ApoE knockout and knockin mice: the history of their contribution to the understanding of atherogenesis

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Abstract ApoE is a multifunctional protein that is expressed by many cell types that influence many aspects of cardiovascular physiology. In humans, there are three major allelic variants that differentially influence lipoprotein metabolism and risk for the development of atherosclerosis. Apoe-deficient mice and human apoE isoform knockin mice, as well as hypomorphic Apoe mice, have significantly contributed to our understanding of the role of apoE in lipoprotein metabolism, monocyte/macrophage biology, and atherosclerosis. This brief history of these mouse models will highlight their contribution to the understanding of the role of apoE in these processes. These Apoe−/− mice have also been extensively utilized as an atherosensitive platform upon which to assess the impact of modulator genes on the development and regression of atherosclerosis.—Getz, G. S., and C. A. Reardon. ApoE knockout and knockin mice: the history of their contribution to the understanding of atherogenesis. J. Lipid Res. 2016. 57: 758–766.

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“A golden age for experimental atherosclerosis dawned when Nobuyo Maeda and Jan Breslow knocked out the Apoe gene in mice” (ref. 1, p. 386). These seminal studies were published in 1992, but were preceded by almost 20 years of experimentation on the structure/function of apoE, since the first description of the protein in VLDL by Shore and Shore (2). The protein was initially designated as the arginine-rich peptide, but only for a limited time before being renamed apoE. During this 20 year period, much important research was reported on the human apoE isoforms and their pathophysiological properties. This historical review will summarize these analyses as they relate to cardiovascular disease. We recognize that apoE has functions beyond the cardiovascular system (3, 4), which will not be dealt with here.

Human apoE is found in the plasma associated with VLDL and HDL. It is a 34 kDa protein that is posttranslationally modified, mostly by glycosylation. The mature protein contains 299 amino acids and is encoded on chromosome 19. The gene was first sequenced in 1985 (5). The mouse protein is ~70% homologous. apoE is the primary ligand for the removal of chylomicron remnants and intermediate density lipoproteins by the liver, functioning to ligate these particles to the LDL receptor (LDLR) and the LDLR-related protein 1 (LRP-1) (6).

The understanding of the physiological role of apoE was greatly enhanced by the study of type III hyperlipoproteinemia. Type III hyperlipoproteinemia presents clinically as hypercholesterolemia and hypertriglyceridemia with the presence of cholesterol-enriched VLDL and a high likelihood of premature ischemic heart disease and peripheral vascular disease, as well as xanthomata, especially palmar xanthomata (7). This abnormal VLDL is the result of impaired clearance of remnant lipoproteins; initially demonstrated by the clearance of injected radiolabeled apoB48- and apoB100-containing lipoproteins in these dyslipidemic patients (8). Indeed, the genetics of type III hyperlipoproteinemia or dysfunctional hyperlipoproteinemia revealed the allelic polymorphism of human apoE, initially reported by Utermann, Hees, and Steinmetz (9). This study identified two allelic forms that were designated as E-N for normal apoE and E-D for dysfunctional apoE, with the E-D isoform prevalent in patients with type III hyperlipoproteinemia. Further study of the genetics of the apoE isoforms identified three common allelic variants, eE2 (equivalent to the E-D allele), eE3 (equivalent to the E-N allele), and eE4, encoding apoE2, apoE3, and apoE4, respectively (10). The allele frequencies are 4–13%, 75–85%, and 14–23% for eE2, eE3, and eE4, respectively. APOE2 homozygotes are found in about 2–5% of the human population.
population, but only a small proportion of these individuals exhibit type III hyperlipoproteinemia. It appears that some other genetic or environmental factors are necessary for the full expression of the dyslipoproteinemia.

The contribution of the investigators at the Gladstone Foundation for Cardiovascular Disease to the understanding of the structure and function of the apoE isoforms was reviewed by Mahley, Weisgraber, and Huang (11). The isoforms differ by the amino acid at residues 112 and 158 (Fig. 1). ApoE2 has Cys at both of these sites, apoE3 has a Cys at residue 112 and Arg at residue 158, and apoE4 has Arg at both of these sites. While none of these residues reside within the LDLR binding site (residues 134-150), these amino acid differences influence the structure of the protein to impact on their affinity for the LDLR. Crystallographic studies by Karl Weisgraber and colleagues at the Gladstone Institute demonstrated that the lipid-free protein assumes a four helix bundle. In apoE3 and apoE4, Arg158 forms a salt bridge with Asp154, thus leaving Arg150 available for receptor interaction. On the other hand, in the apoE2 isoform, the uncharged Cys158 cannot form a salt bridge with Asp154. Instead Asp154 forms a salt bridge with Arg150, thus disrupting the binding of the ligand with the receptor and accounting for the poor clearance of apoE2-containing remnants from the plasma.

These amino acid differences between isoforms also influence their preferential association with VLDL and HDL. ApoE3 preferentially associates with HDL and apoE4 with VLDL. The preferential association of apoE4 with VLDL is believed to be due to Glu255 interacting with Arg61, thus forming an interaction between the N and C termini of the protein, a domain interaction not observed in apoE3. This conformation allows for the unfolding of the protein that contributes to its enhanced lipid binding affinity (12). The preferential association of apoE4 with apoB-containing particles and the fact that its affinity for the LDLR is equal to or greater than that of apoE3 may allow for efficient uptake of cholesterol-containing lipoprotein remnants leading to downregulation of LDLR expression. This may account for the fact that, in the presence of apoE4, plasma cholesterol levels and VLDL:HDL ratios are elevated and that individuals with the e4 allele have increased incidence of atherosclerosis. Viral-mediated expression of apoE isoforms in Apoe−/− mice also suggests that the preferential association of apoE4 with VLDL leads to impaired lipolysis resulting in reduced clearance of the remnants (13).

ApoE deficiency is very rare. Members of six kindreds have been reported with complete or almost complete absence of apoE in the plasma (14–21). The nature of the apoE mutation has been characterized in several of the kindreds; this includes a 10 bp deletion, a premature stop codon, an acceptor splice site mutation in intron 3, and a frameshift mutation. All of these patients exhibit type III hyperlipoproteinemia and show palmar, ear, elbow, and knee xanthomata. Interestingly, despite this clinical picture, coronary artery disease was modest, especially in the female probands.

### Apoe DEFICIENCY AND ATHEROSCLEROSIS IN MICE

In contrast to the situation with human apoE deficiency, apoE deficiency in mice results in a profound susceptibility to atherosclerosis. The year 1992 was the beginning of the “golden” era referred to by Hansson (1). It was in this year that two separate laboratories reported on the creation of the Apoe-deficient mouse; Breslow and colleagues (22) and Maeda and colleagues (23, 24). The background of the two laboratories was somewhat different. Jan Breslow, at Rockefeller University, had spent most of the prior 15–17 years studying lipoproteins and their regulation and genetics. Nobuyo Maeda, on the other hand, had been studying homologous recombination and its use to target genes in collaboration with Oliver Smithies, originally at the University of Wisconsin and later at the University of North Carolina. As she outlines in a review (25), she was introduced to lipoproteins by Jan and Judith Rapacz, who had been studying hypercholesterolemia in domestic pigs at the University of Wisconsin. She also had the help of Alan Attie in her introduction to experimenting with plasma lipoproteins. There were some modest differences in the creation of the targeted apoE mice in the two laboratories. The same ES cells (from 129/Ola mice) were used, but the source of the apoE gene was the BALB/c strain in New York and STO cells (embryonic fibroblasts from SIM mice) in North Carolina. The derived animals were fully viable and the homozygotes were produced in the expected Mendelian ratios. In the initial reports of the mice, Maeda’s animals were F2 derivatives and, hence, were chimeric for C57BL/6 and 129 strains, while the animals in the Breslow laboratory were more extensively backcrossed to C57BL/6 mice. This may account for some of the initial differences reported in HDL and triglyceride levels between the two sets of mice. But homozygous

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**Fig. 1.** Human apoE isoforms and humanized murine apoE. Top: Amino acid differences in the human apoE isoforms and the location of the salt bridges (dashed lines) in the isoforms identified in the crystal structures. Bottom: Amino acid residues at the equivalent position in mouse apoE and the substitution of Arg61 for Thr61 to humanize murine apoE and generate an apoE4-like protein.
deficient (Apoe<sup>−/−</sup>) animals in both laboratories were hypercholesterolemic (~400 mg/dl) while being fed standard chow diet, with most of the cholesterol in chylomicron and VLDL remnants (enriched in apoB48). In Apoe<sup>−/−</sup> mice fed a Western type diet containing 21% fat and 0.2% cholesterol, the hypercholesterolemia was much greater than was found in the few subjects with human apoE deficiency (14–21). Heterozygotes had half as much plasma apoE, but very little difference from wild-type animals in their lipids or lipoproteins. Apoe<sup>−/−</sup> mice generated in the laboratory of Louis Havekes were reported in 1994 (26). When these heterozygous mice (also chimeric for C57BL/6 and 129) were stressed by the inclusion of 1% cholesterol and 0.5% sodium cholate in the diet, they exhibited a clear increment in serum cholesterol and VLDL contrasted to wild-type mice.

In all cases of Apoe deficiency, spontaneous atherosclerosis was noted at an age of 3–4 months in chow-fed animals, mostly in the proximal aorta with lesions at the origins of the coronary artery and also in the pulmonary artery (22, 24). The development of lesions was accelerated by feeding the Western type diet (22). More extensive analysis of lesions in these mice was reported (27–29). With age or increased time of feeding the atherogenic Western diet or an atherogenic diet containing 16% fat, 1.25% cholesterol and 0.5% sodium cholate, lesions were observed at many sites in large- and medium-sized arteries. The lesions in the atherogenic diet-fed animals appeared earlier and were larger than in the animals fed the chow diet. Lesions at various stages of development were seen; ranging from foam cell accumulation to fibrous plaques and lesions with necrotic cores and calcification. While in a few animals coronary lesions were noted, this was not a regular feature of this model. With advanced age or prolonged feeding of an atherogenic diet, lesions in the innominate artery (also called the brachiocephalic trunk) demonstrated intraplaque hemorrhage, probably from disruption of the fibrous cap that is usually associated with plaque erosion.

However, no thrombosis was seen (30, 31).

The two most widely employed mouse models of atherosclerosis are those lacking the Apoe gene and those lacking the Ldlr gene, which models familial hypercholesterolemia in humans (32). Investigators often regard these models as similar, though there are clear differences. The comparative features of these models are summarized by Getz and Reardon (33) and are briefly mentioned here. The two models differ in their lipoprotein profile. The predominant lipoproteins of Apoe<sup>−/−</sup> mice are remnant VLDL particles, rich in cholesteryl esters and apoB48. On the other hand, the predominant lipoproteins in Ldlr<sup>−/−</sup> mice are apoB100-containing LDL and VLDL. The VLDL in the Ldlr<sup>−/−</sup> mice is also richer in triglycerides than is the case for the Apoe<sup>−/−</sup> mice. Atherosclerosis in mouse models is driven by hyperlipidemia. For Ldlr<sup>−/−</sup> mice this requires the use of a cholesterol-rich diet with or without high fat, while in the Apoe<sup>−/−</sup> mice atherosclerosis develops while being fed chow, although the rate of lesion development is accelerated by the atherogenic diet. The distribution of lesions is similar across the vascular tree for the two models.

The atherosclerosis of apoE mice represents quite complex lesions, especially when studied in older animals on chow diet. Hepatic LDLR expression is critical for the clearance of apoB100- and apoE-containing lower density lipoprotein. Consequently transplantation of LDLR-expressing bone marrow to Ldlr<sup>−/−</sup> recipients has relatively little impact on the hyperlipidemia and the development and progression of atherosclerosis. In contrast, while apoE produced by the liver is important in regulating cholesterol and lipoprotein homeostasis, in addition, apoE produced by macrophages and other hematopoietic cells plays an important regulatory role in atherogenesis. Thus, in contrast to the Ldlr<sup>−/−</sup> mice, transplantation of apoE-expressing bone marrow into Apoe<sup>−/−</sup> recipients is able to repair both the hyperlipidemia and atherosclerosis.

**GENETIC APPROACH TO MEDIATORS OF ATHEROSCLEROSIS**

Even though the mutation of a single gene, Apoe, in an atherosclerosis-susceptible murine genetic background is sufficient to engender the development of spontaneous atherosclerosis, its pathogenesis as a complex inflammatory disorder involves the participation of many cells and genes [for example, see reviews by Lichtman et al. (34) and Wolf, Zirlik, and Ley (35)]. The lesions in the Apoe<sup>−/−</sup> mice are characterized by vascular inflammation associated with the infiltration of macrophages and other immune cells. They have been widely employed as a platform for the study of various potential mediators or inhibitors of atherosclerosis (Fig. 2). The findings have been summarized in two recent valuable reviews (36, 37). In the review by Stylianou et al. (36), the plasma lipid and atherogenic phenotype of a total of 66 gene knockouts on the Apoe<sup>−/−</sup> background are tabulated, a third of which are associated with changes in plasma lipid or lipoprotein levels, with the remainder showing an atherosclerosis phenotype without any change in plasma lipids compared with the Apoe<sup>−/−</sup> controls. When plasma lipids are decreased, lesions are almost invariably reduced. Apoe<sup>−/−</sup> mice have also been useful in identification of genes that influence regression of atherosclerotic lesions (38).

These gene knockout studies have involved the selection by the investigators of the specific genes to be studied. However a series of studies have adopted an unbiased approach to identify genes associated with atherosclerosis. The Apoe-null gene has been transferred into the genetic background of inbred strains of mice. Using aortic root lesions as indicators, the order of decreasing atherosclerosis susceptibility is DBA/[2] > C57BL/6 > 129 > AKR/J = BALB/c = C3H/HeJ (39–41). Apoe deficiency in the FVB background is also relatively resistant to the development of atherosclerosis (42). The segregation of quantitative trait loci (QTLs) associated with atherosclerosis has been sought by crossbreeding Apoe<sup>−/−</sup> mice in the atherosusceptible and atheroresistant genetic backgrounds, again summarized by Stylianou et al. (36). While many QTLs have been identified, each locus covers many genes, so the
identification of specific risk genes has been difficult and time consuming (43). These crosses have hitherto very seldom resulted in the identification of single susceptibility genes. However, in at least one case, a specific risk gene has been identified on chromosome 10b from a cross between C57BL/6 Apoe and the 129 strain (48). In the parental strains, the DBA/2J and 129/Ola ES cells used for the creation of the targeted Apoe-null gene may confound the phenotype analysis.

We have been interested in the fact that many genetic manipulations and pharmacological treatments have a site-selective effect on murine atherosclerosis (45), and have suggested that hemodynamic profiles may be major determinants of the selectivity. Of particular interest for this site-selective effect is the comparison of atherosclerosis in C57BL/6 Apoe/–/– mice and in the 129 Apoe/–/– mice (46). Though aortic root atherosclerosis develops more slowly in the 129 Apoe/–/– mice, this is not the case for aortic arch lesions, which develop earlier in the 129 strain than in the C57BL/6 Apoe/–/– mice. An intercross between these two strains indicated that QTLs for aortic root and aortic arch atherosclerosis were not the same and that a QTL for the aortic arch atherosclerosis overlapped with a QTL associated with the aortic arch curvature and likely affected the precise hemodynamics at that site (47). Additional QTLs differentially affecting aortic root and aortic arch atherosclerosis were identified in an intercross between ApoE/–/– mice in the DBA/2J and 129 genetic backgrounds (48). In the parental strains, the DBA ApoE/–/– mice had 10 times more aortic root atherosclerosis than the 129 ApoE/–/– strain (49), but lesions in the aortic arch were of similar size (48).

MURINE MODELS OF APOE ISOFORMS

As noted in the earlier segment, the functional properties of the human apoE isoforms are distinct. In order to probe this in a manageable experimental system, Maeda and her collaborators developed mice in which the endogenous murine apoE was replaced by one of the human isoforms (50). The APOE2 replacement mice exhibit a phenotype, both with respect to plasma lipoprotein accumulation and clearance and enhanced atherosclerosis, as is seen in human type III hyperlipoproteinemia (51). The APOE4 replacement mice exhibit a complex phenotype that includes increased plasma lipid levels with an accumulation of apoE-poor remnants relative to APOE3 replacement mice when fed a Western type diet and expressing high levels of the human LDLR. The mice are also more susceptible to the development of atherosclerosis. This phenotype is postulated to be attributable to the higher affinity of human apoE4 for the LDLR than is apoE3 so that apoE4 is sequestered on the surface of hepatocytes and not transferred to nascent lipoprotein. In addition, apoE4-containing VLDLs are not internalized. Together this leads to a decreased clearance of VLDL and increased susceptibility to atherosclerosis (52, 53). When fed the atherogenic diet containing cholate, all of the human APOE gene replacement mice exhibited more atherosclerosis than did wild-type C57BL/6 mice expressing murine apoE (51, 54). The extent of atherosclerosis was correlated with the level of VLDL retention in the plasma, with the most extensive lesions being found in the APOE2 replacement mice. In the absence of the LDLR, streptozotocin-induced diabetes in APOE4 replacement mice results in dyslipidemia and enhanced atherosclerosis in contrast to the absence of such findings in APOE3 replacement animals (55). In an alternative approach, murine apoE was “humanized” by taking advantage of the fact that, like human apoE4, mouse apoE has an Arg at residue 112, but unlike it, it has a Thr instead of an Arg at residue 61. In human apoE4, Arg112 allows for the domain interaction of Arg61 and Glu255. Thus, conversion of Thr61 to Arg by Weisgraber and colleagues results in a humanized apoE4-type lipoprotein phenotype (Fig. 1) (56). A heterozygote Arg61/wild-type (Thr61) apoE mouse fed an atherogenic diet has a lipoprotein phenotype similar to that in human apoE3/apoE4 heterozygotes with low levels of the apoE4-like protein in the plasma and

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Fig. 2. ApoE/–/– mice, APOE knockin mice, and hypoE mice have contributed to our understanding of pathogenesis of atherosclerosis and the genes and gene products involved, and have been used to develop models of coronary atherothrombosis and myocardial infarction. WTD, Western type diet.
its preferential association with VLDL compared with mouse apoE.

The creation of the Arg-61 apoE mice by Raffai and Weisgraber (57) involved the insertion of a neomycin gene between two loxP sites in intron 3 of the murine Apoe gene. The presence of the neomycin gene reduced the level of expression of apoE to 2–5% of the normal apoE levels, so these mice were designated as hypomorphic or hypoE mice (57). HypoE mice have a normal lipoprotein profile on chow diet. A cross between hypoE mice and Apoe-/- mice indicates that this low level of apoE is sufficient for efficient remnant clearance in chow-fed animals, but the mice are highly susceptible to diet-induced hyperlipidemia. Restoration of the expression of Arg-61 apoE by Cre recombinase-dependent excision of the neomycin marker results in a normal lipoprotein profile in the atherogenic diet-fed mice (57). A comparison of hypoE mice expressing either Arg-61 apoE or Thr-61 apoE revealed no difference in lipoproteins while on chow, but an increased hyperlipidemia and accelerated lesion development in hypoE-61 mice on an atherogenic diet with cholate (58). These differences were particularly clear in the early lesions in the aortic root and aortic arch. The inducible repair of the hypomorphic phenotype can promote regression and stabilization of atherosclerosis (59, 60). The hypoE mice and Apoe-/- mice were crossed with Ldlr-/- mice, with the genetic background of the mice being 85% C57BL/6 and 15% 129SvJ (61). Less atherosclerosis was seen in the hypomorphic animals on chow diet. Despite the difference in plasma apoE levels, total plasma lipid levels were comparable, but the Apoe-/-/Ldlr-/- mice had lower VLDL levels and higher LDL and HDL levels than the double knockouts. In addition, apoA-I was present in all lipoproteins in the Apoe-/-/Ldlr-/- mice, but in the presence of apoE in the hypomorphic mice, apoA-I was only in HDL. The HDL particles in the Apoe-/-/Ldlr-/- mice were enriched in apoA-I and were more potent in promoting cholesterol efflux from macrophages. This may be an additional mechanism for the anti-atherogenic functions of apoE beyond plasma lipid lowering.

CORONARY ATHEROSCLEROSIS AND MYOCARDIAL INFARCTION IN Apoe-DEFICIENT MICE

One of the features of the distribution of atherosclerosis in Apoe-/- mice was the low extent of coronary lesions, which were confined to the origins of these arteries. However, a few models of obstructive coronary lesions have been reported. Mice doubly deficient in apoE and the LDLR, when fed the Western type diet, develop significant hyperlipidemia with high levels of VLDL and LDL and obstructive coronary lesions and myocardial infarction and have a shortened lifespan (62). The expression of a urokinase transgene in macrophages in the Apoe-/- background results in premature mortality and myocardial infarction arising from obstructive lipid-rich proximal coronary lesions (63). A much more dramatic model of coronary atherosclerosis and myocardial infarction is seen in mice deficient in apoE and the HDL receptor SR-B1 (64). Chow-fed Apoe-/-/Srb1-/- animals exhibit profound occlusive coronary atherosclerosis, myocardial fibrosis, and cardiac enlargement and die by 8 weeks of age. The life time of these animals can be extended by treatment with probucol, an anti-oxidant drug, and, if given before 5 weeks of age, the cardiac pathology and red blood cell pathology is corrected (65). Also, adding hepatic lipase deficiency to this model to produce a triple knockout delays the onset of the pathology; such that the cardiac phenotype is similar in 6-week-old Apoe-/-/Srb1-/- mice and 9-week-old triple knockout Apoe-/-/Srb1-/-/Hl-/- mice (66). A similar phenotype of coronary heart disease is induced in Apoe-/-/Srb1-/- mice fed an atherogenic diet with cholate (67).

NON-HEPATIC APOE AND ATHEROSCLEROSIS

ApoE, in addition to being predominantly produced by the liver, is widely expressed in many other cell types, including macrophages, astrocytes, adipocytes, adrenals, and ovary (68–71). In all the studies of apoE pathology discussed above, global deficiency of apoE expression was involved. Apoe-/- mice have been very useful in the examination of the role of macrophage apoE on atherosclerosis (Fig. 3). As macrophages are core cells in atherogenesis, these cells and the bone marrow-derived monocytes that give rise to them have been the focus of much research in the last 20 years.

Monocytes/macrophages are the primary apoE-expressing cell derived from bone marrow precursors. The transplantation of bone marrow from wild-type mice (i.e., Apoe+/+) into Apoe-deficient recipients reduces plasma lipid levels to near wild-type levels and substantially reduces lesions in the proximal aorta (72, 73). This is accomplished with plasma levels of only 40 pg/dl, ~1–2% of the percentage of wild-type apoE levels (74). Crossbreeding of animals expressing human apoE only in macrophages with Apoe-/- mice also reduces plasma cholesterol. However, when normalized for plasma cholesterol, a reduction of atherosclerosis is observed, suggesting that macrophage apoE is atheroprotective beyond its influence on plasma cholesterol homeostasis (75). This suggestion is strongly reinforced by experiments with transgenic mice expressing apoE in the adrenals and crossed with Apoe-/- mice. Mouse lines were derived with different levels of plasma apoE (76), some of which had attenuated atherosclerosis even though they had no effect on plasma cholesterol.

These findings tell us two important things. Macrophage-derived apoE is not obligatorily required for atherosclerosis protection, and atheroprotection may be independent of plasma cholesterol control by apoE. However, in the hypomorphic Apoe model, conditional repair of the Apoe gene only in macrophages increases expression of macrophage apoE in atherogenic diet-fed mice, while reducing plasma cholesterol and atherosclerosis to similar extents (77). Interestingly, because the normal level of plasma apoE is 30 times that required for the remnant lipoprotein
clearance and atheroprotection, this suggests that plasma apoE has functions beyond lipoprotein clearance. So what are the functions of the apoprotein that require these physiological levels of the apoprotein in the plasma? Despite much research, this is not yet clear. One of the functions may be the promotion of cholesterol efflux from cholesterol-loaded cells. But this too is improved by low levels of the apolipoprotein (78). In any event, the reported influence of apoE on cholesterol homeostasis in macrophages is complex, depending upon how it is investigated. In cell culture, exogenous apoE promotes macrophage cholesterol efflux in an ABCA1-dependent fashion, but endogenous apoE is more efficient, especially in promoting ABCA1-independent efflux (79). In addition, apoE apparently interacts with heparin sulfate proteoglycans (HSPGs) on the cell surface and promotes efflux in a cell-autonomous and ABCA1-dependent fashion (80, 81).

Macrophage expression of apoE, not hepatic-derived apoE, appears to be required for in vivo reverse cholesterol transport (82), as demonstrated using macrophages from Apoe−/− mice. On the other hand, the apoE in plasma cholesterol acceptors may have a contrasting influence. apoE-containing HDL impairs ex vivo cholesterol efflux from cells devoid of apoE expression in an ABCA1-dependent fashion, especially when corrected for HDL particle number (83).

ApoE expression has other atherogenesis-relevant effects on the behavior of cells of the monocyte/macrophage lineage. Apoe−/− mice exhibit neutrophilia and monocytes that is increased upon feeding an atherogenic diet. This led to the discovery that apoE expression exerts a negative influence on proliferation of hematopoietic progenitor cells (HPSCs) in the bone marrow. The anti-proliferative effect is likely due to endogenous apoE bound to the surface of HPSCs interacting with ABCA1 and ABCG1 to promote lipid efflux, resulting in decreased signaling in response to growth factors (80). Monocytosis, particularly of the Ly6C+ subclass, is a risk factor for atherosclerosis (84). Total leukocytes and the Ly6C+ monocytes were decreased in the Apoe−/−/Ldlr−/− mice compared with the Apoe−/−/Ldlr+/− mice (61). This could be a response to an improved cholesterol efflux from the HPSCs in the hypomorphic mice in whom there was a redistribution of some of the plasma apoA-I from VLDL to HDL, making for a more efficient cholesterol efflux acceptor. The reduction in blood monocytes, especially of the Ly6C+ subclass, and a decrease in monocyte lipid content were associated with reduced early, but not late, atherosclerosis in the Apoe−/−/Ldlr−/− mice compared with the Apoe−/−/Ldlr+/− mice (61). This was accompanied by a reduction in lesion endothelial cell expression of the cell surface adhesion molecules, ICAM-1, PECAM-1, and JAM-A. Using a bone marrow transplantation approach in which Apoe−/− mice were transplanted with bone marrow from wild-type or Apoe−/− mice, Baitsch et al. (85) have reported that apoE promotes the conversion of macrophages from M1 to M2 phenotype. In vitro studies suggest that this is due to apoE interacting with apoE receptors on the cell surface of macrophages. Not to be outdone, Li et al. (86) demonstrated that monocytes and macrophages from Apoe−/− mice had reduced levels of miRNA146a, a miRNA that reduces NF-kB expression. The expression of even a small amount of apoE in monocytes and macrophages increased miRNA146a levels and attenuated the pro-inflammatory response of the cells, as demonstrated using the hypomorphic Apoe mice. Thus, these apoE models have contributed to our understanding of the anti-inflammatory functions of apoE.

While much research has focused on the monocyte/macrophage lineage, apoE also has actions in other processes and cells. For example, apoE produced by macrophages relieves the inhibition of eNOS by caveolin in endothelial cells by displacing the caveolin from its complex with the NO synthase (87). Exogenous apoE inhibits macrophage activation by TLR3 and TLR4 agonists through distinct mechanisms (88). There is also an effect of apoE on arterial smooth muscle cells that is most clearly demonstrated in the increased formation of neoimal expansion in Apoe−/− mice in response to endothelial denudation (89). The mouse strain dependence is unlike that for atherosclerosis, with wild-type FVB mice being much more susceptible to this injury-induced neointimal formation than wild-type C57BL/6 mice. The formation of the neointima involves the migration and proliferation of smooth muscle cells. The suppression of smooth muscle cell migration is mediated by interaction of apoE with LRP-1, while the suppression of proliferation is mediated by apoE interaction with HSPG.

In addition to its roles in lipoprotein clearance, cholesterol efflux, and modulation of inflammation, apoE also has anti-oxidative activity. Oxidative stress, especially oxidized LDL, is thought to be an important initiator of

**Fig. 3.** Apoe−/− and HypoE mice have been used to demonstrate the beneficial effect of apoE expression in the liver, macrophages, and adrenal gland on dyslipidemia and atherosclerosis. Note: Brain- and adipose tissue-expressed apoE do not repair atherosclerosis and dyslipidemia.
atherogenesis. As an indicator of increased oxidative stress in Apoe \(^{-/-}\) mice, a notable increase in the F2 isoprostane iPF2\(\alpha\)-VI was found in plasma and urine (90). Isoprostanes are an end product of the oxidation of arachidonic acid and are found within cells in the atherosclerotic plaque. The viral vector-mediated expression of human apoE3 in the liver of Ldlr \(^{-/-}\) mice substantially attenuated atherosclerosis without affecting plasma lipid levels (91). This was accompanied by a profound reduction of urinary, LDL, and aortic iPF2\(\alpha\)-VI.

**CONCLUSIONS**

After about 40+ years since apoE was first described and almost 25 years since the seminal description of Apoe \(^{-/-}\) mice as a model for the study of atherosclerosis, much has been learned about the many roles of apoE in systemic lipid metabolism, macrophage cholesterol homeostasis, and the less well-characterized influences of the apolipoprotein on other cells and processes relevant to atherogenesis through the use of the Apoe \(^{-/-}\) mice. The study of the human apoE isoform replacement mice has added to our understanding of these processes with particular relevance to the role of human apoE in cardiovascular physiology. Apoe \(^{-/-}\) models have also been developed for coronary artery occlusion and myocardial infarction. Recent studies of APOE isoform gene replacement and hypomorphic apoE variants have enhanced our understanding of the cell-specific role of apoE in atherosclerosis. The study of bone marrow from Apoe \(^{-/-}\) mice has highlighted the importance of monocytopoiesis as a risk factor for atherosclerosis. Yet in few of these cases do we have full clarity about the actions of apoE. There is indeed a great deal still to be learned, especially in relation to the time and concentration dependence of the apolipoprotein as it influences the evolution of atherosclerosis and its various phases.

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