A major role for VCAM-1, but not ICAM-1, in early atherosclerosis

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VCAM-1 and ICAM-1 are endothelial adhesion molecules of the Ig gene superfamily that may participate in atherogenesis by promoting monocyte accumulation in the arterial intima. Both are expressed in regions predisposed to atherosclerosis and at the periphery of established lesions, while ICAM-1 is also expressed more broadly. To evaluate functions of VCAM-1 in chronic disease, we disrupted its fourth Ig domain, producing the murine Vcam1D4D allele. VCAM-1D4D mRNA and protein were reduced to 2–8% of wild-type allele (Vcam1+) levels but were sufficient to partially rescue the lethal phenotype of VCAM-1–null embryos. After crossing into the LDL receptor–null background, Vcam1+/+ and Vcam1D4D/D4D paired littermates were generated from heterozygous intercrosses and fed a cholesterol-enriched diet for 8 weeks. The area of early atherosclerotic lesions in the aorta, quantified by en face oil red O staining, was reduced significantly in Vcam1D4D/D4D mice, although cholesterol levels, lipoprotein profiles, and numbers of circulating leukocytes were comparable to wild-type. In contrast, deficiency of ICAM-1 either alone or in combination with VCAM-1 deficiency did not alter nascent lesion formation. Therefore, although expression of both VCAM-1 and ICAM-1 is upregulated in atherosclerotic lesions, our data indicate that VCAM-1 plays a dominant role in the initiation of atherosclerosis.

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A major role for VCAM-1, but not ICAM-1, in early atherosclerosis

Myron I. Cybulsky,1 Kaeko Iiyama,1 Hongmei Li,2 Suning Zhu,1 Mian Chen,1 Motoi Iiyama,1 Vannessa Davis,2 Jose-Carlos Gutierrez-Ramos,3 Philip W. Connelly,4 and David S. Milstone2

1Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto General Research Institute, Toronto, Ontario, Canada
2Vascular Research Division, Departments of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts, USA
3Millennium Pharmaceuticals Inc., Cambridge, Massachusetts, USA
4Departments of Laboratory Medicine and Pathobiology, Medicine, and Biochemistry, University of Toronto, St. Michael’s Hospital, Toronto, Ontario, Canada

Address correspondence to: Myron I. Cybulsky, Toronto General Research Institute, 200 Elizabeth Street, CCRW 1-855, Toronto, Ontario, Canada, M5G 2C4. Phone/Fax: (416) 340-3578; E-mail: myron.cybulsky@utoronto.ca.

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VCAM-1 and ICAM-1 are endothelial adhesion molecules of the Ig gene superfamily that may participate in atherogenesis by promoting monocyte accumulation in the arterial intima. Both are expressed in regions predisposed to atherosclerosis and at the periphery of established lesions, while ICAM-1 is also expressed more broadly. To evaluate functions of VCAM-1 in chronic disease, we disrupted its fourth Ig domain, producing the murine Vcam1D4D allele. VCAM-1D4D mRNA and protein were reduced to 2–8% of wild-type allele (Vcam1+) levels but were sufficient to partially rescue the lethal phenotype of VCAM-1-null embryos. After crossing into the LDL receptor–null background, Vcam1+/+ and Vcam1D4D/D4D paired littermates were generated from heterozygous intercrosses and fed a cholesterol-enriched diet for 8 weeks. The area of early atherosclerotic lesions in the aorta, quantified by en face oil red O staining, was reduced significantly in Vcam1D4D/D4D mice, although cholesterol levels, lipoprotein profiles, and numbers of circulating leukocytes were comparable to wild-type. In contrast, deficiency of ICAM-1 either alone or in combination with VCAM-1 deficiency did not alter nascent lesion formation. Therefore, although expression of both VCAM-1 and ICAM-1 is upregulated in atherosclerotic lesions, our data indicate that VCAM-1 plays a dominant role in the initiation of atherosclerosis.

superfamily adhesion molecules in the formation of early atherosclerotic lesions throughout the entire aorta of LDL receptor–null (Ldlr–/–) mice.

Methods

Animals. Mice were group-housed and fed standard chow in a specific pathogen-free environment in accordance with all institutional and governmental guidelines.

Production of VCAM-1 D4D mice. To replace the 5′ portion of Vcam1 exon 6 (encoding Ig domain 4) and the adjacent intron (Figure 1), a targeting construct included the murine phosphoglycerate kinase-1 promoter/neomycin resistance gene (NEO) in the opposite transcriptional orientation and 7.1 kb of 5′ and 1.7 kb of 3′ homology. A mutant polyoma virus enhancer/herpes simplex virus thymidine kinase (TK) gene allowed selection against random integration. Electroporation and drug selection of J1 ES cell clones were performed as described previously (15). Germline transmission was achieved with chimeric mice derived from two of six targeted ES cell lines. Both lines of mice had indistinguishable phenotypes.

Genotyping. Mice were genotyped by Southern blotting (Figure 1, b and c) or PCR. DNA was isolated from tail biopsies (18) with or without (for PCR) isopropanol precipitation. PCR products were 300 and 500 bp for Vcam1+ and Vcam1D4D alleles. A common forward primer (5′-ATACATTGGGTATGGTGTGATATG) was used that bound to the 3′ end of exon 5 [phosphatidylinositol-associated (PI) domain]. The wild-type allele reverse primer (5′-AACTTAAAATCCATTGTTCATAGG) recognized an intron replaced by NEO in the mutant allele. The mutant allele reverse primer (5′-CAAGCAAAACCAAATTAAGGG) recognized the 3′ untranslated region of phosphoglycerate kinase-1 in NEO. PCR conditions were (94°C/90 s, 56°C/60 s, 72°C/120 s) × 3, (94°C/30 s, 56°C/30 s, 72°C/60 s) × 27, and 72°C/7 min.

Flow cytometry. Heparinized blood was obtained from mice by cardiac puncture and 50 µl was incubated with 50 µl PBS containing Fc block (anti-mouse CD16/CD32) and rat IgG for 20 minutes at 22°C. Primary antibodies (rat monoclonal) were incubated for 30 minutes at 22°C. They included biotin-conjugated anti-CD19, fluorescein-conjugated anti-CD3, fluorescein-conjugated anti-GR1, and phycoerythrin-conjugated anti-CD11b (BD PharMingen, San Diego, California, USA). Cells were washed with PBS containing 2.5% FBS, and incubated with streptavidin-fluorochrome if required (4°C for 30 minutes), and erythrocytes were removed by hypotonic lysis. Flow cytometry was carried out immediately after the final wash using an Epics XL-MCL flow cytometer (Coulter Corp., Miami, Florida, USA).

Northern blotting. Total RNA (10 µg) isolated by ultracentrifugation through a cesium chloride gradient or mRNA (3 µg) purified using oligo d(T) cellulose was analyzed by Northern blotting (19).

RT-PCR. RT (SuperScript kit; Life Technologies Inc., Rockville, Maryland, USA) for alternatively spliced 7 and 6 Ig VCAM-1 used a primer complementary to...
GATTGAATTACGAGGG in D5. For 3 Ig VCAM-1, the RT primer was complementary to CATCATTTG-CATGGGGTCA in the PI domain. PCR reactions [(94°C/1 s, 92°C/29 s, 50°C/30 s, 72°C/60 s) × 35] used a common forward primer CAATGACCTGTTCCAGCGAG from D3 and domain-specific reverse primers complementary to CCAAATCCACGCTTGTGTT (in D4), CAAGCATTCCCTGAAGATCC (spanning the junction of D3–D5), and CTTGCTCTGTGAG-GAAGCTG (in the PI domain). The 382-, 217-, and 319-bp products for 7, 6, and 3 Ig VCAM-1, respectively, were resolved on 1.5% agarose gels.

**Immunoprecipitation and immunohistochemistry.** Enzymatically isolated lung cells (20) were depleted of macrophages using anti–Fc receptor magnetic beads. For this purpose, rat monoclonal antibodies to mouse Fcγ III and II receptors (CD16 and CD32) (PharMingen, San Diego, California, USA) were bound to M-450 magnetic beads coupled to sheep anti-rat IgG antibodies (Dynal Biotech Inc., Lake Success, New York, USA). Cells from subculture 2 were treated with media or murine TNF-α (20 ng/ml) for 4 hours, and cell-surface proteins were biotinylated (21). VCAM-1 and ICAM-1 proteins were quantitatively immunoprecipitated from centrifugation-cleared Triton X-100 whole cell lysates using monoclonal antibodies (rat IgG1) MK-2 and YN1.1, respectively, and goat anti-rat IgG-sepharose. After SDS-PAGE and transfer to polyvinylidene difluoride membranes, biotinylated cell-surface proteins were visualized using peroxidase-conjugated streptavidin and chemiluminescent emission (Amersham Pharma-acia Biotech Ltd., Piscataway, New Jersey, USA) and quantified using a Molecular Dynamics (Sunnyvale, California, USA) densitometer. Immunoperoxidase staining of cryostat-cut sections (15) used MK-2 and YN1.1, with nonimmune rat IgG as the negative control.

**Atherosclerosis studies.** Vcam1D4D/D4D and Ldlr–/– (22) mice were bred and their progeny were bred back to Ldlr–/– to obtain Vcam1+/D4DLdlr–/– mice. These were intercrossed to obtain sex-matched paired littermates (Vcam1+/+Ldlr–/– and Vcam1D4D/D4DLdlr–/–) for experiments. Icam1–/– mice (23) were backcrossed into the Ldlr–/– background and intercrossed in a similar fashion. C57BL/6-129 hybrid mice were used because the viability of C57BL/6 Vcam1D4D/D4D embryos was very low (see Results). Also, recombination between Icam1 and Ldlr alleles, which are within 3 centimorgans on mouse chromosome 9 (24), could not be achieved in the C57BL/6 background (not shown).

Paired littermates were fed a semipurified cholate-free diet (no. D12108; Research Diets Inc., New Brunswick, New Jersey, USA) containing 40% kcal lipid, 1.25% cholesterol (25). After 8 weeks of the cholesterol-enriched diet, blood was obtained from the retroorbital plexus or by cardiac puncture and aortae were isolated. The percent of the surface area occupied by atherosclerotic lesions in the whole aorta and in specific regions (aortic arch, descending thoracic aorta, and abdominal aorta) was quantified by en face oil red O staining as described (25). Serum cholesterol levels and leukocyte counts (hemocytometer and differentials) were determined for each mouse. Plasma lipoprotein profiles were determined by gel filtration fast performance liquid chromatography (25) from random animals in each group.

### Table 1

Embryonic survival of VCAM-1–deficient mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Vcam1 genotype:</th>
<th>Live births</th>
<th>Expected births</th>
<th>Embryonic survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/-</td>
<td>+/D4D</td>
<td>D4D/D4D</td>
<td>D4D/D4D</td>
</tr>
<tr>
<td>129-BALB/c and 129-C57BL/6+/+</td>
<td>217</td>
<td>431</td>
<td>62</td>
<td>216</td>
</tr>
<tr>
<td>BALB/c (BC# 6 and 7)</td>
<td>155</td>
<td>245</td>
<td>33</td>
<td>133</td>
</tr>
<tr>
<td>BALB/c (BC# 9, 10, and 11)</td>
<td>243</td>
<td>436</td>
<td>54</td>
<td>226</td>
</tr>
<tr>
<td>129/SvEv</td>
<td>59</td>
<td>137</td>
<td>4</td>
<td>65</td>
</tr>
<tr>
<td>C57BL/6 (BC# 4, 5, and 8)</td>
<td>67</td>
<td>93</td>
<td>3</td>
<td>53</td>
</tr>
</tbody>
</table>

*Vcam1D4D/D4D* embryonic survival was comparable in mixed 129-BALB/c and 129-C57BL/6 strains, and the data were pooled. BC, backcross.

### Table 2

Total and differential leukocyte counts in VCAM-1–deficient mice and littermate controls maintained on a standard diet without elevated cholesterol

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total ± SEM</th>
<th>Monocytes ± SEM</th>
<th>Lymphocytes ± SEM</th>
<th>Neutrophils ± SEM</th>
<th>Eosinophils ± SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vcam1+/+</td>
<td>6.7 ± 0.9</td>
<td>0.3 ± 0.1</td>
<td>5.2 ± 0.7</td>
<td>1.2 ± 0.2</td>
<td>0.06 ± 0.04</td>
<td>6</td>
</tr>
<tr>
<td>Vcam1+/D4D</td>
<td>6.1 ± 1.0</td>
<td>0.4 ± 0.1</td>
<td>5.0 ± 0.7</td>
<td>0.8 ± 0.2</td>
<td>0.02 ± 0.01</td>
<td>6</td>
</tr>
<tr>
<td>Vcam1D4D/D4D</td>
<td>5.8 ± 0.9</td>
<td>0.6 ± 0.1</td>
<td>4.4 ± 0.7</td>
<td>0.8 ± 0.3</td>
<td>0.07 ± 0.02</td>
<td>6</td>
</tr>
</tbody>
</table>

Hybrid 129-C57BL/6 and 129-BALB/c strain mice, age 2–4 months, were used. Values are expressed as mean ± SEM of cells per milliliter of blood × 10⁶. Differential counts were based on 200 cells evaluated in each blood film. *P < 0.05 versus Vcam1+/+ and Vcam1+/D4D groups (Fisher’s PLSD).
Table 3
Flow cytometry analysis of blood leukocytes in VCAM-1-deficient mice and littermate controls maintained on a standard diet without elevated cholesterol

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total</th>
<th>Monocytes (CD11b+, GR1−)</th>
<th>T Lymphocytes (CD3+, CD19+)</th>
<th>B Lymphocytes (CD19+, CD3−)</th>
<th>Neutrophils (CD11b+, GR1+high)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vcam1+/+</td>
<td>4.2 ± 0.7</td>
<td>0.4 ± 0.04</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>7</td>
</tr>
<tr>
<td>Vcam1D4D/D4D</td>
<td>6.9 ± 0.8</td>
<td>(14.1 ± 1.2%)</td>
<td>(32.7 ± 3.2%)</td>
<td>(25.4 ± 3.5%)</td>
<td>(17.4 ± 2.6%)</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of cells per ml of blood (×10⁶), with percent of total cells in parentheses. BALB/c strain mice with a mean age of 8 months. *P < 0.005 (paired t test). **P < 0.05 (paired t test).

Statistics. A paired Student’s t test (Startview 4.5; Abacus Concepts Inc., San Francisco, California, USA) was used when data were obtained from paired littermates. Data from multiple groups were analyzed by ANOVA followed by Fisher’s protected least significant difference (PLSD) test to calculate pairwise comparisons.

Results

Of three distinct VCAM-1 polypeptides (Figure 1a) derived by alternative splicing (26, 27), the predominant form in mice and humans contains seven Ig domains and two α4 integrin–binding sites, in D1 and D4 (28, 29). By disrupting D4 we generated mice that expressed only alternatively spliced VCAM-1 with one ligand-binding site. Embryonic survival of Vcam1D4D/D4D mice was reduced and strain-dependent: 29% in hybrid 129-C57BL/6 and 129-BALB/c, 24% in BALB/c, 6% in C57BL/6, and 6% in 129 (Table 1). At 9.5 and 12.5 days gestation, more than 50% of Vcam1D4D/D4D embryos displayed chorioallantoic fusion defects and embryonic demise similar to that found in Vcam1+/− mice (15).

Viable Vcam1D4D/D4D mice were healthy and fertile and had normal organs and body weight. This is interesting in light of the potential roles proposed for VCAM-1 and its ligand α4β1 integrin in embryonic development, including maintenance of pericardial integrity (17), myogenesis (30), and hematopoiesis (31–33). The number of blood leukocytes obtained at age 2–4 months from 129-C57BL/6 and 129-BALB/c Vcam1D4D/D4D mice was comparable to Vcam1+/+ and Vcam1+/− littermates, but the differential revealed a small yet significant elevation in monocytes (Table 2). In somewhat older animals (average age 8 months) backcrossed over six times into the BALB/c background, blood leukocytes were elevated 1.6-fold in Vcam1D4D/D4D mice (Table 3). Flow cytometry showed that the percent of different leukocyte types was comparable, but absolute numbers of monocytes, T lymphocytes, and B lymphocytes were elevated, whereas the number of neutrophils was not significantly different (Table 3). In very old BALB/c-strain Vcam1D4D/D4D mice (average age 15 months, range 8–19 months), blood leukocytes were also elevated by 1.4-fold (9.7 ± 0.7 × 10⁶ versus 6.9 ± 0.7 × 10⁶ cells/ml, P < 0.05, n = 6) due to elevated mononuclear leukocytes. These data suggest that strain rather than age differences cooperate with adhesion molecule deficiency to account for elevated circulating mononuclear leukocytes in BALB/c Vcam1D4D/D4D mice. Previously, in vitro studies using Dexter-type long-term bone marrow cultures derived from Vcam1D4D/D4D, Vcam1+/−, and Vcam1+/+ littermates demonstrated comparable stromal cell composition and myeloid differentiation, as well as similar flow cytometry profiles of bone marrow, blood, and lymphoid organs (34). VCAM-1 is expressed in lymphoid follicular dendritic cells and may participate in the

Table 4
Age, body weight, total serum cholesterol, and leukocyte counts in adhesion molecule-deficient and control mice in the LDLr−/− background determined after feeding a cholesterol-enriched diet for 8 weeks

<table>
<thead>
<tr>
<th>Genotype (all LDLr−/−)</th>
<th>Aortae analyzed</th>
<th>Sex (M/F)</th>
<th>Age (weeks)</th>
<th>Body weight (g)</th>
<th>Cholesterol (mmol/l)</th>
<th>Total</th>
<th>Mono.</th>
<th>Lym.</th>
<th>Neut.</th>
<th>Eos.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vcam1+/+</td>
<td>12</td>
<td>4/8</td>
<td>17 ± 2</td>
<td>24.3 ± 2.5</td>
<td>15.7 ± 3.8</td>
<td>4.8 ± 0.8</td>
<td>0.5 ± 0.1</td>
<td>3.7 ± 0.7</td>
<td>0.6 ± 0.2</td>
<td>0.07 ± 0.03</td>
<td>11</td>
</tr>
<tr>
<td>Vcam1D4D/D4D</td>
<td>12</td>
<td>3/9</td>
<td>17 ± 2</td>
<td>22.0 ± 1.9</td>
<td>23.6 ± 2.4</td>
<td>4.5 ± 1.1</td>
<td>0.4 ± 0.1</td>
<td>3.5 ± 0.8</td>
<td>0.5 ± 0.1</td>
<td>0.09 ± 0.04</td>
<td>10</td>
</tr>
<tr>
<td>Icam1+/+</td>
<td>8</td>
<td>4/4</td>
<td>31 ± 2</td>
<td>32.5 ± 2.5</td>
<td>27.4 ± 1.3</td>
<td>4.8 ± 1.0</td>
<td>0.3 ± 0.1</td>
<td>3.1 ± 0.8</td>
<td>1.3 ± 0.2</td>
<td>0.07 ± 0.03</td>
<td>8</td>
</tr>
<tr>
<td>Icam1+/−</td>
<td>8</td>
<td>4/4</td>
<td>30 ± 2</td>
<td>21.4 ± 1.5</td>
<td>17.1 ± 3.9</td>
<td>4.1 ± 1.0</td>
<td>0.5 ± 0.2</td>
<td>2.9 ± 0.7</td>
<td>0.6 ± 0.1</td>
<td>0.10 ± 0.03</td>
<td>11</td>
</tr>
<tr>
<td>Vcam1+/+Icam1+/+</td>
<td>11</td>
<td>3/8</td>
<td>15 ± 1</td>
<td>21.6 ± 0.6</td>
<td>16.8 ± 2.5</td>
<td>4.8 ± 0.7</td>
<td>0.5 ± 0.1</td>
<td>3.1 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>0.04 ± 0.02</td>
<td>12</td>
</tr>
<tr>
<td>Vcam1D4D/D4DIcam1+/−</td>
<td>12</td>
<td>3/9</td>
<td>15 ± 1</td>
<td>20.0 ± 0.7</td>
<td>8.2 ± 1.6</td>
<td>10.9 ± 2.0</td>
<td>1.5 ± 0.5</td>
<td>7.0 ± 1.2</td>
<td>2.1 ± 0.4</td>
<td>0.19 ± 0.07</td>
<td>12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. 129-C57BL/6 strain mice were used. Blood samples were obtained after 8 weeks of cholesterol diet feeding, and immediately prior to perfusion-fixation of aortae. The age and body weight of mice at this time point are indicated. Leukocytes are expressed as cells per milliliter of blood (×10⁶). Differentials were based on 200 cells evaluated in each blood film. *P < 0.005 (paired t test). **P < 0.05 (Fisher’s PLSD). Mono., monocytes; Lym., lymphocytes; Neut., neutrophils; Eos., eosinophils.
The spleen, lymph nodes, and Peyer’s patches displayed comparable weight (e.g., spleen: 0.14 ± 0.07 g Vcam1D4D/D4D vs. 0.11 ± 0.01 g Vcam1+/+) and numbers of T, B, and myeloid cells, determined by flow cytometry (not shown).

Constitutive and inducible VCAM-1 mRNA and protein expression in Vcam1D4D/D4D mice was reduced to less than 10% of wild-type, whereas ICAM-1 was unaltered (Figure 2). The predominant mRNA transcript corresponded to 6 Ig VCAM-1 and was only 2–8% as abundant as 7 Ig VCAM-1 in wild-type littermates (Figure 2a). Northern blots and RT-PCR (Figure 2b) did not detect 7 Ig VCAM-1 in Vcam1D4D/D4D mice. Immunoprecipitation revealed reduced (7–8% band density) TNF-α-inducible expression of 6 Ig VCAM-1 (80–85 kDa) in Vcam1D4D/D4D cultured lung cells (Figure 2c). Immunostaining of Vcam1D4D/D4D tissues 4 hours after endotoxin injection (50 µg intraperitoneally) showed markedly reduced VCAM-1 and normal ICAM-1 expression. Weak VCAM-1 staining was detected in endothelium of some blood vessels (not shown).

Atherosclerosis studies were carried out after breeding 129-C57BL/6 VCAM-1– and ICAM-1–deficient mice into the Ldlr–/– background and feeding a cholesterol-enriched diet for 8 weeks. In all genotypes, the body weight of mice was comparable, as was the extent of hypercholesterolemia except for mice with combined VCAM-1 and ICAM-1 deficiency (Table 4). The plasma lipoprotein profile, which we reported previously (25), was similar in all genotypes (not shown). Circulating leukocytes were comparable in hypercholesterolemic Vcam1D4D/D4D and Vcam1+/+ controls (Table 4), similar to normocholesterolemic mice that were 129-C57BL/6 or 129-BALB/c strain hybrids (Table 2), and distinct from mice in the BALB/c background (Table 3), which was not used for atherosclerosis studies. The numbers of circulating monocytes, lymphocytes, and eosinophils were also comparable in mice with ICAM-1 deficiency, but neutrophils were elevated (Table 4), consistent with previous reports (23, 36). Circulating monocytes, lymphocytes, and neutrophils were elevated in mice with combined VCAM-1 and ICAM-1 deficiency (Table 4).

Atherosclerotic lesions in aortae of Ldlr–/– mice fed a cholesterol-enriched diet for 8 weeks consisted of macrophage foam cell–rich fatty streaks. Immunostaining of lesions in the aortic arches of Vcam1D4D/D4D Ldlr–/– mice showed markedly reduced staining for VCAM-1 and strong ICAM-1 staining, whereas ICAM-1 immunostaining was not detected in lesions from Icam1–/– Ldlr–/– mice (not shown). En face oil red O staining demonstrated that these lesions were most prevalent in the lesser curvature of the ascending aorta and arch. The distribution of lesions
in mice deficient in adhesion molecules was identical (Figure 3). Data from male and female mice were combined, because differences in lesion area were not found between sexes. The surface area occupied by lesions was reduced by 40% in Vcam1D4/D4D Ldlr−/− mice (Figure 4). This effect was prominent in the arch, but differences were not significant in the descending thoracic and abdominal aorta because the extent of lesions in this region was relatively low (<2% of surface area) and variability was high. Interanimal variability within each genotype may have been partly the result of genetic background heterogeneity but was unavoidable (see Methods).

The area of lesions was not reduced in aortae or any aortic region of Icam1+/−Ldlr−/− mice compared with Icam1+/+Ldlr−/− sex-matched littermates (Figure 4b) and was similar to that of Vcam1+/+ mice in Figure 4a, supporting the reproducibility of the model. The effect of combined VCAM-1 and ICAM-1 deficiency was assessed by crossing Vcam1D4/D4D Ldlr−/− with Icam1+/− Ldlr−/− mice and intercrossing the progeny to produce Vcam1D4/D4D and Vcam1+/+ litter-mates in the Icam1−/−Ldlr−/− background. Vcam1+/+Icam1+/+Ldlr−/− mice derived from this breeding served as a control group but were not littermates. The whole aorta and arch lesion area in Vcam1D4/D4DIcam1+/−Ldlr−/− mice was reduced by 31% and 45%, respectively, versus Vcam1+/+Icam1−/−Ldlr−/− littermates, and by 48% and 38%, respectively, versus the Vcam1+/+Icam1+/+Ldlr−/− group (Figure 4c). These reductions in lesion area were comparable to those found with VCAM-1 deficiency alone (Figure 4a). These data should be interpreted with caution, because significant differences in total serum cholesterol and circulating leukocytes were found in Vcam1D4/D4DIcam1+/−Ldlr−/− mice (Table 4). ICAM-1 deficiency did not alter atherosclerotic lesion area in any of our experiments (Figure 4, b and c).
Discussion

We developed mice that express 6 Ig VCAM-1 (with one ligand-binding site) at levels less than 10% of wild-type, and used them to directly compare the roles of VCAM-1 and ICAM-1 in atherosclerosis. Our data indicate that deficiency of VCAM-1 significantly diminishes early foam cell lesion formation throughout the aorta of Ldlr−/− mice, and suggest that VCAM-1 has a major role in the initiation of atherosclerosis. The expression of VCAM-1 in our model was markedly reduced but not abolished, and it is possible that complete deficiency of VCAM-1 may have caused even more dramatic effects. Our data are consistent with a recent study by Dansky et al. (37). These investigators bred our VCAM-1 mutant mice into the Apoe−/− background and demonstrated marked reductions in aortic root lesions, which were critically dependent on Vcam1D4D gene dosage.

ICAM-1, an adhesion molecule with similar structure, function, and expression patterns, may compensate for VCAM-1 or cooperate with it in various inflammatory conditions, as is the case with P- and E-selectin (4). However, this was not observed in our experiments. Instead, we found that ICAM-1 deficiency did not influence early foam cell lesion formation either alone or when combined with VCAM-1 deficiency. ICAM-1 deficiency reduced aortic lesion area in Apoe−/− mice (5, 14), although another study did not observe an effect (38). These studies used mice bearing a mutation in exon 5 (36), which encodes the nonfunctional fourth Ig domain of ICAM-1. In contrast, we used mice bearing a mutation in exon 4 (23), which encodes the third Ig domain of ICAM-1 containing the Mac-1 binding site. Both exon 5 and exon 4 ICAM-1 mutants are not null alleles but express markedly reduced levels of alternatively spliced mRNA such that the corresponding variant polypeptides are present at low levels on the cell surface, and at different levels in the circulation (39–41). Relative to wild-type mice, basal levels and the TNF-α stimulation ratio of circulating variant ICAM-1 polypeptides are reduced twofold and sevenfold, respectively, in the exon 5 mutant mice (40, 41), whereas these parameters are not altered by the exon 4 mutation (41). It is thus possible that the differences in circulating ICAM-1 between mice bearing the exon 5 and mice bearing the exon 4 mutations may influence atherogenesis and account for the absence of a significant ICAM-1 effect in our study. Alternatively, this might reflect less dependence on adhesion molecules in Ldlr−/− mice compared with Apoe−/− mice, as was suggested by experiments examining P-selectin deficiency (3, 5, 6).

In Apoe−/− mice, hypercholesterolemia occurs spontaneously and lesions form shortly after birth, whereas in Ldlr−/− mice these processes are initiated by feeding a cholesterol-enriched diet. This diet is usually delayed, as in our study, until the animals are mature, such that the elapsed time since lesion initiation is usually shorter in Ldlr−/− mice compared with Apoe−/− mice of the same age. A preferential role for ICAM-1 in lesion progression as compared with lesion initiation may thus explain the lack of effect of ICAM-1 deficiency on lesions in Ldlr−/− mice in our current study. This hypothesis is supported by our preliminary experiments, in which we extended the duration of cholesterol feeding to 20 weeks and found that ICAM-1 deficiency reduced aortic lesion area by 25% in Icam1−/−Ldlr−/− mice (not shown). Similar inhibition of lesion progression, but not initiation, was observed in CD154-deficient (CD40 ligand–deficient) mice in the Apoe−/− background (42).

Many leukocyte adhesion molecules may contribute to atherosclerosis, yet the relatively large reductions of early foam cell lesions that we observed in Vcam1D4D/D4DLdlr−/− mice and that Dansky and colleagues found in Vcam1D4D/D4DApoe−/− mice (37) suggest a major role for VCAM-1 in the initiation of this disease process. This likely reflects an important function for VCAM-1 in recruitment of monocytes to the arterial intima. Reduced monocyte adhesion found in the aortic arch of Vcam1D4D/D4DApoe−/− mice (37) is consistent with this mechanism, as are observations that antibody blockade of VCAM-1 significantly reduced monocyte rolling and adhesion in perfused carotid arteries isolated from Apoe−/− mice (43, 44). Interaction with VCAM-1 may modulate subsequent monocyte/macrophage activation, and the role of VCAM-1 in activation, proliferation, and apoptosis of intimal monocytes/macrophages, as well as in lesion progression and expansion, remains to be determined.

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