LDL receptor deficiency or apoE mutations prevent remnant clearance and induce hypertriglyceridemia in mice[S]

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Abstract **We have used adenovirus-mediated gene transfer and bolus injection of purified apolipoprotein E (apoE) in mice to determine the contribution of LDL receptor family members in the clearance of apoE-containing lipoproteins in vivo and the factors that trigger hypertriglyceridemia. A low dose [5×10^8 plaque-forming units (pfu)] of an adenovirus expressing apoE4 did not normalize plasma cholesterol levels of apolipoprotein E-deficient ($apoE^{-/-}$) × low density lipoprotein receptor-deficient ($LDLr^{-/-}$) mice and induced hypertriglyceridemia. A similar phenotype of combined dyslipidemia was induced in $apoE^{-/-}$ or $apoE^{-/-} \times LDLr^{-/-}$ mice after infection with a low dose (4×10^8 pfu) of an adenovirus expressing the apoE4[R142V/R145V] mutant previously shown to be defective in receptor binding. In contrast, a low dose of 5×10^8 pfu of the apoE4-expressing adenovirus corrected hypercholesterolemia in $apoE^{-/-}$ mice and did not trigger hypertriglyceridemia. Bolus injection of purified apoE in $apoE^{-/-} \times LDLr^{-/-}$ mice did not clear plasma cholesterol levels and induced mild hypertriglyceridemia. In contrast, similar injection of apoE in $apoE^{-/-}$ mice cleared plasma cholesterol and caused transiently mild hypertriglyceridemia. These findings suggest that *a)* the LDL receptor alone can account for the clearance of apoE-containing lipoproteins in mice, and the contribution of other receptors is minimal, and *b)* defects in either the LDL receptor or in apoE that affect its interactions with the LDL receptor, increase the sensitivity to apoE-induced hypertriglyceridemia in mice.**—Kypreos, K. E., and V. I. Zannis. **LDL receptor deficiency or apoE mutations prevent remnant clearance and induce hypertriglyceridemia in mice.** ***J. Lipid Res.*** **2006.** 47: **521–529.**

Supplementary key words apolipoprotein E • low density lipoprotein receptor • adenovirus-mediated gene transfer • bolus apolipoprotein E injection

Apolipoprotein E (apoE) is a polymorphic protein in humans (1) and promotes the clearance of lipoprotein remnants (2, 3). However, at high concentrations, it induces hyperlipidemia (4–9). In a series of recent studies, we have used adenoviruses expressing full-length and truncated genomic apoE sequences to correct the high cholesterol profile of apolipoprotein E-deficient ($apoE^{-/-}$) mice (2) and low density lipoprotein receptor-deficient ($LDLr^{-/-}$) × $apoE^{-/-}$ double-deficient mice (10). It was shown that low levels of expression of apoE normalized the high cholesterol levels of $apoE^{-/-}$ mice. In contrast, overexpression of full-length apoE2, apoE3, or apoE4 [by infection of mice with $1–2 \times 10^9$ plaque-forming units (pfu)] did not correct the high cholesterol levels of the $apoE^{-/-}$ mice, increased VLDL secretion, inhibited lipolysis, and induced hypertriglyceridemia (5–8, 11). However, the high cholesterol profile of $apoE^{-/-}$ mice or the apoE2 knockin mice was corrected by infection with truncated apoE forms lacking different segments of the C-terminal domain (5–8, 11, 12).

Overexpression of full-length apoE2, apoE3, or apoE4 in C57BL/6 mice induced combined hyperlipidemia characterized by high cholesterol and high triglyceride levels, whereas truncated apoE forms did not change the plasma lipid and lipoprotein levels of these mice (6). Truncated apoE forms could not correct the high cholesterol profiles of the $apoE^{-/-} \times LDLr^{-/-}$ double-deficient mice but did not induce hypertriglyceridemia, indicating that the C-terminal region of apoE is responsible for the hypertriglyceridemia (8, 11). The residues of apoE responsible for the induction of hypertriglyceridemia included the hydrophobic amino acids 261, 264, 265, 268, and 269 (13).

In vitro experiments have shown that lipoprotein-bound apoE is the ligand for the LDL receptor as well as other LDL receptor family members (14–18) and scavenger receptor class B type I (19). The in vivo contribution of different types of lipoprotein receptors in the clearance of lipoprotein remnants is not fully understood (20–23). It has been suggested that heparan sulfate proteoglycan (HSPG) may participate in the clearance of apoE-containing lipoproteins (21, 23, 24).

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Here, we present three pieces of evidence indicating that the LDL receptor may represent the only physiological route of clearance of apoE-containing lipoproteins in mice. It was found that low doses of apoE4-expressing adenovirus corrected the high plasma cholesterol of apoE$^{-/-}$ mice but did not correct the high cholesterol levels and induced hypertriglyceridemia in apoE$^{-/-}$ × LDLr$^{-/-}$ mice, which lack the LDL receptor. Combined dyslipidemia was also caused in apoE$^{-/-}$ mice, which express the LDL receptor, by infection with a low dose of an adenovirus expressing an apoE4 mutant defective in receptor binding. This apoE mutant exacerbated dyslipidemia in double-deficient apoE$^{-/-}$ × LDLr$^{-/-}$ mice. Finally, a bolus injection of apoE transiently corrected the hypercholesterolemia of apoE$^{-/-}$ mice but not of apoE$^{-/-}$ × LDLr$^{-/-}$ mice. These studies also establish that deficiency in the LDL receptor or mutations in apoE, which inhibit its interactions with the LDL receptor, increase the sensitivity and severity of apoE-induced hypertriglyceridemia.

METHODS

Construction of recombinant adenoviruses expressing the wild-type human apoE4 form

The construction of the recombinant adenovirus expressing the wild-type human apoE4 form has been described previously (5–8). The apoE4[R142V/R145V] mutant was generated using the mutagenesis kit QuickChange-XL (Stratagene). The vector pGEM7-apoE4 containing exons II, III, and IV of human apoE was used as a template for the amplification reaction. The mutagenic sense and antisense primers used were apoE-mut3 sense (5′-CTC GCC TCC CAC CTG ***GTC*** AAG CTG ***GTT*** AAG CGG CTC CTC C-3′) and apoE-mut3 antisense (5′-GGA GGA GCC GCT T***AA C***CA GCT T***GA C***CA GGT GGG AGG CGA G-3′) (letters in boldface indicate the sites of mutations). After 18 cycles of PCR amplification of the template DNA using the mutagenic primers, the PCR product was treated with *Dpn*I to digest plasmids containing methylated DNA in one or both of their strands. After digestion, the reaction product, consisting of plasmids containing newly synthesized DNA in both strands carrying the mutations of interest, was used to transform competent XL-10 gold bacteria cells (Stratagene). Ampicillin-resistant clones were selected, and plasmid DNA was isolated from these clones and subjected to sequencing to confirm the presence of the point mutations. The *Hind*III-*Xba*I fragment was excised from the PGEM7-apoE vector and cloned into the corresponding sites of the pAd-Track CMV vector. The pAd-Track CMV vector places the apoE gene under the control of the cytomegalovirus (CMV) promoter. Recombinant adenovirus DNA was generated in bacteria BJ-5183 cells and used to transfect 911 cells (25, 26). After large-scale infection of HEK293 cell cultures, the recombinant adenoviruses were purified by two consecutive CsCl ultracentrifugation steps, dialyzed, and titrated (7).

Adenoviral infection of large-scale cultures of infected HTB-13 cells and purification of apoE

For apoE production, human HTB-13 cells (SW 1783 human astrocytoma) grown to 80% confluence in Leibovitz L-15 medium containing 10% (v/v) FBS were infected with adenoviruses expressing apoE4 at a multiplicity of infection of 20. After 24 h of infection, cells were washed twice with serum-free medium and preincubated in serum-free medium for 30 min. The medium was then removed and fresh serum-free medium was added. After 24 h, the medium was harvested and fresh serum-free medium was added to the cells. The harvest was repeated 8 times. Yields of 50–100 mg/l apoE were obtained. ApoE was purified from the culture medium of adenovirus-infected HTB-13 cells using dextran-sulfate Sepharose ion-exchange chromatography as described (27).

Animal studies

Mice were purchased from Jackson Laboratories (www.jax.org). Female apoE$^{-/-}$ (3) and apoE$^{-/-}$ × LDLr$^{-/-}$ (10) mice (4–6 weeks old) were used in these studies. Groups were formed, after determining the fasting cholesterol and triglyceride levels of the individual mice, to ensure similar average cholesterol and triglyceride levels among groups.

For the adenovirus infections, groups of three to six mice were injected intravenously through the tail vein with doses of 4×10^8 to 2×10^9 pfu of apoE-expressing adenoviruses or the control adenovirus expressing the green fluorescent protein. Blood was obtained daily after a 4 h fasting period for up to 9 days after injection. Aliquots of plasma were stored at 4°C and −20°C.

Injection of mice with PBS-containing purified apoE

For the bolus injection of purified apoE4, after a 4 h fast, groups of three female mice with comparable fasting cholesterol and triglyceride levels were injected intravenously through the tail vein with a solution containing 1.6 mg of apoE4 in PBS, diluted 2:1 in serum that was freshly derived from apoE$^{-/-}$ × LDLr$^{-/-}$ mice. This apoE solution was incubated for 30 min at 37°C before the injection. Control mice were injected with the same solution containing BSA. The total injected apoE or BSA was 1.6 mg in 300 μl. Blood samples of 15 μl were collected from the tail of the injected mice from 1 min to 10 h after injection. Five microliters of plasma was diluted 5-fold in PBS and analyzed for plasma cholesterol and triglyceride levels as described below. Plasma samples were also isolated 30 min before injection of the pure apoE4 and were used as a control.

Determination of plasma lipids and human apoE

Triglycerides and cholesterol were measured using the GPO-Trinder Kit (Sigma) and the CHOL-MPR3 kit (Boehringer-Mannheim), according to the manufacturers' instructions. The triglyceride and cholesterol concentrations of the serum were determined spectrophotometrically at 540 and 492 nm, respectively, as described previously (5–8). Human apoE concentrations were measured using sandwich ELISA (5–8).

Density gradient ultracentrifugation

To assess the ability of apoE forms to associate with different lipoproteins, 0.3 ml of serum from apoE$^{-/-}$ or apoE$^{-/-}$ × LDLr$^{-/-}$ mice infected with adenovirus expressing the wild-type apoE4 was brought to a volume of 0.5 ml with PBS and adjusted to a density 1.23 g/ml with KBr. This solution was then overlaid with 1 ml of 1.21 g/ml KBr, 2.5 ml of 1.063 g/ml KBr, 0.5 ml of 1.019 g/ml KBr, and 0.5 ml of normal saline. The mixtures were centrifuged for 22 h in a SW-41 rotor at 30,000 rpm. After ultracentrifugation, 10 fractions of 0.5 ml were collected and analyzed by SDS-PAGE.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego CA; www.graphpad.com).

RESULTS

Low steady-state plasma apoE levels clear plasma cholesterol in apoE$^{-/-}$ mice, and high apoE levels result in hypertriglyceridemia

It has been established that high levels of plasma apoE are associated with high triglyceride levels in humans and in experimental animal models (4–9). To assess the steady-state apoE concentrations that can induce hypertriglyceridemia, apoE$^{-/-}$ mice were infected with either a low (5×10^8 pfu) or a high (2×10^9 pfu) dose of a recombinant adenovirus expressing the wild-type apoE4. Plasma samples collected from day 1 to day 8 after infection were analyzed for cholesterol, triglyceride, and apoE levels.

In mice infected with 5×10^8 pfu of the apoE4-expressing adenovirus, cholesterol levels were normalized on days 1–8 after infection without induction of hypertriglyceridemia, whereas the steady-state plasma levels of human apoE4 were in the range of 3–5 mg/dl (**Fig. 1A–C**). These findings indicate that low levels of apoE production by the liver suffice for the clearance of lipoprotein remnants.

Consistent with previous findings, infections with high doses (2×10^9 pfu) of apoE aggravated the hypercholesterolemia and induced severe hypertriglyceridemia. Plasma triglyceride levels showed a linear correlation with plasma apoE levels ($r^2 = 0.916$) (Fig. 1D).

Gene transfer of low or high doses of apoE4 does not clear cholesterol and induces hypertriglyceridemia in apoE$^{-/-} \times$ LDLr$^{-/-}$ mice

The plasma lipid and apoE profiles of apoE$^{-/-} \times$ LDLr$^{-/-}$ mice infected with low doses (5×10^8 pfu) of the apoE-expressing adenovirus were drastically different from the lipid profiles of the apoE$^{-/-}$ mice infected with the same adenovirus dose. Plasma cholesterol levels remained high 1–9 days after infection (Fig. 1F). In addition, hypertriglyceridemia and increased steady-state plasma apoE levels were observed 2–7 days after infection (Fig. 1G, H, lower curves).

The increase in the steady-state plasma apoE levels (Fig. 1H) reflects defective clearance of apoE-containing triglyceride-rich lipoproteins (Fig. 1F, G). This is in contrast to the apoE$^{-/-}$ mice infected with low doses (5×10^8 pfu) of the apoE4-expressing adenovirus, which showed very low steady-state apoE levels (Fig. 1C, lower curve) and efficient clearance of lipoprotein remnants (Fig. 1A, B, lower curves).

Infection of apoE$^{-/-} \times$ LDLr$^{-/-}$ mice with a high dose (2×10^9 pfu) of the apoE4-expressing adenovirus aggravated the hypercholesterolemia, induced hypertriglyceridemia, and greatly increased plasma apoE levels (Fig. 1F–H, upper curves).

Density gradient ultracentrifugation showed that the apoE that accumulates in the plasma of apoE$^{-/-}$ and apoE$^{-/-} \times$ LDLr$^{-/-}$ mice after infection with high doses of adenovirus has similar distribution to different lipoprotein classes. Approximately two-thirds of apoE is found in HDL and one-third in the VLDL/IDL/LDL region (Fig. 1E, J).

In apoE$^{-/-} \times$ LDLr$^{-/-}$ mice infected with wild-type apoE4, plasma triglyceride levels showed a linear correlation with plasma apoE levels ($r^2 = 0.861$) (Fig. 1I). The slope of the graph in Fig. 1I is steeper than that in Fig. 1D, indicating that the LDL receptor deficiency increased the sensitivity to apoE-induced hypertriglyceridemia.

Infection of apoE$^{-/-}$ or apoE$^{-/-} \times$ LDLr$^{-/-}$ mice with a control adenovirus expressing only the green fluorescent protein did not have any significant effect on plasma lipid levels of the mice (Fig. 1A, F).

Gene transfer of low doses of an apoE mutant defective in receptor binding do not clear cholesterol and induce hypertriglyceridemia in apoE$^{-/-}$ and apoE$^{-/-} \times$ LDLr$^{-/-}$ mice

We tested the hypothesis that apoE mutations shown previously to cause defective binding to the LDL receptor (28–30) may mimic the phenotype caused by the LDL receptor deficiency after adenovirus-mediated apoE gene transfer. For this purpose, we generated a double mutation in apoE4 by changing the residues arginine 142 and arginine 145 into valines. Previous studies have shown that mutations in either of these amino acids have diminished receptor binding and are associated with dominant forms of type III hyperlipoproteinemia (28–35).

This analysis showed that infection of apoE$^{-/-}$ mice with a low dose (4×10^8 pfu) of adenovirus expressing the apoE4[R142V/R145V] mutant did not correct the high cholesterol levels of the apoE$^{-/-}$ mice and induced high triglyceride levels 1–7 days after infection (**Fig. 2A, B**). Induction of hypertriglyceridemia was associated with an increase in plasma apoE levels (Fig. 2C). Plasma triglyceride levels showed a linear correlation with plasma apoE levels ($r^2 = 0.758$) (Fig. 2D). The slope of the graph in Fig. 2D is steeper than that in Fig. 1D, I, indicating that the receptor-blocking apoE mutation increased the sensitivity to hypertriglyceridemia. Similar experiments showed that a dose of 4×10^8 pfu of adenovirus expressing the apoE4[R142V/R145V] mutant exacerbated the hypercholesterolemia, induced severe hypertriglyceridemia, and increased plasma apoE levels in apoE$^{-/-} \times$ LDLr$^{-/-}$ mice 1–7 days after infection (Fig. 2E–G). Plasma triglyceride levels showed a linear correlation with plasma apoE levels ($r^2 = 0.950$) (Fig. 2H). The slope of the graph in Fig. 2H is steeper than that in Figs. 1D, I and 2D, indicating that the combined mutation in apoE and the LDL receptor further increased the sensitivity to hypertriglyceridemia.

The lipid profiles of apoE$^{-/-}$ or apoE$^{-/-} \times$ LDLr$^{-/-}$ mice infected with adenovirus expressing the apoE4[R142V/R145V] mutant (Fig. 2A–H) resemble those obtained in apoE$^{-/-} \times$ LDLr$^{-/-}$ mice after injection with adenoviruses expressing wild-type apoE4 (Fig. 1F–I). In both situations, the deficiency in LDL receptor or defects in the receptor binding domain of apoE appears to prevent the clearance of apoE-containing lipoproteins and results in combined dyslipidemia characterized by high cholesterol and triglyceride levels. In addition, the deficiency in the LDL receptor or the mutations in the LDL receptor bind-

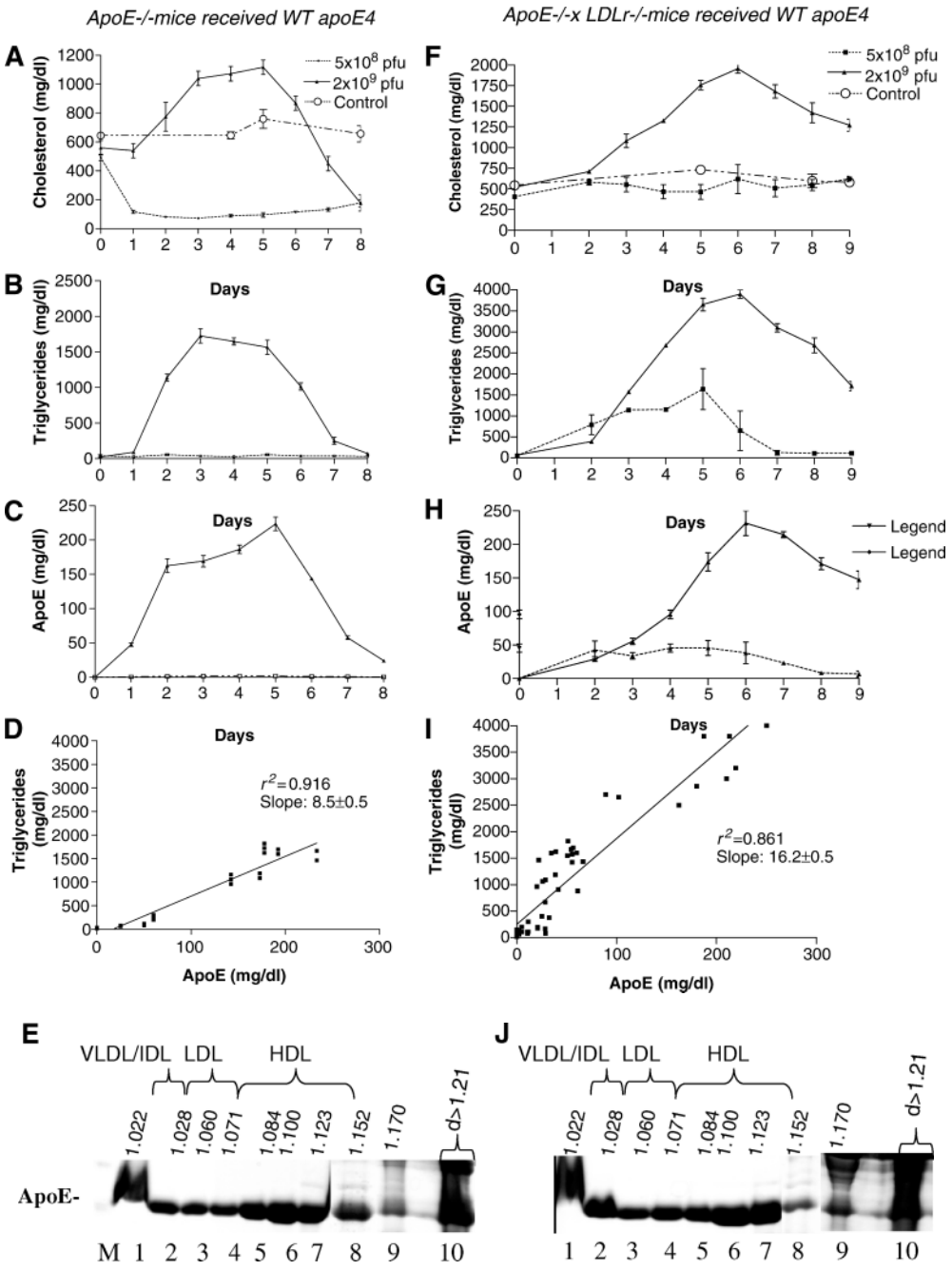

Fig. 1. Plasma cholesterol, triglyceride, and apolipoprotein E (apoE) levels of apolipoprotein E-deficient ($apoE^{-/-}$) and $apoE^{-/-} \times$ low density lipoprotein receptor-deficient ($LDLr^{-/-}$) mice infected with a recombinant adenovirus expressing wild-type (WT) apoE4. Cholesterol (A, F), triglyceride (B, G), and apoE (C, H) levels at different days after infection of the $apoE^{-/-}$ (A–C) or $apoE^{-/-} \times LDLr^{-/-}$ (F–H) mice with the indicated doses of the wild-type apoE4-expressing adenovirus. D: Correlation of plasma apoE to plasma triglyceride levels in $apoE^{-/-}$ mice. H: Correlation of plasma apoE to plasma triglyceride levels in $apoE^{-/-} \times LDLr^{-/-}$ mice. E, J: Distribution of apoE in different lipoprotein fractions after density gradient ultracentrifugation. Plasma obtained from $apoE^{-/-}$ mice (E) or $apoE^{-/-} \times LDLr^{-/-}$ mice (J) after infection with 2×10^9 plaque-forming units of an apoE4-expressing adenovirus was fractionated by density gradient ultracentrifugation and analyzed by SDS-PAGE, as described in Methods. The fraction numbers, the density of each fraction, and the positions of apoE are shown in each panel. Three mice were analyzed for each set of experiments that involved treatment with low or high doses of adenoviruses, and six mice were analyzed for experiments that involved the control adenoviruses expressing green fluorescent protein. The difference in cholesterol triglycerides and apoE of mice infected with low and high doses of the apoE4-expressing adenoviruses between day 0 and days 2–7 is obvious (A–C) and statistically significant ($P < 0.001$). Statistically significant differences in cholesterol triglycerides and apoE are observed between mice infected with high and low doses of an apoE4-expressing adenovirus 4–9 days after infection ($P < 0.0001$) (A–C). Compared with day 0, the average change in cholesterol in F (lower graph) on days 2–9 is statistically significant ($P < 0.001$). Similarly, compared with day 0, the average change in plasma triglycerides in G (lower graph) on days 2–6 is statistically significant ($P < 0.001$). IDL, intermediate density lipoprotein; pfu, plaque-forming units. Error bars indicate standard deviation from the mean.

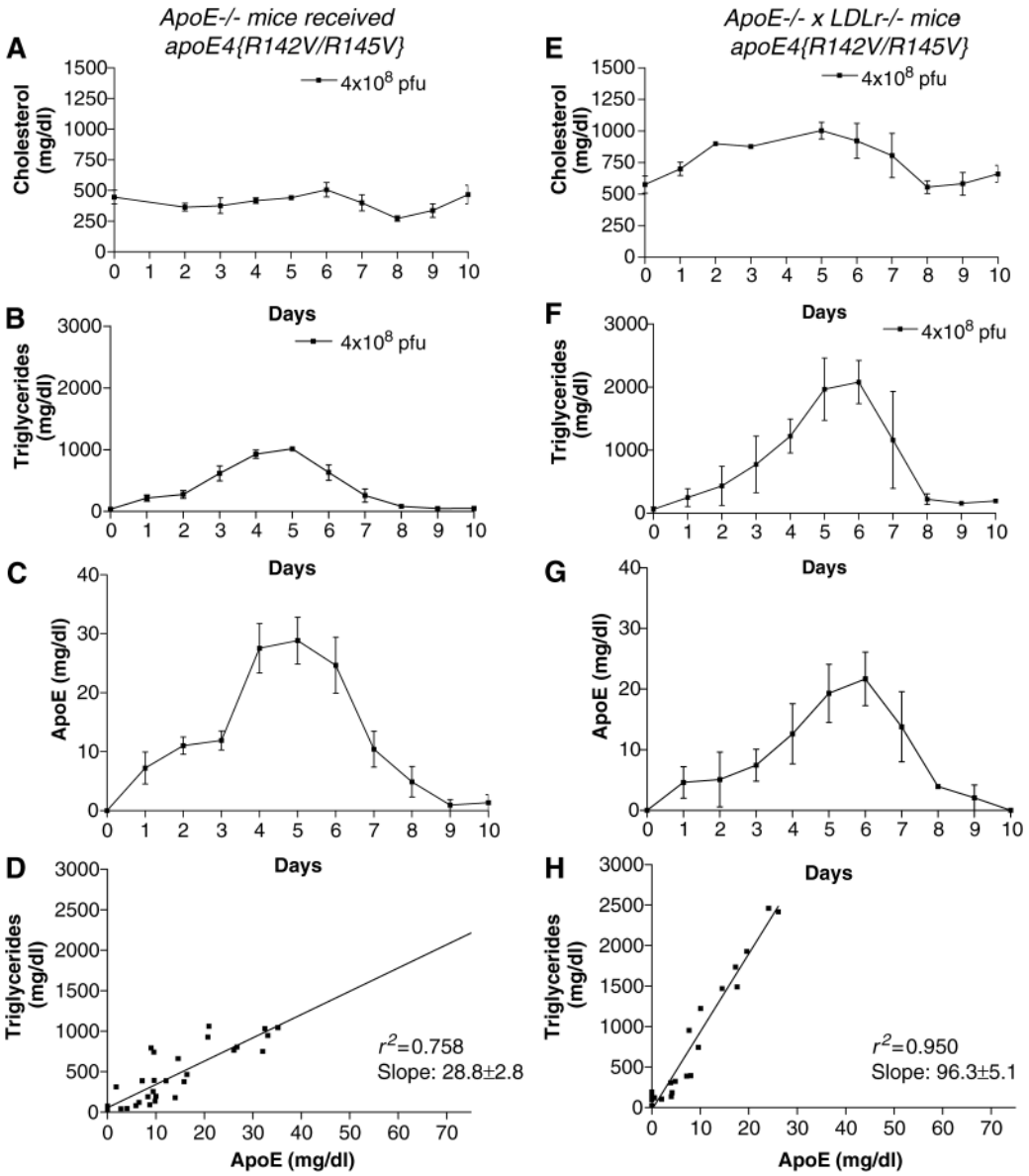

Fig. 2. Plasma cholesterol, triglyceride, and apoE levels of apoE$^{-/-}$ and apoE$^{-/-}$ × LDLr$^{-/-}$ mice infected with a recombinant adenovirus expressing the apoE4[R142V/R145V] mutant. Cholesterol (A, E), triglyceride (B, F), and apoE (C, G) levels at different days after infection of the apoE$^{-/-}$ (A–C) or apoE$^{-/-}$ × LDLr$^{-/-}$ (E–G) mice with the indicated doses of the apoE4[R142V/R145V]-expressing adenovirus. D: Correlation of plasma apoE to plasma triglyceride levels in apoE$^{-/-}$ mice. H: Correlation of plasma apoE to plasma triglyceride levels in apoE$^{-/-}$ × LDLr$^{-/-}$ mice. Three mice were analyzed for each set of experiments that involved treatment with low doses of adenoviruses. Compared with day 0, the average change in cholesterol in A on days 2–10 after infection is not statistically significant ($P > 0.6$). In contrast, compared with day 0, the average change in plasma triglycerides in B on days 2–7 after infection is statistically significant ($P < 0.0001$). Compared with day 0, the average change in cholesterol in E on days 2–7 after infection is statistically significant ($P > 0.002$). Similarly, compared with day 0, the average change in plasma triglycerides in F on days 2–7 after infection is statistically significant ($P < 0.01$). Error bars indicate standard deviation from the mean.

ing domain of apoE, or both, increases the sensitivity to apoE-induced hypertriglyceridemia (compare Fig. 1D with Figs. 1I and 2D, H).

Bolus injection of apoE differently affects plasma lipid levels in apoE$^{-/-}$ and apoE$^{-/-}$ × LDLr$^{-/-}$ mice

The ability of apoE to clear lipoprotein remnants was assessed by a bolus injection of a solution of 1.6 mg of purified apoE4 in PBS serum (at a ratio of 2:1) and collection of blood at different time points, as described in Methods.

Bolus injection of apoE corrected plasma cholesterol levels of the apoE$^{-/-}$ mice over a period of 10 h (**Fig. 3A**) but did not affect plasma cholesterol levels of the apoE$^{-/-}$ × LDLr$^{-/-}$ double-deficient mice (Fig. 3D). Bolus injection of apoE also caused a transient modest

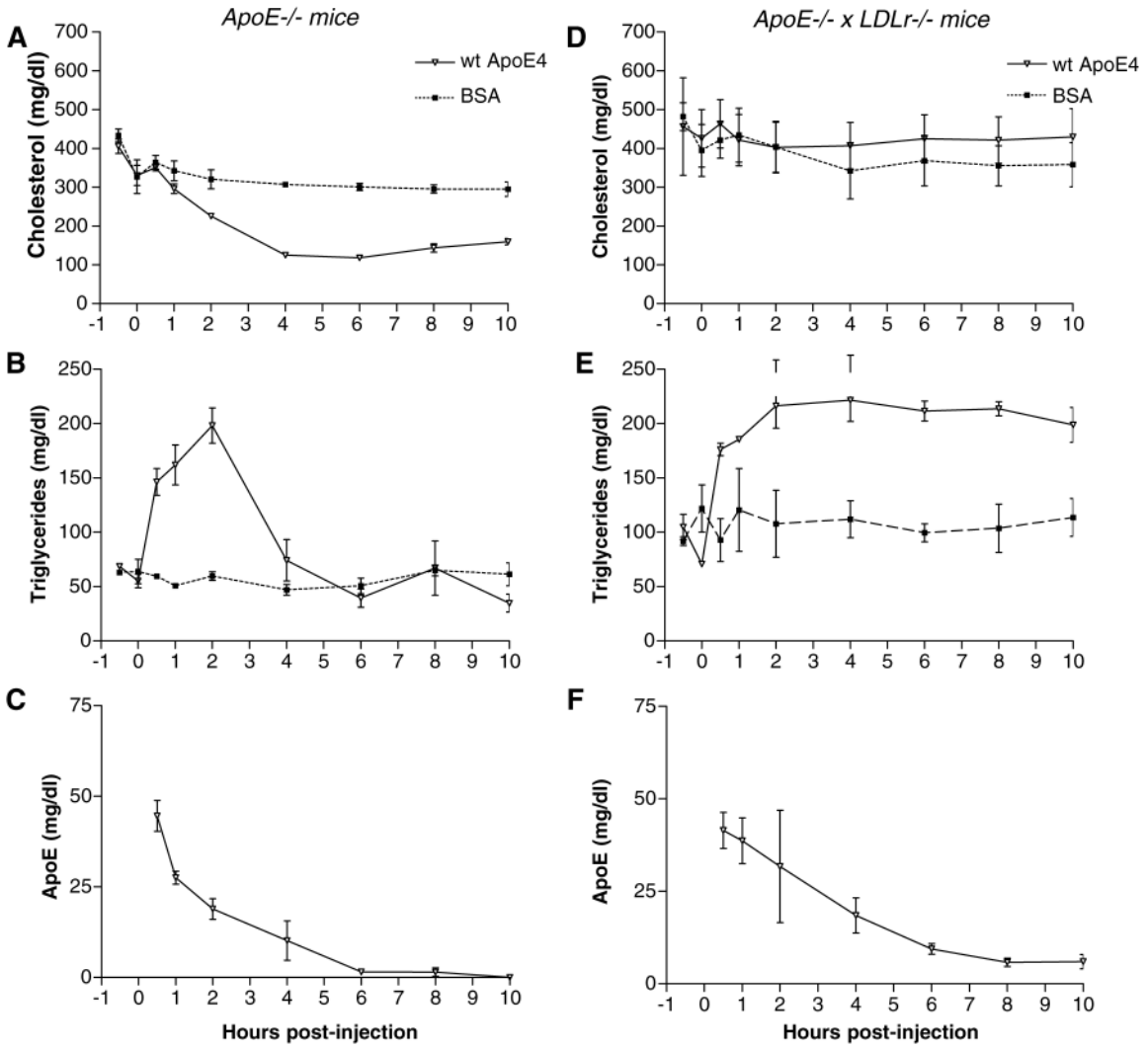

Fig. 3. Changes in plasma, cholesterol, triglyceride, and apoE levels of apoE$^{-/-}$ mice (A–C) and apoE$^{-/-}$ × LDLr$^{-/-}$ mice (D–F) injected with a solution containing pure apoE4 or BSA. A, D: Cholesterol levels. B, E: Triglyceride levels. C, F: ApoE levels. Note that injection of apoE corrected only the high cholesterol levels of the apoE$^{-/-}$ mice. Three mice were analyzed for each set of experiments. Compared with control apoE$^{-/-}$ mice injected with BSA, the average decrease in the cholesterol levels in apoE$^{-/-}$ mice injected with apoE proteoliposomes in A at 1–10 h after injection is statistically significant ($P < 0.006$). Similarly, compared with control apoE$^{-/-}$ mice injected with BSA, the average increase in plasma triglycerides in apoE$^{-/-}$ mice injected with apoE proteoliposomes in B at 1–4 h after injection is statistically significant ($P < 0.02$). For later time points, there is no statistically significant change in plasma triglycerides compared with control apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with BSA. Compared with control apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with BSA, the average increase in cholesterol levels in apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with apoE proteoliposomes in D on days 1–10 after injection is statistically significant ($P < 0.03$). Similarly, compared with control apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with BSA, the average increase in plasma triglycerides in apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with apoE proteoliposomes in E at 1–10 h after injection is statistically significant ($P < 0.005$). There are statistically significant differences in apoE levels between apoE$^{-/-}$ and apoE$^{-/-}$ × LDLr$^{-/-}$ mice at 6, 8, and 10 days after injection (C, F). The significance values are $P < 0.02$ for day 6, $P < 0.035$ for day 8, and $P < 0.0002$ for day 10. wt, wild type. Error bars indicate standard deviation from the mean.

increase in plasma triglycerides in apoE$^{-/-}$ mice 2–4 h after infection and a similar but sustained increase in plasma triglycerides over a 10 h period in apoE$^{-/-}$ × LDLr$^{-/-}$ mice (Fig. 3B, E).

Injected apoE was totally undetectable in the plasma of the apoE$^{-/-}$ mice within 10 h after injection (Fig. 3C); however, apoE levels of 5–10 mg/dl were detected in the plasma of the apoE$^{-/-}$ × LDLr$^{-/-}$ mice up to 6–10 h after injection (Fig. 3F). In both apoE$^{-/-}$ and apoE$^{-/-}$ × LDLr$^{-/-}$ mice, bolus injection of BSA as a control caused a small decrease in plasma cholesterol levels but did not have any significant effects on the plasma triglyceride levels of the mice (Fig. 3A, B, D, E).

DISCUSSION

Receptors involved in lipoprotein clearance in vivo

ApoE at physiological concentrations is required for the catabolism of lipoprotein remnants (2, 3, 36) via the LDL receptor. Other types of lipoprotein receptors (21, 23) and

HSPG (24) have also been suggested to participate in this process. In addition to its role in cholesterol homeostasis, apoE affects plasma triglyceride homeostasis. Thus, in humans and experimental animals, increased plasma apoE levels are associated with increased plasma triglyceride levels (4–9). In mice, the effects of apoE on the induction of hypertriglyceridemia were independent of apoE phenotype or mouse strain (5–8, 11).

In this study, we used adenovirus-mediated apoE gene transfer or a bolus injection of purified apoE to assess the contribution of apoE-recognizing receptors as well as receptor-independent mechanisms in the in vivo clearance of lipoprotein remnants. Gene transfer experiments were performed in apoE$^{-/-}$ mice that express the LDL receptor and in apoE$^{-/-}$ × LDLr$^{-/-}$ double-deficient mice, which lack the LDL receptor. The expectation was that if receptors different from the LDL receptor [such as LDLr related protein (LRP)] were significantly involved in the clearance of apoE-containing lipoproteins, then gene transfer or bolus injection of apoE would correct, at least partially, the high cholesterol profiles of the apoE$^{-/-}$ × LDLr$^{-/-}$ double-deficient mice.

Our experiments established that apoE produced by the liver after infection with a low dose of an apoE-expressing adenovirus or bolus injection of apoE into the plasma can transiently clear cholesterol in apoE$^{-/-}$ mice that express the LDL receptor. However, similar treatments of double-deficient apoE$^{-/-}$ × LDLr$^{-/-}$ mice cannot clear cholesterol from the plasma of these mice.

When apoE$^{-/-}$ mice that express the LDL receptor were infected with low doses of an apoE4-expressing adenovirus (5×10^8 pfu), their cholesterol levels were normalized and their steady-state plasma apoE levels were very low (3–5 mg/dl), indicating fast catabolism of apoE-containing lipoprotein remnants.

Under similar conditions, the high cholesterol levels of the apoE$^{-/-}$ × LDLr$^{-/-}$ double-deficient mice that lack the LDL receptor were not corrected, and the steady-state plasma apoE concentration increased in the range of 35–40 mg/dl, indicating a defective catabolism of the apoE-containing lipoprotein remnants despite the fact that these mice still express the LRP in the liver. These findings indicate that under the experimental conditions used, the LDL receptor alone can account for the clearance of apoE-containing lipoprotein remnants in mice.

If our interpretation is correct, one would expect that infection of apoE$^{-/-}$ mice, which express the LDL receptor, with an adenovirus expressing a mutant apoE form that does not recognize the LDL receptor would create a similar phenotype to the one observed in apoE$^{-/-}$ × LDLr$^{-/-}$ double-deficient mice after infection with a low dose of the adenovirus expressing the wild-type apoE4.

To test this hypothesis, we used a double mutant of apoE in which residues arginine 142 and arginine 145, which are involved in receptor binding, were changed to valines (28–35). Consistent with our hypothesis, we found that low doses of the adenovirus expressing this dominant apoE4[R142V/R145V] mutant did not correct the high cholesterol profiles of the apoE$^{-/-}$ mice, increased plasma apoE levels, and induced hypertriglyceridemia. Furthermore, hypercholesterolemia was exacerbated and hypertriglyceridemia became more severe when the apoE$^{-/-}$ × LDLr$^{-/-}$ mice were infected with adenoviruses expressing the dominant apoE[R142V/R145V] mutant. This treatment also greatly increased plasma apoE levels. These findings establish that the inability of apoE to bind to the LDL receptor, either because of defects in the LDL receptor or defects in apoE, or both, may trigger combined dyslipidemia (**Fig. 4**).

Previous studies using Cre-Lox recombination showed that liver-specific inactivation of LRP resulted in the accumulation of lipoprotein remnants enriched in apoE and apoB-48, suggesting indirectly that LRP may be involved in remnant clearance (20). If LRP (20, 22, 23) or HSPG (24) were involved in the clearance of apoE-containing lipoproteins, one would expect the treatments used in this study would reduce the plasma cholesterol levels of the double-deficient apoE$^{-/-}$ × LDLr$^{-/-}$ mice. However, this study did not show any significant contribution of LRP to lipoprotein catabolism in mice. Consistent with our findings are recent data showing that triple-deficient LDLr$^{-/-}$ × LRP$^{-/-}$ × apoE$^{-/-}$ mice have decreased, rather than increased, cholesterol and triglyceride levels compared with double-deficient LDLr$^{-/-}$ × apoE$^{-/-}$ mice (37).

Defective interactions of apoE-containing lipoproteins with the LDL receptor increase the sensitivity to apoE-induced hypertriglyceridemia

The inability of apoE$^{-/-}$ × LDLr$^{-/-}$ mice to clear lipoprotein remnants also results in an increase in steady-state

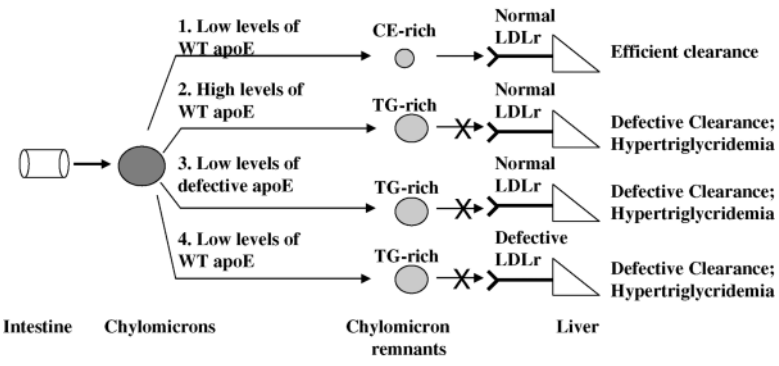

Fig. 4. Schematic representation of the role of apoE and LDL receptor (LDLr) in remnant clearance and the influence of apoE overexpression, receptor binding mutations in apoE, and deficiency in the LDL receptor on the induction of hypertriglyceridemia in mice. CE, cholesteryl ester; TG, triglyceride; WT, wild type.

plasma apoE levels after infection with the apoE-expressing adenovirus. Similar increases in apoE associated with hypertriglyceridemia were observed when apoE$^{-/-}$ or apoE$^{-/-}$ × LDLr$^{-/-}$ mice were treated with a dominant apoE[R142V/R145V] mutant that cannot bind to the LDL receptor. Our studies also establish that LDL receptor deficiency or mutations in apoE that impede its interaction with the LDL receptor increase the sensitivity to hypertriglyceridemia and that there is a linear correlation between plasma apoE and plasma triglyceride levels. The slope of the graphs [apoE] versus triglycerides] was steeper when the mice used for the gene transfer experiments lacked the LDL receptor (apoE$^{-/-}$ × LDLr$^{-/-}$ mice) or when apoE4[R142V/R145V], the mutant that cannot bind to the LDL receptor, was used in gene transfer experiments, or both. The defect in the clearance of apoE-containing lipoproteins is also reflected in the significant levels of apoE that remain in the plasma of apoE$^{-/-}$ × LDLr$^{-/-}$ mice compared with apoE$^{-/-}$ mice 6–10 h after bolus injection of pure apoE. It is well established that lipid-free apoE is not a ligand for lipoprotein receptors (38); thus, receptor-mediated clearance will require the incorporation of lipid-free apoE into preexisting lipoproteins (27). The kinetics of disappearance of apoE from the plasma of the apoE$^{-/-}$ and apoE$^{-/-}$ × LDLr$^{-/-}$ mice suggests that only a fraction of the injected apoE is incorporated into plasma lipoproteins, and in the case of the apoE$^{-/-}$ mice, it is subsequently cleared via the LDL receptor. The observed clearance of apoE in apoE$^{-/-}$ mice is associated with a decrease in plasma cholesterol and a transient increase in plasma triglyceride levels. On the other hand, in apoE$^{-/-}$ × LDLr$^{-/-}$ mice that lack the LDL receptor, there is no decrease of plasma cholesterol and induction of mild hypertriglyceridemia as well as delayed clearance of apoE.

This situation may exist in human patients who have mutations in the 136–147 region of apoE (28). These mutations are associated with dominant forms of type III hyperlipoproteinemia, which is characterized by high plasma cholesterol and triglyceride levels (28). The increased sensitivity of these patients to hypertriglyceridemia, in contrast to the apoE$^{-/-}$ patients (36) and the apoE$^{-/-}$ mouse model (2, 3), may be the result of defective interactions of these apoE mutants with the LDL receptor or abnormal interactions of apoE with other proteins of the lipoprotein transport system.

Overall, this study establishes that the LDL receptor appears to be the only physiological receptor involved in the clearance of apoE-containing lipoproteins in mice and that defects in either the LDL receptor or in apoE that affect their functional interaction increase the sensitivity to, and the severity of, apoE-induced hypertriglyceridemia in mice.

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ERRATA

In the article "LDL receptor deficiency or apoE mutations prevent remnant clearance and induce hypertriglyceridemia in mice," published in the March 2006 issue of the Journal of Lipid Research (Volume 47, pages 521–529), the legend to Figure 3 contained errors. The Figure 3 legend for this article should read:

Fig. 3. Changes in plasma, cholesterol, triglyceride, and apoE levels of apoE$^{-/-}$ mice (A–C) and apoE$^{-/-}$ × LDLr$^{-/-}$ mice (D–F) injected with a solution containing pure apoE4 or BSA. A, D: Cholesterol levels. B, E: Triglyceride levels. C, F: ApoE levels. Note that injection of apoE corrected only the high cholesterol levels of the apoE$^{-/-}$ mice. Three mice were analyzed for each set of experiments. Compared with control apoE$^{-/-}$ mice injected with BSA, the average decrease in the cholesterol levels in apoE$^{-/-}$ mice injected with apoE in A at 1–10 h after injection is statistically significant ($P < 0.006$). Similarly, compared with control apoE$^{-/-}$ mice injected with BSA, the average increase in plasma triglycerides in apoE$^{-/-}$ mice injected with apoE in B at 1–4 h after injection is statistically significant ($P < 0.02$). For later time points, there is no statistically significant change in plasma triglycerides compared with control apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with BSA. Compared with control apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with BSA, the average increase in cholesterol levels in apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with apoE in D on days 1–10 after injection is statistically significant ($P < 0.03$). Similarly, compared with control apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with BSA, the average increase in plasma triglycerides in apoE$^{-/-}$ × LDLr$^{-/-}$ mice injected with apoE in E at 1–10 h after injection is statistically significant ($P < 0.005$). There are statistically significant differences in apoE levels between apoE$^{-/-}$ and apoE$^{-/-}$ × LDLr$^{-/-}$ mice at 6, 8, and 10 days after injection (C, F). The significance values are $P < 0.02$ for day 6, $P < 0.035$ for day 8, and $P < 0.0002$ for day 10. wt, wild type. Error bars indicate standard deviation from the mean.