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Rupture of the Atherosclerotic Plaque
Does a Good Animal Model Exist?

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Abstract—By its very nature, rupture of the atherosclerotic plaque is difficult to study directly in humans. A good animal model would help us not only to understand how rupture occurs but also to design and test treatments to prevent it from happening. However, several difficulties surround existing models of plaque rupture, including the need for radical interventions to produce the rupture, lack of direct evidence of rupture per se, and absence of convincing evidence of platelet- and fibrin-rich thrombus at the rupture site. At the present time, attention should therefore focus on the processes of plaque breakdown and thrombus formation in humans, whereas the use of animal models should probably be reserved for studying the function of particular genes and for investigating isolated features of plaques, such as the relationship between cap thickness and plaque stability. **(*Arterioscler Thromb Vasc Biol*. 2003;23:535-542.)**

Key Words: atherosclerosis ■ plaque rupture ■ pathophysiology ■ animal models

Most of the middle-aged and elderly population in the developed world and in many parts of the developing world have atherosclerosis, and about a quarter will die of it. Much has been achieved by conventional measures to prevent the development of atherosclerosis, but the outcome of persons suffering a coronary occlusion has not changed that much over the years. Nearly half of all people who develop a first acute myocardial infarction will be dead within a month. For this reason we urgently need to understand what leads to the fatal event and to identify and test treatments that prevent it from occurring. Up to quite recently, it was assumed that the risk of death was directly related to the burden of disease: the greater the extent of atherosclerosis, the higher the risk. About 10 years ago, a paradigm shift occurred when it was realized that the fatal complications of atherosclerosis, myocardial infarction, and stroke do not necessarily occur in those with the heaviest burden of disease. Rather, acute blockage of an artery in the brain or heart is often caused by a clot that occurs at the site of rupture of a so-called "vulnerable plaque." Such vulnerable plaques consist of a lipid-rich thrombogenic core that is separated from the arterial bloodstream only by a slender and fragile layer of tissue, the

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fibrous cap. These lesions need not be large, nor need they be particularly old. No longer is the final event seen as "the straw that breaks the camel's back," the last link in an inexorable process taking place over a very long time, but as a catastrophe resulting from an acute imbalance of stabilizing and destabilizing forces within the lesion. Such ruptures recur over many years but do not cause usually complete occlusion of the vessel, resulting instead in mural thrombi that are incorporated into the plaque. As a result, rupture of the coronary vasculature is often clinically silent. In addition, thrombosis may occur at the site of an eroded atherosclerotic plaque even without a tear in the fibrous cap of the lesion.[1]

The search for an animal model of atherosclerotic plaque rupture should be seen against this background. Also, it should be remembered that it has never been easy to find a good animal model of the atherosclerotic process per se. Atherosclerosis is a disease that really only affects humans and 1 or 2 other species, such as the pig and certain primates, with the result that researchers have had to resort to genetically modified models to even partially reproduce the condition.

Several reviews dealing with plaque instability in animal models have been published within the last few years,[2–5] including 1 in this journal.[6] The Macrophage Function and Stability of the Atherosclerotic Plaque (MAFAPS) consortium was established as part of the Fifth Framework Program of the European Union with the aim of better understanding the role of the macrophage in plaque stability.[7]

In the present work, the members of the MAFAPS consortium will not attempt to duplicate these reviews, but rather, in the light of their own research experience, describe their particular view of the current state of play in the search for a viable animal model of plaque stability. The MAFAPS consortium will also give its impression of the direction in which research in this area might proceed in the future.

Why Do We Need a Good Animal Model of Plaque Rupture?

By its very nature, rupture of the atherosclerotic plaque is difficult to study in humans. If at all, its presence in the living person can be divined only indirectly by means of angiography or by newer techniques, such as fast multi-slice computerized tomography. Because rupture of the atherosclerotic plaque occurs in a stochastic fashion, it is also exceedingly difficult to identify trigger factors and equally hard to investigate treatments that directly stabilize the plaque.

A good animal model would help us not only to understand how rupture occurs but also to design and test treatments to prevent it from happening. In addition, animal models generally have the advantage of a shorter time to lesion development and allow the investigation of pathology in a large number of individuals.

Importance of Plaque Rupture in Humans

Plaque Rupture and Thrombosis

As recently pointed out elsewhere,[1] in 1858 the incomparable Rudolf Virchow noted that the term "atheroma" is borrowed from the description of a dermal cyst, that is, a fatty pultaceous mass ("Grützbalg") enclosed within a fibrous capsule.[8] The current paradigm of plaque rupture suggests that this capsule can rupture, exposing the thrombogenic core, analogous to the bursting of a cyst or abscess. This interpretation is based both on autopsy[1,9–11] and angiographic[12,13] studies. This model has undoubtedly been of great use in conceptualizing the pathophysiology of myocardial infarction in particular. However, the charm of its compelling imagery should not blind us to important contradictions that exist within it. These have been outlined in recent excellent reviews by Virmani[1] et al and Badimon[3] and are summarized in Table 1.

TABLE 1. Contradictions That Exist Within the Existing Paradigm of Plaque Rupture

1. Autopsies have shown that a substantial minority of victims of sudden coronary death show superficial plaque erosion without plaque rupture.[15,87–90]
2. In more than 200 cases of 'sudden coronary death,' only one third of all lesions showed signs of plaque rupture. Even among those lesions with thrombi, only about two thirds showed rupture. Many of the thrombotic nonruptured lesions did not even show infiltration of macrophages and lymphocytes as signs of inflammation, displaying instead abundant smooth muscle cells in a proteoglycan-rich matrix.[9,11,15,91]
3. Although plaque disruption accounts for up to two thirds of patients in whom unstable angina or acute myocardial infarction develops, in most cases plaque disruptions are asymptomatic, although they may contribute to the growth of the atherosclerotic plaque.[3]
4. In addition to rupture or erosion of an atherosclerotic plaque, the occurrence, composition and size of a thrombus are regulated by mechanical hemodynamic effects, the thrombogenicity of the arterial surface, the relative concentration of cellular blood components, and the efficiency of fibrinolysis.[3]

Based on current research, therefore, 2 things are clear. First, thrombotic occlusion of a coronary artery may occur without rupture of an atherosclerotic plaque. Second, rupture of an atherosclerotic plaque within a coronary artery does not usually lead to thrombotic occlusion of that artery.

What Are the Factors Governing Plaque Stability?

Despite the absence of a clear-cut link between plaque rupture and occlusive thrombosis, there is no doubt that a defect in the surface of the atherosclerotic plaque is almost always associated with mural thrombosis, which at the least leads to a growth of the atherosclerotic plaque. It is therefore important to examine the current state of knowledge on factors regulating plaque stability. This information will be important later when we come to consider the applicability of currently available animal models.

The atherosclerotic plaque is not a static structure. Rather, its status at any given moment is the result of the complex and dynamic interplay of a very large number of cellular and humoral factors. Here, we will confine ourselves to the factors thought to affect the stability of the fibrous cap, the distinct layer of connective tissue overlying the lipid core of the vulnerable lesion.[1]

The acellular component of the fibrous cap consists largely of filamentous type I collagen and proteoglycans. However, other forms of collagen, such as reticular type VIII collagen, are also present, particularly in the so-called shoulder regions where the cap joins the remainder of the arterial wall. These

TABLE 2. A Proposed List of Ideal Features of an Animal Model of Plaque Rupture

1. The atherosclerotic process in the animal model should be histologically identical to that in humans.
2. The atherosclerotic plaque in the animal model should show the same vulnerability to rupture as its human counterpart.
3. The events leading to vulnerability and rupture: cap/core ratio, cellular composition, collagen production and breakdown, should as far as possible be identical to those in humans.
4. Plaque rupture in the animal model should occur without the need for manipulations such as cuffing of the artery or insertion of balloon catheters.
5. Plaque rupture should at least in some cases be accompanied by the formation of platelet-rich fibrin thrombi.
6. Response to treatment in the animal model should have the potential for duplication in humans.
7. The species should be available for research and easy and economical to maintain, and plaque rupture should occur under reproducible conditions within a reasonable time frame.

are the regions where fissures and tears are most likely to occur. The fibrous cap is said by some authorities to be "thin" when it is less than 65 μm in thickness.[1]

The cellular composition of the fibrous cap may vary considerably. It may be rich in inflammatory cells, chiefly T lymphocytes and macrophages, and may contain many smooth muscle cells, or it may contain almost no cells at all.

Erosion of the fibrous cap is said to be present when endothelium is absent and the exposed intima consists mainly of smooth muscle cells and proteoglycans. Typically, eroded fibrous caps do not show marked signs of inflammation.[14,15]

What Causes Atherosclerotic Plaques to Rupture?

The reasons for thinning and rupture of the fibrous cap are not known. Two main hypotheses have been proposed. The first is that rupture is a result of loss of smooth muscle cells, thought to be the main producers of cap-stabilizing collagen. This is caused by the loss of cells from apoptosis. Bennett and others[16,17] have suggested that such apoptosis may be mediated by the interaction between smooth muscle cells and monocyte/macrophages. Apoptosis of smooth muscle cells has also been shown to be promoted by chymase produced by mast cells, which are present in the plaque in significant numbers.[18–21] It may even be the case that smooth muscle cells in the atherosclerotic plaque undergo apoptosis by default unless their viability is maintained, for example, by the presence of insulin-like growth factor 1 in the interstitial milieu.[22] In addition to undergoing apoptosis, smooth muscle cells within the plaque are very slow to divide[23–25] and stop proliferating altogether after 2 to 5 passages.[26]

The second hypothesis is that plaque rupture is the result of an imbalance between the production of plaque-stabilizing collagen on the one hand and the action of corrosive enzymes on the other. These enzymes are present in the form of metalloproteinases, which derive mainly from macrophages[27] and cathepsins, which are produced by smooth muscle cells.[28,29] There is much experimental evidence for the presence of active metalloproteinases, chiefly matrix metalloproteinases 1 and 9, within the atherosclerotic plaque.[30–32] However, it is not entirely clear how these metalloproteinases are regulated. Tissue inhibitors of metalloproteinase activity are widespread within the lesion.[33] Moreover, macrophages have been shown not only to produce collagen-degrading enzymes but themselves to be a source of collagen.[34] Finally, mast cells are sources not only of degrading enzymes,[35,36] but also, like macrophages, of collagen.[37]

Animal Models of Atherogenesis and Plaque Rupture

Much has been achieved with the use of animal models, particularly in unraveling the function of particular genes. Animal models have been of less use, however, in understanding the pathophysiology of human disease. From the foregoing, it is clear that plaque rupture in humans and, more importantly, the thrombotic occlusion of the artery that sometimes accompanies it, are the final events in a long and complex pathophysiological process that is not completely reproduced in any other animal. In assessing animal models of plaque rupture, it is therefore useful first to consider what would be the ideal features of such a model (Table 2).

It is clear that it will be very difficult if not impossible to find an animal model that exhibits all of the features shown in Table 2. Nevertheless, it is sobering to reflect on how few of these features are exhibited by most of the existing models. Over the years, many animal models of atherosclerosis have been developed. In latter years, models of plaque rupture have also begun to emerge. Each of these models has specific advantages and disadvantages, as will be described in the following sections.

Nonmouse Animal Models of Atherogenesis

Rabbits develop lipid-rich arterial lesions with some of the features of atherosclerosis only if they are fed large amounts of cholesterol and fat—components that are usually lacking in their vegetarian diet. Indeed, it was in cholesterol-fed rabbits that aortic cholesterol accumulation was first noted by Anitschkow in St. Petersburg 90 years ago.[38] Such diets result in cholesterol levels many times greater than those seen in humans. The lesions that rabbits develop bear only a superficial resemblance to human atheroma, being more fatty and macrophage rich.[3]

White Carneau pigeons develop lesions that are morphologically and ultrastructurally more similar to human atherosclerosis.[39–42] However, in contrast with humans, susceptibility to atherosclerosis in these birds lies entirely at the level of the arterial wall. Cholesterol levels are normal and other risk factors are absent,[39] with the lesions in the pigeons thought to be entirely the result of an inherited[43] defect in cholesterol efflux from macrophages.[44,45]

On a high-cholesterol diet, primates, including chimpanzees, squirrel monkeys, howler monkeys, rhesus monkeys, and cynomolgus monkeys, develop a form of atherosclerosis that is very similar to that of humans.[46–49] However, the cost of primates is prohibitive, and many of these species are protected. Thus, work on atherosclerosis in primates is today generally confined to the study of complex issues, such as the effects of psychological stress.[50]

The pig is one of the most useful currently available animal models of atherosclerosis. In time, pigs develop atherosclerosis even on a normal porcine diet.[51–55] When fed cholesterol, they develop plasma cholesterol levels and atherosclerotic lesions that are similar to those seen in humans. The white Belgian pig variety also exhibits sudden coronary death when under stress.[3] However, maintenance of pigs is expensive and difficult, requiring special facilities that are beyond the capabilities of most laboratories.

Dogs and rats are generally very resistant to atherosclerosis and only develop it when their diets are extensively modified.[3] In recent years, however, some transgenic rat models have been produced that develop lesions resembling atherosclerosis.[56–59]

Of Mice and Men, or Why Small Is Not Always Beautiful

Because of ease in handling, the wide knowledge base concerning mouse physiology, and the large amount of mouse genetic information available, most researchers in recent years have focused on mouse models for the study of atherosclerosis.[60–70]

Before proceeding to a description of the individual models, it is important first to recall the fundamental limitations of the mouse model. Mice do not develop atherosclerosis without genetic manipulation. They have a lipid physiology that is radically different from that in humans, most of the cholesterol being transported in HDL-like particles. Furthermore, mice weigh about 25 g, some 3000 times less than the average human. Because mouse cells are about the same size as human cells, this means that a section of coronary artery in the mouse contains about 3000 times fewer cells than an equivalent section of human coronary artery. This is reflected in the histology of mouse arteries, in which the endothelial layer lies directly on the internal elastic lamina and the media consists of only a few layers of smooth muscle cells. In contrast with their counterparts in humans, atherosclerotic lesions in the mouse coronary artery often extend beyond the elastic lamina.[64] Remodeling of the media and aneurysms are also common in mice.[71–74] Furthermore, it is difficult in mice to make a distinction between plaque erosion, as defined by endothelial denudation, and complete rupture of the fibrous cap.[64] Although classic eccentric atheromas with a single fibrous cap exist in lesion-prone mouse models, multiple necrotic core areas with or without separate fibrous caps are the norm.[66,75,76] As pointed out by Calara and colleagues,[64] disruption of these lesions may not mimic plaque rupture in humans, placing a fundamental limit on the applicability of mouse models for investigation of rupture mechanisms.

In addition to these difficulties arising from the differences between mouse and human biology, there are important issues that need to be remembered in interpreting the results obtained in mouse models that have been derived by genetic manipulation. Problems may, for example, occur when 2 different genetic models of a particular illness are used to investigate the effect of a third genetic manipulation. An important example in the field of atherosclerosis research concerns studies investigating knocking out the gene for the type A scavenger receptor in different genetic models of atherosclerosis. Suzuki and colleagues[77] reported that deleting this scavenger receptor in apoE knockout mice reduced atherosclerotic lesion size by 60%. However, de Winther et al[78] found that in the apoE3 Leiden mouse model of atherosclerosis, inactivation of the scavenger receptor actually increases lesion size. A possible explanation for this difference relates to the role of apoE in the vessel wall. ApoE has been shown to mediate efflux of cholesterol from macrophages, and it is therefore possible that deficiency in apoE predisposes to macrophage foam cell formation. This process of foam cell formation might be expected to be inhibited by deletion of the scavenger receptor, the main route by which cholesterol loading of macrophages occurs. By contrast, macrophages from mice bearing the apoE3 Leiden gene show normal apoE-mediated cholesterol efflux, so that scavenger receptor-mediated cholesterol uptake does not lead to enhanced foam cell formation, allowing other presumably antiatherogenic functions of the scavenger receptor to come to the fore.

As indicated by Sigmund,[79] a second major problem is the genetic heterogeneity that exists among the strains used to generate transgenic and knockout mice. This may lead to a situation where animals containing exactly the same genetic manipulation exhibit profoundly different phenotypes when present on diverse genetic backgrounds. For example, the extent of atherosclerosis among apoE knockout mice on a standard atherosclerosis-prone C57BL/6 background was found to be 7 times greater than apoE knockout mice with an atherosclerosis-resistant FVB genetic background.[80] The ideal solution to this problem is to use inbred isogenic strains in which the experimental and control mice differ only at the target locus. The next best alternative is to develop a program of inbreeding to a common, congenic strain, that is, one that is genetically identical to the control strain except for the single region of the chromosome containing the target gene. This is time-consuming and expensive. Six generations, or 2 years, of backcross breeding are required before the genetic backgrounds are more than 99% homogenous, with rapidly diminishing returns thereafter. For example, 4 more generations are needed to increase genetic homogeneity from 99.2% to 99.9%.[79] These problems make it imperative that a detailed description of the genetic background of all mouse models used in transgenic experiments be published, and that the genetic background should always be taken into account when assessing experimental results.

Animal Models of Plaque Instability and Rupture

Despite the drawbacks mentioned above, several models have been reported recently that plausibly reproduce many of the salient features of plaque ruptures in humans. The only nonmouse model of plaque rupture was presented by Rekhter et al in 1998.[81] The aim of this model was not to investigate the pathophysiological mechanisms underlying the development of the vulnerable lesion but rather to design a model "to evaluate plaque mechanical strength/vulnerability characteristics." In this model, 2 balloon catheters were used to mechanically injure and thus produce a lesion in the thoracic aorta of a cholesterol- and fat-fed rabbit. A third indwelling balloon catheter was then inflated and deflated to produce rupture of the lesion. From this description, it is clear that this

animal model may be suitable for measuring the mechanical strength of a plaque and, perhaps, for investigating thrombotic sequelae, but cannot be expected to provide much information about the pathophysiology of plaque rupture in humans.

The first indirect evidence of plaque rupture in the apoE knockout mouse model of atherosclerosis appeared in 1998, when Reddick et al[82] reported thrombus formation in the aortas of mice that were injured by squeezing with a forceps. This rather unphysiological model was followed up by a report by Rosenfeld et al[65] that elderly apoE knockout mice (60 weeks old) develop lesions in the brachiocephalic artery that are characterized by the presence of collagen-rich fibrofatty nodules and xanthomas. These nodules contained necrotic cores and displayed evidence of intramural bleeding. This bleeding was interpreted as possibly being due to plaque rupture. Moreover, from 42 weeks onward, mice exhibited layered lesions, implying, the authors suggested, multiple events. In a more recent report, Rosenfeld's group reported that in 30-week-old apoE-deficient mice, administration of a large dose of simvastatin (50 mg/kg/day) reduced the frequency of bleeding and calcification within lesions in the brachiocephalic artery, which was interpreted as evidence for "stabilizing effects [of simvastatin] on advanced atherosclerotic lesions."[83] Calara and colleagues[64] followed 82 cholesterol-fed apoE and LDL receptor knockout mice for up to 12 months and 33 chow-fed apoE knockout mice for up to 20 months. Of the 82 cholesterol-fed animals, 3 showed aortic plaque rupture and/or thrombi, whereas of the 33 chow-fed mice, 18 showed atherosclerosis of the coronary arteries. In 3 of these 18 animals, blood-filled channels were seen within the coronary lesions. This was taken to indicate the presence of previous plaque disruption and thrombosis, followed by recanalization. These 3 mice also showed deep ruptures and thrombosis of the aortic origin.

Finally, much interest was generated by 2 recently reported models of plaque rupture in apoE knockout mice. In the first of these, from Bristol in the United Kingdom, apoE knockout mice with an unusual mixed C57BL6/129SvJ genetic background were fed a diet containing 21% lard and 0.15% cholesterol for up to 14 months.[62,63] Most of these mice developed atherosclerotic plaque rupture associated with luminal thrombus at the point where the brachiocephalic artery branches into the right common carotid artery. The ruptures were characterized by fragmentation and loss of elastin and smooth muscle cells in the fibrous caps of relatively small and lipid-rich plaques overlying large complex lesions. Of 98 such mice, 51 had an acutely ruptured plaque in the brachiocephalic artery and 64 died suddenly. However, the incidence of sudden death did not differ between those with brachiocephalic rupture and those without. An undisclosed number of mice in this study also suffered myocardial infarction. In the second study, lesions were induced in apoE knockout mice by placement of a silastic collar around the carotid artery.[70] The resultant plaques were then incubated transluminally with adenovirus bearing a p53 transgene. Overexpression of p53, a tumor suppressor gene that promotes apoptosis, reduced the cellular and extracellular content of the cap of the lesion, with a reduced cap/intima ratio. When these mice were made hypertensive by treatment with phenylephrine, 40% developed rupture of the p53-treated plaques. Several articles have also appeared in recent years of myocardial infarction in apoE knockout mice without definite evidence of plaque rupture.[68,69,84] For the sake of brevity, therefore, these models will not be discussed further here, even though some have enthused that their existence should "finally put to rest the notion that mice cannot be models of plaque rupture."[85]

Usefulness of Current Animal Models of Plaque Instability and Rupture

Of the models of plaque rupture presented thus far, none can be regarded as ideal. Both the rabbit model presented by Rekhter et al[81] and the apoE knockout mouse p53/silastic cuff model[70] required such heroic measures to produce evidence of plaque rupture that they can tell us little about the pathophysiology of this condition. The usefulness of these models is thus more or less confined to studies of the mechanical process of rupture itself. More interesting from the etiological and therapeutic points of view are the apoE mouse models in which plaque rupture was seen in elderly fat- and cholesterol-fed mice.[62–65] However, these models too are surrounded by caveats. In the report of Calara et al,[64] evidence of rupture was indirect and was seen much less frequently (about 5% of the animals) than occurs in human atherosclerosis. In the Rosenfeld et al[65] model, evidence of rupture was also indirect and was seen in the brachiocephalic artery in particular. Finally, in the Bristol model,[62,63] plaque rupture was again focused on the brachiocephalic artery, and was seen only in older mice after prolonged feeding with a very high-fat diet. The Bristol group have speculated that the predilection for plaque rupture in the brachiocephalic artery may reflect the high degree of tension in the wall of this artery in the mouse. A more general drawback of both the Rosenfeld and Bristol models is that neither shows convincing evidence of the formation of platelet and fibrin-rich thrombus at the site of presumed rupture. This is a very important limitation because infarction of the heart or brain in humans is not caused by rupture of the artery per se, but by the formation of an occlusive blood clot that is rich in platelets and fibrin. Perhaps as a reflection of this lack of thrombosis, death of the mice in Bristol was not related to plaque rupture. Furthermore, in the absence of thrombosis, intraplaque hemorrhage in these models has been presumed to reflect prior plaque rupture, but this is not necessarily the case.[5] The Rosenfeld and Bristol models also have the disadvantages of the expense required to maintain the mice for more than a year and the variable incidence of plaque rupture.

Conclusions, Directions for the Future

Atherosclerosis in humans is a multifactorial condition that develops over many years, and modeling it in animals is a notoriously tricky business. Reproducing the events leading up to occlusive thrombosis, the event that actually kills and disables humans, is even more challenging. In humans, thrombosis and plaque rupture are linked, yet either one can occur without the other. We still do not know why an occlusive clot occurs at a particular location at a particular

time, but it is certain that this is only the last act in a complex drama, which, like some modern theater, is likely to contain the occasional random event. In view of these deficiencies, can animal models be regarded as a help? The answer is a qualified maybe. Existing models are useful for exploring certain features of the pathogenesis of atherosclerosis, particularly in relation to unraveling the function of particular genes. They are also useful for investigating isolated features of plaques such as the relationship between cap thickness and mechanical stability. However, they are less helpful in modeling the complications of atherosclerosis, such as rupture and thrombosis. Because of the complexity of the processes involved, it is not certain that a good model of these complications will ever be found, particularly when the narrow species specificity of atherosclerosis is taken into account. This is also true of studies of potential treatments intended to stabilize the plaque, any results of such studies obtained in animals must be regarded as strictly preliminary and require verification in humans.

Directions for the Future

In studies of plaque rupture, despite the difficulties involved, more attention should be paid to the study of the processes of plaque breakdown and thrombosis formation in humans themselves. A good example of such work is a recent investigation of Faber et al,[86] who used a subtractive hybridization technique to identify genes that are differentially regulated in human atherosclerotic lesions that showed evidence of plaque rupture. The currently available animal models seem unlikely to play more than a supporting role in this research.

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