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# Retention and Activation of Blood-Borne Proteases in the Arterial Wall

## Implications for Atherothrombosis

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All forms of atheroma are characterized by a risk of arterial wall rupture leading to clinical complications. This involves medial and adventitial ruptures in abdominal aortic aneurysm (AAA) and intimal cap rupture in vulnerable atherothrombotic plaques. Extracellular proteases, including metalloproteinases, locally generated plasmin, and leukocyte elastase, are important molecular mediators of atheroma progression via their matrix degradation properties. The pathological evolution of AAA is linked to the biology of its associated mural thrombus. Indeed, in aneurysmal segments lined by a thrombus, the wall is thinner, the extracellular matrix more degraded, and the adventitial inflammatory response greater than in segments that are not. Several lines of evidence highlight the role of the thrombus, in AAA, as a reservoir of blood-borne proteases that conveys them from the lumen to the diseased wall. In stenosing atheroma, both previous and recent studies provide evidence that recurrent intraplaque hemorrhages play a dominant role in the evolution of the lesion toward vulnerability. In this review, we draw a parallel between the role of protease conveyance and activation of the mural thrombus in AAA and of intraplaque hemorrhages in stenosing atheroma. We hypothesize that intraplaque hemorrhages convey blood-borne proteases into lesions, where they are retained and activated upon thrombus/hematoma formation, thus contributing significantly to their deleterious action. (J Am Coll Cardiol 2006;48:A3–9)


Atherothrombotic plaques evolve toward partial or total wall rupture, causing arterial thrombosis in the case of stenosing forms, and hemorrhage in the dilating aneurysmal forms. Dilatation and rupture of the arterial wall are linked to the degradation of the extracellular matrix (ECM).

The proteases involved in atherosclerotic diseases and their complications belong mainly, but not exclusively, to the matrix metalloproteinase (MMP) and serine-protease families. Among serine-proteases, plasmin and leukocyte elastase probably play a dominant role. A role for mast cell chymase (1) and cyteine-proteases cannot be excluded. Matrix metalloproteinases constitute a family of numerous zinc-dependent metalloproteinases generally secreted as pro-(inactive) forms that are activated extracellularly by several proteinases. They are produced by a variety of cell types, including, for example, mesenchymal cells (smooth muscle cells [SMCs] and fibroblasts), which constitutively secrete MMP-2 and -7 and macrophages and polymorphonuclear neutrophils in which inducible expression and/or secretion of MMP-9 occurs (2). Besides their action on fibrillar ECM, MMPs participate in pathogenic processes, by degrading other ECM constituents. Degradation of ECM not only modifies the cellular environment per se, but also generates degradation products that possess specific biological activities (matrikins) and liberates ECM-bound growth factors. Matrix metalloproteinases also directly regulate proteases and antiprotease activities (3). For example, MMP-3 can activate proMMP-9 (4), and together they cleave plasminogen and inactivate plasminogen activator inhibitor-1, $\alpha_2$-antiplasmin, and $\alpha_1$-antitrypsin (5–7).

The serine-protease plasmin is specifically activated via conversion from plasminogen, a plasma-rich zymogen synthesized by the liver, by serine-proteases, urokinase and tissue-type (t-PA) activators. Binding of plasminogen and plasmin to fibrin, to other natural polymers, or to the cell surface facilitates plasminogen activation enhances the proteolytic activity, and prevents interactions with inhibitors (6), thus focusing the in situ action of plasmin within the tissue. In contrast, the soluble form of plasmin is immediately inactivated by binding to serpins. Besides proteases, several antiproteases can also bind to tissue. $\alpha_2$-antiplasmin covalently binds to fibrin (8,9), and protease nexin-1 (a tissue inhibitor of thrombin and plasmin) is strongly bound to ECM (10).

Leukocyte elastase is mainly released by polymorphonuclear neutrophils (2) but has been recently shown to be also expressed and secreted by macrophages (11). The importance of neutrophils in thrombus evolution has long been recognized (12). Neutrophils are trapped at the site of fibrin formation, but more importantly, accumulate later and invade the thrombus, being 12 times more numerous in

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**Abbreviations and Acronyms**

AAA = abdominal aortic aneurysm
ECM = extracellular matrix
HSP = heat shock protein
MMP = matrix metalloproteinase
MRI = magnetic resonance imaging
SMC = smooth muscle cell
t-PA = tissue-type activators

clots than in circulating blood. Neutrophils have a high affinity for the fibrin-fibronectin network (13) and bind to platelet-exposed P-selectin via the expression of PSGL-1 (14). Neutrophil activation participates in fibrinolysis via both the release of urokinase and direct actions of elastase and cathepsin G (15,16). Plasmin and elastase are involved in tissue remodeling through direct proteolysis of ECM components and, importantly, via the activation of proMMPs. An important consequence of protease activity on the ECM is the suppression of cell matrix interactions, which triggers cell apoptosis in a process called anoïkis (17). Such a phenomenon has been described for both plasmin (18) and elastase (19).

One of the main challenges concerning the understanding of atherothrombotic complications is, therefore, to determine the hierarchy of the proteases involved, to localize their sources, and to elucidate the means of their retention and activation within tissues, thus defining new diagnostic and therapeutic targets. Our recent results suggest that proteases conveyed by blood may be retained and activated by mural thrombus formation in abdominal aortic aneurysm (AAA) as well as by intraplaque hemorrhages in vulnerable plaques.

## ACQUIRED AAA

Abdominal aortic aneurysm formation involves degradation of the medial layer (17), including both disappearance of SMCs by apoptosis (20) and absence of healing by cell recolonization, together with ECM degradation by proteases, adventitial angiogenic and immunoinflammatory responses, and mural thrombus formation.

The dominant role of proteases—MMPs, serine- and cysteine-proteases—in the evolution of AAAs toward enlargement and rupture is well established. Abdominal aortic aneurysms are usually characterized by the presence of a non-occlusive thrombus, through which blood flow continues, permanently maintaining interfaces between blood and thrombus at the luminal pole and thrombus and wall at the abluminal pole. The most recently formed mural thrombus/hematoma, at the luminal pole, is composed of patchy areas of red blood cells and fibrin, retaining leukocytes (mainly neutrophils), aggregated platelets, and plasma components (Fig. 1). The oldest, abluminal part is composed of a loose network of degraded fibrin, in which the specific initial components, including red blood cells and leukocytes, cannot be identified. Thus, the mural thrombus in AAA offers a unique opportunity to study the different stages of arterial thrombotic compositions and evolution within the same sample.

**Arguments for thrombus involvement in AAA.** Several clinical observations have pointed to the involvement of the coagulation and fibrinolytic cascades in the evolution of aneurysms (21,22). Earlier, the morphologic change (crescent sign) of the mural thrombus (23) was correlated with an impending risk of rupture (24,25). Furthermore, the risk of rupture was shown to correlate with the size of the thrombus (26,27). That biological activities of the thrombus participate in aneurysmal evolution was further supported by Kazi et al. (28) who observed that the thrombus-associated aneurysmal wall was thinner and showed more frequent signs of inflammation, SMC apoptosis, and degraded ECMs as compared with adjacent blood flow-lined wall. Their findings suggested that thrombus formation may compromise the structural integrity and stability of the arterial wall (29).

**Storage, activation, and release of blood-borne proteases by the mural thrombus.** The first clues to the presence of proteolytic activities in the mural thrombus came from the findings of fibrin degradation products in AAA mural thrombi (30). Comparing mural thrombus to a retracted blood clot for the presence of proteases, Gacko and Glowinski (31) and Gacko et al. (32) showed an enrichment of cathepsins, elastase, tissue factor, plasminogen, and t-PA in the former. Gelatinase activities (MMP-2 and -9) are also higher in the thrombus than in serum (33).

The mural thrombus of AAA is a complex laminated structure (30), containing several layers of thick, mixed, brown fibrin clot, underlying a red thrombus on the luminal surface (Fig. 1A). Neutrophils, platelets, and red blood cells are present in the luminal layer (34) (Figs. 1B to 1D). In contrast, monocytes/macrophages are rare (Fig. 1E). The few that are present display signs of cell death such as cell contraction, suggesting apoptosis (Fig. 1E, inset). Localization of neutrophils in the luminal part of the thrombus is associated with the detection of increased levels of MMP-8, -9, and elastase, as compared with the other layers (Figs. 1F and 1G) (35). Matrix metalloproteinase-9 is, in part, complexed to lipocalin, providing evidence for its neutrophil origin.

In an initial report, we showed that spontaneous thrombus formation and degradation in vitro was associated with the release of plasmin, responsible for clot lysis, and proMMP-9 from neutrophils trapped within the clot (36). In vivo, we next demonstrated that large amounts of plasminogen accumulate in the mural thrombus. In parallel, we showed that neutrophil-derived elastase, at the luminal pole, prevents detersion and recolonization of the thrombus by SMCs or circulating progenitors (35).

These different observations suggest that the mural thrombus, which permanently interfaces with circulating blood, is biologically active. It undergoes continuous

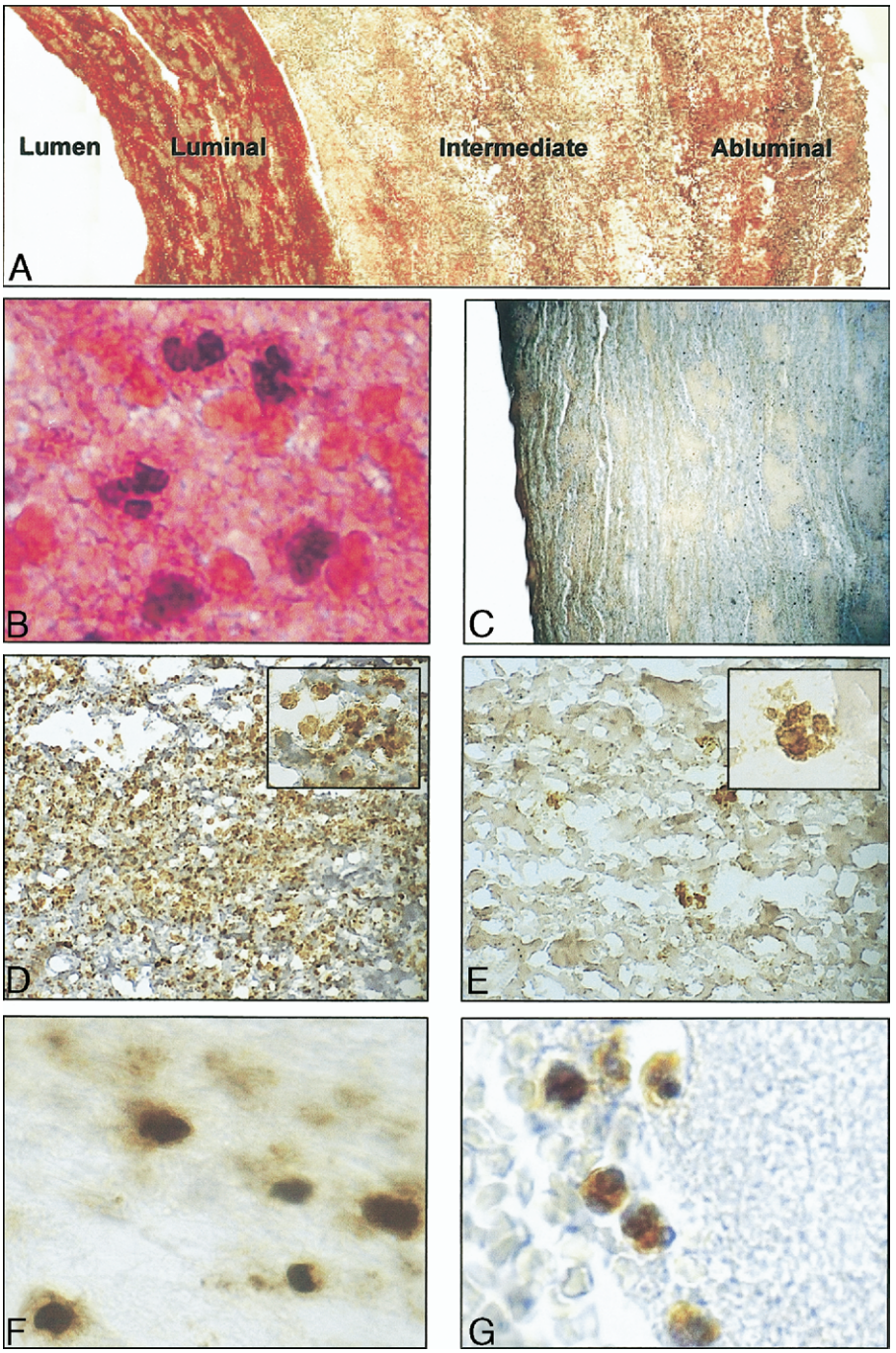


**Figure 1.** Histologic aspect of the mural thrombus in abdominal aortic aneurysm. **(A)** Section through the thrombus showing the **red** luminal layer corresponding to a newly formed clot, associating patchy areas of red blood cells and fibrin. The intermediate layer represents a structured fibrin gel in which cellular components can no longer be recognized. The abluminal layer is composed of a loose network of degraded fibrin (hematoxylin/eosin, ×10). **(B)** Presence of polymorphonuclear leucocytes in the luminal layer; these cells predominate in the fibrin-rich areas (hematoxylin/eosin, ×60). **(C)** Glycoprotein IIb/IIIa immunostaining showing the predominance of platelet aggregation at the luminal pole of the thrombus in fibrin-rich areas (×10). **(D)** Anti-CD66b antibody stains degranulating neutrophils that accumulate in the luminal layer (×20). **Inset** shows neutrophils at different stages of degranulation (×100). **(E)** Immunostaining with anti-CD68 antibody demonstrates the paucity of monocyte/macrophages in the luminal layer of the thrombus (×20). **(Inset)** A macrophage that appears contracted and apoptotic (×100). **(F)** Matrix metalloproteinase-9 immunostaining of gelatinase granules of neutrophils present at the luminal pole of the thrombus (×100). **(G)** Neutrophil elastase immunostaining at the luminal pole of the thrombus colocalizes with polymorphonulear leukocytes (×100).

renewal and fibrinolysis. In this concept, centrifugal conveyance of plasminogen and activators from the lumen across the thrombus play an important role in fibrinolysis (37) and MMP activation. Luminal thrombus renewal is the main determinant of neutrophil trapping and plasminogen storage, causing protease release, plasmin formation, and MMP activation, all of which contribute to the expansive arterial wall remodeling, which

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inexorably, sooner or later, leads to rupture. The thrombus is also probably the main source of plasma markers of AAA evolution, such as for example MMP-9 (38,39), thrombin-antithrombin, and plasmin-antiplasmin complexes (22). These observations may have clinical consequences in terms of diagnosis and therapeutics in AAA; thrombus morphology, functional imaging, and plasma biological markers could be of prognostic value.

**Angiogenesis and inflammatory responses in the adventitia.** In the arterial wall, mass transport of diffusible mediators occurs unidirectionally by convection (40), due to transmural volume flow, from the lumen to the adventitia. This outward convection of mediators, from the lumen across the wall, is probably the physiological determinant of the adventitial response to arterial wall injury (41). The adventitia responds to proteolytic injury by promoting angiogenesis, resulting in the external localization of inflammation and lymphoid neogenesis (42). Fibroblast activation, mediated by inflammation, probably limits AAA evolution toward rupture through the stimulation of peripheral sclerosis. The observation of periaortic inflammation, possibly leading to the clinical entity of the "inflammatory AAA" and to retroperitoneal fibrosis (43), has focused investigations on adventitial inflammation in AAA (44,45). Therefore, in AAA, mediators generated inwardly mainly by proteolysis could be centrifugally convected toward the adventitia, where they induce angiogenic, immunoinflammatory, and fibrotic responses.

## INTRAPLAQUE HEMORRHAGE AND THROMBUS FORMATION IN PLAQUE VULNERABILITY

As compared with aneurysms, vulnerable stenosing plaques (Stary type V and VI lesions) are characterized by the retention of biomaterials in the core of the lesion, encapsulated between the luminal fibrous cap and the remaining media (46). The nature of this biological gruel is heterogeneous and depends on the stage of the lesion. The core includes components of different ages (unesterified lipid accumulation, frequent calcifications, macro- or microscopic hematoma) that can colocalize within the same lesion. This highlights the discontinuous evolution of the plaque, from the initial lipid retention to the formation of a more complex core, leading to plaque instability and complications (Fig. 2).

**Historical background.** The proteolytic nature of the core in atheroma was first suggested by Galen (131 to 201 CE) ($\alpha\theta\eta\rho\omega\mu\alpha$ = atheroma = gruel). The involvement of repeated intraplaque hemorrhages in the evolution of atherosclerotic lesions toward complications was proposed as early as 1938 (47), and the importance of thrombotic material in the generation of the pultaceous core has been emphasized in pulmonary hypertension-induced plaque formation secondary to thromboembolic events (48). The crucial role of repeated intraplaque hemorrhages in the evolution of stenosing atheroma toward complications has been recently revisited by Kolodgie et al. (49). They linked unesterified cholesterol accumulation in the necrotic core of the lesion to specific red

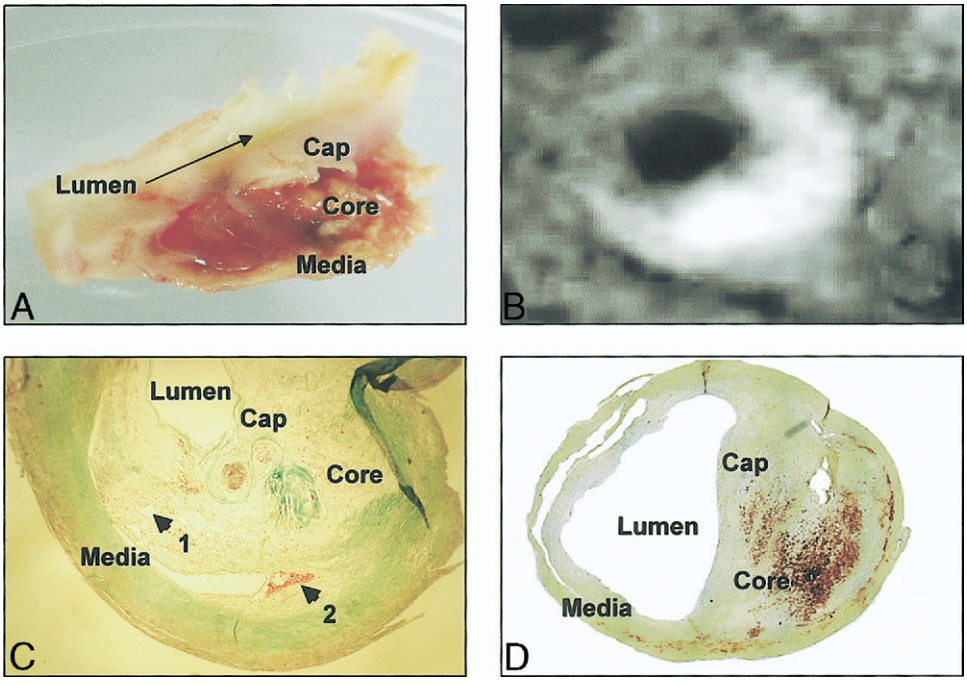


**Figure 2.** Histologic aspect of culprit lesions in carotid atheroma. **(A)** Macroscopic aspect of a carotid culprit lesion showing the hemorrhagic nature of the core. **(B)** Magnetic resonance imaging of a carotid plaque hemorrhage. Intraplaque hemorrhages are characterized by a focal post-contrast enhancement in T1 spin echocardiography, giving a hyperintensity signal. **(C)** Masson's trichrome staining of a culprit carotid lesion, showing the presence of: 1) neocapillaries within the lesion; and 2) intraplaque hemorrhage in the core. This example highlights the heterogeneity of culprit lesions, involving pathologies at different stages: fibrosis and hemorrhages. **(D)** Alizarin **red staining** of a culprit carotid lesion revealing the presence of calcification within the core and at the interface between the core and the media.

blood cell antigen expression (glycophorin). These histologic observations were corroborated by magnetic resonance imaging (MRI) of human carotid atheroma (50,51). Takaya et al. (52) demonstrated that only plaques presenting hemorrhage within the lesion, detected by MRI, showed progression over a period of 18 months, including a decrease in lumen area and an increase in core volume. Although the mechanisms leading to intraplaque hemorrhage are still not well established, numerous studies focus on the involvement of angiogenesis and the infiltration of microvessels from the adventitial vasa vasorum to invade the shoulder region of the plaque (53).

**From intraplaque hemorrhage to plaque vulnerability.** Intraplaque hemorrhages convey into the lesion all the blood components, including red blood cells, leukocytes (of which 80% are neutrophils), platelets, and plasma proteins. Plasma membranes of circulating cells, including red blood cells (49), activated platelets (54,55), and probably dead leukocytes, participate in unesterified cholesterol retention within the core (Fig. 3). Platelet aggregation, prothrombin activation, and fibrinogen proteolysis all induce fibrin polymerization, which is potentially capable of trapping neutrophils and other leukocytes, and of binding plasmin and elastase (Fig. 3). In order to further explore the deleterious potential of the core contents, the release of activated proteases by carotid plaque endarterectomy specimens at different stages of evolution (type III to VI lesions of the Stary classification) were analyzed and compared with normal endarteries (mammary arteries). The greater the size and the complexity of the core, the greater was the release of activated proteases, including plasmin and the activated gelatinases MMP-2 and -9 (56). In parallel, proteolytic degradation of secreted heat shock protein (HSP) 27 occurs in diseased tissues (57). Heat shock protein 27 was previously identified using a differential proteomics approach as a very sensitive biomarker, which is decreased in plasma from atherosclerotic patients (58). Microdissection of the lesion to separate cap, core, and media demonstrated that, among these different lesion compartments, the core is the major site of plasminogen/plasmin and gelatinase storage and activation (56). Plasmin accounts for a major part of the proteolytic degradation of HSP27 (57).

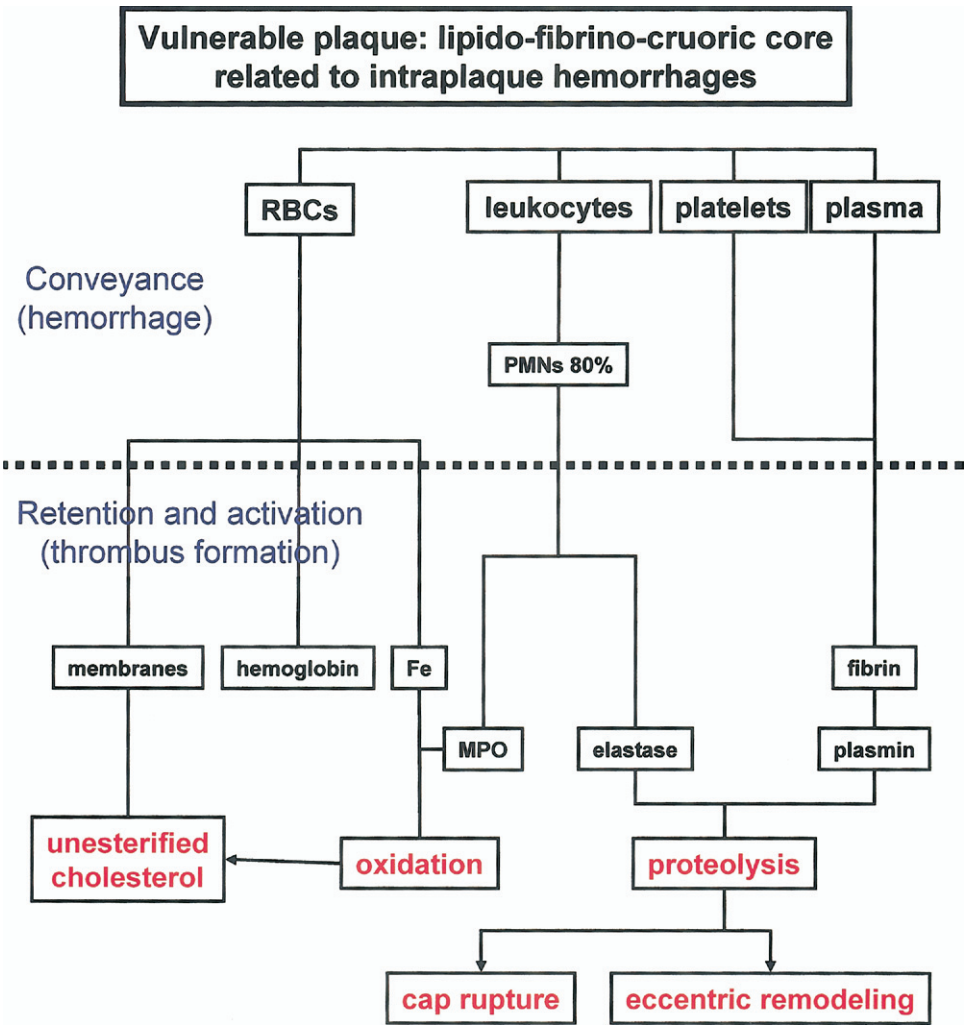


**Figure 3.** Representative diagram of the biological components conveyed by the intraplaque hemorrhage to the core and their roles in plaque progression. Fe = iron; MPO = myeloperoxidase; PMN = polymorphonuclear leukocyte; RBC = red blood cell.

Other enzymatic activities associated with the core of the atheromatous lesion have also been described, including phospholipase A2 (59) and sphingomyelinase (60), which are able to modify low-density lipoprotein particles and generate unesterified cholesterol from cell membranes. This enzymatic modification of low-density lipoprotein is a key to understanding how cholesterol retention within the arterial wall could be the driving force for the inflammatory response in atheroma (61,62). This concept of the response to lipoprotein retention could be extrapolated to apply also to the phenomenon of core retention of proteolytic activities, which may also play a crucial role in the different mechanisms of plaque progression.

The core of the lesion is a cytotoxic environment, essentially acellular, containing cell debris and only a few active macrophages (preferentially located in the cap). Detersion of cell debris by macrophages is a prerequisite for a normal healing process. This main function of macrophages is prevented in atherosclerosis, probably because they undergo apoptosis, a hallmark of human atherosclerotic lesions (63,64). Macrophage survival is dependent on adhesion (65), and proteases trigger cell apoptosis via ECM proteolysis (18,19). Proteases could thus represent central mediators of the cytotoxicity of the core. Such a function of proteases was previously proposed in AAA to explain the absence of detersion and recolonization of the mural thrombus by mesenchymatous cells (35).

The lesional core in vulnerable atheroma is, therefore, the main site of retention and activation for proteases conveyed by repeated intraplaque hemorrhages. These blood-borne proteases likely represent a major determinant for cap rupture on the luminal side, leading to occlusive thrombus, as well as for outward expansive remodeling (66). The proteolytic nature of the lesional core could thus explain the link observed between the expansive remodeling and plaque hemorrhages (67) or vulnerability (68). Therefore, the eccentric growth of the plaque and its possible rupture into the lumen, both suggest a central proteolytic role of the core in atheroma progression (69).

**Conclusions.** Abdominal aortic aneurysm and atheromatous plaques are 2 clinically and etiopathogenic different forms of atherosclerosis that are characterized by the formation of a mural thrombus in the former and the recurrence of intraplaque hemorrhages in the latter. Both play a crucial role in the progression of the disease. Mural thrombus is the site of blood-borne storage and activation of proteases necessary for the proteolytic aggression of the wall. Intraplaque hemorrhages convey blood-borne proteases into the lesional core. In this review, we argue for a similar role of conveyance, storage, and activation of proteases between mural thrombus in AAA and recurrent intraplaque hemorrhages and thrombus/hematoma formation in atheromatous plaques. The concept of "proteolytic retention" within the diseased arterial wall offers new diagnostic and therapeutic targets in atheroma.

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## REFERENCES

1. Lindstedt KA, Kovanen PT. Mast cells in vulnerable coronary plaques: potential mechanisms linking mast cell activation to plaque erosion and rupture. Curr Opin Lipidol 2004;15:567–73.
2. Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 1997;89:3503–21.
3. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001;17:463–516.
4. Ramos-DeSimone N, Hahn-Dantona E, Sipley J, Nagase H, French DL, Quigley JP. Activation of matrix metalloproteinase-9 (MMP-9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. J Biol Chem 1999;274:13066–76.
5. Patterson BC, Sang QA. Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). J Biol Chem 1997;272:28823–5.
6. Lijnen HR. Elements of the fibrinolytic system. Ann N Y Acad Sci 2001;936:226–36.
7. Liu Z, Zhou X, Shapiro SD, et al. The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. Cell 2000;102:647–55.
8. Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. J Clin Invest 1980;65:290–7.
9. Lee KN, Lee CS, Tae WC, Jackson KW, Christiansen VJ, McKee PA. Cross-linking of wild-type and mutant alpha 2-antiplasmins to fibrin by activated factor XIII and by a tissue transglutaminase. J Biol Chem 2000;275:37382–9.
10. Farrell DH, Wagner SL, Yuan RH, Cunningham DD. Localization of protease nexin-1 on the fibroblast extracellular matrix. J Cell Physiol 1988;134:179–88.
11. Dollery CM, Owen CA, Sukhova GK, Krettek A, Shapiro SD, Libby P. Neutrophil elastase in human atherosclerotic plaques: production by macrophages. Circulation 2003;107:2829–36.
12. Kluft C. The fibrinolytic system and thrombotic tendency. Pathophysiol Haemost Thromb 2003;33:425–9.
13. Kuijper PH, Gallardo Torres HI, Lammers JW, Sixma JJ, Koenderman L, Zwaginga JJ. Platelet and fibrin deposition at the damaged vessel wall: cooperative substrates for neutrophil adhesion under flow conditions. Blood 1997;89:166–75.
14. Moore KL, Patel KD, Bruehl RE, et al. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. J Cell Biol 1995;128:661–71.
15. Plow EF. The contribution of leukocyte proteases to fibrinolysis. Blut 1986;53:1–9.
16. Plesner T, Ploug M, Ellis V, et al. The receptor for urokinase-type plasminogen activator and urokinase is translocated from two distinct intracellular compartments to the plasma membrane on stimulation of human neutrophils. Blood 1994;83:808–15.
17. Michel JB. Anoikis in the cardiovascular system: known and unknown extracellular mediators. Arterioscler Thromb Vasc Biol 2003;23:2146–54.
18. Meilhac O, Ho-Tin-Noe B, Houard X, Philippe M, Michel JB, Angles-Cano E. Pericellular plasmin induces smooth muscle cell anoikis. FASEB J 2003;17:1301–3.
19. Mtairag el M, Houard X, Rais S, et al. Pharmacological potentiation of natriuretic peptide limits polymorphonuclear neutrophil-vascular cell interactions. Arterioscler Thromb Vasc Biol 2002;22:1824–31.
20. Lopez-Candales A, Holmes DR, Liao S, Scott MJ, Wickline SA, Thompson RW. Decreased vascular smooth muscle cell density in medial degeneration of human abdominal aortic aneurysms. Am J Pathol 1997;150:993–1007.

21. Yamazumi K, Ojiro M, Okumura H, Aikou T. An activated state of blood coagulation and fibrinolysis in patients with abdominal aortic aneurysm. Am J Surg 1998;175:297–301.
22. Lindholt JS, Jorgensen B, Fasting H, Henneberg EW. Plasma levels of plasmin-antiplasmin-complexes are predictive for small abdominal aortic aneurysms expanding to operation-recommendable sizes. J Vasc Surg 2001;34:611–5.
23. King PS, Cooperberg PL, Madigan SM. The anechoic crescent in abdominal aortic aneurysms: not a sign of dissection. AJR Am J Roentgenol 1986;146:345–8.
24. Siegel CL, Cohan RH, Korobkin M, Alpern MB, Courneya DL, Leder RA. Abdominal aortic aneurysm morphology: CT features in patients with ruptured and nonruptured aneurysms. AJR Am J Roentgenol 1994;163:1123–9.
25. Mehard WB, Heiken JP, Sicard GA. High-attenuating crescent in abdominal aortic aneurysm wall at CT: a sign of acute or impending rupture. Radiology 1994;192:359–62.
26. Satta J, Laara E, Juvonen T. Intraluminal thrombus predicts rupture of an abdominal aortic aneurysm. J Vasc Surg 1996;23:737–9.
27. Wolf YG, Thomas WS, Brennan FJ, Goff WG, Sise MJ, Bernstein EF. Computed tomography scanning findings associated with rapid expansion of abdominal aortic aneurysms. J Vasc Surg 1994; 20:529–38.
28. Kazi M, Thyberg J, Religa P, et al. Influence of intraluminal thrombus on structural and cellular composition of abdominal aortic aneurysm wall. J Vasc Surg 2003;38:1283–92.
29. Kazi M, Zhu C, Roy J, et al. Difference in matrix-degrading protease expression and activity between thrombus-free and thrombus-covered wall of abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 2005;25:1341–6.
30. Francis CW, Markham RE Jr., Marder VJ. Demonstration of in situ fibrin degradation in pathologic thrombi. Blood 1984;63:1216–24.
31. Gacko M, Glowinski S. Activities of proteases in parietal thrombus of aortic aneurysm. Clin Chim Acta 1998;271:171–7.
32. Gacko M, Worowska A, Glowinski S. Coagulative and fibrinolytic activity in parietal thrombus of aortic aneurysm. Rocz Akad Med Bialymst 1999;44:102–10.
33. Sakalihasan N, Delvenne P, Nusgens BV, Limet R, Lapiere CM. Activated forms of MMP2 and MMP9 in abdominal aortic aneurysms. J Vasc Surg 1996;24:127–33.
34. Adolph R, Vorp DA, Steed DL, Webster MW, Kameneva MV, Watkins SC. Cellular content and permeability of intraluminal thrombus in abdominal aortic aneurysm. J Vasc Surg 1997;25:916–26.
35. Fontaine V, Touat Z, Mtairag el M, et al. Role of leukocyte elastase in preventing cellular re-colonization of the mural thrombus. Am J Pathol 2004;164:2077–87.
36. Fontaine V, Jacob MP, Houard X, et al. Involvement of the mural thrombus as a site of protease release and activation in human aortic aneurysms. Am J Pathol 2002;161:1701–10.
37. Diamond SL, Anand S. Inner clot diffusion and permeation during fibrinolysis. Biophys J 1993;65:2622–43.
38. Lindholt JS, Vammen S, Fasting H, Henneberg EW, Heickendorff L. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms. A preliminary study. Eur J Vasc Endovasc Surg 2000;20:281–5.
39. Sangiorgi G, D'Averio R, Mauriello A, et al. Plasma levels of metalloproteinases-3 and -9 as markers of successful abdominal aortic aneurysm exclusion after endovascular graft treatment. Circulation 2001;104: I288–95.
40. Tedgui A, Lever MJ. The interaction of convection and diffusion in the transport of 131I-albumin within the media of the rabbit thoracic aorta. Circ Res 1985;57:856–63.
41. Thaunat O, Field AC, Dai J, et al. Lymphoid neogenesis in chronic rejection: evidence for a local humoral alloimmune response. Proc Natl Acad Sci U S A 2005;102:14723–8.
42. Kratz A, Campos-Neto A, Hanson MS, Ruddle NH. Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. J Exp Med 1996;183:1461–72.
43. Warnatz K, Keskin AG, Uhl M, et al. Immunosuppressive treatment of chronic periaortitis: a retrospective study of 20 patients with chronic periaortitis and a review of the literature. Ann Rheum Dis 2005;64: 828–33.
44. Rose AG, Dent DM. Inflammatory variant of abdominal atherosclerotic aneurysm. Arch Pathol Lab Med 1981;105:409–13.
45. Schonbeck U, Sukhova GK, Gerdes N, Libby P. T(H)2 predominant immune responses prevail in human abdominal aortic aneurysm. Am J Pathol 2002;161:499–506.
46. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part I: evolving concepts. J Am Coll Cardiol 2005;46:937–54.
47. Wartman WB. Occlusion of the coronary arteries by hemorrhage into their walls. Am Heart J 1938;5:459–70.
48. Arbustini E, Morbini P, D'Armini AM, et al. Plaque composition in plexogenic and thromboembolic pulmonary hypertension: the critical role of thrombotic material in pultaceous core formation. Heart 2002;88:177–82.
49. Kolodgie FD, Gold HK, Burke AP, et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003;349:2316–25.
50. Kampschulte A, Ferguson MS, Kerwin WS, et al. Differentiation of intraplaque versus juxtaluminal hemorrhage/thrombus in advanced human carotid atherosclerotic lesions by in vivo magnetic resonance imaging. Circulation 2004;110:3239–44.
51. Leiner T, Gerretsen S, Botnar R, et al. Magnetic resonance imaging of atherosclerosis. Eur Radiol 2005;15:1087–99.
52. Takaya N, Yuan C, Chu B, et al. Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: a high-resolution magnetic resonance imaging study. Circulation 2005;111:2768–75.
53. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 2005;25:2054–61.
54. Chandler AB, Hand RA. Phagocytized platelets: a source of lipids in human thrombi and atherosclerotic plaques. Science 1961;134:946–7.
55. Kruth HS. Cholesterol accumulation in vascular smooth muscle cells incorporated into platelet-rich plasma clots. Lab Invest 1985;53:634–8.
56. Leclercq A, Houard X, Loyau S, et al. Topology of protease activities reflects atherothrombotic plaque complexity. Atherosclerosis 2006. In press.
57. Martin-Ventura JL, Nicolas V, Houard X, et al. Biological significance of HSP27 in human atherosclerosis. Arterioscler Thromb Vasc Biol 2006;26:1337–43.
58. Martin-Ventura JL, Duran MC, Blanco-Colio LM, et al. Identification by a differential proteomic approach of heat shock protein 27 as a potential marker of atherosclerosis. Circulation 2004;110:2216–9.
59. Menschikowski M, Kasper M, Lattke P, et al. Secretory group II phospholipase A2 in human atherosclerotic plaques. Atherosclerosis 1995;118:173–81.
60. Oorni K, Posio P, Ala-Korpela M, Jauhiainen M, Kovanen PT. Sphingomyelinase induces aggregation and fusion of small very low-density lipoprotein and intermediate-density lipoprotein particles and increases their retention to human arterial proteoglycans. Arterioscler Thromb Vasc Biol 2005;25:1678–83.
61. Williams KJ, Tabas I. Lipoprotein retention—and clues for atheroma regression. Arterioscler Thromb Vasc Biol 2005;25:1536–40.
62. Torzewski M, Suriyaphol P, Paprotka K, et al. Enzymatic modification of low-density lipoprotein in the arterial wall: a new role for plasmin and matrix metalloproteinases in atherogenesis. Arterioscler Thromb Vasc Biol 2004;24:2130–6.
63. Bjorkerud S, Bjorkerud B. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. Am J Pathol 1996;149:367–80.
64. Kolodgie FD, Narula J, Burke AP, et al. Localization of apoptotic macrophages at the site of plaque rupture in sudden coronary death. Am J Pathol 2000;157:1259–68.
65. Judware R, McCormick TS, Mohr S, Yun JK, Lapetina EG. Propensity for macrophage apoptosis is related to the pattern of expression and function of integrin extracellular matrix receptors. Biochem Biophys Res Commun 1998;246:507–12.
66. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. N Engl J Med 1987;316:1371–5.
67. Burke AP, Kolodgie FD, Farb A, Weber D, Virmani R. Morphological predictors of arterial remodeling in coronary atherosclerosis. Circulation 2002;105:297–303.
68. Pasterkamp G, Schoneveld AH, van der Wal AC, et al. Relation of arterial geometry to luminal narrowing and histologic markers for plaque vulnerability: the remodeling paradox. J Am Coll Cardiol 1998;32:655–62.
69. Ward MR, Pasterkamp G, Yeung AC, Borst C. Arterial remodeling. Mechanisms and clinical implications. Circulation 2000;102:1186–91.