls, although there is a possibility that an influence from other components has been made on the arterial wall before transplantation that could affect subsequent atherosclerosis in the new environment. This study also suggests that variations of VCAM-1 expression and structure may contribute to the resistance of C3H mice to atherosclerosis.


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**CLINICAL PERSPECTIVE**

Atherosclerotic cardiovascular disease is a multifactorial disorder with a strong heritable component. Although environmental factors such as diet and smoking play a role in atherosclerosis, genetic factors are a major determinant for development of the disease. Only a small subset of atherosclerosis cases are caused by rare mutants that are observable as mendelian traits. The common forms of atherosclerosis involve multiple genes and exhibit significant gene-environment interactions. Identification of genes involved in atherosclerosis has not been readily achieved in humans. The difficulties in human studies suggest the use of animal models to identify genes for atherosclerosis. Atherosclerosis is a complex disease of the large and medium arteries that results from interactions among lipids, blood cells, and arterial walls. If a major genetic variation leading to susceptibility to atherosclerosis could be attributed to ≥1 defined components, it would represent a major advance in the search for genes influencing atherosclerosis. Inbred mouse strains C57BL/6J (B6) and C3H/HeJ (C3H) exhibit marked differences in atherosclerosis susceptibility. This study determined whether the difference in atherosclerosis susceptibility resides at the level of arterial walls. Thoracic aortic segments from B6 and C3H apolipoprotein E−deficient mice were transplanted into the infrarenal aorta of F1 mice. After transplantation, recipients were maintained on a chow diet for 16 weeks. The donor aortic segments of B6 mice developed significantly larger atherosclerotic lesions than those of C3H. This study provides the first direct proof that factors operating in the vessel wall can serve as atherosclerosis modifiers.
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