Loss of Collagen XVIII Enhances Neovascularization and Vascular Permeability in Atherosclerosis

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Background—Plaque neovascularization is thought to promote atherosclerosis; however, the mechanisms of its regulation are not understood. Collagen XVIII and its proteolytically released endostatin fragment are abundant proteoglycans in vascular basement membranes and the walls of major blood vessels. We hypothesized that collagen XVIII in the aortic wall inhibits the proliferation and intimal extension of vasa vasorum.

Methods and Results—To test our hypothesis, we bred collagen XVIII– knockout (Col18a1−/−) mice into the atherosclerosis-prone apolipoprotein E– deficient (ApoE−/−) strain. After 6 months on a cholesterol diet, aortas from ApoE−/−; Col18a1−/− and ApoE−/−:Col18a1+/− heterozygote mice showed increased atheroma coverage and enhanced lipid accumulation compared with wild-type littermates. We observed more extensive vasa vasorum and intimal neovascularization in knockout but not heterozygote aortas. Endothelial cells sprouting from Col18a1−/− aortas were increased compared with heterozygote and wild-type aortas. In contrast, vascular permeability of large and small blood vessels was enhanced with even heterozygous loss of collagen XVIII but was not suppressed by increasing serum endostatin to wild-type levels.

Conclusions—Our results identify a previously unrecognized function for collagen XVIII that maintains vascular permeability. Loss of this basement membrane proteoglycan enhances angiogenesis and vascular permeability during atherosclerosis by distinct gene-dose–dependent mechanisms. (Circulation. 2004;110:1330-1336.)

Key Words: angiogenesis ■ atherosclerosis ■ extracellular matrix ■ permeability ■ vasa vasorum

Neovascularization in the wall of large blood vessels may promote the entry of inflammatory cells and growth of atheromas.1,2 Atherosclerotic but not normal aortas stimulate angiogenesis in the chick chorioallantoic membrane and corneal micropocket assays of angiogenesis.3 Several angiogenic growth factors are highly expressed in atherosclerotic lesions.4,5 However, not all atheromas develop neovascularization, and the distribution of growth factors does not spatially correlate with regions of neovascularization.6 Thus, the complex regulation of plaque angiogenesis must extend beyond an analysis of positive regulators. Factors that antagonize angiogenesis may also be present in atheromas, and loss of endogenous inhibitors may be a potential mechanism for the initiation of plaque angiogenesis.7

Collagen XVIII is an abundant heparan sulfate proteoglycan in vascular and epithelial basement membranes.8 Three different N-terminal ends distinguish short and long isoforms (migration weights range, 220 to 350 kDa) that have different tissue distributions.9 Collagen XVIII molecules contain 10 triple-helical collagen domains that are flanked by 11 non–triple-helical (NC) domains (Figure 1).10 Endostatin (ES), a 20-kDa proteolytic fragment from the C-terminal NC1 domain of collagen XVIII, has been shown to inhibit angiogenesis and atherosclerosis.2,11 The aorta is among the most abundant tissue sources of collagen XVIII and proteolytically released ES.12 We hypothesized that collagen XVIII is degraded during atherosclerosis and that loss of this vessel wall proteoglycan will promote the proliferation of vasa vasorum (VV) into the intima of atheromas.

To test this hypothesis, we bred collagen XVIII–deficient mice (Col18a1−/−) into the atherosclerosis-susceptible apolipoprotein E–deficient (ApoE−/−) strain. Col18a1−/− mice back-crossed several generations into the C57BL6 strain are viable and fertile but show defective hyaloid vessel regression in the eye, resulting in abnormal retinal vessel development.13 We provide evidence for distinct functions of collagen XVIII in the vascular basement membrane that regulate vascular permeability and angiogenesis during atherosclerosis.

Methods

Materials

Rabbit polyclonal antibodies for collagen XVIII were prepared against the C-terminus ES fragment (amino acids 132 to 315,
Collagen type XVIII (mouse CR form)

Figure 1. Differential loss of collagen XVIII in aortas with atherosclerosis. Structure of collagen XVIII shows collagen (solid bars) and noncollagen (NC) domains. Collagen XVIII in ApoE−/− atheromas was detected with ES-IgG (A–C) and NC11-IgG (D–F). A, Media and intima show strong ES-IgG reactivity. B, ES-IgG staining is reduced in media that is severely attenuated (M) but remains along surface endothelium (arrow). C, ES+ capillaries filled with erythrocytes (arrow) located along intima/media border (>400). D, Same atheroma (A) is stained for collagen XVIII using NC11-IgG. Normal aorta shows similar staining patterns with both antibodies, but NC11-IgG reactivity is significantly reduced in atheromas. E, NC11-IgG reactivity in media is markedly reduced beneath atheromas (*). F, Both antibodies do not stain aK0 tissues. Magnifications: ×100 (A, D, F) and ×200 (B, E).

counted from the first residue of NC1 or the most proximal NC11 domain (amino acids 487 to 785, counted from the methionine start codon of mouse collagen XVIII CR isoform).14 NC11-IgG and ES-IgG cross-react with human collagen XVIII but show no reactivity to tissues from Col18al−/− mice. Recombinant murine ES was expressed in Pichia pastoria and supplied by EntreMed, Inc.

Immunohistochemistry

We stained frozen coronal aortic arch sections with Mac3, α-smooth muscle actin, and CD31 to quantify cells and neovascularization in atheromas.1 Lipid content was determined after oil red O staining. We analyzed percent area of lipid staining on 5 atheroma sections from >15 mice per genotype. Collagen XVIII in atheromas was detected in paraffin sections stained with NC11-IgG or ES-IgG (1:500 dilution), followed by biotinylated goat anti-rabbit IgG (1:500), avidin-peroxidase (Vector Laboratories), and the red substrate 3-amino-9-ethylcarbazole.

In Vivo Studies on Atherosclerosis

The generation of Col18al−/− mice has been described.13 We bred Col18al−/− mice (C57BL6/J background, n=15) into the ApoE−/− strain (C57BL6/J, n=10, Jackson Laboratory, Bar Harbor, Me).

ApoE−/−:Col18al−/− matings generated littermates with wild-type (WT), heterozygote (Het), and null collagen XVIII deficiencies. Mice were fed a 0.15% cholesterol diet (Harlan-Fedklad) from age 8 to 32 weeks to develop advanced lesions. We measured the percent area of Sudan IV+ atheromas in the descending aorta and the mean wall thickness of atheromas along the inner curve of the aortic arch.3 Serum cholesterol levels were measured in an automated colorimetric assay (Children’s Hospital, Boston). We screened blood for genotype-related differences in blood cell counts and liver, renal, and thyroid function tests (Anlytics, Inc). Mouse pathology was evaluated at the Harvard Mouse Histopathology Core. Animal studies were performed in compliance with the Animal Care and Use Committee guidelines.

VV and Intimal Neovascularization

We counted the number of CD31+ VV networks per descending aorta and measured the vascular density of each VV (percent area of CD31+ capillaries per 200× field) detected in flat mounted aortas from each genotype (n=15).4 We compared aortic arches across all genotypes to determine the incidence of intimal neovascularization in the same region of high disease susceptibility, which we reported as the percentage of aortic arches that contained intimal capillaries.

Aorta Explant Sprouting Assay

We adapted the rat aortic ring assay for mouse aortas to test the effects of targeted mutations on endothelial sprouting.3,15 Aorta rings from age-matched WT, Het, and knockout (KO) mice were embedded in rat collagen I (2 mg/mL) and cultured with serum-free media (Invitrogen, No. 17601) and vascular endothelial cell growth factor (VEGF) (0, 2, 4, or 8 ng/mL, n=4 rings per dose).3,15 We measured sprout area in images captured through a Nikon SMZ microscope (50× magnification). Maximum sprout area was normalized to a value=1. Sprouting for each genotype was compared at all VEGF doses by ANOVA based on pooled data from 3 independent experiments.

Permeability of Aortas and Skin Vessels

We measured the permeability of aortas and skin vessels to albumin-bound Evans blue dye (EBD).16 We sedated WT and KO mice (8 to 10 weeks old) in the C57BL6/J, ApoE−/−, and LDL receptor-deficient (LDLR−/−) strains with avertin and intravenously injected 0.1 mL of 1% EBD per 20 g body weight. After 10 minutes, we made 2 intradermal injections (50 μL) each of PBS+0.05% BSA containing 0 or 10 ng VEGF in dorsal skin. After another 20 minutes, aortas were removed, briefly rinsed clear of blood, and eluted in 100 μL of formamide. Punch biopsies (1 cm) around dorsal skin injection sites were eluted in 0.5 mL formamide. EBD concentration was determined by absorption at 605 nm. We reported EBD content of KO tissues as the fold difference relative to WT.

Permeability to LDL was performed in WT and KO mice in the LDLR−/− background. Human LDL (Biomedical Technologies, Inc) was labeled with 125I.17 We intravenously injected 100 μL buffer containing 5×107 cpm 125I-LDL and 1% EBD. Aorta and blood were collected 30 minutes later.18 The level of 125I-LDL in each rinsed aorta was normalized for the peak blood radioactivity. To test whether systemic ES modifies the permeability of KO mice, we delivered 25 or 100 μg/d of murine ES by subcutaneous osmotic pumps (Durect Corp). We measured serum ES levels 4 days after pump implantation and measured LDL permeability on day 5.

Statistical Analysis

Data for lesion content and areas, plaque thickness, body weight, and serum cholesterol fit a normal distribution. Overall probability values for genotype differences were based on the ANOVA. A significant F test indicating overall group differences was followed with post hoc Bonferroni comparisons between groups. Data for VV were compared by Kruskal-Wallis followed by Mann-Whitney U tests for pairwise comparisons.
Results

Collagen XVIII Proteolysis in Atherosclerosis

Atherosclerosis remodels the artery wall through the action of several proteases that could modify collagen XVIII. We detected collagen XVIII in normal and atherosclerotic aortas with either ES-IgG or NC11-IgG, which respectively recognize ES or NC11 domains common to all collagen XVIII isoforms (Figure 1). Aorta without atheromas from C57BL6/J and apoE/H11002 mice showed similar staining patterns, with either antibody recognizing collagen XVIII proteins in the media and endothelium. In atheromas, the staining patterns for ES and NC11 antibodies diverged. NC11-IgG reactivity was greatly reduced in the artery wall beneath the expanded intima of atheromas, whereas ES-IgG reactivity was preserved except where the media was severely attenuated. These findings suggest that collagen XVIII proteins are differentially reduced during atherosclerosis. The more rapid loss of NC11-containing collagen XVIII proteins is consistent with the presence of a protease-sensitive hinge region upstream of ES and the known susceptibility of this region to proteolytic cleavage by cathepsins, metalloproteases, and elastases, which are abundantly expressed by macrophages and other cells in atheromas.19,20

Collagen XVIII Deficiency Enhances Atherosclerosis

We crossed Col18al−/− mice into the ApoE−/− strain. Congenic analysis confirmed that >99% of chromosomal markers matched C57BL6/J markers. ApoE−/−;Col18al−/− matings produced littermates with different Col18al genotypes, which we designated as aWT, aHet, and aKO to indicate their ApoE−/− genotype and distinguish them from WT, Het, and KO mice in the C57BL6/J strain. After cholesterol feeding for 24 weeks, we observed that the mean percent aortic area with Sudan IV stains (red) are increased in aWT, aHet, and aKO aortas compared to aWT (left to right). Area and intensity of Sudan IV+ lesions (red) are shown for aWT (circles, n=28), aHet (diamond, n=30), and aKO (triangle, n=24) aortas. aHet and aKO aortas have increased atherosclerosis.

Effects of Collagen XVIII Deficiency on Lesion Size and Composition

<table>
<thead>
<tr>
<th>Variable</th>
<th>aWT</th>
<th>aHet</th>
<th>aKO</th>
<th>Overall</th>
<th>Het vs KO</th>
</tr>
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<tbody>
<tr>
<td>Plaque area, %</td>
<td>24.4±8.9</td>
<td>37.7±11.2*</td>
<td>40.7±18.0†</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Wall thickness, μm</td>
<td>212±30</td>
<td>293±60*</td>
<td>299±51†</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>863±273</td>
<td>942±293</td>
<td>906±208</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>29±5</td>
<td>27±5</td>
<td>23±4†</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ORO, % area</td>
<td>7.4±1.8</td>
<td>19.0±4.6*</td>
<td>19.1±3.7†</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>VV foci, n</td>
<td>3 (0–9)</td>
<td>4 (1–8)</td>
<td>8 (2–13)†</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>VV density, % area</td>
<td>17.6±9.2</td>
<td>16.0±3.6</td>
<td>32.0±8.2†</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intimal neovascularization, %</td>
<td>13</td>
<td>20</td>
<td>53‡</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>SMC, % area</td>
<td>6.9±2.6</td>
<td>1.7±0.8*</td>
<td>1.8±0.7†</td>
<td>&lt;0.01</td>
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<tr>
<td>MAC3, % area</td>
<td>6.8±3.8</td>
<td>9.3±2.3§</td>
<td>9.8±2.7§</td>
<td>0.03</td>
<td></td>
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</table>

Data are mean±SD except for vasa vasaorum, which are medians with ranges. P values are based on ANOVA with post hoc Bonferroni comparisons, except for vasa vasaorum (Kruskal-Wallis followed by Mann-Whitney U tests) and intimal neovascularization (χ2 analysis followed by Fisher’s exact tests for binomial proportions). ORO indicates oil red O; VV, vasa vasaorum; and SMC, smooth muscle cell.

*aWT vs aHet, P<0.01.
†aWT vs aKO, P<0.01.
‡aWT vs aKO, P<0.05.
§aWT vs aHet, P=0.07.
genotype groups (Table). These findings show that loss of even one Col18a1 allele increased atherosclerosis.

Absence of Collagen XVIII Increases Plaque Angiogenesis

We next evaluated the effect of collagen XVIII deficiency on the extent of VV and intimal neovascularization. We identified CD31⁺-VV networks in the entire descending aorta by in situ staining. The median number of VV was 2-fold higher in aKO than in aHet and aWT aortas (Table). In addition, the mean capillary density of VV in aKO aortas was 2-fold increased (Figure 3, A and B).

Serial sections demonstrate the continuity between erythrocyte-filled intima capillaries and media capillaries (Figure 1C). In advanced atherosclerosis, aortic arches uniformly contain atheromas along the lesser curve and in all major artery branches. We compared the incidence of CD31⁺ intimal neovascularization in aortic arches with different Col18a1 genotypes. Intimal neovascularization was detected in 53% of aKO arches compared with 20% of aHet and 13% of aWT (Table), which represents a 4-fold higher incidence than previous studies. Intimal capillaries were most often seen in extensive atheromas of the right brachiocephalic artery (Figure 3, C and D).

Collagen XVIII Deficiency Increases Atheroma Lipids

Sudan IV stains of aHet and aKO aortas were more intense and readily distinguished from aWT (Figure 2a). We determined the relative area of oil red O lipids in atheromas from

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**Figure 3.** Absence of collagen XVIII increases plaque angiogenesis. A, Clarified flat-mounted aWT aorta contains CD31⁺-VV (brown). B, VV networks in aKO aortas are more numerous and have increased capillary density. C, Coronal section of aKO aortic arch contains atheromas affecting its major branches (×40). D, Right brachiocephalic artery atheroma contains several CD31⁺ capillaries (red) located along intima-media border.

**Figure 4.** Partial loss of collagen XVIII increases lipids. Representative oil red O lipid stains of aWT (A), aHet (B), and aKO (C) atheromas. Both aHet and aKO atheromas have increased foam cells and lipid. Magnification ×200. D, Relative lipid area per measured intima (y axis) was determined for aWT (black bar, n = 15), aHet (gray, n = 15), and aKO (white, n = 17) atheromas. Lipid areas for aKO and aHet are 2.5-fold increased above aWT.
but reached only borderline statistical significance for aHet aKO compared with aWT lesions (9.8% versus 6.8%, Table) 

XVIII deficiency altered the lipid and cellular composition of SMCs in aKO and aHet atheromas (Table). Thus, collagen phage contents were accompanied by significantly fewer P in C57, LDLR, and ApoE, and 

Vascular permeability in aorta and skin. A, EBD content of aortas from WT (black bar), Het (gray bar), and KO (open bar) mice (each strain, hatched bars) and EBD axis, hatched bars) and EBD 

Collagen XVIII Deficiency Increases the Permeability of Large and Small Blood Vessels

The visibly pronounced lipids in aHet and aKO aortas were also seen in skin and neural tissues. Lipid and cholesterol accumulation in the middle ear and adjacent 7th and 8th cranial nerves produced late-onset gait and balance disturbances in >25% of cholesterol-fed aHet and aKO but not aWT mice. To determine whether Col18a1 genotype alters vascular permeability, we measured permeability to albumin-bound EBD in large and small blood vessels for each genotype. EBD contents of aHet and aKO aortas harvested 30 minutes after EBD injection were increased 2.3- and 2.2-fold, respectively, compared with aWT (Figure 6A, P<0.01). We also compared EBD permeability of KO and WT aortas in the absence of hypercholesterolemia.21 Aortas from chow-fed KO mice in C57BL6/J and LDLR−/− strains both showed 1.9-fold increases in EBD contents compared with WT aortas (each strain, P<0.02). Het aortas from C57 and LDLR−/− strains had enhanced EBD permeability similar to the ApoE−/− strain (data not shown).

We performed the Miles assay in the same mice to compare small blood vessel permeability in the skin. A submaximal VEGF dose (10 ng) was used to detect a potential increase in permeability. Interestingly, EBD skin contents after buffer injections were 2.1-, 2.3-, and 1.9-fold higher in KO compared with WT mice in the C57BL6/J (P<0.01), LDLR−/− (P=0.02), and ApoE−/− (P<0.01) strains, respectively. KO from each strain showed a further VEGF-stimulated increase in EBD, but the levels of enhancement above baseline were proportional for KO and WT (Figure 6B). In separate studies, we observed increased EBD contents of lung, liver, and brain

Figure 5. Increased endothelial cell sprouting activity of Col18a1−/− aorta explants. Endothelial cell sprouting from KO (dashed line, open circles), Het (gray line, squares), and WT (solid line, closed circles) aorta explants stimulated by VEGF (0, 2, 4, 8 ng/mL). A representative curve (n=4 rings/dose) from 3 independent assays is shown. Sprouting from KO aortas is increased at all VEGF doses (P<0.05 for all VEGF doses) but is similar for Het and WT.

Angiogenic Sprouting From Col18a1−/− Aorta Is Enhanced

Because the aorta contains abundant collagen XVIII, we predicted that endothelial cell sprouting from WT aortas would be reduced compared with KO explants. WT aorta explants develop few sprouts when cultured in basal serum-free media but show a dose-dependent increase in sprouting that is maximal at VEGF=10 ng/mL.3 We compared the sprouting response of aorta explants from age-matched KO, Het, and WT mice over a range of VEGF doses (0, 2, 4, and 8 ng/mL). Sprout areas from KO aorta explants were significantly higher than Het or WT aortas (Figure 5, P<0.05 for all VEGF doses). In contrast, the sprouting responses of Het and WT aortas were similar. Therefore, sprout formation in vitro and plaque-associated angiogenesis in vivo were enhanced in aortas with complete but not heterozygous loss of collagen XVIII.

Figure 6. Vascular permeability in aorta and skin. A, EBD content of aortas from WT (black bar), Het (gray bar), and KO (open bar) mice in C57, LDLR−/−, and ApoE−/− strains (n=6 mice per group). EBD contents of KO and Het aortas are 2-fold increased compared with WT (C57, P=0.02; LDLR and ApoE, P<0.01). B, Miles assay results from dorsal skin of KO and WT. Basal EBD content after control buffer injections without VEGF (−) are 2-fold increased in KO compared with WT mice for all strains (C57, P<0.01; LDLR, P=0.02; apoE, P<0.01). Injection of VEGF (+, 10 ng/mL) increased EBD above basal levels, but VEGF responses of WT and KO are proportional. C, WT and KO (LDLR−/−) were injected with 125I-labeled LDL and EBD. Relative radioactivity (right y axis, hatched bars) and EBD contents (left y axis) of KO aortas are increased 3.2-fold. Increased permeability in KO is not suppressed after osmotic pumps increased serum ES to levels similar to or 5-fold higher (shown) than WT mice.
from Het and KO mice (not shown). Thus, baseline vascular permeability to albumin-bound EBD is increased in large and small vessels of mice with loss of one or both Col18al alleles.

Because aHet and aKO aortas accumulated more lipids during atherosclerosis, we measured the permeability of the aorta to LDL. EBD permeability in KO mice was similar for all strains, so we used chow-fed LDLR−/− mice to control for confounding variables affecting 125I-LDL deposition, such as competition by high endogenous LDL and LDLR-mediated cell uptake. Mice were coinjected with 5×10⁷ cpm of 125I-human LDL and EBD. After 30 minutes, LDL radioactivity deposited in aortas was 3.2-fold higher in KO relative to WT controls (Figure 6C, P<0.01). These same KO aortas had a 2-fold increase in EBD content, as seen previously.

Increased vascular permeability in KO mice could be because of loss of collagen XVIII proteins in the basement membrane and/or loss of ES in the blood. To determine whether circulating ES modifies the permeability of KO aortas, we administered ES protein to KO mice by continuous delivery from a subcutaneous osmotic pump. After 4 days, KO mice that received 25 or 100 µg/d of ES achieved serum levels of 60±12 or 353±80 ng/mL, respectively. Despite correction of serum ES to similar or 5-fold higher levels than WT mice, aorta permeability to EBD and LDL was not altered (Figure 6C).

**Discussion**

These studies provide genetic evidence that loss of collagen XVIII promotes atherosclerosis. Loss of collagen XVIII increases plaque angiogenesis and vascular permeability to lipids by distinct mechanisms that develop at different gene doses. Atherosclerotic involvement in arteries results in differential loss of collagen XVIII proteins. Binding of ES with laminin, nidogen, and perlecan could account for the relative preservation of ES-containing collagen XVIII proteins in blood vessels or could modify its exposure to matrix metalloproteinases, elastase, and cathepsin L. These proteases are active in atheromas, in which they may have redundant activities for processing collagen XVIII.

Complete or heterozygous loss of collagen XVIII increased lipid deposition and vascular permeability, which was not related to genotype differences in serum cholesterol. Increased lipid deposits in the vessel wall enhanced foam cells and atherosclerosis. Increased permeability of small vessels resulted in lipid accumulation in skin and other tissues of mice fed an atherogenic diet. The permeability defect was observed even in the absence of atherosclerosis and hypercholesterolemia, which indicates that it correlated with the Col18al genotype. Our results demonstrate a previously unrecognized function for collagen XVIII in basement membranes that is required for the maintenance of vascular permeability.

Increased retention or reduced clearance rates of lipoproteins are other potential mechanisms for accumulating lipids in tissues. Lipid retention in the subendothelium is an early event in atherosclerosis. Areas of EBD accumulation in the aorta contain proteoglycans that also retain lipids. Loss of the heparan sulfate proteoglycan collagen XVIII could indirectly alter lipid retention by other proteoglycans, such as biglycan. However, labeled LDL levels retained in KO aortas after 72 hours were not higher than levels consistent with a 2-fold rise in permeability (data not shown). In a quantitative macrophage recruitment assay, we found that KO and WT mice recruited similar numbers of macrophages, which degraded oxidized LDL at equal rates. ES exerted no effect on macrophage functions important for foam cell generation or degradation of modified LDL (data not shown). Together, these findings suggest that the mechanism of increased lipid in atheromas is in part because of increased vascular permeability and lipid deposition.

Short-term restoration of serum ES to physiological or 5-fold higher levels did not compensate for the permeability defect associated with loss of the entire gene. It is possible that other domains of collagen XVIII are also necessary for normal permeability function or that longer periods of ES delivery are needed to reverse the defect. Because Het mice were affected, we speculate that the permeability functions of collagen XVIII are sensitive to intermediate reductions of native protein in the vascular basement membrane. Although we show that collagen XVIII deficiency affects the permeability of large and small vessels, ultrastructural studies in KO mice fail to show an anatomic defect. There is little functional compensation for loss of collagen XVIII by highly homologous collagen XV or other major matrix components.

Loss of collagen XVIII resulted in more extensive VV and a higher incidence of intimal neovascularization in aKO but not aHet atheromas. These in vivo findings are consistent with the enhanced in vitro sprouting response of KO but not Het and WT aortas and suggest that more severe collagen XVIII deficiency is needed to enhance plaque angiogenesis. The increased angiogenesis in aKO was accompanied by a modest but not statistically significant increase in plaque size compared with aHet. Intimal neovascularization is more frequent in lesions of >250-µm thickness, but neovascular density and intima thickness are poorly correlated. During regression of atherosclerosis, neovascularization can increase significantly with only a small decrease in plaque size. Plaque neovascularization strongly correlates with the regional content of inflammatory cells. Consistent with this, we observed increased lesion macrophages in aKO.

This study reports the effects of deletion of the entire Col18al gene, not just the ES domain, which constitutes only a small 20-kD fragment of this >220-kDa trimeric molecule. Exogenous ES treatments inhibited plaque angiogenesis in ApoE−/− mice, and ES doses similar to serum levels inhibit sprouting from KO, Het, and WT aortas (not shown). However, full-length collagen XVIII or other domains could contribute to the angiogenesis and permeability phenotypes. Putative receptors or cofactors for ES, including glypican, α5β1 integrin and E-selectin, and the β-catenin signaling pathway, have been implicated in ES effects on angiogenesis, but their role in the phenotype of KO mice has not yet been determined. We can address the specific contribution of ES by raising variant Col18al mutant mice that endogenously express a truncated collagen XVIII lacking ES. Interestingly, some Knobloch syndrome patients have Col18al mutations predicted to encode similar truncated...
Our results have important implications for future investigations. First, the inhibition of angiogenesis by a component of normal blood vessels raises questions as to whether replacement or preservation of collagen XVIII in the vessel wall is a desirable therapeutic outcome. Second, the acquired loss of an artery component that increases lipid deposition and then angiogenesis at late stages suggests a potential property of endothelium.

Acknowledgments

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References

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