Rapid Regression of Atherosclerosis Induced by Liver-Directed Gene Transfer of ApoE in ApoE-Deficient Mice

Kazuhisa Tsukamoto, Rajendra Tangirala, Sam H. Chun, Ellen Puro, Daniel J. Rader

Abstract—Apolipoprotein E (apoE) is a multifunctional protein synthesized by the liver and tissue macrophages. ApoE-deficient mice have severe hyperlipidemia and develop accelerated atherosclerosis on a chow diet. Both liver-derived and macrophage-derived apoEs have been shown to reduce plasma lipoprotein levels and slow the progression of atherosclerosis in apoE-deficient mice, but regression of atherosclerosis has not been demonstrated in this model. We utilized second-generation adenoviruses to achieve hepatic expression of human apoE in chow-fed, apoE-deficient mice with established atherosclerotic lesions of different stages. As expected, hepatic expression of human apoE3 significantly reduced plasma cholesterol levels. Liver-derived apoE also accumulated substantially within preexisting atherosclerotic lesions, indicating that plasma apoE gained access to the arterial intima. Hepatic expression of human apoE3 for 6 weeks resulted in significant quantitative regression of both early fatty streak lesions as well as advanced, complex lesions in both the aortic root and the aortic arch. In addition, hepatic expression of apoE induced substantial morphological changes in lesions, including decreased foam cells and increased smooth muscle cells and extracellular matrix content. In parallel, human apoE4 and apoE2 were also expressed in the liver by using recombinant adenoviruses. ApoE4 reduced cholesterol levels to the same extent as did apoE3 and also prevented progression but did not induce significant regression of preexisting lesions. ApoE2 reduced cholesterol levels to a lesser degree than did apoE3 and apoE4 and lesion progression was reduced, but regression was not induced. In summary, (1) regression of preexisting atherosclerotic lesions in apoE-deficient mice can be rapidly induced by hepatic expression of apoE, despite the absence of macrophage-derived apoE; (2) the morphological changes seen in this model of regression resemble those in other animal models, induced over longer periods of time; (3) liver-derived apoE gained access to and was retained by intimal atherosclerotic lesions; and (4) apoE4 was less effective in inducing regression, despite its effects on plasma lipoproteins that were similar to those of apoE3. The rapid regression of preexisting atherosclerotic lesions induced by apoE gene transfer in apoE-deficient mice could provide a convenient murine model for investigation of the molecular events associated with atherosclerosis regression.

Key Words: atherosclerosis n regression n gene transfer n adenoviral vectors n apolipoproteins

Regression of atherosclerosis has been demonstrated in several animal models,1–6 largely through dietary manipulation of cholesterol levels. Regression is characterized by morphological changes in lesions, such as increased fibrotic components and decreased lipid and inflammatory components. These changes are consistent with the concept of lesion stabilization,7,8 which is thought to be responsible for the reduction in acute coronary events associated with cholesterol reduction in humans. However, the cellular and molecular mechanisms of atherosclerosis regression and stabilization are not yet well understood.

ApoE is a multifunctional protein that is synthesized in a variety of tissues, including the liver and macrophages.9 ApoE-deficient mice have hypercholesterolemia and develop extensive atherosclerosis on a chow diet.10–13 Expression of human apoE in either the liver14,15 or macrophages16,17 of apoE-deficient mice reduces cholesterol levels and slows progression of atherosclerosis. In addition, macrophage-derived apoE has been shown to slow progression of atherosclerosis, independent of plasma lipoprotein levels.18,19 However, neither liver- nor macrophage-derived apoE has been shown to induce regression of preexisting atherosclerotic lesions in apoE-deficient mice. Furthermore, it is not known whether regression of established atherosclerotic lesions is dependent on macrophage production of apoE. Finally, the ability of liver-derived plasma apoE to gain access to the arterial intima has been suggested by the identification of lipid-poor forms of apoE in plasma but has not been directly investigated.

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ApoE occurs in 3 major, common isoforms in humans. ApoE3 is the most common isoform. ApoE2 has a single substitution of 158 Cys for Arg. It has impaired binding to the LDL receptor in vitro and slower turnover in humans in vivo compared with apoE3. Homozygosity for apoE2 is associated with familial dysbeta lipoproteinemia due to impaired clearance of remnant lipoproteins.

ApoE4 has a single substitution of 112 Arg for Cys. It has normal binding to the LDL receptor in vitro and faster turnover in humans in vivo compared with apoE3. Heterozygosity for apoE4 is associated with an increased risk of atherosclerotic cardiovascular disease. ApoE4 has certain cellular effects in vitro that differ substantially from those of apoE3.

We previously demonstrated that hepatic expression of the 3 human apoE isoforms in apoE-deficient mice had effects on lipoproteins in vivo that were predicted on the basis of receptor-binding and human kinetic studies.

In the current experiments, we utilized recombinant, second-generation adenoviruses to achieve liver-directed gene transfer of apoE in apoE-deficient mice to test the following hypotheses: (1) that hepatic expression of apoE can induce rapid regression of and morphological changes in established atherosclerotic lesions, even in the absence of macrophage-derived apoE; (2) that liver-derived apoE can gain access to the arterial wall via the plasma compartment; and (3) that apoE isoforms differ in their ability to induce regression of preexisting atherosclerotic lesions in apoE-deficient mice.

Methods

Construction of Recombinant, Second-Generation Adenoviruses

We utilized second-generation adenoviruses, which result in longer transgene expression in the mouse liver, with which we previously demonstrated prolonged expression of human apoE isoforms in apoE-deficient mice. Recombinant adenoviruses were constructed as previously described. Recombinant plasmids were cotransfected into 293 cells with DNA of H5.110CMVlacZ digested with restriction enzyme HpaI. These viral DNAs were subjected to polymerase chain reaction–restriction fragment length polymorphism analysis, and the genotypes were confirmed. The recombinant plasmids were cotransfected into 293 cells with DNA of H5.110CMVlacZ digested with Clal. Transfected cells were overgrown with agar and kept at 32°C for 15 days, and positive plaques for expression of human apoE, detected using a Quantimet 500 image analysis system in a blinded manner.

Experimental Protocols in Animals

The first series of experiments were performed in 12-week-old, male and female, apoE-deficient mice fed a chow diet. Groups of mice were matched on the basis of plasma cholesterol levels before initiation of the experiment to ensure similar mean cholesterol levels in each group. At 12 weeks of age, 1 group of mice (n = 13) was killed for baseline quantitation and examination of atherosclerotic lesions. The remaining mice were injected with 2.5 × 10⁸ plaque-forming units per gram of body weight of AdapoE2 (n = 12), AdapoE3 (n = 13), AdapoE4 (n = 12), a control adenovirus encoding the lacZ gene (AdlacZ, n = 12), or sterile saline (n = 12). Mice were killed 6 weeks after injection (18 weeks of age) for quantitation and morphological characterization of atherosclerotic lesions.

Plasma was obtained on days 0, 7, 14, 28, and 42 after adenovirus injection for analysis of transgene expression and lipids. Mice were anesthetized with Rompun/ketamine, ip, and after the aorta was gently perfused with saline via the left ventricle, the heart was cut off at the base and embedded in OCT. The rest of the aorta was removed and fixed in 10% formalin/PBS for at least 3 days. Aortic arch lesion area was quantitated using a method similar to that previously described. After the adventitial and adipose tissue was removed, the aortic arches were stained with oil red O solution (1.8% oil red O, wt/vol, in 60% isopropanol, filtered twice through a 0.2-μm filter) for 15 minutes and destained with 60% isopropanol for 5 minutes to eliminate background staining. The outer curvature of the arch was cut longitudinally, and the arch was laid open on a glass slide and mounted in Supermount (BioGenex). The image was captured with the use of a Leica MZ12 microscope and digitized, and the oil red O–stained lesion area was quantitated using the Quaintmet 500 image analysis system. All data capture and quantitation were performed in a blinded fashion.

En Face Quantitation of Atherosclerotic Lesions in the Aortic Arch

For quantitation of lesions in the aortic root, sections (8 μm) of the aortic root were mounted on masked slides. Sections were fixed in acetone, air-dried, rehydrated in PBS containing 0.02% NaN₃, and blocked with 1% BSA in PBS/NaN₃. For detection of human apoE, β₂-integrin, and vascular cell adhesion molecule (VCAM)-1, sections were reacted with polyclonal goat anti-human apoE antibody (Jackson Immuno Research Labs), monoclonal hamster anti-murine CD18 antibody (clone 2E6), or monoclonal rat anti-murine VCAM-1 (clone 429, Pharmingen), followed by incubation with biotinylated mouse anti-goat IgG antibody, goat anti-hamster IgG antibody, or mouse anti-rat IgG antibody, respectively, in the presence of 200 μg/mL normal mouse IgG. Antibody reactivity was detected with horseradish peroxidase–conjugated biotin-streptavidin complexes and developed with diaminobenzidine tetrahydrochloride as the substrate. Immunostained sections were photographed on a Leica microscope.

For quantitation of lesions in the aortic root, 5 sections stained with antibody to β₂-integrin, each separated by 40 μm (thus spanning a total of 200 μm of the root), were analyzed from each mouse by using methods similar to those previously described. The entire intimal lesion area in each section was manually traced and quantitated using a Quaintmet 500 image analysis system in a blinded fashion. The mean lesion area per section was determined for each mouse.

Analytical Methods

The plasma total cholesterol levels were measured in individual mice at each time point with an enzymatic assay on a Cobas Fara II (Roche Diagnostics).
Liver-Directed Gene Transfer of ApoE-Influenced Plasma Lipids in ApoE-Deficient Mice

After injection of adenoviruses into 12-week-old, apoE-deficient mice fed a chow diet, the plasma concentrations of apoE2 were considerably higher than those of apoE3, which in turn were somewhat higher than those of apoE4 over the course of the 6-week experiment (the Table). These differences in plasma concentrations were not due to differences in the abundance of hepatic mRNA and may have been due to the known differences in plasma catabolism among the apoE isoforms.24,26 As expected, expression of apoE3 and apoE4 resulted in sustained, marked reductions in plasma cholesterol levels (Figure 1A) in chow-fed, apoE-deficient mice. Cholesterol levels were increased to a similar degree by injections in mice expressing apoE2 (data not shown). HDL cholesterol levels were increased to a similar degree by expression of all 3 apoE isoforms (Figure 1B).

Liver-Derived ApoE Accumulated Within Established Atherosclerotic Lesions in ApoE-Deficient Mice

Immunostaining of aortic root sections with antibody against human apoE established that liver-derived apoE accumulated in substantial amounts within the vessel wall, predominantly localized within atherosclerotic lesions (Figure 2). This was true of all 3 isoforms, although lesions were rare and very small in mice expressing apoE3 (see below). Lesions from mice injected with AdlacZ had no evidence of apoE immunoreactivity (Figure 2). Importantly, there was no evidence of lacZ expression in the vessel wall, suggesting that intravenous injection of the adenovirus vectors did not result in direct transduction of macrophages or other cells in the arterial wall. To definitively test whether macrophages expressed apoE after apoE adenovirus injection, apoE-deficient mice were injected with the apoE3 adenovirus, and peritoneal macrophages and aortas were harvested 2, 4, and 6 weeks after adenovirus injection for reverse transcription–polymerase chain reaction analysis of human apoE mRNA. We found no evidence of human apoE mRNA in peritoneal macrophages or in the vessel wall at any of the time points after virus injection (data not shown). We conclude that intravenous administration of recombinant, replication-defective adenovirus to apoE-deficient mice did not result in expression of apoE by macrophages or other cells in the vessel wall. Instead, liver-derived apoE gained access to the intima via the plasma compartment and was deposited at sites of preexisting atherosclerotic lesions, where it may have been

### Results

#### Quantitation of Plasma Human ApoE Concentrations in Mice Injected With ApoE Adenoviruses

<table>
<thead>
<tr>
<th>Adenovirus</th>
<th>ApoE2</th>
<th>ApoE3</th>
<th>ApoE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>613(236)</td>
<td>306(132)</td>
<td>198(179)</td>
</tr>
<tr>
<td>14</td>
<td>80(10)</td>
<td>15.6(5.0)</td>
<td>11.5(2.7)</td>
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<tr>
<td>28</td>
<td>83(13)</td>
<td>6.4(0.9)</td>
<td>5.9(1.1)</td>
</tr>
<tr>
<td>42</td>
<td>80(10)</td>
<td>5.9(0.6)</td>
<td>5.3(1.0)</td>
</tr>
</tbody>
</table>

ND indicates not determined. Plasma was obtained from mice at the indicated times after adenovirus injection, and human apoE levels were quantitated with an immunoturbidimetric assay (Wako Chemicals USA, Inc) on a Cobas Fara autoanalyzer. Data are expressed as the means and SDs in μg/mL. No apoE was detectable in the apoE-deficient mice before injection.

| Figure 1. Liver-directed gene transfer of all 3 apoE isoforms resulted in significantly decreased plasma total cholesterol levels. A, Plasma total cholesterol levels after gene transfer. Squares denote AdapoE2; diamonds, AdapoE3; circles, AdapoE4; triangles, AdlacZ; and inverted triangles, saline. Plasma cholesterol was measured in each mouse at each time point, and the data are the mean±SEM. Where error bars are not visible, they are smaller than the height of the data points themselves. B, Plasma HDL cholesterol levels after gene transfer. Squares denote AdapoE2; diamonds, AdapoE3; circles, AdapoE4; and triangles, AdlacZ. HDL cholesterol levels were determined by FPLC in pooled plasmas from 2 separate pools for all experimental groups. The data are the means of HDL cholesterol levels in the 2 pools for each experimental group, and therefore SEMs were not obtained.

### Statistical Analysis

Atherosclerotic lesion area data were subjected to a 1-way ANOVA. Experimental groups were compared with the baseline group by using the Dunnett test. Repeated-measures ANOVA was used to compare cholesterol levels among different groups of mice over time after gene transfer. Statistical significance for all comparisons was assigned at P<0.05. Graphs represent mean±SEM values.
retained through binding to components of the extracellular matrix.

Liver-Directed ApoE Gene Transfer Caused Regression and Morphological Changes of Preexisting Fatty Streak Lesions

Atherosclerosis was quantified using 2 independent methods: quantitation of lesion area in multiple sections through the aortic root (Figure 3A) and en face quantitation of lesion area in the aortic arch (Figure 3B). Compared with mice killed at baseline, significant lesion progression occurred in mice injected with the control AdlacZ virus. There were no significant differences in lesion area per section for each group shown. In comparison with the baseline group, the AdapoE3-injected group was significantly lower (*P<0.05), but the AdapoE4 and AdapoE2 groups were not significantly different from baseline. B. En face quantitation of atherosclerotic lesion area in aortic arches. In comparison with the baseline group, the AdapoE3-injected group was significantly lower (*P<0.05), but the AdapoE4 and AdapoE2 groups were not significantly different from baseline. Base indicates mice killed at baseline; AdlacZ, mice injected with AdlacZ; AdE3, mice injected with AdapoE3; AdE4, mice injected with AdapoE4; and AdE2, mice injected with AdapoE2. Error bars represent SEM.

Figure 2. Liver-directed gene transfer of apoE resulted in substantial deposition of apoE in atherosclerotic lesions. Shown are representative atherosclerotic lesions from 12-week-old apoE-deficient mice injected with AdlacZ or AdapoE2 and killed 6 weeks later. Fresh-frozen sections were stained with a goat polyclonal antibody to human apoE. Similar findings were observed in AdapoE3- and AdapoE4-injected mice, although lesions in AdapoE3-injected mice were very small and made it more difficult to discern specific staining.

Figure 3. Liver-directed gene transfer of apoE3 caused regression and of apoE4 and apoE2, lack of progression of fatty streak lesions in 12-week-old, apoE-deficient mice in both the aortic root and aortic arch. A. Quantitation of atherosclerotic lesion area in cross sections of the aortic root. The mean lesion area per section was determined for each mouse, and the mean lesion area per section for each group is shown. In comparison with the baseline group, the AdapoE3-injected group was significantly lower (*P<0.05), but the AdapoE4 and AdapoE2 groups were not significantly different from baseline. B. En face quantitation of atherosclerotic lesion area in aortic arches. In comparison with the baseline group, the AdapoE3-injected group was significantly lower (*P<0.05), but the AdapoE4 and AdapoE2 groups were not significantly different from baseline. Base indicates mice killed at baseline; AdlacZ, mice injected with AdlacZ; AdE3, mice injected with AdapoE3; AdE4, mice injected with AdapoE4; and AdE2, mice injected with AdapoE2. Error bars represent SEM.
dense reactivity with the anti–β2-integrin that was not associated with discrete cells were observed. Interestingly, although expression of apoE4 and apoE2 did not induce regression of total lesion area, the morphology of the lesions revealed reduced numbers of foam cells and development of prominent, smooth muscle–derived fibrous caps that stained for antibodies to laminin (data not shown) as well as VCAM-1 (Figure 5). Control virus–injected mice had an abundance of typical foam cells (which do not stain for VCAM-1), whereas apoE virus–injected animals had a substantial increase in VCAM-1–positive smooth muscle cells, including cells within thick, fibrous caps (Figure 5). Only 6% of lesions from mice injected with AdlacZ had fibrous caps, whereas 68% of the detectable residual lesions in the AdapoE3-injected mice, 42% of the lesions in the AdapoE4-injected mice, and 39% in the AdapoE2-injected mice had discrete fibrous caps.

Liver-Directed Gene Transfer of ApoE Influenced the Extent and Morphology of Advanced, Complex Lesions in 6-Month-Old ApoE-Deficient Mice

To determine whether gene transfer of apoE would induce regression of advanced, complex lesions, we performed a similar experiment with AdapoE3 in 26-week-old, chow-fed, apoE-deficient mice. By this age, chow-fed apoE-deficient mice develop complex lesions containing smooth muscle cells, extracellular matrix, and necrotic lipid cores.10–13 Hepatic expression of apoE3 in 26-week-old, apoE-deficient mice resulted in a significant decrease in plasma cholesterol levels comparable to those in younger mice (data not shown). Significant regression of atherosclerotic lesion area in the aorta (Figure 6A) and the aortic root (Figure 6B) in the AdapoE3-injected mice was noted, compared with mice killed at baseline. For both atherosclerosis assays, 1-way ANOVA indicated that the groups were significantly different (P<0.0001). In comparison with the baseline group, the control adenovirus–injected group was significantly higher (P<0.01) and the AdapoE3-injected group significantly lower (P<0.01). The magnitude of regression of advanced lesions was somewhat less than that seen with fatty streak lesions.

Hepatic apoE3 expression also induced morphological changes in advanced lesions (Figure 7). In mice killed at baseline, lesions contained abundant foam cells and necrotic lipid cores in addition to smooth muscle cells and extracellular matrix. Lesions from AdlacZ-injected control mice generally progressed in size and abundance of β2-integrin–positive foam cells compared with lesions in baseline mice. In contrast, lesions in mice expressing apoE3 had substantially fewer foam cells, smaller lipid cores, and a relatively higher fibrous content, similar morphological changes to those seen in the younger mice. Therefore, the reduction in lesion size associated with apoE3 expression was primarily due to the loss of foam cell mass. As was noted in younger mice, substantial liver-derived apoE was found within atherosclerotic lesions 6 weeks after adenovirus injection (data not shown). These results indicate that liver-derived apoE-induced regression of advanced, complex lesions and accumulated in lesions in aging apoE-deficient mice as it did in younger apoE-deficient mice.

Discussion

Liver-directed gene transfer and hepatic expression of human apoE3 in chow-fed, apoE-deficient mice reduced plasma cholesterol levels and resulted in rapid and marked regression of preexisting atherosclerotic lesions. Six weeks after gene transfer of apoE3, virtually complete regression of preexisting fatty streak atherosclerotic lesions at 2 different sites within the aorta was noted. Substantial regression of advanced, complex lesions in older mice was seen as well.

Figure 4. Liver-directed gene transfer of apoE resulted in substantial morphological changes in fatty streak lesions in 12-week-old, apoE-deficient mice. Mice were killed 6 weeks after injection, and aortic root sections were analyzed for β2-integrin–bearing leukocyte content. Immunostaining was recorded by photography on a Leica microscope at ×200 magnification. The first 2 panels represent lesions from 2 different baseline mice and demonstrate the β2-integrin–positive foam cells composing the majority of the fatty streak lesions at baseline. Other panels are representative of their respective groups.
Regression occurred despite the absence of macrophage-derived apoE, indicating that macrophage-derived apoE is not required for induction of atherosclerotic lesion regression. Although regression of atherosclerosis has been demonstrated in other animal models,1–6 those studies mostly involved dietary manipulations over several months to years, and the degree of quantitative regression was generally modest. In contrast, regression induced by hepatic expression of apoE3 in apoE-deficient mice was rapid and the extent of regression was marked. Similar to previous regression studies, regression in this model was characterized by loss of macrophage-derived foam cells and a relative increase in smooth muscle cells and extracellular matrix. Therefore, this approach provides a model for rapid regression of preexisting atherosclerotic lesions in mice and may be useful in elucidating cellular and molecular events associated with regression and morphological changes in atherosclerotic lesions.

The reduction in plasma cholesterol induced by hepatic expression of apoE undoubtedly played a major role in mediating the regression seen in these experiments. However, macrophage-specific transgenic expression of apoE in apoE-deficient mice reduced progression of atherosclerosis even after controlling for changes in plasma cholesterol levels.18 Furthermore, when apoE-deficient bone marrow was transplanted into wild-type mice, atherosclerotic lesion formation was increased despite a lack of effect on plasma cholesterol levels.19 Thus, macrophage-derived apoE appears sufficient to inhibit the progression of atherosclerotic lesions, independent of its effects on plasma lipoproteins. In the current study, liver-derived apoE gained access to and accumulated within atherosclerotic lesions in the absence of macrophage-derived apoE.

Figure 5. Liver-directed gene transfer of apoE resulted in increased smooth muscle cell–rich fibrous cap formation as detected by VCAM-1 expression. Aortic root sections were analyzed for smooth muscle content by immunostaining with rat anti-murine VCAM-1 (clone 429). Stained sections were photographed on a Leica microscope at ×300 magnification. Shown are representative atherosclerotic lesions from mice injected with AdlacZ or AdapoE2 (AdE2). Abundant foam cells (VCAM-1−negative) are seen in the AdlacZ–injected animal, whereas the AdapoE2–injected animal has a substantial increase in VCAM-1–positive smooth muscle cells, including cells within the thick, fibrous cap.

Figure 6. Liver-directed gene transfer of apoE3 caused regression of atherosclerosis in 26-week-old, apoE-deficient mice in both the aortic root and aortic arch. A, En face quantitation of atherosclerotic lesion area in the entire aorta and normalized to aortic surface area. In comparison with the baseline group, the AdapoE3–(AdE3) injected group was significantly lower (*P<0.01). B, Quantitation of atherosclerotic lesion area in cross sections of aortic root. The mean lesion area per section was determined for each mouse, and the mean lesion area per section for each group is shown. In comparison with the baseline group, the AdapoE3–injected group was significantly lower (*P<0.05).
apoE. Therefore, it is possible that apoE deposited within the lesions may have had additional direct effects that, together with the reduction in plasma cholesterol, promoted regression of lesions.

That liver-derived apoE can access the vessel wall via the plasma compartment is itself not surprising, given that many plasma proteins are known to gain access to the arterial intima. Plasma apoE may gain access to the vessel wall in the context of small lipoprotein particles such as \( \gamma-LpE \), and pre-\( \beta \)-LpE. Once within the intima, apoE would be expected to associate with components of the extracellular matrix, such as heparan sulfate proteoglycans and laminin, for which it has known affinity. In fact, the distribution of the plasma-derived apoE detected by immunohistochemistry in lesions in these experiments was consistent with its being primarily extracellular. Consistent with the concept that plasma apoE may gain access to the vessel wall is the report by Fazio et al., in which wild-type mice reconstituted with apoE-deficient bone marrow were found to have some apoE present within the vessel wall despite the lack of macrophage production of apoE.

Although the direct antiatherogenic mechanisms of apoE are poorly understood, apoE has been shown in vitro to have a variety of direct cellular effects, including stimulation of cellular cholesterol efflux, inhibition of platelet activation, inhibition of T-lymphocyte proliferation, inhibition of proliferation and chemotaxis of tumor cells, effects on neurite outgrowth, and antioxidant effects. ApoE4 is less effective at promoting cholesterol efflux than is apoE3 in vitro, and differs from apoE3 in other cellular effects in vitro.

In the current experiments, expression of apoE4 reduced plasma cholesterol levels to the same extent as did apoE3 but did not result in significant regression of atherosclerotic lesions over a 6-week period. Therefore, differences between apoE3 and apoE4 in their cellular effects could potentially explain some of the differences in their effects on atherosclerotic lesions. However, further experiments will be required to definitively test this hypothesis.

In summary, regression of preexisting atherosclerotic lesions in apoE-deficient mice was rapidly induced by hepatic expression of apoE3, despite the absence of macrophage-derived apoE. The morphological changes in lesions seen in this model of regression resemble those in other animal models, induced over longer periods of time. Liver-derived apoE gained access to and was retained by intimal atherosclerotic lesions. ApoE4 was less effective in inducing regression despite similar effects on plasma lipoproteins as apoE3. The rapid regression of preexisting atherosclerotic lesions induced by apoE gene transfer in apoE-deficient mice provides a murine model for the investigation of the cellular and molecular events associated with atherosclerosis regression.

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References


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