Monoclonal Antibody Against Vascular Cell Adhesion Molecule-1 Inhibits Neointimal Formation After Periadventitial Carotid Artery Injury in Genetically Hypercholesterolemic Mice

Sumito Oguchi,* Paul Dimayuga,* Jenny Zhu, Kuang-Yuh Chyu, Juliana Yano, Prediman K. Shah, Jan Nilsson, Bojan Cercek

Abstract—Vascular cell adhesion molecule (VCAM)-1 is induced in smooth muscle cells after arterial injury, in which it has been implicated in the recruitment of inflammatory cells to the site of injury. To investigate the effect of hypercholesterolemia on VCAM-1 induction after injury and the role of VCAM-1 in neointimal response to injury, we injured the carotid artery of wild-type and apolipoprotein E null (KO) mice fed normal and high cholesterol chow. We demonstrate a graded response of VCAM-1 induction as well as monocyte/macrophage infiltration by immunohistochemistry 3 days after injury that correlated with increasing circulating cholesterol levels. Three weeks after injury, KO mice fed high cholesterol chow (KO HC group) had a significantly greater neointimal formation compared with wild-type and KO mice fed normal chow ($P < 0.05$). Inhibition of VCAM-1 function in the KO HC group by monoclonal antibody treatment significantly reduced monocyte/macrophage infiltration and neointimal formation. There was reduced α-actin expression in KO HC mice 7 days after injury that was partially inhibited by VCAM-1 antibody treatment. Cell migration in an in vitro injury model was partially inhibited by monoclonal VCAM-1 antibody treatment. We propose an additional role for VCAM-1 in smooth muscle cell activation and neointimal formation after injury. (Arterioscler Thromb Vasc Biol. 2000;20:1729-1736.)

Key Words: vascular cell adhesion molecule-1 ■ inflammation ■ apoE knockout mice ■ neointimal formation

Vascular cell adhesion molecule (VCAM)-1 is a member of the immunoglobulin superfamily known to be expressed by vascular endothelial cells for the recruitment of leukocytes during inflammation.$^{1-3}$ VCAM-1 is also expressed by cytokine-treated vascular smooth muscle cells (SMCs) in vitro and by balloon-injured arteries in vivo, in which a concomitant increase in monocyte/macrophage cells at the site of injury was described.$^{4-6}$ There is increasing evidence that an atherogenic diet induces inflammatory genes potentially through increased oxidative stress.$^{7,8}$ Accordingly, hypercholesterolemia may increase VCAM-1 expression and play an important role in the arterial response to injury. Immune-mediated inflammation has also been implicated in this response, characterized by the influx of leukocytes, activation of SMCs, and neointimal formation.$^{9,10}$ We used the arterial wall injury model in the genetically mutated hypercholesterolemic apoE knockout mice$^{11}$ and monoclonal VCAM-1 antibody treatment$^{12}$ to assess the role of VCAM-1 in intimal formation after arterial injury. Our findings suggest that hypercholesterolemia potentiates the response to injury by increasing VCAM-1 expression, leading to increased macrophage infiltration and subsequent neointimal formation. Neutralization of VCAM-1 function by antibody treatment inhibited neointimal formation in vivo and SMC migration in vitro, suggesting an important role for VCAM-1 in the arterial response to injury.

Methods

Animals

Wild-type (WT) mice and apoE knockout (apoE KO) mice with a genetic background of C57BL/6J were purchased from Jackson Laboratory (Bar Harbor, Me). At the age of 25 weeks, the mice were anesthetized with Avertin (0.016 mL/g of 2.5% solution IP), and the right carotid artery was carefully isolated under a dissection microscope. A 3-mm-long tube was applied around the carotid artery, and the skin incision was closed, as previously described.$^{11}$ At the time of euthanasia, the carotid artery was perfused with 0.9% saline for 10 minutes and frozen at $-70^\circ$C after embedding in OCT compound (Tissue-Tek, Miles Inc). Serial 4-μm-thick sections of arteries were collected. Experimental protocols involving these animals were approved by the Institutional Animal Care and Use Committee.

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From the Atherosclerosis Research Center, Burns and Allen Research Institute, Division of Cardiology, Cedars-Sinai Medical Center/UCLA School of Medicine, Los Angeles, Calif, and the Department of Medicine (J.N.), Lund University, University Hospital MAS, Malmö, Sweden.

*S.O. and P.D. contributed equally to the article.

Correspondence to Jan Nilsson, MD, PhD, Department of Medicine, Malmö University Hospital, 205 02 Malmö, Sweden. E-mail jan.nilsson@medforsk.mas.lu.se

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Experimental Groups
WT mice were divided into 2 groups. One group was fed normal chow, injured, and euthanized after 3 (n=5), 7 (n=5), and 21 (n=7) days. The second group served as uninjured controls. The apoE-KO mice were maintained on a normal or high cholesterol diet (n=34 each); half of this group was injured, and the other half served as uninjured controls. The time points were the same as those used in the WT mice. For neutralization studies, purified rat anti-mouse VCAM-1 monoclonal antibody or rat IgG isotype was administered (1 μg/g body wt, Pharmingen) by tail vein injection to apoE KO mice on cholesterol chow (n=8) on the day of injury and every other day for the duration of the experiment. This antibody has been shown previously to inhibit VCAM-1 function in vivo.12 The tissues were harvested for morphometric analysis. Presence of the antibody in serum was demonstrated by dot-blot analysis of serum from mice 21 days after injury. Blots were incubated in anti-rat IgG (DAKO Corp) and detected by enhanced chemiluminescence (ECL, Amersham).

Immunohistochemistry
Immunohistochemical stains were carried out with the following antisera: biotinylated anti-α-smooth muscle actin (Sigma Chemical Co), anti-CD4, anti-CD8a, anti–VCAM-1 (Pharmingen), MOMA-2 (Serotec), and Mac-1 (Roche). Biotinylated secondary antibody (Pierce) was used with the AEC chromogen detection kit (DAKO Corp). Nonimmune serum or isotype IgG was used as a negative control. Sections from mice 3 days after injury were used for computer-assisted image analysis as a semiquantitative assessment of immunohistochemical stains as previously described.13 Briefly, images were captured and analyzed by use of Optimas 6.1 (Optimas System, Bioscan). Color detection was accomplished by sampling, and threshold masking defined the positive area. The same threshold was applied to all sections. The area was then standardized against the medial area and expressed as percent stained area of the media. For morphometric analysis, serial sections were stained with eosin and hematoxylin, and the intimal and medial areas of 4 to 6 sections from the middle portion of the injured segment from each animal were measured by the Optimas System. Results were expressed as millimeters squared (mean±SD). Representative sections were stained for elastin by using the Accustain Elastic Stain Kit (Sigma) for photography.

Plasma and Tissue Cholesterol
EDTA plasma from all groups was collected at the time of euthanasia, and cholesterol levels were measured by using a commercially available kit (Sigma). For tissue cholesterol levels, a modification of a previously described protocol was adapted.14 Briefly, aortic tissue was weighed and homogenized for 1 minute in a mixture of 160 μL distilled water, 200 μL chloroform, and 400 μL methanol. After homogenization, 200 μL chloroform was added, and the mixture was blended for 30 seconds. Water (200 μL) was then added and blended again for another 30 seconds. The mixture was centrifuged briefly to separate the chloroform layer, which was carefully aspirated into another tube. The lipid remained in the bottom of the tube after the chloroform was vaporized in a vacuum trap. Lipid was then resuspended in 50 μL of 100% ethanol, and 10 μL was used for the cholesterol assay. Results were expressed as micrograms total cholesterol per milligram tissue.

Cell Culture and Injury
Aortic SMCs were cultured by an explant method using 25-week-old adult C57BL/6J mice as described previously.15 Briefly, mice were anesthetized, and the aortas were isolated. The adventitia and endothelium were removed with the aid of microscopy. The aortic media was cut into 2-mm pieces and placed into 6-well plates containing DMEM/F-12 with 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B (Fungizone), 2 mmol/L L-glutamine (GIBCO-BRL), and 10% FBS (Omega Scientific) incubated at 37°C in a humidified atmosphere of 5% CO2/95% air incubator. Cells that had migrated out of the explants were grown in 20% FBS to confluence. Verification of cell type was accomplished by using anti–smooth muscle α-actin clone 1A4 (Sigma) immunocytochemistry. Cells were subcultured into 75-cm2 plates and grown to confluence. After incubating in 1% FBS for 48 hours, cell injury was performed by use of a 4-mm-wide sterile rubber tube gently pressed for 10 seconds as described previously.16,17 Injured cells were harvested after 24 and 48 hours. All experiments were performed within the first 8 passages.

Western Blot
Cytosolic protein was extracted by lysing cells with cold hypotonic buffer (10 mmol/L HEPES, 10 mmol/L KCl, 1.5 mmol/L MgCl2, 1 mmol/L dithiothreitol, 1 mmol/L phenylmethylsulfonyl fluoride, 1 μg/mL leupeptin, 15 μg/mL aprotinin, and 0.4% NP-40). After spinning at 9000g for 30 seconds, the supernatant was collected as a cytosolic fraction. For detection of VCAM-1 protein after injury, equal amounts of the extracted cytosolic protein were electrophoresed on 7.5% SDS-PAGE gel and transferred to nitrocellulose membrane. The membrane was blocked with 1% milk in PBS with 0.1% Tween 20 overnight at 4°C. The membrane was subsequently probed with VCAM-1 antibody (goat polyclonal, 1:1500, Santa Cruz Biotechnology), followed by horseradish peroxidase–conjugated anti-goat antibody. Detection was accomplished by using the ECL kit (Amersham). Computer-assisted densitometric analysis was performed to quantify the detected bands.

Migration Studies
Cells were subcultured into 2-chamber Permanox slides (Laboratory-Tek) and grown to confluence. After synchronizing growth in 1% FBS for 48 hours, cell injury was performed, and the medium was replaced with either 1% FBS medium or 1% FBS with 50 μg/mL rat anti-mouse VCAM-1 antibody. Rat isotype IgG at the same concentration was used as a control. After 48 hours, cells were fixed in acetone and stained with hematoxylin and eosin. Distance between the nuclei of migrating cells and the margin of injury was measured by use of the Optimas System. The distance of 50 cells migrating from a clear border of injury was averaged and counted as 1 experimental value.17

Statistics
Numeric data are expressed as mean±SD. Differences among the groups were determined by 1-way ANOVA, followed by the Tukey-Kramer test for multiple comparisons unless otherwise noted. A value of P<0.05 was considered significant.

Results
Plasma and Vascular Cholesterol Levels
Plasma cholesterol levels were increased in apoE KO mice on normal chow and those on high cholesterol chow compared with WT mice (567±252 and 1348±318 mg/dL versus 164±47 mg/dL, respectively; P<0.01, ANOVA).

In the KO mice fed normal chow, tissue cholesterol was ≈50% higher compared with WT mice (3.24±0.55 versus 2.04±0.22 μg/mg). Tissue cholesterol was increased severalfold (11.2±2.1 μg/mg; P<0.01, ANOVA) in mice fed high cholesterol chow compared with WT and KO mice fed normal chow.

Arterial Response to Injury
No intima was apparent in uninjured carotid arteries from C57 WT and apoE KO mice. Three days after cuff placement, the injured segments were mostly devoid of endothelial cells (data not shown), as we have previously described.18 Twenty-one days after cuff placement, there was neointimal formation observed (Figure 1A and 1D). Injury to the carotid artery of the apoE KO mice fed normal chow (KO N group) resulted in 100% increased neointimal formation compared with the intimal area in the WT mice after 21 days, which was even greater when the apoE KO mice were fed high cholesterol chow (KO HC, Figure 1B through 1D). Vessel diameter and medial area were not different
between the WT and KO N mice. The luminal area was slightly
less in KO N mice compared with WT mice, whereas the
intima-to-media ratio was significantly increased in KO N mice.
Vessel diameter in KO HC mice was similar to that in the other
groups, but the medial area was significantly increased com-
pared with KO N and WT mice. The luminal area was
significantly reduced in the KO HC group compared with the
WT group. The intima-to-media ratio was also significantly
increased in the KO HC mice (Table).

VCAM-1 Expression and Monocyte/Macrophage
Infiltration After Injury
There was no apparent VCAM-1 staining in the media of
uninjured mice. Injury resulted in increased VCAM-1 expres-

<table>
<thead>
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<th>Group</th>
<th>EEL, mm²</th>
<th>Media, mm²</th>
<th>Neointima, mm²</th>
<th>Lumen, mm²</th>
<th>I/M Ratio</th>
</tr>
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<tr>
<td>WT (n=7)</td>
<td>0.0955±0.0282</td>
<td>0.0234±0.0045</td>
<td>0.0115±0.0066</td>
<td>0.062±0.018</td>
<td>0.49±0.22</td>
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<td>KO N (n=5)</td>
<td>0.0973±0.0245</td>
<td>0.0259±0.0097</td>
<td>0.0245±0.0101</td>
<td>0.052±0.018</td>
<td>0.96±0.19*</td>
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<tr>
<td>KO HC (n=4)</td>
<td>0.1170±0.0378</td>
<td>0.0402±0.0149*</td>
<td>0.0451±0.0204**</td>
<td>0.032±0.011*</td>
<td>1.16±0.49*</td>
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<tr>
<td>KO HC+Ab (n=5)</td>
<td>0.0855±0.0592</td>
<td>0.0348±0.0155</td>
<td>0.0064±0.0072‡</td>
<td>0.092±0.018‡</td>
<td>0.16±0.11‡</td>
</tr>
<tr>
<td>KO HC+IgG (n=5)</td>
<td>0.0946±0.0392</td>
<td>0.0453±0.0231</td>
<td>0.0277±0.0193</td>
<td>0.022±0.009§</td>
<td>0.58±0.19‡</td>
</tr>
</tbody>
</table>

Values are mean±SD. EEL indicates external elastic lamina; Ab, antibody.
The arterial response 21 days after cuff injury is summarized above. Measurements were made as described in Methods. The effect of VCAM-1 antibody treatment in KO HC mice (KO HC+Ab) is also summarized. Reduction in neointimal and luminal measurements as well as the intima-to-media (I/M) ratio is shown.

*P<0.05 vs WT; †P<0.05 vs KO N. Statistical analyses on the effects of the treatments were performed without WT and KO N groups. ‡P<0.05 vs KO HC; §P<0.001 vs KO HC+Ab.
sion in the media of WT mice, as shown by immunohistochemical staining, which peaked after 3 days of cuff placement and persisted for 7 days. At 3 days, this increase was slightly augmented in the KO N group and significantly more so in the KO HC group at the same time point (Figure 2A through 2C). Isotype control antibody stain was negative. Semiquantitative computer analysis indicated that the VCAM-1–stained area was significantly increased in injured KO HC mice compared with KO N and WT mice (27±5% versus 14±3% and 11±2%, respectively; P<0.01; n=3). MOMA-2 staining 3 days after injury showed minimal presence of monocytes/macrophages in the injured vessels of WT mice. Injured KO mice stained positively for MOMA-2, which was increased in high cholesterol–fed mice (Figure 2D through 2F). Confirmation of MOMA-2 stains were accomplished by using Mac-1, which yielded a similar but fainter stain pattern. Isotype control antibody stain was negative. Computer-assisted analysis of the MOMA-2–stained area showed a trend similar to VCAM-1 expression, with the lowest stained area in the WT mice, increasing in KO N and HC mice (1.3±1% versus 10.4±1% and 15.3±6%, respectively; P<0.05). VCAM-1 expression was correlated with monocyte/macrophage infiltration, as shown by MOMA-2 staining at the site of injury (r=0.85, P<0.05; Figure 3A). The VCAM-1–stained area 3 days after injury was also correlated with the intimal area 21 days after injury (Figure 3B). All groups had minimal stains with CD4 and CD8 antibodies.

VCAM-1 Antibody Neutralization
To determine the function of VCAM-1 in an injury model of hypercholesterolemia, injured KO mice on a high cholesterol diet were injected with VCAM-1 antibody. A slot-blot analysis of serum from treated mice after 21 days indicated the presence of rat IgG (Figure 4A). After 3 days, the injured carotid arteries were harvested. Localization of the antibody in the injured arteries was confirmed by use of biotinylated anti-rat IgG secondary antibody. Staining for rat IgG was observed only in the VCAM-1 antibody–treated mice (Figure 4B). The 3-day injured arteries were stained for VCAM-1 and MOMA-2. By use of computer quantitative measurements, anti–VCAM-1 treatment did not alter VCAM-1 expression (Figure 4C) but significantly reduced the MOMA-2–stained area compared with the area in KO mice fed high cholesterol chow (3±2% versus 15±6%, P<0.05; Figure 4D). Administration of the anti-mouse VCAM-1 antibody significantly reduced neointimal formation in the KO HC group (Figure 5A and 5B). Rat IgG isotype control did not have the same effect on neointimal formation after injury (Figure 5C and Table). The tissue cholesterol level was not affected by the injection of VCAM-1 antibody (11.2±2.1 versus 12.8±3.8 μg/mg, P=NS).

α-Actin Expression After Injury
To identify the cells that express VCAM-1 in the media after injury, double staining for α-actin and VCAM-1 was performed on sections. There was staining for both proteins 3 days after injury for WT and KO HC mice (Figure 6A and 6B). Seven days after injury, there was a conspicuous reduction in α-actin expression in the media (Figure 6C). Treatment with the anti–VCAM-1 antibody partially inhibited this α-actin reduction (Figure 6D).

VCAM-1 Expression and Migration of Cultured SMCs
To test whether mechanical injury alters VCAM-1 expression by SMCs, we used a previously described cell injury model.16 Uninjured SMCs showed baseline expression, which was increased 48 hours after injury (2.2±0.9-fold versus before injured, P<0.05; n=4) determined by densitometric analysis of Western blots (Figure 7A).
To investigate the role of VCAM-1 in the migration of SMCs, injured cells were incubated in 50 μg/mL anti-VCAM-1 antibody immediately after injury for 48 hours. The distance migrated from the injury border was approximately 35% less in antibody-treated compared with untreated and isotype IgG-treated cells (0.107 ± 0.012 versus 0.176 ± 0.016 and 0.164 ± 0.021 mm, respectively; P<0.05, ANOVA; Figure 7C).

Discussion
This report identifies VCAM-1 as a mediator of neointimal formation after carotid injury in mice. Inhibition of VCAM-1 function by antibody blockade profoundly reduced neointimal formation after injury. VCAM-1 in SMCs has been demonstrated in atherosclerotic plaques and more recently in injured rat carotid arteries.4–6,19 Because neointimal formation after injury primarily involves SMC activation, we sought to examine the effect of hypercholesterolemia on VCAM-1 expression in medial SMCs after injury in vivo. We show a graded response in VCAM-1 expression after injury, which was associated with different cholesterol profiles. The apoE KO mice on high cholesterol chow showed the greatest expression, whereas the least expression was in WT mice. The difference in tissue cholesterol parallels plasma cholesterol among the groups of mice. The results suggest that hypercholesterolemia increases lipid accumulation in tissue augmenting VCAM-1 expression after injury; this increased accumulation is perhaps due to increased oxidative stress8 and may cause exacerbated intima thickening in response to injury. This notion is further supported by the correlation between VCAM-1 expression and neointimal formation (Figure 3B). Recent work from our laboratory shows the presence of malondialdehyde-modified lipoprotein in injured arteries of apoE KO mice,11 which may partially mediate the inflammatory response by increasing VCAM-1 expression. One can speculate that VCAM-1 expression might be a predictor of intimal thickening after injury. This suggestion is further strengthened by a recent report showing increased VCAM-1 expression on endothelial cells at sites prone to lesion formation in hypercholesterolemic apoE KO mice compared with WT mice.20

Reports correlating inflammatory infiltrates with neointimal thickening have substantially strengthened the notion that inflammation plays a significant role in the arterial response to injury.9,10 Adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and VCAM-1, expressed by endothelial and smooth muscle cells, mediate inflammatory cell recruitment to sites of injury.1,6 Arterial injury has been shown to increase ICAM-1 expression in rats. Treatment of rats with anti-inflammatory drugs, such as aspirin, reduced ICAM-1 expression and neointimal formation after injury.21 Antibody blockade experiments against ICAM-1 inhibited intimal formation in balloon-injured rat arteries. The mechanism of the effects of ICAM-1 inhibition was not clear; however, monocyte/macrophage accumulation was suggested not to play a role.22 A recent report describing increased VCAM-1 expression after rat arterial injury was associated with the adhesion of monocytes/macrophages.6 Several experimental models of inflammation have correlated the expression of VCAM-1 with monocyte recruitment.1–3,23 Therefore, we tested the hypothesis that VCAM-1 facilitates the recruitment of inflammatory cells after arterial injury. Our results show a correlation between VCAM-1 expression and monocyte/macrophage infiltration after injury. The augmented VCAM-1 expression in hypercholesterolemic mice in response to injury is associated with increased monocyte/macrophage recruitment and increased neointimal formation. A rabbit cuff-injury model using antibody blockade of leukocytes showed that polymorphonuclear influx had little effect on SMC migration and intimal thickening.24 It is notable that mononuclear cells were present in significant quantity only after prolonged exposure of the artery to LDL in vivo. This exposure to LDL also significantly increased intimal thickening.25 In a cuff model with electrical stimulus-induced injury, mononuclear cell but not polymorphonuclear influx was shown to influence intimal thickening.26 It is reasonable to speculate that polymorphonuclear influx does not significantly affect intimal thickening in the cuff-injury model. In the present study, the reduction of neointimal formation with antibody treatment was associated with reduced inflammatory infiltrates, suggesting further that monocyte/macrophage accumulation plays a role in promoting neointimal formation in injured arteries of apoE KO mice.
There was a slight reduction in neointimal formation by IgG treatment. Although suggestive of a protective effect, the result was not significantly different compared with the result in untreated KO HC mice.

The interaction between monocytes/macrophages and SMCs is integrin-ligand–mediated. The integrin α4β1 (VLA-4) is constitutively expressed on monocytes and has been identified to bind to VCAM-1.4–6 Antibody blockade of the VCAM-1/VLA-4 pathway has produced favorable results in animal models of inflammation.2,11 In a different injury model, antibody blockade of VLA-4 reduced intimal hyperplasia in endarterectomized carotid arteries.26 More recently, treatment of hyperlipidemic mice with an antibody against the immune mediator CD40L resulted in reduced aortic atherosclerosis, which was attributed to inhibition of inflammatory cell accumulation. This was shown to occur because of the reduction of VCAM-1 expression.27 The observation in the present study that VCAM-1 antibody treatment blocked the recruitment of monocytes/macrophages indicates a neutralization of VCAM-1 function. Our results concerning the role of VCAM-1 in inflammation concur with their report.

The partial loss of immunodetectable smooth muscle α-actin on arterial sections concomitant with the expression of VCAM-1 several days after injury suggests an association between phenotypic modulation and VCAM-1 expression. This association is further strengthened by the partial inhibition of the observable decrease in α-actin staining in VCAM-1 antibody–treated mice (Figure 6C and 6D). The process of losing α-actin expression after injury has been described in the rat balloon deendothelialization model.28 The most conspicuous changes took place at an early time point (5 days after injury), which coincides with our 7-day time point. This observation may be another possible mechanism by which VCAM-1 may affect SMC activation, leading to

Figure 4. VCAM-1 antibody is detectable in 21-day injured mouse serum dot-blotted on membrane. Detection was accomplished by using biotinylated anti-rat IgG incubated in streptavidin (A). Sections from 3-day injured mice treated with VCAM-1 antibody (Ab) show localized presence of the Ab as detected by stain for rat IgG (B, bottom panel). Untreated and IgG-treated mice were negative for rat IgG stain (B, top and middle panels). Computer-assisted morphometry shows a lack of significant effect of VCAM-1 Ab treatment on detectable VCAM-1 stain (C). The treatment reduced the presence of monocytes/macrophages in the injured arteries of KO HC mice (D). *P<0.05 vs KO HC mice (n=3).

Figure 5. Low-power magnification of elastic stain shows the extent of intimal formation in the carotid of KO HC mice 21 days after injury (A). Treatment with VCAM-1 antibody (1 μg/g body wt, B) inhibits the formation of intima in injured carotid artery of KO HC mice. IgG treatment had no significant effect (C). Bar=100 μm.
migration and intimal formation. Li et al. described a similar finding in atherosclerotic lesions of rabbits, in which SMCs positive for VCAM-1 had reduced α-actin staining. More recently, Duplaà et al. described the inhibitory effects on SMC markers by VCAM-1/VLA-4 blockade in an in vitro model of SMC differentiation. However, inhibition of inflammatory cell infiltration in our model may also lead to reduced local release of growth factors by monocytes/macrophages known to modulate α-actin expression.

VCAM-1 was shown to stimulate the chemotaxis of endothelial cells, which was inhibited by the blockade of VLA-4. Corneal angiogenesis was shown to be mediated by VCAM-1 in the same study. Similar results have been reported on the adhesion, spreading, and subsequent motility of a human melanoma cell line on VCAM-1. Reduction of the distance migrated by injured SMCs produced by the antibody against VCAM-1 in the present study suggests the involvement of VCAM-1 in cell motility. The mechanism for the effect of VCAM-1 on migration was not addressed; however, integrin-ligand interaction is likely involved in this process via VLA-4. A recent report using a similar in vitro injury model inhibited SMC migration by using an antibody against VLA-4. That report, in connection with the present findings, suggests a novel role for VCAM-1 in SMC migration after injury that may contribute to neointimal formation. The signaling pathway involving VLA-4 in this process is currently unknown.

The injury-induced thickening of the neointima occurred without changes in the vessel diameter. The observable change occurred in the medial area of the KO HC group. The mechanism driving this seems to point toward the combination of injury and substantial increase in total circulating and arterial tissue cholesterol. Although the neointima was reduced, the increase in medial area was not significantly affected by antibody treatment of KO HC mice.

Our results indicate that the inhibition of VCAM-1 function after carotid injury in hypercholesterolemic mice profoundly reduces neointimal formation. The mechanism may be the increased monocyte/macrophage infiltration. There is also evidence that VCAM-1 is involved in phenotypic modulation that may influence neointimal formation after arterial injury. Furthermore, in vitro migration of SMCs after injury is partially mediated by VCAM-1. Inhibition of VCAM-1 may be an interesting target for future therapeutic intervention against vaso-occlusive disease.
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