Adhesion of Monocytes to Arterial Endothelium and Initiation of Atherosclerosis Are Critically Dependent on Vascular Cell Adhesion Molecule-1 Gene Dosage

Hayes M. Dansky, Courtenay B. Barlow, Chris Lominska, John L. Sikes, Catherine Kao, Jonathan Weinsaft, Myron I. Cybulsky, Jonathan D. Smith

Abstract—Vascular cell adhesion molecule-1 (VCAM-1/Vcam1) is a cytokine-inducible member of the immunoglobulin gene superfamily that is expressed by arterial endothelial cells in regions predisposed to atherosclerosis and at borders of atherosclerotic plaques. To determine whether VCAM-1 expression regulates atherosclerotic lesion formation, we crossed Vcam1 domain 4–deficient (D4D) mice, which partially circumvent the embryonic lethality of Vcam1 null mice, with apolipoprotein E null (Apoe<sup>−/−</sup>) mice, which spontaneously develop hypercholesterolemia and atherosclerosis. In the Apoe<sup>−/−</sup> background, mice homozygous for the Vcam1 D4D allele had markedly reduced arterial VCAM-1 expression, monocyte adherence in the aortic root, and fatty streak formation. Heterozygous Vcam1 D4D mice revealed a Vcam1 gene-dosage effect and had intermediate, yet significant, reductions in these parameters. Our data demonstrate that VCAM-1 plays a pivotal role in the initiation of atherosclerosis in Apoe<sup>−/−</sup> mice. (Arterioscler Thromb Vasc Biol. 2001;21:1662-1667.)

Key Words: vascular cell adhesion molecule-1  •  apolipoprotein E  •  hypercholesterolemia  •  gene targeting  •  monocytes

Atherosclerosis begins as a focal process at specific regions of the vasculature, so-called lesion-prone areas, where hemodynamic flow is altered. The arterial endothelium expresses numerous adhesion molecules, such as P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), at these lesion-prone areas before lesion development<sup>1,2</sup> and at the borders of atherosclerotic lesions.<sup>3,4</sup> Whereas ICAM-1 is also abundantly expressed at lesion-prone areas in wild-type mice with normal cholesterol levels,<sup>1,2</sup> VCAM-1, a cytokine-inducible member of the immunoglobulin gene superfamily,<sup>4</sup> is specifically upregulated in arterial endothelial cells at lesion-prone areas in hypercholesterolemic mice and rabbits.<sup>1,2,5</sup> This specific upregulation of VCAM-1 at lesion-prone areas in hypercholesterolemic animals suggests that VCAM-1 may regulate monocyte adhesion in early atherogenesis.

To circumvent the embryonic lethality of Vcam1 null mice,<sup>6,7</sup> mutant mice were generated with a targeted disruption of the exon encoding the fourth immunoglobulin domain of VCAM-1, which codes for an α<sub>l</sub> integrin binding site.<sup>8</sup> Domain 4–deficient (D4D) mice (Vcam1<sup>Δ<sub>D4D</sub></sup>) express only a 6-immunoglobulin domain form of Vcam1, with VCAM-1 mRNA and protein levels <10% of those found in wild-type mice.<sup>8</sup> Reduced expression of VCAM-1 protein resulted in decreased embryonic survival of Vcam1<sup>Δ<sub>D4D</sub></sup> mice.<sup>8</sup> The frequency of embryonic survival was strain dependent, ranging from 29% in 129-C57BL/6 hybrids to 6% of expected in C57BL/6 Vcam1<sup>Δ<sub>D4D</sub></sup> mice.<sup>8</sup> When Vcam1<sup>Δ<sub>D4D</sub></sup> of mixed genetic background were bred with LDL receptor–deficient (Ldlr<sup>−/−</sup>) mice, the aortic surface area occupied by atherosclerosis was reduced by 40% compared with that of Ldlr<sup>−/−</sup> mice.<sup>8</sup> In the present study, Vcam1<sup>Δ<sub>D4D</sub></sup> mice were bred with hypercholesterolemic apoE null (Apoe<sup>−/−</sup>) mice<sup>9</sup> to determine whether relative deficiency in VCAM-1 would also attenuate lesion formation in the apoE-deficient background. Mice homozygous for the Vcam1 D4D allele (Apoe<sup>−/−</sup> and Vcam1<sup>Δ<sub>D4D</sub></sup> ) had markedly reduced arterial VCAM-1 expression, monocyte adherence, and an 84% decrease in aortic root lesion area. We also demonstrate a significant Vcam1 gene-dosage effect on these parameters. Our data indicate that endothelial VCAM-1 plays a critical role in monocyte entry into the subendothelial space in early atherogenesis.

Methods

Mice

Because of the high incidence of embryonic lethality of Vcam1<sup>Δ<sub>D4D</sub></sup> mice on the C57BL/6 genetic background,<sup>8</sup> all studies were performed with littermate-controlled outbred mice. Apoe<sup>−/−</sup> mice on an outbred C57BL/6-129 mixed genetic background<sup>8</sup> were first crossed to Vcam1<sup>Δ<sub>D4D</sub></sup> mice on the same outbred background, and progeny were bred back to Apoe<sup>−/−</sup> mice to obtain Apoe<sup>−/−</sup>, Vcam1<sup>Δ<sub>D4D</sub></sup> mice. Apoe<sup>−/−</sup>, Vcam1<sup>Δ<sub>D4D</sub></sup> mice were then intercrossed to obtain the 3 possible Vcam1 genotypes: Vcam1<sup>+/−</sup>, Vcam1<sup>Δ<sub>D4D</sub></sup>, and Vcam1<sup>Δ<sub>D4D</sub></sup>. Because of...
the partially penetrant lethality of mice homozygous for the Vcam1 D4D-targeted allele, we did not obtain a sufficient number of Vcam1D4D/D4D mice from Vcam1D4D/D4D intercrosses. Only ~6% of the progeny were Vcam1D4D/D4D instead of the expected 25%. Therefore, 2 parallel littermate-controlled experiments were established to obtain sufficient numbers of Vcam1D4D/D4D, for cross 1, Vcam1D4D mice were bred with Vcam1D4D mice to generate Vcam1D4D/D4D and Vcam1D4D/D4D littermate mice; for cross 2, Vcam1D4D mice were bred to Vcam1D4D/D4D mice to obtain Vcam1D4D/D4D and Vcam1D4D/D4D littermates. Twenty-five percent of the progeny from cross 2 were ApoeD4D/D4D mice, instead of the 50% expected by mendelian inheritance. Progeny from both crosses were fed a chow diet and were euthanized at 16 weeks of age. Vcam1D4D/D4D mice appeared healthy and were indistinguishable by appearance from the wild-type or heterozygous Vcam1 D4D mice. There were no significant differences in body weight, total cholesterol, HDL cholesterol, or median aortic atherosclerotic lesion area in Vcam1D4D/D4D mice of both sexes from cross 1 compared with sex-matched Vcam1D4D/D4D mice obtained from cross 2 (data not shown). Therefore, data from Vcam1D4D/D4D mice from both crosses were pooled, and these mice are referred to as Vcam1D4D/D4D mice in the tables and figures.

**TABLE 1. Plasma Lipids and Body Weight Measurements in ApoeD4D/D4D Mice**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Genotype</th>
<th>Weight, g</th>
<th>TC, mg/dL</th>
<th>HDL-C, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>ApoeD4D/D4D, Vcam1D4D/D4D</td>
<td>32±3 (14)</td>
<td>483±174 (12)</td>
<td>38±5 (12)</td>
</tr>
<tr>
<td></td>
<td>ApoeD4D/D4D, Vcam1D4D/D4D</td>
<td>31±3 (36)</td>
<td>618±199 (35)</td>
<td>37±7 (35)</td>
</tr>
<tr>
<td></td>
<td>ApoeD4D/D4D, Vcam1D4D/D4D</td>
<td>33±2 (6)</td>
<td>542±179 (4)</td>
<td>36±9 (4)</td>
</tr>
<tr>
<td>Female</td>
<td>ApoeD4D/D4D, Vcam1D4D/D4D</td>
<td>26±2 (14)</td>
<td>509±143 (9)</td>
<td>30±3 (12)</td>
</tr>
<tr>
<td></td>
<td>ApoeD4D/D4D, Vcam1D4D/D4D</td>
<td>26±2 (24)</td>
<td>455±162 (25)</td>
<td>32±1 (25)</td>
</tr>
<tr>
<td></td>
<td>ApoeD4D/D4D, Vcam1D4D/D4D</td>
<td>25±1 (10)</td>
<td>502±152 (5)</td>
<td>34±3 (6)</td>
</tr>
</tbody>
</table>

TC indicates total plasma cholesterol; HDL-C, HDL cholesterol. Values are mean±SD, with the number of mice assayed in parentheses. No statistical differences among genotypes were obtained by ANOVA.

**Leukocytes Counts**

Total blood leukocyte counts were measured with a particle and size analyzer (Beckman Coulter) after red blood cell lysis with Zapto-globin II (Beckman Coulter). The percentage of monocytes was determined by PCR assay as previously described. For immunohistochemistry, the aortic root and arch were stained with a monoclonal rat anti-mouse VCAM-1 antibody (M/K-2, Southern Biotechnology) that does not bind to domain 4. Quantification of the area occupied by CD11a-positive cells was performed by using a monoclonal rat anti-mouse CD11a antibody (2D7, Pharmingen) as described previously. Briefly, hearts and aortic arches were removed after perfusion with PBS and were frozen in OCT embedding medium (VWR International). Aortic root sections (8 μm) were obtained from an anatomically defined area of the aortic root, starting with the appearance of the aortic valve leaflets and ending when the aortic leaflets were no longer visualized. Aortic root and aortic arch sections were fixed with acetone, treated with 0.1% hydrogen peroxide, and blocked with normal goat serum. VCAM-1 (dilution 1:250) or CD11a (dilution 1:100) antibody was applied, and sections were washed with PBS and incubated with a biotinylated rabbit anti-rat mouse- absorbed secondary antibody (1:200, Vector Laboratories). After a wash with PBS, avidin-biotin-complex linked to horseradish peroxidase (Vector-Elite, Vector Labs) was applied, sections were washed with PBS, and peroxidase was detected with NovaRed substrate (Vector Labs). Controls were performed with an irrelevant isotype-matched monoclonal antibody or in the absence of primary antibody, and no background staining was visualized. The area occupied by adherent CD11a mononuclear cells was measured on 1 section per slide for a total of 5 slides per animal. The 5 areas were then averaged. Total plasma cholesterol and HDL cholesterol were isolated and measured as described previously. Statistical Analysis

The Kolmogorov-Smirnov test with the Dalal and Wilkinson approximation was used to determine whether distributions were gaussian. When data were gaussian, ANOVA with a Newman-Keuls multiple comparison test was performed. Atherosclerosis measurements often were not gaussian, so comparisons between group median values were made by using a nonparametric ANOVA (Kruskal-Wallis) with the Dunn multiple comparison post test, or for comparisons of 2 groups, the Mann-Whitney t test was used. Statistics were performed with Prism 3.0 software (GraphPad).

**Results**

We evaluated the role of Vcam1 in early atherogenesis by breeding Vcam1D4D/D4D mice to ApoeD4D/D4D mice, with the subsequent generation of ApoeD4D/D4D mice with 2, 1, or 0 wild-type Vcam1 alleles. All viable mice appeared healthy, and the number of Vcam1 wild-type alleles did not affect body weight, total cholesterol, or HDL cholesterol (Table 1). Total leukocyte count and numbers of circulating monocytes were not altered in mice harboring the D4 mutation (Table 2). The extent of VCAM-1 immunostaining was assessed at lesion-prone sites in the aortic arch of 16-week-old ApoeD4D/D4D mice (Figure 1A through 1C). Atherosclerotic lesions were not present in the aortic arch at this time point, inasmuch as atherosclerosis is temporally delayed in the aortic arch compared with the aortic root. In Vcam1+/+/ mice, VCAM-1 staining in the aortic arch was found primarily on endothelial cells with less abundant staining in the media. VCAM-1

**Pathology and Cholesterol Measurements**

The heart containing the aortic root was processed for the aortic root quantitative atherosclerosis assay as previously described. For immunochemistry, the aortic root and arch were stained with a monoclonal rat anti-mouse VCAM-1 antibody (M/K-2, Southern Biotechnology) that does not bind to domain 4. Quantification of the area occupied by CD11a-positive cells was performed by using a monoclonal rat anti-mouse CD11a antibody (2D7, Pharmingen) as described previously. Briefly, hearts and aortic arches were removed after perfusion with PBS and were frozen in OCT embedding medium (VWR International). Aortic root sections (8 μm) were obtained from an anatomically defined area of the aortic root, starting with the appearance of the aortic valve leaflets and ending when the aortic leaflets were no longer visualized. Aortic root and aortic arch sections were fixed with acetone, treated with 0.1% hydrogen peroxide, and blocked with normal goat serum. VCAM-1 (dilution 1:250) or CD11a (dilution 1:100) antibody was applied, and sections were washed with PBS and incubated with a biotinylated rabbit anti-rat mouse-absorbed secondary antibody (1:200, Vector Laboratories). After a wash with PBS, avidin-biotin-complex linked to horseradish peroxidase (Vector-Elite, Vector Labs) was applied, sections were washed with PBS, and peroxidase was detected with NovaRed substrate (Vector Labs). Controls were performed with an irrelevant isotype-matched monoclonal antibody or in the absence of primary antibody, and no background staining was visualized. The area occupied by adherent CD11a mononuclear cells was measured on 1 section per slide for a total of 5 slides per animal. The 5 areas were then averaged. Total plasma cholesterol and HDL cholesterol were isolated and measured as described previously. Statistical Analysis

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staining of endothelial cells in lesion-prone areas of the aortic arch was gene-dosage dependent. The extent of immunohistochemical staining for VCAM-1 was greatest for Apoe<sup>−/−</sup>, Vcam1<sup>1/2+</sup> mice, intermediate for Apoe<sup>−/−</sup>, Vcam1<sup>1/4D</sup> mice, and nearly absent in Apoe<sup>−/−</sup>, Vcam1<sup>2/4D</sup> mice (Figure 1A through 1C).

Monocyte adhesion to the aortic root was assessed in 8-week-old chow-fed mice at a time point before atherosclerotic lesion development. CD11a immunostaining was performed at the aortic root in male Apoe<sup>−/−</sup> mice of the 3 Vcam1 genotypes. Representative sections of CD11a staining are shown Figure 1D through 1F. No CD11a<sup>+</sup> cells were observed in this Vcam1<sup>2/4D</sup> aortic root section (Figure 1F). Quantification of the area occupied by CD11a<sup>+</sup> cells was performed by using sections from multiple mice from each genotype (Figure 2A). Adherent CD11a<sup>+</sup> cells were detected in only 1 of 8 Apoe<sup>−/−</sup>, Vcam1<sup>1/4D</sup> mice. The area occupied by CD11a-positive cells was significantly higher in Apoe<sup>−/−</sup>,

![Image](https://via.placeholder.com/150)

Figure 1. Immunohistochemical staining for VCAM-1 in representative aortic arch sections (A through C) and CD11a in representative aortic root sections (D through F) from Apoe<sup>−/−</sup>, Vcam1<sup>1/4+</sup> (A and D), Apoe<sup>−/−</sup>, Vcam1<sup>1/2D</sup> (B and E), and Apoe<sup>−/−</sup>, Vcam1<sup>1/4D</sup> (C and F) mice (left, original magnification ×40; right, original magnification ×100). The extent of endothelial VCAM-1 staining (red color) at lesion-prone areas was greatest for Apoe<sup>−/−</sup>, Vcam1<sup>1/4+</sup>, intermediate for Apoe<sup>−/−</sup>, Vcam1<sup>1/2D</sup>, and lowest for Apoe<sup>−/−</sup>, Vcam1<sup>1/4D</sup> mice (n=4 of each genotype). CD11a<sup>+</sup> adherent cells are noted (arrowheads) in aortic root sections taken from Apoe<sup>−/−</sup>, Vcam1<sup>1/4+</sup> (D) and Apoe<sup>−/−</sup>, Vcam1<sup>1/2D</sup> (E) mice, but CD11a<sup>+</sup> cells were absent from nearly all sections from Apoe<sup>−/−</sup>, Vcam1<sup>1/4D</sup> mice (F).

![Image](https://via.placeholder.com/150)

Figure 2. Effect of VCAM-1 on monocyte adherence and atherosclerotic lesion area in apoE<sup>−/−</sup> mice. A, Quantification of CD11a<sup>+</sup> mononuclear cells bound to the aortic root endothelium of male Vcam1<sup>+/+</sup> (n=5), Vcam1<sup>1/4D</sup> (n=16), and Vcam1<sup>2/4D</sup> (n=8) Apoe<sup>−/−</sup> mice. Values are mean±SD. B and C, Aortic root atherosclerotic lesion areas in male (B) and female (C) Apoe<sup>−/−</sup> mice according to Vcam1 genotype.

Vcam1<sup>1/2+</sup> mice and Apoe<sup>−/−</sup>, Vcam1<sup>1/4D</sup> mice than in Apoe<sup>−/−</sup>, Vcam1<sup>2/4D</sup> mice (Figure 2A). Linear regression analysis revealed a significant correlation between the areas of CD11a<sup>+</sup> cells and Vcam1 genotype (r<sup>2</sup>=0.24, P<0.01), suggesting an overall Vcam1 gene-dosage–dependent effect on mononuclear cell adherence. This suggests that VCAM-1 plays an important role in monocyte adherence to arterial endothelium during early atherogenesis. This is consistent with the observation that antibody blockade of VCAM-1

TABLE 2. Leukocyte Counts, Monocyte Counts, and Percentage of Monocytes in Peripheral Blood in Apoe<sup>−/−</sup> Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Leukocytes, 10&lt;sup&gt;6&lt;/sup&gt;/mL</th>
<th>Monocytes, 10&lt;sup&gt;6&lt;/sup&gt;/mL</th>
<th>Monocytes, % Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;, Vcam1&lt;sup&gt;1/4+&lt;/sup&gt;</td>
<td>18.4±2.8</td>
<td>1.2±0.6</td>
<td>6.5±2.6</td>
</tr>
<tr>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;, Vcam1&lt;sup&gt;1/2D&lt;/sup&gt;</td>
<td>15.0±4.3</td>
<td>0.8±0.3</td>
<td>5.4±2.0</td>
</tr>
<tr>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;, Vcam1&lt;sup&gt;1/4D&lt;/sup&gt;</td>
<td>14.4±1.3</td>
<td>0.9±0.3</td>
<td>6.5±1.8</td>
</tr>
</tbody>
</table>

Values are mean±SD (n=5). No statistical differences among genotypes were obtained by ANOVA.

A, Percentages of leukocytes, monocytes, and monocytes obtained by ANOVA. (1C).
Figure 3. Representative oil red O staining for lipid in aortic root sections from male 16-week-old chow-fed Apoe<sup>–/–</sup>, Vcam1<sup>1/1</sup> mice (A and B), Apoe<sup>–/–</sup>, Vcam1<sup>1/0D</sup> mice (C and D), and Apoe<sup>–/–</sup>, Vcam1<sup>1/1D</sup> mice (E and F). Images of the aortic root were taken at low and intermediate magnification (×4 objective on the left and ×20 objective on the right, respectively).

function reduced mononuclear cell adhesion by 75% in isolated-perfused carotid arteries from Apoe<sup>–/–</sup> mice.14

A Vcam1 gene-dosage–dependent effect on atherosclerotic plaque formation was noted in mice of both sexes (Figure 2). In males, there was a 54% decrease in median lesion area in Apoe<sup>–/–</sup>, Vcam1<sup>1/0D</sup> mice and a 74% decrease in Apoe<sup>–/–</sup>, Vcam1<sup>1/1D</sup> mice compared with the median lesion area in Apoe<sup>–/–</sup>, Vcam1<sup>1/1</sup> mice (Figure 2B). In females, there was a 55% decrease in median lesion area in Apoe<sup>–/–</sup>, Vcam1<sup>1/0D</sup> mice and an 89% decrease in Apoe<sup>–/–</sup>, Vcam1<sup>1/1D</sup> mice (Figure 2C). Because there were no significant differences in lesion size between male and female mice of a given Vcam1 genotype, male and female data were combined. In the combined sex groups, the median lesion areas were 37×10<sup>3</sup>, 17×10<sup>3</sup>, and 6×10<sup>3</sup> µm<sup>2</sup> for Apoe<sup>–/–</sup>, Vcam1<sup>1/1</sup> mice, Apoe<sup>–/–</sup>, Vcam1<sup>1/0D</sup> mice, and Apoe<sup>–/–</sup>, Vcam1<sup>1/1D</sup> mice, respectively (P<0.001 for Vcam1<sup>1/0D</sup> versus Vcam1<sup>1/1</sup>, P<0.01 for Vcam1<sup>1/1D</sup> versus Vcam1<sup>1/0D</sup>, and P<0.05 for Vcam1<sup>1/0D</sup> versus Vcam1<sup>1/1D</sup>). Thus, the presence of 1 and 2 Vcam1 D4D alleles was associated with significant (56% and 84%, respectively) reductions in lesion areas. These data reveal a robust Vcam1 gene-dosage effect on lesion cross-sectional area.

Microscopic examination of atherosclerotic lesions revealed that the majority of the lesions in mice with 2, 1, or 0 wild-type Vcam1 alleles were fatty streaks composed of macrophage foam cells (Figure 3). Lesions in Apoe<sup>–/–</sup>, Vcam1<sup>1/0D</sup> mice were limited to very small nascent fatty streak lesions (Figure 3E and 3F), and fatty streak lesions of progressively increased size were noted in Apoe<sup>–/–</sup>, Vcam1<sup>1/1D</sup> mice (Figure 3C and 3D) and in Apoe<sup>–/–</sup>, Vcam1<sup>1/1</sup> mice (Figure 3A and 3B). The extent of VCAM-1 immunostaining was also assessed in aortic root fatty streak lesions of 20-week-old Apoe<sup>–/–</sup> mice. In Vcam1<sup>1/1</sup> mice, VCAM-1 staining was observed on the endothelium and even more prominently within the intimal lesions (Figure 4A). In Vcam1<sup>1/1D</sup> mice, aortic lesions were smaller, and VCAM-1 staining was predominantly observed on the endothelium but also visible in the intima (Figure 4B). In the lesion from a Vcam1<sup>1/1D</sup> mouse shown in Figure 4C, VCAM-1 staining was not visualized in lesions from an Apoe<sup>–/–</sup>, Vcam1<sup>1/1</sup> mouse (A). Less abundant staining was found in lesions from an Apoe<sup>–/–</sup>, Vcam1<sup>1/1D</sup> mouse (B), and no staining was visualized in lesions from an Apoe<sup>–/–</sup>, Vcam1<sup>1/0D</sup> mouse (C).

Discussion

The pathogenesis of early atherosclerosis in Apoe<sup>–/–</sup> mice involves the accumulation of lipoprotein aggregates in the subendothelial space,15 upregulation of specific endothelial adhesion molecules and chemokines,1,2,16,17 monocyte recruitment, and subsequent foam cell formation.18 Deficiencies of P-selectin, E-selectin, and ICAM-1 have been shown to decrease atherosclerosis in hypercholesterolemic mice,19–23 although a gene-dosage–dependent effect has not been documented for any of these deficiencies, and results in ICAM-1–deficient mice have not been consistent.8,24 Embryonic demise of Vcam1 null mice6,7 has made it difficult to study the role of VCAM-1 in atherogenesis with the use of mutant mouse models. Cybulsky et al8 created Vcam1 D4D mice that...
had dramatic decreases in VCAM-1 expression and an improved survival rate compared with those in Vcam1 null mice. The effect of very low levels of VCAM-1 expression on survival was strain dependent, with very low rates of survival of C57BL/6 D4D homozygous mice and better survival rates of D4D homozygous mice on a mixed genetic background.

To generate sufficient numbers of mice for atherosclerosis studies, we bred C57BL/6-129 hybrid Vcam1/D4D mice onto the apoE-deficient background to determine the effect of decreased VCAM-1 expression on atherosclerosis.

Vcam1 gene dosage affected endothelial VCAM-1 expression and the subsequent monocyte adherence to lesion-prone areas of the arterial wall. Decreased VCAM-1 expression in Vcam1DN/DND mice resulted in an overall 84% decrease in fatty streak formation in the aortic root. Cybulsky et al.8 reported a 40% decrease in the percentage of aortic surface area occupied by lesions in Ldr−/−, Vcam1/D4D homozygous mice. There are several potential differences that may explain the varying magnitude of reduction of atherosclerosis between these 2 studies. The varying lipoprotein profiles in Ldr−/− and Apoe−/− mice may have different downstream effects on lipoprotein deposition and endothelial activation.

The high-fat high-cholesterol diet used in the study by Cybulsky et al might have induced a VCAM-1–independent inflammatory response that may not be present in the chow-fed Apoe−/− mice used in the present study. In addition, Cybulsky et al measured atherosclerosis as the percentage of the entire aorta occupied by lesions, whereas in the present study, atherosclerosis was assessed in cross sections through the aortic root. Taken together, these studies demonstrate that arterial expression of VCAM-1 plays an important role in atherosclerotic lesion formation in the context of varying lipoprotein profiles and at multiple sites in the vasculature. The interanimal variation in aortic root lesion size within each genotype may have been partly due to genetic heterogeneity. Despite this variation, the Vcam1 genotype had a highly significant effect on atherosclerosis.

The reduction in endothelial VCAM-1 expression most likely led to a decrease in monocyte adhesion and fatty streak formation in the Vcam1DN/DND mice. An alternative explanation for these findings is that decreased VCAM-1 expression affected the number of circulating monocytes and, by this mechanism, attenuated atherogenesis. There are several reasons why we do not favor this alternative explanation. It has been previously shown that the D4D mutation does not affect sons why we do not favor this alternative explanation. It has been previously shown that the D4D mutation does not affect the number of circulating monocytes and, by this mechanism, attenuated atherogenesis. There are several potential differences that may explain the varying magnitude of reduction of atherosclerosis between these 2 studies. The varying lipoprotein profiles in Ldr−/− and Apoe−/− mice may have different downstream effects on lipoprotein deposition and endothelial activation.

Acknowledgments

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References


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