Editorial

Contrasting Outcomes of Atheroma Evolution
Intimal Accumulation Versus Medial Destruction

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Although not all forms of aneurysm, which may vary in location, are of atherosclerotic origin, atheroma is probably the main cause of acquired abdominal aneurysms in humans. In 1992, Reed et al described the similarity of risk factors for occlusive and aneurysmal diseases in a cohort of 8000 men and concluded that atheroma was a causal pathway to aneurysm development. This common initial pathway has been recently confirmed through a postmortem morphological approach in humans. The usual occlusive forms of atheroma involve intimal accumulation of material including lipids, matrix proteins, and cells, whereas the medial layer remains largely uninjured, consisting of smooth muscle cells (SMCs) and insoluble extracellular matrix. In contrast, aneurysm development involves proteolytic injury to the medial layer, including the degradation of elastin and collagen, SMC rarefaction, and compensatory fibrosis of the adventitia. Therefore, the question raised is this: What are the biological determinants that preferentially switch the outcome of atheroma from intimal accumulation of biological materials to medial destruction? The involvement of protease and antiprotease systems in the evolution of atheroma is now well documented both in aneurysm formation and in plaque rupture (see Libby for a review). These systems involve matrix metalloproteinases (MMPs), which are able to degrade the insoluble extracellular matrix, their activation and inhibition by tissue inhibitors (TIMPs), and the plasminogen activators and their inhibitors (PAIs).

In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Silence and coworkers have explored this interesting question of the switch from intimal accumulation to medial destruction in atheroma and implicate MMP-3 activity in this phenomenon with an experimental genetic approach in mice by cross-breeding ApoE/−/− mice and mice deficient for the MMP-3 gene. The authors show that disruption of the MMP-3 gene in hypercholesterolemic atherosclerotic mice increases the area of intimal plaques, enriches them in collagen, decreases their lipid content, and partially prevents aneurysm formation by limiting localized elastolysis, SMC loss, macrophage infiltration, and urokinase-type plasminogen activator (u-PA) activity. Therefore, this study confirms the possible etiological role of atheroma in experimental aneurysm formation and highlights the involvement of inflammatory cells as a source of proteinases, focusing on MMP-3 and u-PA in this complex pathophysiological process within the arterial wall.

Because the insoluble extracellular matrix is the main structural component responsible for the function of blood content by the arterial wall and because its degradation requires specific enzyme activities, Busuttil et al as early as 1982 suggested a role for matrix proteinases in human aneurysms. By analogy with the local use of elastase for generating experimental emphysema in the lung, we developed the first pathophysiological model of aneurysms in rats. In that study, we also showed that plasmin and inflammatory cell infiltration enhanced aneurysm formation. Since 1990, this model has been adapted in various animal species (rats, mice, rabbits, dogs) and has largely been used to explore in more detail the pathophysiology and therapy of aneurysms. The arterial grafting of xenogenic, decellularized, extracellular matrix also leads to experimental aneurysm formation through inflammatory injury of the arterial xenogenic matrix.

More recently, the genetic disruption of targeted genes in mice, including those involved in lipid metabolism leading to atherosclerosis and genes coding for proteolytic enzymes, have provided new opportunities to explore the cellular and molecular bases of aneurysmal outcome in atheroma. Extensive clinical investigations of the proteinase systems in the aneurysmal arterial wall have been developed in parallel, confirming the main role of matrix proteinases and the plasminogen activation cascades in the genesis of matrix degradation leading to aneurysm formation in humans.

MMP-9 (gelatinase-β) gene disruption in mice has been used to demonstrate the involvement of this important MMP in the development of aneurysms. Disruption of the MMP-9 gene induces resistance to elastase-dependent aneurysm formation, whereas bone marrow transplantation of wild-type hematopoietic cells in these knockout mice restores the sensitivity to aneurysm formation, providing evidence of the predominant role of inflammatory cells in MMP-9 release. Evidence of MMP-9 activity in the aneurysmal wall and endoaneurysmal thrombus was also reported several years ago in human aneurysms. MMP-9 is released from tertiary granules of neutrophils, and its expression is induced in macrophages. Active MMP-9 is able to degrade denatured collagens and elastin. Because MMP-9 is secreted but present only in low levels in plasma under nonpathological conditions, the plasma level of MMP-9 activity could be used as a marker for aneurysm evolution and for the efficacy of treatment in humans. Nevertheless, the possible use of this...
biological marker should be investigated in a larger human population. In parallel, the presence of gelatinase A (MMP-2) constitutively secreted by mesenchymatous cells, physiologically present as a latent form in the plasma, has also been documented in human aneurysms. The presence of macrophage elastase (MMP-12) was also identified in the aneurysmal wall, colocalizing with the remaining elastin fibers.

Targeted disruption of the u-PA gene in atheroma-sensitive mice also led to the prevention of matrix degradation and aneurysm formation, providing evidence of the involvement of plasminogen activation in the pathophysiology of aneurysms. Plasmin formation has also been demonstrated in human aneurysms, and aneurysmal evolution has been correlated with peripheral markers of active fibrinolysis. Moreover, the presence of an intense, fibrinolytic area within the endoaneurysmal thrombus, described as the “crescent sign,” is considered to be a marker of imminent rupture in humans, suggesting that a peak of fibrinolytic activity could be involved in the mechanism of rupture.

In this well-documented context, the study of Silence and coworkers provides some insights and raises some unresolved questions about the role of MMPs in arterial wall pathology. MMP-3 (stromelysin-1) is also an important protease able to degrade a wide range of extracellular matrix proteins, including proteoglycans, collagens, laminin, fibronecrtin, and elastin. Furthermore, stromelysin-1 could activate other members of the MMP family. MMP-3 is expressed in atherosclerotic lesions in humans, mainly in the areas where rupture is the most commonly detected, colocalizing with macrophages, and thereby supporting the hypothesis of its role in plaque instability. Similarly, the presence of MMP-3 has been demonstrated in the wall of human aneurysms. Experimentally, MMP-3 expression is significantly correlated with the destruction of elastic lamellae and ectasia in LDL receptor–deficient mice. In this model, treatment with an MMP inhibitor had no effect on the extent of aortic plaque development but reduced elastin degradation and the degree of ectasia, fitting well with the data obtained by Silence and coworkers in their double-knockout model (ApoE−/− and MPP-3−/−).

In addition to the direct involvement of macrophage-derived MMP-3 in matrix degradation and in the activation of other MMPs, the data presented by Silence and coworkers suggest that MMPs could also be involved in modulating the ability of macrophages to invade the plaque. Ever since MMPs have been recognized, their involvement in tumor invasiveness is well acknowledged (see Stamenkovic for a review). Therefore, the hypothesis that the expression of MMP-3 and MMP-9 may affect macrophage migration and accumulation in atherosclerotic plaques is of interest, suggesting that MMPs could be involved in the early stages of plaque genesis, foam cell formation and inflammatory cell penetration, as well as in the later steps of plaque complications: plaque rupture and aneurysm formation.

Despite the decreased proteolytic injury, inflammatory cell invasion, foam cell formation, and u-PA and MMP secretion and activation in mice devoid of MMP-3 activity compared with wild-type mice, they showed greater plaque development. However, the composition of the plaque was different, being richer in collagen and providing evidence for the role of SMC migration, proliferation, and matrix secretion in the intima. This SMC response is common to all types of endoluminal injury, whether caused by lipids, mechanical stress, or immune mechanisms, and it corresponds to a healing process. In the arterial wall, vascular SMCs are the main source of antiproteases, including TIMPs, which inhibit MMPs and PAIs, which inhibit u-PA. In particular, when vascular SMCs undergo activation and hypertrophy, as, for example, in response to hypertension, their secretion of antiproteases increases. Therefore, SMCs could be used to prevent proteolytic injury. Because antiproteases diffuse, the plasma levels of PAI-1 and TIMP-1 have been used as biological markers for the diagnosis and treatment of hypertension, providing evidence for arterial wall remodeling in this condition. Therefore, in occlusive atheroma, medial smooth muscle could play a role in limiting the outward remodeling associated with plaque evolution by inhibiting the inflammatory cell infiltration of the media and switching the response toward an accumulation of biological material in the intima. Similar contrasting functions of inflammatory cells (mainly proteolysis) and SMCs (anti-proteolysis) have been reported within the intima.

Indeed, Silence and coworkers reported that medial extracellular matrix degradation was accompanied by a loss of SMCs. A similar loss of SMCs has been previously reported in human aneurysms. Probably, the loss of SMCs is necessary for the development of aneurysms, permitting proteolytic injury of the extracellular matrix. SMC apoptosis is a common feature of arterial aneurysms, plaque rupture, and also varicose veins, which share some similarities with aneurysms. The mechanisms leading to the disappearance of SMCs remain to be described. Nevertheless, a role for proteases, able to induce pericellular proteolysis, is not excluded. Recently, Ikari et al showed that the antiproteases present in serum are antiapoptotic factors for vascular SMCs. However, Ikari et al showed that the antiproteases present in serum are antiapoptotic factors for vascular SMCs in culture, suggesting that proteases could be involved in such a phenomenon. Nevertheless, the implication of proteolytic genes and their products does not exclude the participation of other genes of susceptibility in the development of aneurysms. For example, the inducible NO synthase has been recently demonstrated to be involved in clinical and experimental aneurysm development.

As shown by epidemiological data of familial aggregation in humans and in spontaneous experimental models in animals, the switch of atheroma evolution toward aneurysm development could be partly determined by genetic background. Genetic susceptibility to aneurysm development in atheroma could be associated with polymorphisms located in the promoter of candidate genes, regulating their transcriptional rate in response to risk factors. In this context, genes involved in matrix degradation and the fibrinolytic cascade are good candidates, and their genetic exploration is in progress. MMP-3 is an interesting candidate because a polymorphism has been reported in the promoter that regulates the transcriptional rate of the coding part of the gene in response to stimuli. This polymorphism has been recently reported to be involved in the susceptibility to aneurysm development and to influence carotid geometry. Nevertheless, the biological question is complex because genetic determination of such susceptibility in the context of atheroma is probably determined by the regulated expression of numerous genes, including not only the upregulation of genes...
involved in proteolytic injury but also the downregulation of genes involved in defense. For example, in the context of emphysema, which presents some analogies with atherosclerotic pathology, the promoter regulating the polymorphism of the heme oxygenase-1 gene has been shown to be involved in the lung response to tobacco.\(^\text{56}\)

In summary, our knowledge of the cellular and molecular bases of the dual outcome of atheroma evolution has been largely extended through experimental and clinical approaches in the last 10 years, implicating both the MMPs and fibrinolytic cascades. Nevertheless, some points remain to be investigated in more detail, such as the role of proteinases in SMC disappearance. Last, genetic susceptibility to aneurysm development in atheroma has probably complex polygenic determinants, involving both genes of susceptibility and genes of resistance expressed in both inflammatory cells and SMCs.

References


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