Rupture of advanced human atherosclerotic plaques can precipitate coronary thrombosis, myocardial infarction, and sudden death, but insights into the causes and treatment of plaque rupture have been hampered by lack of a suitable animal model. Particularly desirable would be a model of plaque rupture that would take advantage of current and forthcoming mouse mutant alleles. It is therefore of considerable interest that the study by von der Thüsen et al1 in this issue of Circulation reports the characterization of a model induced plaque rupture in apolipoprotein E–deficient (ApoE^{-/-}) mice secondary to cap thinning produced by overexpression of p53, a proapoptotic stimulus for plaque smooth muscle cells (SMCs).

See p 2064

Vulnerable Atherosclerotic Plaques

Erosion, ulceration, or rupture of the surface of atherosclerotic plaques exposes highly thrombogenic components in the interior of the lesion. SMC apoptosis, loss of extracellular matrix (ECM) integrity, and inflammatory cell accumulation in the fibrous cap are thought to be important pathogenic factors leading to plaque instability.2 Previous attempts to produce an animal model for plaque rupture have been less than optimal. The ability to carry out genetic analyses in mice, together with existing mouse models for atherosclerosis, suggests that a mouse model of plaque rupture would be extremely valuable. Unfortunately, atherosclerotic plaques in mice generally are regarded as resistant to plaque rupture. However, the ability to carry out genetic analyses in mice, together with existing mouse models for atherosclerosis, suggests that a mouse model of plaque rupture would be extremely valuable.

Taken together, the studies described above show that long-term maintenance of ApoE^{-/-} mice can generate lesions in the innominate artery that exhibit thin fibrous caps and a variable incidence of cyclic breakdown and healing that leads to intraplaque hemorrhage. However, the long duration of experiment, the expense required for mouse maintenance for ≥1 year, and the variable incidence of plaque rupture make these models less desirable as a convenient animal model of atherosclerotic plaque rupture.

p53-Mediated Plaque Smooth Muscle Apoptosis

The present approach described by von der Thüsen et al1 was based on the observations of Bennett et al6 that human coronary plaque SMCs are predisposed to p53-mediated apoptosis. The authors used a method previously developed in their laboratory to produce accelerated atherosclerosis in a site-specific manner by placement of a restrictive silastic collar around the common carotid artery of ApoE^{-/-} mice. 7 Collar-induced lesions in ApoE^{-/-} mice are monocytic foam cell lesions at 3 weeks that progress to lesions with a necrotic core and a fibrocellular cap by 6 weeks. To induce conversion to vulnerable plaques, adenoviral vectors expressing either full-length human p53 driven by a cytomegalovirus (CMV) promoter (Ad5-CMV.p53) or a β-galactosidase-expressing reporter gene (Ad5-CMV.lacZ) were introduced into the common carotid artery of ApoE^{-/-} mice bearing collar-induced lesions. LacZ staining suggested efficient infection of endothelial cells and innermost SMCs of lesions that develop just proximal to the collar. Overexpression of wild-type p53, but not lacZ, produced a decrease in cell proliferation and an increase in fibrous cap cell apoptosis as early as 1 day after infection. Most striking were the reductions in cap thickness attained by 14 days after Ad5-CMV.p53 infection, which were characterized by a reduction in collagen content and a loss of cap SMCs. The lesions produced had structural features resembling those used to define unstable plaques in hemorrhage, accompanied by loss of the fibrous cap and fibrotic conversion of the necrotic core, was found in plaques located in the innominate artery (called brachiocephalic artery by Johnson and Jackson).3 By 60 weeks, very thin fibrous caps were present with a discontinuous endothelium and occasional exposed macrophage foam cells suggestive of plaque erosion. Surprisingly, in neither case were platelet-rich fibrin thrombi prominent features of the lesion. The fact that ApoE^{-/-} mice are capable of generating such thrombi was shown in a study by Reddick et al, in which abdominal aortic plaques were deliberately disrupted by gently squeezing the plaque-bearing aortic segment in situ between blunt forceps. Such a mechanical injury exposed foam cells and plaque contents to the circulation, leading quickly to the formation of platelet aggregates and platelet-rich fibrin clots.

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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(Circulation 2002;105:2010-2011.)

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Circulation is available at http://www.circulationaha.org

DOI: 10.1161/01.CIR.0000016964.88228.E0

2010
human coronary arteries. Although spontaneous rupture of these p53-induced lesions was rare (2 of 16 lesions), the incidence of plaque rupture was increased to 8 of 20 lesions after intravenous injection of the vasopressor compound phenylephrine (dose sufficient to raise arterial pressures at least 15 mm Hg for at least 15 minutes). No ruptured plaques were found in the contralateral carotids that were exposed to Ad5-CMV.lacZ and phenylephrine. Unfortunately, the brachiocephalic artery was not examined. Histological criteria for plaque rupture included findings of intraplaque hemorrhage, cap breaks with extrusion of core contents, intracarotid thrombosis, and phagocytosis of erythrocytes by macrophages within the lesion. The authors report that 3 of 16 plaques from Ad5-CMV.lacZ-treated vessels and 8 of 20 plaques from Ad5-CMV.p53-treated vessels exhibited some combination of these histological features.

**Strengths and Weaknesses of the Current Model**

Von der Thüsen et al describe an approach to produce unstable lesions with thin fibrous caps in mice over a short treatment period and at a predetermined arterial site. On introduction of a defined hemodynamic stimulus (phenylephrine injection), ~40% of such lesions exhibited multiple histological end points suggestive of plaque rupture. As such, it is an attractive model with several advantages over previous reports of unstable plaques in mice that required long durations for their appearance. Unfortunately, the lesions described by von der Thüsen et al evidently exhibited little or no fibrin formation or development of fibrin-rich platelet clots. In that respect, they resemble the unstable lesions in ApoE<sup>−/−</sup> mice described by Rosenfeld et al and Johnson and Jackson but not those characteristic of ruptured human vulnerable plaques. Moreover, the criterion of intraplaque hemorrhage cannot necessarily be assumed to reflect plaque rupture. Moulton et al reported that lesions developing in ApoE<sup>−/−</sup> aortic sinus are dependent on an intraplaque capillary supply. If the carotid lesions described by von der Thüsen et al also developed intraplaque capillaries, then disruption or leakage of these microvessels after phenylephrine injection could be an important source of intraplaque hemorrhage. In addition, the use of restrictive collagen-induced accelerated atherosclerosis and overexpression of p53 by adenoviral gene transfer introduce requirements for survival surgery and carotid viral instillation steps in the experimental protocol that are not readily amenable to high-throughput screens. Furthermore, the mechanism for cap thinning after infection with Ad5-CMV.p53 remains to be clarified. On the basis of studies using p53-deficient mice, Guevara et al showed that p53<sup>−/−</sup>/ApoE<sup>−/−</sup> mice had larger lesions than p53<sup>+/−</sup>/ApoE<sup>−/−</sup> mice, primarily because of increased cell proliferation with no evidence for reduced rates of apoptosis. The opposite results were found in p53<sup>−/−</sup>/APOE<sup>−/−</sup>3-Leiden transgenic mice, where the absence of p53 in lesion macrophages was found to correlate with reduced rates of macrophage apoptosis. Thus, considerable work remains to be done to clarify the relative roles of cell proliferation, cell death, protease production, and inflammatory cell activity in cap thinning after introduction of a p53-expressing adenoviral vector into preexisting atherosclerotic plaques. The requirement of phenylephrine treatment to induce plaque rupture most likely reflects the action of biomechanical strain on a structurally weakened fibrous cap. Phenylephrine is a synthetic α<sub>1</sub>-adrenergic agonist with an unusually prolonged duration of vasopressor effects. It remains to be seen whether or not more physiological stimuli for hemodynamic strain on the lesion will produce effects on plaque stability similar to those reported by von der Thüsen et al.

**Summary and Future Directions**

An efficient and reproducible mouse model of atherosclerosis that exhibits the salient features of spontaneous human plaque rupture would be a welcome advance. The model described by von der Thüsen et al is an important step toward that goal. Additional studies of the mechanisms underlying p53-induced thinning of the fibrous cap will lead to improvements in current protocols and more efficient induction of unstable experimental lesions. An important component of human plaque rupture that remains elusive in widely used mouse models of atherosclerosis is the formation of platelet-rich fibrin clots. Future studies will build on the findings of von der Thüsen et al and Reddick et al to develop mouse models that more fully represent the cascade of events that follow destabilization and rupture of advanced human atherosclerotic plaques.

**References**


**Key Words:** Editorials ■ fibrin ■ apoptosis ■ apolipoproteins ■ plaque
Mouse Model for Atherosclerotic Plaque Rupture
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Circulation. 2002;105:2010-2011
doi: 10.1161/01.CIR.0000016964.88228.E0
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/105/17/2010

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