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Genetics

Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells

(homologous recombination/atherosclerosis)

JORGE A. PIEDRAHITA*, SUNNY H. ZHANG, JOHN R. HAGAMAN, PAULA M. OLIVER, AND NOBUYO MAEDA

Department of Pathology and Curriculum in Genetics, University of North Carolina, Chapel Hill, NC 27599-7525

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ABSTRACT **We have inactivated the endogenous apolipoprotein E (*apoE*) gene by using gene targeting in mouse embryonic stem (ES) cells. Two targeting plasmids were used, pJPB63 and pNMC109, both containing a neomycin-resistance gene that replaces a part of the *apoE* gene and disrupts its structure. ES cell colonies targeted after electroporation with plasmid pJPB63 were identified by the polymerase chain reaction (PCR) followed by genomic Southern analysis. Of 648 G418-resistant colonies analyzed, 9 gave a positive signal after PCR amplification, and 5 of them were confirmed as targeted by Southern blot analysis. The second plasmid, pNMC109, contains the negatively selectable thymidine kinase gene in addition to the neomycin-resistance gene. After electroporation with this plasmid, 177 colonies resistant both to G418 and ganciclovir were analyzed; 39 contained a disrupted *apoE* gene as determined by Southern blotting. Chimeric mice were generated by blastocyst injection with 6 of the targeted lines. One of the lines gave strong chimeras, three of which transmitted the disrupted *apoE* gene to their progeny. Mice homozygous for the disrupted gene were produced from the heterozygotes; they appear healthy, even though they have no apolipoprotein E in their plasma.**

The etiology of atherosclerosis is complex. Its development is affected by many different genes, including those related to lipid metabolisms, and is greatly influenced by environmental factors (1, 2). In addition, because the disease can be reproduced only in a living animal, there are no adequate *in vitro* systems available. For studying this type of complex multifactorial disease, gene targeting in mouse embryonic stem cells (ES cells) provides a unique tool [for reviews see Capecchi (3) and Smithies (4)]. It allows the generation of animals with changes in specific genes, and thence the study of the effects of these changes on the physiological state of the animals. We have, therefore, started to make specific alterations in lipid metabolism related genes of otherwise normal mice by homologous recombination in ES cells as another approach to dissecting some of the molecular details of atherogenesis.

Apolipoprotein E (apoE) is a constituent of very low density lipoprotein (VLDL) synthesized by the liver (5), and of a subclass of high density lipoproteins (HDLs) involved in cholesterol transport among cells (6). apoE mediates high-affinity binding of apoE-containing lipoprotein particles to the low density lipoprotein (LDL) receptor and is thus responsible for the cellular uptake of these particles (7). apoE is also a major protein constituent of remnant particles of intestinally synthesized chylomicrons (8). After transport of dietary cholesterol and triglycerides, chylomicron remnants are taken up by the liver by an apoE-dependent receptor-mediated mechanism (9). In humans, a variant form of apoE that is defective in binding to the LDL receptor is associated with familial type III hyperlipoproteinemia, a disease characterized by elevated plasma cholesterol and premature coronary heart disease (10, 11). In addition to its involvement in cholesterol and lipid metabolism, apoE has been implicated in regulation of the immune response (12), in nerve regeneration (13, 14), and in muscle differentiation (15, 16).

In the present paper, we describe the results of experiments that have allowed us to inactivate the mouse *apoE* locus by homologous recombination. Animals homozygous for the modified *apoE* gene are devoid of apoE protein in their sera. Further studies of these animals are expected to lead to a better understanding of the role of apoE in lipid metabolism, atherogenesis, nerve injury, and other biological phenomena.

MATERIALS AND METHODS

Cloning of the *apoE* Gene. Mouse genomic DNA, isolated from STO cells (17) and fully digested with *Eco*RI, was used to make a library in λ phage Charon 32 (18). The library was screened with an *apoE*-specific probe (a *Sac* I/*Bgl* II fragment) isolated from a mouse *apoE* cDNA clone (19), which was kindly provided by A. J. Lusis (University of California, Los Angeles). From one of the strongly hybridizing phage clones obtained in this screening, a 7.8-kilobase (kb) *Eco*RI fragment was isolated. Comparison of the restriction map of this fragment with the nucleotide sequence of the mouse *apoE* gene (20) showed that it contained the complete *apoE* gene in addition to 5.7 kb of its upstream sequence.

Cell Culture and Electroporation. An ES cell line, E14TG2a, obtained from Martin Hooper (21), was used for all experiments. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 15% fetal bovine serum, 0.1 mM 2-mercaptoethanol, and 2 mM glutamine (ES medium). Penicillin (100 international units/ml) and streptomycin (100 μg/ml) were used in some experiments. ES cells were maintained on embryonic fibroblast feeder layers prepared as previously described (22).

Cells were electroporated essentially as described by Reid *et al.* (23), using linearized DNA at a final concentration of 2–5 nM. Electroporated cells were plated at a density of 1–2 × 10^6 cells per 10-cm plate. Twenty-four hours after electroporation, the ES medium was replaced with medium containing 150–200 μg of G418 per ml with or without 2 μM ganciclovir (Ganc; gift from Syntex, Palo Alto, CA). G418 selects for neomycin phosphotransferase (neo) and Ganc selects against herpes simplex thymidine kinase (TK) expression.

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Abbreviations: apoE, apolipoprotein E; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; ES cells, embryonic stem cells; neo, neomycin phosphotransferase; TK, thymidine kinase; Ganc, ganciclovir.

*Present address: Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77840-4458.

Screening for Homologous Recombinants. ES cells carrying an *apoE* gene disrupted by homologous recombination were identified by polymerase chain reaction (PCR) and/or by genomic Southern blotting. Briefly, 10–14 days after electroporation and selection by G418, individual colonies were picked and divided into two parts. One portion was used for further growth and the other was combined with portions from 3–5 other colonies to form pools of 4–6 colonies per pool. DNA was isolated from pooled cells and subjected to PCR as described (23, 24), except that 0.5 μg of each primer and 1.0 unit of *Taq* DNA polymerase were used in the reaction, and that dimethyl sulfoxide was omitted. The mixture was amplified for 55 cycles with 60 sec of denaturation at 92°C followed by 10 min of annealing and extension at 65°C. The primers used were *apoE*-specific, 5′-TGTCTTCCACTATTGGCTCG-3′; and *neo*-specific, 5′-TGGCGGACCGCTATCAGGAC-3′. After PCR, samples were electrophoresed in 1.5% agarose and transferred to nylon membranes (Hybond, Amersham). Filters were hybridized with the ^{32}P-labeled *apoE*-specific probe described above to detect a 1.2-kb diagnostic band (see Fig. 1).

When plasmid pNMC109 was employed as the targeting plasmid, Southern blot analysis was used to screen for homologous recombinants. Individual colonies were expanded and genomic DNA was prepared, most conveniently by the "salting out" procedure of Miller *et al.* (25).

Generation of Germ-Line Chimeras. Chimeras were generated as previously described by Koller *et al.* (26). Animals classified as chimeric by coat color were mated to strain 129 and/or C57BL/6J mice. DNA samples were isolated from tails of ES cell-derived animals by the salting out procedure and were analyzed for the presence of a disrupted *apoE* gene by genomic Southern blotting.

Double Immunodiffusion Test. Double immunodiffusion precipitation (27) on glass slides was carried out for apoE detection in 1.0% agarose in phosphate-buffered saline (PBS) containing 3% (wt/vol) polyethylene glycol (M_r = 8000). Rabbit antiserum to rat apoE was kindly supplied by John Taylor (University of California, San Francisco). Blood samples were collected in EDTA-coated capillaries from the tails of animals between 2 weeks and 3 weeks of age, and plasma was isolated by centrifugation. Plasma (5–8 μl) in the peripheral wells was used with 8 μl of antiserum in the central well. The slides were incubated in a wet chamber for 16 hr at 37°C, washed in PBS for 2 days, and stained with Coomassie brilliant blue G250.

RESULTS

Construction of Targeting Plasmids. Two different targeting plasmids were constructed from part of the 7.8-kb *Eco*RI fragment. Plasmid pJPB63 (Fig. 1*b*) was constructed by removing a 1.1-kb *Sac* I fragment containing part of intron 1 through part of exon 3 and replacing it with the *neo* gene driven by a TK promoter and a mutated polyoma enhancer [pMCneopolyA (28)]. In addition, a 400-base-pair (bp) *Bst*EII fragment corresponding to the 3′ end of exon 4 and 3′ flanking region was deleted to facilitate the choice of a PCR primer sequence unique to the target locus. The plasmid contains 4.9 kb of uninterrupted sequence in the 5′ region and 700 bp in the 3′ region; prior to electroporation it was digested with *Nsi* I and *Cla* I.

The second plasmid, pNMC109 (Fig. 2*b*), was designed so as to be able to use positive-negative selection as described by Mansour *et al.* (29). pNMC109 was constructed from the 7.8-kb *Eco*RI fragment by removing an *Xho* I/*Bam*HI fragment containing part of exon 3 and part of intron 3 and replacing it by the neo gene. In addition, a copy of the herpes simplex virus TK gene was placed at the 3′ end of the construct. Both of these selectable marker genes are inserted in the same transcriptional orientation as the *apoE* gene. The plasmid contained 6.4 and 1.2 kb of uninterrupted sequences in the 5′ and 3′ region, respectively. Prior to electroporation, the plasmid was linearized by digestion at a *Not* I site (not shown) that occurs in the plasmid vector 5′ to the end of the *apoE* insert.

Targeting with pJPB63. Four electroporations were carried out with plasmid pJPB63. The number of cells treated ranged from 2×10^7 to 8×10^7 (Table 1). For the first three electroporations, PCR analysis was carried out on pools of 4–6 colonies per pool. Six of 128 pools analyzed gave a positive PCR signal as judged by amplification of the 1.2-kb fragment diagnostic of targeting. From these 6 pools, four individual PCR-positive clones were identified. For the fourth electroporation of the series, PCR analysis was carried out on individual colonies instead of on pools. From 72 colonies analyzed, 3 were PCR positive, although two of them were lost due to contamination prior to genomic analysis (Table 1).

To confirm the occurrence of the targeted disruption of the *apoE* gene, PCR-positive clones were expanded and DNA isolated from them was digested with *Eco*RI, *Nco* I, or *Bgl* II. Introduction of the *neo* gene in the planned manner creates a new *Eco*RI site in the locus, and a 2.4-kb fragment is thereby generated. An undisrupted *apoE* gene generates a 7.8-kb fragment. Fig. 1*d* illustrates this distinction, with the parental cell line (lanes P) giving only the 7.8-kb fragment and a targeted cell line (lanes T) giving both the 7.8-kb fragment and the new 2.4-kb fragment, thus confirming the presence of one normal and one disrupted *apoE* gene. Similarly, *Nco* I

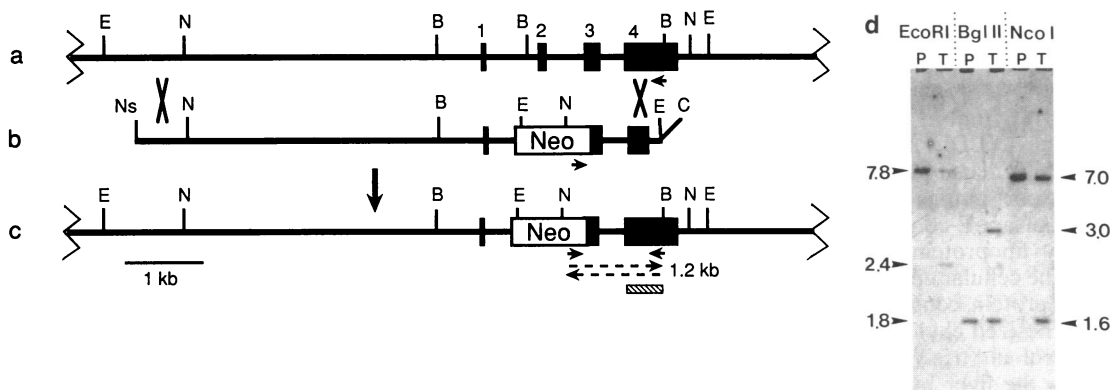

FIG. 1. Targeting of the mouse *apoE* gene by using pJPB63. (*a*) Normal mouse *apoE* locus. (*b*) Targeting *Nsi* I/*Cla* I fragment of pJPB63. (*c*) The *apoE* locus after homologous recombination, indicated by the Xs between *a* and *b*. Black boxes indicate *apoE* exons and the open box indicates a *neo* gene. Small arrows indicate the locations of PCR primers and large dashed arrows indicate the expected PCR fragment. The *apoE*-specific probe is indicated by a hatched box. E, *Eco*RI; N, *Nco* I; B, *Bgl* II; C, *Cla* I; Ns, *Nsi* I. (*d*) Southern blot analysis of genomic DNA from parental (P) and targeted (T) cell lines. Fragment sizes sizes are in kb.

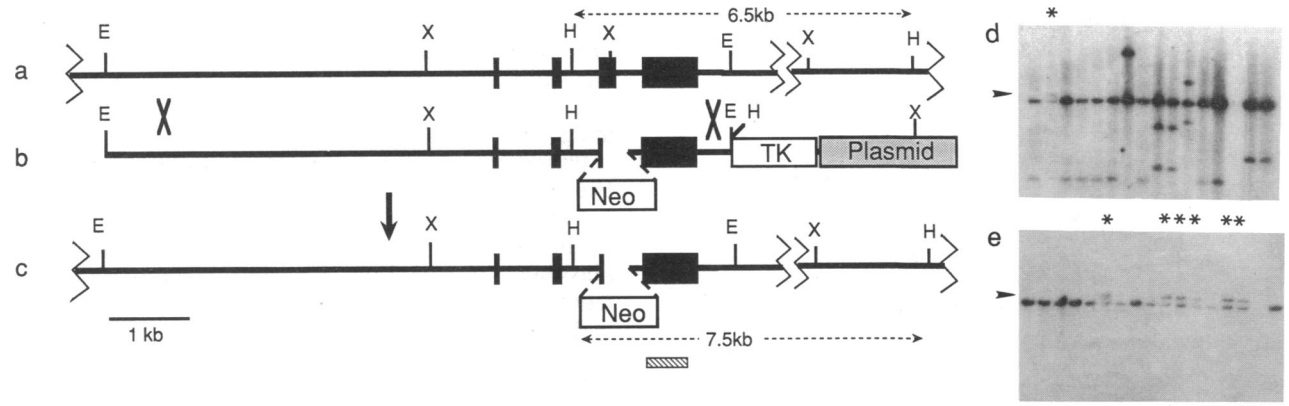

FIG. 2. Targeting of the *apoE* gene by using pNMC109. (*a*) Normal mouse *apoE* locus. (*b*) Linearized targeting plasmid pNMC109; the TK gene and plasmid sequences at the 3′ end of the targeting fragment are not to scale. (*c*) The *apoE* locus after homologous recombination. E, *Eco*RI; H, *Hin*dIII; X, *Xmn* I. The diagnostic *Hin*dIII fragments used for screening by Southern blotting are shown by dashed lines with their sizes. (*d*) Southern blot analysis of *Hin*dIII digests of genomic DNA from fast-growing colonies. (*e*) Southern blot analysis of DNA from slow-growing colonies. In *d* and *e*, arrowheads indicate 7.5-kb bands and asterisks indicate targeted cells.

and *Bgl* II digestions of the targeted line showed 1.6- and 3.0-kb fragments, respectively, indicative of a disrupted *apoE* gene, in addition to the 7.0- and 1.8-kb bands indicative of an undisrupted *apoE* gene, respectively.

When the blot shown in Fig. 1*d* was rehybridized to a probe specific for the *neo* gene, only bands predicted from the structure in Fig. 1*c* were detected, showing that no insertions of the targeting plasmid had occurred at nonhomologous sites (results not shown). Thus all three enzyme digests confirm the PCR results by demonstrating that the cell lines analyzed carry a disrupted *apoE* gene.

The combined targeting frequency for all electroporations was 0.77% (5/648) of the G418-resistant colonies, with a range of 0.5% (1/192) to 1.3% (1/72) (Table 1). The overall targeting frequency at the mouse *apoE* locus when pJPB63 was used was 2.6 in 10^7 treated cells.

Targeting with pNMC109. Five electroporations were carried out as summarized in Table 2. A combined total of 1132 colonies doubly resistant to G418 and Ganc were obtained, and 177 of these colonies were expanded. As illustrated in Fig. 2, if homologous recombination takes place between the endogenous *apoE* locus and the plasmid pNMC109, the size of the *Hin*dIII fragment hybridizing to the *apoE* probe increases from 6.5 to 7.5 kb. Thus the presence of a 7.5-kb band in the Southern blot analysis was used to diagnose homologous recombination (Fig. 2 *d* and *e*).

During this screening, we noted that a higher proportion of slow-growing colonies (picked after 12 days of double selection) were targeted compared with fast-growing colonies (picked after 10 days of selection). As illustrated in Fig. 2 *d* and *e*, only 1 of 15 fast-growing colonies was targeted (6.6%), compared with 6 of 16 slow-growing colonies (37%). Altogether, we identified 39 targeted colonies in 177 of the doubly resistant colonies (a combined frequency of 22%; Table 2). The frequency in individual electroporations ranged from 8.3% (2/24) to 45.8% (11/24). The higher frequency obtained in the fifth electroporation was partly because in this experiment we analyzed only the slower-growing colonies. Excluding the last electroporation, the overall targeting frequency with pNMC109 was estimated to be one in 10^6 treated cells.

The existence of the desired modification of the *apoE* locus in the isolated cell lines was confirmed by Southern blot analysis of DNA digested with various enzymes. Of the 39 cell lines isolated in the pNMC109 experiments 37 showed a simple pattern of two hybridizing bands of equal intensity, one corresponding to the endogenous *apoE* locus and the other to the modified locus (see, as an example, clone SZ-26 in lanes 2 of Fig. 3).

The two remaining clones, T-88 and T-89, showed a third, strongly hybridizing fragment in each digest in addition to the two bands indicative of a targeted cell (see, for example, clone T-89 in lanes 3 of Fig. 3). Digests of the DNA from T-88 always gave patterns identical to those from T-89, and it is likely that they are of identical origin (they were picked one after the other from a single dish). The third hybridizing band in T-89 DNA digested with *Xmn* I is 7.9 kb in length, is approximately 4–5 times as intense as the other two bands, and also hybridizes to plasmid vector DNA. Similarly, extra bands of 12 and 4.3 kb were seen in *Xba* I and *Kpn* I digests, respectively (see Fig. 3).

Analysis of T-89 DNA with several other restriction enzymes (*Apa* I, *Bam*HI, *Hin*dIII, *Not* I, and *Sac* I) with both the *apoE* probe and a plasmid vector probe failed to reveal any bands, suggesting that the extra copies are integrated at nonhomologous locations in the genome. These various results are consistent with the explanation that four to five

Table 1. Targeting the mouse *apoE* gene with pJPB63

Exp.	No. of cells treated	G418^r colonies	No. of pools analyzed*	PCR-positive pools	Targeted colonies†
1	2×10^7	420	36 (4)	2	1
2	4×10^7	660	32 (6)	1	1
3	8×10^7	3216	60 (4)	3	2
4	4×10^7	1808	72 (1)	3	1

All electroporations were carried out at 200 μF and 300 V in ES cell medium. G418^r, G418-resistant.
*The number of colonies in each pool is in parentheses.
†The number of colonies isolated and confirmed by Southern blots.

Table 2. Homologous recombination at the *apoE* locus with pNMC109

Exp.	No. of cells treated	G418^r colonies*	G418^r + Ganc^r colonies	G418^r + Ganc^r colonies analyzed	Targeted colonies
1	4×10^7	690	218	24	2
2	8×10^7	2120	206	29	4
3	4×10^7	1245	412	21	3
4	2.5×10^7	400	120	79	19
5	4×10^7	480	176	24	11

Electroporation experiments 1–3 were carried out at 200 μF and 300 V in ES medium, and experiments 4 and 5 were carried out at 250 μF and 350 V in PBS. G418^r, G418-resistant; Ganc^r, Ganc-resistant.
*Determined on a known fraction of the cells selected with G418 alone.

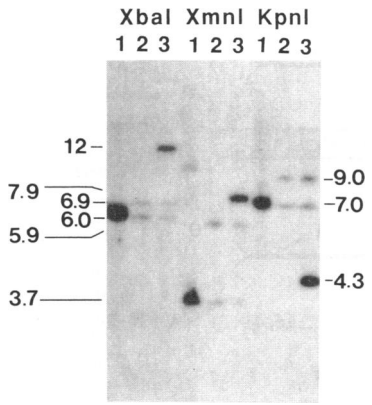

FIG. 3. Southern blot analysis of ES cells targeted with pNMC109. Genomic DNA of parental ES cells (lanes 1), SZ-26 (lanes 2), and T-89 (lanes 3) was digested with *Xba* I, *Xmn* I, or *Kpn* I.

identical copies of incoming plasmid have integrated in a head-to-tail concatemeric orientation at the *apoE* locus. The sizes of fragments obtained with these digests suggest that the incoming linear DNA lost about 1.6 kb of DNA prior to its concatemerization.

Production of Chimeras and Transmission of the Modified Gene. Six different targeted ES cell lines were tested for the production of chimeras after injection into C57BL/6J blastocysts; one (A13-D) had been targeted with the plasmid pJPB63, the other five (T-76, T-84, T-89, SZ-38, and SZ-91) with the pNMC109 plasmid. About 50 embryos were used to test each line. All six generated chimeras ranging from very weak (less than 10% contribution of ES cells to coat color) for chimeras obtained from cell line T-84 to very strong (greater than 80%) for chimeras obtained with cell line T-89. In all, a total of 95 embryos were injected with T-89 cells to obtain 6 male and 4 female chimeras with 60–100% ES cell contribution. Two males and one female were found to be germ-line competent. [Germ-line transmission of the E14TG2a ES cell genome through female chimera has previously been reported by Koller *et al.* (30).] One male chimera, JH126.1, transmitted the ES cell genome to 100% of its offspring. A Southern blot of tail DNA from his pups revealed that 14 of 30 pups (the expected Mendelian proportion) have inherited the disrupted copy of the *apoE* gene (Fig. 4*a*).

(C57BL/6J × 129) F_1 animals heterozygous for the disrupted *apoE* gene are healthy at age 3 months and are breeding normally. From two sets of heterozygous F_1 matings, we have obtained litters of 8 and 6 pups to date. Three of these 14 animals are homozygous for the modified *apoE* locus (the expected Mendelian proportion), as identified by Southern blot of their tail DNA (Fig. 4*b*). All appear healthy at age 4–6 weeks.

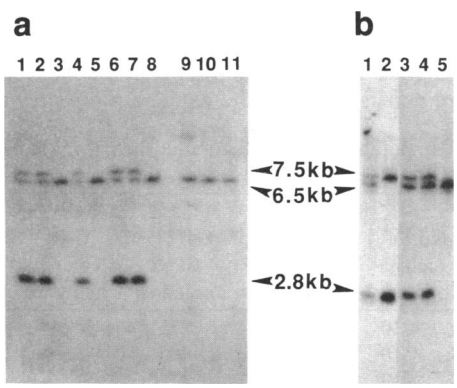

FIG. 4. Transmission of the targeted *apoE* gene. (*a*) Southern blot of *Hin*dIII digests of genomic DNA isolated from tails of agouti offspring of JH126.1 mated with C57BL/6J females. (*b*) DNA from pups of a cross between heterozygous F_1 animals. The 6.5-kb bands indicate transmission of the normal allele and the 7.5-kb bands indicate transmission of the modified allele. The 2.8-kb band is derived from the concatemeric insert in the T-89 cell line (see text).

Plasma was obtained from the offspring of a mating between heterozygotes, and the presence of apoE protein was investigated by the Ouchterlony double immunodiffusion method, using a rabbit antiserum to rat apoE. As Fig. 5 illustrates, plasma from the animal homozygous for the modified *apoE* gene gave no detectable immunoprecipitate with anti apoE antiserum, although precipitation is clearly visible with plasma from animals heterozygous or homozygous for the normal gene. The plasma level of apoE in heterozygotes appears to be less than that in the normal animal as judged by the positions where the precipitates were formed.

DISCUSSION

We have inactivated the *apoE* gene in mouse ES cells by homologous recombination. Colonies targeted with plasmid pJPB63 were identified by PCR and confirmed by genomic Southern blotting. As shown in Table 1, four independently targeted cell lines were isolated from seven PCR-positive pools. This corresponds to a confirmed frequency of 1 targeting in 120 G418-resistant colonies (Table 1). This relatively high frequency encouraged us to screen single colonies by PCR in the fourth experiment. In this experiment, 3 out of 72 individually amplified colonies gave a positive PCR signal, and one of these was confirmed to be targeted by Southern blotting.

The overall frequency of finding targeted cells was considerably improved when a plasmid containing a TK tail, pNMC109, was used in conjunction with the positive–negative protocol (29). The frequency ranged from 1/12 to 1/2 of the doubly selected cells (Table 2). The highest frequency, obtained in the last electroporation, was probably because only slower-growing (late-appearing) colonies were analyzed in this experiment, following our observation that a higher proportion of late-appearing colonies were targeted compared with early-appearing colonies. A possible explanation is that colonies are able to grow in G418 faster when they have multiple copies of the *neo* gene inserted into their genomes. This explanation is supported by our finding that the early-appearing colonies tend to have extra hybridizing bands, indicative of multiple integrations, in their Southern blot analysis (Fig. 2*d*). This agrees with previous observations reported by Reid *et al.* (23) that targeted colonies rarely have multiple inserts.

During our screening for cell lines targeted with pNMC109 we isolated two (probably not independent) cell lines containing an additional restriction fragment indicating that the plasmid had been integrated into the target *apoE* locus as a

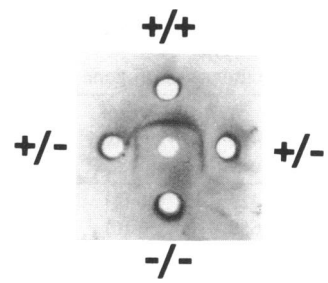

FIG. 5. Double immunodiffusion test of *apoE* expression. Plasma of normal (+/+, 5 μl), heterozygous (+/−, 8 μl), and homozygous (−/−, 8 μl) mice were placed in peripheral wells and 8 μl of rabbit antiserum to rat apoE was placed in the central well.

head-to-tail concatemer. Similar observations have been made by Thomas and Capecchi (31) and Hasty *et al.* (32). Mapping of the T-89 DNA shows that the TK gene in the concatemer appears to be complete, although the cells are resistant to Ganc. Presumably the TK gene was inactivated prior to the integration, but the inactivation was not caused by a gross deletion. Our best estimate is that there are four or five tandem copies of the initially 13.6-kb pNMC109 plasmid, shortened by 1.6 kb from the ends, inserted into the *apoE* locus in the T-89 cell line. The existence of these copies does not materially affect the value of this cell line, which is the one that to date has yielded chimeras able to transmit a disrupted *apoE* gene to their progeny.

The animals that are heterozygous for the disrupted *apoE* gene are apparently in good health at age 3 months. Two pairs of heterozygotes have been mated, and to date we have obtained 14 pups, of which 3 are homozygous and 8 are heterozygous for the targeted disruption of the *apoE* gene. All these animals are apparently healthy at age 4–6 weeks. The disrupted gene is transmitted at a frequency that does not differ significantly from that of the normal gene. A homozygous animal is predicted, from its genotype, to be unable to synthesize apoE. Immunodiffusion tests (Fig. 5) of plasma from one of the homozygous animals confirm this expectation. Thus, judging at the level of genomic DNA structure and at this level of plasma apoE, we have succeeded in generating mice that lack apoE expression.

The facts that homozygous animals have been born at the expected frequency and that they appear to be healthy are important. They demonstrate that lack of apoE is compatible with normal development, and they also provide another tool for studies of the phenotypic consequences of apoE deficiency. Rare examples of apoE deficiencies have been reported in humans (11, 33, 34), where they cause a form of type III hyperlipoproteinemia. In one case, the deficiency was the consequence of a mutation in a splicing site in the *apoE* gene that prevents normal mRNA processing (35). Considering the importance of apoE protein in the binding of remnants of chylomicron and VLDL to receptors, we expect that mice without apoE will in due course show a phenotype similar to type III hyperlipoproteinemia in humans, and they will therefore have a recessively inherited predisposition to coronary heart disease. One caveat is, however, required: mice have substantially different relative lipoprotein levels from humans, and they use HDL as a major cholesterol carrier (36), while humans use LDL. Thus the immediate phenotype of apoE deficiency may be different in mice from that in humans. The progression of our animals and their responses to dietary factors will be of considerable interest. In addition, we expect that combining the deficiency of apoE in these animals with defects in other lipid metabolism related gene defects, arising spontaneously (37) or generated by gene targeting, should help in dissecting genetic and environmental factors that can lead to coronary heart disease.

Mention should also be made of the putative importance of apoE in the repair of injury in peripheral nerve tissue. Following nerve injury, macrophages within the sciatic nerve start to produce apoE, which is probably involved in the removal of debris from damaged cells and in the stimulation of regeneration of the peripheral nerve cells (13). It will be of considerable interest to observe the responses of the apoE-deficient mice to nerve injury.

We thank Dr. John Taylor for supplying us antiserum to rat apoE; Ms. Denise Lee for assistance in tissue culture experiments; Dr. Ron Shehee for maintaining our cell cultures at a critical time; and Mr. Mike Sandlin, Mr. Matt Williamson, Ms. Traci Clegg, and Ms. Beth Gibbs for help in maintenance of mouse colonies and in animal experiments. We are grateful to Drs. Oliver Smithies, Terry Smith, and Beverly Koller for helpful discussions at various steps of executing this work. This work was supported by National Institutes of Health Grant HL42630.

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