Sensory Regeneration Conference (sponsored by the University of Virginia Office of Continuing Medical Education and by the National Institute in Deafness and other Communication Disorders), May 1994, Charlottesville, VA.
29. R. Goodyear *et al.*, *Hear. Res.* **80**, 93 (1994).
30. M. Takumida *et al.*, *Acta Otolaryngol.* **112**, 643 (1992).
31. J. Hasko and G. Richardson, *Hear. Res.* **35**, 21 (1988).
32. We thank J. Saunders, J. Eberwine, G. Massey, J. Kamholz, and D. Navaratnam for assistance and G. Richardson for helpful discussions. We also thank the M. Selzer lab for the space used to maintain the fish. Supported by grants from the Lucille P. Markey Charitable Trust to M.I.G. and from the National Institute on Deafness and other Communication Disorders (K08-DC00069) and the Pennsylvania Lions Hearing Research Foundation (GA-1307) to J.C.O. J.G.D. was supported by grants from the Lucille P. Markey Charitable Fund to M.I.G. and by training grant 5 T32 NS07064-13.

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Prevention of Atherosclerosis in Apolipoprotein E–Deficient Mice by Bone Marrow Transplantation

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Apolipoprotein E (apoE) deficiency causes severe hyperlipidemia and atherosclerosis in humans and in gene-targeted mice. Although the majority of apoE in plasma is of hepatic origin, apoE is synthesized by a variety of cell types, including macrophages. Because macrophages derive from hematopoietic cells, bone marrow transplantation was used to examine the potential of apoE synthesized by bone marrow–derived cells to correct the hyperlipidemia and atherosclerosis caused by apoE deficiency. After transplantation of bone marrow from mice with the normal apoE gene into apoE-deficient mice, apoE was detected in serum and promoted clearance of lipoproteins and normalization of serum cholesterol levels. ApoE-deficient mice given transplants of normal bone marrow showed virtually complete protection from diet-induced atherosclerosis.

ApoE is a 34-kD glycoprotein that serves as the ligand for receptor-mediated clearance of several classes of lipoproteins, including chylomicrons, very low density lipoproteins (VLDL), and lipoprotein remnants (*1*). ApoE is thought to mediate lipoprotein clearance through its interaction with two different receptors: the LDL receptor and a putative remnant receptor (*1*), likely the LDL receptor–related protein (LRP) (*2*). Complete deficiency of apoE is a rare cause of the human genetic disorder type III hyperlipoproteinemia (HLP), a disease characterized by high serum cholesterol and triglyceride levels, accumulation of β-migrating remnant particles, and development of premature atherosclerosis (*3*). An animal model of apoE deficiency has been created by targeted inactivation of the gene locus encoding apoE in the mouse (*4*, *5*). ApoE-deficient ($apoE^{-/-}$) mice have severe hypercholesterolemia with accumulation of chylomicrons, VLDL, and remnant particles, a phenotype closely resembling that of human type III HLP. In addition, $apoE^{-/-}$ mice develop extensive aortic and coronary atherosclerosis with complex lesions that are similar in many respects to those in humans (*4*–*6*).

Studies of humans after liver transplantation have demonstrated that >90% of the apoE in plasma is synthesized by the liver (*7*), but apoE synthesis has been documented in a wide variety of tissues and cell types (*8*), including macrophages (*9*). Proposed functions for apoE synthesized by macrophages include participation in reverse cholesterol transport (*10*), promotion of local redistribution of cholesterol (*1*), and protection against atherosclerotic lesion development (*11*). Direct evidence that apoE synthesized by macrophages contributes to plasma lipoprotein clearance or influences atherosclerosis in vivo is lacking. However, apoE is secreted by mouse peritoneal macrophages in the form of lipoprotein particles in vitro (*9*), and VLDL enriched with apoE secreted by human macrophages display enhanced affinity for the LDL receptor in vitro (*12*). Because macrophages are derived from hematopoietic stem cells, we transplanted bone marrow from $apoE^{+/+}$ mice into $apoE^{-/-}$ mice to examine the capacity of apoE synthesis by bone marrow–derived cells to contribute to the clearance of plasma lipoproteins and to influence atherosclerosis in vivo.

After lethal irradiation [9 grays (Gy)] of 12 apoE-deficient mice, 6 mice received transplants of bone marrow cells from mice with the normal apoE gene ($apoE^{+/+} \rightarrow apoE^{-/-}$) and 6 control mice received marrow from apoE-deficient mice ($apoE^{-/-} \rightarrow apoE^{-/-}$) (*13*, *14*). Donor mice were derived from the ROSA β-geo 26 mice, an engineered strain that shows ubiquitous expression of the *Escherichia coli* β-galactosidase (β-Gal) from a *LacZ* gene promoter trap (*15*), providing a marker for identification of cells of donor origin by flow cytometry (*16*). Two weeks after transplantation, apoE was detectable by protein immunoblot in the serum of two out of six of the $apoE^{+/+} \rightarrow apoE^{-/-}$ mice, and by 3 weeks all six mice had detectable apoE in the serum (*17*). Ultracentrifugation analysis showed that most of the apoE in the serum of $apoE^{+/+} \rightarrow apoE^{-/-}$ mice was associated with lipoproteins, with less than 5% being recovered in the lipoprotein-free bottom fraction (*17*).

The appearance of apoE in the plasma of $apoE^{+/+} \rightarrow apoE^{-/-}$ mice was associated with dramatic changes in serum cholesterol levels (Table 1). Two weeks after transplantation, mean serum cholesterol levels were unchanged, but after 3 weeks they had decreased by almost 50%. Four weeks after transplantation, serum cholesterol levels had decreased more than 70% from baseline values, reaching values close to those in unaffected littermates (125 ± 18 mg/dl, $n = 13$). There were no significant changes in serum cholesterol in the $apoE^{-/-} \rightarrow apoE^{-/-}$ controls.

The change in the distribution of serum lipoprotein cholesterol in the $apoE^{+/+} \rightarrow apoE^{-/-}$ mice was analyzed by fast protein liquid chromatography (FPLC) analysis of serum at serial time points after bone marrow transplantation (BMT) (Fig. 1). The reduction in serum cholesterol was entirely due to a decrease in the levels of VLDL, intermediate-density lipoproteins (IDL), and LDL. The 2-week lipoprotein cholesterol profile resembled that of an apoE-deficient mouse. A marked decrease in VLDL, IDL, and LDL cholesterol was evident at the end of the third week and was more pronounced 4 weeks after transplantation (Fig. 1A). Seven weeks after transplantation (Fig. 1B), the VLDL-IDL peak in apoE-deficient serum was normalized, a small elevation in LDL remained, and HDL increased to levels similar to those of normal mice.

The efficiency of apoE in reducing serum cholesterol levels was examined by transplantation of bone marrow from mice heterozygous for the targeted disruption of the apoE allele into lethally irradiated $apoE^{-/-}$ mice ($apoE^{-/+} \rightarrow apoE^{-/-}$) and comparison of the results to those obtained in

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apoE$^{+/+}$ → apoE$^{-/-}$ mice (*18*). At 4 weeks after transplantation, serum apoE levels in apoE$^{-/+}$ → apoE$^{-/-}$ mice were 5.5% (SD ± 0.6) of normal, or approximately one-half the level achieved in apoE$^{+/+}$ → apoE$^{-/-}$ mice, which reached 12.5% (SD ± 0.6) of normal mouse apoE levels (Fig. 2). Mean serum cholesterol levels decreased by 51% in the apoE$^{-/+}$ → apoE$^{-/-}$ mice as compared with a 74% decrease in the apoE$^{+/+}$ → apoE$^{-/-}$ mice (Table 2). The mean baseline triglyceride level (mg/dl ± SD) of the recipients in these experiments was 156 ± 92 (n = 25), and changes in triglyceride levels after BMT did not reach statistical significance in any of the three groups. These results demonstrate a clear gene-dosage effect of the apoE expressed by bone marrow–derived cells on serum cholesterol levels after transplantation.

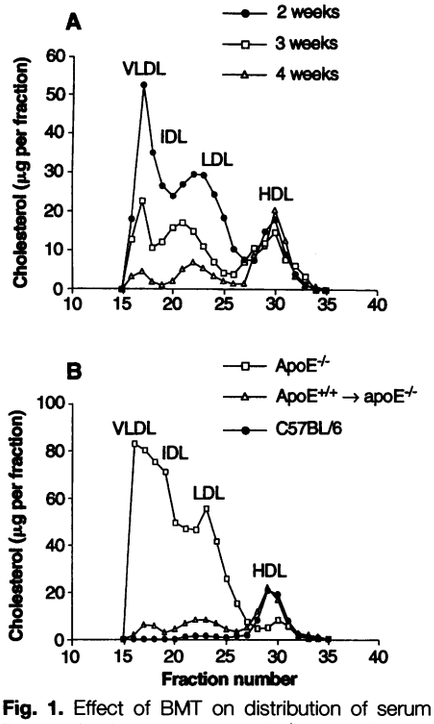

Fig. 1. Effect of BMT on distribution of serum lipoprotein cholesterol in apoE$^{-/-}$ mice. Results of gel filtration analysis of mouse serum by means of a Superose 6 column (Pharmacia) on a Waters 600 FPLC system are shown. A 100-μl aliquot of mouse serum was injected onto the column and separated with a buffer containing 0.15 M NaCl, 0.01 M Na_2HPO_4, and 0.1 mM EDTA (pH 7.5), at a flow rate of 0.5 ml/min. Fifty fractions of 0.5 ml each were collected: fractions 15 to 19, VLDL and chylomicrons; fractions 20 to 26, IDL, LDL, and large HDL; fractions 27 to 33, HDL. Cholesterol determinations were done with Sigma kit 352 in the microplate assay. (**A**) Distribution of cholesterol in apoE$^{+/+}$ → apoE$^{-/-}$ mice at 2, 3, and 4 weeks after transplantation of normal mouse bone marrow (closed circles, open squares, and open triangles, respectively). (**B**) FPLC cholesterol profile in a normal C57BL/6 mouse, an apoE-deficient mouse, and an apoE$^{+/+}$ → apoE$^{-/-}$ mouse 7 weeks after BMT.

The similarity in the timing between the appearance of serum apoE and the decrease in serum cholesterol, together with the absence of any significant lipid or lipoprotein changes in the control group, suggests that after BMT apoE is secreted by bone marrow–derived cells, reaches the plasma compartment, associates with lipoproteins, and ultimately causes a reduction in serum cholesterol levels. Our hypothesis was that apoE achieves this effect by providing the accumulating remnant lipoproteins with a ligand for their receptor-mediated clearance. This hypothesis was tested by study of the turnover of ^{125}I-labeled d<1.019 g/ml (^{125}I-d<1.019) lipoproteins, as tracers of remnant lipoprotein clearance, in four groups of mice: apoE$^{-/-}$, apoE$^{+/+}$ → apoE$^{-/-}$, apoE$^{-/+}$ → apoE$^{-/-}$, and C57BL/6 (Fig. 3). As expected, ^{125}I-d<1.019 lipoprotein turnover was fastest in C57BL/6 mice and slowest in apoE$^{-/-}$ mice. Although the clearance of ^{125}I-d<1.019 lipoproteins was accelerated in both apoE$^{+/+}$ → apoE$^{-/-}$ mice and apoE$^{-/+}$ → apoE$^{-/-}$ mice as compared with apoE$^{-/-}$ mice, it was significantly faster in apoE$^{+/+}$ → apoE$^{-/-}$ mice than in apoE$^{-/+}$ → apoE$^{-/-}$ mice, which indicates a gene-dosage effect of apoE gene expression by donor bone marrow–derived cells on the turnover of d<1.019 lipoproteins (*19*). These results indicate that the reduction in plasma cholesterol levels in apoE-deficient mice after BMT is due to increased lipoprotein clearance.

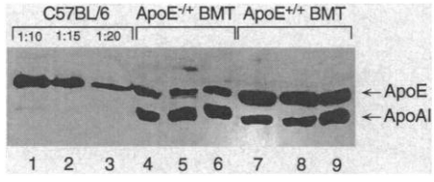

Fig. 2. Protein immunoblot analyses of serum apoE in apoE$^{-/-}$ mice after BMT. Lanes 1 to 3 represent 3 μl of serum dilutions from normal C57BL/6 mice (1:10, 1:15, 1:20, respectively); lanes 4 to 6 represent 3 μl of undiluted serum from apoE$^{-/+}$ → apoE$^{-/-}$ BMT mice; and lanes 7 to 9 represent 3 μl of undiluted serum from apoE$^{+/+}$ → apoE$^{-/-}$ BMT mice.

Flow cytometry was used to evaluate the extent of reconstitution of the recipient's bone marrow and spleen by donor myeloid cells (*20*). Four weeks after transplantation, >95% of the Mac-1$^+$ cells of the bone marrow and >80% of the Mac-1$^+$ cells of the spleen were donor in phenotype (β-Gal$^+$) (*17*). Two apoE$^{+/+}$ → apoE$^{-/-}$ mice and two apoE$^{-/-}$ → apoE$^{-/-}$ mice were analyzed 5 months after BMT. Taken as a group, the mean (±SD) percentage of Mac-1$^+$ cells staining positive for β-Gal was 95.2 ± 0.4 in the bone marrow and 92.9 ± 2.9 in the spleen. These results indicate that the apoE$^{+/+}$ → apoE$^{-/-}$ mice and apoE$^{-/-}$ → apoE$^{-/-}$ mice were similarly reconstituted by myeloid cells of donor origin. The impact of BMT on the composition of various lymphohematopoietic populations in the bone marrow, spleen, and thymus was assessed by flow cytometry. The composition (percent of cells staining positive for the monoclonal antibody) of myeloid cells (Mac-1$^+$) and B cells (CD45R$^+$) in the bone marrow and spleen, and T cells (CD5$^+$) in the thymus, were nearly identical in the mice that had received transplants and those that had not (*17*).

Macrophages of donor origin are known to reconstitute a wide variety of the recipient's tissues, including the spleen, lung, liver, thymus, and brain (perivascular microglial cells) after BMT (*21*). We studied the tissue distribution of apoE gene expression in apoE$^{+/+}$ → apoE$^{-/-}$ mice by ribonuclease (RNase) protection assay (*22*). In these mice, levels of apoE mRNA in the lung, spleen, kidney, and small intestine were greater than or equivalent to the levels of apoE mRNA in C57BL/6 control mice. ApoE mRNA was also detected in the liver and the brain of the apoE-deficient mice with transplants, but the levels were much lower than in C57BL/6 control mice. Lower levels of apoE mRNA in the liver of apoE$^{+/+}$ → apoE$^{-/-}$ mice are expected because hepatocytes are the predominant source of apoE synthesis in normal mice.

Table 1. Total serum cholesterol levels in apoE-deficient mice after transplantation of apoE$^{-/-}$ or apoE$^{+/+}$ bone marrow. Mice were on a diet of 11% fat breeder chow (Mouse Diet 5015, PMI). At the indicated time points, nonfasting mice were anesthetized with methoxyflurane and blood was collected by puncture of the retro-orbital venous plexus with the tip of a sterile Pasteur pipette. Serum cholesterol determinations were done with the Sigma kit 352, adapted for a microplate assay. Absorbance at 490 nm was read on a Molecular Devices microplate reader. Values are in milligrams per deciliter (mean ± SD). The number of mice in each group is indicated by n.

Donor mouse	n	Baseline	2 Weeks	3 Weeks	4 Weeks
ApoE$^{-/-}$	6	458 ± 99	577 ± 126	455 ± 79	472 ± 118
ApoE$^{+/+}$	6	545 ± 92	537 ± 102	290 ± 174	153 ± 43*

*Statistically different (P < 0.0001) compared with the baseline and 2-week values of apoE$^{+/+}$ → apoE$^{-/-}$ mice and to all values of apoE$^{-/-}$ → apoE$^{-/-}$ mice.

The low levels of expression of apoE mRNA in the brain of $apoE^{+/+} \rightarrow apoE^{-/-}$ mice suggest either that reconstitution of the brain with macrophages of donor origin is less extensive or takes longer than that of the other tissues examined, or that cells other than macrophages are responsible for the majority of apoE synthesis in the brain. Examination of hematoxylin-stained sections of the liver, brain, lung, and spleen from $apoE^{+/+} \rightarrow apoE^{-/-}$ mice showed no evidence of a disproportionate number of monocytes or macrophages as a sign of inflammatory reaction (*23*). These results indicate that after the transplantation of $apoE^{+/+}$ marrow into $apoE^{-/-}$ mice, apoE gene expression is reconstituted by bone marrow–derived cells in a number of tissues, and they suggest that macrophages (and possibly other bone marrow–derived cells) contribute most of the apoE gene expression in extrahepatic tissues such as the spleen, lung, kidney, and small intestine. Alternatively, apoE production by $apoE^{+/+}$ macrophages in extrahepatic tissues might be up-regulated in the setting of apoE deficiency.

Two months after BMT, five $apoE^{+/+} \rightarrow apoE^{-/-}$ mice and five $apoE^{-/-} \rightarrow apoE^{-/-}$ control mice were challenged with a high-fat Western-type diet (21% fat and 0.15% cholesterol; Teklad, Madison, Wisconsin). After 3 months on this diet, the mean serum cholesterol rose to 1303 ± 462 mg/dl in the $apoE^{-/-} \rightarrow apoE^{-/-}$ mice and to 318 ± 76 mg/dl in the $apoE^{+/+} \rightarrow apoE^{-/-}$ mice. Gel filtration chromatography of serum showed that the increase in cholesterol in $apoE^{-/-} \rightarrow apoE^{-/-}$ mice was due to an increase in the VLDL-IDL region, whereas the increase in serum cholesterol levels in $apoE^{+/+} \rightarrow apoE^{-/-}$ mice on the diet was due to elevations in LDL and HDL and did not involve the remnant lipoproteins (*17*).

After 3 months on the Western-type diet, the mice were killed for quantitative analysis of aortic atherosclerosis (*24*). The mice were 32 to 35 weeks old and 5 months post-BMT at the time of death. Atherosclerotic lesions in the $apoE^{-/-} \rightarrow apoE^{-/-}$ controls were present in the proximal aorta, beginning at the base of the aortic sinus and extending distally in the aorta (Fig. 4). The lesions were raised and contained a fibrous cap overlying a lipid-rich core, with foam cells, areas of necrosis, and extracellular lipid deposits. The lesion areas in the aortas of the $apoE^{+/+} \rightarrow apoE^{-/-}$ mice were dramatically reduced; the lesions were at a very early stage (low cellularity and absence of fibrous cap) and were localized exclusively to the base of the aortic valves (a region of high variability). Analysis of the atherosclerosis by quantitative morphometry revealed that the mean lesion area per mouse was 52-fold greater in the $apoE^{-/-} \rightarrow apoE^{-/-}$ controls as compared with the $apoE^{+/+} \rightarrow apoE^{-/-}$ mice. The average lesion areas per section (in micrometers squared; mean ± SEM) were 260,642 ± 53,764 in the $apoE^{-/-} \rightarrow apoE^{-/-}$ group ($n = 4$) and 4939 ± 1812 in the $apoE^{+/+} \rightarrow apoE^{-/-}$ group ($n = 5$) ($P < 0.0001$; Student's t test).

The present study demonstrates that transplantation of normal bone marrow into apoE-deficient mice results in correction of the hypercholesterolemia and prevention of aortic and coronary atherosclerosis. We prove that apoE synthesized by bone marrow–derived cells associates with serum lipoproteins and accelerates their

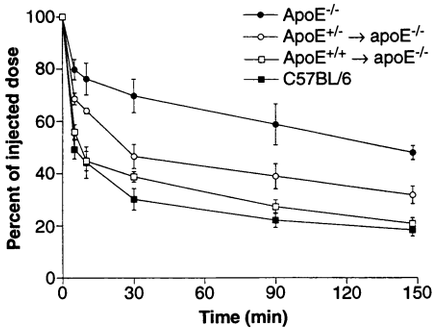

Fig. 3. Turnover of ^{125}I-d<1.019 g/ml lipoproteins after injection in control mice and in $apoE^{-/-}$ mice with transplants. The d<1.019 g/ml lipoproteins were prepared from four different groups of mice: $apoE^{-/-}$ mice, $apoE^{-/+} \rightarrow apoE^{-/-}$ BMT mice, $apoE^{+/+} \rightarrow apoE^{-/-}$ BMT mice, and control C57BL/6 mice. After iodination with $Na^{125}I$, 3 μg of each lipoprotein was injected into mice of the respective group. The radiotracer (400 dpm/ng of protein in a 300-μl volume) was injected into the jugular vein of mice anesthetized by brief exposure to metofane. We used six mice in the C57BL/6 group and three mice in the other groups. Lipoproteins from the $apoE^{-/+} \rightarrow apoE^{-/-}$ and $apoE^{+/+} \rightarrow apoE^{-/-}$ mice were isolated 6 weeks after BMT. Bars represent the standard deviation.

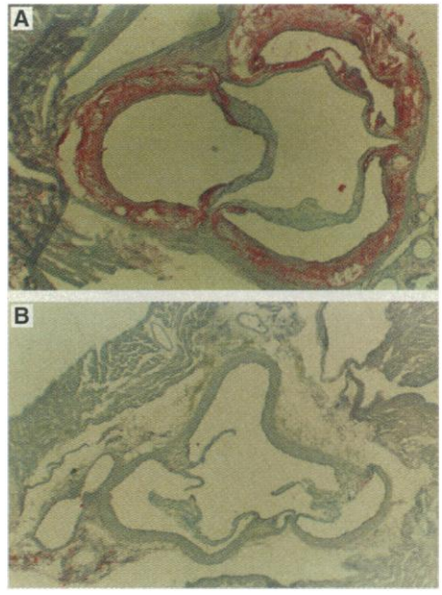

Fig. 4. Cross sections of the aortic sinuses of BMT mice on a Western-type diet. Five $apoE^{+/+} \rightarrow apoE^{-/-}$ and five $apoE^{-/-} \rightarrow apoE^{-/-}$ mice were started on a 21% fat and 0.15% cholesterol diet 2 months after BMT. After 3 months on the diet, the mice were killed and the heart was flushed first with saline, then with 4% paraformaldehyde in phosphate buffer. The inferior vena cava was cut to allow the perfusate to exit. Frozen sections of 10 μm thickness were taken in the region of the proximal aorta starting from the end of the aortic sinus and for 300 μm distally. Sections were stained with Oil Red O and counterstained with hematoxylin. (**A**) and (**B**) show aortic sections from representative $apoE^{-/-} \rightarrow apoE^{-/-}$ and $apoE^{+/+} \rightarrow apoE^{-/-}$ mice, respectively. Large, advanced raised lesions are present in the aortic sinus of the $apoE^{-/-} \rightarrow apoE^{-/-}$ mouse. The $apoE^{+/+} \rightarrow apoE^{-/-}$ mouse shows only a small focal lesion localized to the aortic valve stump. Quantitative morphology was done on sections starting just beyond this location at the end of the aortic sinus.

Table 2. Total serum cholesterol levels in apoE-deficient mice after transplantation of $apoE^{-/-}$, $apoE^{-/+}$, or $apoE^{+/+}$ bone marrow. Mice were on a diet of 4.5% autoclaved mouse chow. The mice had been irradiated and received transplants as described in (*14*), except that they received a larger amount of bone marrow cells (1×10^7). Blood collection and serum cholesterol analysis were done as described in Table 1. The pre-BMT time point was the day before transplantation; the post-BMT time point was 3 weeks. Values are in milligrams per deciliter (mean ± SD). The number of mice in each group is indicated by n.

Donor mouse	n	Pre-BMT		Post-BMT	
		Cholesterol	Triglycerides	Cholesterol	Triglycerides
$apoE^{-/-}$	10	448 ± 98	164 ± 71	504 ± 103	137 ± 26
$apoE^{-/+}$	5	530 ± 78	167 ± 60	259 ± 69	147 ± 50
$apoE^{+/+}$	12	545 ± 117	148 ± 99	140 ± 45*	98 ± 27

*Statistically different ($P < 0.0001$) compared with the post-BMT values of both $apoE^{-/-} \rightarrow apoE^{-/-}$ and $apoE^{-/+} \rightarrow apoE^{-/-}$ mice.

clearance in vivo. Furthermore, these studies demonstrate the efficiency of apoE in promoting lipoprotein clearance, because apoE levels that are only 12.5% of those in normal mice are sufficient to achieve normalization of plasma lipoproteins in apoE-deficient mice after BMT. Finally, the current studies demonstrate that BMT can be used to correct a nonenzymatic metabolic disorder in which the liver is the organ that primarily expresses the genetic defect and the artery is the primary focus of expression of the clinical disease.

The ability of apoE to associate with plasma lipoproteins and to serve as an efficient ligand for receptor-mediated clearance of lipoproteins suggests a potential role for the enrichment of lipoproteins with apoE in the treatment of other causes of hyperlipidemia. Experimental support for this hypothesis includes the demonstration that overexpression of rat apoE in transgenic mice reduces plasma lipids and prevents diet-induced hypercholesterolemia (*25*) and the observation that intravenous administration of apoE reduces serum cholesterol levels (*26*) and atherosclerosis (*27*) in Watanabe heritable hyperlipidemic rabbits, an animal model of familial hypercholesterolemia. These data suggest that overexpression of apoE may provide effective gene therapy for hyperlipidemia and atherosclerosis in conditions other than apoE deficiency. Our current studies suggest the potential of bone marrow to serve as a vehicle for gene therapy to treat hyperlipidemia and atherosclerosis.

REFERENCES AND NOTES

1. R. W. Mahley, *Science* **240**, 622 (1988).
2. T. E. Willnow *et al.*, *ibid.* **264**, 1471 (1994).
3. G. Ghiselli, E. J. Schaefer, P. Gascon, H. B. Brewer Jr., *ibid.* **214**, 1239 (1981); E. J. Schaefer *et al.*, *J. Clin. Invest.* **78**, 1206 (1986); H. Mabuchi *et al.*, *Metabolism* **38**, 115 (1989); S. Fazio, *Trends Cardiovasc. Med.* **3**, 191 (1993).
4. S. H. Zhang, R. L. Reddick, J. A. Piedrahita, N. Maeda, *Science* **258**, 468 (1992).
5. A. S. Plump *et al.*, *Cell* **71**, 343 (1992).
6. Y. Nakashima, A. S. Plump, E. W. Raines, J. L. Breslow, R. Ross, *Arterioscler. Thromb.* **14**, 133 (1994); R. L. Reddick, S. H. Zhang, N. Maeda, *ibid.*, p. 141.
7. M. F. Linton *et al.*, *J. Clin. Invest.* **88**, 270 (1991); H. G. Kraft, H. J. Menzel, F. Hoppichler, W. Vogel, G. Utermann, *ibid.* **83**, 137 (1989).
8. M.-L. Blue, D. L. Williams, S. Zucker, S. A. Khan, C. B. Blum, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 283 (1983); D. M. Driscoll and G. S. Getz, *J. Lipid Res.* **25**, 1368 (1984); D. L. Williams, P. A. Dawson, T. C. Newman, L. L. Rudel, *J. Biol. Chem.* **260**, 2444 (1985).
9. S. K. Basu, M. S. Brown, Y. K. Ho, R. J. Havel, J. L. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7545 (1981); S. K. Basu *et al.*, *J. Biol. Chem.* **257**, 9788 (1982).
10. S. K. Basu, J. L. Goldstein, M. S. Brown, *Science* **219**, 871 (1983).
11. M. I. Rosenfeld *et al.*, *Arterioscler. Thromb.* **13**, 1382 (1993).
12. S. Ishibashi *et al.*, *J. Biol. Chem.* **265**, 3040 (1990).
13. M. F. Linton and S. Fazio, *Circulation* **90**, I-133 (1994).
14. Recipients were prepared and bone marrow was harvested from femurs and tibias of donor mice as described (*28*). Bone marrow cells were washed and resuspended in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 2% fetal bovine serum (FBS) and heparin (5 U/ml). Recipients received 5×10^6 bone marrow cells in 0.3 ml by tail vein injection 4 hours after lethal irradiation (9 Gy) from a cesium gamma source. ROSA β-geo 26 mice (*15*) and the apoE-deficient mice (*4*) used in our experiments were C57BL/6 × 129 hybrids, which were backcrossed for 6 to 8 generations into C57BL/6. The C57BL/6 and 129 strains share the same major histocompatibility complex (H-2^b) (*29*). Donor apoE-deficient mice bearing the ROSA β-geo 26 marker gene were obtained by mating of the two strains. After transplantation, there was no evidence of rejection or graft-versus-host disease (GVHD). Animal care and experimental procedures involving animals were conducted in accordance with institutional guidelines.
15. G. Friedrich and P. Soriano, *Genes Dev.* **5**, 1513 (1991).
16. Bone marrow, spleen, and thymus cells from ROSA β-geo 26 mice show ubiquitous staining for β-Gal activity by the FACS-Gal technique (*30*) (personal communication, W. G. Kerr).
17. M. F. Linton and S. Fazio, unpublished results.
18. Determination of plasma levels of apoE was done by computerized densitometry of apoE immunoreactive bands on SDS–polyacrylamide gel electrophoresis (PAGE) protein immunoblots. Aliquots of 3 μl of serum were separated on a 12% SDS-polyacrylamide gel. After blotting, the filter was incubated with a rabbit antiserum to rat apoE that cross-reacts strongly with mouse apoE and to a lesser degree with mouse apoAI. The secondary antibody was a goat antiserum to rabbit immunoglobulin G (IgG). Detection of bands was achieved by chemiluminescence, with the use of the ECL kit from Amersham. In the experiment presented here, plasma apoE levels in three $apoE^{+/+} \rightarrow apoE^{-/-}$ mice and three $apoE^{-/+} \rightarrow apoE^{-/-}$ mice were compared to plasma dilutions of normal C57BL/6 mice. In addition, protein immunoblots of plasma from all $apoE^{+/+} \rightarrow apoE^{-/-}$ mice 4 weeks after BMT consistently showed that the apoE immunoreactivity in undiluted plasma from mice with transplants was similar to that of 1:10 dilutions of C57BL/6 mouse plasma.
19. The apolipoprotein composition of the ^{125}I-d<1.019 g/ml lipoproteins from each of the four groups of mice was examined by SDS-PAGE. As expected, the d<1.019 g/ml lipoproteins from normal C57BL/6 mice contained apoB-100, apoB-48, apoE, the C apolipoproteins, and traces of apoAI; relative to normal mice, the lipoprotein fraction of $apoE^{-/-}$ mice was deficient in apoE and enriched in apoAI and apoAIV. The d<1.019 g/ml fraction from the BMT mice contained apoE and showed lower levels of apoAI and apoAIV as compared with that of the $apoE^{-/-}$ mice. Results from another turnover study indicated that the differences in lipoprotein composition did not influence the outcome of this experiment. $ApoE^{-/-}$ mice, $apoE^{+/+} \rightarrow apoE^{-/-}$ mice, and control C57BL/6 mice were injected with ^{125}I- d<1.019 g/ml lipoproteins from $apoE^{-/-}$ mice, and the plasma clearance of the radiolabeled lipoproteins was followed for 90 min (*17*). The clearance of $apoE^{-/-}$ d<1.019 g/ml lipoproteins was much more rapid in the C57BL/6 mice than in the apoE-deficient mice. However, the clearance of $apoE^{-/-}$ d<1.019 g/ml lipoproteins in the $apoE^{+/+} \rightarrow apoE^{-/-}$ mice was not significantly different from that in $apoE^{-/-}$ mice. These results also indicate that the differences observed in the lipoprotein turnover study shown in Fig. 3 were not simply a function of pool size. In fact, no difference in clearance was observed between $apoE^{-/-}$ mice and $apoE^{+/+} \rightarrow apoE^{-/-}$ mice, despite the large difference in pool size. Conversely, a significant difference in clearance was observed between normal mice and $apoE^{+/+} \rightarrow apoE^{-/-}$ mice, despite the similarity in pool size.
20. Hematopoietic cell populations from C57BL6/J, ROSA β-geo 26, $apoE^{+/+} \rightarrow apoE^{-/-}$, and $apoE^{-/-} \rightarrow apoE^{-/-}$ mice were analyzed by flow cytometry. Red blood cells in the spleen and bone marrow were lysed by ammonium chloride. Single-cell suspensions from bone marrow, spleen, and thymus were washed twice in phosphate-buffered saline (PBS) with 2% bovine serum albumin. Cells (1×10^6) were incubated with phycoerythrin-conjugated mAb for 30 min. Cells were washed three times, and one- and two-color flow cytometry analyses were done on a FACScan (Beckton-Dickinson, San Jose, CA). Gating for viable cells was done with the use of 7-amino-actinomycin D exclusion (Molecular Probes, Eugene, OR). In each sample, 0.5×10^4 to 1.0×10^4 cells were analyzed. The antibodies to lineage-specific differentiation antigens were: M1/70 for Mac-1, 53-7.3 for CD5, and RA3-62B for CD45R (Pharmingen, San Diego, CA). Activity of β-D-Gal was measured as intracellular accumulation of fluorescein from the hydrolysis of fluorescein di-β-galactopyranoside (FDG; Molecular Probes) by β-D-Gal, as described (*30*). Cells were loaded with FDG by hypotonic shock: 1×10^6 cells in 100 μl of staining media [PBS, 4% FBS, and 10 mM Hepes (pH 7.2)] were incubated with 100 μl of 2 mM FDG in distilled H_2O at 37°C, and the reaction was stopped by addition of 1.8 ml of ice-cold staining media. The cells were incubated on ice before flow cytometry; staining with secondary antibodies was as described above except that all incubations were on ice and centrifugations were at 4°C.
21. D. Longo and R. Schwartz, *J. Exp. Med.* **151**, 1452 (1980); M. A. Brunstetter and J. A. Hardie, *Arch. Intern. Med.* **127**, 1064 (1971); K. Paradis, H. L. Sharp, D. A. Vallera, *J. Pediatr. Gastroenterol. Nutr.* **11**, 525 (1990); D. L. Longo and M. L. Davis, *J. Immunol.* **130**, 2525 (1983); W. F. Hickey and H. Kimura, *Science* **239**, 290 (1988).
22. The riboprobe was a 108–base pair fragment of exon 3 of the mouse apoE complementary DNA (cDNA) inserted in the vector pBSKS (Stratagene, La Jolla, CA) under the control of both the T3 (sense) and T7 (antisense) promoters. The probe was labeled with [^{32}P]UTP with the use of T7 polymerase and the RNA transcription kit from Stratagene. Tissue RNA was prepared by the guanidinium isothyocianate method, followed by CsCl gradient ultracentrifugation. Solution hybridization and RNase protection were carried out with 3×10^5 cpm of probe and 3 μg of RNA, with the use of the RPAII kit from Ambion (Austin, TX).
23. M. F. Linton, J. B. Atkinson, S. Fazio, unpublished results.
24. B. Paigen, A. Morrow, P. A. Holmes, D. Mitchell, R. A. Williams, *Atherosclerosis* **68**, 231 (1987).
25. H. Shimano *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1750 (1992).
26. R. W. Mahley *et al.*, *J. Clin. Invest.* **83**, 2125 (1989); N. Yamada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 665 (1989).
27. N. Yamada *et al.*, *J. Clin. Invest.* **89**, 706 (1992).
28. D. L. Thiele, M. L. Eigenbrodt, S. E. Bryde, *J. Clin. Invest.* **84**, 1947 (1989).
29. J. Klein, F. Figueroa, C. David, *Immunogenetics* **17**, 553 (1983).
30. G. P. Nolan, S. Fiering, J.-F. Nicolas, L. A. Herzenberg, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2603 (1988).

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