Vascular extracellular matrix remodeling in cerebral aneurysms

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Object. The occurrence of cerebral aneurysms has been linked to alterations in the extracellular matrix and to matrix-degrading proteases. The purpose of the present study was to determine whether active extracellular matrix remodeling occurs within cerebral aneurysms.

Methods. Aneurysm tissue was collected from 23 patients (two of whom had a ruptured aneurysm and 21 of whom had an unruptured aneurysm) and compared with 11 control basilar arteries harvested at autopsy. Active proteinases capable of gelatin lysis were identified by performing in situ zymography in the presence and absence of a metalloproteinase inhibitor (ethylenediamine tetraacetic acid) and a serine proteinase inhibitor (phenylmethylsulfonyl fluoride). Immunohistochemical analysis was used to localize plasmin, tissue-type (t)-plasminogen activator (PA), urokinase-type (u)-PA, membranetype (MT1)-matrix metalloproteinase (MMP), MMP-2, MMP-9, and tenascin.

Focal areas of gelatin lysis occurred in most cerebral aneurysm tissue samples (17 of 21), but rarely in control arteries (two of 11) (p = 0.002). Both serine proteinases and MMPs contributed to gelatin lysis; however, the MMPs were the predominant enzyme family. Plasmin (p = 0.04) and MT1-MMP (p = 0.04) were expressed in the aneurysm tissue but were unusual in control tissue. The MMP-2 was also expressed more commonly in aneurysm than in control tissue (p = 0.07). The MMP-9 and t-PA were expressed in both groups; however, different staining patterns were observed between aneurysm and control tissue. Tenascin staining was commonly present in both groups, whereas u-PA staining was rarely present.

Conclusions. Aneurysm tissue demonstrates increased proteolytic activity capable of lysing gelatin and increased expression of plasmin, MT1-MMP, and MMP-2 when compared with normal cerebral arteries. This activity may contribute to focal degradation of the vascular extracellular matrix and may be related to aneurysm formation and growth.

Key Words • cerebral aneurysm • extracellular matrix • matrix metalloproteinase • serine proteinase • plasmin • plasminogen activator • in situ zymography

Aneurysmal subarachnoid hemorrhage produces one of the most severe forms of stroke. Despite decades of clinical research and therapeutic innovation, the incidence of this catastrophic illness has remained unchanged and management results continue to be uniformly poor. As with other illnesses, efforts directed at understanding the basic pathophysiological mechanisms of aneurysm formation and rupture promise to yield therapeutic dividends that may alter these grim statistics. Although enthusiasm for aneurysm research is building, the biology of cerebral aneurysm formation and rupture remains an enigma.

Early investigators hypothesized that aneurysm formation occurs when cerebral blood vessels passively respond to exaggerated hemodynamic forces. Experimental and clinical data, however, both suggest that hemodynamic forces alone are not sufficient to produce cerebral aneurysms, pointing toward an active, rather than a passive role of cerebral arteries in aneurysm formation. Remodeling of the arterial extracellular matrix has been linked to the occurrence of other types of vascular diseases, including atherosclerosis, aortic aneurysm, and postangioplasty restenosis. Interruption of matrix remodeling prevents or reduces the severity of these illnesses.

Diminished arterial structural proteins, exaggerated collagen metabolism, and increased circulating levels of serum gelatinase and elastase, which are observed in some patients, point toward matrix remodeling as a potentially important pathophysiological process occurring in cerebral aneurysms. The purpose of the present study was to investigate molecular mechanisms associated with matrix remodeling in human cerebral aneurysm tissue. For these experiments, cerebral aneurysm tissue was compared with normal cerebral arteries to: 1) determine whether active proteinases capable of degrading the extracellular matrix are present within the cerebral aneu-
rysm wall; 2) characterize further the nature of these proteases; and 3) identify potential tissue-bound site-specific activators of these proteases (targeting mechanisms).

Matrix metalloproteinases (MMPs) are a homologous group of zinc- and calcium-dependent neutral proteases that are secreted as inactive zymogens and require enzymatic cleavage of the N terminus for activation. Matrix metalloproteinases, along with serine proteases, degrade existing matrix elements and are crucial to extracellular matrix remodeling. Although each MMP and serine protease has unique substrate affinities, many are capable of degrading gelatin. Because of this, the ability of aneurysm tissue to lyse gelatin was used to determine whether active proteases capable of degrading the extracellular matrix were present in the cerebral aneurysm wall. Enzymatic activity was further characterized through the use of known protease inhibitors and by studying specific proteases. Both MMP-2 (Gelatinase A) and MMP-9 (Gelatinase B) were selected for study because they have been linked to the occurrence of cerebral aneurysms as well as other types of vascular disease. Tenascin, an extracellular matrix glycoprotein, was studied as a possible biochemical marker of vessel wall stress and injury.

Pathways that may enhance the rate and target the site of matrix remodeling were also studied. Plasmin is formed by the enzymatic cleavage of plasminogen catalyzed by tissue-type (t)-plasminogen activator (PA) or urokinase-type (u)-PA. The PA-plasmin system is able to bind to tissue-type (t)-plasminogen activator receptor, making the cell surface a preferred site for the conversion of plasminogen to plasmin. Although primarily degrading the glycoprotein component of the extracellular matrix, plasmin can activate certain members of the MMD family, thereby directly and indirectly influencing the rate and location of matrix remodeling. Membrane-type (MT1)-MMP, which is active when bound to the cell surface, was also studied. Its primary function appears to be the activation of pro-MMP-2, thereby also influencing the rate and location of extracellular matrix remodeling.

Materials and Methods

Clinical Material

Patients who were undergoing direct microsurgical aneurysm repair for either a ruptured or unruptured intracranial aneurysm were eligible for this study. Informed consent was obtained in accordance with a protocol approved by the Cleveland Clinic Foundation Institutional Review Board. As a routine part of the surgery, the aneurysm dome was opened after clip removal to confirm that the lesion was completely isolated from the circulation. When technically feasible, a small portion of the aneurysm wall was retained for this study. Clinical features of each case were determined by a retrospective review of the patient’s medical records. The size of each aneurysm was determined angiographically.

Basilar arteries were harvested within 24 hours of death in patients at the time of autopsy and served as control tissue. Basilar artery controls were obtained only in patients in whom no diagnosis of cerebral aneurysm had been made before death and in whom the autopsy failed to reveal cerebral aneurysms. The cause of death in each case was determined by a board-certified pathologist.

Aneurysm and control basilar artery tissue were snap frozen in isopentane cooled with liquid nitrogen within 1 hour of collection. Frozen tissue was cut into 5- to 6-μm sections on a cryostat and mounted on microscope slides for immunohistochemical analysis. Slides were allowed to air dry and were stored at −70°C until processing. For in situ zymography, sections were placed on specially prepared substrate slides as described in the following section.

In Situ Zymography

Photographic Emulsion Method. Five- to six-micrometer cryostat sections were collected on frosted microscope slides and stored at −70°C. On the day of the experiment, the slides were thawed to room temperature and coated with a 1:1 mixture of gelatin photographic emulsion/destilled water. These slides were incubated in a humidity chamber set at 37°C for 72 hours and were developed and fixed according to manufacturer’s specifications. Clear areas on an otherwise black background indicated gelatin lysis.

Fluorescent Gelatin Method. Fluorescein was coupled to gelatin by using a fluorescein labeling kit. Glass microscope slides were cleaned with detergent under running distilled water. Fluorescein-coupled gelatin substrate was mixed 1:1 with 1% agarose and incubated at room temperature. The slides were coated with this mixture and allowed to gel at room temperature in darkness. Cryostat sections were mounted on substrate-coated slides and covered with one of the following solutions: TBS, 20 mM ethylenediaminetetraacetic acid (EDTA) in TBS, or 1 mM phenylmethylsulfonyl fluoride. The slides were incubated for 72 hours at 37°C in a tissue-culture incubator and examined with the aid of a fluorescent microscope. Areas of darkness on an otherwise bright-green background indicated areas of gelatin lysis.

Immunohistochemical Analysis

Cryostat sections mounted on frosted microscope slides were acetone fixed for 10 minutes and washed in modified phosphate-buffered saline (mPBS), pH 7.6, for 5 minutes. An avidin-biotin complex was used to suppress endogenous biotin and the slides were incubated for 30 minutes in a non-species-specific protein block. Primary antibodies against MMP-2 (1:500), tenascin (1:250), MMP-9 (1:100), MT1-MMP (1:50), t-PA (1:100,000), t-PA (1:600), and plasmin (1:6000) were diluted in mPBS and incubated with the tissue sections for 30 minutes. A species-specific biotinylated secondary antibody was allowed to react for 30 minutes, after which 33'-diaminobenzidine substrate was added and the color was intensified by addition of 0.04% osmium tetroxide for 30 minutes, producing a dark-brown insoluble precipitate. The slides were counterstained with hematoxylin, dehydrated in a series of graded ethanol, cleared in xylene, coverslipped, and examined microscopically.

Data Interpretation and Analysis. All slides were reviewed by the authors and an independent pathologist. A three-tiered qualitative grading scheme was used to describe the presence, distribution, and localization of immunohistochemical staining. Slides were first examined for brown deposits indicating the presence or absence of immunoreaction. Slides were also classified by the distribution pattern of immunohistochemical staining. Slides in which the bulk of the tissue was homogeneously and contiguously stained were considered to have a diffuse staining pattern. Those that were characterized by more discrete areas of staining were considered to have a focal staining pattern. Finally, immunoreactions were classified according to their histological localization. Staining was classified as being cellular, extracellular matrix, or both cellular and extracellular matrix. In situ zymograms were classified using a similar qualitative grading scheme.

Demographic similarities between patients harboring an aneurysm and individuals from whom control tissue was harvested were determined by comparing age and gender distributions. Patient age in years was expressed as a mean ± standard deviation and differences between the aneurysm and control groups were compared using Student’s t-test. The incidence of men and women in each group was calculated and differences were compared using the Yates-corrected chi-square test. For aneurysm patients, aneurysm size in millimeters was expressed as a mean ± standard deviation. Aneurysm location was described by naming the parent vessel. The
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TABLE 1
Summary of results in control basilar arteries obtained at autopsy in 11 patients*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Cause of Death</th>
<th>Zymography</th>
<th>Immunohistochemical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77, M</td>
<td>heart failure</td>
<td>NR</td>
<td>Plasmin: F-B MMP-9: F-B</td>
</tr>
<tr>
<td>2</td>
<td>50, M</td>
<td>respiratory failure</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>3</td>
<td>73, F</td>
<td>respiratory failure</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>4</td>
<td>48, M</td>
<td>respiratory failure</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>5</td>
<td>63, M</td>
<td>cardiac arrest/angioma</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>6</td>
<td>60, F</td>
<td>heart failure</td>
<td>F-C</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>7</td>
<td>37, M</td>
<td>ALS</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>8</td>
<td>53, F</td>
<td>ovarian tumor</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>9</td>
<td>64, F</td>
<td>diabetes/myocardial infarction</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>10</td>
<td>63, F</td>
<td>ALS</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
<tr>
<td>11</td>
<td>16, M</td>
<td>autoimmune small bowel enteropathy</td>
<td>NR</td>
<td>MMP-2: F-B</td>
</tr>
</tbody>
</table>

* ALS = amyotrophic lateral sclerosis; B = both cellular and extracellular matrix; C = cellular; D = diffuse staining pattern; F = focal staining pattern; NR = no reaction; — = not enough tissue available.

incidence of each type of aneurysm was calculated. The incidences of the various staining and zymographic characteristics were calculated and differences between the groups were compared using the Yates-corrected chi-square test. Differences were considered statistically significant if the probability value was 0.05 or less.

Sources of Supplies

Eastman Kodak (Rochester, NY) manufactured the gelatin photographic emulsion (NTB2), developer (D-19), and fixer. The fluorescein was coupled to gelatin provided by BioRad Laboratories (Hercules, CA) by using a fluorescein labeling kit obtained from Boehringer Mannheim (Indianapolis, IN).

In the immunohistochemical analyses, we used a nonspecies-specific protein block purchased from Dako (Carpinteria, CA). Primary antibodies were obtained from a number of manufacturers. A polyclonal antibody against MMP-2 was obtained from Triple Point Biologics (Forest Grove, OR); a monoclonal antibody against tenascin from Novacastra (Newcastle, UK); monoclonal antibodies against MMP-9 and MT1-MMP from Calbiochem (Cambridge, MA); a polyclonal antibody against u-PA from Chemicon (Temecula, CA); and a monoclonal antibody against t-PA and a polyclonal antibody against plasmin from American Diagnostica (Greenwich, CT).

Results

Aneurysm tissue was collected from 23 surgical patients. For a comparison, basilar arteries were harvested at the time of autopsy in 11 individuals known to be free of cerebral aneurysms. The clinical and experimental data are summarized for control subjects in Table 1 and for patients with aneurysms in Table 2. Individuals with and without aneurysms were similar in age (54.2 ± 12.2 years compared with 54.9 ± 17.2 years, respectively; p = 0.191) and gender distribution (52.2% compared with 45.5%, respectively; p = 1). Most samples were obtained from unruptured aneurysms (21 of 23). The mean aneurysm diameter was 8.7 ± 3.7 mm (range 3.5–15 mm). Ten aneurysms arose from the anterior communicating artery, seven from the middle cerebral artery, five from the internal carotid artery, and one from the basilar artery.

In Situ Zymography

In situ zymography was performed on 21 aneurysm and 11 basilar artery samples by using the photographic emulsion method (10 aneurysms and five control arteries) and/or the fluorescein-coupled gelatin method (19 aneurysms and 11 control arteries). Using the photographic emulsion technique, gelatin lysis resulted in areas of white on an otherwise black background; using the fluorescein-conjugated gelatin method, areas of lysis appeared black on an otherwise bright-green background. Gelatin lysis occurred in 17 (81%) of the 21 aneurysm samples but in only two (18%) of the 11 basilar artery samples (p = 0.002). In each instance, areas of gelatin lysis were heterogeneously distributed within the aneurysm wall occurring exclusively in focal pockets of increased activity (Fig. 1). By comparing immunohistochemical slides with similar sections that had undergone in situ zymography, gelatin lysis appeared to occur in both cellular and acellular areas.

To characterize the molecular mechanisms responsible for gelatin lysis further, in situ zymography was performed by adding EDTA (a known inhibitor of MMPs) or PMSF (a known inhibitor of serine proteinases) to 11 aneurysm samples. Both MMPs and serine proteinases appeared to be responsible for gelatin degradation. In seven (64%) of 11 proteolysis-capable aneurysm samples, EDTA produced more inhibition than PMSF, indicating that the majority of gelatin lysis was due to MMPs in these cases. In four (36%) of 11 aneurysm samples, PMSF produced more inhibition than EDTA, indicating that the majority of gelatin lysis was due to serine proteininas in these cases.

Immunohistochemical Studies

Plasmin and Plasmin Activators. Immunoreactive plasmin was present in seven (70%) of 10 aneurysms but not in control samples (zero [0%] of five [p = 0.04]). As in the case of gelatin lysis, plasmin was heterogeneously distributed within the aneurysm wall occurring in focal areas of increased expression in five of seven plasmin-positive samples (Fig. 2 upper). In two of seven plasmin-positive aneurysm samples, a homogeneous, diffuse distribution of plasmin immunoreactivity was observed. In aneurysm samples, plasmin staining was associated with cellular
TABLE 2
Summary of results in aneurysm tissue obtained at surgery in 23 patients

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Size (mm)</th>
<th>SAH</th>
<th>Location</th>
<th>Zymography</th>
<th>Immunohistochemical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasmin</td>
</tr>
<tr>
<td>A1</td>
<td>42, M</td>
<td>5</td>
<td>absent</td>
<td>MCA</td>
<td>F-B</td>
<td>NR</td>
</tr>
<tr>
<td>A2</td>
<td>67, M</td>
<td>15</td>
<td>absent</td>
<td>ACoA</td>
<td>F-C</td>
<td>F-B</td>
</tr>
<tr>
<td>A3</td>
<td>57, M</td>
<td>12</td>
<td>absent</td>
<td>ACoA</td>
<td>F-C</td>
<td>NR</td>
</tr>
<tr>
<td>A4</td>
<td>63, F</td>
<td>15</td>
<td>absent</td>
<td>MCA</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A5</td>
<td>53, M</td>
<td>15</td>
<td>absent</td>
<td>ACoA</td>
<td>F-C</td>
<td>F-B</td>
</tr>
<tr>
<td>A6</td>
<td>63, F</td>
<td>8</td>
<td>absent</td>
<td>ICA</td>
<td>F-C</td>
<td>F-B</td>
</tr>
<tr>
<td>A7</td>
<td>63, F</td>
<td>8</td>
<td>absent</td>
<td>ACoA</td>
<td>F-B</td>
<td>F-B</td>
</tr>
<tr>
<td>A8</td>
<td>50, M</td>
<td>8</td>
<td>absent</td>
<td>ACoA</td>
<td>NR</td>
<td>F-B</td>
</tr>
<tr>
<td>A9</td>
<td>40, F</td>
<td>4</td>
<td>present</td>
<td>ACoA</td>
<td>F-C</td>
<td>NR</td>
</tr>
<tr>
<td>A10</td>
<td>66, M</td>
<td>9</td>
<td>present</td>
<td>ACoA</td>
<td>F-C</td>
<td>D-B</td>
</tr>
<tr>
<td>A11</td>
<td>63, F</td>
<td>5</td>
<td>absent</td>
<td>MCA</td>
<td>F-C</td>
<td>D-B</td>
</tr>
<tr>
<td>A12</td>
<td>52, M</td>
<td>10</td>
<td>absent</td>
<td>MCA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A13</td>
<td>37, F</td>
<td>4</td>
<td>absent</td>
<td>ICA</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A14</td>
<td>56, M</td>
<td>7</td>
<td>absent</td>
<td>MCA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A15</td>
<td>46, F</td>
<td>6</td>
<td>absent</td>
<td>MCA</td>
<td>NR</td>
<td>—</td>
</tr>
<tr>
<td>A16</td>
<td>36, F</td>
<td>3.5</td>
<td>absent</td>
<td>ACoA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A17</td>
<td>48, M</td>
<td>7</td>
<td>absent</td>
<td>BA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A18</td>
<td>33, F</td>
<td>10</td>
<td>absent</td>
<td>ICA</td>
<td>NR</td>
<td>—</td>
</tr>
<tr>
<td>A19</td>
<td>67, F</td>
<td>12</td>
<td>absent</td>
<td>ACoA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A20</td>
<td>59, F</td>
<td>5</td>
<td>absent</td>
<td>MCA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A21</td>
<td>42, F</td>
<td>7</td>
<td>absent</td>
<td>ICA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A22</td>
<td>63, M</td>
<td>10</td>
<td>absent</td>
<td>ICA</td>
<td>F-C</td>
<td>—</td>
</tr>
<tr>
<td>A23</td>
<td>80, M</td>
<td>14</td>
<td>absent</td>
<td>ACoA</td>
<td>NR</td>
<td>—</td>
</tr>
</tbody>
</table>

*ACoA — anterior communicating artery; BA — basilar artery; ICA — internal carotid artery; M — extracellular matrix; MCA — middle cerebral artery; PCA — posterior cerebral artery; — not enough tissue available.

Elements near the luminal surface, whereas staining of acellular matrix components was observed near the abluminal surface.

The plasminogen activators u-PA and t-PA had distribution patterns significantly different from plasmin in all samples. Immunoreactive t-PA was present in all aneurysm (10 of 10) and control (five of five) samples tested (p = 1). Staining was seen throughout all layers of the aneurysm tissue whereas only the media of the basilar arteries exhibited immunoreactive t-PA. In contrast, u-PA was present in only two (18%) of 11 aneurysm and none (zero to five [0%]) of the control samples (p = 0.84). When present, u-PA was heterogeneously distributed in focal, predominantly cellular areas.

Matrix Metalloproteinases. Immunoreactive MMP-2 was identified in 14 (64%) of 22 aneurysms but in only one (14%) of seven control arteries. This difference exhibited a trend toward, but did not reach statistical significance (Yates-corrected chi-square test; p = 0.066). In 10 (71%) of 14 MMP-2-positive aneurysm samples, the staining was distributed in focal pockets, with the bulk of the reaction product at the cell membrane surface (Fig. 2 lower).

By using immunohistochemical analysis, we found that MMP-9 was present in 10 (77%) of 13 aneurysms and in seven (100%) of seven control arteries (p = 0.47). The distribution of MMP-9, however, differed significantly between aneurysm and control tissues. In seven (100%) of seven control basilar arteries, MMP-9 staining was diffusely distributed, whereas in four (40%) of 10 MMP-9-positive aneurysms, MMP-9 was heterogeneously distributed in focal pockets of increased expression (Fig. 3) (p = 0.05).

Immunoreactive MT1-MMP was observed in seven (54%) of 13 aneurysm but no (zero of eight [0%]) control samples (p = 0.04). As in the case of gelatin lysis activity...
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FIG. 3. Photomicrograph showing immunohistochemical localization of MMP-9 (arrows) within an aneurysm wall (upper) and control basilar artery (lower). Focal areas of MMP-9 expression are seen within the aneurysm. Immunoreactive MMP-9 appears dark brown. Original magnification × 1980.
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and plasmin, MT1-MMP was heterogeneously distributed within the aneurysm wall, occurring in focal areas of increased expression in five of seven MT1-MMP-positive samples (Fig. 4). In two of seven MT1-MMP-positive aneurysm samples, a homogeneous, diffuse distribution of MT1-MMP immunoreactivity was observed. In all positive samples, MT1-MMP was observed both in association with cells and in acellular regions.

Tenascin. Immunoreactive tenascin was observed in all aneurysm (eight of eight) and basilar (seven of seven) samples studied (p = 1). In six (74%) of eight tenascin-positive aneurysms and in seven (100%) of seven tenascin-positive control arteries, tenascin staining was heterogeneously distributed in focal pockets of increased expression (Fig. 5). Tenascin staining was markedly increased in two of eight aneurysm samples compared with other aneurysm or control specimens.

Discussion

In contrast with normal cerebral arteries, we observed the molecular signature of vascular extracellular matrix remodeling within most aneurysms. Unruptured aneurysms, which served as the source of most study material, were previously believed to be static or stable lesions. Our findings do not support this hypothesis. Unruptured cerebral aneurysms are biochemically dynamic lesions with a remarkable potential for matrix remodeling. Active proteases that can degrade gelatin were commonly found within the aneurysm tissue. Inhibitor studies revealed that both serine proteinases and MMP accounted for this activity; however, MMPs appeared to be the predominant participants. Both MMP-2 and MMP-9 may have contributed to the observed gelatin lysis. Both have been linked to the occurrence of cerebral aneurysms \(^{15,23,48}\) and other types of vascular diseases. \(^{15,23,48}\) In our study, MMP-2 was rarely found in normal cerebral arteries but was focally expressed in more than half of the aneurysms studied. In contrast, we identified MMP-9 in both normal cerebral arteries and aneurysms. This enzyme was diffusely expressed in normal cerebral arteries, but showed focal areas of increased expression within some aneurysms.

Interpretation of our results must take into account the fact that cerebral aneurysms develop at discrete loci and usually do not affect entire blood vessels. \(^{8}\) Although a variety of different mechanisms may be responsible for this targeting, our findings have identified two enzymatic mechanisms capable of targeting matrix remodeling. Plasmin was found to be focally present in cerebral aneurysms but in not normal cerebral arteries. Plasmin is known to degrade glycoprotein components of the extracellular matrix including fibronectin and tenascin. \(^{14,18}\) Although plasmin does not degrade the fibrillar components of the extracellular matrix, destruction of the matrix glycoproteins may make these fibrillar components more vulnerable to the MMPs. \(^{14}\) Fragments formed by fibro-
nectin and tenascin digestion upregulate MMP transcription.\textsuperscript{32} Plasmin activates several members of the MMP family (MMP-3, MMP-4, and MMP-9), which in turn activate other family members in a cascade that can lead to MMP-2 activation.\textsuperscript{33,40}

Plasmin is formed by the enzymatic cleavage of plasminogen by t-PA and u-PA. When bound to its cell surface receptor, u-PA is the preferred site of plasminogen activation, whereas t-PA functions best when bound to fibrin.\textsuperscript{14} Although u-PA has been identified as a key plasminogen activator in other site-specific types of vascular disease,\textsuperscript{16,33,43,45} our data do not provide a clear understanding of the predominant mechanism responsible for plasmin production in cerebral aneurysms.

A newly described membrane-bound MMP, MT1-MMP was expressed in aneurysm tissue but not in control arteries. Unlike other members of this family, MT1-MMP is functional when bound to the cell surface and its regulation is thought to be at the level of transcription. It is responsible for activation of pro-MMP-2.\textsuperscript{21} and thus with the plasmin system, MT1-MMP may be a regulator of the rate and location of matrix remodeling in cerebral aneurysms.

Tenascin was commonly expressed in both control arteries and cerebral aneurysms. In both groups, tenascin was heterogeneously distributed. Like fibronectin, tenascin is a matrix glycoprotein that exists in several isoforms.\textsuperscript{9} Tenascin production is upregulated in fibroblasts exposed to stress\textsuperscript{37,38} and is an important component of healing tissues.\textsuperscript{13} Although two aneurysms exhibited intense focal staining that appeared consistent with injury to the vessel wall, tenascin, as a biological marker, did not appear to distinguish aneurysms from normal cerebral arteries.

We hypothesize that a variety of different and presently unknown triggers lead to remodeling of the arterial extracellular matrix, a final common pathway of cerebral aneurysm formation and growth. Identification of the initial events that lead to aneurysm formation in humans may be difficult because "preaneurysmal" lesions are rarely encountered and the age of an aneurysm is usually unknown. Matrix remodeling is believed to lead to atherosclerosis\textsuperscript{10,19,28,32} as well as to other forms of vascular disease.\textsuperscript{15,22,42} Why matrix remodeling leads to atherosclerosis in one vessel and to aneurysms in another remains a crucial, but unanswered question.

Acknowledgments

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References


FIG. 5. Photomicrograph showing immunohistochemical localization of tenascin focally expressed in an aneurysm. Original magnification × 990.
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35. Reilly JM, Siciar GA, Lacore CL: Abnormal expression of...


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