The study reported in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* by Rosenfeld et al.¹ is very important in experimental atherosclerosis for 2 reasons. First, the study shows that there is an available murine model that expresses many features of the human plaque that are very relevant to the pathogenesis of clinically significant disease. Second, the study shows that there is a site of the arterial tree other than the aortic root that (in the appropriate models) almost invariably develops a lesion that begins with the accumulation of foam cells and progresses to the advanced lesion of the sort shown in the study. Many recent studies of atherosclerosis report on results exclusively based on the aortic root, not a frequent site of clinically significant lesions in humans. The innominate artery is an alternate site for the study of the pathogenesis of atherosclerosis. There has been shown to be a high frequency of extensive lesions at this site in apolipoprotein E (apoE)–null mice by 24 to 28 weeks of age, even if these mice are maintained on a chow diet (Reardon et al, unpublished observations, 2000), and this could be shortened if the mice are fed a Western-type diet. This makes it a site worthy of study even for shorter-term experiments. Although the aortic root may be easy to study, its lesion responses are not invariably reflective of the situation elsewhere in the vascular tree. We note that the aortic root and the innominate artery respond differently to immune deficiency (see below). It has recently been shown that the aortic root responds differently to probucol than do more distal regions of the aorta.³

The lesions that Rosenfeld et al.¹ have described have many, though not all, of the signal features of the advanced human atherosclerotic plaque. They have examined male animals aged 8 to 14 months maintained throughout on chow. In many of the arteries, the lumina are substantially and in some cases almost completely occluded. Much of the lesion area is occupied with a fibrofatty nodule consisting of an acellular core with cholesterol crystals and fibrous scarring, which may be attributable to the chondrocyte-like cells seen frequently in these plaques. These acellular areas are often flanked by xanthomatus collections of foamy macrophages in the shoulders of the lesions. Many of these lesions contain hemorrhage, which is considered transient because it is not accompanied by signs of thrombosis despite the presence of much potentially prothrombotic matrix and, in some cases, the loss of endothelium; ie, no platelets and little fibrin were noted. The hemorrhage, as illustrated in the article of Rosenfeld et al.,¹ is in the base of the lesion or appears to be associated with the lateral xanthomata. Although these are static observations, there appears to be evidence that the fibrous cap thins as the animals and the lesions age. Finally, the authors note medial changes that they term medial atrophy with erosion of the lesion into the media.

In recent years, much attention has been paid to abrupt changes that may occur in the plaque and thus lead to acute coronary syndromes. Such changes are most likely to occur in the potentially unstable advanced plaque. Among these changes are hemorrhage, which could acutely expand the plaque, and its obstructive effects: rupture, erosion, or ulceration of the surface of the plaque; in each case, the underlying highly thrombogenic matrix components are exposed,⁴ and this frequently leads to a catastrophic thrombosis. Several of the findings of Rosenfeld et al.¹ suggest plaque instability. The thinning of the fibrous cap, the loss of the endothelium in some cases, the occasional evidence of endothelial rupture, the presence of lipid-rich shoulders of the lesion, and the occurrence of hemorrhage within the lesion are all signs that this model presents many of the features of unstable plaque. However, the exposure of the subendothelial matrix, which should be highly thrombogenic, is not accompanied by thrombosis in this model. Nor is there much of a sign of fibrin or platelet deposition despite the hemorrhage. It is not clear why this model does not progress to thrombosis despite the presence of most of the preconditions. Some aberration in the regulation of the coagulation cascade or of the regulation of fibrinolysis in the apoE-null mice may contribute to this lack of thrombosis. Fibrin is not required for the development of the apoE-deficient lesion even when more complex lesions are examined.⁵ On the other hand, the presence of plasminogen and its activation seem to be required for development of the full lesion in these animals.⁶ The lack of thrombosis is surprising in this model, given previous observations that apoE inhibits platelet aggregation in vitro in response to a variety of agonists⁷ and that this effect appears to be receptor-mediated.⁸

The origin of the hemorrhage in these arteries is not clear. It could either arise from some continuity with the lumen of the artery, the channel for which is not always obvious, although loss of endothelium was observed in some cases, and endothelial disruption with emerging macrophages is seen on occasion. In the preparation of the tissue, the arterial tree was perfused with lactated Ringer’s solution for only 20 seconds before fixation was initiated. If there is a functional continuity with the lumen, especially in those cases in which the red blood cells are seen in the shoulders of the lesion, one wonders whether a longer Ringer’s perfusion might have “washed out” some of this hemorrhage giving “functional”
evidence of this continuity. An alternate source of the hemorrhage is the new vessels formed in these advanced plaques. It has been strongly suggested that new vessels increase in the intimal lesion as it expands in apoE-deficient mice. As these vessels increase in number, the possibilities of their disruption might increase, perhaps under the influence of the proteases that are found especially in the macrophage-rich shoulder areas.

In our study (Reardon et al, unpublished observations, 2000), we described lesions in the innominate artery of the male apoE-deficient mouse at 10 months of age also maintained on chow, and our morphological observations are strikingly similar to those described in the study of Rosenfeld et al. The only difference is that we did not observe the obvious hemorrhage described by these authors. Furthermore, we noted the same changes in apoE-deficient animals that are also immune deficient (ie, Rag 2 deficient), with respect to the size and the character of the intimal lesions. This is in contrast to the situation in the aortic root of the same animals, in which we found a substantial reduction in lesions in the immune-incompetent mice. However, we did differ with Rosenfeld et al in the interpretation of the medial changes. The media of the innominate artery in the mouse is made up of 4 or 5 lamellae, and we and Rosenfeld et al regularly observed an apparent expansion of the inner 1 to 3 lamellae, often containing increased amounts of collagen. This lamellar expansion is found under the lesion but not in the portion of the arterial circumference not containing the lesion, and it is most often overlaid by an apparently intact internal elastic lamina and even intact inner elastic laminae. We also frequently observe this expansion of the inner lamellae in young apoE-deficient animals before significant lesions were observed in these arteries. Like the expansion of the advanced lesions in the arteries of younger animals, it is not seen circumferentially but only on the face of the artery where the plaque will develop. The expansion is also seen in the left carotid and the left subclavian, as well as on the inner curvature of the aorta at early time points. Therefore, we believe that this change is much more likely to be a response to the hyperlipidemia or the altered metabolism of these mice rather than a reaction to the presence of the plaque. This possibility makes it an attractive observation worthy of more study, especially on the cellular and matrix composition of these lamellae in the context of hyperlipidemia.

As valuable as this apoE-deficient model may be, it is important to repeat these long-term studies in other mouse models of atherosclerosis. The lipoproteins of the apoE-null mice are unlike those seen with most human atherosclerosis, in which LDL elevation is probably the most important contributor to the atherosclerosis. The remnants that predominate in the apoE-deficient mouse also have an abnormal lipid composition with a high ratio of sphingomyelin to lecithin. The local action of apoE in the plaque is likely to be very relevant given the large amount of data that are accumulating on the evolution of the aortic root lesion as a result of the transgenic or transplantation exchange of apoE-expressing or -deficient monocyte/macrophages. Smooth muscle cells in culture and neointimal formation in the injured vessel wall are influenced by lipid-free apoE. Also, a recent article in *Arteriosclerosis, Thrombosis, and Vascular Biology* shows that levels of apolipoprotein expression that are insufficient to modify plasma lipid levels nevertheless have a substantial impact on the progression of aortic atherosclerosis. All of these observations provocatively point to the likely local effect of apoE. Perhaps the best LDL model for a comparable study is the apo bec/LDL receptor double-knockout model, which develops an elevated LDL and advanced atherosclerosis while being maintained on chow.

Finally, an attractive aspect of these studies is that they present the possibilities for further exploration of the factors that contribute to the progression of the lesions from the intimal xanthomata to the advanced plaques described in the study of Rosenfeld et al. The complex lesion with a compromised endothelial barrier may present a unique opportunity for the in vivo virus-mediated gene transfer to modify this pathogenesis. For example, the factors that account for the death of the lesion macrophages, the chondrocyte differentiation, the thinning of the fibrous cap, and the disposition of the hemorrhage within the lesion would all be worthy of further study. With a lesion that develops with great regularity in this particular site, such investigations should provide valuable new experimental information.

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2. Deleted in proof.


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