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Expression of human apolipoprotein A-I/C-III/A-IV gene cluster in mice reduces atherogenesis in response to a high fat-high cholesterol diet

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Abstract We have previously generated transgenic (Tg) mice expressing the human apolipoprotein (apo) A-I/C-III/A-IV gene cluster. This expression induced hyperlipidemia but reduced atherosclerotic lesions in genetically modified mice lacking apoE. Atherosclerosis is a multifactorial process and environmental factors such as diet play significant roles in its development. We examined here how an atherogenic diet influences the expression of the human genes and the characteristics of the Tg mice. Our results indicate that a high fat-high cholesterol diet up-regulates the intestinal expression of the three genes and the concentration of the three proteins in plasma. Cholesterol concentration was highly increased in the non-high density lipoprotein (HDL) fraction, and less, although significantly, in the HDL fraction. Tgs showed a 65% reduction in diet-induced aortic lesions compared with non-Tg mice. Atherogenic diet increases the expression of the genes encoding the scavenger receptor class B type I (SR-BI) and ATP binding cassette transporter 1 (ABCA1) proteins. As cholesterol efflux mediated by SR-BI or by ABCA1 was enhanced in Tg mice fed an atherogenic diet, we can hypothesize that increased reverse cholesterol transport is the basis of the protective mechanism observed in these animals. In conclusion, we present evidence that the expression of the human gene cluster in mice protects against atherogenesis in response to an atherogenic diet. **© 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Apolipoprotein; Atherosclerosis; Transgenic mouse; Atherogenic diet; Cholesterol efflux

1. Introduction

Coronary heart disease (CHD), the principal cause of mortality in the developed countries, is mainly caused by atherosclerosis. The atherogenic process is known to be strongly influenced by lipoprotein metabolism, and the association between low high density lipoprotein (HDL) concentration and premature atherosclerosis is well known [1]. Two important protein components of HDLs are apolipoproteins (apo) A-I and A-IV. Their genes are grouped together with the gene of apoC-III in a cluster of 17 kb on human chromosome 11 [2]. ApoA-I and apoA-IV have essential roles in the reverse cholesterol transport (RCT) pathway [3], and their overexpression protects against atherosclerosis [4–7]. ApoC-III, by inhibiting triglyceride-rich lipoprotein catabolism, induces hypertriglyceridemia [8], and its overexpression results in increased atherosclerosis [9,10]. In humans, a DNA inversion within the apoA-I/C-III/A-IV-encoding gene cluster causes premature atherosclerosis [11]. In family studies, familial apoA-I/C-III/A-IV deficiency has been associated with premature atherosclerosis [12], and genetic variations of the gene cluster have also been detected in hypertriglyceridemic subjects [13]. All these observations indicate that the study of the apoA-I/C-III/A-IV gene cluster is of major interest in relation to atherogenesis.

We have recently generated transgenic (Tg) mice using a 33 kb human genomic fragment containing the entire cluster and its 5′ and 3′ flanking regions [14]. These mice provide an interesting model for studies of the regulation of the three genes in combination. They are also useful for studies of the function of the proteins encoded by the three genes. Expression of the entire human gene cluster induced hyperlipidemia but reduced atherogenesis in genetically modified mice lacking apoE [14]. The observed resistance to atherosclerosis could be attributable to an enhanced RCT. RCT is the process by which cholesterol in peripheral tissues is redistributed to sites of excretion and metabolism, and is thought to be mediated by HDL. The initial step in this process is the release of unesterified cholesterol (UC) and phospholipid from the plasma membrane of cells to acceptor particles. Efflux of UC occurs via a number of mechanisms, including: (i) aqueous diffusion to a phospholipid-containing acceptor, and (ii) efflux to HDL mediated by receptors such as scavenger receptor class B type I (SR-BI). The flux of the UC between cells and HDL is bi-directional; depending on the direction of the UC concentration gradient between cells and lipoproteins, either net efflux or net influx of cholesterol can occur. The creation of a cholesterol gradient depends upon many properties of the acceptors and the cell plasma membrane. Such factors include the cholesterol and phospholipid content and the size and number of the acceptor particles. Another pathway (iii) is the release of membrane phospholipid and cholesterol to apolipoproteins. The identification of defects in the ATP binding cassette transporter 1 (ABCA1) as the molecular basis of Tangier disease [15] has highlighted its crucial role for the loading of nascent apolipoprotein particles with phospholipids and cholesterol. ApoA-I and apoA-IV have essential roles in the reverse cholesterol pathway, mainly by their property of forming small lipid-poor particles, characterized by

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their pre-β electrophoretic mobility, that are necessary to interact with ABCA1 at the cell membranes.

To deepen our understanding of the properties developed by the Tg mice, we decided to examine how an atherogenic diet influences their characteristics. Atherosclerosis is a multifactorial process and environmental factors such as diet play significant roles in its development. It is known that diets high in saturated fat and cholesterol increase the risk of CHD when administered to mice with a susceptible background [16]. In the current study, we examined how a high fat-high cholesterol diet (atherogenic diet) influences the expression of the human apoA-I, C-III and A-IV genes, the plasma apolipoprotein and lipid concentrations and the development of aortic lesions in the Tg mice. We also analyzed the hepatic expression of the SR-BI and ABCA1 genes, and the capacity of the HDL and of the $d > 1.21$ g/ml density fraction from Tg mice fed chow or the atherogenic diet to promote cholesterol efflux from Fu5AH hepatoma cells [17] and from J774 macrophage cells [15,18,19].

2. Materials and methods

2.1. Animal models

Female Tg mice for the human apoA-I/C-III/A-IV gene cluster line 12 (10 weeks old) were used. Non-Tg littermates were used as controls. Mice had free access for 20 weeks to a regular mouse chow (UAR, France) or to a high fat-high cholesterol diet (chow diet supplemented with 10% cocoa butter and 1.25% cholesterol (TD*8805; Teklad Premier Laboratory Diets, Madison, WI, USA). Animals were housed in a temperature-controlled room with alternating 12 h light (7.00–19.00 h) and dark periods (19.00–7.00 h). All procedures involving animal handling and care were conducted in accordance with the Pasteur Institute Guidelines for Husbandry of Laboratory Mice.

2.2. RNA analyses

Total RNAs were extracted from different tissues with RNA-plus (Quantum), purified and electrophoresed on 1% agarose gels containing formaldehyde. Samples were blotted onto Hybond-N^+ membranes (Amersham Pharmacia Biotech), and hybridized with ^{32}P-labeled cDNA probes, as previously reported [14].

For SR-BI and ABCA1 mRNA analyses, total RNA was extracted from livers by the acid guanidinium thiocyanate/phenol/chloroform method [20]. Dot blot analysis was performed by loading 10 μg of total RNA onto Hybond C extra membranes (Amersham Pharmacia Biotech). Membranes were hybridized using rodent SR-BI and ABCA1 ^{32}P-labeled cDNA probes. A ubiquitin probe was used as control. After 24 h exposure, spots were quantified on a GS525 phosphorimager. Values were normalized to the internal control probe and expressed as a percent of control.

2.3. Plasma apolipoprotein quantification

Human apolipoproteins were quantified by immunoelectrophoresis using specific polyclonal antibodies (Hydragels SEBIA).

2.4. Plasma lipid analyses

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 was used as a calibrator.

2.5. Evaluation of atherosclerotic lesions

Fatty streak lesions were quantified in seven Tg and seven control mice, by evaluating cross-sectional lesion sizes in the aortic sinus [21]. Procedures were as reported [14].

2.6. Lipoprotein fractionation

HDL and the fraction of density $d > 1.21$ g/ml used for cholesterol efflux studies were obtained by sequential ultracentrifugation from pooled mouse samples. HDL was isolated at d between 1.063 and 1.21 g/ml.

2.7. Cell culture and cholesterol efflux

2.7.1. Fu5AH cells. Cellular cholesterol efflux from Fu5AH rat hepatoma cells to HDL was determined following the procedure described by de la Llera Moya et al. [22]. Briefly, the cells were maintained in minimal essential medium containing 5% fetal calf serum. 25 000 Fu5AH cells/ml were plated on 24 mm multiwell plates (Inbro, Polylabo) using 2 ml/well. Two days after plating, cellular cholesterol was labeled during a 72 h incubation with [^{3}H]cholesterol (NEN, Dupont de Nemours) (1 μCi/well). To allow equilibration of the label, the cells were washed and incubated for 24 h in minimal essential medium with 0.5% bovine serum albumin (BSA). Then, the cells were washed with phosphate-buffered saline (PBS) and incubated at 37°C for 3 h with HDL at a protein concentration of 50 μg/ml. At the end of the incubation, the medium was removed and centrifuged; the cell monolayer was washed three times with PBS and harvested with 0.5 ml of 0.1 M NaOH. Radioactivity was then measured in both medium and cells, and the percentage of cholesterol efflux calculated. All efflux values are averages of three determinations.

2.7.2. J774 macrophage cells. Cellular cholesterol efflux from J774 cells to poorly lipidated apolipoproteins was determined following the procedure described by Fournier et al. [23]. Cells were maintained in RPMI, containing 10% fetal calf serum. 150 000 cells/ml were plated on 24 mm multiwell plates, using 2 ml/well. One day after plating, cells were washed and cellular cholesterol labeled during a 48 h incubation with [^{3}H]cholesterol (NEN, Dupont de Nemours) (1 μCi/well) in medium containing 2.5% BSA. On the day of the experiment, confluent cells were washed three times with PBS and incubated for 2 h at 37°C with RPMI containing 1% BSA to allow equilibration of the [^{3}H]cholesterol in the membranes. For determination of cholesterol efflux, the cells were washed once with PBS and incubated at 37°C for 3 h with the plasma fraction of $d > 1.21$ g/ml at a protein concentration equivalent to 0.2% dilution of plasma concentration. To specifically measure the cholesterol efflux, J774 macrophages were pretreated, after the equilibration period, with 0.2 mmol/l 8-bromo-cAMP for 12 h. All efflux values are averages of three determinations. The rest of the procedure is the same as described for Fu5AH cells.

3. Results

The effect of the high fat-high cholesterol diet on apoA-I/C-III/A-IV Tg mice was determined in female mice fed a chow or an atherogenic diet for 20 weeks.

3.1. Human apoA-I/C-III/A-IV transgene expression

We have previously shown that Tg mice fed a chow diet expressed the three human genes in liver and intestine [14]. In this report, Northern blot analyses indicated that feeding an atherogenic diet resulted in an eightfold elevation of intestinal apoA-I and apoC-III mRNA levels and a threefold elevation of an intestinal apoA-IV mRNA level over those in Tg fed a chow diet (Fig. 1B). The hepatic levels of the three apolipoprotein mRNAs were diminished but the decrease was statistically significant only for apoA-I mRNA (Fig. 1A).

3.2. Plasma apolipoprotein concentrations

Plasma concentrations of human apolipoproteins were examined by rocket immunoelectrophoresis using specific antibodies. The three human apolipoprotein concentrations were

Table 1
Human apolipoprotein concentrations in plasma of Tg mice after an atherogenic diet

	Chow diet	Atherogenic diet
ApoA-I	199 ± 11	233 ± 12*
ApoC-III	5.4 ± 0.5	10.6 ± 1.0**
ApoA-IV	0.4 ± 0.1	9.7 ± 1.4**

Values are mean ± S.E.M. ($n = 11$) and expressed in mg/dl. *$P < 0.05$ and **$P < 0.0001$ vs. mice fed a chow diet.

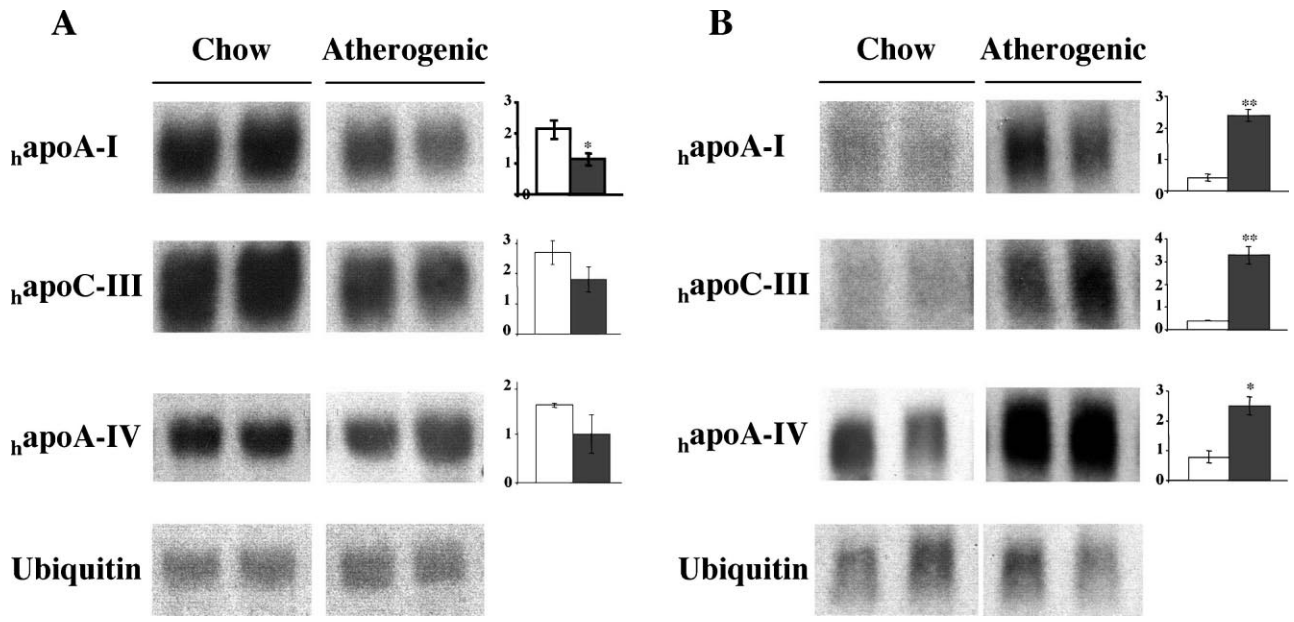

Fig. 1. Northern blot analysis. Quantification of the expression of the corresponding human mRNA in Tg mice fed a chow (open bars) or an atherogenic (filled bars) diet in liver (A) and intestine (B). Values are reported as arbitrary units normalized to ubiquitin mRNA levels. Means were obtained from RNA preparations from at least 5 different mice. $^{*}P < 0.05$ and $^{**}P < 0.0001$ vs. mice fed a chow diet.

significantly higher in animals on an atherogenic diet, principally that of apoA-IV which was increased more than 20 times (Table 1).

3.3. Plasma lipid levels

When Tg mice were fed the atherogenic diet, triglyceride levels dropped to 22% of the levels in chow diet Tg mice (Table 2). In contrast, a sixfold increase in total cholesterol concentration was observed, due mostly to an increase in the non-HDL subclass. However, HDL cholesterol was also significantly raised (Table 2). In control littermate mice fed the atherogenic diet, triglyceride levels remained low and only a twofold increase in total cholesterol concentration, exclusively in the non-HDL lipoproteins, was observed.

3.4. Diet-induced aortic lesions

We analyzed aortic lesions in Tg and control littermate mice maintained on an atherogenic diet. Lesion scores in Tg were reduced to 35% of those in control mice ($10\,104 \pm 6364$ μm^2 versus $29\,498 \pm 8086$ μm^2; $P < 0.0003$).

3.5. Expression of SR-BI and ABCA1 genes

No significant differences were observed in the mRNA levels of both genes in Tg and non-Tg animals fed a chow diet. An atherogenic diet provoked a significant increase in the transcription of the two genes, but at similar levels in Tg and control mice (Fig. 2A,B).

3.6. Cholesterol efflux studies

HDL from Tg mice maintained on an atherogenic diet was more efficient than HDL from non-Tg mice in promoting cholesterol efflux from Fu5AH cells (Fig. 3A). Interestingly, the phospholipid content of the HDL from the first group was about 70% higher than that of the control group (data not shown).

In the J774 mouse macrophage system, in the presence of cAMP, the isolated fraction of $d > 1.21$ g/ml from Tg mice fed an atherogenic diet was also more efficient in promoting cholesterol efflux than the fraction from control mice (Fig. 3B).

In both systems, cholesterol efflux was increased in Tg and control mice fed an atherogenic diet, when compared to efflux measured in Tg and control mice fed a chow diet (data not shown).

4. Discussion

To study the effect of the coordinated expression of the three genes of the apoA-I/C-III/A-IV cluster, we recently generated Tg mice containing the human cluster. We have demonstrated that expression of the entire cluster induces hyperlipidemia but protects against atherogenesis in mice in an apoE-deficient background [14]. In the present report, we analyze the effect of this expression in mice in a normal genetic background, when subjected to an induced atherogenic process provoked by a high fat-high cholesterol diet. This diet

Table 2
Lipid concentrations in control and Tg mice after an atherogenic diet

	Triglycerides		Total cholesterol		HDL cholesterol	
	Chow diet	Atherogenic diet	Chow diet	Atherogenic diet	Chow diet	Atherogenic diet
Control	69 ± 3	61 ± 7	75 ± 2	$182 \pm 30^{*}$	55 ± 3	55 ± 5
Transgenic	$475 \pm 51^{\dagger\dagger}$	$105 \pm 7^{**\dagger}$	$115 \pm 9^{\dagger}$	$565 \pm 69^{**\dagger\dagger}$	$74 \pm 4^{\dagger}$	$96 \pm 6^{*\dagger\dagger}$

Values are mean ± S.E.M. ($n = 12$) and expressed in mg/dl. $^{*}P < 0.05$ and $^{**}P < 0.0001$ vs. mice fed a chow diet. $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.0001$ vs. control mice.

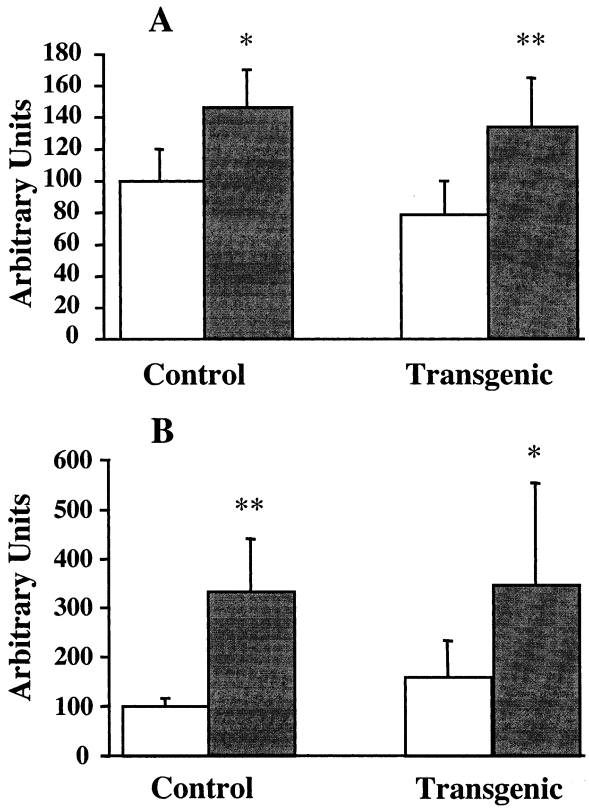

Fig. 2. Analyses of SR-BI (A) and ABCA1 (B) mRNAs in control and Tg mice fed a chow (open bars) or an atherogenic (filled bars) diet. Values are reported as arbitrary units normalized to ubiquitin mRNA levels. Means were obtained from RNA preparations from five different mice from each type of sample. $*P < 0.05$ and $**P < 0.001$ vs. mice fed a chow diet.

results in a clear elevation of intestinal apoA-I, apoC-III and apoA-IV mRNA levels; in contrast, the hepatic levels are unmodified or decreased. These results indicate that lipids up-regulate the intestinal expression of the three genes. This is accompanied by an increased concentration of the corresponding proteins in plasma. The increase is particularly significant in the case of apoA-IV in accordance with previous observations indicating that apoA-IV plasma concentration in rats and humans was enhanced after fat- or cholesterol-rich diets [24–26].

In control mice fed an atherogenic diet, triglyceride levels remained low, whereas total cholesterol concentration was increased twofold, exclusively in the non-HDL lipoproteins. In contrast, in Tg mice, triglyceride concentrations dropped dramatically. This has also been observed in mouse apoA-IV transgenic mice when fed an atherogenic diet [4]. In cluster Tg mice, the cholesterol concentration was highly increased, mainly in the non-HDL fraction, and less, although significantly, in the HDL population. These higher HDL cholesterol levels, as well as the overexpression of the human gene cluster, may have implications in the response of these animals to diet-induced aortic lesions, and indeed we have found that lesion scores in Tgs were reduced to 35% of those in control mice.

ApoA-I and apoA-IV have been shown to protect against the formation of aortic lesions, and both apolipoproteins participate in RCT and promote cholesterol efflux from cultured cells [23,27,28]. The initial step in the RCT process is the release of free cholesterol and phospholipids from the cellular plasma membrane to acceptor particles. Cholesterol efflux from cells may occur by both SR-BI- and ABCA1-dependent mechanisms [15,18,29]. It was previously observed that in SR-BI Tg mice in an inbred mouse background hemizygous for a human apoB transgene, endogenous SR-BI expression levels were not affected by a high fat-high cholesterol diet [30]. It was also shown that ABCA1 mRNA levels were increased upon cholesterol treatment of immortalized macrophages and fibroblasts [31]. In the present work we observed that in the Tg and non-Tg animals fed a chow diet, hepatic mRNA levels of the two SR-BI and ABCA1 genes were similar in each case. It should be noted that an atherogenic diet provokes a significant but similar increase in the transcription of the two genes, in both Tg and control mice. This seems to indicate that the expression of the human apoA-I/C-III/A-IV cluster in the mice has no influence on the transcription of the genes encoding SR-BI and ABCA1.

Efflux from Fu5AH hepatoma cells or from J774 macrophage cells was greater to Tg than to non-Tg acceptors in mice fed an atherogenic diet. As phospholipid is a major component of HDL that modulates cell cholesterol efflux, it

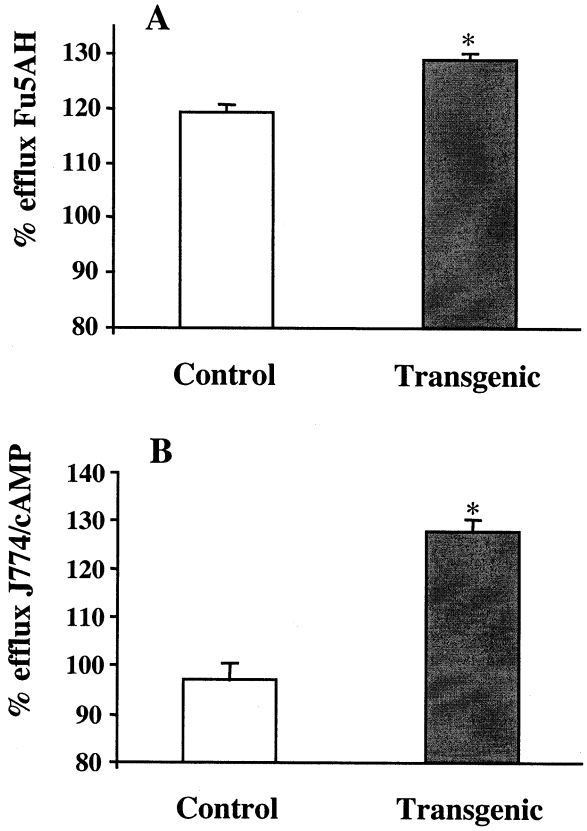

Fig. 3. A: Efflux of [^{3}H]cholesterol from Fu5AH to HDL from control (open bars) or Tg (filled bars) mice fed an atherogenic diet. $*P < 0.02$ vs. control mice. Data are from a representative experiment with triplicate wells ($n = 3$). Values are expressed as mean ± S.D. B: Efflux of [^{3}H]cholesterol from J774 to particles of $d > 1.21$ g/ml from control (open bars) or Tg (filled bars) mice fed a chow or an atherogenic diet. $*P < 0.035$ vs. control mice. Data are from a representative experiment with triplicate wells ($n = 3$). Values are expressed as mean ± S.D. Values for control mice are taken as 100% to allow comparison.

was interesting to observe that on an atherogenic diet the phospholipid content of the Tg HDL was about 70% higher than that of non-Tg mice. Thus, in the apoA-I/C-III/A-IV Tg animals fed an atherogenic diet, cholesterol efflux is mediated by phospholipid-rich HDL through the SR-BI receptor pathway [32] and by the interaction between the lipid poor $d > 1.21$ g/l fraction with the ABCA1 transporter [18].

As cholesterol efflux, the first step in RCT, is enhanced in Tg mice, we can hypothesize that RCT is the basis of the protective mechanism detected in these mice. However, at least part of the protection against atherogenesis could also be attributable to properties of apoA-I and apoA-IV unrelated to their role in RCT, such as their ability to protect low density lipoprotein (LDL) from oxidative modification [33,34]. It is interesting to note that in the Tg mice, after an atherogenic diet, we detected a diminished level of autoantibodies directed against oxidized LDL compared to that present in normal counterparts; however, the difference observed was not statistically significant (data not shown).

In conclusion, here we present evidence that the human apoA-I/C-III/A-IV gene cluster in Tg mice protects against atherogenesis in response to an atherogenic diet. We have previously observed such a protection in the Tg mice established in an apoE-deficient background [14]. ApoA-I and apoA-IV are known to have antiatherogenic properties, whereas apoC-III overexpression resulted in increased atherosclerosis. Thus, the apoA-I/C-III/A-IV Tg mice provide a new model for the design and screening of products that selectively modulate the expression of the three human genes, in order to favor the synthesis of apoA-I and/or apoA-IV.

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References

[1] Reichl, D. and Miller, N.E. (1989) Arteriosclerosis 9, 785–797.
[2] Karathanasis, S.K. (1985) Proc. Natl. Acad. Sci. USA 82, 6374–6378.
[3] Miller, N.E. and Tall, A.R. (1992) Excerpta Medica, Amsterdam.
[4] Cohen, R.D., Castellani, L.W., Qiao, J.H., Van Lenten, B.J., Lusis, A.J. and Reue, K. (1997) J. Clin. Invest. 99, 1906–1916.
[5] Duverger, N., Tremp, G., Caillaud, J.M., Emmanuel, F., Castro, G., Fruchart, J.C., Steinmetz, A. and Denefle, P. (1996) Science 273, 966–968.
[6] Paszty, C., Maeda, N., Verstuyft, J. and Rubin, E.M. (1994) J. Clin. Invest. 94, 899–903.
[7] Rubin, E.M., Krauss, R.M., Spangler, E.A., Verstuyft, J.G. and Clift, S.M. (1991) Nature 353, 265–267.
[8] Ito, Y., Azrolan, N., O'Connell, A., Walsh, A. and Breslow, J.L. (1990) Science 249, 790–793.
[9] Masucci-Magoulas, L., Goldberg, I.J., Bisgaier, C.L., Serajuddin, H., Francone, O.L., Breslow, J.L. and Tall, A.R. (1997) Science 275, 391–394.
[10] Hayek, T., Masucci-Magoulas, L., Jiang, X., Walsh, A., Rubin, E., Breslow, J.L. and Tall, A.R. (1995) J. Clin. Invest. 96, 2071–2074.
[11] Karathanasis, S.K., Ferris, E. and Haddad, I.A. (1987) Proc. Natl. Acad. Sci. USA 84, 7198–7202.
[12] Ordovas, J.M., Cassidy, D.K., Civeira, F., Bisgaier, C.L. and Schaefer, E.J. (1989) J. Biol. Chem. 264, 16339–16342.
[13] Hong, S.H., Park, W.H., Lee, C.C., Song, J.H. and Kim, J.Q. (1997) Clin. Chem. 43, 13–17.
[14] Vergnes, L., Baroukh, N., Ostos, M.A., Castro, G., Duverger, N., Nanjee, M.N., Najib, J., Fruchart, J.C., Miller, N.E. and Zakin, M.M. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 2267–2274.
[15] Oram, J.F. and Vaughan, A.M. (2000) Curr. Opin. Lipidol. 11, 253–260.
[16] McNamara, D.J. (2000) Biochim. Biophys. Acta 1529, 310–320.
[17] Ji, Y., Jian, B., Wang, N., Sun, Y., Moya, M.L., Phillips, M.C., Rothblat, G.H., Swaney, J.B. and Tall, A.R. (1997) J. Biol. Chem. 272, 20982–20985.
[18] Wang, N., Silver, D.L., Costet, P. and Tall, A.R. (2000) J. Biol. Chem. 275, 33053–33058.
[19] Bortnick, A.E., Rothblat, G.H., Stoudt, G., Hoppe, K.L., Royer, L.J., McNeish, J. and Francone, O.L. (2000) J. Biol. Chem. 275, 28634–28640.
[20] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[21] Paigen, B., Morrow, A., Holmes, P.A., Mitchell, D. and Williams, R.A. (1987) Atherosclerosis 68, 231–240.
[22] de la Llera Moya, M., Atger, V., Paul, J.L., Fournier, N., Moatti, N., Giral, P., Friday, K.E. and Rothblat, G. (1994) Arterioscler. Thromb. 14, 1056–1065.
[23] Fournier, N., Atger, V., Paul, J.L., Sturm, M., Duverger, N., Rothblat, G.H. and Moatti, N. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 1283–1292.
[24] Green, P.H. and Glickman, R.M. (1981) J. Lipid Res. 22, 1153–1173.
[25] Delamatre, J.G. and Roheim, P.S. (1983) Biochim. Biophys. Acta 751, 210–217.
[26] Bisgaier, C.L., Sachdev, O.P., Megna, L. and Glickman, R.M. (1985) J. Lipid Res. 26, 11–25.
[27] Castro, G., Nihoul, L.P., Dengremont, C., de Geitere, C., Delfly, B., Tailleux, A., Fievet, C., Duverger, N., Denefle, P. and Fruchart, J.C. (1997) Biochemistry 36, 2243–2249.
[28] Duverger, N., Kruth, H., Emmanuel, F., Caillaud, J.M., Viglietta, C., Castro, G., Tailleux, A., Fievet, C., Fruchart, J.C. and Houdebine, L.M. (1996) Circulation 94, 713–717.
[29] Gu, X., Kozarsky, K. and Krieger, M. (2000) J. Biol. Chem. 275, 29993–30001.
[30] Ueda, Y., Gong, E., Royer, L., Cooper, P.N., Francone, O.L. and Rubin, E.M. (2000) J. Biol. Chem. 275, 20368–20373.
[31] Lawn, R.M., Wade, D.P., Garvin, M.R., Wang, X., Schwartz, K., Porter, J.G., Seilhamer, J.J., Vaughan, A.M. and Oram, J.F. (1999) J. Clin. Invest. 104, R25–31.
[32] Fournier, N., Paul, J.L., Atger, V., Cogny, A., Soni, T., de la Llera-Moya, M., Rothblat, G. and Moatti, N. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 2685–2691.
[33] Ostos, M.A., Conconi, M., Vergnes, L., Baroukh, N., Ribalta, J., Girona, J.M., Caillaud, A., Ochoa, A. and Zahin, M.M. (2001) Arterioscler. Thromb. Vasc. Biol. (in press).
[34] Garner, B., Waldeck, A.R., Witting, P.K., Rye, K.A. and Stocker, R. (1998) J. Biol. Chem. 273, 6088–6095.