Human apolipoprotein A-I prevents atherosclerosis associated with apolipoprotein[a] in transgenic mice

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Abstract Elevated levels of apolipoprotein[a] (apo[a]) and apolipoprotein A-I (apoA-I) are associated, respectively, with increased and decreased atherosclerosis risk, in both humans and transgenic mice. To investigate the interactions of these two important lipid-associated proteins, we assessed the effect of expression of human apoA-I and apo[a] transgenes, both singularly and together, on murine atherogenesis. Mice expressing the apo[a] transgene have a lipoprotein profile similar to non-transgenic controls, yet have significantly increased susceptibility to diet-induced atherosclerosis. Compared to mice expressing only the apo[a] transgene, mice expressing both apo[a] and apoA-I transgenes have twofold greater high density lipoprotein (HDL) concentrations and approximately a 20-fold decrease in development of early atherosclerotic lesions.


Supplementary key words lipoprotein[a] • high density lipoprotein

The development of atherosclerosis is a complex, multifactorial process involving a variety of environmental and genetic interactions. A number of factors have been identified that predispose or protect against atherosclerosis, but the complex interaction of these factors make studies of atherogenesis difficult to design and interpret. The transgenic mouse model is a powerful tool in studies on the genetic basis of atherosclerosis susceptibility as it utilizes reductionist strategies that allow focused examination of specific gene products.

HDL cholesterol and its major protein constituent, apolipoprotein A-I (apoA-I), have been shown through clinical and epidemiological studies to have a strong inverse correlation with the development of atherosclerosis and myocardial infarction (1). Transgenic mice expressing human apoA-I have previously been created (2) in the C57BL/6 background, an atherosclerosis-susceptible inbred strain that differs from resistant inbred strains in that its HDL concentration decreases when an atherogenic diet is fed. The C57BL/6 mice expressing the human apoA-I transgene had higher plasma concentrations of human apoA-I and HDL than non-transgenic C57BL/6 mice and were resistant to diet-induced atherogenesis (3). Hence, the observation that an increase in HDL concentration is associated with a decrease in atherosclerosis susceptibility of C57BL/6 mice correlates well with clinical and epidemiological human data.

Elevated lipoprotein[a] (Lp[a]) is an established independent risk factor for cardiovascular disease (4, 5). Lp[a] differs from low density lipoprotein (LDL) by the presence of an additional glycoprotein, apolipoprotein[a] (apo[a]). Apo[a] shares remarkable homology with plasminogen, a protease zymogen involved in fibrinolysis and other metabolic pathways (6). The atherogenic mechanism of Lp[a] is not known, but much speculation centers on the apo[a] moiety and the potential pathological consequences of its homology to plasminogen. We previously developed a line of transgenic mice that express human apo[a] and showed that these mice develop atherosclerosis when fed a high fat diet, while control mice do not (7, 8). Immunostaining for apo[a] within vessel walls demonstrated concentrations of apo[a] in localized regions of atherosclerosis, but not in “unaffected” regions of the vessel wall. The focal nature of apo[a] deposition correlates with human atherosclerotic plaque studies (9, 10), supporting the suitability of apo[a] transgenic mice for studying the in vivo properties of apo[a].

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein.
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To examine the interactions of human apoA-I and apo[a] in the development of atherosclerosis, transgenic mice expressing these genes were crossed. Four groups of mice were studied: those expressing apo[a], apoA-I, both apo[a] and apoA-I (apo[a]/apoA-I), and non-transgenic controls. The different groups of mice were placed on an atherogenic diet and its effect on the animal's lipoproteins and atherosclerosis in the proximal aorta was examined.

MATERIALS AND METHODS

Production of mice

Transgenic mice expressing apoA-I and apo[a] have previously been described and characterized (2, 3, 7, 8). The apoA-I line was created and maintained in the C57BL/6 background, while the apo[a] line was created and maintained by continued crossing to C57BL/6 x SJL mice.

Homozygous apoA-I mice were mated with hemizygous apo[a] mice to produce two of the four experimental mice groups. The resultant offspring were thus either apo[a]/apoA-I double hemizygotes or apoA-I hemizygotes. A second mating between apo[a] hemizygotes and C57BL/6 mice produced apo[a] hemizygotes and non-transgenic controls. The murine genetic background in both mating schemes is thus C57BL/6 crossed with C57BL/6 x SJL.

Apolipoprotein and lipoprotein measurements

Apo[a] mice were screened by measuring plasma levels of apo[a] using an ELISA kit standardized against human Lp[a] (Strategic Diagnostic). ApoA-I mice were screened by a previously described radial immunodiffusion assay using anti-human apoA-I antibodies (3). Total cholesterol concentration was measured by the procedure of Rudel and Morris (11). HDL cholesterol was determined after the removal of non-HDL lipoproteins by selective precipitation with polyethylene glycol (12). Only female mice were subject to analysis, as gender differences in susceptibility to murine atherosclerosis have been noted (13).

Assessment of atherogenesis

At 10 weeks of age, the four groups of mice described above were switched from a normal mouse chow diet (Purina Mills, 4.5% fat, rodent chow fl 5001) to a high fat diet containing 15% dairy butter fat and 1.25% cholesterol and were maintained on this diet for 18 weeks duration before being killed. Aortic sectioning, lipid staining, and lesion scoring were performed blindly according to the methods described previously (3, 8).

Briefly, the heart and attached aorta were fixed in 10% phosphate-buffered formalin and 10-μm sections separated by 70 μm each were prepared. The first and most proximal section of the aorta was taken 80 μm distal to where the aorta becomes rounded and the aortic valves are distinct. Sections were stained with oil red O and hematoxylin and counterstained with light green. The amount of oil red O staining lesions was measured at 100 x magnification by a calibrated eyepiece with square micron units. The mean lesion area per section per animal was then calculated for each individual and group of animals.

RESULTS

Apolipoprotein and lipoproteins

Apo[a] levels were similar in the groups expressing apo[a] alone and apo[a]/apoA-I transgenic animals (Table 1). ApoA-I levels were also similar in the apoA-I and the apo[a]/apoA-I transgenic mice. As has previously been shown (2, 3), high level expression of human apoA-I was associated with a 5- to 8-fold reduction in endogenous murine apoA-I plasma concentrations in both the apoA-I and the apo[a]/apoA-I mice (data not shown).

Total cholesterol and HDL cholesterol were measured for each group of animals (Table 1). The apoA-I and apo[a]/apoA-I groups had approximately double the HDL concentration of the apo[a] and nontransgenic control animals. The marked elevation in HDL concentrations in mice expressing human apoA-I transgenes is con-

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Apo[a] (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>Non-HDL Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo[a]</td>
<td>3.6 ± 0.2</td>
<td>171.0 ± 10.0</td>
<td>43.6 ± 4.0</td>
<td>128</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0</td>
<td>178.2 ± 8.7</td>
<td>99.7 ± 7.0</td>
<td>79</td>
</tr>
<tr>
<td>Apo[a]/apoA-I</td>
<td>3.2 ± 0.2</td>
<td>197.2 ± 17.0</td>
<td>115.2 ± 11.2</td>
<td>82</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>180.0 ± 13.2</td>
<td>33.5 ± 9.2</td>
<td>126</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM.

* Differences in HDL between lines containing the apoA-I transgene (apoA-I and apo[a]/apoA-I) versus control and apo[a] mice were significant (P < 0.001).
sistent with prior studies in mice (2, 3) as well as in rats (14). The non-HDL cholesterol tended to be higher in the apo[a] transgenic and control mice than in the two groups of animals containing the human apoA-I transgene, although this difference was not statistically significant ($P < 0.1$). Decreases in non-HDL cholesterol levels have not been previously associated with expression of this apoA-I transgene (3, 15). One possible explanation is that the latter studies were performed in a different genetic background than that of this study.

Atherosclerotic lesions

The area of oil red O staining lesions for the four study groups are represented graphically in Fig. 1. By far the most significant degree of atherogenesis was observed in the apo[a] transgenic mice. Animals expressing the apo[a] transgene alone had a mean lesion area greater than 25-fold that of mice expressing either the human apoA-I transgene alone or both the apoA-I and the apo[a] transgene. Compared to non-transgenic control mice, lesion area was more than 5-fold greater in the apo[a] transgenic mice. Conversely, in the groups expressing both the apoA-I and apo[a] transgenes, or the apoA-I transgene alone, lesion areas were approximately 5-fold smaller compared to non-transgenic control mice. (Due to the significant contribution of the atherosclerosis-susceptible C57BL/6 strain to the genome of these animals, it is not surprising that the non-transgenic control mice develop some atherosclerosis after being fed the atherogenic diet.) Among the 32 apo[a] animals examined, 27 had a significant degree of atherosclerosis (mean lesion area > 2000 $\mu^2$) (Fig. 2). Of the apoA-I (n = 23) and the apo[a]/apoA-I (n = 22) mice examined, only one animal demonstrated a significant degree of atherosclerosis (mean lesion area > 2000 $\mu^2$).

Histological analysis of the lesions in these animals revealed primarily fatty streak lesions identical to that previously reported for these animals (3, 8). There were no histological differences noted between the apo[a] transgenics and other groups, other than the fact that lesions were increased both in size and number in the apo[a] animals.

DISCUSSION

This study supports prior results demonstrating the pro- and anti-atherogenic properties, respectively, of hu-
human apo[a] and human apoA-I in mice (8, 16). It extends these studies by showing that the presence of both transgenes (apo[a] plus apoA-I) results in animals protected against diet-induced atherosclerosis, essentially to the same extent as in mice expressing the apoA-I transgene alone. Although the mechanism of action of apo[a] and apoA-I in the pathogenesis of atherosclerosis is not clear, these results provide clues to how these apolipoproteins participate in determining atherosclerosis susceptibility.

Consistent with human epidemiological studies and with studies in transgenic mice that suggest a direct antiatherogenic role for apoA-I and HDL, Badimon, Badimon, and Fuster (17) demonstrated that infusion of HDL into rabbits with experimentally induced atherosclerosis resulted in reduction of cholesterol deposition within vessel walls. Several mechanisms have been proposed to explain the antiatherogenic properties of elevated HDL and apoA-I. The most widely held hypothesis is that HDL plays a key role in reverse cholesterol transport, a pathway by which cholesterol is carried from extracellular sites such as artery walls to the liver (18). In this model, the HDL particle functions as an acceptor of tissue cholesterol, perhaps aided by cell-surface binding proteins in the mobilization of intracellular cholesterol pools (19, 20).

Numerous hypotheses have been proposed to explain the atherogenic properties of Lp[a]. In humans, virtually all apo[a] is lipoprotein-associated. The transgenic apo[a] mouse develops fatty lesions in the aorta when fed an atherogenic diet, despite the fact that at least 95% of apo[a] exists free in plasma and not bound to LDL in an Lp[a]-like particle (8). This suggests that apo[a] itself may be atherogenic, at least in the apo[a] transgenic mice, independent of its association with apolipoprotein-B100 or lipid particles. The observation that the apo[a] transgenic mice develop lipid deposits in their aortas suggests that the presence of apo[a] in the plasma of mice either acts on the vessel wall, or on molecules that come in contact with the vessel, leading to accelerated accumulation of lipid when animals are confronted with a diet high in fat and cholesterol.

If the protective mechanism of apoA-I is due to an acceleration of cholesterol transport out of the vessel wall, then the present study suggests that deposition of lipid in the vessel wall may be an important step in the pathogenesis of apo[a]-induced atherosclerosis. Several other studies support the concept that cholesterol delivery and availability to the vessel wall may play an important role in apo[a]-induced disease. Lawn et al. (8) noted that while transgenic apo[a] mice readily develop atherosclerosis on a high fat, high cholesterol diet, they do not develop lesions on a low fat diet. Furthermore, several human clinical studies show that the cardiovascular risk associated with Lp[a] is greatly potentiated by elevated levels of LDL cholesterol. Armstrong et al. (21) reported that the cardiovascular risk of elevated Lp[a] is nearly fourfold higher in persons with LDL levels above, compared to below, the median population value. In studies of individuals with elevated LDL due to familial hypercholesterolemia, it was found that elevated Lp[a] levels greatly potentiate the risk for cardiovascular disease, again demonstrating a synergistic effect of the two variables (22).

In prior studies with apoA-I transgenic mice, it was demonstrated that increasing apoA-I levels in atherosclerosis-susceptible C57BL/6 mice, a strain with lower HDL concentrations than resistant inbred strains, resulted in elevated HDL concentrations and lower atherosclerosis susceptibility. Recently, the effect of expression of a human apoA-I transgene and elevation of HDL in the setting of massive hypercholesterolemia and heightened atherosclerosis susceptibility present in apoE knockout animals was studied (23). ApoE knockout mice that expressed human apoA-I and had elevated HDL also had significantly less atherosclerosis than apoE knockout mice without human apoA-I and lower HDL. The present study, in contrast to the studies in relatively HDL-deficient C57BL/6 (3) and hypercholesterolemic apoE knockout mice (23), demonstrates that elevation of apoA-I and HDL can prevent atherosclerosis in mice that have heightened susceptibility not associated with alterations of their lipoprotein profile. Thus, these findings further support a direct antiatherogenic role for apoA-I and HDL and extend them to settings of heightened atherosclerosis susceptibility due to the presence of apo[a] in the plasma of mice.

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REFERENCES


Liu et al. Atherosclerosis in apoA-I/apo[a] transgenic mice