Subphysiologic Apolipoprotein E (ApoE) Plasma Levels Inhibit Neointimal Formation After Arterial Injury in ApoE-Deficient Mice


Objective—Apolipoprotein E (apoE) reduces mouse atherosclerosis progression independent of plasma cholesterol level effects. A mouse artery injury model was used to examine whether apoE exhibits beneficial lipid-independent effects on neointimal formation.

Methods and Results—ApoE-deficient (apoE−/−), wild-type (WT), and transgenic apoE−/− mice (secrating apoE at different levels from adrenal glands) underwent femoral artery injury. Mice with low expression of plasma apoE (0.1% of WT) had cholesterol levels approximately half those of apoE−/− littermates (but still ∼6 times WT). Mice with higher expression (HE; 2% to 3% of WT) of plasma apoE had cholesterol levels approximately twice those of WT. Injured WT mouse (versus apoE−/−) arteries had a smaller mean intima-to-media (I/M) ratio (0.87 versus 1.96; P=0.05). HE mice tended to have lower mean I/M ratios (1.3; P>0.05 versus apoE−/− mice). Multiple regression analysis indicated that apoE levels were significantly associated with reduced I/M ratios, but plasma cholesterol levels were not, before or after adjusting for apoE. In addition, foam cell content of the neointima and media of injured arteries, a negative prognostic indicator in postangioplasty human lesions, was inversely related to plasma apoE levels.

Conclusions—Similar to its effects on atherosclerosis progression, in a mouse model of restenosis, a subphysiological level of apoE was associated with beneficial effects on lesion size/composition. (Arterioscler Thromb Vasc Biol. 2004; 24:1460-1465.)

Key Words: apolipoprotein E ■ arterial injury ■ neointimal formation ■ restenosis ■ mouse

Atherosclerosis complications are the most common causes of death in Western societies. In the United States, heart disease and stroke account for almost half of all deaths.1,2 Atherosclerosis is a chronic process resulting from interaction between normal and modified lipoproteins, monocyte-derived macrophages, lymphocytes, and normal cellular elements of the arterial wall. Endothelial dysfunction and the retention and oxidation of cholesterol ester-rich lipoproteins in the subendothelial space initiate complex inflammatory reactions, which can ultimately lead to plaque formation and rupture.3,4 Restenosis after angioplasty is a common clinical problem, even with the widespread use of stents. In certain aspects, restenosis represents an accelerated form of atherosclerosis, in which neointima formation is a prominent feature. After endothelial denudation, there is proliferation and migration of vascular smooth muscle cells (SMCs) from the tunica media to the intima.5 In addition, abnormal vascular remodeling associated with inefficient compensatory enlargement or even constriction of the arterial wall contributes to luminal stenosis.6 Despite the use of stents, restenosis occurs in 20% to 30% of patients in the first 6 months after coronary angioplasty procedures.7,8

Apolipoprotein E (apoE) plays a critical role in cholesterol homeostasis. It serves as the ligand for receptor-mediated clearance of several classes of lipoproteins, including chylomicrons, very low-density lipoprotein (LDL), high-density lipoprotein subclasses, and lipoprotein remnants.9 In apoE-deficient (apoE−/−) mice (in which the apoE gene is inactivated), the hyperlipidemia is marked and results in the spontaneous development of atherosclerotic lesions throughout the arterial tree.10,11 Because of similarities in histopathologic characteristics between lesions of apoE−/− mice and patients, these mice are widely used as a model of human atherosclerosis and restenosis.

Previous studies have suggested that apoE has antiatherogenic properties independent from its lipid-lowering effect.
For example, low levels of human apoE expressed in macrophages of apoE−/− mice were associated with decreased atherosclerosis without correcting hyperlipidemia. The proposed explanation was the positive effect of macrophage-derived apoE on reverse cholesterol transport in the arterial wall. We have reported previously that in apoE−/− mice that express transgenic apoE in the adrenal glands at levels too low to correct the hypercholesterolemia of their apoE−/− background, there was almost complete suppression of atherosclerotic lesion development. Zhu et al were able to show beneficial effects of apoE overexpression (in transgenic FVB/N mice) on neointimal formation after arterial injury. In contrast to Zhu et al, we examined the effects of plasma apoE levels that varied from nil to WT on neointimal formation after arterial injury. This was accomplished by performing femoral artery injuries not only in apoE−/− and WT mice, but also in apoE−/− mice with a range of expression of transgenic mouse apoE in the adrenal glands. As will be presented, multiple regression statistical analysis showed that plasma apoE but not cholesterol levels were significantly associated with the intima-to-media (I/M) ratio, a standard measure of neointimal formation after arterial injury. In addition, the macrophage foam cell content of the lesions (a negative prognostic clinical indicator) decreased as plasma apoE levels increased. Similar to our previous report on atherosclerosis progression, these results suggest that subphysiological levels of plasma apoE have beneficial effects on the arterial wall.

**Methods**

The Methods section is available online at http://atvb.ahajournals.org.

**Results**

**Plasma Cholesterol and ApoE Levels**

Plasma cholesterol and apoE levels at the time of death are summarized in Table 1. The data show that only very small apoE levels were apparently necessary to reduce plasma cholesterol levels. For example, at 0.1% of WT apoE, plasma cholesterol in low expressors was half that in apoE−/− littersmates (but still ~6-fold more than the WT level). In higher expressors, at 2% to 3% of WT apoE, plasma cholesterol levels were lower by 80%, to 2.3-fold higher than the WT level.

**Figure 1.** Histological appearance of mouse femoral arteries. A, Analysis at 4 weeks after injury. Representative photomicrographs (combined Masson–elastic stain) of lesions from mice in each group are displayed. EKO indicates the apoE−/− sample. Arrows indicate the junctions between the intimal and medial layers. B, Appearance of an apoE−/− femoral artery just before injury. Staining is with hematoxylin and eosin. Magnification for all is ×40.
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immunostaining, the arteries from apoE−/− mice showed large
accumulations of foam cells in the immediate subendothelial
region of the intima and in deeper regions on both sides of the
internal elastic lamina (IEL). Foam cells were less prominent in
the intima of the LE artery and appeared to be in deeper regions
of the intima and in the media near the IEL. In contrast, no foam
cells were observed in any of the media or intima of WT or HE
mice. This was confirmed by an independent and blinded
pathologist, who was able to accurately distinguish between LE
and HE mice on the basis of foam cell amount and distribution,
with only 1 exception after examining 2 sections from each
artery of every mouse in the study.

In contrast to the pattern of macrophage staining,
α-actin staining of SMCs showed a direct relationship with
apoE expression levels (Figure 2, α-actin panels). α-Actin
staining was present and layered in the media of WT
arteries, less prominent in the HE arteries, sparse and
dispersed throughout the neointima of the LE arteries, and
barely detectable in the neointima of the apoE−/−
arteries. The media of LE and apoE−/− mice contained only
traces of α-actin stained cells.

For the sections shown from the HE and LE groups, the
mice used were matched for similar cholesterol levels (HE
211 mg/dL; LE 276 mg/dL). Therefore, histological changes
between these 2 mice illustrate the greater effect of apoE
(versus cholesterol) on lesion characteristics.

<p>| TABLE 2. Summary of Morphometric Data and Statistical Analyses     |</p>
<table>
<thead>
<tr>
<th>A. Morphometric Data</th>
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<tbody>
<tr>
<td>IA</td>
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<tr>
<td>IA 11.19±2.8</td>
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<tr>
<td>MA 13.62±0.99</td>
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<tr>
<td>I/M ratio 0.87±0.2</td>
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<td>HS 21.0±5.5</td>
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<th>B. One-Way ANOVA</th>
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<td>Dependent Variable</td>
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<tr>
<td>IA</td>
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<tr>
<td>MA</td>
</tr>
<tr>
<td>I/M</td>
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<tr>
<td>HS</td>
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Data are mean±SEM. Units are mm², except for HS, which is percentage.
One-way ANOVA with the Bonferroni Multiple Comparison Test was used to determine statistically
significant effects of the different genotypes (WT, HE, LE, and apoE−/−) on the indicated dependent
variables.
IA indicates intimal area; MA, medial area; HS, histopathological stenosis.

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<th>TABLE 3. Stepwise Regression Analysis of the I/M Ratio (Dependent Variable) and the Plasma Levels of ApoE and Cholesterol (Independent Variables)</th>
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<tbody>
<tr>
<td>Outcome</td>
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<td>ApoE enters the model first</td>
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<td>Cholesterol enters the model first</td>
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ApoE and cholesterol values were transformed logarithmically because they were not normally
distributed. In both models, P values associated with the coefficient for the cholesterol term were
nonsignificant (P>0.25).
Hasty et al reported similar results using apoE/H11002 mice was half of the average level of their apoE associated with reductions in plasma cholesterol. For exam-
summarized in Results, even low apoE plasma levels were
plasma level. A potential contributing factor to both findings
distribution were also influenced negatively by the apoE
associated with changes in the plasma apoE but not choles-
terol plasma levels were lower by 60%.12 Notably, in a
2-step, forward multiple regression model, whether the level
of apoE or cholesterol was entered first into the model,
plasma cholesterol did not add statistical significance. In
contrast, the inclusion of apoE in the model was consistently
significant (Table 3).

Because of the presumed overlap in some of the mecha-
nisms underlying restenosis and atherosclerosis, results from
a number of mouse atherosclerosis studies may be relevant to
our finding that the level of apoE but not plasma cholesterol
was strongly associated with the I/M ratio. For example, in
our previous studies,13 levels of apoE too low to result in
reduced plasma cholesterol were still associated with retarded
atherosclerosis progression. Chronic intravenous administra-
tion of apoE in the Watanabe heritable hyperlipidemic rabbit
inhibited atherosclerosis without a reduction in total plasma
cholesterol.17 Also, apoE overexpressed in the arterial wall by
either transgenic means18 or retroviral transduction of bone
marrow before transplantation19 protected the aorta from
early atherosclerosis, although plasma cholesterol levels and
lipoprotein profiles remained unchanged from those seen in
control mice. Furthermore, Zhu et al were able to show that
not only atherosclerosis but also neointimal formation after
arterial injury was reduced in normocholesterolemic WT
mice overexpressing apoE, independent of further cholesterol
lowering.14 Except in our report,13 in all of these studies,
supraphysiological apoE concentrations were used (either
systemically or locally in the vessel wall). Notable, then, is
our present finding that very low plasma apoE levels are
sufficient to observe a beneficial effect on the I/M ratio after
arterial injury.

The second major finding of our present studies was the
altered quantity and distribution of cell-specific immuno-
staining in the neoIntima and media of the LE and apoE/−/−
mice. In apoE/−/− and LE mice, foam cells positive for the
macrophage marker MOMA-2 were present in the intima and
media near the IEL (Figure 2). In contrast, the level of apoE
in HE mice (=2% to 3% of WT) was apparently sufficient to
prevent foam cell accumulation in either the media or intima.
In a previous study, we reported the presence of macrophages
adjacent to the media in the injured femoral arteries of
apoE/−/− but not WT mice20 and speculated that this was
associated with hyperlipidemia-related induction of adhesion
molecules on SMCs, such as vascular cell adhesion molecules
and intercellular adhesion molecules. The present study now
puts forward the exciting prospect that the expression level of
apoE may make a contribution independent of hyperlipidemia
on macrophage accumulation in the injured arterial wall. In
turn, the importance of the macrophage content in the injured
artery is suggested by the finding in patients who have
undergone angioplasty that neointimal macrophage content is
a strong risk factor for restenosis.21

Another histopathologic finding of interest was the loss of
α-actin staining in the media of apoE/−/− and LE mice. α-
Actin expression in SMCs is known to be labile and is lost,
for example, when cells shift from a “contractile” to a proliferative phenotype, which would precede their migration into the neointima. Thus, given the larger neointima formed in the apoE−/− and LE mice, perhaps the loss of α-actin staining represents a high degree of recruitment of medial SMCs to the neointima.

There have been several suggestions about the molecular mechanisms by which apoE protects against atherosclerosis and restenosis. Similar to our observation that apoE may influence the cellular composition of lesions, other authors have demonstrated inhibition of early monocyte invasion and, therefore, reduction in foam cell deposition and atherogenesis.19,22 Previous studies showed that apoE has a variety of cytokine-like or hormonal effects in steroidogenic cells,23,24 platelets,25 and lymphocytes.26 More recently, Hui et al showed that apoE inhibits the platelet-derived growth factor (PDGF) stimulation of SMC proliferation and migration.27

Inhibition of PDGF-stimulated cell migration appears to be mediated via suppression of signal transduction through the LDL receptor–related protein (LRP). LRP participates in signal transduction pathways in the brain and other cells.28 LRP is tyrosine phosphorylated on its cytoplasmic tail, leading to recruitment of downstream signaling components.29–31 LRP tyrosine phosphorylation is stimulated by PDGF, and this activity is inhibited by apoE.30,31 These findings suggest that low apoE levels might inhibit neointima formation after arterial injury via suppression of vascular SMC migration and proliferation. In support of this hypothesis, inactivation of the LRP gene in vascular SMCs caused activation of PDGF signaling, SMC proliferation, and enhanced aortic lesion development in LDL receptor–deficient mice.32

We find it interesting that very little circulating apoE is apparently needed to reduce the cholesterol plasma level or to defend postinjury arterial morphology in apoE−/− mice. Why then is apoE expressed in such abundance? There is evidence that apoE plays a part in many normal and pathological processes beyond its traditional role in lipoprotein metabolism, such as in cerebral and cognitive functions, nerve regeneration, tumor cell proliferation, steroidogenesis, Alzheimer’s disease, gallstone formation, and proximal colon carcinoma.33,34 This suggests that a level of apoE effective in one context may not be relevant to another.

In summary, our results demonstrate that a very low apoE level provided systemically was associated with a major beneficial impact on the quantity (as assessed by the I/M ratio) and quality of the neointima after arterial injury in mice. Furthermore, the quantitative effects were statistically independent of changes in plasma cholesterol levels. Taken with the beneficial effects of apoE on atherosclerosis progression,13 the data suggest that apoE has a generally important protective function in the arterial wall.

Acknowledgments

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References


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