Short Communication

Macrophages Contain 92-kd Gelatinase (MMP-9) at the Site of Degenerated Internal Elastic Lamina in Temporal Arteritis

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Inflammation precedes erosion and rupture of atherosclerotic atheromas and aneurysms. Inflammatory infiltrates of macrophages have been shown to secrete proteolytic enzymes, including matrix metalloproteinases (MMPs), that weaken the arterial wall. The effect of inflammation on arterial structure and remodeling can be studied in primary vascular inflammatory diseases such as in temporal arteritis. We examined the 72-kd gelatinase (MMP-2) and the 92-kd gelatinase (MMP-9) in inflamed and uninvolved temporal arteries from 10 patients with temporal arteritis and 5 controls by immunobistochemistry. The substrates of these enzymes, type IV collagen and elastin, were detected by immunobistochemistry and histochemical staining, respectively. Both diseased and normal artery specimens had moderate staining for immunoreactive MMP-2. Temporal arteritis specimens had clearly enhanced immunostaining for MMP-9 compared with normal arteries. MMP-9 was specifically localized to macrophages in regions of internal elastic lamina disruption, which may thus be of pathological significance. (Am J Pathol 1996, 149:1427–1433)

Inflammation has been shown to play an important role in the erosion and rupture of atheromas, contributing to the clinical complications of atherosclerosis, such as myocardial infarction, stroke, and arterial aneurysms. Sites of rupture in the margins of fibrous caps of atherosclerotic plaque are rich in macrophages. Release by these cells of proteolytic enzymes, particularly matrix metalloproteinases (MMPs), has been suggested as a mechanism for plaque rupture. In fact, macrophages in vulnerable regions of the plaque have been shown to express interstitial collagenase (MMP-1), 72-kd gelatinase (MMP-2), stromelysin (MMP-3), and 92-kd gelatinase (MMP-9).

Effects of inflammation on vascular remodeling can be studied in primary inflammatory arterial diseases. Temporal arteritis is the most common vasculitis of medium-sized arteries leading to extensive intimal fibrosis and arterial occlusion. This syndrome affects most frequently temporal arteries, but occasionally also coronary arteries, aorta, and other arterial beds are involved.

Recent data have indicated a link between the occurrence of temporal arteritis and aortic aneurysm, often a complication of atherosclerosis, suggesting a common pathogenesis. The survival rate of temporal arteritis patients with pre-existing angina pectoris is decreased compared with patients without previous circulatory diseases. The pathogene-
sis of temporal arteritis is currently unknown. Biopsy specimens usually show mononuclear cell infiltration or granulomatous inflammation, often with macrophage-derived giant cells.10

Disintegration of arterial elastic lamellae and cell basement membranes by macrophage-derived proteolytic enzymes may be an important mechanism compromising arterial stability and promoting excessive cell migration and proliferation, thus enhancing intimal hyperplasia. Because MMP-2 and MMP-9 may contribute to arterial remodeling in arterial inflammation, we examined their location in temporal arteritis arterial wall in relation to matrix and cellular components.

Materials and Methods

Human Arterial Tissue

Temporal artery biopsy specimens were obtained from ten patients with temporal arteritis (nine women and one man; mean age, 67 years; range, 61 to 75 years). Temporal arteritis was diagnosed on the basis of classical clinical and histological criteria.10 Five arterial biopsies of uninvolved temporal arteries were used as control arterial tissue (four women and one man; mean age, 64 years; range, 54 to 80 years). Tissues were fixed in 10% neutral buffered formalin. After paraffin embedding, 5-μm transverse sections were cut and used for immunohistochemistry and histochemistry.

Histochemistry and Immunohistochemistry

To detect elastic laminae, the samples were stained with orcein. Cell nuclei were stained with hematoxylin. Immunohistochemical staining was performed by the streptavidin-biotin/horseradish peroxidase method with 3,3′-diaminobenzidine as a chromogen, as described previously.5 The following cell types were identified with the use of their respective monoclonal antibodies: human macrophages by HAM-56 (titer = 1:100; Dako Corp., Carpinteria, CA), smooth muscle cells by anti-smooth muscle α-actin (titer = 1:250; Sigma Chemical Co., St. Louis, MO), and T lymphocytes by anti-human T cell CD45RO (titer = 1:100; Dako). MMP-9 protein was localized by a mouse monoclonal antiserum to human MMP-9 (GE-213; 1:70).11 MMP-2 protein was detected by a mouse monoclonal antiserum to human MMP-2 (CA-4001; 1:1000).12 Type IV collagen was identified by a mouse monoclonal antibody to human type IV collagen (Sigma). Negative controls included substitution of mouse IgG for monoclonal antibodies as well as omission of the primary antibody.

In double immunostaining, the sections were treated after the first peroxidase plus nickel chloride (black) staining for 15 minutes with an avidin/biotin blocking kit (Vector Laboratories, Burlingame, CA) and then processed for the second staining with alkaline phosphatase (red) using the appropriate ABC kits (Vector).

Western Blot

Recombinant proteins for MMP-2 and MMP-9 were produced by the Vaccinia system as described previously for MMP-2.13 Conditioned serum-free media from human fibrosarcoma HT-1080 cells (CCL-121, American Type Culture Collection, Rockville, MD) was used as an additional source of MMP-9 antigen. Recombinant and cell culture media MMP-9 were activated by 1 mmol/L p-aminophenylmercuric acetate (Sigma) at 37°C for 30 minutes. Samples and biotinylated molecular weight standards (BioRad, Hercules, CA) were separated on 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred electrophoretically to nitrocellulose sheets (Schleicher and Schuell, Keene, NH). The blots were blocked with 1% bovine serum albumin in 50 mmol/L Tris/HCl (pH 7.4), 150 mmol/L NaCl, 0.05% Tween-20 (TBST). Incubations were done with monoclonal antibody GE-213 (5 μl/ml) diluted in bovine serum albumin/TBST. A biotinylated anti-mouse IgG (1 μg/ml; Vector) diluted in TBST was used as the second antibody. Subsequently, Vectastain ABC reagent (Vector) was added, and peroxidase activity was visualized using dianinobenzidine and H2O2 as substrate.

Results

All temporal artery specimens exhibited strong medial and adventitial inflammation, characterized by numerous macrophages (Figure 1A), lymphocytes, and occasional accumulation of neutrophils (data not shown). The artery lumen was severely narrowed by intimal hyperplasia consisting mostly of smooth muscle cells and macrophages (Figure 1, A and B). The hyperplasia appeared to be superimposed on a pre-existing diffuse intimal thickening (Figure 1E). Macrophage-derived foam cells were most frequently located in the boundary between intima and media. Six of the ten temporal arteritis specimens had characteristic infiltrates of giant cells (Table 1). The internal elastic lamina (IEL) was scanty and dis-
Figure 1. Histological cross sections of temporal arteritis showing intimal thickening, intimal and medial inflammation, and fragmented IEL. Arrows point to the intima-media boundary and have been placed at the same location in adjacent sections. A: Identification of macrophages by immunostaining with antibodies to HAM-56. B: Identification of smooth muscle cells by immunostaining with antibodies to α-actin. C: Immunostaining with antiserum to MMP-9. D: Immunostaining with antiserum to MMP-2. E: Staining of elastic lamellae with orcein; counterstaining with hematoxylin to show cell nuclei. Arrowheads show the border between the initial diffuse intimal thickening and the more recent intimal hyperplasia; the degenerated IEL is at the level of the arrow. F: Immunostaining with antiserum to type IV collagen. Original magnification, ×200. Nearly occluded lumen is to the top; i, intima; m, media.

continuous in all temporal arteritis specimens (Figure 1E), in contrast to that in the control arteries (Figure 2C).

Arteritis and control arteries both contained focal areas of MMP-2 protein in all artery layers (Figures 1D and 2B). There was no clear relationship between the staining intensities of MMP-2 and its main substrate, type IV collagen (Figures 1F and 2D).

Six of the ten temporal arteritis specimens stained for MMP-9 (Figure 1C; Table 1) in locations of degenerated IEL (Figure 1E). The staining was always in areas containing macrophages and macrophage foam cells (Figure 1, A and C). Localization of MMP-9 protein to macrophages was confirmed by double-label immunohistochemistry in a subpopulation of macrophage foam cells at the interface of media and fragmented IEL (Figure 3). Macrophages deeper in the media were negative for MMP-9. MMP-9 protein was present in specimens irrespective of the occurrence of macrophage-derived giant cells in individual patients (Table 1).

All uninvolved temporal arteries exhibited moderate diffuse intimal thickening (Figure 2) and stained ubiquitously for SMCs in all layers (data not shown). There were only a few scattered macrophages (data not shown). No immunostaining was seen for MMP-9 (Figure 2A). The IEL was intact in all control specimens (Figure 2C).
Table 1. Temporal Arteritis Patient Data and the Presence of MMP-9 and Giant Cells in Individual Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>MMP-9*</th>
<th>Giant cells†</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>F</td>
<td>2+</td>
<td>+</td>
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<tr>
<td>2</td>
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<td>10</td>
<td>61</td>
<td>F</td>
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*Semiquantitative analysis of MMP-9 on the basis of abundancy of immunostaining: -, no staining; +, moderate staining; 2+, abundant staining.
†Presence (+) or absence (−) of giant cells in the temporal artery biopsy.

As MMPs contain conserved domains, the specificity of the GE-213 antibody was further verified to recognize both pro and activated forms (92 and 78 kd, respectively) of recombinant human MMP-9 (Figure 4, lanes 2 and 3). The GE-213 antibody was also shown to recognize the pro and activated forms of MMP-9 from cultured human fibrosarcoma cells (HT-1080) cell culture medium (Figure 4, lanes 6 and 7). HT-1080 cells also secrete the tissue inhibitor of metalloproteinases-1 (TIMP-1), and the complex of MMP-9 with TIMP-1 is seen at approximately 200 kd (Figure 4, lanes 6 and 7). The GE-213 antibody did not stain human recombinant MMP-2 (72 kd; Figure 4, lanes 4 and 5).

**Discussion**

Temporal arteritis is the most common example of primary large artery vasculitis. Diagnosis of temporal arteritis is hampered by limited involvement of the artery, and several sections are often necessary before the typical lesion is found. Characteristic giant cells are present in two-thirds of cases, and the diagnosis is frequently also made on the basis of nonspecific granulomatous lymphocyte and eosinophil infiltration and intimal fibrosis. In patients with temporal arteritis, inflammation is sometimes seen in the coronary arteries and the aorta as well. Generalized primary granulomatous arteritis is probably an underdiagnosed condition, because the specific inflammatory changes seen at autopsy might easily be attributed to atherosclerosis. Temporal arteritis has generally received little attention in vascular biology despite readily available tissue material. There appear to be similarities between atherosclerosis and...
temporal arteritis in that chronic inflammation also plays an important role in atherosclerotic plaque rupture, resulting in complications such as myocardial infarction, stroke, and arterial aneurysms. Increased mortality has been reported in patients with a combination of temporal arteritis and coronary disease. There is also a positive correlation between the occurrence of temporal arteritis and aortic aneurysm, suggesting a common pathogenesis.

MMP-9 is a member of the MMP family of enzymes that, as a group, can degrade a variety of extracellular matrix components. MMPs are secreted as zymogens, which need to undergo activation, usually proteolytic. MMP-9 and MMP-2 are closely related gelatinases that can degrade gelatin, collagen types IV, V, and XVII, and elastin. In six of our ten temporal arteritis specimens, MMP-9 stained specifically in areas of degenerated IEL. In control arteries, there was no staining for MMP-9 and no IEL fragmentation. This agrees with previous data that cultured human monocytes and macrophages secrete MMP-9 capable of degrading elastin.

In addition to epidemiological data, MMP-9 appears to provide another connection between the mechanisms of vasculitis and complications of atherosclerosis, as MMP-9 has been suggested to play a role in unstable coronary atherosclerosis and has been reported to be present in human carotid atherosclerotic lesions. Growth and rupture of aneurysm has recently been implicated to be partly mediated by MMP-9, as human abdominal aortic aneurysms produce 10-fold more of this enzyme than normal aorta and MMP-9 activity increases with aneurysm size.

In our temporal arteritis specimens, the staining for MMP-9 was always in areas containing macrophages and macrophage-derived foam cells. This is in accordance with previous data from aortic aneurysms, but in coronary and carotid atherosclerosis also smooth muscle cells have been shown to stain positively for MMP-9. Localization of MMP-9 protein to a subpopulation of macrophage foam cells at the interface of media and fragmented IEL in the present work supports its suggested role in IEL disruption. Whether the expression of this enzyme is the primary cause of IEL degradation remains to be shown. It cannot be ruled out that other elastin-degrading enzymes, such as the human metalloprotease metalloelastase and the serine protease neutrophil elastase, might participate in IEL degradation.

Growth factors and cytokines possibly capable of up-regulating the expression of MMP-9 in vivo in atherosclerotic lesions include interleukin-1, tumor necrosis factor-α, and platelet derived growth factor. There might be unique properties in the IEL that stimulate MMP-9 expression. MMP-9 may also be proteolytically activated by cytokines that stimulate plasmin activity, which has been speculated to activate MMPs in vivo.

In contrast to MMP-9, MMP-2 stained ubiquitously in both normal and arteritic arterial specimens, which is in accordance with previous studies. The presence of MMP-2 has been shown by zymography in both diseased and normal arterial tissue, and it is constitutively expressed by vascular cells in tissue culture conditions. It is unlikely that staining for
MMP-2 is a consequence of a pathological mechanism in arterial specimens, as it is also present in normal arterial tissue. The staining will show enzymatically active and inactive MMP-2 alike. It might be that activation plays a more important role in the functional regulation of MMP-2 than that of MMP-9. Despite extensive efforts, the mechanism of MMP-2 activation is not clear.28

In all temporal arteritis specimens, the artery lumen was severely narrowed by intimal hyperplastic thickening, which consisted mostly of smooth muscle cells. It is possible that an increase of MMPs is necessary for such vascular remodeling to occur. Up-regulation of MMP-9 has been reported in extracts of rat carotid arteries during the intimal hyperplastic response after damage to the IEL by catheter ballooning.29 In temporal arteritis, the inflammatory disruption of IEL, possibly by MMP-9, may promote migration of smooth muscle cells from the media to the intima.

In conclusion, this study demonstrates that MMP-9 is specifically seen in areas of disrupted IEL in temporal arteritis. Normal arteries do not stain for MMP-9, but it has been demonstrated in complicated atherosclerosis and arterial aneurysms. The similar presence of MMP-9 in primary vasculitis as in atherosclerosis and aneurysm formation suggests a common inflammatory pathogenic mechanism. The luminal obstruction in temporal arteritis resembles accelerated atherosclerosis or restenosis in that most lesions had evolved from intimal thickening to near occlusion of the lumen with inflammation. We suggest that macrophage staining for MMP-9 represents a marker for arterial remodeling, which may be of pathological significance.

Acknowledgment
We thank Mrs. Tarja Arvela for expert technical assistance.

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