Production and Localization of 92-Kilodalton Gelatinase in Abdominal Aortic Aneurysms

An Elastolytic Metalloproteinase Expressed by Aneurysm-infiltrating Macrophages

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Abstract

Abdominal aortic aneurysms (AAA) are characterized by disruption and degradation of the elastic media, yet the elastolytic proteinases involved and their cellular sources are undefined. We examined if 92-kD gelatinase, an elastolytic matrix metalloproteinase, participates in the pathobiology of AAA. Gelatin zymography of conditioned medium from normal, atheroocclusive disease (AOD), or AAA tissues in organ culture showed that all tissues produced 72-kD gelatinase. AOD and AAA cultures also secreted 92-kD gelatinase, but significantly more enzyme was released from AAA tissues. ELISA confirmed that AAA tissues released ~ 2-fold more 92-kD gelatinase than AOD tissue and ~ 10-fold more than normal aorta. Phorbol ester induced a 5.3-fold increase in 92-kD gelatinase secretion by normal aorta and AOD and an 11.5-fold increase by AAA. By immunohistochemistry, 92-kD gelatinase was not detected in normal aorta and was only occasionally seen within the neointimal lesions of AOD tissue. In all AAA specimens, however, 92-kD gelatinase was readily localized to numerous macrophages in the media and at the adventitial–medial junction. The expression of 92-kD gelatinase mRNA by aneurysm-infiltrating macrophages was confirmed by in situ hybridization. These results demonstrate that diseased aortic tissues secrete greater amounts of gelatinolytic activity than normal aorta primarily due to increased production of 92-kD gelatinase. In addition, the localization of 92-kD gelatinase to macrophages in the damaged wall of aneurysmal aortas suggests that chronic release of this elastolytic metalloproteinase contributes to extracellular matrix degradation in AAA. (*J. Clin. Invest.* 1995. 96:318–326.) Key words: aortic aneurysm • metalloproteinases • macrophages • elastin • 92-kilodalton gelatinase

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Received for publication 29 August 1994 and accepted in revised form 6 March 1995.

Introduction

Abdominal aortic aneurysms (AAA)[1] are an increasingly significant clinical problem for which the only effective treatment is surgical replacement of the aorta (1, 2). Efforts to develop alternative treatment stategies for small asymptomatic AAA are presently limited by incomplete understanding of the biologic factors causing and promoting aneurysmal degeneration, and current concepts of aneurysm disease include the possibilities that atherosclerosis, hypertension, inflammatory and immune responses, gender, and genetic factors all play roles in the pathogenesis of AAA (3–10). AAA are characterized by marked destruction and functional loss of elastin in the aortic media (11, 12); however, the cellular and molecular events underlying the specific destruction of the elastic lamellae remain largely unknown. Because elastin is normally a highly stable extracellular matrix protein (13, 14), locally active elastolytic proteinases likely contribute to aneurysmal dilatation by progressive degradation of this structurally important aortic wall component (15, 16). In this context, increased proteolytic activities have been described in AAA, including serine elastases (17), plasminogen activators (18), and matrix metalloproteinases (MMPs) (19–22), some or all of which may be involved in aortic wall elastin degradation (23).

MMPs represent a family of connective tissue–degrading enzymes that participate in a variety of normal and pathologic processes (24, 25). 92-kD gelatinase is the largest member of this structurally related family, which also includes interstitial collagenase, 72-kD gelatinase/type IV collagenase, stromelysin-1, matrilysin, macrophage metalloelastase, and other enzymes. Like other MMPs, 92-kD progelatinase is secreted in zymogen form, and is thought to be activated by cleavage within a conserved amino-terminal propeptide (24–28). Its activity is further dependent upon zinc and calcium ions as essential cofactors. Secreted progelatinase is activated within the extracellular space, perhaps by other proteases, to yield the active 80-kD form (26, 27), and its activity can be sequestered by at least one tissue inhibitor of metalloproteinases (TIMP-1) (28). 92-kD gelatinase is prominently produced and secreted by blood monocytes/tissue macrophages (29–31), eosinophils (32, 33), and neutrophils (34), indicating that this MMP plays an important role in inflammatory processes.

Because 92-kD gelatinase can degrade numerous extracellular matrix components of the vessel wall, such as elastin and type IV collagen (35), and because it is produced by inflammatory cells associated with aneurysms, this enzyme may contribute to the etiology of aneurysm disease. Indeed, a metallogelatinase of 80–92 kD has been described in extracts of aneurysm tissue (19–23). Although 92-kD gelatinase is considered to be a relatively weak elastase as compared to serine enzymes, such

1. *Abbreviations used in this paper:* AAA, abdominal aortic aneurysm; AOD, athrosclerotic occlusive disease; CM, conditioned medium; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases.

J. Clin. Invest.

0021-9738/95/07/0318/09 $2.00
Volume 96, July 1995, 318–326

as human neutrophil elastase (35, 36), sustained local production may result in pronounced and progressive degradation of elastin in the aortic media. The purpose of this study was to evaluate the production of elastolytic MMPs by human aortic tissues based on the idea that significant differences in metalloproteinase production between normal, atherosclerotic occlusive disease (AOD), and AAA tissues might be involved in the pathophysiology of aneurysmal degeneration. We report that 92-kD gelatinase is actively produced in AAA by macrophages that localize to sites of tissue damage.

Methods

Human aortic tissues. Full-thickness specimens of normal infrarenal abdominal aorta, without visible evidence of atherosclerosis, were obtained in the operating room from organ transplantation donors. Tissue specimens were also obtained from patients undergoing aortic reconstruction for AOD or nonspecific AAA, following a protocol approved by the Washington University School of Medicine Human Research Subjects Committee. For each specimen obtained, one portion of the aortic wall was used for aortic organ cultures and an adjacent portion was processed for histologic studies.

Aortic organ cultures. Aortic tissue was transported to a sterile tissue culture hood in cold (4°C) DME supplemented with 1% BSA and antibiotics. Each specimen was divided with a scalpel into multiple 2-mm^2 segments of full-thickness aortic wall, and these segments were placed into separate wells of 6-well tissue culture plates. After 20 min to allow adherence to the tissue culture plastic, each well was supplemented with 1.5 ml medium, and the plates incubated at 37°C in a humidified 5% CO_2 atmosphere. After 72 h, conditioned medium (CM) was collected and stored at −20°C. Samples then received 1.5 ml fresh medium containing 0.4% (vol/vol) DMSO vehicle or 40 nM PMA in DMSO. After an additional 72-h incubation, CM was harvested and stored at −20°C. For each CM sample total protein concentration was determined using a Bradford assay kit from Bio Rad Laboratories (Hercules, CA).

Substrate gel electrophoresis. CM samples were examined by SDS-gelatin zymography as described (37). Equivalent amounts of CM (8 μg protein per lane) were resolved by nondenaturing electrophoresis through a 10% SDS-polyacryamide gel copolymerized with 1 mg/ml gelatin. Purified 72-kD progelatinase from human dermal fibroblast–conditioned medium and 92-kD progelatinase from PMA-differentiated U937 cell-conditioned medium were included as internal controls. SDS was removed, and gels were incubated overnight at 37°C and stained with 0.1% Coomassie brilliant blue R-250. In some experiments, gels were incubated in the presence of 10 mM EDTA, 2 mM 1,10-phenanthroline, or 5 mM PMSF, or latent metalloproteinases were activated by incubating CM with 2 mM aminophenylmercuric acetate (APMA) for 1 h at 37°C before gelatin zymography.

Gelatin-Sepharose purification. To determine if gelatinase activities secreted by aortic cultures degraded elastin, CM was partially purified by gelatin-Sepharose chromatography. CM was added to gelatin-Sepharose (Sigma Chemical Co., St. Louis, MO) equilibrated with 20 mM Tris-HCl, pH 7.5, 1.0 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35, and the flow-through fraction was saved. Gelatinase activities were eluted with the same buffer containing 10% DMSO, and the column was stripped with 8 M urea. All fractions were examined by κ-elastin zymography as described (36).

Immunologic assays. The 92-kD gelatinase content in CM samples was quantitated by a competitive-binding indirect ELISA as described (38). This assay has nanogram sensitivity, is specific for the 92-kD metalloproteinase, and measures total enzyme present whether free or bound to inhibitor or substrate, or whether in inactive or active forms. Standard curves were included in each assay using purified human 92-kD gelatinase. The concentration of TIMP-1 in CM samples was quantitated by ELISA (39).

Immunohistochemistry. Fresh aortic tissues were rinsed free of blood with normal saline, fixed in 10% neutral buffered formalin at 4°C for 24 h, and processed for routine paraffin embedding. 5-μm sections were deparaffinized and rehydrated, and endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 for 30 min at room temperature. Samples were incubated with affinity-purified rabbit anti–human 92-kD gelatinase IgG or preimmune rabbit serum as described (40), and immune complexes were detected by immunoperoxidase using a Vectastain Elite kit (Vector Laboratories, Inc., Burlingame, CA). Tissue macrophages were detected with CD-68 antibodies, vascular smooth muscle with smooth muscle α-actin antibodies, vascular endothelium with *Ulex europaeus* Type I lectin and monospecific antiagglutinin, and lymphocytes with a panel of human lymphocyte-specific antibodies, all from Dako Corporation, (Carpenteria, CA).

In situ hybridization. Probes for in situ hybridization were prepared as described (33). Briefly, a 560-bp BamHI-XbaI fragment of the 3′ end of human 92-kD gelatinase cDNA, subcloned in a Bluescript KS transcription vector (Stratagene Inc. La Jolla, CA), was linearized to allow transcription of antisense or sense RNA. This part of the cDNA encodes for the carboxyl terminus of 92-kD gelatinase which displays considerable sequence divergence from similar domains of other metalloproteinases. RNA probes were transcribed from linear cDNA templates and labeled with digoxigenin-11-UTP under conditions recommended by and with reagents from Boehringer Mannhein Corp. (Indianapolis, IN). In situ hybridization was performed essentially as described (33). Briefly, sections (5 μm) were treated with proteinase K (Sigma Chemical Co.) and were washed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. Sections were covered with 25–50 μl of hybridization buffer containing digoxigenin-labeled antisense or sense RNA probe and incubated at 42°C for 18 h in a humidified chamber. After hybridization, slides were washed under stringent conditions, including treatment with RNase-A. Sections were treated with alkaline phosphatase-conjugated antidigoxigenin antibody and 10% normal sheep serum, followed by 5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolium (NBT) as chromogens. After development for 4–24 h in the dark, the color reaction was stopped with Milli-Q water and the slides were stained with nuclear fast red.

Results

Aortic tissues release elastolytic gelatinases. CM of normal aorta released a gelatinase which comigrated with authentic 72-kD progelatinase (Fig. 1 *A*). Equivalent levels of 72-kD gelatinase activity were observed in CM from AOD and slightly greater levels were detected in AAA organ cultures, suggesting that this MMP is not selectively increased in aneurysm disease. CM from normal and AOD tissues produced little to no 92-kD gelatinase, whereas AAA organ cultures consistently produced prominent gelatinolytic activity which comigrated with purified human 92-kD progelatinase (Fig. 1 *A*). The higher molecular weight gelatinolytic bands observed in AAA CM probably represent complexed forms of 92-kD progelatinase with itself and with TIMP-1 which remain undissociated under the nondenaturing conditions of substrate zymography (28). Indeed, when these bands were isolated and resolved under denaturing conditions, only two distinct protein bands at 92 kD and 28 kD were seen, consistent with 92-kD progelatinase and 28-kD TIMP-1 (data not shown). We used gelatin-Sepharose chromatography to partially purify gelatinases from CM and κ-elastin zymography to detect elastolytic activities. Elastolytic activities migrating at ~ 72-kD from normal aortic CM and both 72 kD and 92 kD from AAA CM were consistently seen in fractions eluted with 10% DMSO (Fig. 1 *B*). No other elastolytic activity was detected in this fraction, in the column flow-through, or in a dialyzed urea strip fraction. For all CM samples examined,

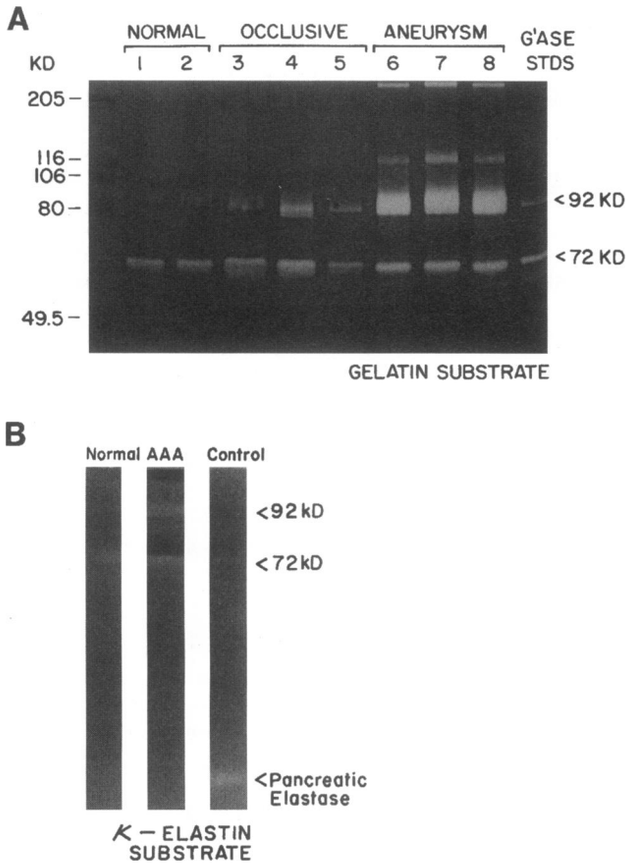


Figure 1. Substrate zymography of aortic organ culture CM. (*A*) Gelatin zymography of 72-h serum-free CM from cultures of normal aorta (lanes *1* and *2*), AOD (lanes *3–5*), and AAA (lanes *6–8*). The migration of undenatured molecular weight standards is shown on the left and authentic gelatinase standards were run in the right lane. Equivalent amounts (8 μg protein) of CM were analyzed for each sample, and substrate gels were incubated overnight to reveal gelatinolytic activity. Gelatinolytic activities were observed as clear bands against a dark staining background of intact gelatin, and their relative molecular weights estimated by comparison with the migration of known molecular weight and gelatinase standards. Relatively uniform amounts of 72-kD gelatinase are seen for each aortic tissue type. 92-kD gelatinase is seen in CM from AOD and AAA cultures, with a marked increase in all samples derived from AAA cultures. (*B*) κ-elastin zymography of partially purified CM. In the right lane (*control*) the migration of porcine pancreatic elastase is shown. Equivalent amounts of normal or AAA CM samples were resolved, and elastolytic activity was revealed by overnight incubation. A single band of 72-kD elastolytic activity is seen in normal CM, while both 72-kD and 92-kD bands of activity, corresponding to the gelatinases previously isolated, are seen in AAA CM. These elastolytic activities were abolished by incubation of gels in EDTA or 1,10-phenanthroline but not PMSF, while porcine pancreatic elastase had the inhibitory profile expected for a serine protease (data not shown).

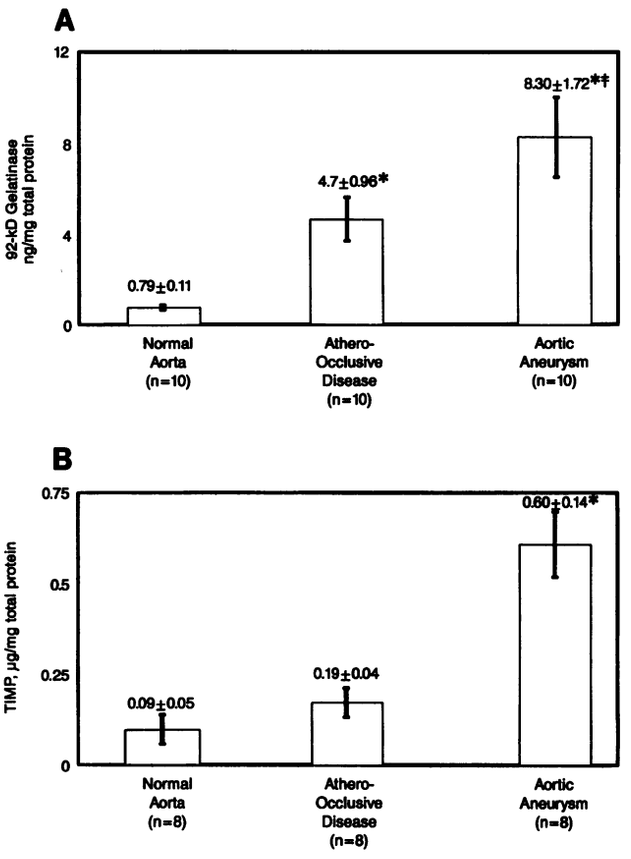


Figure 2. Production of 92-kD gelatinase and TIMP-1 in aortic organ cultures. 92-kD gelatinase (*A*) and TIMP-1 (*B*) were quantified by ELISA in the 72-h serum-free CM from organ cultures of normal, AOD, and AAA aortic tissues. (*A*) Data shown are the mean±SEM for $n = 10$ samples derived from each tissue type (ANOVA, $P < 0.05$ vs * normal aorta, vs ‡AOD). B. Data shown are the mean±SEM for $n = 8$ samples derived from each tissue type (ANOVA, $P < 0.05$ vs * normal aorta).

the gelatin- and elastin-degrading activities were abolished by incubation of the gels in EDTA or 1,10 phenanthroline, but were unaffected by PMSF, confirming their identity as metalloproteinases (data not shown). Thus, 92-kD gelatinase is the principle elastolytic activity that is selectively produced in AAA tissue.

AAA produce increased amounts of 92-kD gelatinase and TIMP-1. In agreement with the gelatin zymography results, cultures of AAA and AOD tissues released ~ 10.5-fold and 5.9-fold more 92-kD gelatinase, respectively, than that secreted by normal aortic organ cultures (Fig. 2 *A*). Similarly, six-fold higher levels of TIMP-1 protein were detected in CM from AAA cultures relative to normal aorta, while AOD cultures secreted only two-fold more TIMP-1 (Fig. 2 *B*). These findings indicate that markedly elevated levels of 92-kD gelatinase and TIMP-1 are produced in AAA tissues.

PMA stimulates 92-kD gelatinase production by aortic organ cultures. To assess if the production and secretion of 92-kD gelatinase and TIMP-1 can be modulated in aortic tissue, we treated cultures with phorbol ester, which is a potent stimulator of 92-kD gelatinase and TIMP-1 in various cell types (41, 42). Gelatin zymography demonstrated that PMA markedly increased 92-kD gelatinase expression in CM from all tissue types and had little effect on the production of 72-kD gelatinase (Fig. 3). When quantified by ELISA, normal and AOD aortic cultures secreted 5.3-fold more 92-kD gelatinase after PMA stimulation than unstimulated cultures, whereas PMA-stimulated AAA cultures secreted 11.5-fold more 92-kD gelatinase than unstimulated cultures (Fig. 4 *A*). TIMP-1 was not significantly affected by PMA-treatment in any aortic tissue type (Fig. 4 *B*). The marked up-regulation of 92-kD gelatinase secretion

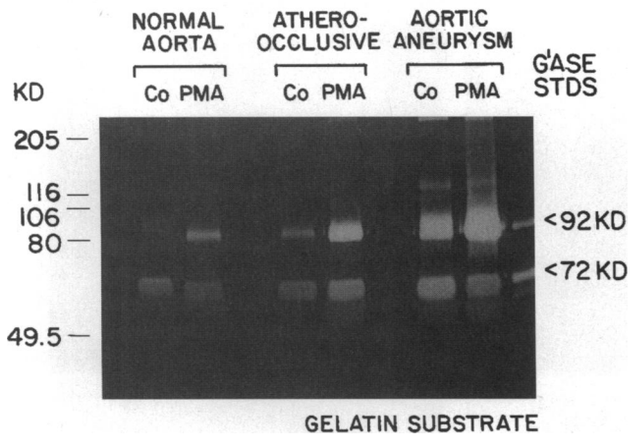


Figure 3. Effect of PMA on aortic tissue production of gelatinolytic activities. Gelatin zymography of CM from cultures treated with 50 ng/ml PMA or vehicle control (*Co*). Equivalent amounts (8 μg protein) of CM were analyzed for each sample. Relatively uniform production of 72-kD gelatinase is seen for each type of aortic tissue, with minimal stimulation by PMA. In cultures of normal aortic tissue, PMA induces the secretion of 92-kD gelatinase activity not present in unstimulated CM. In cultures of both AOD and AAA tissue, the amount of 92-kD gelatinase activity secreted is markedly increased by PMA.

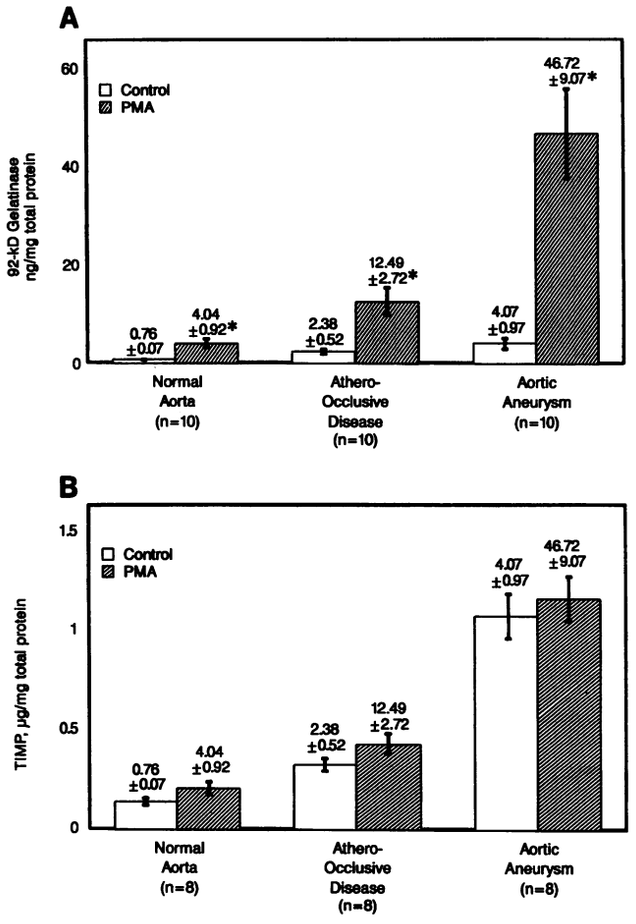


Figure 4. Effect of PMA on the production of 92-kD gelatinase and TIMP-1 in aortic organ cultures. 92-kD gelatinase (*A*) and TIMP-1 (*B*) were quantified by ELISA in the 72-h serum-free CM from organ cultures of normal, AOD, and AAA aortic tissues treated with 50 ng/ml PMA or vehicle control. (*A*) Data shown are the mean±SEM for $n = 10$ samples derived from each tissue type and treatment (*ANOVA, $P < 0.05$, PMA vs control). (*B*) Data shown are the mean±SEM for $n = 8$ samples derived from each tissue type and treatment (*ANOVA, $P < 0.05$, PMA vs control).

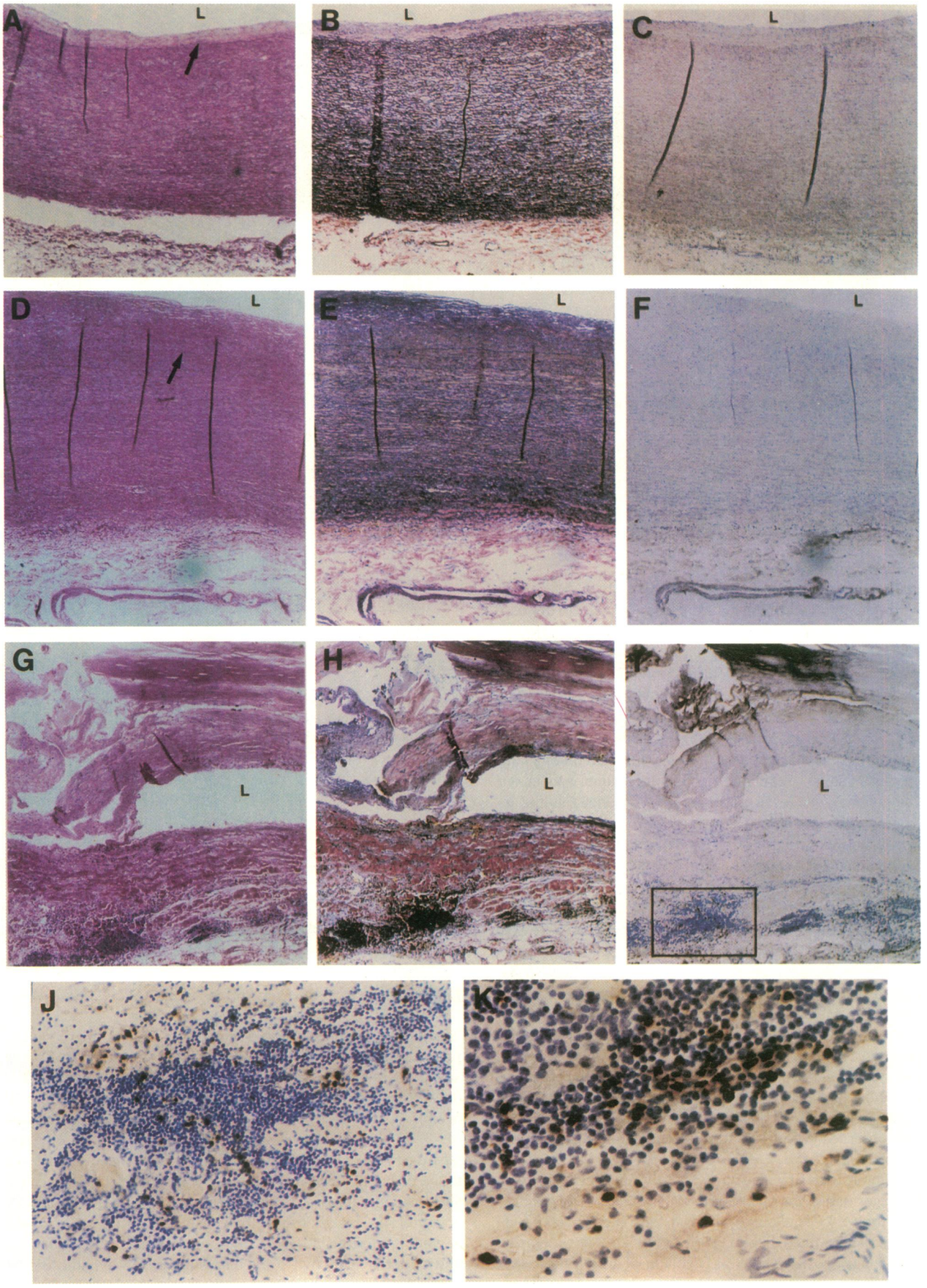
by tissue segments exposed to PMA, particularly in AAA, indicates the potential for stimulated expression by cells in diseased aorta upon exposure to MMP-inducing agents.

92-kD gelatinase is localized to aneurysm-infiltrating macrophages. Using immunohistochemistry with affinity-purified antibody, 92-kD gelatinase was not detected in the normal aortic wall (Fig. 5). In AOD specimens, 92-kD gelatinase was detected in only 3 of 12 samples. In two of these tissues weak staining for the enzyme was seen in scattered inflammatory cells within the thickened intimal atheromatous plaque, and in one specimen signal was localized to an area of periadventitial inflammation (data not shown). In contrast to normal and AOD tissues, 92-kD gelatinase was readily detected in all AAA specimens examined and staining was typically seen in severely damaged areas of the aortic wall (Figs. 5 and 6). Staining of serial sections of AAA for various cell type–specific antigens demonstrated that 92-kD gelatinase-producing cells were also positive for macrophage-specific CD-68 antigen (Fig. 6). Distinctly different patterns were observed for lymphocyte, vascular smooth muscle cell, and endothelial cell antigens (data not shown). In no case did nonmacrophage cell types stain for 92-kD gelatinase. 92-kD gelatinase–positive macrophages represented a subset (~ 10–20%) of the inflammatory cells seen in the aneurysm wall and were spatially associated with periadventitial vascularization and lymphocytic infiltration. Despite the presence of severe aortic atherosclerosis, the enzyme was generally not observed within the degenerated intima of the AAA tissues examined.

As demonstrated by in situ hybridization, the majority of macrophages within the outer aortic wall of AAA were strongly positive for 92-kD gelatinase mRNA, and signal for 92-kD gelatinase was confined to this cell type (Fig. 7). The proportion of macrophages positive for 92-kD gelatinase mRNA was substantially greater than those that were seen by immunohistochemistry, suggesting that this enzyme is actively being secreted. No significant signal was seen on sections hybridized with sense RNA probes (Fig. 7 *B*).

Discussion

Traditional concepts of AAA have generally considered aneurysmal degeneration to be a manifestation or complication of advanced atherosclerosis (4). However, clinical and pathologic differences between patients with aneurysms and those with occlusive atherosclerosis have challenged these assumptions (1–3, 43, 44), and investigations of the pathobiology of AAA have begun to assess the role of aneurysm-related connective tissue degrading enzymes in this disease (7, 15–23). Because aneurysmal change is characterized by a marked decrease in aortic elastin, much of this effort has focused on known elastin degrading enzymes, such as the serine protease human neutrophil elastase. Although some data support the hypothesis that serine elastases are involved in the pathophysiology of aneurysms (16, 17), our results suggest that MMPs, and in particular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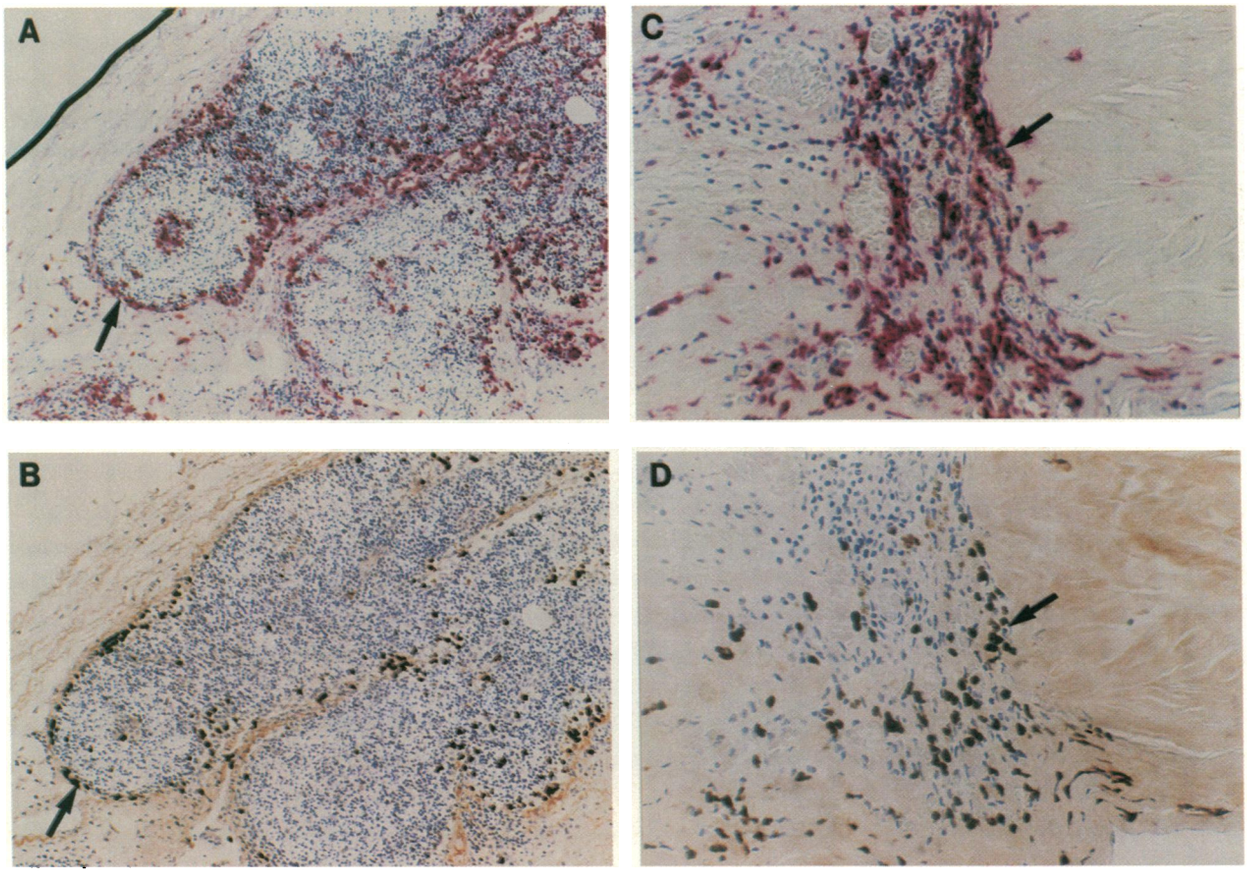

Figure 6. 92-kD gelatinase is localized to macrophages in AAA. Direct comparison of immunolocalization patterns for tissue macrophages and 92-kD gelatinase in serial sections of AAA. In each pair of sections (*A* and *B, C* and *D*), the population of the tissue macrophages stained by CD-68 monoclonal antibody also stained for 92-kD gelatinase (*arrows*). Alkaline phosphatase/antialkaline phosphatase using CD-68 monoclonal antibody (red reaction product) and hematoxylin counterstain (*A* and *C*); immunoperoxidase using affinity-purified rabbit anti-human 92-kD gelatinase antibody (brown reaction product) and hematoxylin counterstain (*B* and *D*). ×100 (*A* and *B*); ×200 (*C* and *D*).

92-kD gelatinase, are the principle elastases present in human aneurysmal tissue.

The identification of other connective tissue enzymes with elastolytic activity, particularly members of the MMP family (35, 36, 45, 46), has suggested that metalloelastases might participate in the connective tissue degeneration underlying aneurysm disease. Indeed, using zymography or substrate-degradation assays, various investigators have found elastolytic metalloenzymes in extracts of AAA (19–23), and, in agreement with our findings, these earlier studies found prominent activity at ~ 80–92 kD (20, 21). We have shown that 92-kD gelatinase is actively secreted by aortic tissues using substrate zymography and a highly specific ELISA, and that the production of this elastolytic metalloproteinase is markedly increased in cultures of AAA compared to normal and atheroocclusive disease tissues. Further, our results demonstrate that release of 92-kD gelatinase by aortic tissues is increased by PMA, with a significantly greater effect on AAA tissues. Taken together with previous studies, our findings implicate an important role for the regulated, local production of 92-kD gelatinase in the pathophysiology of aortic aneurysm disease.

In many tissues, MMPs produced by normal, migratory, and cancer cells are involved in connective tissue degradation and remodeling (24, 25). In the diseased aorta, resident or inflammatory cells might produce MMPs, mediate their activation, or localize their enzymatic activity to adjacent matrix protein substrates. Our findings indicate that 92-kD gelatinase was localized to nonfoamy tissue macrophages present in the outer aortic wall in AAA; however, the enzyme was not seen in normal aorta or atheroocclusive disease tissues. Thus, the ex-

Figure 5. Immunohistology of abdominal aortic tissues. (*A–I*) Serial sections of formalin-fixed, paraffin-embedded specimens representing normal aorta (*A–C*), AOD aorta (*D–F*), and AAA (*G–I*). Sections were stained with hematoxylin and eosin (*A, D,* and *G*), with Verhoeff-van Geisen elastin (*B, E,* and *H*), or affinity-purified rabbit anti-human 92-kD gelatinase antibody and Harris-hematoxylin counterstain (*C, F,* and *I*). The presence of elastic lamellae in AOD is contrasted with their virtual absence in AAA. 92-kD gelatinase was seen only in sections of AAA, and the positive-staining cells were located within the prominent outer aortic wall inflammatory infiltrate (*outlined*). In each section the aortic lumen (*L*) is oriented at the top, and the internal elastic lamina is marked by arrows. ×40. (*J* and *K*) High-power views of the outer aortic wall inflammatory infiltrate outlined in *I*, demonstrating localization of 92-kD gelatinase to mononuclear inflammatory cells. ×100 (*J*); ×200 (*K*).

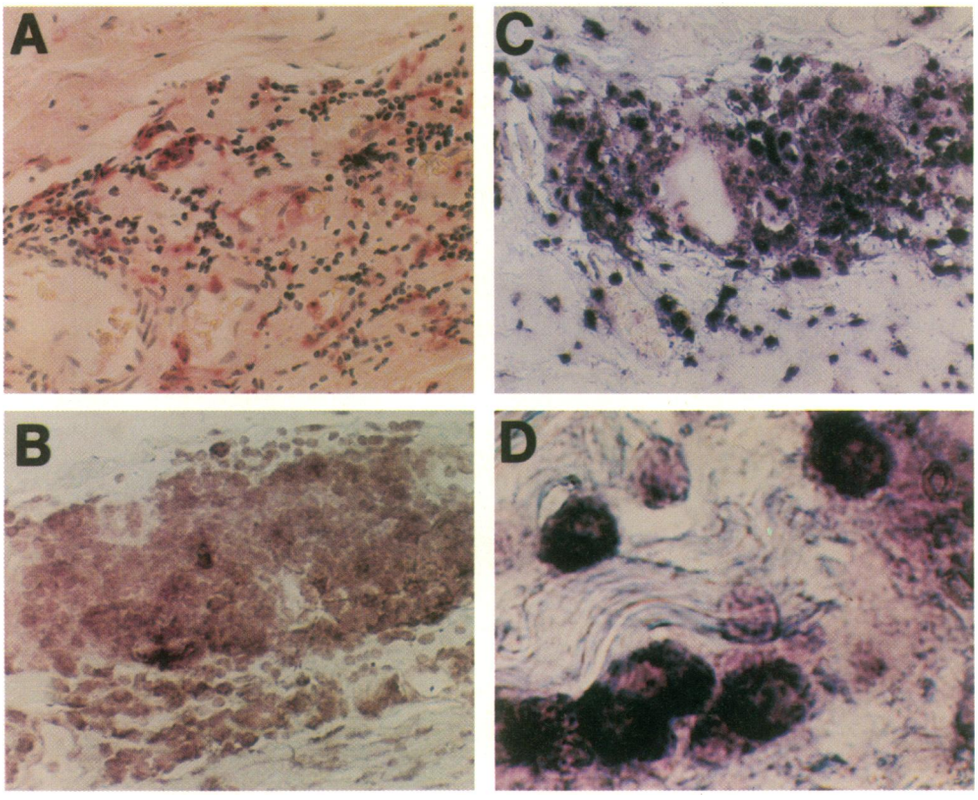

Figure 7. Macrophages express 92-kD gelatinase mRNA in AAA. Immunolocalization of tissue macrophages (*A*) and in situ hybridization for 92-kD gelatinase (*B–D*) in sections of AAA. Whereas no significant hybridization is observed for the control (sense) RNA probe (*B*), nearly all macrophages within the outer aortic wall of AAA display hybridization with 92-kD gelatinase antisense RNA (*C* and *D*). Alkaline phosphatase/antialkaline phosphatase using CD-68 monoclonal antibody (red reaction product) and hematoxylin counterstain (*A*); alkaline phosphatase-conjugated antidigoxigenin using digoxigenin-labeled sense (*B*) and antisense (*C* and *D*) 92-kD gelatinase cRNA probes, with nitro blue tetrazolium chromogen (dark purple reaction product) and nuclear fast red counterstain. ×100 (*A–C*); ×400 (*D*).

pression of 92-kD gelatinase, which can degrade diverse matrix proteins, such as gelatin, collagen types IV, V, and XVII, and elastin, by aneurysm-infiltrating macrophages is a potential mechanism of aortic structural protein degradation. Although 92-kD gelatinase may be a somewhat weaker elastase than serine proteases, sustained local production and proteolysis by this MMP could mediate significant and progressive aortic elastin degradation to promote aneurysmal degeneration.

Chronic inflammation within the outer aorta is a prominent feature of AAA (5, 6), suggesting that immune and inflammatory cells participate in the connective tissue destruction associated with aneurysmal degeneration. As shown here, the production and localization of 92-kD gelatinase to tissue macrophages in human aortic aneurysms provides strong evidence that these cells and this elastolytic metalloproteinase participate in the progressive pathophysiology of this disease. Whether there are unique features of the tissue environment in aneurysms that specifically affect macrophage function in the elastic media remains unknown. For example, human alveolar macrophages express several elastolytic proteinases, principally 92-kD gelatinase and the 22-kD human macrophage metalloelastase (46). However, using similar methods to detect elastin-degrading activity, we were unable to detect an elastolytic proteinase in the molecular weight range of human macrophage metalloelastase produced by AAA tissues. Thus, our findings indicate that 92-kD gelatinase is the main elastolytic MMP released in AAA, and suggests that aneurysm-infiltrating macrophages secrete a unique spectrum of proteinases that differs from the pattern of MMPs secreted by these cells in other tissues.

The factors that attract macrophages to the outer aortic wall of AAA are unknown. However, the destruction of insoluble elastic fibers may lead to production of soluble elastin degradation peptides that are chemotactic for several cell types, including monocytes (47, 48). Additional factors, such as cytokines, growth factors, and soluble peptides derived from other matrix protein substrates, might also act to further recruit and influence macrophage migration (49–51). Such a cyclical pattern of matrix degradation and effector cell recruitment would be consistent with the progressive nature of aortic wall destruction that occurs in AAA.

All known members of the MMP family are secreted in proenzyme form, requiring extracellular modification to achieve full activity (24–28). For 92-kD gelatinase, this proteolytic cleavage results in an 80-kD active enzyme that binds tightly to its matrix substrates, and thus, would be difficult to isolate from tissue extracts. Because we measured soluble enzyme in CM from aortic cultures, active (80 kD) enzyme would be predictably underrepresented in this system. Nevertheless, the substantial levels of 92-kD gelatinase secreted by aneurysmal tissues may indicate that significant and possibly greater levels of the active enzyme would be present in the tissue.

Metalloenzyme activity is further regulated by specific inhibitors, and we found that TIMP-1 is also released by aortic CM. However, in other studies of diseased tissues with evidence of ongoing remodelling, we have usually found that TIMP-1 and metalloenzymes, including 92-kD gelatinase, are produced in distinct locations and often by different cell types (33, 52–54). This spatially distinct pattern of expression suggests that metalloproteinases act in a pericellular fashion without impedence from TIMP-1. Thus, the inhibitor may act principally to neutralize excess activated enzyme that is free in the tissue fluid after substrate degradation, but the presence of 92-kD gelatinase and TIMP-1 in AAA CM does not mean that these proteins had interacted at sites of tissue destruction.

The findings presented here suggest that increased production of 92-kD gelatinase by tissue macrophages participates in the etiology and progression of aneurysmal disease. We cannot fully exclude that other elastin-degrading metalloenzymes, such as human macrophage metalloelastase, might have some role in the pathophysiology of this disease. Furthermore, medial degeneration may ultimately be the result of multiple enzymes acting in concert, including serine proteases and plasminogen activators, as well as other metalloenzymes. In turn, resident aortic wall cells, such as endothelium, smooth muscle cells, and fibroblasts may contribute to this process. Our results indicate that 92-kD gelatinase is the predominant metalloelastase present in established AAA. The factors controlling the apparent selective expression of this enzyme in aneurysm disease remain to be discovered.

Acknowledgments

We gratefully acknowledge the assistance of Drs. Brent T. Allen, Charles B. Anderson, M. Wayne Flye, Todd K. Howard, Gregorio A. Sicard, and Steven M. Strasberg in procuring tissue specimens for this study; Leslie Cottle and Catherine Flizsar for ELISA determinations; and Teresa Tolley and Jill Roby for expert assistance with immunohistology and in situ hybridization studies.

This work was supported by a grant from the Washington University School of Medicine New Investigator Research Support Program and an American College of Surgeons Faculty Fellowship (R. W. Thompson), a Genentech/American Lung Association Career Investigator Award (W. C. Parks), and Grants HL-29594 (W. C. Parks, H. G. Welgus, and R. P. Mecham) and HL-48762 (W. C. Parks) from the National Institutes of Health.

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