Growth Factors Stimulate Neointimal Cells In Vitro and Increase the Thickness of the Neointima Formed at the Neck of Porcine Aneurysms Treated by Embolization

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**Background and Purpose**—Growth factors (GFs) may favor the healing of aneurysms treated with endovascular techniques by stimulating neointima formation.

**Methods**—Bilateral carotid aneurysms were constructed with venous pouches in 50 pigs and embolized intraoperatively with collagen sponges with and without GFs (platelet-derived growth factor-BB [PDGF-BB] 0.15 or 1.5 μg or transforming growth factor-β₁ [TGF-β₁] 60 or 600 ng) in each animal. DNA synthesis, cell proliferation, and collagen secretion assays were performed to assess the in vitro effects of GFs on neointimal cells harvested from the treated aneurysms. ¹²⁵I-PDGF-BB was used to study in vivo GF release from sponges. The thickness of the neointima at the surface of the sponges was measured 2 weeks after surgery. Since porcine aneurysms tend to heal after collagen sponge embolization, this experiment was repeated in dogs, which have shown a propensity for recurrence with the same technique, with 600 ng TGF-β₁ or platelet extracts.

**Results**—PDGF-BB stimulated DNA synthesis and cell proliferation, while TGF-β₁ strongly increased collagen synthesis of neointimal cells in vitro. Clearance of ¹²⁵I-PDGF-BB from the sponges followed a biphasic curve, with 1.5% of exogenous PDGF-BB remaining at 1 week. The local delivery of PDGF-BB (0.15 or 1.5 μg) and TGF-β₁ (600 ng) significantly increased neointimal thickness at the neck of porcine aneurysms, while 60 ng of TGF-β₁ had no demonstrable effect. TGF-β₁ (600 ng) or platelet extracts had no influence on canine aneurysms.

**Conclusions**—PDGF-BB and TGF-β₁ can stimulate neointimal cells in vitro and neointima formation in vivo, but TGF-β₁ and platelet extracts do not compensate for deficient thrombosis in canine aneurysms. Effects on the long-term results of embolization remain speculative. (Stroke. 2000;31:498-507.)

*Key Words:* cerebral aneurysm • growth factors • pathology

Endovascular treatment of cerebral aneurysms is effective in preventing rebleeding after subarachnoid hemorrhage.¹ ² However, recurrences are observed frequently, which limits more widespread application of this less invasive treatment.³ To improve the long-term results of endovascular treatment, aneurysmal healing can be stimulated by promoting cell migration and proliferation within the thrombus that forms after embolization.³ We have demonstrated previously that experimental aneurysms heal with the formation of a thick neointima at the neck of treated lesions.³,⁴ Conversely, aneurysms that recur harbor a thin neointima.⁴,⁵

A variety of growth factors (GFs), including platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-β₁ (TGF-β₁), have been implicated in neointima formation and vascular remodeling phenomena.⁶–¹⁸ We have shown that the neointima that develops at the neck of treated aneurysms can be significantly thickened 2 weeks after embolization by the local delivery of a porcine platelet extract (PE) rich in PGDF-BB and TGF-β₁.⁴ The goals of the present work were to better define which single GF could reach this stimulation as well as to explore in vitro the mechanisms involved. We studied the in vitro effects of PDGF-BB and TGF-β₁ on proliferation, thymidine incorporation, and collagen secretion by cells explanted from the neointima that formed at the surface of the embolic agent. Clearance of GFs delivered with collagen sponges used for embolization was also determined in vivo with ¹²⁵I-labeled PDGF-BB. The local delivery of 2 doses of PDGF-BB and TGF-β₁ was tested in vivo with the collagen sponge model in porcine lateral wall venous pouch aneurysms. We demonstrate that neointimal cells respond to GF stimulation by enhanced DNA synthesis and proliferation with PDGF-BB as well as collagen secretion with TGF-β₁. Both PDGF-BB and...
TGF-β₁ can increase the thickness of the neointima formed at the neck of treated porcine aneurysms 2 weeks after embolization.

We have previously shown with this model that the neointima formed at the neck of canine aneurysms is very thin and that lesions recur consistently.₅ We finally assessed whether PE or TGF-β₁, both capable of thickening the neointima in porcine aneurysms,⁴ can improve healing of canine aneurysms treated by sponge embolization.

**Materials and Methods**

**Experimental Aneurysms**

All animal experiments were performed in accordance with guidelines of the Canadian Council on Animal Care, and protocols were approved by the local animal care committee. Seven-week-old pigs (Yorkshire Swine, Primipor, St-Gabriel-de-Brandon, Quebec, Canada) weighing 25 to 30 kg (n=50) and adult mongrel dogs (Laka, St-Basile, Quebec, Canada) weighing 20 to 25 kg (n=7) were used. All procedures were conducted under general anesthesia. The animals were sedated with atropine (0.04 mg/kg IM), azaperone (4 mg/kg IM), and ketamine (25 mg/kg IM), followed by thiopental (20 mg/kg IV). After endotracheal intubation, animals were ventilated with a Harvard respirator and kept under anesthesia with 1.5% to 2% buprenorphine (0.01 mg/kg IM). Bilateral common carotid lateral wall aneurysms were constructed according to the technique of German and Black,¹⁰ as modified previously. Brieﬂy, 2 segments from the same external jugular vein were harvested. After temporary occlusion of the common carotid artery, an oval 5-mm arteriectomy was created in the arterial wall, to which the open venous pouch was sutured with 8-0 polypropylene. One 8×8-mm absorbable gelatin sponge (Gelfoam, Upjohn Canada) fragment with or without GFs or PE was inserted inside the aneurysm to completely occlude it. Before the experiments, the absorbable gelatin sponge fragments were rehydrated in PBS for 5 minutes at 100°C, and then PDGF-BB (recombinant human PDGF-BB, 0.15 or 1.5 µg in 200 µL PBS), TGF-β₁ (recombinant human TGF-β₁, R and D Systems, 60 or 600 ng in 200 µL PBS), PE (Theratechnology Inc, 2×10³ platelet equivalent per milliliter), or an equal volume of PBS was added to an equal volume of PBS. TGF-β₁ and PDGF-BB were shown to contain <0.1 ng/µg endotoxin. The amounts of TGF-β₁, PDGF-BB, and other proteins measured in the PE have been previously reported.⁴ Treated and control sponges were placed randomly, and the surgeon was blinded to their contents. The extent of aneurysmal obliteration was adjusted by decreasing aneurysm size with additional sutures at the fundus to completely occlude the aneurysm. Immediate postoperative angiography, with the use of 5F catheters introduced by femoral puncture, was performed while the animals were still under general anesthesia, to study the symmetry of the 2 aneurysms and to detect neck remnants, in the first 20 pigs and in all dogs. Healing of aneurysms was assessed in vivo by angiography before the animals were killed, and results were scored according to a classification described previously.₄ A score of 0 was attributed to complete obliteration, 1 to “dog ears,” 2 to recurrent necks, and 3 to recurrent aneurysms.

**Pathological Studies**

Histopathological and morphometric studies were performed at 1 week in 4 pigs treated on 1 side with 1.5 µg PDGF-BB and at 2 weeks in all other pigs (n=28). Dogs were studied at 3 weeks (n=7). Carotid arteries and aneurysms were resected en bloc in fresh Dulbecco’s minimal essential medium (DMEM) (Wisent), and the carotid arteries were opened longitudinally opposite the aneurysms to examine the luminal surface of the neointima covering the neck. A 2-mm axial section of the aneurysms, taken in the middle of the neck, was sliced from the specimen and used for neointimal measurement after formalin fixation and paraffin embedding. The remainder of the aneurysms was used for cell culture, as described below. Five-micrometer axial sections were stained with hematoxylin-phloxin-safran and Movat’s pentachrome, and the neointimal layer at the neck of each aneurysm was measured (without knowledge of the nature of the sponge) in 5 locations, as previously developed and standardized in our laboratory.³⁻⁵ Direct measurements were taken with a computerized image analysis system (Vision 2.0, Clemex Technologies). Immunohistochemical methods were used to characterize neointimal cells and cells inside the sponges.³⁻⁵

**In Vivo Clearance of ¹²⁵I-PDGF-BB**

In 18 pigs, experimental aneurysms were embolized with collagen sponges containing a mixture of radioactive PDGF-BB (¹²⁵I-PDGF-BB 200 ng; Amersham-Pharmacia) and nonradioactive PDGF-BB (800 ng). After 10 minutes to 1 week, the collagen sponges were removed and homogenized with PBS, and the remaining radioactivity was determined by liquid scintillation counting for comparison with control sponges kept in vitro for identical time periods.

**Cell Cultures, DNA Synthesis, Proliferation, and Collagen Synthesis**

Porcine carotid arteries and aneurysms were harvested under sterile conditions at autopsy and maintained in cold DMEM. Twenty-four explants (1×1 mm) were prepared from the neointima at the luminal surface of the sponge (neointimal cells) and from the carotid media, 2 cm distal to the aneurysmal neck (carotid smooth muscle cells), according to a method modified from Ross.³ Cells between passages 1 and 3 were used for in vitro experiments.

DNA synthesis was measured by ³H-thymidine (Amersham-Pharmacia) incorporation assays exactly as described elsewhere.²⁹ Cell proliferation was measured by WST-1 assay (Roche Diagnostics) according to the manufacturer’s instructions. Brieﬂy, cells were seeded in 96-well plates at a density of 5×10³ cells per well in DMEM (100 µL) supplemented with 10% fetal bovine serum (FBS). After 24 hours, the medium was removed, and the cells were treated with appropriate concentrations or combination of GFs or FBS for 48 hours. At the end of the incubation period, 10 µL of WST-1 was added for 2 hours. During this time, viable cells converted WST-1 in a water-soluble formazan dye. Absorbance was then determined at 450 nm with an automated optical density reader.

Collagen synthesis was measured according to the method of Koyano et al.²¹ Cellular outgrowths were seeded in 24-well dishes at a density of 5×10³ cells per well in DMEM supplemented with 10% FBS, followed by 24 hours in DMEM containing 0.5% FBS. After 24 hours, the cells were treated with GFs or FBS for 48 hours and labeled with 10 µCi/mL ³H-proline (Amersham-Pharmacia) supplemented with 50 µg/mL ascorbic acid for the last 24 hours. The labeling medium was removed, and the cell layer was extracted with 500 µL acetic acid (0.5 mol/L) for 24 hours at 4°C on a rocking platform, after brief sonication with a Branson Sonifier (Branson Ultrasonics). The day after, each membrane of a 96-well multiscreen filtration plate (0.65-µm pore size, Millipore) was soaked with 50 µL of 25% trichloroacetic acid (TCA), then 50 µL of cell extract and 50 µL of 50% TCA were added to each well. The plates were incubated for 1 hour at 4°C. The precipitate formed was collected on the filter membranes with a vacuum source, and the filters were washed 3 times with 100 µL of 10% TCA. After drying, the membranes were put into scintillation vials, and the filter-bound radioactivity was quantified by liquid scintillation counting.

**Statistical Analysis**

All data are expressed as mean±SD. The in vitro effects of GFs on neointimal and carotid smooth muscle cells were compared by 1-way ANOVA followed by Student’s t tests. A value of P<0.05 was considered significant.

Two-way repeated-measures ANOVA with sides (GF-treated or control) as a repeated factor and 4 groups (PDGF-BB 0.15 or 1.5 µg and TGF-β₁ 60 or 600 ng) as the other factor was used to compare.
neointimal thickness at the neck of porcine aneurysms. In the presence of a side-by-group interaction, multiple comparisons were made by the least significant difference procedure. A linear regression model fitted the 125 I-PDGF-BB clearance from sponges. We included dummy variables to adjust separate regressions for the 10-minute to 24-hour period and the 1- to 7-day period and compared the 2 slopes. To stabilize the variance, logarithmic transformation was first applied to the percentage of radioactivity remaining within the aneurysms.

Results

In Vitro Studies
Spindle cells were consistently recovered from carotid and aneurysmal explants, and proliferating cells grew according to a “hill and valley” pattern. Virtually all cells were α-actin positive after the first passage.

GFs and DNA Synthesis
DNA synthesis of neointimal and carotid smooth muscle cells was increased significantly ($P<0.005$) by 20 ng/mL of PDGF-BB (Figure 1). Sixty nanograms per milliliter of TGF-β1 decreased thymidine incorporation in neointimal and carotid smooth muscle cells ($P<0.05$), while no significant change was observed with 6 ng/mL of TGF-β1. Moreover, 60 ng/mL of TGF-β1 significantly decreased PDGF-BB–induced DNA synthesis ($P=0.05$). Finally, there was no difference in the basal or FBS-stimulated incorporation of thymidine between neointimal and carotid smooth muscle cells (data not shown).

GFs and Cell Proliferation
Proliferation of neointimal and carotid smooth muscle cells was increased significantly with 20 ng/mL of PDGF-BB compared with basal values (Figure 2). The lower dose of PDGF (2 ng/mL) had no significant effect on cell proliferation. Both doses of TGF-β1 had no significant impact on cell proliferation, as shown in Figure 2. However, 60 ng/mL of TGF-β1 significantly decreased ($P=0.01$) PDGF-BB–induced cell proliferation of both cell types. Finally, no differences were observed in the basal and FBS-stimulated proliferation of both neointimal and carotid smooth muscle cells (data not shown).

GFs and Collagen Synthesis
A 48-hour incubation with either PDGF-BB or TGF-β1 significantly increased collagen synthesis by neointimal and carotid smooth muscle cells (Figure 3). TGF-β1 was more potent than PDGF-BB. No synergistic effect was observed...
between PDGF-BB and TGF-β₁. Both cell types responded well to GFs, but basal collagen synthesis by carotid cells was twice that of neointimal aneurysmal cells (data not shown).

In Vivo Studies

Angiographic Results

Porcine Aneurysms

Angiographic results initially or at 2 weeks showed no significant difference between PDGF-BB- or TGF-β₁-treated and control aneurysms. The mean angiographic scores immediately after embolization were 0.25 ± 0.44 and 0.06 ± 0.25 for PDGF-BB- or TGF-β₁-treated and control aneurysms, while at 2 weeks they were 0.0 ± 0.6 and 0.36 ± 0.8, respectively. Because >80% of animals had symmetrical results and >80% of aneurysms (GF-treated or control) were completely obliterated immediately after surgery and remained obliterated at 2 weeks, we stopped performing angiography after the first 20 animals.

Canine Aneurysms

Angiographic results in canine aneurysms are presented in Table 1. The mean angiographic scores immediately after surgery were 1.8 ± 0.8 and 0.7 ± 0.6 for TGF-β₁– or PE-treated and control aneurysms, while at 2 weeks they were 2.75 ± 0.8 and 2.5 ± 0.7, respectively. The only significant difference (P=0.003) was between the mean immediate score (1.29 ± 1.3) and mean score at 3 weeks (2.6 ± 0.9) of all aneurysms, confirming the tendency of recurrence in canine aneurysms treated with sponges. TGF-β₁ or PE did not significantly improve the angiographic scores at 3 weeks.

In Vivo Clearance of 125I-PDGF-BB

125I-PDGF-BB clearance from collagen sponges in vivo followed a biphasic curve (Figure 4). After logarithmic transformation, the first rapid clearance phase (between 10 minutes and 24 hours) fitted the equation 2.86−1.79×(time), while the second slower phase corresponded to the equation 2.32−0.27×(time). The difference between the 2 slopes (−1.79 and −0.27) was statistically significant (P=0.0001).

GFs and Neointimal Thickness

Porcine Aneurysms

Table 2 presents the data on mean neointimal thickness at the surface of the sponge in control and GF-treated aneurysms for each group of animals. The neointimal layer at the neck of porcine aneurysms was thick (185 to 439 μm). There was no significant difference between the control aneurysms of all groups. In addition, no significant difference was noted between the control and GF-treated aneurysms in animals treated with 60 ng TGF-β₁. The neointima in aneurysms treated with 600 ng TGF-β₁ or 0.15 or 1.5 μg PDGF-BB was significantly thicker than the respective controls (P<0.005).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neointimal Thickness, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF 1.5 μg (n=6)</td>
<td>324.2±91.6*</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>184.7±107.1</td>
</tr>
<tr>
<td>PDGF 0.15 μg (n=6)</td>
<td>360.5±148.7*</td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>226.0±140.2</td>
</tr>
<tr>
<td>TGF-β 600 ng (n=6)</td>
<td>438.5±187.3*</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>294.7±180.9</td>
</tr>
<tr>
<td>TGF-β 60 ng (n=8)</td>
<td>251.1±193.7</td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>193.6±194.7</td>
</tr>
</tbody>
</table>

Values are mean±SD. n indicates number of aneurysms.

*GF-treated aneurysm has significantly thicker neointima than contralateral aneurysm (P<0.05).

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**TABLE 1. Angiographic Results and Neointimal Thickness in Canine Aneurysms**

<table>
<thead>
<tr>
<th>Canine Aneurysms</th>
<th>Immediate Angiographic Score</th>
<th>Follow-Up Angiographic Score</th>
<th>Neointimal Thickness, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE (n=4)</td>
<td>1.75±1.2</td>
<td>2.5±0.6</td>
<td>61.2±35.4</td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>1.0±1.2</td>
<td>2.0±1.4</td>
<td>36.5±27.7</td>
</tr>
<tr>
<td>TGF-β (n=3)</td>
<td>2.00±1.7</td>
<td>3.0±0.0</td>
<td>28.6±15.5</td>
</tr>
<tr>
<td>Control (n=3)</td>
<td>0.33±0.6</td>
<td>3.0±0.0</td>
<td>27.0±23.4</td>
</tr>
<tr>
<td>All canine aneurysms (n=14)</td>
<td>1.29±1.3</td>
<td>2.6±0.9*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD. n indicates number of aneurysms.

*Score at 3 weeks greater than immediate score (P=0.0031).
A significant difference ($P<0.05$) in neointimal thickness was observed between aneurysms treated with 0.15 mg PDGF-BB or 600 ng TGF-$\beta_1$ and those treated with 60 ng TGF-$\beta_1$.

**Canine Aneurysms**

The neointimal layer at the surface of sponges in canine aneurysms was thin or absent (mean thickness, 27 to 61 mm). There was no significant difference in neointimal thickness between control aneurysms, aneurysms treated with 600 ng TGF-$\beta_1$ (n=3), and aneurysms treated with PE (n=4) (Table 1).

**Pathological Studies**

**Porcine Aneurysms**

One week after surgery, control sponges were covered by a thick clot containing mainly fibrin, platelets, blood cells, or inflammatory cells and few $\alpha$-actin-positive spindle cells. Sponges treated with 1.5 $\mu$g of PDGF-BB were covered with a more advanced organizing thrombus, infiltrated with and covered by a layer of $\alpha$-actin-positive spindle cells (Figure 5).

At 2 weeks, the collagen sponges were invaded by inflammatory cells and $\alpha$-actin-positive cells, and a thick neointima was constantly found at the neck of porcine aneurysms. The neointima was composed of $\alpha$-actin-positive and -negative spindle cells and extracellular matrix, including collagen and macrophages, and was covered by a continuous layer of von Willebrand factor-positive cells. There was no significant qualitative difference between histological or immunohistochemical appearance of the neointima found at the surface of sponges treated or not treated with GFs (Figure 6).

**Canine Aneurysms**

Collagen sponges were almost intact in canine aneurysms, even at 3 weeks. The neointimal layer was very thin, was again composed of $\alpha$-actin-positive cells (1 to 3 layers) and collagen, and was covered with von Willebrand factor-positive cells (Figure 7). Histological findings were unchanged by the presence or absence of TGF-$\beta_1$ or PE.

**Discussion**

The experiments reported here are parts of a project aimed at the development of a new embolic agent that could locally deliver GFs, stimulate neointima formation, and might improve the long-term results of embolization. A number of issues are relevant to this project. The causes of recurrences after embolization are poorly understood and may be multiple. The relevance of animal models is uncertain, and mechanisms involved in aneurysmal healing after embolization remain speculative. Few human pathological studies have been performed on aneurysms treated by endovascular techniques. Deficient thrombosis and poor neointima formation, as seen in our canine model, have been documented in many cases. On the other hand, thrombosis, fibrosis, and neointima formation, as routinely found in porcine aneurysms, are also encountered in human aneurysms. Questions addressed in the present work include the follow-
Are neointimal cells present at the neck of embolized aneurysms responsive to GF stimulation? Which platelet factor is effective in increasing neointima thickness in porcine aneurysms? How effective are collagen sponges as slow-release vehicles for GFs? Finally, is this strategy effective in animals that heal poorly after embolization?

Animal Models and Response to GFs

Venous pouch carotid aneurysm models have been widely used in experimental studies on healing mechanisms after embolization of aneurysms. We have used collagen sponges (Gelfoam) as embolic agents, and we have previously shown that healing mechanisms are basically similar to those found in aneurysms embolized with platinum coils. Although these 2 embolic agents are certainly different in nature, major species-related differences are witnessed in animal models, irrespective of the embolic agent used. Gelfoam has been successfully used as a slow-release vehicle for PE that resulted in thicker neointima at 2 weeks in treated porcine aneurysms. The main drawback of the porcine model is a strong tendency to heal after embolization, which does not permit assessment of the effects of GFs on the incidence of angiographic recurrences. This characteristic seems to be linked to exuberant thrombosis and thick neointimal formation in this animal, 2 phenomena also found after angioplasty. In the present study we chose to aim at the complete obliteration of aneurysms to minimize asymmetries in residual blood flow between aneurysms. Angiography performed in the first 20 animals served to demonstrate that the surgical technique led to consistent results and that differences in neointimal thickness found at 2 weeks were not caused by asymmetries in blood flow. Angiographic cure of porcine aneurysms at 2 weeks was virtually constant in GF-treated or control aneurysms. In contrast, recurrences can reliably be seen after collagen sponge embolization of aneurysms in dogs, and this was confirmed in our study.

Deficient healing of canine aneurysms is associated with thin or absent neointima formation, probably related to deficient thrombus and consequently the lack of a provisional matrix, which permits physical support to cell migration and colonization. This deficiency could not be compensated with TGF-β1 or PE, which were not able to increase neointimal thickness or improve the morphological results at follow-up, at least with the doses and techniques used in the presentstudy.

Figure 6. GFs and neointimal thickness in porcine aneurysms. Histological and immunohistochemical findings 2 weeks after surgery in a pig with bilateral carotid aneurysm, treated on 1 side with control sponge (a-d) and on the other side with sponge containing 1.5 μg PDGF-BB (e-h). The GF-treated sponge is covered by a thicker neointima (e-h) than the control sponge, but neointimas are qualitatively similar, being composed of α-actin-positive spindle cells (c, g) and collagen (b, f) and covered with a layer of von Willebrand-positive cells (d, h). Immunohistochemical staining was performed with anti-α-actin (c, g, i) and anti-von Willebrand factor (d, h, j) antibodies; a normal artery is shown for comparison (i, j). Slides were also stained with hematoxylin-phloxin-safran (a, e) and Movat’s pentachrome (b, f) (magnification ×150).
study. However, fibrinogen and vascular smooth muscle cell grafts have been successful in increasing neointima formation in the same canine model. Thus, thrombus formation and deposition of a sufficient provisional matrix at the surface of the embolic agent may be sine qua non prerequisites to cellular colonization, and only then can GF stimulation of neointima formation be demonstrated. Alternatively, some thrombus-related signal may be essential for GF stimulation of neointima formation.

Neointimal Cells and GFs
We have previously reported that cells involved in aneurysmal healing after embolization can be cultured in vitro with the collagen sponge model. Their in vitro response to PDGF-BB and TGF-β1 is reported here for the first time. The nature and origin of cells involved in neointima formation after vascular injury or in atherosclerosis remain a matter of debate. Although circulating stem cells, monocytes/macrophages, and adventitial myofibroblasts have been evoked as potential actors in neointima formation, migration and proliferation of vascular smooth muscle cells from the adjacent media is a commonly accepted mechanism. These cells are thought to undergo phenotypic modulation to switch from a quiescent contractile stage to a secretory phenotype, specialized in proliferation and extracellular matrix secretion. An alternative hypothesis is that the arterial media is composed of multiple cell types and a specific population of cells is responsible for neointima formation. Cells recovered at the surface of the embolic agent in this model are α-actin–positive smooth muscle–like cells that present in vitro characteristics identical to carotid medial smooth muscle cells. Neointimal cells recovered from vascular injury models have been reported as cells that proliferate faster and secrete more extracellular matrix proteins than medial vascular smooth muscle cells, characteristics that persist for many passages in vitro. Aneurysmal neointimal cells manifested no significant increase in DNA synthesis or cell proliferation compared with carotid smooth muscle cells and presented decreased basal collagen synthesis in the present study. Reports have suggested that neointimal cells recovered after balloon injury have a blunted response to GFs compared with normal vascular smooth muscle cells. In the present experiments, aneurysmal neointimal cells and carotid smooth muscle cells responded in a fashion similar to exogenous GFs added to the culture media. Thus, the present study supports the theory that neointimal cells are related to smooth muscle cells migrating from the arterial media. An alternative hypothesis is that in vitro cultures by explantation techniques select a subpopulation of medial cells that, if proper conditions arise, are also involved in neointima formation in vivo. This second hypothesis may be supported by the observation of a thicker neointima at the surface of sponges seeded with vascular smooth muscle cells harvested from explants prepared from the femoral artery.

The mitogenic effect of PDGF-BB is well known. Conversely, the in vitro effects of TGF-β1 are remarkably diverse and sometimes paradoxical. Depending on cell type, density, and culture conditions, TGF-β1 may stimulate or inhibit proliferation. In this study as well as other studies, TGF-β1 slightly inhibited the growth of both neointimal and carotid smooth muscle cells. TGF-β1 also inhibited the proliferative response to PDGF-BB, a phenomenon also observed with medial or neointimal cells in other models. Both factors could increase collagen secretion by both cell types, but TGF-β1 was more potent, a phenomenon documented previously.

The present in vitro studies were performed to better understand the potential mechanisms of GF stimulation of neointima formation. We demonstrated that cells involved in neointima formation at the neck of aneurysms treated by embolization and cultured in vitro respond to PDGF-BB by enhanced DNA synthesis and to TGF-β1 and PDGF-BB by increased collagen synthesis. These 2 phenomena could potentially stimulate the formation of a thicker or “stronger” neointima, which is hoped could decrease the risks of recurrence after endovascular treatment.

GFs and Neointima Formation
PDGF-BB and TGF-β1 have been shown to stimulate neointima formation after balloon injury. The in vitro response...
of neointimal cells to PDGF-BB and TGF-β1 are distinct, and yet in vivo effects on neointima formation cannot be distinguished. The in vivo actions of GFs are complex and likely part of a cascade phenomenon. In vitro effects on cells in artificial culture conditions represent, by necessity, a simplistic approach to the mechanisms involved. Other hypotheses, which were not studied here, can also be considered, such as the chemotactic actions of GFs on inflammatory cells and vascular smooth muscle cells, effects on cell differentiation, and thrombogenic properties of TGF-β1 in vivo, or even induction of tissue factor expression. We have shown in earlier studies that neointima formation at the neck of porcine aneurysms could be significantly increased at 2 weeks by the local delivery of PDGF-BB and PDGF-BB. The present study demonstrates that this response can be duplicated by the local delivery of 2 different doses of PDGF-BB and by a high dose of TGF-β1. The in vivo response to GFs witnessed in our model seems to be nonspecific, and one hypothesis is that exogenous GFs encourage recruitment of inflammatory cells, which are themselves a more persistent source of GFs and cytokines important to healing phenomena, as proposed in other experimental studies.  

**Local GF Delivery**

If the thicker neointima observed in our model is a nonspecific response, perhaps mediated through recruitment of inflammatory cells, a large quantity of GFs, released within 24 hours, may be an effective strategy. The study of the in vivo release of 125I-PDGF-BB may support this hypothesis since added GFs are rapidly liberated from sponges at a stage when few neointimal cells are detected histologically (Figure 5). GF release from sponges showed at least 2 distinct phases. The initial loss of GFs may be caused by in vitro preparation and surgical manipulations. The first rapid clearance phase may be explained by rapid washout with circulating blood flow at the level of the sponge. The second slower phase could be related to protection from dilution by clot formation at the surface of the sponge and to nonspecific binding of PDGF-BB to collagen or other matrix molecules. Thus, an alternative theory to account for a thicker neointima at 2 weeks is that a minimal fraction of GFs still bound in situ to cells and vascular smooth muscle cells, effects on cell differentiation, and thrombogenic properties of TGF-β1 in vivo, or even induction of tissue factor expression. We have shown in earlier studies that neointima formation at the neck of porcine aneurysms could be significantly increased at 2 weeks by the local delivery of PDGF-BB and PDGF-BB. The present study demonstrates that this response can be duplicated by the local delivery of 2 different doses of PDGF-BB and by a high dose of TGF-β1. The in vivo response to GFs witnessed in our model seems to be nonspecific, and one hypothesis is that exogenous GFs encourage recruitment of inflammatory cells, which are themselves a more persistent source of GFs and cytokines important to healing phenomena, as proposed in other experimental studies.  

**Conclusion**

Neointimal cells harvested from the neck of embolized porcine aneurysms respond to PDGF-BB by enhanced proliferation and to TGF-β1 and PDGF-BB by increased collagen secretion. In vivo, both factors can significantly augment the thickness of the neointima formed at the neck of embolized porcine aneurysms. The strategy was not effective in correcting deficient healing observed in canine aneurysms.

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**References**

The authors present a myriad of experiments, in pigs and in dogs, in vivo and in vitro, sometimes with a single growth factor, other times with platelet extract rich in multiple growth factors. However, all of these experiments are interconnected in a logical way, and I applaud the authors for providing us with one comprehensive paper rather than two or three separate ones.

The authors have used gelfoam sponges for the embolization of the experimental aneurysms. Although clinically the sponges are clearly inferior to GDCs, for the purpose of this paper this could have been an advantage if the sponges with growth factors had led to a better healing of the aneurysms in dogs than without the growth factors (quod non).

Interesting ideas and results are presented here, but obviously a lot of work still needs to be done before these or similar techniques can be applied to humans, especially in view of the large differences between dogs and pigs (I suspect that humans are closer to pigs, at least in the respects at stake here).

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Growth Factors Stimulate Neointimal Cells In Vitro and Increase the Thickness of the Neointima Formed at the Neck of Porcine Aneurysms Treated by Embolization
Anne-Cécile Desfaits and Jean Raymond

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