Apolipoprotein E and Atherosclerosis
Beyond Lipid Effect

Jean Davignon

In humans, apolipoprotein E (apoE) is a polymorphic multifunctional protein. It is coded by three alleles (ε2, ε3, ε4) of a modulator gene (level, variability, and susceptibility gene) at the apoE locus on chromosome 19, determining six apoE genotypes and plasma phenotypes. Its pleiotropic effects are exerted on plasma lipoprotein metabolism, coagulation, oxidative processes, macrophage, glial cell and neuronal cell homeostasis, adrenal function, central nervous system physiology, inflammation, and cell proliferation.2,3 ApoE polymorphism modulates susceptibility to many diseases. It is, however, particularly notorious for its role in neurodegenerative disorders4 and atherosclerotic arterial disease.5,6 The ε4 allele (phenotypes E4/4 and E4/3) that is associated with higher low density lipoprotein cholesterol (LDL-C) is considered proatherogenic, whereas the presence of the ε2 allele (E3/2, E2/2), being associated with lower LDL-C levels, is deemed to have the opposite effect (although it may be associated with increased plasma triglycerides and lipoprotein remnants). This simple equation, however, is an oversimplification because these properties are subject to many environmental and genetic influences. ApoE has allele- and gender-dependent effects on reverse cholesterol transport, platelet aggregation, and oxidative processes that are likely to affect the overall atherogenic potential ascribed to modulation of lipoprotein metabolism.2,3,6 Notwithstanding the context dependency, a recent meta-analysis fully supports the presence of the ε4 allele as a significant risk factor for coronary artery disease.7 Several mechanisms have been evoked to link apoE with atherosclerosis, but the relationship is not fully unraveled in humans. Nevertheless, some apoE mimetic peptides that promote LDL clearance are currently tested in animals for potential clinical applications.8,9

See page 436

The situation is relatively simpler in animals. The mouse model has been prominently useful to test mechanisms of atherogenesis and apoE-driven atherosclerosis modulation. The apoE (−/−) mouse has been used to this end in a wide variety of experiments to study the effect of diet and drugs, the role of inflammation, oxidation, immunomodulation, coagulation, and plaque composition, as well as atheroma progression and regression.6,10 The apoE knockout mouse reproduces many of the features of human apoE deficiency and develops spontaneous atherosclerosis which is enhanced by a high-cholesterol diet to the extent that the animal exhibits xanthomas. In recent years, this model has been used to determine the threshold of circulating apoE above which atherosclerosis develops and whether it is possible to distinguish between lipoprotein effects and effects related to other antiatherogenic properties of apoE.6 The heterozygous apoE (+/−) mouse does not spontaneously exhibit the lipid phenotype of the apoE knockout mouse but remains susceptible to lipoprotein abnormalities on an atherogenic diet. Very little circulating apoE is necessary to rescue the apoE−/− phenotype. After transplantation of bone marrow from normal mice into apoE-deficient mice, apoE is detected in serum, promotes clearance of lipoproteins, normalized serum cholesterol concentrations, and is associated with virtually complete protection from diet-induced atherosclerosis.11 ApoE produced by macrophages, resulting in plasma levels ≈10% of normal (or ≈0.5 mg/dL), was sufficient to induce these changes. The source of circulating apoE is not a determinant factor. In apoE-null mice that express apoE in the adrenal glands at levels too low (<1% to 2% of wild type) to correct the hypercholesterolemia of their apoE−/− background, there is almost complete suppression of atherosclerotic lesion development.12 This implies that mechanisms other than improvement of the lipoprotein profile could account for the benefit of a small amount of circulating apoE but did not exclude effect of a change in a high turnover minor lipid component or modulation of other non-lipid abnormalities associated with apoE deficiency.13 Tangirala et al14,15 have shown that liver-specific adenoviral transfection of human apoE3 in LDL receptor−knockout mice markedly reduces atherosclerosis without improvement of the lipoprotein profile but with significant reduction of 8-iso-PGF2α-VI levels in LDL, arterial wall, and urine. This was a major contribution in support of the antioxidant properties of apoE and their relevance to atherosclerosis. Recently, Wientgen et al16 have expressed apoE in the adrenals of apoE-deficient mice resulting in subphysiological plasma levels and submitted them to femoral artery injury. Even when concentrations of plasma apoE were as low as 0.1% of that of the wild type, apoE had the ability to inhibit neointimal formation, but plasma cholesterol concentration, though half that of the apoE-null littermates, was still 6× higher than that of the wild type. Multiple regression analysis indicated that apoE but not cholesterol levels were significantly associated with mean intima-to-media ratio reduction before and after adjusting for apoE, giving further credence to a non-lipid effect of apoE.
Elsewhere in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Raffai et al. have taken a new approach to isolate the apoE antiatherosclerotic effect from the lipid effect in a normolipidemic mouse with a very low expression of an E4-like murine apoE variant (Arg-61 apoE) resulting in apoE levels 2% to 5% of normal.18 This hypomorphic apoeh/hMxl-Cre mouse is normolipidemic on a chow diet but develops hypercholesterolemia on a high-fat diet which is reversed when the chow diet is resumed. Hypercholesterolemia was induced by a high-fat cholesterol-rich diet supplemented with cholic acid that resulted in severe aortic atherosclerosis after 18 weeks. The animals were then placed on a regressive chow diet for 16 weeks but half were induced to express physiological levels of plasma apoE while the others stayed with their original low ApoE expression. Although plasma cholesterol concentrations fell rapidly to similar levels with elimination of the aortic foam cell layer in both groups, physiological levels of apoE enhanced removal of neutral lipids from the fibrotic core in the induced animals. This is of importance, first because the authors demonstrate for the first time that physiological levels of apoE can induce atherosclerosis regression independently of lowering of plasma cholesterol. Second, in earlier experiments macrophage-derived apoE expression in apoE null mice has halted atherosclerosis progression but had not caused regression as in this study.6 Regression has been observed, however, with human apoE gene transfer in the apoE-deficient/nude mice19 and with other types of intervention.20,21 Third, this new model provides good opportunities for further research into the mechanisms responsible for a non-lipid antiatherogenic effect of apoE. Indeed the study raises a large number of questions that will need to be addressed. The regression was mainly ascribable to a neutral lipid loss in the fibrotic core; was this associated with reduction of the other markers of plaque instability, including macrophage and T-cell number, increase in collagen content, changes in matrix metalloproteinases or their inhibitors, or any other subtle modification of the fibrotic component? How much of this beneficial effect of apoE results from its antioxidant properties? Measurement of markers of oxidation need to be done in future experiments. The same applies to inflammatory markers, helper T cell balance (Th1/Th2 ratio), macrophage and endothelial cell activation, as well as to many other established pleiotropic effects of apoE that are likely to affect the atherogenic process. Does the interferon induced by the pI:pC injections to activate Cre impact on atherosclerotic changes? What is the effect of such low levels of apoE on endothelial dysfunction, an early injury conducive to atherogenesis? Finally, an important mechanism worth studying in this model is the role of the apoE-LRP interaction. There is recent evidence that

![Diagram of Macrophage apoE secretion and antiatherogenic effects.](http://atvb.ahajournals.org/)

ApoE secreted after differentiation of monocytes into macrophages is associated with expression of the scavenger receptor type A (SR-A) and modulated by positive (sterols) or negative (cytokines) factors. Secreted apoE forms lipoprotein particles that promote reverse cholesterol transport, some containing apoAI (pre-β-LpAI, α-LpAI) whereas others are devoid of apoAI (γLpE, pre-β-LpE, αLpE). Cholesterol is thus mobilized from peripheral tissues towards the liver for excretion into the bile through the scavenger receptor B type 1 (SR-B1) or the remnant receptor (LDL-receptor related protein, LRP1). Although plasma cholesterol concentrations fell rapidly to similar levels with elimination of the aortic foam cell layer in both groups, physiological levels of apoE enhanced removal of neutral lipids from the fibrotic core in the induced animals. This is of importance, first because the authors demonstrate for the first time that physiological levels of apoE can induce atherosclerosis regression independently of lowering of plasma cholesterol. Second, in earlier experiments macrophage-derived apoE expression in apoE null mice has halted atherosclerosis progression but had not caused regression as in this study.6 Regression has been observed, however, with human apoE gene transfer in the apoE-deficient/nude mice19 and with other types of intervention.20,21 Third, this new model provides good opportunities for further research into the mechanisms responsible for a non-lipid antiatherogenic effect of apoE. Indeed the study raises a large number of questions that will need to be addressed. The regression was mainly ascribable to a neutral lipid loss in the fibrotic core; was this associated with reduction of the other markers of plaque instability, including macrophage and T-cell number, increase in collagen content, changes in matrix metalloproteinases or their inhibitors, or any other subtle modification of the fibrotic component? How much of this beneficial effect of apoE results from its antioxidant properties? Measurement of markers of oxidation need to be done in future experiments. The same applies to inflammatory markers, helper T cell balance (Th1/Th2 ratio), macrophage and endothelial cell activation, as well as to many other established pleiotropic effects of apoE that are likely to affect the atherogenic process. Does the interferon induced by the pI:pC injections to activate Cre impact on atherosclerotic changes? What is the effect of such low levels of apoE on endothelial dysfunction, an early injury conducive to atherogenesis? Finally, an important mechanism worth studying in this model is the role of the apoE-LRP interaction. There is recent evidence that
LRP, its ligand apoE, and the platelet-derived growth factor receptor cooperate in the remodeling of the vascular wall and protect against atherosclerosis.22,23

References

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