Expansive Remodeling Is a Response of the Plaque-Related Vessel Wall in Aortic Roots of ApoE-Deficient Mice

An Experiment of Nature

Jacob F. Bentzon, Gerard Pasterkamp, Erling Falk

Objective—In the present study, we (1) evaluated the apolipoprotein E–deficient (apoE<sup>−/−</sup>) mouse aortic root as a model for expansive remodeling in atherosclerosis and (2) examined whether remodeling at this site was due to dilation of the vessel wall beneath or flanking the plaque.

Methods and Results—Plaque area and length of the internal elastic lamina (IEL) were measured in aortic roots of apoE<sup>−/−</sup> mice at 11 weeks, 6 months, and 13 months of age. Expansive remodeling, indicated by IEL lengthening exceeding that of normal growth in wild-type C57BL/6 mice, was evident at 6 months of age and showed overcompensation (luminal enlargement) by 13 months of age. When the left coronary (LC), right coronary (RC), and noncoronary (NC) sinuses of the aortic root were looked at individually, remodeling was located to the LC at 6 months of age and to the LC and RC at 13 months of age. The remodeling response was closely related to local plaque area but unrelated to plaque formation in flanking sinuses.

Conclusions—The aortic root of the apoE<sup>−/−</sup> mouse features a consistent remodeling response. In this model, expansive remodeling was a strictly local response of the plaque-related vessel wall. (Arterioscler Thromb Vasc Biol. 2003;23:257-262.)

Key Words: atherosclerosis ■ remodeling ■ apolipoprotein E–deficient mice ■ animal model ■ media degeneration

Arterial remodeling strongly influences the effect of atherosclerotic plaque formation on luminal narrowing. Initially, atherosclerosis is an intimal disease, but the tunica media and adventitia may secondarily either dilate to preserve the lumen (expansive remodeling) or shrink and thus aggravate the obstruction caused by plaque formation (constrictive remodeling).<sup>1–3</sup> In a recently published autopsy study, Var- nava and Davies<sup>4</sup> examined 389 coronary segments with plaque formation, but no (acute) thrombotic complications, in men dying of sudden cardiac death. They found expansive remodeling of the vessel wall in 317 (81%) of the segments examined and constrictive remodeling in the remaining 72 segments (19%). In these cases, the mode and extent of remodeling were stronger determinants of lumen stenosis than plaque size, as has also been found by others.<sup>3</sup>

A number of animal models have been described in which expansive remodeling occurs,<sup>5–10</sup> including the apolipoprotein E–deficient (apoE<sup>−/−</sup>) mouse.<sup>11–14</sup> However, no simple, reliable model featuring a consistent response suitable for studies on remodeling mechanisms has been reported. The apoE<sup>−/−</sup> mouse is extensively used to study the development of atherosclerotic lesions,<sup>15,16</sup> and arterial remodeling has been reported at several sites of the arterial tree in this model.<sup>11–14</sup> The aortic root is a predilection site for atherosclerosis in apoE<sup>−/−</sup> mice and the object of standardized methods of histological processing and plaque quantification.<sup>17</sup> In the present study, we report that the aortic root features a consistent remodeling response with low variation.

Little is known about the mechanisms of expansive remodeling, including even the fundamental question of whether remodeling is a local pathophysiological process driven by factors of the atherosclerotic plaque or a homeostatic response of the endothelium to maintain normal shear stress and wall tension.<sup>18</sup> The finding of medial thinning and disruption of elastic laminae beneath plaques in humans and animal models<sup>19,20</sup> supports the first hypothesis, although it is unknown whether these media changes actually take part in the remodeling response. The latter hypothesis has been inferred from the observation that both normal<sup>1</sup> and atherosclerotic<sup>21</sup> arteries undergo outward remodeling when exposed to increased wall shear stress. By analogy, a plaque encroaching on the lumen could elicit expansive remodeling through a circumferential increase in wall shear stress. This response would be expected to be less marked at the actual...
site of plaque formation (plaque-related vessel wall) because of dysfunction of the endothelium overlying the plaque. Being divided into 3 sinuses by the cusps, the aortic root of the apoE \(^{-/-}\) mouse allows distinction of remodeling of the plaque-related vessel wall from that of the flanking vessel wall, and thereby the aortic root constitutes an experiment of nature to evaluate these hypotheses.

**Methods**

**Animals**

Forty-eight male apoE \(^{-/-}\) mice, back-crossed 9 generations into the C57BL/6 background, were obtained from Bomholtgaard Breeding and Research Center, Ry, Denmark. These mice originally served as control groups of other studies conducted in our research facility in the period 1997 to 1999. Animals were killed at 11 weeks (n=13), 6 months (n=21), or 13 months (n=14) of age. Twenty-two male wild-type (WT) C57BL/6 mice of similar ages (n=6, 3, and 11 for the 3 time points, respectively) were included to control for normal growth. Mice were housed in groups and fed normal chow (Altromin 1314) and water. The Danish National Ethics Committee approved all procedures.

**Histological Processing**

Mice were anesthetized (midazolam 20 mg/kg IP, fluanisone 40 mg/kg IP, fentanyl citrate 1.5 mg/kg IP or pentobarbital 150 mg/kg IP) and killed by exsanguination. The arterial system was flushed with St. Thomas’ cardioplegic solution containing heparin and pressure-fixed with phosphate-buffered 4% formaldehyde (pH 7.2) through the left ventricle. Mice were then immersed in the fixative for 6 hours before storage in cold phosphate buffer. The heart, including the ascending aorta, was removed and cut in half, followed by paraffin embedding. The half containing the aortic root was sectioned serially at 4-μm intervals. Once the aortic sinuses appeared, every other cross section of 4-μm thickness was collected on glass slides. The first section showing the commissures of the aortic cusps was stained with orcein (for elastin) and evaluated microscopically by the same observer (J.F.B.) for plaque type, media changes, and morphometry.

**Morphometry**

The attachments of the aortic cusps mark the division of the aortic root into left coronary (LC), right coronary (RC), and noncoronary (NC) sinuses (Figure 1). The main stem of the left coronary artery and the right coronary artery originate from the LC and RC, respectively. In each sinus, the plaque area and length of the internal elastic lamina (IEL length) were measured by computer-assisted image analysis (Olympus BX50 light microscope, Sony DXC-151P, Imagraph Precision frame grabber, and SigmaScan Pro from Jandel Scientific Software). Increase in IEL length was used as a measure of expansive remodeling. To avoid the influence of folding artifacts, the vessel area was not measured directly but calculated from the arterial circumference (total IEL length) as follows: (IEL lengths of all coronary sinuses)\(^2\)/4. Subsequently, the lumen area was calculated: vessel area minus total plaque area of the 3 sinuses.

**Statistical Analysis**

All values are presented as mean±SD. ANOVA was performed to study differences in plaque area over time. An independent sample t test was used to assess differences in plaque area and IEL length between apoE \(^{-/-}\) and WT mice, except for 1 time point (6 months of age), where nonparametric analysis (Mann-Whitney) was used because of the low number of mice in the WT group (n=3). For each remodeled sinus (LC at 6 and 13 months of age, RC at 13 months of age), linear regression analysis was performed to study the relation between local plaque area and local IEL length after confirming compliance with the appropriate assumptions. To determine the influence of plaque in flanking sinuses on local IEL length, the sum of plaque areas in flanking sinuses was entered into the regression analysis. SPSS 10.0 was used for statistical analysis. A probability value <0.05 was considered significant.

**Results**

**Atherosclerosis**

At 11 weeks of age, only fatty streaks and individual foam cells were seen (Figure 1A), whereas plaques in mice of 6 or 13 months of age were of the advanced type, containing extracellular lipids (cholesterol crystals) and fibrous connective tissue (Figure 1B and 1C). In some of the specimens, the right coronary or main stem of the left coronary artery left the aortic root at the level of sectioning, leading to slight underestimation of the plaque area (Figure 1C).

**Media**

At the level of the commissures of the aortic cusps, the media was homogenous with circumferentially intact elastic laminae in all WT mice and in apoE \(^{-/-}\) mice at 11 weeks of age. At 6 or 13 months of age, the normal lamellar structure of the media in apoE \(^{-/-}\) mice was severely degenerated beneath plaques (Figure 2A), but the observed pathology was not uniform or simple. In some sites, media thinning with loss of elastic layers was predominant (Figure 2B), whereas other
sites showed increased spacing between laminae with intercalated amorphous material (Figure 2C). In both these situations, fragmented elastic laminae were frequently seen, but thinning and increased spacing also occurred without fragmentation. The staging systems used by others to quantify atherosclerotic media degeneration rely solely on the extent of lamina fragmentation or the thickness of the media. None of these were found to be applicable to the media of the aortic root because of the heterogenous appearance. By close inspection (by J.F.B.), the medial changes did not appear to be more pronounced in mice 13 months of age than in those 6 months of age, but the changes were not quantified. Full disruption of the media was observed in only 2 mice: 1, 6 months of age and 1, 13 months of age (Figure 2D).

Remodeling May Overcompensate
Mean vessel area in apoE−/− mice was enlarged compared with WT mice at 6 months of age with preservation of luminal area. At 13 months, the lumen areas in the apoE−/− mice were significantly even larger than those of WT mice (Table 1). The severely atherosclerotic aortic root may resemble a cloverleaf more than a circle in cross section and thereby not meet the assumptions for calculating the lumen area (see Methods). Therefore, we also measured the lumen area directly in 13-month-old apoE−/− mice. This method is subject to folding artifacts but leads to an only slightly lower mean lumen area (1713±283×10^3 μm² for 13-month-old apoE−/− mice), which was still significantly larger than the calculated lumen areas of WT mice (P<0.001). At 11 weeks, no differences in mean vessel or lumen areas were observed between groups.

Remodeling Response in Each Coronary Sinus
When each sinus was looked at individually, the remodeling response was located to the LC at 6 months of age and to the LC and RC at 13 months of age (Figure 3). The NC did not exhibit atherosclerotic remodeling at any time point. The asymmetrical remodeling response was paralleled by differences in plaque growth between sinuses, as shown in Table 2. Plaque growth was largest in the LC and least in the NC (P<0.05). The relations between local plaque area and the local IEL length are illustrated in Figure 4 for sinuses exhibiting remodeling.

In the LC at 6 months of age, a consistent increase in IEL length was observed in response to local plaque growth (IEL length [μm]=1256+3.2×plaque area [in 10^3 μm²], r²=0.63, P<0.001). Also at 13 months of age, a significant relation was observed in the LC (IEL length=1862+1.4×plaque area, r²=0.38, P<0.001). For the RC, a significant relation was observed between local plaque area and IEL length at 13 months.

### TABLE 1. Vessel, Plaque, and Lumen Areas in ApoE−/− and WT Mice

<table>
<thead>
<tr>
<th></th>
<th>WT Mice</th>
<th>ApoE−/− Mice</th>
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<tbody>
<tr>
<td>Vessel Area</td>
<td>Lumen Area</td>
<td>Vessel Area</td>
</tr>
<tr>
<td>weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>893±88</td>
<td>914±29</td>
</tr>
<tr>
<td>6 months</td>
<td>1033±91</td>
<td>1342±52*</td>
</tr>
<tr>
<td>13 months</td>
<td>1344±185</td>
<td>2482±72†</td>
</tr>
</tbody>
</table>

Vessel area denotes the area circumscribed by the IEL. Lumen area = vessel area − plaque area.

*P<0.05 or †P<0.001 vs WT. All numbers in 1000 μm².
In arteries that are often diffusely affected by atherosclerosis, the difficulty in finding a proper nondiseased reference site becomes even more critical.

The same observation was made for the left coronary sinus at 6 and 13 months of age and of the right coronary sinus at 13 months of age. The noncoronary sinus is least affected, both by atherosclerosis and remodeling. WT mice. Bars indicate SD. *P<0.01, †P<0.001.

**Table 2. Plaque Area in Aortic Sinuses of ApoE−/− Mice**

<table>
<thead>
<tr>
<th></th>
<th>Left Coronary Sinus</th>
<th>Right Coronary Sinus</th>
<th>Noncoronary Sinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 weeks</td>
<td>7.1±2.6</td>
<td>1.5±1.4</td>
<td>4.1±2.6</td>
</tr>
<tr>
<td>6 months</td>
<td>121.7±54.4</td>
<td>26.6±30.6</td>
<td>44.2±43.6</td>
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<tr>
<td>13 months</td>
<td>277.2±74.5</td>
<td>188.1±67.7</td>
<td>121.5±39.9</td>
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</tbody>
</table>

All numbers in 1000 μm².

**Figure 3.** Compared with normal aortic roots of wild-type (WT) mice, apoE−/− mice display expansive atherosclerotic remodeling of the left coronary sinus at 6 and 13 months of age and of the right coronary sinus at 13 months of age. The noncoronary sinus is least affected, both by atherosclerosis and remodeling.

**Discussion**

**Methods to Study Arterial Remodeling**

The mode of remodeling, expansive or constrictive, is an important determinant of luminal narrowing in de novo atherosclerotic disease. Insight into the mechanisms that direct the remodeling response may lead to new interventional strategies to treat arterial occlusive disease. Most human studies on the mechanisms of constrictive or expansive remodeling are hampered by their cross-sectional design. The essential problem of such nonprospective studies is the difficulty in finding a proper nondiseased reference site in arteries that are often diffusely affected by atherosclerosis. This limitation can partly be overcome by using an animal model with little variation and high reproducibility in expansive remodeling response over time. Expansive enlargement has been reported in several animal models of atherosclerosis. However, these previously described animal models suffered from limitations. Often, lesions do not develop spontaneously but in response to mechanical injury. Furthermore, the extent of atherosclerotic disease and subsequent remodeling at predetermined sites often vary widely.

**The ApoE−/− Mouse Aortic Root Model**

The present study shows that the aortic root of the apoE−/− mouse has unique characteristics that may facilitate studies on the mechanisms of expansive remodeling in atherosclerosis. Within 6 months of age, advanced atherosclerosis was present, and the fragmented media beneath the plaques resembled what has been described for human coronary arteries undergoing expansive remodeling.

Expansive remodeling was evident in the aortic root at 6 months of age and even led to overcompensation at 13 months of age: the lumen became significantly larger despite increasing plaque area. Overcompensation is a feature of the remodeling response in human coronary arteries as well.

Expansive remodeling was restricted to those sinuses with the most extensive atherosclerosis. Furthermore, overcompensation was closely correlated to the extent of local plaque formation. These observations strongly suggest a causal relation between lesion formation and expansive remodeling. However, because lesion size was not manipulated experimentally, it remains to be shown that altering lesion size by intervention will also alter remodeling.

Expansive remodeling is a local, plaque-related phenomenon. In normal and atherosclerotic arteries, wall shear stress is a major determinant of lumen size, and it has been suggested that expansive remodeling in atherosclerosis is due to a similar endothelium-dependent enlargement of the arteries. If local plaque formation were to encroach on the lumen, increased shear stress would elicit an endothelium-dependent...
dilatory response. This effect would be anticipated to be least pronounced in the plaque-related vessel wall because of endothelial dysfunction, thereby explaining the observation of some investigators, though not all,25 that outward remodeling is more prominent in the setting of eccentric than concentric plaques.4,10 Conversely, Glagov et al,1 using detailed geometrical characterization of human coronary arteries, concluded that the dilatory response is mainly associated with outward bulging beneath plaques.

The present study is the first to locate expansive remodeling to the vessel wall underlying atherosclerotic plaques by using a direct approach. This result does not exclude the possibility that remodeling of the flanking vessel wall elicited by increases in shear stress may be important at other sites of the arterial system, because the special hemodynamic environment of the aortic root may well have interfered with this mechanism. Also, it is possible that the presence of the cusps may have formed a kind of internal strut that potentially distorted the remodeling response.

Matrix Metalloproteinases
Proposed mechanisms for local expansive remodeling underneath plaques have focused on matrix metalloproteinases (MMPs). It has been shown that expansive remodeling is associated with increased macrophage and lymphocyte counts in human plaques.23,26 Although the implications for de novo atherosclerosis are uncertain, a similar correlation was recently suggested in an apoE−/− mouse model of neointima formation.27 In plaques with ongoing inflammation, macrophages may locally release MMPs that weaken the skeleton of the arterial wall, thereby promoting outward bulging of the plaque,18 and several MMPs, including MMP-2, MMP-3, and MMP-9, have been found in excess in plaques at sites of expansive remodeling compared with plaques at constrictive sites.28,29 The same processes may weaken fibrous caps, thereby explaining the association of expansive remodeling with culprit plaques of acute coronary syndromes observed by several investigators.30,31

The presented apoE−/− remodeling model may prove valuable for studies on the causality of the relations between macrophages, MMP expression, and expansive remodeling. The association of expansive remodeling with plaque rupture is not likely to be elucidated in this model, because plaques in the aortic root rarely rupture in these mice, at least not in those back-crossed into the C57BL/6 background.32,33

Expansive Remodeling or Aneurysm Formation?
Media degeneration is often observed beneath remodeled plaques, including those examined in the present study. This phenomenon bears a strong resemblance to aneurysm formation,44 and with present knowledge, there seems to be little reason for choosing 1 designation over the other except for the scope of individual research and the magnitude of vessel dilation (by definition, true aneurysms are localized dilations of >50% diameter increase34). For example, when apoE−/− mice are fed an atherogenic diet for 26 weeks, the induced dilations of the atherosclerotic abdominal aorta are described as aneurysms (vessel diameter increased by >50%),22 whereas in the present study, we denote changes with similar histological description as expansive remodeling (diameter increase of 27% to 49% compared with WT mice; data not shown). However, few indications exist to suggest that in expansive remodeling, the disruption of a normal lamellar structure of the media may be coincidental to rather than implicated in the remodeling process. Although not quantified because of their heterogeneous appearance, there was no apparent correlation between the severity of media changes and the remodeling response in the present study. ApoE−/−, apoE−/− t-PA−/−, and apoE−/− u-PA−/− mice all exhibit vascular remodeling in the abdominal aorta, but only in apoE−/− and apoE−/− t-PA−/− mice is this followed by media degeneration and aneurysm formation.35 Also, in a previously
published study, we observed (but did not report) remodeling with lumen preservation without laminar fragmentation (but with medial hypertrophy) in the brachiocephalic trunk of chow-fed apoE−/− mice at 6 months of age. Better understanding of the role of media degeneration in expansive remodeling may aid in establishing the demarcation (if any) between this phenomenon and aneurysm formation.

Conclusions

The aortic root of the apoE−/− mouse is a model in which expansive remodeling consistently occurs in response to plaque formation and is therefore suitable to study its mechanism. In this model, expansive remodeling was a local, plaque-related response that did not involve the flanking vessel wall.

References

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Arterioscler Thromb Vasc Biol. 2003;23:257-262; originally published online December 12, 2002;
doi: 10.1161/01.ATV.0000051387.70962.79
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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