Comparison of human apoA-I expression in mouse models of atherosclerosis after gene transfer using a second generation adenovirus

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Abstract Gene transfer and expression of apolipoprotein A-I (apoA-I), the major protein component of high density lipoproteins (HDL), is a potentially attractive method for investigating the effects of apoA-I on atherosclerosis. We constructed a second generation recombinant adenovirus encoding the human apoA-I cDNA. This adenoviral vector or a control vector was injected intravenously into apoE-deficient mice fed a chow diet and low density lipoprotein (LDL) receptor (LDLR)-deficient mice fed Western diet, as well as control wild-type C57BL/6 mice. The mean peak plasma human apoA-I concentrations were 235, 324, and 276 mg/dL in apoE-deficient, LDLR-deficient, and wild-type mice, respectively. Human apoA-I concentrations decreased rapidly in apoE-deficient mice and were barely detectable 6 weeks after injection. In contrast, substantially higher levels of human apoA-I were sustained in LDLR-deficient mice. In wild-type mice, human apoA-I levels decreased more rapidly than in LDLR-deficient mice, but could still be detected in plasma for up to 8 months after virus injection. In apoE-deficient mice, a substantial fraction of human apoA-I was found associated with triglyceride (TG)-rich lipoproteins; in contrast, in LDLR-deficient and wild-type mice the majority of human apoA-I was found in the HDL fraction. Finally, expression of human apoA-I caused a transient but significant increase in triglyceride levels in all three mouse models. In summary: *1*) a second generation recombinant adenovirus resulted in high-level expression of human apoA-I in mice; *2*) significantly higher levels of human apoA-I persisted for a longer time in LDLR-deficient mice compared with apoE-deficient mice; and *3*) substantial human apoA-I was found associated with TG-rich lipoproteins in apoE-deficient but not LDLR-deficient mice.—**Tsukamoto, K., K. G. Hiester, P. Smith, D. C. Usher, J. M. Glick, and D. J. Rader.** Comparison of human apoA-I expression in mouse models of atherosclerosis after gene transfer using a second generation adenovirus. *J. Lipid Res.* 1997. **38:** 1869–1876.

Supplementary key words apolipoprotein A-I • gene transfer • adenoviral vector • lipoprotein metabolism • hyperlipoproteinemia • atherosclerosis

Plasma concentrations of high density lipoprotein (HDL) cholesterol and its major protein constituent apolipoprotein A-I (apoA-I) are strongly inversely associated with atherosclerotic cardiovascular disease (1), but it is not known whether this association is causal. Animal studies have provided some support for the concept that HDL and apoA-I may directly inhibit atherogenesis. Repeated intravenous injection of human HDL (2) or rabbit apoA-I (3) into cholesterol-fed rabbits resulted in reduced progression of atherosclerotic lesions. Transgenic overexpression of human apoA-I in hyperlipidemic WHHL rabbits reduced the development of aortic atherosclerosis (4). Transgenic overexpression of human apoA-I partially inhibited progression of atherosclerotic lesions in C57BL/6 mice fed a high fat/cholic acid-containing diet (5) and in chow-fed apoE-deficient mice (6, 7). However, specific methods to substantially raise apoA-I levels in humans do not currently exist. Preliminary studies have been performed by infusing purified apoA-I into human subjects, demonstrating little impact on HDL cholesterol levels (8).

There is substantial interest in the concept of using somatic gene transfer as a strategy for expressing apoA-I in vivo (9, 10). Replication-defective recombinant adenoviruses have been used in the last few years for liver-directed somatic gene transfer in animals (11, 12). A first generation recombinant adenovirus was reported to result in transient expression of human apoA-I in normal mice for up to 2 weeks (13). However, the use of first generation adenoviruses is limited by the short duration of transgene expression, which is due to a cellular immune response leading to hepatic inflammation and destruction of the genetically modified hepatocytes (11). To address the hypothesis that somatic gene

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein.

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transfer and expression of apoA-I reduces atherosclerosis in animals, longer gene expression is required. Second generation recombinant adenoviruses have been developed in which a temperature-sensitive mutation in the adenoviral E2A gene results in less adenoviral gene expression at body temperature. As a result, there is less viral gene transcription in vivo, less inflammation, and substantially longer transgene expression in mouse liver after intravenous injection (14–16). We constructed a second generation recombinant adenovirus encoding human apoA-I to test whether we can achieve longer term expression in mouse models of atherosclerosis.

In this study, we used a second generation recombinant adenovirus to express apoA-I in the two major mouse models of atherosclerosis, the chow-fed apoE-deficient mouse (17, 18) and the LDL receptor (LDLR)-deficient mouse fed a Western-type diet (19); as a control, wild-type C57BL/6 mice were also used. We found that the second generation virus resulted in high level expression of human apoA-I in all three mouse models. However, levels of human apoA-I remained much higher and persisted for a longer period in LDLR-deficient mice than in apoE-deficient mice.

MATERIALS AND METHODS

Construction of recombinant adenoviruses

The human apoA-I cDNA (kindly provided by Dr. J. Breslow of Rockefeller University) was subcloned into the shuttle plasmid vector pAdCMV-Link (15). After screening for the appropriate orientation by restriction analysis, the resulting plasmid was designated as pAdCMVapoAI. The adenoviral DNA used for subsequent cotransfection was purified from H5.110CMVlacZ (15) (lacZ virus) which contains a temperature-sensitive mutation (ts125) in the E2A adenoviral gene. Recombinant second generation adenovirus was generated as previously described (20). Briefly, the plasmid pAdCMVapoAI was linearized with NheI and contransfected into 293 cells along with adenoviral DNA digested with ClaI, and cells were overlaid with agar and incubated at 32°C for 15 days. Plaques were picked and screened by PCR; those positive for the apoA-I cDNA were subjected to a second round of plaque purification. After confirmation of the presence of apoA-I cDNA and the absence of wild type adenovirus, the recombinant adenovirus was expanded in 293 cells at 32°C. Cell lysates were used to infect HeLa cells for confirmation of the expression of human apoA-I by Western blotting of media. The recombinant adenovirus, designated as H5.110CMVAI (apoA-I virus), was further expanded in 293 cells and purified by cesium chloride ultracentrifugation. The lacZ virus encoding the β-galactosidase cDNA was also subjected to plaque purification and purified as described above. The purified viruses were stored in 10% glycerol/PBS at −80°C.

Animal studies

Four to six-week-old wild-type C57BL/6 mice, apoE-deficient mice (back crossed with C57BL/6 mice), and LDLR-deficient mice (back crossed with C57BL/6 mice) were obtained from Jackson Laboratory. Wild-type C57BL/6 mice and apoE-deficient mice were fed normal chow; LDLR-deficient mice were fed a Western-type diet (19) (normal chow supplemented with 0.15% cholesterol and 21% butter fat). Four weeks after initiation of the diet, all mice were injected on the same day intravenously with the same preparations of apoA-I virus or lacZ virus at a dose of 6.0×10^9 particles (2.0×10^8 pfu) per gram body weight. Each group contained 4 to 6 mice. Blood was obtained from the retro-orbital plexus after a 4-h fast 1 day prior to injection, 3 days after injection, and weekly over the course of the next 6 weeks. Blood was collected into a tube containing EDTA, NaN_3, gentamicin, PMSF, and benzamidine (the final concentrations were 2 mM, 0.2%, 0.77%, 1 mM, and 1 mM, respectively). Some wild-type C57BL/6 mice injected with the apoA-I virus were maintained beyond the 6-week experimental period to determine the long-term duration of human apoA-I expression.

Detection of human apoA-I in mouse plasma by Western blotting

One μL of plasma sample was subjected to 12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH), and human apoA-I was detected by a monoclonal antibody specific for human apoA-I and peroxidase-labeled goat anti-mouse IgG (Jackson ImmunoResearch Lab. Inc., West Grove, PA) as a secondary antibody.

Lipoprotein analysis

Pooled plasma samples (120 μL) from each group were subjected to fast protein liquid chromatography (FPLC) gel filtration (Pharmacia LKB Biotechnology, Uppsala, Sweden) using two Superose 6 columns in series as described (21). Five hundred-μL fractions were collected, and cholesterol concentrations were determined using an enzymatic assay (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The cholesterol in the HDL fractions was divided by the total cholesterol recovered from the column and this fraction was multiplied by the total plasma cholesterol concentration in order to determine the plasma HDL cholesterol concentration.

Distribution of human apoA-I in lipoprotein classes

After separating the pooled plasma samples on FPLC, 40-μL samples from two adjacent fractions were pooled

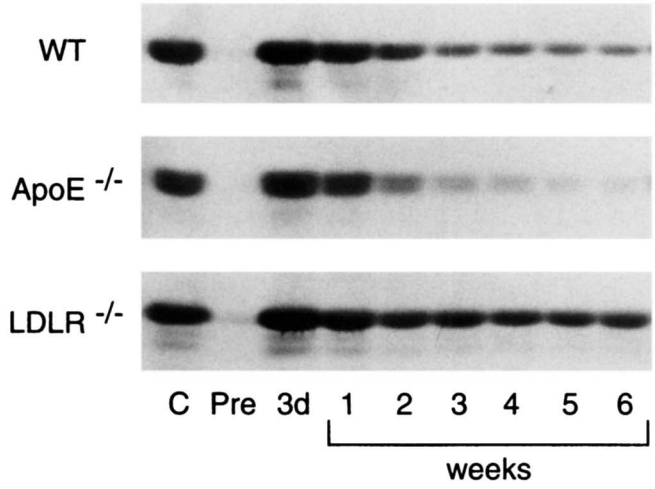

Fig. 1. Western blotting analysis of the plasma samples of mice injected with recombinant human apoA-I adenoviruses through 6 weeks. After separating 1 μL of pooled plasma sample by SDS-PAGE, proteins were transferred to a nitrocellulose membrane, and human apoA-I was detected with a monoclonal antibody against human apoA-I. Panels WT, ApoE$^{-/-}$, and LDLR$^{-/-}$ represent the time course changes in plasma concentration of the human apoA-I proteins in pooled plasma samples from wild-type C57BL6 mice, apoE-deficient mice, and LDLR-deficient mice, respectively. Lane C: control human plasma; lane Pre: mouse plasma drawn before injection of the virus; lane 3d: 3 days after injection.

and subjected to 12% SDS-PAGE. Human apoA-I was detected by Western blotting as described above. After color development, the membranes were subjected to densitometric analysis.

General methods

DNA and RNA manipulations were performed by standard techniques (22) and immunoblotting was done by commonly used methods (23) unless otherwise indicated. The plasma total cholesterol and triglyceride levels were measured enzymatically on a Cobas Fara (Roche Diagnostic Systems Inc., Montclair, NJ) using Sigma reagents (Sigma Chemical Co., St. Louis, MO). ApoA-I was quantitated using an immunoturbidometric assay (Sigma) on a Cobas Fara autoanalyzer. Densitometric analysis was performed by using an Imaging densitometer (Bio-Rad Lab., Hercules, CA) and Molecular Analyst software (Bio-Rad Lab.).

RESULTS

Expression of human apoA-I

High peak plasma levels of human apoA-I were achieved in all three mouse models as shown by Western blotting of plasma (**Fig. 1**) and immunoassay (**Table 1**). Peak plasma concentrations were achieved on day 3 after the injection of virus. However, the rate of decrease in plasma human apoA-I levels over time differed widely in the three types of mice (Fig. 1 and Table 1). By 6 weeks after injection, human apoA-I was barely detectable in the apoE-deficient mice; in contrast, abundant human apoA-I remained in LDLR-deficient mice 6 weeks after injection. Levels of apoA-I in wild-type C57BL/6 mice declined faster than in LDLR-deficient mice (Fig. 1), but low levels of human apoA-I were detectable for up to 8 months post injection (**Fig. 2**).

TABLE 1. Plasma human apoA-I levels in wild-type, apoE-deficient, and LDLR-deficient mice

Day	Wild-Type	ApoE-Deficient	LDLR-Deficient
		mg/dL	
3	276 ± 34	235 ± 28	324 ± 108
7	153 ± 22	156 ± 51	218 ± 42
14	76 ± 4	37 ± 32	120 ± 8
28	27 ± 11	1.8 ± 5	68 ± 11
42	23 ± 3	0.2 ± 4	61 ± 12

Data are means ± SD; n = 5–6.

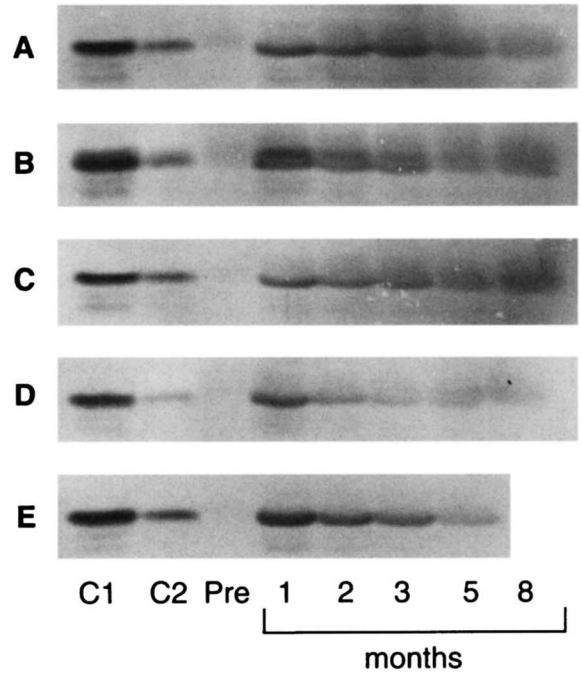

Fig. 2. Western blotting analysis of the plasma samples of wild-type mice injected with recombinant human apoA-I adenoviruses up to 8 months. After separating 1 μL of plasma sample by SDS-PAGE, proteins were transferred to a nitrocellulose membrane, and human apoA-I was detected with a monoclonal antibody against human apoA-I. Panels A, B, C, D, and E represent the long term analysis of plasma human apoA-I in an individual wild-type C57BL/6 mouse. Lane C1: one tenth volume of control human plasma; lane C2: 1/100 volume of control human plasma; lane Pre: mouse plasma drawn before injection of the virus.

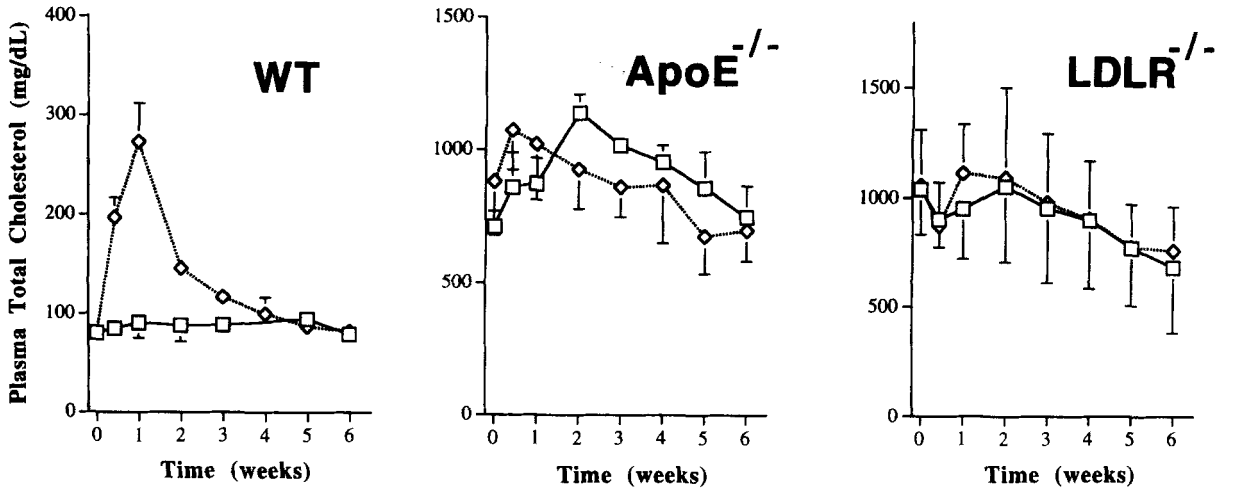

Fig. 3. Changes in plasma total cholesterol. Plasma total cholesterol levels were measured enzymatically as described in Materials and Methods. Panels WT, ApoE$^{-/-}$, and LDLR$^{-/-}$ present the data from wild-type C57BL6 mice, apoE-deficient mice, and LDLR-deficient mice, respectively. (□), mice injected with LacZ virus (n = 4–5); (◇), mice injected with apoA-I virus (n = 5–6). Data are mean ± standard deviation.

Effects on lipids and lipoproteins

The effect of apoA-I gene transfer and expression on plasma lipoproteins was different in each of the mouse models studied. In wild-type C57BL/6 mice, the plasma cholesterol level increased by 3.4-fold to a peak of 273 mg/dL on day 7 (**Fig. 3**). FPLC gel filtration of pooled plasma samples indicated that this increase was due to increased HDL cholesterol (**Fig. 4**), levels of which were increased by 3.5-fold to 234 mg/dL on day 7 compared to baseline (**Fig. 5**). By day 7, the FPLC HDL peak was eluted earlier than at baseline or on day 3, indicating larger HDL particle size (Fig. 4). This is consistent with the observation that HDL cholesterol levels were higher on day 7 compared with day 3 despite the fact that human apoA-I levels were lower and suggests that the newly formed HDL particles containing human apoA-I may have undergone maturation and enlargement between days 3 and 7. There were no changes in total or HDL cholesterol in wild-type mice injected with the lacZ adenovirus.

The effect of apoA-I expression in apoE-deficient mice revealed a very different pattern. Total cholesterol levels were increased slightly on day 3 and quickly returned to baseline (Fig. 3). FPLC gel filtration (Fig. 4) indicated an increase in HDL cholesterol on day 3 to 132 mg/dL which returned to baseline by day 7 (Fig. 5). There was no effect of human apoA-I expression on levels of cholesterol in apoB-containing lipoproteins in apoE-deficient mice (Fig. 4). The lacZ adenovirus resulted in an increase in total cholesterol levels in apoE-deficient mice which returned to baseline by 6 weeks (Fig. 3); this was due to an increase in apoB-containing lipoproteins (data not shown) and not to an effect on HDL cholesterol levels (Fig. 5).

Despite similar baseline total cholesterol levels in LDLR-deficient mice fed a Western diet, the effect of human apoA-I expression was substantially different in these mice compared with that in apoE-deficient mice. There was relatively little effect of apoA-I expression on total cholesterol levels (Fig. 3). However, on day 3, LDLR-deficient mice injected with the apoA-I virus had increased HDL and decreased LDL compared with baseline (Fig. 4), which by day 7 were beginning to return to baseline. The mean HDL cholesterol level was increased by 90% to 309 mg/dL on day 3 and remained elevated for considerably longer than in apoE-deficient mice (Fig. 5). In LDLR-deficient mice injected with the lacZ virus, there were no significant changes in total (Fig. 3) or HDL (Fig. 5) cholesterol levels.

Plasma triglyceride levels were acutely increased in all three mouse models as a result of human apoA-I expression (**Fig. 6**). The changes differed in magnitude (170% in wild-type mice, 300% in apoE-deficient mice, and 250% in LDLR-deficient mice), but demonstrated a consistent pattern: triglyceride levels were elevated through day 7 but returned to baseline by day 14 in all three models. This effect appeared to be a direct result of the high levels of human apoA-I expression, as injection of the lacZ virus had no major effect on triglyceride levels in all three groups (Fig. 6).

Lipoprotein distribution of human apoA-I

To determine the distribution of human apoA-I on lipoproteins, the proteins in the FPLC-fractionated

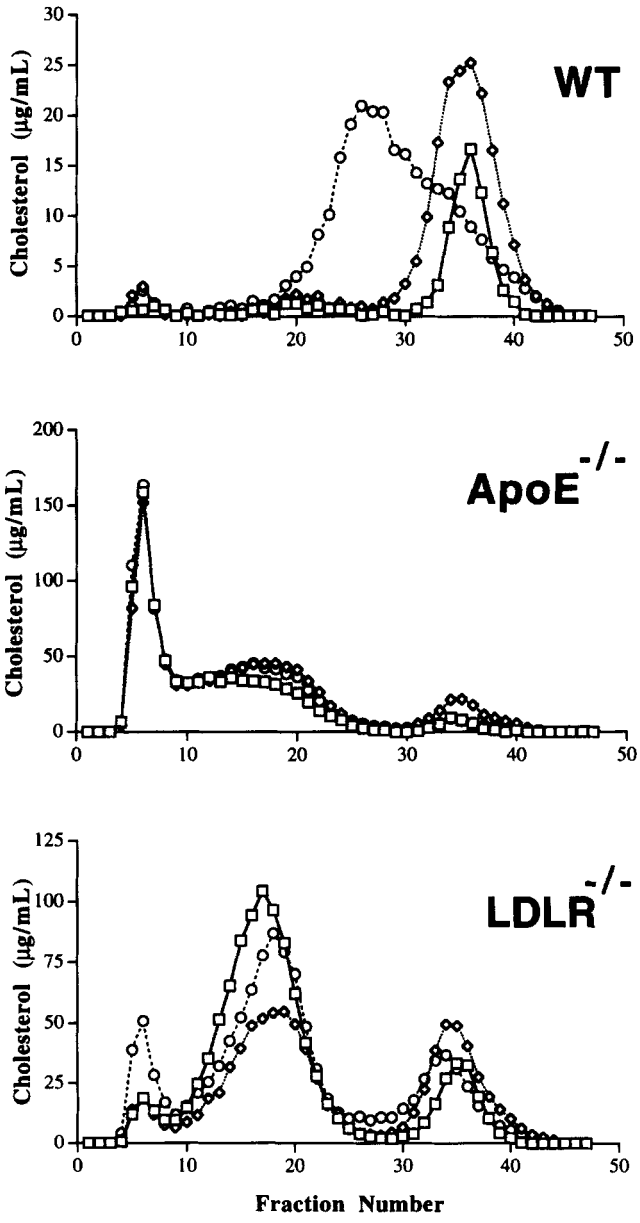

Fig. 4. FPLC lipoprotein profile. Pooled plasma samples were subjected to gel filtration using Superose 6 columns and the cholesterol level in each fraction was measured. Panels WT, ApoE$^{-/-}$, and LDLR$^{-/-}$ present data from wild-type C57BL/6 mice, apoE-deficient mice, and LDLR-deficient mice, respectively. (□), before injection of virus; (◇), 3 days after injection; (○) 7 days after injection.

samples were separated by SDS-PAGE and human apoA-I was detected by Western blotting (**Fig. 7**). In wild-type C57BL/6 mice on day 3, most of the human apoA-I was found in the HDL fraction. In contrast, in the apoE-deficient mice a substantial amount of human apoA-I was associated with apoB-containing particles, especially with VLDL and IDL, although some apoA-I was found on HDL as well. By comparison, the LDLR-deficient mice had almost no human apoA-I associated with VLDL or IDL, a small amount within the LDL fraction, and most on HDL. **Table 2** presents the concentrations of human apoA-I in lipoprotein fractions on days 3 and 7 after injection. In wild-type mice, the apoA-I was almost exclusively associated with HDL particles. In apoE-deficient mice, there was a shift in the distribution of human apoA-I from HDL to apoB-containing lipoproteins and by day 7 there was more human apoA-I associated with apoB-containing lipoprotein particles than with HDL particles. In LDLR-deficient mice, although some human apoA-I was found within the LDL fraction, the majority remained associated with HDL. In addition, some human apoA-I was consistently found in a "lipid-poor" fraction; LDLR-deficient mice were noted to have a higher fraction of human apoA-I in this fraction, especially by day 7.

DISCUSSION

Somatic gene transfer of apoA-I could theoretically be used to test hypotheses regarding the effect of apoA-I on atherosclerotic lesions in animal models of atherosclerosis. This report addresses some of the important issues in attempting to demonstrate efficacy of apoA-I gene transfer in mouse models of atherosclerosis. We report that the use of a second generation recombinant adenovirus resulted in higher levels of human apoA-I expression and for considerably longer than previously reported using a first generation adenovirus (13). Furthermore, we describe the use of this vector to express human apoA-I in two of the major mouse models of atherosclerosis, chow-fed apoE-deficient mice and LDLR-deficient mice fed a Western diet. There were substantial differences in the plasma concentrations and lipoprotein distribution of the expressed human apoA-I between these two types of mice.

The injection of second generation adenovirus encoding human apoA-I cDNA in wild-type C57BL/6 mice resulted in high levels and prolonged expression of human apoA-I. Kopfler and colleagues (13) reported that injection of first generation adenovirus resulted in a mean peak level of human apoA-I of 168 mg/dL on day 5; however, it declined quickly and human apoA-I was undetectable by day 21. Using our second generation adenovirus, a mean level of human apoA-I of 276 mg/dL on day 3 was achieved and its levels declined more slowly. A low level of human apoA-I could be detected in the plasma of wild-type C57BL/6 mice by Western blotting as long as 8 months after adenovirus injection. Expression of human apoA-I in wild-type C57BL/6 mice raised HDL cholesterol levels from 67 mg/dL to 178 mg/dL on day 3. Interestingly, HDL cholesterol levels increased even further to 234 mg/dL by

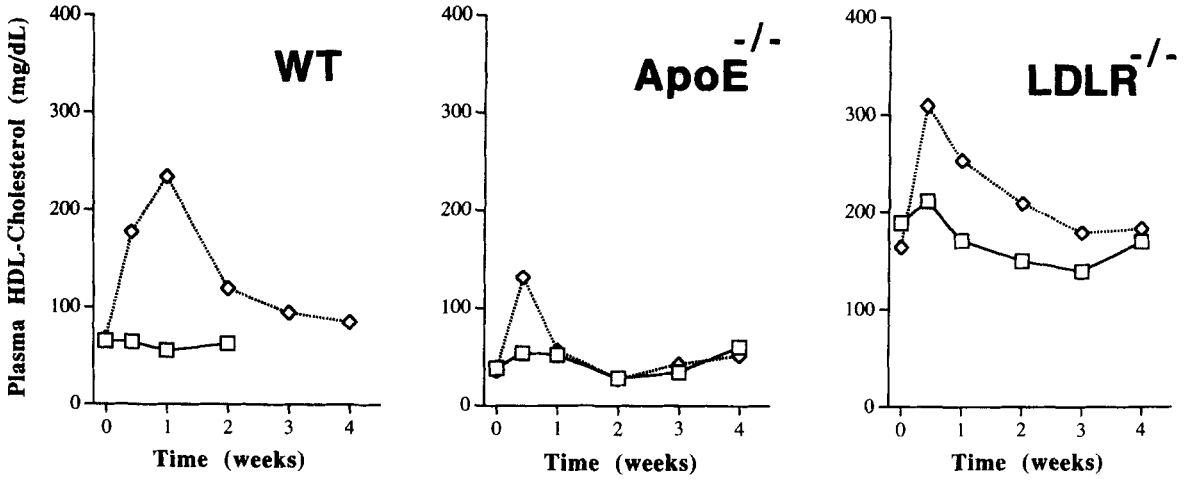

Fig. 5. Changes in HDL cholesterol. Panels WT, ApoE $^{-/-}$, and LDLR$^{-/-}$ present data from wild-type C57BL/6 mice, apoE-deficient mice, and LDLR-deficient mice, respectively. (□), mice injected with lacZ virus; (◇), mice injected with apoA-I virus.

day 7 even as human apoA-I levels declined. This was accompanied by a clear shift in HDL size to larger particles, suggesting that the nascent HDL formed by expression of human apoA-I underwent maturation to larger, more cholesterol-enriched particles.

We tested the ability of this same vector to express human apoA-I in two major mouse models of atherosclerosis, chow-fed apoE-deficient mice and LDLR-deficient mice fed a Western diet. There were substantial differences in the pattern of human apoA-I expression, including magnitude and duration of expression, distribution on lipoproteins, and impact on HDL cholesterol levels. Peak plasma levels of human apoA-I were significantly lower in apoE-deficient mice compared with LDLR-deficient mice, and they declined much more rapidly in apoE-deficient mice. A much higher fraction of human apoA-I was found to be associated with apoB-containing lipoproteins (particularly VLDL and IDL) in apoE-deficient mice compared with LDLR-deficient mice. On day 7, more than 50% of the plasma human apoA-I was associated with apoB-containing lipoproteins in apoE-deficient mice. ApoA-I is known to be associated with triglyceride (TG)-rich lipoproteins in humans and this is thought to accelerate the catabolism of apoA-I (24). We speculate that the association of human apoA-I with TG-rich lipoproteins in apoE-deficient mice resulted in faster catabolism and therefore lower peak plasma levels and a faster decline in plasma levels of human apoA-I. The increase in catabolism of apoA-I is not necessarily due to direct uptake of TG-

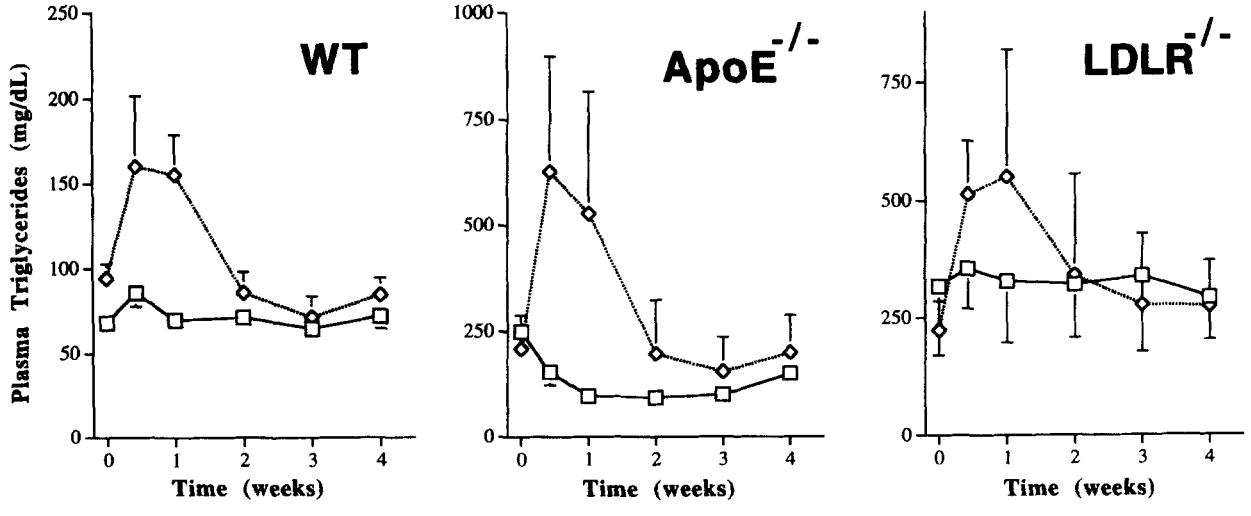

Fig. 6. Changes in plasma triglycerides. Plasma triglyceride levels were measured enzymatically as described. Panels WT, ApoE$^{-/-}$, and LDLR$^{-/-}$ represent data from wild-type C57BL/6 mice, apoE-deficient mice, and LDLR-deficient mice, respectively. (□), mice injected with lacZ virus (n = 4–5); (◇), mice injected with apoA-I virus (n = 5–6). Data are mean ± standard deviation.

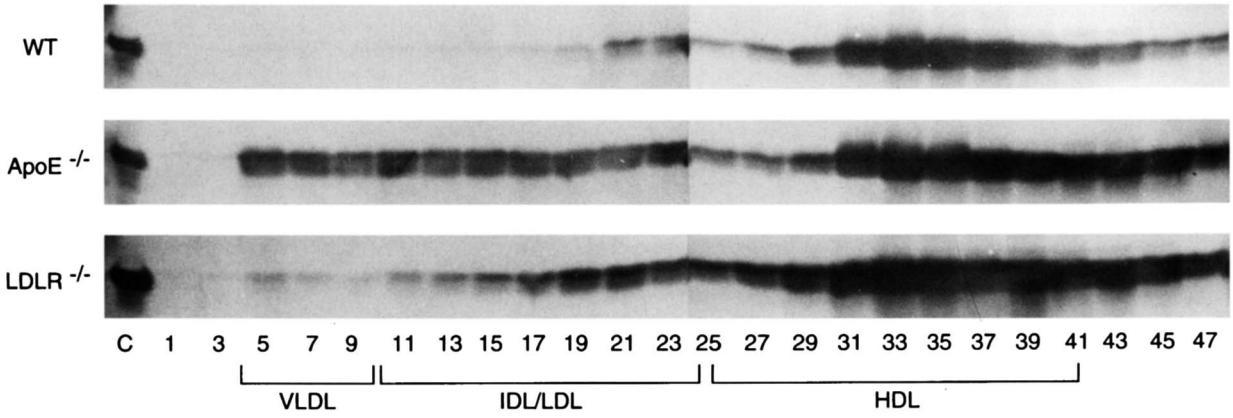

Fig. 7. Distribution of apolipoprotein A-I among lipoprotein classes on day 3. FPLC fractions were subjected to SDS-PAGE, followed by Western blotting analysis with a monoclonal antibody against human apoA-I. Panels WT, ApoE$^{-/-}$, and LDLR$^{-/-}$ represent data from wild-type C57BL/6 mice, apoE-deficient mice, and LDLR-deficient mice, respectively. Lanes 5–9 represent VLDL fractions, lanes 11–25 represent IDL/LDL fractions, lanes 25–41 represent HDL fractions, and lanes 43–47 represent "lipid-poor" fractions. Lane C is 1 μL of a human control plasma sample.

rich lipoproteins; apoA-I is thought to become dissociated from TG-rich lipoproteins, generating a "lipid-poor" form of apoA-I that is then rapidly catabolized (24). In addition to causing faster catabolism, association of apoA-I with apoB-containing lipoproteins may result in less availability of apoA-I to the vessel wall. These findings could have important implications regarding the use of apoA-I gene transfer to influence atherosclerosis in apoE-deficient mice and other animal models involving elevated levels of triglyceride-rich lipoproteins.

In contrast to apoE-deficient mice, plasma levels of human apoA-I were higher at peak and remained higher for much longer in LDLR-deficient mice. Much less human apoA-I was found associated with apoB-containing lipoproteins in LDLR-deficient mice compared with apoE-deficient mice. Even though their total cholesterol levels are similar to apoE-deficient mice, LDLR-deficient mice fed a Western diet have an elevation primarily in LDL, and apoA-I is known to have less affinity for LDL than for the triglyceride-rich lipoproteins (VLDL and IDL). The effect of apoA-I expression on HDL cholesterol levels was also much greater in LDLR-deficient mice compared with apoE-deficient mice. In addition, the fraction of "lipid-pool" apoA-I was consistently higher in LDLR-deficient mice. Because this fraction may have better access to the vessel wall, apoA-I gene transfer may be expected to have a greater impact on atherosclerosis in LDLR-deficient mice than in apoE-deficient mice. It is interesting that levels of human apoA-I remained higher in LDLR-deficient mice than even those in wild-type C57BL/6 mice during the 6 weeks of this experiment. It is possible but unlikely that the Western diet itself is directly responsible for this difference. The decrease in levels of human apoA-I over time in all three models could be due to decreased hepatic apoA-I gene transcription and protein production (14) and/or generation of antibodies to human apoA-I. Hepatic human apoA-I mRNA levels were not quantitated in these experiments and antibodies to human apoA-I could not be detected in mouse plasma 6 weeks after virus injection. Further experiments will be required to address these important questions.

Finally, we noted that expression of human apoA-I caused significant increases in plasma triglyceride levels in all three mouse models. Triglyceride levels were elevated for the first week but normalized by day 14. Plasma levels of human apoA-I also fell substantially between days 7 and 14, suggesting a threshold effect of apoA-I in causing triglyceride elevation. Gene transfer of human apoA-I in C57BL/6 mice using a first generation adenovirus was also reported to be associated with increased triglyceride levels (13). It is interesting that

TABLE 2. Distribution of apoA-I on lipoproteins in mice on days 3 and 7 after injection

	Wild-Type		ApoE-Deficient		LDLR-Deficient	
	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7
	mg/dL					
VLDL	0.8	0.1	17	8.8	2.2	1.6
I/LDL	17	9.6	53	67	64	47
HDL	224	132	125	66	190	122
Lipid-poor	34	11	40	13	68	47

The percent of apoA-I in each fraction was determined from the Western blots of FPLC fractions by densitometer and multiplied by the plasma human apoA-I concentration to obtain the concentration of apoA-I in each fraction.

the only effect on plasma lipids of infusing purified human apoA-I into human subjects was a modest increase in triglyceride levels (8). It is possible that a high level of apoA-I causes increased triglyceride levels by inhibiting lipolysis or lipoprotein uptake, or even potentially by enhancing VLDL secretion. Testing of these hypotheses will also require further investigation.

In summary, a second generation recombinant adenovirus resulted in high level, prolonged expression of human apoA-I in mice. Levels of human apoA-I persisted at a significantly higher level for a longer time in LDLR-deficient mice fed a Western diet compared with chow-fed apoE-deficient mice. This appeared to be due at least in part to substantial association of human apoA-I with apoB-containing lipoproteins in apoE-deficient mice. This study indicates that prolonged transgene expression from second generation adenoviruses may allow the investigation of the effect of apoA-I gene transfer on atherogenesis, but that the success of this approach may be highly dependent on the type of hyperlipidemia in the animal model of atherosclerosis being utilized.

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