Plasminogen Activators, Matrix Metalloproteinases, and Their Inhibitors in Implanted Vascular Prostheses

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Objectives: to examine the role of plasminogen activators (PAs) and matrix metalloproteinases (MMPs) in the healing of prosthetic grafts.

Methods: thirty explanted grafts (16 Dacron and 14 PTFE) were studied immunohistochemically using antibodies to PAs, MMPs, and their inhibitors. The percentages of immunostain-positive multinucleated giant cells (MGC) were related to duration of implantation (early vs late), type of lesion (stenosis vs false aneurysm), graft material (Dacron vs PTFE), and graft status (occluded vs patent).

Results: all specimens were positive for PAs and MMPs. There were no significant differences in the percentages of MGCs positive for PAs, MMPs, or tissue inhibitor type 2 of MMP (TIMP-2) between the groups. The percentage of TIMP-1 in the aneurysm group (mean, 26%) was significantly lower than that of the stenosis group (mean, 46%) (p<0.05).

Conclusion: after the implantation of a vascular prosthesis, PAs and MMPs are expressed in cell migration, proliferation and matrix construction. Under-expression of TIMP-1 may be related to the formation of an anastomotic aneurysm.

Key Words: Vascular prosthesis; Intimal hyperplasia; Anastomotic aneurysm; Matrix metalloproteinase; Plasminogen activator.

Introduction

Direct vascular anastomosis elicits acute inflammation and then heals completely.1–3 In implanted prostheses, however, acute inflammation is followed by an immunological reaction to the foreign body.4 Thrombosis occurs in direct sutures at the anastomotic site and is replaced by re-endothelialisation. However, the luminal surface of the implanted prosthesis is not covered with endothelium except at the anastomotic site.2

As a result of incomplete healing prosthetic grafts are prone to occlusion by stenosis and anastomotic aneurysm formation.5,6 Plasminogen activators (PAs) and matrix metalloproteinases (MMPs) play a role in intimal hyperplasia, plaque rupture, and wall dilatation.7–10 In wound healing, PAs and MMPs degrade damaged tissues and play a role in cell migration.11 In the healing process of implanted prostheses, multinucleated giant cells (MGCs) surround the prosthetic material. MGCs create a barrier-like structure,12 and the immunological reaction to the foreign body is controlled and stabilised in most implanted grafts.4 Loss of such control may result in excessive intimal hyperplasia or anastomotic aneurysm.

The aim of this study was to examine the expression of PAs, MMPs, and their inhibitors by MGCs in the anastomotic region of explanted prosthetic grafts.

Materials and Methods

Thirty vascular prostheses were retrieved at operation or autopsy. Seven Dacron grafts had been implanted for aortic aneurysm, and nine for arteriosclerotic occlusive disease. Fourteen PTFE grafts had been implanted for occlusive disease. Implanted grafts were obtained at revision surgery from four patients with anastomotic stenosis (implantation periods were 8, 54, 111, and 166 months), and from four patients with anastomotic aneurysms (implantation periods were 28, 54, 97 and 139 months). The observation periods ranged from 5 days to 166 months (median, 52 months).

For autopsy specimens, the delay between death...
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Immunostaining for MMP-1 (A), u-PA (B), and u-PAR (C) in a PTFE graft 21 days after implantation. Macrophages and fibroblasts, which migrated from the outside of the graft, are positive for MMP-1 and u-PA; some are also positive for u-PAR. Immunostaining for t-PA at the anastomotic region (D) and on the luminal surface (E), and PAI-1 (F) in a Dacron graft 22 months after implantation. Endothelial cells and smooth muscle cells at the anastomotic region are positive for t-PA. Macrophages and the fibrin layer are positive for t-PA, but only a few macrophages are positive for PAI-1 on the luminal surface of the graft. The bar represents 20 μm.

and graft retrieval was always less than 60 min. Samples were immersion-fixed in 10% neutral buffered formalin and then embedded in paraffin. Sections were stained with haematoxylin and eosin, azan, and Verhoeff–van Gieson stains. Serial sections were incubated with monoclonal antibodies (source; DAKO Japan, Tokyo, Japan); elastin (Sigma, St. Louis, Mo., U.S.A.); collagen types I to V (SBA, Birmingham, Ala., U.S.A.); α-actin (cells identified; smooth muscle cell) (DAKO); macrophages (DAKO), von Willebrand factor (endothelial cell) (DAKO); CD3 (T cell) (DAKO), and CD20 (B cell) (DAKO). MMP types 1 (collagenase), MMP-2 (gelatinase-A), MMP-3 (stromelysin-1), MMP-7 (matrilysin), MMP-9 (gelatinase-B); tissue inhibitor type 1 of MMPs (TIMP-1), TIMP-2, tissue type PA (t-PA), urokinase type PA (u-PA), u-PA receptor (u-PAR),
Fig. 2. Multinucleated giant cells are positive for MMPs and TIMPs. Immunostaining for MMP-3 (a), MMP-7 (b), and TIMP-2 (c) in a Dacron graft at 57 days after implantation. (d), immunostaining for MMP-2 in a Dacron graft 12 months after implantation. (e), immunostaining for MMP-9 in a PTFE graft 41 months after implantation. (f), immunostaining for TIMP-1 in a Dacron graft 148 months after implantation. Immunostaining for MMP-2 (g) and for MMP-9 (h) in a Dacron graft with an anastomotic aneurysm 54 months after implantation. MGCs are stained for MMP-2 and -9, and fibroblasts are stained for MMP-2. The bar represents 20 μm.
### Table 1. Percentages of multinucleated giant cells immunostained positive for t-PA, u-PA, u-PAR and PAI-1.

<table>
<thead>
<tr>
<th></th>
<th>Early retrieval</th>
<th>Late retrieval</th>
<th>Dacron</th>
<th>PTFE</th>
<th>Occluded</th>
<th>Patent</th>
<th>Stenosis</th>
<th>Aneurysm</th>
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<tbody>
<tr>
<td>t-PA</td>
<td>81 ± 9</td>
<td>87 ± 7</td>
<td>88 ± 7</td>
<td>85 ± 7</td>
<td>87 ± 6</td>
<td>87 ± 7</td>
<td>84 ± 5</td>
<td>88 ± 8</td>
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<tr>
<td>u-PA</td>
<td>84 ± 4</td>
<td>86 ± 10</td>
<td>88 ± 9</td>
<td>85 ± 10</td>
<td>86 ± 10</td>
<td>86 ± 10</td>
<td>77 ± 16</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>u-PAR</td>
<td>85 ± 6</td>
<td>79 ± 10</td>
<td>81 ± 11</td>
<td>77 ± 9</td>
<td>78 ± 10</td>
<td>80 ± 11</td>
<td>75 ± 12</td>
<td>85 ± 11</td>
</tr>
<tr>
<td>PAI-1</td>
<td>77 ± 13</td>
<td>66 ± 17</td>
<td>67 ± 17</td>
<td>65 ± 17</td>
<td>66 ± 17</td>
<td>76 ± 14</td>
<td>77 ± 11</td>
<td></td>
</tr>
</tbody>
</table>

Early retrieval, specimens of grafts retrieved within 3 months after implantation; Late retrieval, specimens retrieved more than 3 months after implantation; Dacron, specimens of Dacron graft; PTFE, specimens of polytetrafluoroethylene; Occluded, specimens of occluded grafts; Patent, specimens of patent grafts; Stenosis, specimens of anastomotic stenosis; Aneurysm, specimens of anastomotic aneurysm. Values are means ± SD.

### Table 2. Percentages of multinucleated giant cells immunostained positive for MMP-1, -2, -3, -7, -9 and TIMP-1 and -2.

<table>
<thead>
<tr>
<th></th>
<th>Early retrieval</th>
<th>Late retrieval</th>
<th>Dacron</th>
<th>PTFE</th>
<th>Occluded</th>
<th>Patent</th>
<th>Stenosis</th>
<th>Aneurysm</th>
</tr>
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<tr>
<td>MMP-1</td>
<td>89 ± 7</td>
<td>86 ± 13</td>
<td>86 ± 14</td>
<td>87 ± 12</td>
<td>89 ± 7</td>
<td>84 ± 16</td>
<td>89 ± 7</td>
<td>92 ± 5</td>
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<tr>
<td>MMP-2</td>
<td>88 ± 6</td>
<td>78 ± 21</td>
<td>78 ± 25</td>
<td>77 ± 17</td>
<td>76 ± 17</td>
<td>79 ± 25</td>
<td>82 ± 10</td>
<td>85 ± 9</td>
</tr>
<tr>
<td>MMP-3</td>
<td>44 ± 19</td>
<td>55 ± 29</td>
<td>59 ± 36</td>
<td>31 ± 18</td>
<td>52 ± 27</td>
<td>58 ± 33</td>
<td>41 ± 25</td>
<td>58 ± 16</td>
</tr>
<tr>
<td>MMP-7</td>
<td>71 ± 17</td>
<td>84 ± 16</td>
<td>86 ± 19</td>
<td>83 ± 10</td>
<td>81 ± 15</td>
<td>87 ± 16</td>
<td>74 ± 11</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>MMP-9</td>
<td>82 ± 7</td>
<td>82 ± 13</td>
<td>83 ± 13</td>
<td>79 ± 15</td>
<td>79 ± 15</td>
<td>83 ± 13</td>
<td>83 ± 7</td>
<td>87 ± 6</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>42 ± 20</td>
<td>35 ± 28</td>
<td>50 ± 31</td>
<td>61 ± 23</td>
<td>54 ± 28,0</td>
<td>69 ± 23</td>
<td>46 ± 18</td>
<td>26 ± 9*</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>53 ± 19</td>
<td>65 ± 22</td>
<td>69 ± 24</td>
<td>59 ± 19</td>
<td>59 ± 21</td>
<td>69 ± 23</td>
<td>60 ± 24</td>
<td>51 ± 16</td>
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</tbody>
</table>

Values are means ± SD. *p<0.05 compared to the value of stenosis.
and PA inhibitor type 1 (PAI-1). The antibodies against MMPs and TIMPs were provided by the Department of Virology, Cancer Research Institute, Kanazawa University. The antibodies against PAs, u-PA, and PAI-1 were obtained from American Diagnostica, Inc. (Greenwich, Ct., U.S.A.). Avidin-biotin immunoperoxidase was used for immunolabelling.

The percentage of positively staining MGCs was calculated. These data were related to duration of implantation; graft material (Dacron vs PTFE), graft status (occluded vs patent), and type of lesion (stenosis vs false aneurysm). The data are presented as means ± SD and analysed using the Student’s t-test. Significance was defined as p<0.05.

**Results**

Macrofages in anastomotic thrombi stained for t-PA, u-PA, u-PAR, PAI-1, MMP-1, -2, -3, -7, -9, and TIMP-1 and -2. The immunostaining for PAI-1, TIMP-1, and TIMP-2 was weak in the macrophages. In the midportion of the PTFE graft 21 days after implantation, fibroblasts had migrated from the outside to the luminal surface and were positive for t-PA, u-PA, u-PAR, MMP-1, and MMP-2. At the anastomotic site, the smooth muscle cells had migrated from the host artery to the luminal surface of the graft and were positive for t-PA, u-PA, u-PAR, MMP-1, and MMP-2. MGCs were found around the graft and stained for t-PA, u-PA, u-PAR, PAI-1, MMP-1, -2, -3, -7, -9, and TIMP-1 and -2. The immunostaining for PAI-1, TIMP-1, and TIMP-2 was weak in MGCs. The endothelial cells in the anastomosis of the Dacron graft 24 days after implantation were immunostained for t-PA, u-PA, and MMP-1, -2, -3 and -9.

At later times, connective tissues were formed at the anastomosis and at some parts of the luminal surface of the grafts. Most of the collagen in the connective tissue was type III, and some type I collagen was present with collagen type III. Type IV collagen was detected around the smooth muscle cells at the anastomosis and around the fibroblasts in the midportion of the grafts. In most parts of the connective tissue on the luminal surfaces, the smooth muscle cells, fibroblasts, and macrophages were not stained for MMPs, but some were stained for t-PA and u-PA. T-PA was also detected in the endothelial cells at the anastomosis and in the connective tissues and fibrin layers on the luminal surface of the graft. The endothelial cells in the small vessels around the grafts were positive for t-PA, u-PA, and MMP-1, -2, -3, and -9. Some T cells in the perigraft tissues were positive for u-PA, u-PAR, and MMP-2. No B cells were detected. Some macrophages and fibroblasts in surrounding tissues and between the graft fibres were positive for t-PA, u-PA, u-PAR, MMP-1, MMP-9, and TIMP-2. T-PA, u-PA, PAI-1, MMP-1, -2, -3, -7, -9, and TIMP-1 and -2 were present in the MGCs surrounding the grafts.

In samples of anastomotic hyperplasia, some smooth muscle cells were positive for t-PA, u-PA, MMP-1, -3, -7, -9, and TIMP-2. In the fibrin layer of anastomotic stenosis, some macrophages were positive for t-PA, u-PA, u-PAR, and MMP-1, -2, -3, and -9. In samples of anastomotic aneurysm, t-PA, u-PA, u-PAR, PAI-1, MMP-1, -2, -3, -7, -9, TIMP-1, and -2 were present in MGCs surrounding the graft fibres and sutures. The immunostaining for PAI-1 was weak in the MGCs (Figs 1, 2).

The mean values of immunostained MGCs at anastomotic regions were 86% for t-PA, 84% for u-PA, 81% for u-PAR, 72% for PAI-1, 88% for MMP-1, 82% for MMP-2, 51% for MMP-3, 78% for MMP-7, 83% for MMP-9, 45% for TIMP-1, and 59% for TIMP-2. There were no significant differences in PAs, u-PAR, PAI-1, MMPs, or TIMP-2 between early retrieval vs late retrieval, stenosis vs false aneurysm, Dacrons vs PTFEs, or occluded grafts vs patent grafts. The percentage of TIMP-1 in the aneurysm group (mean, 26%) was significantly lower than that in the stenosis group (mean, 46%) (p<0.05) (Tables 1, 2).

**Discussion**

In the healing of implanted vascular prostheses, PAs and MMPs are expressed during cell migration and matrix remodelling. Thrombi are formed on the luminal surface of the graft, and monocytes/macrophages migrate into the thrombi to remove them. Smooth muscle cells or fibroblasts migrate from the host artery or surrounding tissues into the luminal surface of the graft and produce collagen and other matrix components. MGCs cluster around the graft fibres and express PAs and MMPs as in wound healing. Neo-vascularisation in the connective tissues around the graft is also regulated by PAs and MMPs. In the late phase of graft healing, a thin layer of fibrin and collagen covers the luminal surface and gains anti-thrombotic properties. The MGCs surrounding the graft fibres express PAs and MMPs, degrading the connective tissues to allow the passage of cells into the graft and luminal surface. The migrated macrophages become phagocytes in the fibrin, and the
migrated fibroblasts produce collagen.\textsuperscript{16} Neovascularisation into the graft sprouts new capillaries. Thus, the MGCs surrounding the graft act not as a barrier, but as a modulator for continuous remodelling of the connective tissues, in which the production of extracellular matrix is correlated to the extent of proteolysis. The endothelial cells at the anastomotic site and fibroblasts at other parts of the graft express PAs, contributing to the anti-thrombogenesis of the luminal surface.\textsuperscript{17}

The grafts implanted for aneurysmal disease did not differ from those implanted in patients for occlusive disease. Nor was there any difference between Dacron and PTFE,\textsuperscript{2} or between occluded and patent grafts.

The percentages of MGCs immunostained for PAs and MMPs in the grafts with anastomotic stenosis did not differ from those in the grafts without stenosis. In spite of activated MGCs, smooth muscle cells and macrophages at the anastomotic site were inactive in the late phase, and our results confirmed that normal healing of the graft does not cause excessive intimal hyperplasia.\textsuperscript{4} However, deleterious events such as thrombosis or mechanical stress may cause migration and proliferation of smooth muscle cells, fibroblasts, and macrophages, resulting in anastomotic stenosis.\textsuperscript{9,20}

In an atherosclerotic aneurysm, MMPs in smooth muscle cells and macrophages are upregulated and degrade elastin and collagen.\textsuperscript{21} In the anastomotic aneurysms, the expression of MMPs was not increased in smooth muscle cells, macrophages, or MGCs at the anastomosis, but expression of TIMP-1 was decreased in MGCs. This suggests that in an anastomotic aneurysm the continuous remodelling of connective tissues may deviate from balanced reconstruction to degradation, and some mechanisms other than those in the atherosclerotic aneurysm may have caused the deviation. This difference in percentage of TIMP-1 between the aneurysm and the stenosis group should, however, be interpreted cautiously, as it could be a result of multiple comparisons.

In this study, we found that PAs and MMPs are expressed in cell migration and proliferation and in matrix remodelling after the implantation of a vascular prosthesis. The most outstanding finding was the great activity of MGCs surrounding the graft. They play an important role in the continuous remodelling of the connective tissues as the graft heals. As a therapeutic approach for small anastomotic aneurysms, TIMP-1 may be increased through gene delivery.\textsuperscript{22} When new vascular prostheses are developed, the immunological reaction to a foreign body may be regulated to target MGCs.

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