Growth factor production after polytetrafluoroethylene and vein arterial grafting: An experimental study

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Purpose: Occlusion caused by myointimal hyperplasia appears to be the main reason of late failure of polytetrafluoroethylene (PTFE) arterial bypass grafts. Evidence exists that growth factors are involved in the genesis of myointimal hyperplasia. The aim of this study was to assess the release of platelet-derived growth factor (PDGF) and basic fibroblastic growth factor (bFGF) by PTFE arterial grafts.

Methods: In 15 inbred Lewis rats a 1 cm long segment of PTFE was interposed at the level of the abdominal aorta. In a control group of another 15 Lewis rats a vein graft was implanted at the level of the abdominal aorta. Animals were killed 4 weeks after implantation and the tissue was studied in organ culture for release of PDGF AA, PDGF BB, and bFGF.

Results: PTFE grafts released a greater quantity of PDGF AA than did control vein grafts (28 ± 4 ng/cm²/72 hr vs 7 ± 2 ng/cm²/72 hr). Similarly, PTFE grafts released a greater quantity of bFGF than did arterial vein grafts (308 ± 22 ng/cm²/72 hr vs 204 ± 20 ng/cm²/72 hr).

Conclusions: We conclude that PTFE arterial grafts release a high quantity of growth factor, which could explain, in part, the occurrence of distal anastomotic myointimal hyperplasia. (J VASC SURG 1996;23:453-60.)

The development of anastomotic myointimal hyperplasia (MH) is the major obstacle in bypass grafting to small arteries. The phenomenon has been noted in infrapuinal artery bypass with any prosthetic material and, to a much lesser degree, with autologous saphenous vein.1-3 Factors involved in the genesis of anastomotic MH include local anastomotic factors such as trauma, compliance mismatch between graft and artery, and abnormal flow hemodynamics.4-7 Probably biologic events occurring during transit of blood through the synthetic graft may contribute to the formation of anastomotic MH and accelerated atherosclerosis often seen in the arterial tree below a synthetic graft.8

Growth factors, cytokines, and other chemicals induce and regulate numerous cell functions during the process of MH and atherogenesis.9,10 Basic fibroblastic growth factor (bFGF) and platelet-derived growth factor (PDGF) are two of the best-characterized mitogens for smooth muscle cells. Several reports have demonstrated increased expression of PDGF in association with naturally occurring atherosclerosis, experimentally induced atherosclerosis, and MH associated with failure of vascular grafts.9,11-16

bFGF is a member of the heparin-binding mitogens, characterized by their affinity for heparin and ability to stimulate both endothelial and smooth muscle cell proliferation.17-20 Thus PDGF and bFGF may stimulate smooth muscle cell proliferation and eventually lead to formation of anastomotic MH.

The purpose of our study was to analyze the release of PDGF and bFGF by polytetrafluoroethylene (PTFE) grafts once inserted in the arterial circulation. An increased release of growth factors could explain, at least in part, the occurrence of anastomotic MH in synthetic grafts. Arterial vein
grafts, in which anastomotic MH occurs much less frequently, were used as control grafts.

MATERIAL AND METHODS

Experimental design. In 15 male inbred Lewis rats (average weight, 250 gm) a 1 cm long segment of nonreinforced PTFE (30 μm fibril length, 2 mm internal diameter, and 0.39 mm thick; W.L. Gore & Associates Inc., Flagstaff, Ariz.) was inserted in the arterial system, at the level of the abdominal aorta. Four weeks after surgery the animals were killed and the PTFE grafts removed. The aorta above and below the graft and a segment of supradiaphragmatic inferior vena cava were also removed.

The control group consisted of 15 male inbred Lewis rats (average weight, 250 gm) in which a 1 cm long segment of inferior vena cava was inserted at the level of the abdominal aorta. The segments of inferior vena cava were obtained from syngeneic Lewis rats (same genetic pattern). Similarly, 4 weeks after surgery the animals were killed and the arterial vein graft, the aorta above and below the graft, and a segment of similar dimensions of endogenous inferior vena cava were explanted.

Animal care complied with the Principles of Laboratory Animal Care as formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (US Department of Health and Human Science, National Institutes of Health publication 80.23, revised 1985).

Operative procedures. The animals were anesthetized with intramuscular xylazine (3 mg/kg) and intramuscular ketamine (50 mg/kg) supplemented by intraperitoneal ketamine for maintenance. Surgery was performed with the use of an operating microscope (Zeiss OPMI-7D; Carl Zeiss, Inc., Thornwood, N.Y.).

In the PTFE group the abdomen was opened and the abdominal aorta identified, and both proximal and distal control was obtained. The PTFE graft was inserted with end-to-end anastomoses with 10-0 monofilament nylon sutures (Ethicon Inc.). Throughout each procedure, care was taken to avoid unnecessary damage of the vein tissue. Four weeks after surgery, the animals were anesthetized and the abdomen and chest were opened. The aorta above the graft, the grafts, and a 1 cm long segment of supradiaphragmatic inferior vena cava were exposed and excised.

Histologic examination. Grafts (n = 3) were perfused with an initial infusion of Hanks’ balanced salt solution, followed by 10% formaldehyde. Following standard procedures, the specimens were stained with hematoxylin and eosin. For scanning electron microscopy, specimens were fixed in 2.5% glutaraldehyde made up in 0.1 mol/L cacodylate buffer (pH 7.2), rinsed several times, and left 1 hour in the same buffer. Next the specimens were fixed 1 hour in osmium 1%, dehydrated in ascending concentrations of ethanol (15% to 100%), critical point dried in CO2, mounted in specimen stubs, and sputter coated with gold-palladium according to standard technique. All specimens were examined in a scanning electron microscope (S 570; Hitachi Ltd., Tokyo) at the accelerating voltage of 15 kV.

Organ culture. The PTFE grafts, arterial vein grafts, control veins, and aortic segments were opened longitudinally and rinsed thoroughly for 10 minutes with DMEM supplemented with antibiotics (gentamycin, 200 μg/ml; streptomycin, 100 μg/ml, and penicillin 100 IU/ml). The specimens (n = 12) were placed in 48-well Costar tissue culture plates (Costar, Cambridge, Mass.) for organ culture. The tissue was incubated in 1 ml DMEM supplemented with antibiotics at pH 7.4. The tissue was incubated for 5 days at 37°C in a 5% CO2 atmosphere. Aliquots of conditioned media were collected at 72 hours and centrifuged for 5 minutes at 15,000 rpm and the supernatant was stored at -80°C for assay of mitogenic activity and assay of PDGF AA, PDGF BB, and bFGF release.

Assay for mitogenic activity. The presence of mitogenic activity in conditioned medium suggests that the medium contain mitogens that could stimulate smooth muscle cell proliferation and lead to formation of MH.

Swiss 3T3 cells were plated in 96-well plates (Falcon Plastics) at a density of 4 × 10^4 cells/ml in 200 μl DMEM supplemented with 0.1% fetal calf serum. Seventy-two-hour conditioned serum-free media from PTFE grafts, vein grafts, and aortic segments were added to Swiss 3T3 cells (20 μl). Positive controls received an equivalent volume of DMEM plus human recombinant bFGF (20 ng/ml) or human recombinant PDGF (50 ng/ml) (Genzyme...
Co., Boston, Mass.); negative controls received only DMEM. After 2 days, tritiated thymidine (0.5 μCi per well plate) was added and the cultures were incubated for 18 hours and collected on Skatron Filters (Skatron Instruments, Sterling, Va.) for determination of radioactivity in an LKB scintillation counter (LKB Instruments, Inc., Gaithersburg, Md.).

Analysis of reduction of mitogenic activity by anti-PDGF and anti-bFGF antibody. Reduction of mitogenic activity by antibody to specific growth factor suggests that the mitogenic activity is due to that particular growth factor. Measurement of the Swiss 3T3 cells DNA synthesis-stimulating activity of the conditioned media from PTFE grafts was repeated in the presence of an excess of monospecific antibody to human PDGF (50 μg/ml) (Genzyme Co.) and an excess of monoclonal antibody to human bFGF (produced in our laboratory), which cross-react with rat growth factor. Tritiated thymidine was again added and the cultures were incubated for 18 hours. After further processing, the radioactivity was measured.

Assay of PDGF AA, PDGF BB, and bFGF in the conditioned media. The presence of bFGF, PDGF AA, and PDGF BB molecules in the serum-free conditioned media from vein grafts, PTFE grafts, control veins, and aortic segments were determined by inhibition antibody-binding assay. A dilution of anti-bFGF mouse monoclonal antibody that showed 50% maximal reactivity against bFGF (4 μg/ml) was incubated with various dilutions of conditioned media in 400 μl tubes precoated with phosphate-buffered saline (PBS) gelatin 2%. After 20 hours of incubation at 4° C, Staphylococcus aureus protein A was added, and the immunoaggregates were removed by centrifugation. The residual antibody-binding activity in the supernatant was measured by enzyme-linked immunosorbennt assay (ELISA). Plastic wells (96 wells; Falcon Plastics) were coated with bFGF (10 ng per well) for 8 hours at 4° C. Plates were then washed twice with PBS and saturated with PBS-gelatin 1% for 2 hours at 37° C. Washed wells were then filled with 50 μl per well supernatant obtained after immunoprecipitation. After 2 hours of incubation at 37° C, the wells were washed with PBS-gelatin 0.1%. Peroxidase-labeled goat antimouse immunoglobulin antibody was added. After 60 minutes of incubation at 37° C, the plate was washed three times in PBS-gelatin 0.1% and once in distilled water. Finally, O-phenylene-diamine dihydrochloride (0.4 mg/ml) (Sigma Chemical Co., St. Louis, Mo.) was added as substrate for the enzyme. Bound specific antibody was measured quantitatively by optical density reading at 492 nm with a spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.). The amount of bFGF in the conditioned media was determined by use of a reference curve obtained with known quantities of human recombinant bFGF. We used mouse anti-bFGF monoclonal antibody as positive control and antibody without specificity as negative control. Similar experiments were used to determine the presence and amount of PDGF AA and PDGF BB in the conditioned media, with rabbit polyclonal antibody to PDGF AA and rabbit polyclonal antibody to PDGF BB, respectively.

Statistical analysis. Statistical analysis was performed with Statview software (Abacus Concepts, Inc., Berkeley, Calif.) on a Macintosh Powerbook microcomputer (Apple Computer, Inc., Cupertino, Calif.). Data were expressed as mean ± SD. Differences between the groups were analyzed by use of the two-tailed Student t test and analysis of variance.

RESULTS

All grafts were patent at the time of death.

Histology. At harvest, PTFE grafts (n = 3 for each group) showed evidence of a minor degree of MH at the level of the anastomosis. Endothelial cells recognizable for their cobblestone appearance extended from the adjacent artery across the anastomosis and into the graft for only a few millimeters (Fig. 1). The midgraft region was covered by thrombus and fibrin. Few platelets and leukocytes were present in the midportion of the PTFE grafts (a mean of four for each field examined). The tissue surrounding the PTFE graft penetrated the interstices of the graft. Rare tufts of ingrowth tissue arrived to the luminal surface. Vein grafts were completely covered by endothelial cells as recognized by their cobblestone appearance. Few scattered platelets and leukocytes were present in the lumen (a mean of four for each field examined).

Mitogenic activity of the conditioned media. Addition of serum-free conditioned media from PTFE grafts collected at 72 hours (n = 12 for each group) produced a statistically significant increase of trititated thymidine uptake of Swiss 3T3 cell cultures compared with conditioned media from vein grafts. Fig. 2 shows the mitogenic activity of conditioned media from PTFE grafts, vein grafts, and thoracic aorta. These differences were statistically significant (p < 0.01), and the higher quantity of mitogens released could stimulate the growth of smooth muscle cells and lead to formation of MH.
Reduction of mitogenic activity by anti-bFGF and anti-PDGF antibody. Reduction of mitogenic activity by anti-bFGF and anti-PDGF antibodies (n = 12 for each group) means that the mitogenic activity is due to that particular growth factor. Addition of monospecific anti-bFGF antibody to the medium of 3T3 cell cultures exposed to conditioned medium from PTFE grafts decreased the uptake of tritiated thymidine by 65% (p < 0.001) (Fig. 3). Addition of monospecific anti-PDGF antibody to the medium of 3T3 cell cultures exposed to conditioned medium from PTFE grafts decreased the uptake of tritiated thymidine by 20% (p < 0.05) (Fig. 4).  

PDGF assay by ELISA. Tables I and II show the release of PDGF AA and PDGF BB from control veins, arterial vein grafts, aortic segments, and PTFE grafts (n = 12 for each group). The release of PDGF BB was similar in arterial vein grafts and PTFE grafts. Release of PDGF AA was statistically higher in PTFE grafts (p < 0.05).  

bFGF assay by ELISA. Table III shows the release of bFGF by control veins, arterial vein grafts, aortic segments, and PTFE grafts (n = 12 for each group). The release of bFGF was statistically higher in arterial vein grafts and PTFE grafts than in aortic segments (p < 0.01). PTFE grafts released a significantly higher amount of bFGF than did arterial vein grafts (p < 0.05).  

DISCUSSION  
Anastomotic MH is more common in cases of synthetic grafts and at the level of the distal anastomosis. Many theories have been proposed regarding the biogenesis and cause of this pathologic entity. Local anastomotic factors probably play a major role. Bassiouny et al. found anastomotic MH
Fig. 2. Addition of serum-free conditioned media from PTFE grafts collected at 72 hours produced significant increase of tritiated thymidine uptake of Swiss 3T3 cell cultures compared with conditioned media from vein grafts and conditioned media from thoracic aorta. All these differences were statistically significant (n = 12 for each group) (DMEM: negative control; human recombinant PDGF and bFGF: positive controls).

in two zones: suture line, which represents a consequence of vascular healing, and arterial floor, which develops in a region of flow oscillation and relatively low shear. In either situation the response was associated with altered flow conditions. Abbott et al. attributed compliance mismatch between the graft and the host artery as being the cause of anastomotic MH. Ojha et al. demonstrated greater flow disturbances at this level. They found low wall shear stress at the level of the toe and heel of the anastomosis and high shear stress on the bed. How these different factors may lead to MH is ill defined.

Several studies have shown that abnormal hemodynamic conditions may lead to an increased release of bFGF and PDGF by endothelial and smooth muscle cells. Many other studies have shown a correlation between PDGF and bFGF and MH. Jawien et al. found that PDGF infused into rats subjected to balloon carotid artery injury produced a twofold to threefold increase in medial smooth muscle cell proliferation, and a twentyfold increase in migration of smooth muscle cells from the media to the intima during the first 7 days. In a similar experimental model, administration of antibodies to PDGF resulted in a 40% reduction in area of MH. A similar role has been attributed to bFGF. Lindner and Reidy have shown that proliferation of smooth muscle cells was reduced significantly in animals that
received anti-bFGF antibodies before balloon angioplasty. Thus many studies support a role for bFGF and PDGF in the genesis of MH. It is possible that the graft itself generates a substance that could contribute to distal anastomotic MH. In our study we found that PTFE grafts release growth factors. The release of PDGF AA and especially bFGF was much higher than in arterial vein grafts. Zacharias et al.28 reported that perfusates from PTFE grafts implanted in baboons contained more mitogenic activity than perfusates from arteries. Experimental studies from the same laboratory29 showed that the intima of PTFE grafts implanted in baboons expressed higher levels of PDGF A mRNA than the native aorta. Kaufman et al.30 analyzed the production of PDGF by PTFE grafts seeded with autologous endothelial cells and implanted in dogs. PDGF production increased with time and was greater in the distal portion of the graft. Interestingly, PDGF production increased with time also in the control, unseeded PTFE graft group. Again, PDGF production was greater at the level of the distal anastomosis where intimal thickness was more evident. In our study we confirmed the increased release of PDGF. However, the most striking difference between PTFE and vein grafts was the amount of bFGF released by synthetic grafts. The source of bFGF and PDGF is speculative.

Platelets, leukocytes, endothelial cells, smooth muscle cells, and macrophages are able to release PDGF and bFGF. Platelet survival is shortened after

### Table I. Release of PDGF BB

<table>
<thead>
<tr>
<th>Graft</th>
<th>PDGF BB (ng/cm²/72 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vein</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Arterial vein</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>PTFE</td>
<td>66 ± 7</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>25 ± 4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 12 separate experiments.

### Table II. Release of PDGF AA

<table>
<thead>
<tr>
<th>Graft</th>
<th>PDGF AA (ng/cm²/72 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vein</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Arterial vein</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>PTFE</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>7 ± 4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 12 separate experiments.

### Table III. Release of bFGF

<table>
<thead>
<tr>
<th>Graft</th>
<th>bFGF (ng/cm²/72 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vein</td>
<td>55 ± 6</td>
</tr>
<tr>
<td>Arterial vein</td>
<td>295 ± 20</td>
</tr>
<tr>
<td>PTFE</td>
<td>404 ± 22</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>46 ± 8</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 12 separate experiments.
graft implantation and platelet deposition on prosthetic grafts is well documented. 31

Endothelial cells migrate from the adjacent artery onto the graft. This is a slow process limited to a few millimeters from the anastomosis. In vitro studies suggest that endothelial cells in culture are activated and produce a higher quantity of PDGF. 32 Similarly, endothelial cells migrating onto a synthetic graft may activate and release a greater quantity of growth factors. Smooth muscle cells proliferate beneath endothelial cells and, similarly, could be the source of growth factors. Ingrowth of capillaries through graft interstices is a well-known phenomenon in high-porosity (60 to 90 μm) PTFE, especially in baboons. 33, 34 We found ingrowth of capillaries through the graft, even if very few reached the lumen. This ingrowth of tissue might contribute to growth factor release. Leukocyte and macrophage infiltration characterizes the chronic inflammatory response to vascular prostheses.

Organ culture was used in our study. This technique has many advantages, including maintenance of the normal tissue structure in anatomic and functional terms. The shortcomings of the technique are that it does not allow identification of the cells responsible for the production of growth factors and does not exclude the contribution from cells residing outside the graft.

In our study we have found that PTFE grafts release a greater quantity of growth factors than do arterial vein grafts. This phenomenon could contribute to the development of distal anastomotic MH and the progression of atherosclerosis in the distal arterial tree that is often seen when synthetic grafts are implanted.

Agents able to reduce the release of these growth factors may contribute to the prevention of anastomotic MH. 35

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