Matrix Metalloproteinase-2 Production and Its Binding to the Matrix Are Increased in Abdominal Aortic Aneurysms

Valerie Davis, Raisa Persidskaia, Lisa Baca-Regen, Yoshifumi Itoh, Hideaki Nagase, Yuri Persidsky, Anuja Ghorpade, B. Timothy Baxter

Abstract—Degradation of the elastic media is a hallmark of abdominal aortic aneurysms (AAAs). We examined the expression of 2 elastolytic matrix metalloproteinases (MMPs), MMP-2 and MMP-9, in AAA aortic tissues compared with those from atherosclerotic occlusive disease (AOD) and nondiseased control tissues. Quantitative competitive reverse transcription–polymerase chain reaction and gelatin zymography showed increased MMP-9 mRNA and protein in both AAA and AOD tissues compared with those in control tissue, but there was no significant difference between AAA and AOD. In contrast, MMP-2 mRNA and protein levels were significantly higher in AAA than in AOD or control tissues. Sequential extraction of the MMPs from the aortic tissue with a physiological salt solution, 2% dimethylsulfoxide (DMSO), and 10 mol/L urea showed that large amounts of MMP-2 and MMP-9 were bound to the matrix. The most conspicuous finding was that the levels of MMP-2 were significantly elevated in the DMSO fraction in AAA tissues compared with AOD and control tissues. Sequential extraction of the MMPs from the aortic tissue with a physiological salt solution, 2% dimethylsulfoxide (DMSO), and 10 mol/L urea showed that large amounts of MMP-2 and MMP-9 were bound to the matrix. The most conspicuous finding was that the levels of MMP-2 were significantly elevated in the DMSO fraction in AAA tissues compared with AOD and control tissues. In addition, a large portion of MMP-2 found in the DMSO and urea fractions was in the active 62-kDa form, indicating that the precursor of MMP-2 in AAA is largely activated locally and binds to the tissue matrix tightly. By immunolocalization, MMP-9 was found to be primarily produced by macrophages and MMP-2 by mesenchymal cells. The production of MMP-2 was prominent when mesenchymal cells were surrounded by inflammatory cells, suggesting paracrine modulation of MMP-2 expression in AAAs. These observations emphasize that MMP-2 participates in the progression of AAAs by degrading aortic tissue matrix components. (Arterioscler Thromb Vasc Biol. 1998;18:1625-1633.)

Key Words: abdominal aortic aneurysms ■ matrix metalloproteinases ■ smooth muscle cells ■ macrophages ■ lymphocytes

Atherosclerosis develops in the distal aorta of most adults, and with progression it results in 1 of 2 distinct but equally devastating clinical problems: atherosclerotic occlusive disease (AOD) or abdominal aortic aneurysm (AAA). In AOD, atherosclerotic plaque progressively narrows the lumen, compromises distal blood flow, and may lead to lower-extremity gangrene. In AAA, the aorta dilates beyond twice its normal diameter through a complicated remodeling process that culminates in rupture and exsanguination. The anatomic dichotomy between AAA and AOD suggests fundamental differences in matrix metabolism. The most striking histological feature of AAA is the fragmentation and relative decrease in medial elastin. In addition, the fibrillar collagen network of the aortic wall reorganizes, and a marked cellular inflammatory response occurs. In other diseases such as arthritis and periodontal disease, and dermatological disorders, similar destruction of extracellular matrix macromolecules is seen, and invading inflammatory cells exacerbate the disease process. The inflammatory infiltrate in AAA is composed of lymphocytes and macrophages. These immune cells are thought to play an etiologic role through their ability to produce cytokines that induce resident mesenchymal cells to produce matrix metalloproteinases (MMPs). The proinflammatory cytokines secreted from macrophages have been shown to enhance MMP production by human vascular smooth muscle cells (SMCs). Many of these cytokines are present within AAA tissue.

MMPs are a family of related zinc metalloendopeptidases that function in the turnover of components of the extracellular matrix. The elastolytic MMPs that have been implicated in AAA are MMP-2 (gelatinase A, 72-kDa gelatinase) and MMP-9 (gelatinase B, 92-kDa gelatinase), which are the products of resident mesenchymal cells and macrophages, respectively. In vivo, MMPs may bind to matrix and both MMP-2 and MMP-9 contain a fibronectin-like domain that mediates their binding to substrate. The extent of their matrix binding may have important physiological significance in bringing the MMP to its substrate and potentially facilitating matrix breakdown. Understanding the expression, synthesis, binding to the matrix, cellular source, and location of these MMPs in AAA is

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the first step in planning preventative strategies for aneurysm formation.

In this article, we report the levels of MMP-2 and MMP-9 in AAA tissues compared with normal aortic control tissue and with atherosclerotic aortas affected by occlusive disease without dilation (AOD). First, we determined the mRNA levels of these enzymes by quantitative competitive reverse transcription–polymerase chain reaction (QCRT-PCR). The extent of MMP binding to the matrix was investigated by using a series of progressive extraction procedures of the enzymes and detection by zymography. Tissue immunohistochemistry was used to identify in situ location of the MMPs and their associated cell types. The elevated MMP-2 production and the increased activation and matrix binding in AAA tissue suggest its participation in degrading the medial elastin and fibrillar collagen of the aorta in the process of this disease.

Methods

Tissue Procurement and Cell Culture

Infrarenal aortic tissues were obtained at surgical repair of AAA, bypass of AOD, or organ procurement for transplantation (control). The average age of the control group (mean ± SE, 50.0 ± 6.2 years) was less than that in the AAA (65.1 ± 6.2) or AOD (61.1 ± 2.8) group. There were no differences in the ratio of men to women in the control (8/2), AOD (8/2), or AAA (9/1) groups. The mean size of the aneurysms was 6.2 cm, with a range from 3.5 to 11 cm. Informed consent was obtained for tissue collection in accordance with a protocol approved by the Institutional Review Board and Research Committee of the University of Nebraska Medical Center and the Omaha Veterans Administration Medical Center. The specimens were transported to the laboratory on ice in serum-free Dulbecco’s modified Eagle’s medium and either processed for histological studies or frozen in LN2 and stored (−80°C). All reagents were from Sigma Chemical Co unless otherwise specified.

Cultures of human aortic SMCs and adventitial fibroblasts were established from control aortas by using previously described techniques.27 Human monocytes and peripheral blood lymphocytes were separated and purified by countercurrent centrifugal elutriation of peripheral blood mononuclear cells from leukapheresis of healthy donors. The monocytes were allowed to differentiate in culture.

Peripheral blood mononuclear cells from leukapheresis of healthy donors. The monocytes were allowed to differentiate in culture.

TABLE 1. Primers for RT-PCR

<table>
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<tr>
<th>Amplification Product</th>
<th>Nucleotide Position</th>
<th>Sequence (5′ to 3′)</th>
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</thead>
<tbody>
<tr>
<td>MMP-2, 347 bp</td>
<td>Sense</td>
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<tr>
<td></td>
<td>Antisense</td>
<td>CTCGAGAAATTGTCTCCAGG</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>TACTGGCTACTGACCGG</td>
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<tr>
<td>MMP-9, 199 bp</td>
<td>Sense</td>
<td>GT6CGTCTCCCTCCCTACCTTCCT</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>GGAATGATCTAAGCCCAGCG</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>GCACTTCCTCGCGCTGCTCA</td>
</tr>
</tbody>
</table>

MMP-2 and MMP-9 mRNAs

Table 1 lists the primer sequences used in these studies. Aortic tissue was pulverized and solubilized in 1 mL of Trizol (Life Technologies) extraction. The aortic tissues were powdered in LN2 in a freezer mill (salt fraction). Preliminary analysis demonstrated that a

Extractions of Gelatinases From Aortic Tissue

MMP-2 and MMP-9 in the tissue were sequentially extracted with salt buffer, DMSO, and urea to remove soluble salt and matrix-bound (DMSO, urea) MMPs. Preliminary experimental work was done to ensure that these buffers did not activate MMP-2 or MMP-9 during extraction. The aortic tissues were powdered in LN2, in a freezer mill and lyophilized. Aliquots (50 mg) of each sample were homogenized in 100 μL of TNC buffer [50 mmol/L Tris-HCl (pH 7.5), 0.15 mol/L NaCl, 10 mmol/L CaCl2, 10 μmol/L EDTA, 0.05% Brij 35, 2 mmol/L PMSF, and 0.02% NaN3]. The homogenates were centrifuged (12,000g for 10 minutes at 4°C) and the extract removed. This salt extraction was then repeated on the pellet, and the extracts were combined (salt fraction). Preliminary analysis demonstrated that a

TABLE 2. Primers for Synthesizing rRNA for QCRT-PCR

<table>
<thead>
<tr>
<th>Amplification Product</th>
<th>Nucleotide Position</th>
<th>Sequence (5′ to 3′)</th>
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<tr>
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<td>Sense</td>
<td>TTAATACGACTCATATAGGGatgatgcatcctagcatatgctcc</td>
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<tr>
<td></td>
<td>Antisense</td>
<td>TTTTTTTTTTTTTTTTctccagaattgtctccagcTACTGAGATCT</td>
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<tr>
<td></td>
<td>Probe</td>
<td>cctATTGAGAACCGC</td>
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<tr>
<td>MMP-9 rRNA, 295 bp</td>
<td>Sense</td>
<td>TTAATACGACTCATATAGGGatgatgcatcctagcatatgctcc</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>TTTTTTTTTTTTTTTTggaatgtaagccagcgcCCACTTCT</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>TGTCGCTGTCAcgcaagctagacttccagc</td>
</tr>
</tbody>
</table>

"RT-PCR was used to make MMP-2 and MMP-9 rRNAs.29 The sense primer contains a T7 promoter sequence (upper-case italics), the target sense sequence (lower case), and the sense spacer sequence (upper-case). The antisense primer contains a poly(dT) sequence, the target antisense sequence (lower case), target probe sequence (upper case), and the antisense spacer sequence (lower-case italics)."
Finally, the residual materials were further extracted with 100
\( \text{mol/L} \) urea in TNC buffer (urea fraction).

An equivalent volume of each fraction was used for zymography.
Zymography was conducted with SDS polyacrylamide gels containing
gelatin (0.8 mg/mL) as described previously.\(^3\) Enzymatic activity
was visualized as negative staining with Coomassie Brilliant Blue
R-250. The molecular sizes of gelatinolytic activities were determined
by using Kaleidoscope PreStained Molecular Weight Markers
(Bio-Rad). Conditioned media from human macrophages and from
fibroblasts served as positive controls for MMP-9 and MMP-2,
respectively. Purified MMP-2 was used as a standard for the 62-kDa
form of MMP-2. The gelatinolytic activities were quantified by
densitometry (Molecular Dynamics).

Several steps were taken to ensure that MMP-2 and MMP-9 were
not activated during the extraction process. Purified pro–MMP-2 was
processed by the same protocol used for tissue extraction with salt,
DMSO, and urea. In comparison with the purified pro–MMP-2
before processing, there was no increase in the activated form by
zymography, which would remove other proteinase inhibitors. Ad-
ditionally, the proportion of active and latent MMP-2 did not change
in samples that were stored for several weeks at
\( 80^\circ \text{C} \), demonstrating that under these conditions, no additional activation occurred.

### Immunohistochemistry
Human aortic tissues embedded in OCT compound (Baxter) were cut
into 8-\( \mu \text{m} \) sections. The sections were treated with 1% BSA in PBS
(pH 7.4, 30 minutes) before incubation with mouse anti-human
MMP-2 or anti-human MMP-9 monoclonal antibodies (Oncogene
Science). Sections were double-labeled with mouse anti–HAM 56
(monocyte lineage), rabbit anti-CD3 (T cells), or mouse anti-CD20
(B cells) antibodies (Dako). Primary antibody binding was visualized
by incubating the sections with FITC-conjugated anti-mouse IgG
antibody F(ab')\(_2\), TRITC-conjugated anti-mouse IgM antibody
F(ab')\(_2\), fragment, or rhodamine-conjugated anti-rabbit IgG antibody

**Figure 1.** MMP-2 mRNA levels in aortic tissue. RNA extracted from control (CON), AOD, and AAA tissue (\( n=5 \) per group) was analyzed
by QCRT-PCR. A, With progressive dilution of competitor RNA (left to right), smaller competitor product (open arrow) decreases and
MMP-2 product (closed arrow) increases. Points of equivalent expression of competitor and MMP-2 amplification products (of repre-
sentative samples) are indicated (\( \triangle \)). B, Mean±SEM MMP-2 mRNA levels (pg/\( \mu \text{g} \) RNA) from each group are shown in bar graphs, as
determined by QCRT-PCR. *Different from control, ‡different from AOD at \( P<0.05 \).

**Statistical Methods**
Values are expressed as mean±SEM. Differences between groups
were analyzed by ANOVA. QCRT-PCR results were additionally
compared by Student’s \( t \) test for equality of slopes. The correlation
between MMP-2 protein levels and aneurysm size was analyzed by linear regression. A value of \( P<0.05 \) was considered
significant.

**Results**

**Analyses of MMP-2 and MMP-9 mRNAs by QCRT-PCR**
We assessed mRNA levels of MMP-2 and MMP-9 in AAA,
AOD, and normal control aortas (\( n=5 \) per group) by QCRT-
PCR. The inverse relationship between the target PCR
products and serial dilutions of the rcRNA was observed, as
shown in representative samples (Figure 1A). MMP-2 and
MMP-9 concentrations were determined by QCRT-PCR for
each sample, and the mean±SEM was determined for each
group. By intergroup statistical analysis of MMP-2 mRNA
(Figure 1B), all 3 groups were statistically different
(\( P<0.05 \)), with the highest mRNA levels in AAA (control,
2.7±0.8; AOD, 4.0±0.4; and AAA, 27.3±6.3 pg MMP-2/\( \mu \text{g} \)
RNA). In contrast to MMP-2, MMP-9 transcripts did not
differ between AOD (6.6±4.6) and AAA (5.9±2.3 pg MMP-
9/\( \mu \text{g} \) RNA), but both were higher than that in the control
aortas (0.04±0.01). There was a high degree of variability of
MMP-9 expression in the AOD group, as demonstrated by the
large SE within this group. These data demonstrate that
MMP-2 and MMP-9 transcripts are increased in both disease
states (AOD and AAA) compared with the control aorta.
However, only the levels of MMP-2 mRNA distinguished
between AOD and AAA, with a higher transcript level of this
gelatinase in AAA.

**Gelatin Zymography**
Control, AOD, and AAA aortic tissues (\( n=10 \) per group) were
lyophilized, and the equivalent amounts of tissue were
serially extracted with salt buffer, DMSO, and urea to ensure
removal of both the soluble and the matrix-bound MMPs. Preliminary analysis demonstrated that after duplicate extractions with the salt buffer, no additional MMP could be removed with this buffer alone, whereas additional MMP could be extracted with DMSO and urea. These findings suggested that a portion of gelatinases are bound to the tissue or matrix. The fractions extracted with each different buffer were analyzed by zymography for MMP-2 (Figure 2A) and MMP-9 (Figure 2B). The predominant gelatinolytic bands in the salt extractions occurred at 92, 72, and 62 kDa in all 3 groups, corresponding to MMP-9 and the pro- and active forms of MMP-2, respectively. The identity of these bands was verified by Western blot analysis and a comparison with purified MMP-2 by zymography (data not shown).

The 62-kDa form of MMP-2 was more apparent in the DMSO fraction, and it was also the predominant form in the urea fraction. The percentage of MMP-2 in the active form in each fraction increased with increasing harshness of the extraction conditions (Table 3). These results indicated that the active form of MMP-2 binds to the matrix more tightly than does pro–MMP-2. In AAA, for example, only 19.7% ± 5.5% of the total MMP-2 was in the active form in the salt fraction, whereas more than half (52.2 ± 2.6%) of the urea-extractable MMP-2 was in the active form. When the amount of tissue-bound, active, 62-kDa MMP-2 in the DMSO and urea fractions was combined and compared among the 3 different groups, the largest amount of the 62-kDa MMP-2 was found in AAA tissue (Figure 3). It is particularly notable that the DMSO fraction from AAA tissues has the highest levels of active MMP-2 compared with AOD and control tissues (Table 3). These observations indicate that AAA aortas not only produce an increased amount of MMP-2 but also enhance the activation of pro–MMP-2. The activated MMP-2 then binds to the tissue more tenaciously than does the 72-kDa precursor form. MMP-9 was also extracted with DMSO and urea, but it was primarily in the pro-form.

Simple addition of the MMP content in each fraction showed differences in the total MMP-2 among the 3 groups. For a better comparison of the total extractable MMP-2 and MMP-9, 5 samples from each group (control, AOD, and AAA) were randomly chosen. The salt, DMSO, and urea fractions from each of these samples were then combined in a ratio proportional to the extract volume (salt/DMSO/urea, 2:2:1), and aliquots were used for zymography. This procedure allowed the 15 samples to be analyzed on the same gel to minimize the error associated with normalizing data from multiple gels. The results indicated that both the 62- and 72-kDa forms of MMP-2 were significantly higher in AAA compared with AOD and control (Figure 4A). The total extractable amount of MMP-2 (pro- and active forms) in the AAA tissues was ~3-fold higher than that of control tissues. By linear regression analysis, there was no significant correlation (P=0.65) between MMP-2 levels and aneurysm size. MMP-9 was also elevated in the diseased aorta (AAA and AOD) compared with the control (Figure 4B), but it did not differ between AAA and AOD.

### Table 3. Distribution of Active MMP-2 (62 kD) in Each Fraction*

<table>
<thead>
<tr>
<th></th>
<th>Salt</th>
<th>DMSO</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.4±3.7%</td>
<td>27.6±3.5%</td>
<td>44.5±5.4%</td>
</tr>
<tr>
<td>AOD</td>
<td>16.4±5.5%</td>
<td>29.9±5.2%</td>
<td>42.2±2.5%</td>
</tr>
<tr>
<td>AAA</td>
<td>19.7±5.5%</td>
<td>41.6±1.5%†</td>
<td>52.2±2.6%†</td>
</tr>
</tbody>
</table>

*Control, AOD, and AAA tissue (n=10 per group) was serially extracted with salt, DMSO, and urea buffers and analyzed by gelatin zymography. Densitometry was used to determine the total amount of gelatin lysis (100%) from both the 72-kD (inactive) and the 62-kD (active) MMP-2 band of each group. Percent active MMP-2 (62-kD/total MMP-2) was then calculated. †AOD>AOD and control, P<0.05.
Immunolocalization of MMP-9 and MMP-2 in Aortic Tissue

To identify cell types that produce MMP-2 and MMP-9, immunolocalization studies were performed on AAA, AOD, and control aortic tissue. Consecutive sections from each of the 3 groups (n = 10) were subjected to coimmunolocalization by incubation with monoclonal antibodies to MMP-2 or MMP-9 and with either HAM-56 (monocyte lineage) or a marker for T (anti-CD4) or B (anti-CD20) lymphocytes. Control immunohistochemical staining was performed by using mouse IgG to evaluate background for nonspecific staining (Figure 5D). MMP-9 and HAM-56 collocalized to cells within the outer media and adventitia of the aneurysmal aortic wall, indicating that macrophages were the primary source of MMP-9 within diseased aortic tissue (Figure 5A). These findings were corroborated by in vitro analysis with RT-PCR, which demonstrated high levels of MMP-9 transcripts in both inactivated and LPS-activated macrophages in culture (Figure 5E, lanes 1 and 2). Neither aortic SMCs nor adventitial fibroblasts in culture expressed MMP-9 mRNA (Figure 5E, lanes 5 and 6).

Tissue sections incubated with anti–MMP-2 antibody showed that MMP-2 was rarely localized to macrophages. The majority of cells that stained with the MMP-2 antibody in the outer media and adventitia (Figure 5B) were fibroblasts or SMCs, by hematoxylin and eosin staining (data not shown). It is notable that MMP-2 expression was highest in mesenchymal cells surrounded by intense inflammation, suggesting that paracrine modulation participates in MMP-2 expression in resident mesenchymal cells (Figure 5B). Areas of AAA tissue with fewer invading lymphocytes or macrophages had less MMP-2 expression (Figure 5C). Using QCR-PCR, we detected MMP-9 in control, AOD, and AAA tissues. MMP-9 transcripts found in the control aorta may reflect the presence of subclinical atherosclerosis in some transplant specimens. These atherosclerotic changes begin in early adulthood, and previous work has invariably shown increased MMP-9 production in the diseased aorta.21,23,33–37 However, the differences in MMP-9 expression between AOD and AAA have been less consistent. We detected a high degree of variability in MMP-9 mRNA in aortic SMCs and adventitial fibroblasts were the primary cellular source of MMP-2 mRNA (Figure 5E, lanes 5 and 6), although lower levels of expression were seen in both LPS-activated and unactivated macrophages (Figure 5E, lanes 1 and 2). Coimmunolocalization of MMP-2 or MMP-9 with markers for T or B lymphocytes indicated that these cells did not express MMP-2 and MMP-9 (data not shown). Additionally, MMP mRNA was not detected in unactivated or PHA-activated lymphocytes by RT-PCR (Figure 5E, lanes 3 and 4).

Discussion

Aneurysm development is a complex process that involves synthesis and degradation of the extracellular macromolecules of the matrix. Proteinases that degrade the structural matrix are likely to have a pivotal role in this process. We have investigated 2 MMPs that account for the majority of the elastolytic activity found in AAA tissue by using 3 different techniques. Our quantitative analyses of transcript and protein levels demonstrate that both MMP-2 and MMP-9 are increased in the diseased aorta. However, only MMP-2 distinguishes between AAA and AOD, with increased production found in AAA. Additionally, there was increased activation and matrix binding of this gelatinase in AAA. MMP-9 transcript and protein were increased in both diseases to a similar extent.
AOD, but there was no difference between AOD and AAA at the mRNA level. These findings are consistent with Northern and in situ hybridization results of McMillan et al.23 Zymographic analyses also indicated that MMP-9 proteins were elevated in both AOD and AAA, but again there were no significant differences between the 2 groups. These results support the earlier work of Vine and Powell,38 who reported similar gelatinolytic activities in AAA and AOD. On the other hand, Thompson et al21 reported that AAA explants after 72 hours in culture produced more MMP-9 than did AOD explants. These findings indicate the ability of AAA tissue to produce increased MMP-9 under specific culture conditions that other investigators have shown to activate tissue macrophages.11

MMP-9 is found in neutrophils,39 macrophages,40 and malignant carcinomas.41 Some mesenchymal cells also produce this enzyme in response to certain stimuli.42–44 Our coimmunolocalization studies of MMP-9 and HAM-56 in AAA aortic tissue showed that MMP-9 is a product of macrophages. Although these results are consistent with those of in situ hybridization studies by Thompson et al21 and McMillan et al,23 Patel et al34 reported that AAA-derived SMCs in culture produce MMP-9. The production of MMP-9 in cultured aortic SMCs may be mediated by proinflamma-

Figure 5. Immunolocalization and in vitro expression of MMP-2 and MMP-9 by cellular components of aorta. Sections of AAA tissue were double-immunolabeled with antibodies to MMP-2 or MMP-9 and anti–HAM 56 (macrophages). Primary antibody was visualized with secondary antibody conjugated to FITC (MMP-2, MMP-9) or TRITC (HAM-56). MMP-9 colocalized with HAM-56–staining cells (A, original magnification, $\times$200). MMP-2 localized to spindle-shaped cells in outer media and adventitia and rarely to macrophages (B). MMP-2 expression was distinctly decreased in areas with little inflammation (C). Sections treated with IgG alone served as immunohistochemical control (D). Bar$=30\mu m$. MMP-2 and MMP-9 mRNA expression was determined by RT-PCR (E) in unactivated macrophages (lane 1), LPS-activated macrophages (lane 2), unactivated T lymphocytes (lane 3), PHA-activated T lymphocytes (lane 4), aortic SMCs (lane 5), and adventitial fibroblasts (lane 6). Equal levels of GAPDH amplification product for each cell type indicate equivalence of total RNA for RT-PCR.
tory cytokines.\textsuperscript{15,45} Therefore, although SMCs have the potential to produce MMP-9 in vitro, tissue immunohistochemical analysis indicates that SMCs are not a primary source of MMP-9 in diseased aortic tissue. Nevertheless, our studies indicate that increased MMP-9 content in aortic tissue occurs as a result of the macrophage infiltration that accompanies severe atherosclerosis, rather than being a unique phenomenon associated with aneurysm formation.

MMP-2 is expressed constitutively by many cell types in culture.\textsuperscript{24} Unlike that of other MMPs, the synthesis of MMP-2 is not regulated by inflammatory cytokines such as interleukin-1 and tumor necrosis factor-\(\alpha\), although transforming growth factor-\(\beta\) increases the production of MMP-2 in fibroblasts\textsuperscript{46} and keratinocytes.\textsuperscript{47} Furthermore, expression of the MMP-2 gene is regulated in stromal tissues around carcinomas\textsuperscript{48,49} and in mouse endometrial stroma during the peri-implantation period.\textsuperscript{50} Our studies indicated that AAA mesenchymal cells (SMCs and fibroblasts) produce elevated levels of MMP-2 in vivo. Although no other study has investigated the expression, content, and matrix binding of MMP-2, several studies have examined specific aspects of MMP-2 in AAAs. Thompson et al\textsuperscript{51} did not find increased soluble MMP-2 in conditioned media from AAA explants. This report is consistent with our inability to find differences when we examined only salt-soluble MMP-2. Both Freestone et al\textsuperscript{19} and Knox et al\textsuperscript{52,53} found trends toward increased MMP-2 levels in AAAs by zymography and immunohistochemistry, respectively. Although Freestone at al reported high MMP-2 levels in small aneurysms, we found no correlation between MMP-2 level and aneurysm size. Our study further emphasizes that the expression of MMP-2 is particularly upregulated in mesenchymal cells in close proximity to inflammatory infiltrates, indicating that inflammatory cells may produce stimulatory factors. Such MMP-2 stimulatory factors are found in the conditioned medium of human breast adenocarcinoma cell lines MCF-7 and BT-20.\textsuperscript{51} A similar paracrine stimulatory system may therefore take place between the resident mesenchymal cells and invading macrophages and lymphocytes in AAAs.

One of the most notable findings of this study is the remarkable amount of MMP-2 and MMP-9 that was resistant to extraction by a physiological salt buffer. A significant proportion of these MMPs was extracted from the aortic tissue with the solvent DMSO and finally, under extremely harsh conditions, with 10 mol/L urea. The differences in MMP-2 levels were not apparent with salt extraction alone but became significant when all extractable MMPs were combined. Furthermore, a larger percentage of active (62-kDa) MMP-2 was found in the DMSO fractions, especially from AAA tissues. The reasons for the differential distributions are not clear, as both pro–MMP-2 and MMP-2 can bind to matrix components through the fibronectin type II–like domain.\textsuperscript{25,52,53} This may be due in part to exposure of certain extracellular matrix components as a result of tissue matrix degradation. Nonetheless, these findings indicate that AAA tissue has the greater ability to activate pro–MMP-2 locally. Such activation may be mediated by recently characterized plasma membrane–anchored MT-MMPs,\textsuperscript{54,55} other proteases such as MMP-1 and MMP-7,\textsuperscript{56} thrombin,\textsuperscript{57} or reactive oxygen species.\textsuperscript{58} The large AAA inflammatory infiltrate could influence MMP-2 activation through these mechanisms, particularly by expressing\textsuperscript{59} or inducing these MT-MMPs in resident mesenchymal cells. In contrast to the significant amounts of active MMP-2 bound to the matrix, MMP-9 found in the tissue extracted by DMSO and urea was primarily in the proenzyme form. Further studies to investigate which extracellular matrix components interact with activated MMP-2 as well as pro–MMP-2 will provide insights into a possible role of this enzyme in the pathogenesis of AAAs.

Considering that both MMP-9 and MMP-2 have potent elastolytic ability, both could play a major role in the remodeling that occurs with AAAs. The increased levels of MMP-2 and its increased binding and activation suggest an important role in the progression of aortic aneurysm pathogenesis. MMP-2 is synthesized by the same mesenchymal cells that synthesize collagen and elastin,\textsuperscript{52} the major structural macromolecules of the aorta. Furthermore, MMP-2 is a more active elastase than is MMP-9,\textsuperscript{56} and the former has the ability to degrade interstitial collagen.\textsuperscript{60} Future work will be directed toward understanding the regulation, activation, and role of inhibitors of MMP-2. Identification of such inhibitors within the aneurysm wall or development of pharmacological approaches that block these proteolytic enzymes could prevent aneurysm formation or inhibit growth of existing aneurysms.

Acknowledgments

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Increased MMP-2 Levels and Binding in Aneurysms


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