Who are the proteolytic culprits in vascular disease?

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The extracellular matrix of the medial and adventitial layers of arteries serves several functions essential for vessel homeostasis. Beyond being an adhesive substrate for resident endothelial and smooth muscle cells and fibroblasts, vascular wall matrix proteins, such as elastin and collagen types I and III, provide resiliency, strength, and structural integrity to a tissue that is continually subjected to constant pulsatile pressure. Key to the structural function of these matrix proteins is their organization into large bundles of polymeric fibers welded together by covalent crosslinks. In healthy tissue, matrix proteins, especially fibrillar proteins, are quite stable and turn over slowly, if at all. In contrast, excessive degradation of vascular wall matrix is a key causative process in the progressive dilation of arteries during aneurysm formation (1) and in the breakdown, weakening, and eventual rupture of atherosclerotic lesions (2, 3).

Although a variety of proteinases, mostly released by infiltrating macrophages, have been implicated in the destruction of vessel wall matrix, the actual enzymatic culprits have not yet been conclusively fingered. Naming the guilty proteinase or proteinases is not straightforward for two main reasons. First, vascular lesions, or any inflamed tissue, are filled with many potentially destructive, extracellular proteinases of diverse gene families, principally metallo-, serine, and cysteine proteinases. Second, and more important, the actual in vivo function and scope of functions of most extracellular proteinases are not known. It is likely that many so-called matrix-degrading enzymes actually serve other functions, such as processing secreted precursor proteins, in a tissue environment. Thus, we cannot yet conclude if a specific proteinase in an inflammatory or injury setting is contributing to a reparative or disease process. Compounding this problem is a lack of animal models that fully duplicate human vascular disease. Although neointimal lesions form in injured and apolipoprotein E–deficient mice, these do not progress to rupture, the often fatal event that is generally thought to be a consequence of matrix breakdown.

Of the various proteinases present in vascular diseases, members of the matrix metalloproteinase family have received much attention, and several investigators, including myself, have proposed that these are the enzymes tearing down critical protein structures. Indeed, blocking metalloproteinase activity by treating animals with hydroxamate or tetracycline-based inhibitors or by over-expressing TIMPs (tissue inhibitors of metalloproteinases) slows or prevents aortic dilation in elastase-induced models of aneurysm formation (4–7). Inhibiting metalloproteinase activity by similar strategies also reduces neointimal formation in injury models of restenosis and in animal models of atherosclerosis, such as apolipoprotein E–deficient mice (8–11). The inhibition seen in the restenosis and atherosclerosis models implicates metalloproteinases in the development of neointimal lesions rather than in the weakening and rupture of the vessel wall.

Serine proteinases, mostly plasminogen and its activators, u-PA and t-PA, also have a role in vascular pathology, but they seemingly function indirectly by activating the latent forms of certain prometalloproteinases, which, in turn, degrade matrix. This concept was demonstrated in a series of convincing studies by Carmeliet, Collen, and coworkers (12, 13). For this work, they injured arteries in Plasminogen and u-PA knockout mice or crossed these animals with apolipoprotein E–deficient mice, which, in addition to neointimal formation, apparently develop destruction of their aortic media. In both models, they found that reduced activity of some metalloproteinases correlated with protection from neointimal formation and from breakdown of medial lamellar matrix. Because elastin is the main matrix component of aortic lamellae and because it is not degraded by plasmin or u-PA, these findings suggest that metalloproteinases are destructive, at least in these mouse models of vascular disease. However, the mechanisms in human disease appear to be more complex.

In this issue of the JCI, Shi et al. (14) report that cystatin C, the most abundant extracellular inhibitor of cysteine proteinases, is markedly reduced in human atherosclerotic and aneurysmal lesions. Furthermore, they find that the circulating levels of cystatin C are significantly lower in patients with dilated abdominal aortas, as determined by ultrasonography, compared with the levels in patients with a normal range of aortic diameter. Together with previous observations from this same group showing that cathepsins S and K are expressed in human vascular disease (15), these findings implicate cysteine proteinases in the pathogenesis of human vascular disease.

Several characteristics of cysteine proteinases make them intriguing suspects for causing matrix destruction in vascular disease. Although they were long thought to be strictly lysosomal enzymes that could not function in the extracellular space, many cysteine proteinase are now known to be secreted from cells, including macrophages (16), and to be active at neutral pH (17, 18). In addition, cysteine proteinases, such as the two enzymes found in diseased arteries, efficiently degrade extracellular matrix proteins, including elastin and fibrillar collagens. Cathepsin S is one of the most, if not the most potent elastase known (19), and cathepsin K cleaves the triple helix of collagens I and III (20, 21), an activity previously thought to be restricted to collagenases of the metalloproteinase family. Inherited deficiency of cathepsin K in humans — and targeted deletion of its mouse homolog — results in the sclerotic bone abnormality osteopetrosis.
vascular disease.

2. Libby, P., et al. 1996. Macrophages and athero-
3. Libby, P. 1995. Molecular bases of the acute coro-
4. Allaire, E., Forough, R., Cloves, M., Starcer, B., and
Clowes, A.W. 1998. Local overexpression of TIMP-1
prevents aortic aneurysm degeneration and rupture
Thompson, R.W. 1998. Pharmacologic suppress-
ion of experimental abdominal aortic aneurysms: a comparison of doxycycline and four
28:1082–1083.
teinase inhibitor BB-94 limits expansion of exper-
29:130–138.
imental abdominal aortic aneurysms by systemic
treatment with a hydroxamate-based matrix met-
Inhibition of matrix metalloproteinase activity
inhibits smooth muscle cell migration but not
neointimal thickening after arterial injury. Circ.
Res. 78:38–43.
9. George, S.J., Johnson, J.L., Angelini, G.D., Newby,
gene transfer of the human TIMP-1 gene inhibits
smooth muscle cell migration and neointimal
formation in human saphenous vein. Hum. Gene
Ther. 9:867–877.
10. George, S.J., Baker, A.H., Angelini, G.D., and
Newby, A.C. 1998. Gene transfer of tissue
inhibitor of metalloproteinase-2 inhibits meta-
lloproteinase activity and neointima formation in
expression of tissue inhibitor of metallopro-
teinase-1 reduces atherosclerotic lesions in
100:533-540.
plasmin activates matrix metalloproteinases dur-
minogen/plasmin and matrix metalloproteinase
systems after vascular injury in mice with target-
ed inactivation of fibrinolytic system genes. Arte-
human atherosclerotic and aortic aneurysms. J.
15. Sukhova, G.K., Shi, G.-P., Simon, D.J., Chapman,
ic cathespins S and K in human atheroma and
regulation of their production in smooth muscle
Pericellular mobilization of the tissue-destructive
cysteine proteinases, cathespins B, L, and S, by
human cathespin S in Saccharomyces cerevisiae.
Purification and characterization of the recom-
18. Dehmann, F.M., Coetzer, T.H., Pike, R.N., and
Dennison, C. 1995. Mature cathespin L is sub-
stantially active in the ionic milieu of the extracel-
and Chapman, H.A. 1992. Molecular cloning and
expression of human alveolar macrophage
cathespin S, an elastolytic cysteine proteinase. J.
20. Garrero, P., et al. 1998. The collagenolytic activ-
ity of cathespin K is unique among mammalian
21. Kafemah, W., Bromme, D., Buttle, D.J., Croucher,
K cleaves native type I and II collagens at the
terminal end of the triple helix. Biochem. J.
22. Gelb, B.D., Shi, G.-P., Chapman, H.A., and
Desnick, R.J. 1996. Pyconodysostosis, a lysosomal
disease caused by cathespin K deficiency. Science.
273:1236–1238.
resorption leads to osteopetrosis in cathespin K-defi-
spin L degrades extracellular matrix proteins in the