Expression of Human Apolipoprotein A-I/C-III/A-IV Gene Cluster in Mice Induces Hyperlipidemia but Reduces Atherogenesis

Laurent Vergnes, Nadine Baroukh, Maria A. Ostos, Graciela Castro, Nicolas Duverger, M. Nazeem Nanjee, Jamila Najib, Jean-Charles Fruchart, Norman E. Miller, Mario M. Zakin, Alberto Ochoa

Abstract—The apolipoprotein (apo)A-I/C-III/A-IV gene cluster is involved in lipid metabolism and atherosclerosis. Overexpression of apoC-III in mice causes hypertriglyceridemia and induces atherogenesis, whereas overexpression of apoA-I or apoA-IV increases cholesterol in plasma high density lipoprotein (HDL) and protects against atherosclerosis. Each gene has been studied alone in transgenic mice but not in combination as the entire cluster. To determine which phenotype is produced by the expression of the entire gene cluster, transgenic mice were generated with a 33-kb human DNA fragment. The results showed that the transgene contained the necessary elements to direct hepatic and intestinal expression of the 3 genes. In the pooled data, plasma concentrations were 257 ± 9, 7.1 ± 0.5, and 1.0 ± 0.2 mg/dL for human apoA-I, apoC-III, and apoA-IV, respectively (mean ± SEM). Concentrations of these apolipoproteins were higher in males than in females. Human apoA-I and apoC-III concentrations were positively correlated, suggesting that they are coregulated. Transgenic mice exhibited gross hypertriglyceridemia and accumulation of apoB48–containing triglyceride-rich lipoproteins. Plasma triglyceride and cholesterol concentrations were correlated positively with human apoC-III concentration, and HDL cholesterol was correlated with apoA-I concentration. In an apoE-deficient background, despite being markedly hypertriglyceridemic, cluster transgenic animals compared with nontransgenic animals showed a 61% reduction in atherosclerosis. This suggests that apoA-I and/or apoA-IV can protect against atherosclerosis even in the presence of severe hyperlipidemia. These mice provide a new model for studies of the regulation of the 3 human genes in combination. (Arterioscler Thromb Vasc Biol. 2000;20:2267-2274.)

Key Words: transgenic mice ■ hypertriglyceridemia ■ cholesterol ■ lipoproteins ■ atherosclerosis

Plasma lipoproteins influence the development of atherosclerosis, and their concentrations are associated with the risk of coronary heart disease.1 The genes for 3 apolipoproteins, apoA-I, apoC-III, and apoA-IV, are grouped together in a cluster on 17 kb of human chromosome 11.2 ApoA-I is the major protein component of HDL. Through its ability to promote cholesteryl efflux from cultured cells and to activate lecithin:cholesterol acyltransferase (LCAT), it is involved in reverse cholesterol transport (RCT).1 Expression of the human apoA-I (apoA-I) gene decreases the development of fatty lesions in cholesterol-fed transgenic (Tg) mice3 and in apoE-deficient (apoE−/−) mice.4 ApoA-I deficiency in mice did not increase atherogenesis in a normal background5 but did so in hypercholesterolemic mice expressing human apoB.6

ApoC-III is a component of HDL and triglyceride-rich lipoproteins (TGRLs). Plasma apoC-III concentration is positively correlated with triglyceride concentration. By inhibiting TGRL catabolism, apoC-III induces hypertriglyceridemia in Tg mice.7 Expression of the gene was not atherogenic in apoE−/− mice.8 In contrast, apoC-III expression, in normal or in LDL receptor–deficient mice, resulted in increased atherosclerosis.9,10 ApoA-IV, involved in RCT-like apoA-I,1 also has antiatherogenic properties, inasmuch as overexpression of apoA-IV reduced aortic lesions.11,12 The foregoing observations indicate that the apoA-I/C-III/A-IV gene cluster is of major interest in relation to atherogenesis.

A series of DNA elements seems to coordinately regulate the transcription of all 3 genes.13−15 Indeed, analyses of intestinal mRNA levels have shown that the expression of the 3 genes is coregulated in this organ in vivo.16 Tg mice have already been developed for apoA-I,1,13,17 apoC-III,8,13,18 and apoA-IV7,11,12,19,20 and have provided valuable information on

Received November 3, 1999; revision accepted February 14, 2000.
Accepted for publication January 3, 2000.
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the properties and functions of each gene in isolation. However, none of these animal models is suitable for studies of the effect of the coordinated expression of the 3 genes in combination. Moreover, new therapeutic agents that increase apoA-I concentration are currently being sought. Whether such agents will change the expression of the other genes of the cluster and produce an atherogenic or a nonatherogenic effect is not known. A model expressing the entire human apoA-I/C-III/A-IV cluster would be of considerable value for the investigation of its properties and functions as an integrated unit. In the present study, we report the generation and characterization of such Tg mice.

Methods

Animals

The transgene was obtained from the DMPC-HF81-1412-B12 cosmid isolated from a human genomic library (DuPont Merck Pharmaceutical Co) screened for the presence of apoA-IV sequences by polymerase chain reaction. A 33-kb NotI/SalI fragment, containing 8.3 kb of the 5' region of apoA-I, the 17-kb entire apoA-UC-III/A-IV gene cluster, and 7.5 kb of the 3' region of apoA-IV, was microinjected into fertilized mouse eggs. Tg mice were identified by the presence of the 3 human genes. Three Tg lines were established in the C57Bl/6j genetic background (Tg11, Tg12, and Tg21). Transgene copy numbers were determined by Southern blotting. Mice, housed in a temperature-controlled room with alternating 12-hour light (7:00 AM to 7:00 PM) and dark (7:00 PM to 7:00 AM) periods, had access to regular mouse chow and water ad libitum. All procedures involving animal handling and care were conducted in accordance with Pasteur Institute Guidelines for Husbandry of Laboratory Mice. Procedures involving animal handling and care were conducted in accordance with Pasteur Institute Guidelines for Husbandry of Laboratory Mice.

Northern Blot Experiments

After an overnight fast, the animals were killed by cervical dislocation, and tissues (liver, small intestine, spleen, kidney, lung, brain, and stomach) were removed. Total RNAs were extracted with a TRIzol reagent (Life Technologies, Grand Island, NY). After precipitation with 2 volumes of 2-propanol, RNAs were dissolved in water and subjected to electrophoresis in a 1%–2% agarose gel that had been stained with 0.5 µg/mL ethidium bromide. Membranes were hybridized with [32P]adenosine 5'-triphosphate-labeled cDNA probes, as previously described. Apolipoprotein Quantification

Apolipoproteins were quantified by immunoelectrophoresis with specific polyclonal antibodies (Hydragels SEBIA); mouse apolipoproteins did not cross-react. Mouse plasma apoA-I concentrations were measured by immunonephelometry with the use of specific polyclonal antibodies (Hydragels SEBIA); mouse plasma apoA-I concentrations were measured by immunonephelometry with the use of specific polyclonal antibodies (Hydragels SEBIA). Mouse plasma apoA-I, apoC-III, or apoA-IV antibodies, followed by secondary peroxidase–labeled antibodies directed against rabbit IgG. The antisera directed against human or mice apolipoproteins were highly specific and did not show any cross-species reactivity, with the exception of the anti-mouse apoC-III, which recognized human apoC-III. Membranes were developed by use of a Chemiluminescence Western blotting detection kit (ECL System, Amersham Pharmacia Biotech). Experiments were repeated 3 times with samples from different control and Tg animals.

Triglyceride concentrations were determined by an enzymatic GPO-PAP method, and total and HDL cholesterol concentrations were determined by enzymatic CHOD-PAP methods with commercial kits (Boehringer-Mannheim). Precinorm L (Boehringer-Mannheim) was used as a calibrator.

Cholesterol Efflux and Esterification Studies

Cellular cholesterol efflux was determined with rat Fu5AH hepatoma cells incubated with 5% diluted serum. Values are reported as the average of at least 3 different determinations. Purified human LDL samples were included in each assay as positive controls. LCAT activity was determined by use of the exogenous enzymolipoprotein substrate method with 25 µL of plasma from female mice. Experiments were repeated twice with 3 and 5 different pools of samples for control and Tg mice, respectively. All measurements were made in triplicate (coefficient of variation <5%). Cholesterol esterification rate was determined as the decrease in unesterified cholesterol mass (measured enzymatically) during in vitro incubation of whole plasma at 37°C for periods up to 4 hours. Only female mouse plasma was analyzed, and all measurements were made in triplicate (coefficient of variation <1.5%).

Evaluation of Atherosclerotic Lesions

The spontaneous atherosclerosis model used in the present study was the apoE-/- mouse. Fatty streak lesions were quantified by evaluating cross-sectional lesion sizes in the aortic sinus. A randomly chosen subset of 10 Tg and 11 control female mice, both with the
apoE−/− background, were euthanized at 20 weeks of age. Hearts and proximal aortas were removed, cleaned of pericardial fat, and fixed in PBS formalin solution (5%) for at least 48 hours. Then the hearts were cut directly under and parallel to the aortic leaflet, and the upper portions were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek) and frozen at −80°C. Ten-micron-thick sections were cut through the aortic sinus, recognized by 3 valve cusps at the junction of the left ventricle and the aorta. Eighty sections per animal were stained for lipids with oil red O and counterstained with hematoxylin. Cross-sectional areas were analyzed by computerized planimetry. Statistical analysis was performed on 4 different cross-sectional lesion areas per animal, separated by 160 μm. The first section analyzed for each animal corresponded to the lower origin of the aortic sinus.

**Statistical Analyses**

Results are reported as mean±SEM In all experiments non-Tg littersmates were used as controls. Statistical differences in apolipoprotein and lipid levels between different groups of animals were evaluated by ANOVA. Other statistical differences were tested by the Student t test or Mann-Whitney U test for nonparametric analysis. Group differences or correlations with P<0.05 were considered statistically significant.

**Results**

**ApoA-I/C-III/A-IV Transgene Expression**

Tg mice were generated with a 33-kb human genomic fragment containing the entire cluster and flanking sequences. Three Tg lines, Tg11, Tg12, and Tg21 containing ≈9, 1, and 2 copies, respectively, of the transgene, were established. All Tg lines showed hepatic and intestinal expression of the 3 human genes (Figure 1). Human mRNA levels were higher in the liver than in the intestine. No expression was detected in other analyzed tissues, with the exception of traces of all 3 human mRNAs in the kidney, of apoA-I mRNA in the brain, spleen, and stomach, and of apoA-IV mRNA in the stomach (data not shown). Because apoA-IV expression was reported to be mainly intestinal in humans and in apoA-IV Tg mice, we have compared by Northern blot analysis the mRNA levels in apoA-IV Tg mice with those in the cluster Tg mice. Although the hepatic levels were similar in both strains, the intestinal mRNA levels were 2.3-fold lower in the cluster Tg mice (15.5±3.2 arbitrary units for apoA-IV Tg mice versus 6.6±4.8 arbitrary units for cluster Tg mice, P<0.05), suggesting intestinal downregulation in the latter.

**Human Plasma Apolipoproteins**

All 3 human apolipoproteins were present in plasma from each line, and their concentrations were higher in Tg males than in Tg females (Table 1). The means in the different lines were very similar. Average apoA-I levels ranged from 241 to 283 mg/dL, with an overall mean for the 3 lines of 257±9 mg/dL (n=47); average apoC-III levels were between 4.8 and 8.8 mg/dL, with an overall mean of 7.1±0.5 mg/dL; and apoA-IV concentrations averaged 0.8 to 1.4 mg/dL, with an overall mean of 1.0±0.2 mg/dL. The plasma concentration of apoA-I was positively correlated with that of apoC-III (r=0.67, P<0.001). Because of the low concentration of apoA-IV, it was difficult to assess the strength of its association with the concentrations of apoA-I or apoC-III.

**TABLE 1. Human Apolipoprotein Concentrations in Plasma of Tg Mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Human ApoA-I</th>
<th>Human ApoC-III</th>
<th>Human ApoA-IV</th>
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<tbody>
<tr>
<td></td>
<td>Concentration, mg/dL</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Tg11</td>
<td>241±14</td>
<td>279±15*</td>
<td>197±12</td>
</tr>
<tr>
<td>Tg12</td>
<td>247±20</td>
<td>336±29*</td>
<td>199±11</td>
</tr>
<tr>
<td>Tg21</td>
<td>283±08</td>
<td>300±11</td>
<td>263±09</td>
</tr>
<tr>
<td>Tg12/apoE−/−</td>
<td>163±13‡</td>
<td>202±44‡</td>
<td>108±9‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=4 to 11).

*P<0.001 and †P<0.01 vs females; ‡P<0.001 and §P<0.01 vs Tg12.
Lipid Concentrations

Mean plasma triglyceride and total cholesterol concentrations were higher in Tg males than in Tg females (Table 2). A similar sex effect was seen in control littersmates. Expression of the cluster produced a 4- to 10-fold increase in triglyceride concentration relative to those in control animals and a moderate increase in total cholesterol concentration due mostly to an increase in the non-HDL subclass. However, HDL cholesterol was also significantly raised in 3 of the 3 Tg lines (Tg11 and Tg12). Plasma triglyceride concentrations in individual mice correlated with non-HDL cholesterol concentrations (r = 0.92, P < 0.001). In addition, apoC-III concentrations were positively correlated with triglyceride (r = 0.80, P < 0.001) and non-HDL cholesterol (r = 0.64, P < 0.001) concentrations. These associations suggest that the increased concentrations of triglycerides and cholesterol in Tg mice were mainly dependent on the concentration of apoC-III. Relatively low concentrations of apoC-III were sufficient to induce marked changes in the lipid profiles (compare data in Tables 1 and 2). Finally, apoA-I concentration was correlated with HDL cholesterol concentration (r = 0.66, P < 0.001). Triglycerides showed a low but significant correlation with apoA-I (r = 0.36, P = 0.012). The correlation between HDL cholesterol and apoC-III was not significant (r = 0.14, P = 0.92), nor was the correlation between HDL cholesterol and triglyceride concentrations (r = 0.16, P = 0.28).

Lipid and ApoB Distributions in Lipoprotein Density Fractions

Lipoproteins were isolated by sequential ultracentrifugation from plasma samples from Tg mice of line 12 and control littersmates. Increases of triglycerides and cholesterol in Tg mice were observed in lipoproteins of d<1.006 g/mL and d=1.006 to 1.019 g/mL (Figure 2A and 2B). Analysis of apoB showed a major increase in total apoB₄₈, whereas total apoB₁₀₀ remained unchanged. The apoB₄₈ increase in the Tg mice was detected in all fractions analyzed; in particular, an 11-fold increase was observed in particles of d<1.006 g/mL (Figure 2C). Although the total apoB₁₀₀ level remained unchanged, lipoprotein fraction analysis showed a significant decrease in the d=1.019 to 1.063 g/mL fraction in Tg mice (Figure 2D). Thus, the increases in triglycerides and cholesterol observed in Tg mice were due to an accumulation of apoB₄₈-rich lipoproteins of d<1.006 g/mL. Similar results were obtained with mice from line 21.

Cholesterol and Apolipoprotein Distributions in Size Subclasses of Lipoproteins

The distributions of the cholesterol and human apolipoproteins in size subclasses of lipoproteins were analyzed by HP-SEC of whole plasma. Results for representative male and female animals are shown in Figure 3. Human apoC-III and apoA-IV were present in TGRLs plus LDLs (fractions 1 to 14, molecular mass >670 kDa) as well as in lipid-rich HDLs (fractions 15 to 35, 100 to 670 kDa), whereas apoA-I was almost exclusively present in HDL particles of 70 to 350 kDa (fractions 20 to 40). In addition, a minor proportion of apoA-I and a substantial proportion of apoA-IV were present in small lipid-poor particles (fractions 40 to 50 of 35 to 70 kDa and fractions 35 to 45 of 50 to 100 kDa). Male mice had significantly higher concentrations of apoA-IV in all fractions compared with females (0.84±0.22 versus 0.10±0.03 mg/dL, respectively, in TGRLs plus LDLs, P<0.001; 5.55±4.18 versus 0.82±0.35 mg/dL, respectively, in lipid-rich HDLs, P<0.07; and 3.61±0.75 versus 2.27±0.55 mg/dL, respectively, in lipid-poor forms, P<0.04). The higher concentrations of apoA-I, apoC-III, and cholesterol characteristic of males were also observed in the different lipoprotein fractions, although an insufficient number of animals was studied for statistical comparison.

Human and Mouse Apolipoprotein Distribution in HDL Particles

In addition to the quantitative differences in non-HDL and HDL cholesterol between Tg and non-Tg animals demonstrated by precipitation assays, there were marked differences between the 2 groups in the number and sizes of HDL subpopulations analyzed in nondenaturing polyacrylamide gradient gels. An essentially monodisperse population of large HDLs present in the control mice was replaced by multiple HDL subspecies in the cluster Tgs, accompanied by a redistribution of core lipids into small HDLs (Figure 1, which can be accessed online at www.ahajournals.org).

Bidimensional electrophoresis and Western blotting with species-specific anti-apolipoprotein antisera (Figure 4) showed that apoA-I was contained mostly in α-migrating particles and to a lesser degree in multiple pre-β particles.

### TABLE 2. Lipid Concentrations in Control and Tg Mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Triglycerides</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Control</td>
<td>78±0.2</td>
<td>86±0.03*</td>
<td>71±0.3</td>
</tr>
<tr>
<td>Tg11</td>
<td>374±40‡</td>
<td>517±37‡</td>
<td>247±29§</td>
</tr>
<tr>
<td>Tg12</td>
<td>683±77‡</td>
<td>850±190‡‡</td>
<td>597±48‡</td>
</tr>
<tr>
<td>Tg21</td>
<td>443±38‡</td>
<td>497±48‡‡</td>
<td>381±56§</td>
</tr>
<tr>
<td>apoE⁻⁻/⁻⁻</td>
<td>122±1.5</td>
<td>150±18</td>
<td>71±9</td>
</tr>
<tr>
<td>Tg12/E⁻⁻/⁻⁻</td>
<td>1665±265¶</td>
<td>2491±234¶†</td>
<td>530±65¶</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=6 to 25).

*P<0.01 and †P<0.001 vs females; ‡P<0.001 and §P<0.01 vs control mice; ††P<0.001 vs apoE⁻⁻/⁻⁻ mice; and †††P<0.001 vs Tg12.
Mouse apoA-I was localized predominantly in \( \alpha \)-HDL particles in control mice, whereas in Tg mice, it was reduced and distributed almost exclusively in pre-\( \beta \) HDL particles. This reduction in mouse apoA-I concentrations was consistent with the values measured in whole plasma by immunoassay (114 \( \pm \) 6 mg/dL in control mice versus 20 \( \pm \) 6 mg/dL in Tg mice, \( P < 0.001 \)). Mouse apoC-III in control mice and h apoC-III in Tg mice were localized mainly in \( \alpha \) particles. We did not localize mouse apoC-III in Tg mice, because antibodies directed against the mouse protein also recognized h apoC-III. Finally, human apoA-IV and mouse apoA-IV were localized only in pre-\( \beta \) HDLs. In Tg mice, mouse apoA-IV was displaced from certain pre-\( \beta \) HDL particles, presumably by hapoA-I. Thus, hapoA-I and hapoA-IV were able to form pre-\( \beta \) particles in these mice.

**Cholesterol Efflux, LCAT Activity, and Cholesterol Esterification Rate**

Plasma from Tg mice was 29% more efficient than plasma from non-Tg mice (12.4 \( \pm \) 0.8% versus 9.6 \( \pm \) 0.5%, respectively; \( P < 0.05 \)) in promoting cholesterol efflux from Fu5AH cells. Whereas no significant difference in plasma LCAT activity was observed between Tg and control mice (7.3 \( \pm \) 0.5 versus 6.6 \( \pm \) 0.4, respectively; \( P = 0.324 \)), the plasma cholesterol esterification rate was reduced in the Tg mice (Figure II, which can be accessed online at www.ahajournals.org).

**Expression of the Human ApoA-I/C-III/A-IV Gene Cluster in ApoE\(^{-/-}\) Mice**

Concentration of hapoA-I was significantly lower in an apoE null background (Tg12/apoE\(^{-/-}\)) than in the original background (Tg12). In Tg12/apoE\(^{-/-}\) mice, the hapoC-III concentration was increased in males but not in females, and the hapoA-IV concentration was unchanged (Table 1). In this apoE\(^{-/-}\) background, concentrations of human apolipoproteins were also higher in males than in females. There was a large increase in plasma triglycerides in Tg compared with control mice (Table 2): an 18-fold increase in males and a 7-fold increase in females. Total cholesterol, which was already very high in apoE\(^{-/-}\) mice, was even more elevated in the Tg12/apoE\(^{-/-}\) strain because of an increase in non-HDL and HDL fractions. Cholesterol efflux capacity and LCAT activity were both higher in apoE\(^{-/-}\) mouse plasma than in the Tg12/apoE\(^{-/-}\) mouse plasma (8.31 \( \pm \) 1.57% versus 6.14 \( \pm \) 1.10%, \( P < 0.05 \), and 2.5 \( \pm \) 0.2 versus 1.4 \( \pm \) 0.1 mmol \( \cdot \) mL\(^{-1}\) \( \cdot \) h\(^{-1}\), \( P < 0.01 \), respectively). Previous studies have shown that the apoE\(^{-/-}\) mouse model develops atherosclerotic lesions spontaneously.\(^{21}\) In the present study, all the control and cluster Tg mice with an apoE\(^{-/-}\) background developed spontaneous lesions in the aortic sinus at 20 weeks of age. Characteristic sections from each group showed that the lesions were morphologically similar. However, the average cross-sectional areas of lesions were 61% lower in Tg12/apoE\(^{-/-}\) mice than in apoE\(^{-/-}\) mice (89 797 \( \pm \) 16 386 versus 232 279 \( \pm \) 16 273 \( \mu \)m\(^2\), respectively; \( P < 0.001 \); Figure III, which can be accessed online at www.ahajournals.org).

**Discussion**

To study the effect of the coordinated expression of the 3 genes of the apoA-I/C-III/A-IV cluster, we have created Tg mice with this human cluster. In the present study, we demonstrate that expression of the entire cluster in mice produces a hyperlipidemic profile but protects against atherosclerosis.
Regulatory regions of the 3 genes have been localized throughout the entire gene cluster, and all known regulatory elements were present in their natural positions in the genomic fragment used to generate the Tg animals. Northern blot analyses showed the presence of the 3 human mRNAs in the liver and intestine. These results suggest that the transgene contains all the regulatory regions required to direct a tissue-specific expression of the 3 genes. Hepatic and intestinal expressions of apoA-I and apoC-III were roughly similar to those observed in humans. ApoA-IV expression was mainly hepatic, in contrast to the predominant intestinal expression observed in humans or in apoA-IV Tg mice. Because the latter were generated with a DNA fragment contained within that used to generate the cluster Tg mice, the apoA-IV expression observed in the present study does not seem to be due to the absence of a DNA regulatory element. Comparison between intestinally expressing apoA-IV Tg mice and cluster Tg mice showed that hepatic mRNA levels were similar in both types of mice, whereas intestinal mRNA levels were much lower in the cluster Tg mice. We hypothesize that the overexpression of apoA-I or apoC-III genes or the subsequent alterations induced in the lipid profile might reduce intestinal apoA-IV expression in cluster Tg mice.

All Tg lines showed the presence of the 3 human apolipoproteins in plasma. The mean plasma concentrations of each human apolipoprotein were rather similar in the 3 lines, suggesting that their concentrations were precisely controlled in these animals. This control seems to be independent of transgene copy number, inasmuch as Tg11, Tg12, and Tg21 lines contained 9, 1, and 2 copies, respectively, of the transgene. The control also seems independent of the insertion site, inasmuch as the 3 lines were independently generated.

Concentrations of apoA-I and apoC-III in plasma were ~2-fold greater than the values in humans. In contrast, much lower concentrations of apoA-IV were detected in the cluster Tg mice, probably because of the low level of intestinal expression observed. Size exclusion chromatography demonstrated that most apoA-I was present in particles of a size similar to that of cholesterol-rich HDLs in humans. Minor amounts of apoA-I were present in larger particles as well as in small lipid-poor species. Human apoC-III and apoA-IV were found in cholesterol-rich HDLs and in the non-HDL fraction. A substantial proportion of apoA-IV was also present in small lipid-poor subclasses. These profiles were very similar to those observed in normal humans.

Analysis of the lipid profile of the Tg mice showed gross hypertriglyceridemia and a moderate increase in plasma cholesterol, mainly in the non-HDL fraction. This lipid profile and the changes in apoB48 and apoB100 concentrations in lipoprotein subclasses show that the major increases in plasma lipids in the Tg mice reflect an accumulation of apoB48-rich particles. The observed association between the triglyceride, cholesterol, and apoC-III concentrations and the
The presence of severe hyperlipidemia. Both apolipoproteins participate in RCT and promote cholesterol efflux from cultured cells.\textsuperscript{1,2,3,5} In a normal background, new populations of HDLs appeared in the cluster Tg mice. All the human apoA-IV and a proportion of human apoA-I migrated to the pre-β position. Efflux of radiolabeled cholesterol from Fu5AH hepatoma cells was greater to Tg than to non-Tg plasma. All these results suggest that RCT might be increased in these mice. The lower LCAT activity in Tg compared with control mice may have been related to an effect of apoC-III, which others have shown can inhibit LCAT activity in the presence of an excess of apoA-I.\textsuperscript{30} In Tg apoE\textsuperscript{−/−} mice, cholesterol efflux and LCAT activity were reduced, but we cannot rule out the possibility that enhanced RCT is the basis of the protective mechanism. The extent to which the result of these in vitro assays, when applied to Tg apoE\textsuperscript{−/−} sera, reflect the flux of cholesterol through the RCT pathway in vivo is uncertain. At least part of the resistance to atherosclerosis induced by cluster expression could have been attributable to properties of HDLs unrelated to their role in RCT, such as their ability to protect LDLs from oxidative modification\textsuperscript{31} or to suppress adhesion molecule expression and induce cyclooxygenase-2 in vascular endothelium.\textsuperscript{32} Associated changes in the concentration, size, and composition of apoB-containing lipoproteins may also have contributed. Further work will be needed to clarify the mechanism(s) of the antatherogenic effect of human cluster expression in mice. These apoA-IC-III/A-IV Tg mice should be of value in the search for pharmacological agents that selectively act on the expression of the 3 human genes.

Acknowledgments

This work was supported by the European Community (EC) (BMH4-C1T-96/2597), by the Centre National de la Recherche Scientifique (URA 1129), and by the British Heart Foundation. L. V. was a fellow of the Caisse Nationale d’Assurance Maladie, N. B. was a fellow of the Ministère de la Recherche de l’Espace, and M. A. O. was a fellow of the EC (CEE BMH4-CT98-5125). We would like to thank F. Emmanuel and J.-M. Caillaud for their help in histological analyses of the EC (CEE BMH4-CT98-5125). We would like to thank F. Emmanuel and J.-M. Caillaud for their help in histological analyses of mice and G. N. Cohen for helpful suggestions.

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Arterioscler Thromb Vasc Biol. 2000;20:2267-2274
doi: 10.1161/01.ATV.20.10.2267
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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<td>C</td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Tg</td>
<td></td>
<td>Tg</td>
</tr>
</tbody>
</table>
Mean cross-sectional area of lesions ($10^5 \mu m^2$)

- apoE$^{-/-}$
- Tg12/apoE$^{-/-}$

***