Accelerated Neointima Formation After Vascular Injury in Mice With Stromelysin-3 (MMP-11) Gene Inactivation

H. Roger Lijnen, Berthe Van Hoef, Ingrid Vanlinhout, Maria Verstreken, Marie-Christine Rio, Désiré Collen

Abstract—The hypothesis that stromelysin-3 (MMP-11), a unique member of the matrix metalloproteinase (MMP) family, plays a role in neointima formation was tested with the use of a vascular injury model in wild-type (MMP-11+/+) and MMP-11-deficient (MMP-11−/−) mice. Neointima formation 2 to 3 weeks after electric injury of the femoral artery was significantly enhanced in MMP-11−/− as compared with MMP-11+/+ mice, in both mice of a pure 129SV genetic background (0.014 versus 0.0010 mm2 at 2 weeks, P<0.001) and those of a 50/50 mixed 129SV/BL6 background (0.030 versus 0.013 mm2 at 3 weeks, P<0.05). The medial areas were comparable, resulting in intima/media ratios that were significantly increased in MMP-11−/− as compared with MMP-11+/+ arteries, in mice of both the 129SV (1.0 versus 0.18, P<0.001) and mixed (1.5 versus 0.70, P<0.05) backgrounds. Nuclear cell counts in cross-sectional areas of the intima of the injured region were higher in arteries from MMP-11−/− mice than in those from MMP-11+/+ mice (210 versus 48, P<0.001, in pure 129SV mice and 290 versus 150, P<0.01, in mice of the mixed genetic background). Immunocytochemical analysis revealed that α-actin–positive and CD45-positive cells were more abundant in intimal sections of MMP-11−/− mice. Degradation of the internal elastic lamina was more extensive in arteries of MMP-11−/− mice than in those of MMP-11+/+ mice (39% versus 6.8% at 3 weeks, P<0.005). The mechanisms by which MMP-11 could impair elastin degradation and cellular migration in this model remain, however, unknown. (Arterioscler Thromb Vasc Biol. 1999;19:2863-2870.)

Key Words: neointima ■ restenosis ■ transgenic mice ■ stromelysin-3

Neointima formation is a wound-healing process that occurs in response to vascular injury. Degradation of the extracellular matrix with migration of smooth muscle cells (SMCs) from the media to the intima, followed by deposition of extracellular matrix, leads to this intimal hyperplasia.1,2 The plasminogen/plasmin and matrix metalloproteinase (MMP) systems are believed to play a role in this phenomenon by degrading the extracellular matrix and thereby allowing SMC migration.3–8 In a balloon-injured rat carotid artery model, a correlation was observed between changes in plasminogen activators3 or MMPs5,6 and SMC migration. This was supported by the observation that in vivo SMC migration in the rat was inhibited by the plasmin inhibitor α2-proteinase inhibitor.17 How- ever, the 28-kDa NH2-terminal domain of mouse MMP-11 has the properties of a weak metalloproteinase,18 and on deletion of 175 COOH-terminal amino acids, it acquires enzymatic activity against casein, laminin, and type IV collagen.19 In addition, the catalytic domain of murine MMP-11 was found to degrade the Aα-chain of fibronogen.20 MMP-11 is expressed at a high level in most invasive carcinomas.21–23 Tissues that normally undergo extensive remodeling, such as the placenta, uterus, and postlactation mammary glands, also express MMP-11.21,22,24 These observations suggest that it may play a role in extracellular matrix remodeling.

In the study described here, we investigated the potential role of MMP-11 in neointima formation after vascular injury in mice. Surprisingly, we found more pronounced neointima formation in mice with MMP-11 gene deficiency.
Methods

Animals
MMP-11+/− and wild-type mice (CNRS-INSERM-ULP, Illkirch, France) of the same genetic background (129SV or 129SV/B6L, 50/50) were obtained and characterized as described elsewhere. Homozygosity of offspring was confirmed by genotyping of tail-tip DNA (digested with SpeI) by using as probe a 0.8-kb SpeI-EcoRI restriction fragment derived from intron 7 and Southern blotting (data not shown). Mice were kept in microisolation cages on a 12-hour-day/12-hour-night cycle and fed standard chow. Animals were anesthetized by intraperitoneal injection of 60 mg/kg Nembutal (Abbott Laboratories), and all experiments were performed in accordance with guidelines of the American Physiological Society and the International Society on Thrombosis and Hemostasis. Mice (males and females) were 12 to 15 weeks old with a body weight (mean±SEM) of 26±0.9 g (n=23) or 25±0.6 g (n=21) for 129SV/B6L MMP-11+/− or MMP-11−/− mice, respectively, and 28±0.9 g (n=10) or 28±1.2 g (n=10) for 129SV MMP-11+/− or MMP-11−/− mice, respectively. Statistical analysis was performed by using a 2-tailed t test (nonparametric Mann-Whitney U test).

Vascular Injury Model
Perivascular electric injury to the femoral artery of mice was performed essentially as described elsewhere. In brief, arteries were exposed by blunt-end dissection and injured by electric current at distances of 1 mm over a total length of 2 to 3 mm. One to three weeks after injury, the animals were killed and vessel segments were analyzed. In situ zymography on fibrin overlay at 37°C for 2 hours, without or with addition to the gel of antibodies against murine tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) (final concentration, 40 μg/mL), or was performed for 48 hours in the presence of anti-tPA and anti-uPA antibodies to determine PA-independent activity. For comparison, the lysis area (mm²) was normalized to the total area of the section. Data are reported as mean±SEM of 6 experiments (using different animals); within each experiment, 6 to 14 sections equally spaced throughout uninjured arteries or throughout the center of the injury (position 3) were analyzed.

Histological and Immunocytochemical Testing
Morphometric measurements of cross-sectional areas and cell counts were made in a blinded manner on transverse arterial sections (stained with hematoxylin and eosin) using a computer-assisted image analysis system as described elsewhere. Measurements were made at equally spaced positions (80 to 100 μm apart) across the artery. After staining for elastin with Verhoeff’s and von Gieson’s stains, the circumference of the internal elastic lamina was measured by computer-assisted image analysis.

For immunostaining, the primary monoclonal antibodies used were rat anti-mouse macrophage-specific Mac3 (clone M3/84, Pharmingen), biotinylated mouse anti-human smooth muscle α-actin (clone 1A4, Sigma Chemical Co), biotinylated rat anti-mouse pan-leukocyte antigen CD45 (clone 30F11.1, Pharmingen), and biotinylated rat anti-mouse polymorphic neutrophils (clone 7/4, Biosource). Immunostaining for Mac3 was performed by using biotinylated rabbit anti-rat immunoglobulins (Dakopatts) and the Tyramide signal amplification kit (DuPont-NEB), whereas for α-actin, CD45, and neutrophils, biotinylated primary antibodies were used in combination with the Vectastain system (ABC Elite kit, Vector Laboratories).

Results

Histological Testing
Hematoxylin and eosin staining of sections of noninjured femoral arteries (from control animals) showed similar findings for MMP-11+/− and MMP-11−/− mice; the adventitial and medial areas were comparable, whereas no significant neointima was detectable (Table 1 and Figure 1a). Nuclear cell counts (mainly endothelial cells) did not reveal differences between MMP-11+/− and MMP-11−/− arteries (23±2 versus 28±2 in 129SV/B6L mice and 25±1 versus 22±1 in 129SV mice, mean±SEM, n=6).

Staining of sections obtained 1 to 3 weeks after injury at equally spaced locations (positions 2 to 4) throughout the damaged artery showed neointima formation in both MMP-11+/− and MMP-11−/− mice (Figure 1c and 1e). In MMP-11+/− mice, the intimal area was always smaller than at corresponding time points in MMP-11−/− mice, whereas the medial areas were comparable, with the exception of a larger medial area at 2 weeks after injury in 129SV MMP-11−/− mice (P<0.01). This result was significantly lower intima/media ratios in MMP-11+/− mice. Also, the adventitial areas of injured arteries were smaller in MMP-11+/− mice than in MMP-11−/− mice (Table 1). This observation was confirmed in mice of 2 different genetic backgrounds, although intima formation in 129SV MMP-11−/− mice was significantly lower than that in 129SV/B6L MMP-11−/− mice. In the different experimental groups, no significant differences in neointimal area were observed between male and female mice (P>0.1).

Nuclear cell counts revealed comparable cell populations in media of control (noninjured) arteries and in normal sections (positions 1 and 5) of injured arteries (not shown). In the injured area (positions 2 to 4), cell counts were overall comparable over time in the media of both genotypes, whereas cell counts in the intima were higher in MMP-11−/− than in MMP-11+/− mice (Table 2). Separate analysis of the data at the borders of injury (positions 2 and 4) revealed somewhat, but not statistically significant, higher nuclear cell counts at 2 weeks after injury in the intima of MMP-11+/− than in MMP-11−/− mice: 61±9 versus 48±7 (mean±SEM, n=11 to 12, P=0.32) in 129SV/B6L mice and 106±26 versus 43±9 (mean±SEM, n=5, P=0.056) in 129SV mice. Anti-PCNA staining did not reveal cell proliferation in the media or intima at 1 week after injury in MMP-11+/− or MMP-11−/− 129SV/B6L mice; at 2 weeks, the rate of cell proliferation was also very low (0.9±0.5% and 2.8±1.7% of the total nuclear cell counts in intima and media, respectively, of MMP-11+/− arteries, with corresponding values of Peroxidase activity was determined by incubating sections in 0.05 mol/L Tris-HCl buffer, pH 7.0, containing 0.06% 3,3′-diaminobenzidine and 0.01% H₂O₂, followed by counterstaining with Harris’ hematoxylin. Specificity of the staining was confirmed by omission of the primary antibody or by replacing it with equivalent amounts of isotype-matched, nonimmune IgG or serum. The number of positive cells was semiquantitatively graded on a scale of − (no positive cells) to ++++ (≥75% cells positive), with ++++, +, and + representing 50% to 75%, 25% to 50%, and <25% of positively stained cells, respectively.

Proliferating cells were identified by immunostaining with monoclonal anti-proliferating cell nuclear antigen (PCNA; clone PC10, Sigma) as recommended by the manufacturer and counted microscopically. According to the manufacturer, anti-PCNA recognizes the acidic nonhistone auxiliary protein of DNA polymerase.
TABLE 1. Morphometric Quantitation of Cross-Sectional Areas of Adventitia, Media, and Intima After Injury of the Femoral Artery in MMP-11/+/+ or MMP-11−/− Mice of a 129SV/BL6 or 129SV Genetic Background

<table>
<thead>
<tr>
<th>Time After Injury</th>
<th>MMP-11/+/+</th>
<th>MMP-11−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adventitial Area, mm²</td>
<td>Intimal Area, mm²</td>
</tr>
<tr>
<td>129SV/BL6</td>
<td>n=6</td>
<td>n=6</td>
</tr>
<tr>
<td>Control</td>
<td>0.016±0.001</td>
<td>ND</td>
</tr>
<tr>
<td>1 week</td>
<td>0.132±0.021</td>
<td>0.0056±0.0014</td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.134±0.014</td>
<td>0.0066±0.0011</td>
</tr>
<tr>
<td>3 weeks</td>
<td>0.186±0.018</td>
<td>0.013±0.0042</td>
</tr>
<tr>
<td>129SV</td>
<td>n=6</td>
<td>n=6</td>
</tr>
<tr>
<td>Control</td>
<td>0.027±0.0026</td>
<td>ND</td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.058±0.0045</td>
<td>0.0010±0.0005</td>
</tr>
<tr>
<td>3 weeks</td>
<td>0.070±0.0066</td>
<td>0.0017±0.0009</td>
</tr>
</tbody>
</table>

Measurements were performed on 5 to 16 sections equally spaced throughout the injured region (positions 2 to 4) of the arteries and were then averaged per artery. Data are mean±SEM of the average values obtained in n arteries. ND indicates not detectable.

*P<0.05 vs MMP-11+/+.
†P<0.01 vs MMP-11+/+.
‡P<0.001 vs MMP-11+/+.
§Mean±SEM of 4 arteries (no neointima was detected in 4 other arteries).

1.5±0.7% and 2.3±0.6% for MMP-11−/− arteries). At 2 weeks after injury, virtually no cell proliferation occurred in the intima or media of 129SV MMP-11+/+ mice; in 129SV MMP-11−/− mice, the rate of cell proliferation was also very low (1.5±0.7% and 1.2±0.8% of the total nuclear cell counts in the intima and media, respectively).

Immunocytochemical Testing

Noninjured control arteries of MMP-11+/+ and MMP-11−/− mice were very similar, with 2 or 3 layers of α-actin–immunoreactive smooth muscle cells in the media but none in the intima or adventitia (Figure 1b).

The cell population in the injured arteries was heterogeneous, as shown by immunostaining for smooth muscle cells (α-actin), leukocytes (CD45), polymorphic neutrophils, and macrophages (Mac3) (Figures 1 and 2). In 129SV/BL6 MMP-11+/+ and MMP-11−/− mice, at 1 week after injury, the neointima in the injured region contained mainly CD45-positive cells, only occasionally Mac3-positive cells, and no α-actin–positive cells. At 2 and 3 weeks after injury, fewer CD45-positive cells (<25%) but more α-actin–positive cells (25% to 50% at 2 weeks and 50% to 75% at 3 weeks) were observed in the neointima of both genotypes. In the media of MMP-11+/+ and MMP-11−/− mice, comparable amounts of α-actin–positive cells were seen at 1 and 2 weeks (≤25%), whereas at 3 weeks, these were more abundant in injured arteries of MMP-11−/− mice (50% versus <25%). It should be kept in mind, however, that the number of SMCs at 1 week after injury may be underestimated because proliferating/migrating SMCs are not efficiently stained for α-actin.28 In the adventitia of MMP-11+/+ or MMP-11−/− arteries, no α-actin–positive cells were detected at any time point, and most cells were CD45– or Mac3–positive (Table 3).

In 129SV mice, at 2 weeks after injury (largest difference in neointima between MMP-11+/+ and MMP-11−/− mice), virtually no α-actin– or CD45-positive cells or polymorphic neutrophils were detected in the neointima of arteries from MMP-11+/+ mice. In the neointima of arteries from MMP-11−/− mice, α-actin–positive cells (<25%), CD45-positive cells (<50%), and polymorphic neutrophils (<25%) were more abundant. In the media, less α-actin–positive cells (<25% versus 50% to 75%) and more CD45-positive cells (50% to 75% versus <25%) were seen in arteries from MMP-11−/− mice than in arteries from MMP-11+/+ mice. Diffuse staining of neutrophils was observed in media of MMP-11−/− but not MMP-11+/+ arteries. In the adventitia, no α-actin–positive cells were observed in MMP-11+/+ or MMP-11−/− arteries, but more CD45-positive cells (≈100% versus <25%) and neutrophils (<25% versus 0%) were observed in sections from MMP-11−/− mice than MMP-11+/+ mice. Thus, in absolute numbers, the amount of α-actin– and CD45-positive cells and of neutrophils in the neointima of arteries from MMP-11−/− mice was higher than in arteries from MMP-11+/+ mice.

Verhoeff and von Gieson staining of elastin indicated that in both genotypes, the external elastic lamina was strongly degraded, whereas the internal elastic lamina remained essentially intact up to 2 weeks. Determination of the circumference by computer-assisted image analysis (6 experiments each with MMP-11+/+ and MMP-11−/− control, noninjured arteries and 6 experiments each at 1 and at 2 weeks after injury; 8 sections analyzed per artery) did not reveal significant differences between the 2 genotypes (≥95% of the internal elastic lamina was intact). Visual inspection of the autofluorescence of the elastic lamina on hematoxylin and eosin–stained sections confirmed this observation (not shown). Three weeks after injury, however, degradation of the internal elastic lamina was significantly more pronounced in arteries from MMP-11−/− mice than in those from MMP-11+/+ mice: 39±4.1% versus 6.8±3.1% (mean±SEM, n=6, P=0.0022) (Figure 2b and 2f). At the sites of extensive elastic lamina degradation, macrophages (Mac3–positive cells) were abundantly present in the media (Figure 2e).

Zymographic Analysis

In situ zymographic analysis of fibrinolytic activity was performed by fibrin overlay of arterial sections. This assay...
detects primarily tPA activity, as shown by the finding that lysis of the fibrin gel (after 2 hours at 37°C) with femoral arterial sections obtained 3 weeks after injury in wild-type mice was virtually abolished on addition of anti-tPA antibodies (residual lysis, \(\leq 1\%\)) but was not significantly affected by addition of anti-uPA antibodies (residual lysis, 90±4%; mean±SEM, n=12). Fibrinolytic activity (lysis zone) in arterial sections obtained 3 weeks after injury in 129SV/BL6 mice was increased 3- to 10-fold as compared with that in noninjured arteries. After normalization for the section areas, however, the activities (mean±SEM, n=6) were comparable both in noninjured arteries (2.2±0.56 versus 3.3±1.1 for arteries from MMP-11\(^{+/+}\) and MMP-11\(^{-/-}\) mice, respectively) and at the center of injury after 3 weeks (2.2±0.16 versus 2.9±0.50 for arteries from MMP-11\(^{+/+}\) and MMP-11\(^{-/-}\) mice, respectively).

Prolonged overlay at 37°C in the presence of anti-tPA antibodies did not reveal a significant difference in lysis of the fibrin gel between injured sections of MMP-11\(^{+/+}\) and MMP-11\(^{-/-}\) arteries (lysis zone, mean±SEM, 0.042±0.007 mm², n=18 versus 0.064±0.010 mm², n=15; \(P=0.053\)). On addition of both anti-tPA and anti-uPA antibodies, the residual lysis

---

**Figure 1.** Light microscopic analysis of femoral arterial sections of noninjured controls (a and b) or from the center of injury after 3 weeks in MMP-11\(^{+/+}\) (c and d) and MMP-11\(^{-/-}\) (e and f) 129SV/BL6 mice. Sections were stained with hematoxylin and eosin (a, c, and e) or antiserum against \(\alpha\)-actin (b, d, and f). Arrows and arrowheads indicate the internal and external elastic lamina, respectively. Scale bars indicate 50 \(\mu\)m.
TABLE 2. Cell Accumulation in Media and Intima After Injury of the Femoral Artery in MMP-111/1 or MMP-112/2 Mice of a 129SV/BL6 or 129SV Genetic Background

<table>
<thead>
<tr>
<th>Time After Injury</th>
<th>129SV/BL6</th>
<th>129SV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMP-111/1</td>
<td>MMP-112/2</td>
</tr>
<tr>
<td></td>
<td>1/1</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>Cells</td>
<td>Cells</td>
</tr>
<tr>
<td></td>
<td>Media</td>
<td>Intima</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>72±5</td>
</tr>
<tr>
<td>1 week</td>
<td>12</td>
<td>44±6</td>
</tr>
<tr>
<td>2 weeks</td>
<td>12</td>
<td>76±7</td>
</tr>
<tr>
<td>3 weeks</td>
<td>6</td>
<td>48±6</td>
</tr>
<tr>
<td>129SV</td>
<td>6</td>
<td>60±2</td>
</tr>
<tr>
<td>2 weeks</td>
<td>8</td>
<td>26±2</td>
</tr>
<tr>
<td>3 weeks</td>
<td>6</td>
<td>33±7</td>
</tr>
</tbody>
</table>

Nuclear cell counts were performed on 5 to 17 sections equally spaced throughout the injured region (positions 2 to 4) of the arteries and were then averaged per artery. Data are mean±SEM of the average values obtained in n arteries.

*P<0.01 vs MMP-111/1.
†P<0.001 vs MMP-112/2.

zones were also comparable for sections from MMP-111/1 and MMP-112/2 mice (0.018±0.003 mm², n=18 versus 0.017±0.002 mm², n=15).

These data indicate that tPA- or uPA-mediated lysis of the fibrin gel, as well as tPA- and uPA-independent lysis (eg, MMP-2-dependent), was not significantly different between sections obtained at the center of injury in MMP-111/1 and MMP-112/2 mice.

Discussion

Remodeling after vessel wall injury may contribute to luminal stenosis.20 It involves migration of SMCs from the media across the internal elastic lamina underneath the endothelium, proliferation in the neointima, and deposition of extracellular matrix.1,2,30 Proteinases of the plasminogen/plasmin and MMP systems may play a role in SMC migration by degrading the extracellular matrix that prevents migration.3-8

Within the MMP family, MMP-11 (stromelysin-3) is an unusual member, because its mature form is unable to hydrolyze the major components of the extracellular matrix, whereas a fragment containing the catalytic domain acquires some enzymatic activity.17-19 The biological role of MMP-11 is not fully understood. MMP-111/1 mice are viable, fertile, and normal in appearance and behavior.25 This is compatible with our finding that vessel formation is normal in MMP-111/2 mice, because no differences were observed in normal, uninjured femoral arteries from wild-type mice. Recently, it was shown that MMP-11, in a paracrine manner, promotes homing of malignant epithelial cells, a key process for primary tumors and metastases; this function requires extracellular matrix-associated growth factors.25 Furthermore, it was reported that MMP-11 hydrolizes the insulin-like growth factor-binding protein-1 and may thus regulate bioavailability of insulin-like growth factor-I, favoring cell survival and proliferation.31

To assess a potential role of MMP-11 in neointima formation, a perivascular electric injury model was applied to mice with targeted inactivation of MMP-11. In this model, wound healing initiates from the adjacent uninjured borders and progresses into the necrotic center and is associated with migration of SMCs and leukocytes.27 Surprisingly, we found accelerated neointima formation in MMP-112/2 mice. Because of previous observations of pronounced differences in atherosclerotic lesion formation between mice of different genetic backgrounds32-34 and in neointima formation after vascular injury,11,12,35 we confirmed our finding in mice of 2 different backgrounds. Neointima formation in wild-type mice with a pure 129SV background up to 3 weeks after injury was more than 5-fold lower than that in mice with the mixed 129SV/BL6 (50/50) background; however, in mice of both genetic backgrounds, neointima formation after vascular injury was significantly enhanced with deficiency of MMP-11.

Because no specific antiserum was available, we were not able to study the temporal and topographic expression pattern of MMP-11 in wild-type mice after vascular injury. However, several previous studies have demonstrated local expression of MMP-11 during remodeling processes, such as tissue involution,24 tissue repair,36 and human carcinoma development,21 but systemic effects were not reported. During the repairation process after skin wound healing, MMP-11 is temporally and locally expressed,36 and its expression has also been observed in human atherosclerotic lesions but not in normal arteries.37 Strong expression has been observed in fibroblasts.22 In MMP-111/1 mice, the absence of MMP-11 mRNA has been clearly demonstrated. Thus, although we cannot conclusively show whether the observed effects in this study are direct or indirect, the presence or absence of MMP-11 is the main difference between both genotypes, suggesting that the absence of MMP-11 contributes to neointimal thickening.

This unexpected observation probably cannot be explained by direct effects of MMP-11 on matrix degradation, as suggested by our previous finding that lysis of a 3 H-proline-labeled subendothelial matrix by in vivo thiglycollate-stimulated macrophages was similar for wild-type and MMP-112/2 mice.20 We have also shown that MMP-11 deficiency does not affect expression and activation of MMP-2 or MMP-9 in segments of aorta.20 Furthermore, it is unlikely that degradation of soluble serpins by MMP-11 plays a role in this model, because lower proteolytic activity in MMP-112/2 mice would be expected to result in reduced neointima formation.

We explored several other potential mechanisms that may be involved in the accelerated neointima formation observed in MMP-112/2 mice. The overall fibrinolytic activity in arterial sections was enhanced after vascular injury, but after normalization for the section area, there was no difference between wild-type and MMP-112/2 mice. Additional quantitative analysis did not reveal significant differences in the medial areas, whereas the adventitial areas after injury were larger in the arteries from MMP-111/2 mice than in those from wild-type mice. Nuclear cell counts in the intima were higher after injury in arteries from MMP-111/2 mice. Anti-PCNA staining revealed virtually no proliferating cells at 1 or 2 weeks after injury at the center of injury in media or intima.
Figure 2. Light microscopic analysis of femoral arterial sections from MMP-11+/− (a–d) and MMP-11−/− (e–h) mice. Sections were obtained from the center of injury after 3 weeks in 129SV/BL6 mice (a, b, e, and f) and after 2 weeks in 129SV mice (c, d, g, and h). Sections were stained with antiserum against Mac-3 (a and e), CD45 (c and g), or polymorphic neutrophils (d and h). Elastin staining (b and f) was performed using Verhoeff’s and von Gieson’s stains. Scale bars indicate 50 μm.
of arteries from MMP-11/+/+ mice, and the cell proliferation rate was also very low in arteries from MMP-11−/− mice, suggesting that mainly invading cells are present. Although anti-PCNA staining on arterial sections may underestimate the actual number of proliferating cells, it is unlikely that the large difference in nuclear cell counts between both genotypes (Table 2) is caused by different proliferation rates. The cell population in the intima was heterogeneous, consisting mainly of α-actin–positive and CD45-positive cells, of which a significant fraction was identified as polymorphonuclear neutrophils in the arteries of MMP-11−/− mice, possibly related to an inflammatory response. In mice of the pure 129SV background, in which the largest increase in intimal area in MMP-11−/− mice was observed, these cells appeared to be more abundant than in wild-type mice. It cannot be excluded that proteinases produced by inflammatory cells contribute to degradation of matrix and other tissue components. It is, however, unclear why a stronger inflammatory response would occur in MMP-11−/− than in wild-type animals. In 129SV/B6 mice, the relative contribution of different cell types in the total population seemed more comparable in arteries of wild-type and MMP-11−/− mice, but the absolute numbers were significantly higher in arteries of MMP-11+/+ mice. Furthermore, degradation of the internal elastic lamina at 3 weeks after injury was significantly more pronounced in arteries from MMP-11−/− mice than in those from MMP-11+/+ mice. Extensive degradation was seen at sites (Figure 2f) where Mac-3–positive cells were abundant in the media (Figure 2e), compatible with a role of macrophage-secreted proteinases in degradation of the elastica lamina.38

Together, these findings suggest that the enhanced neointima formation occurring after vascular injury in MMP-11−/− mice is a complex process in which enhanced elastin degradation and enhanced cellular migration, with participation of inflammatory cells, play a role. The mechanisms by which MMP-11 may impair these processes remain unknown and require further investigation.

### Acknowledgments

This study was supported by grants from the Flemish Fund for Scientific Research (contract G.0293.98) and from the Interuniversitaire Attractiepole (contract P4/34). CNRS-INSERM-ULP was supported by INSERM, CNRS, the Bristol Myers Squibb Pharmaceutical Research Institute, the Association pour la Recherche sur le Cancer, and BIOMED2 (contract BMH 4CT 96-0017). The skillful technical assistance of A. Dewulf is gratefully acknowledged.

### References


Accelerated Neointima Formation After Vascular Injury in Mice With Stromelysin-3 (MMP-11) Gene Inactivation

H. Roger Lijnen, Berthe Van Hoef, Ingrid Vanlinthout, Maria Verstreken, Marie-Christine Rio and Désiré Collen

doi: 10.1161/01.ATV.19.12.2863

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/12/2863

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/