In vivo chemotactic properties and spatial expression of PDGF in developing mesenteric microvascular networks

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Zeller, Peter J., Thomas C. Skalak, Ana M. Ponce, and Richard J. Price. In vivo chemotactic properties and spatial expression of PDGF in developing mesenteric microvascular networks. Am J Physiol Heart Circ Physiol 280: H2116–H2125, 2001.—The recruitment of perivascular cells to developing microvessels is a key component of microvessel assembly. Whereas platelet-derived growth factor (PDGF) signaling is critical for this process during embryonic development, its role from the postnatal stages through adulthood remains unclear. We investigated the potential role of PDGF signaling during microvessel assembly by measuring in vivo the migration of labeled fibroblasts to PDGF in mesenteric connective tissue and by examining PDGF-B and PDGF receptor-β (PGDFR-β) expression in microvascular networks during normal maturation. PDGF-B homodimer (PDGF-BB; 30 ng/ml) application elicited a significant (P < 0.05) increase (7.8 ± 4.1 cells) in labeled fibroblasts within 100 μm of the source micropipette after 2 h. PDGF-A homodimer (30 ng/ml) application and control solution did not elicit directed migration. PDGF-B was expressed in microvessel endothelium and smooth muscle, whereas PDGFR-β was expressed in endothelium, smooth muscle, and interstitial fibroblasts. Given that PDGF-BB elicits fibroblast migration in the mesentery and that PDGF-B and PDGF-β are expressed in a pattern that indicates paracrine signaling from microvessels to the interstitium, the results are consistent with a role for PDGF-B in perivascular cell recruitment to microvessels.

The PDGF family of growth factors consists of homodimers (PDGF-AA and PDGF-BB) or heterodimers (PDGF-AB) that are synthesized and secreted by many cell types, including endothelial cells (7, 28). They are chemotactic for SM cells (16, 2, 10) and fibroblasts (21, 11, 12) in vitro, and coculture studies have shown that endothelial expression of PDGF-B elicits the directed migration of undifferentiated SM precursor cells (7). However, the appearance of normal large arteries such as the aorta has suggested that large artery vasculogenesis might be unaffected by PDGF disruption (24). Further evidence that PDGF mediates arteriolar formation was indicated by examining the net advantage conferred by expression of PDGF receptor-β (PDGF-β) in specific cell lineages through chimeric and analysis (3). Here it was found that cells lacking PDGFR-β composed only 15% of the SM cells in the aorta, suggesting that PDGFR-β is necessary for the SM cell investment associated with large vessel development. This chimeric study did not, however, differentiate between molecular mechanisms occurring during embryogenesis from those occurring during postnatal remodeling. A later study (4) using the same chimeric mouse model began to address this issue by demonstrating that endothelial and fibroblast participation in connective tissue formation is dependent on PDGF-β, thereby providing evidence that PDGF signaling is recapitulated in the adult during wound healing. The role of PDGF-B in normal postnatal development remains, however, unclear. Furthermore, despite the implications of PDGF as a perivascular cell recruitment factor, no direct experiments demonstrating in vivo chemotaxis of fibroblasts in response to PDGF have been performed.

We investigated the potential role of PDGF in recruiting perivascular cells to microvessels by studying the chemotactic properties and spatial expression patterns of PDGF in rat mesenteric microvascular networks that continue to grow during normal maturation (5, 18). To study the in vivo chemotactic properties of PDGF, we employed the novel technique of injecting platelet-derived growth factor; vascular remodeling; microcirculation; cell migration; arterialization

THE FORMATION OF PROPERLY PATTERNELED and functional microvascular networks is critically dependent on the recruitment of perivascular supporting cells to the abluminal surface of preexisting vessels. Microvessels destined to become arterioles and venules require subsequent phenotypic modulation of these precursor cells into smooth muscle (SM). These basic microvessel assembly processes are tightly regulated by the controlled spatial and temporal expression of selected growth factors and their receptors. Members of the platelet-derived growth factor (PDGF) family of growth factors and their receptors have been implicated in the formation of arterioles by stimulating the paracrine recruitment of supporting cells and SM cell progenitors in the in vivo environment.

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labeled fibroblasts into the mesentery to investigate the direct effect of a point source application of PDGF-AA and PDGF-BB on migration. In a previous study (23), labeled fibroblasts were observed to be recruited to abluminal positions on microvessels of various sizes. This approach has two important advantages over previous studies. The first is that by injecting fibroblasts into the animal several days before the experiment, undesirable effects due to the cell culture environment are reduced. The second is that our assays were performed in the mesentery, a true physiological matrix compared with synthetically generated extracellular matrix substrates or polycarbonate filters such as those employed in Boyden-type chambers. This second consideration becomes especially important in light of abundant recent evidence suggesting that the ability of various cell types to migrate in response to the selected growth factors may be affected by interactions between, and the expression of, cellular adhesion molecules, growth factor receptors, and the underlying extracellular matrix substrate (2, 10, 13, 15, 16, 20, 27). To study the spatial patterns of PDGF expression in vivo, we immunofluorescently labeled whole mount mesenteries from normally developing animals for PDGF-B and PDGFR-β and subsequently examined them with the use of confocal microscopy. Markers for endothelial and SM cells were used to establish cell-specific expression of PDGF-B and PDGFR-β in arterioles, capillaries, and venules. Our results from these two studies indicate that the chemotactic properties and spatial expression patterns of PDGF-B and its receptor are consistent with a role for PDGF-B in microvessel assembly.

MATERIALS AND METHODS

Cell harvest, culture, and PKH26-labeling methods. The following animal procedures were approved by the Animal Care and Use Committee of the University of Virginia. Animals were anesthetized with an intramuscular injection of ketamine (80 mg/kg body wt) and xylazine (8 mg/kg body wt), and a midline 3- to 4-cm incision was made with a sterile scalpel through the abdomen after shaving and sterilizing the skin with ethanol. Next, the small intestine was gently exposed, starting at the junction with the large intestine. The enteric fibroblasts (1.5 \times 10^6 cells) were injected intraperitoneally into 10- to 11-wk-old male Fischer 344 rats. After 7–14 days had passed, animals were anesthetized with an intramuscular injection of 1% α-chloralose and 13.3% urethane in saline at 0.6 ml/100 g body wt. The femoral vein was cannulated for supplemental anesthesia, and the mesentery was prepared for intravital microscopy. The mesentery was perfused (150 ml/h) with Ringer solution at 37°C, and the stage was mounted on a Nikon K2 SBIO microscope connected to a Dage model 104722–01 GenIsys image intensifier and a Dage model CCD-72 video camera. The camera output was connected to a Panasonic model AG-1980 videocassette recorder and a Sony PVM-137 monitor.

Mesenteric windows were scanned with the use of a \times 20 objective (0.50 numerical aperture, \times 729 overall magnification) and selected if they had a homogeneous distribution of fluorescently labeled fibroblasts. A micropipette with a tip diameter of 10–12 μm was embedded in the tissue at a 30° angle using a micromanipulator. The micropipette was connected to a Sage Instruments (Cambridge, MA) model 341B
infusion pump, and one of the following solutions was infused at a flow rate of 73 µl/h: 1) control solution (Ringer solution containing 0.6 ng/ml BSA and 18.2 ng/ml acetic acid); 2) PDGF-AA solution [control solution containing 30 ng/ml recombinant human PDGF-AA homodimer (Genzyme; Cambridge, MA)]; or 3) PDGF-BB solution [control solution containing 30 ng/ml recombinant human PDGF-BB homodimer (Genzyme)]. The concentration of PDGF was established by an in vitro migration assay using fibroblasts cultured from fibrotic lungs (25). The micropipette was centered in the field of view (FOV), the infusion was initiated, and the area was videotaped using the ×20 objective under fluorescent illumination and transillumination to provide reference images to locate the exact position of the pipette tip. Thereafter, images were videotaped every 15 min for a period of 3 h.

Fluorescent and transilluminated digitized images were acquired, circles with 50- and 100-µm radii were superimposed on the images centered on the micropipette tip, and cells within these two circles were counted at each time point. The total number of cells within the initial FOV was also recorded. Significance was assessed using repeated measures analysis of variance, and pairwise comparisons were performed using the Bonferroni correction to Student’s t-test, with P < 0.05.

To ensure that PKH26-labeled fibroblasts had become incorporated into the mesenteric tissue and were not attached to the upper or lower mesothelial layers, separate specimens were created using the same cell culture and injection methods as described above. Fourteen days after being injected with PKH26-labeled fibroblasts, rats were anesthetized with an intramuscular injection of ketamine (80 mg/kg body wt) and then euthanized by an intramuscular injection of sodium pentobarbital (8 mg/kg body wt) and xylazine (8 mg/kg body wt). Negative control slides for PDGF-B and PDGFR-β were generated by removing the primary antibodies from the initial incubation and replacing the primary antibodies with the same concentration of rabbit IgG.

### RESULTS

**PDGF-induced fibroblast migration in vivo.** We investigated the in vivo chemotactic potential of PDGF by measuring the migration of PKH26-labeled fibroblasts within 50 µm of the pipette tip. Data are means ± SD and indicate that platelet-derived growth factor (PDGF)-A homodimer (PDGF-AA) and control solution exhibited no significant change. The PDGF-B homodimer (PDGF-BB) values were significantly different than control values after 150 min. *Significantly different than control (P < 0.05).

![Graph](image_url)

**Table 1. Number of PKH26-labeled fibroblasts in selected regions at the start of infusion**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Radius of Pipette Tip</th>
<th>Entire FOV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µm</td>
<td>100 µm</td>
</tr>
<tr>
<td>Control</td>
<td>2.8 ± 1.6</td>
<td>10.8 ± 4.8</td>
</tr>
<tr>
<td>PDGF-AA</td>
<td>3.8 ± 2.6</td>
<td>10.4 ± 4.9</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>3.2 ± 1.7</td>
<td>11.0 ± 6.8</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5 preparations. FOV, field of view; PDGF-AA and PDGF-BB; platelet-derived growth factor homodimers.

Heparinized PBS (pH 7.4) was then infused through the catheter to remove blood from the microvessels. Mesenteric windows were dissected free, dried as whole mounts on a gelatin-coated slide, and fixed in either 100% MeOH at −20°C for 30 min (PDGF-ß staining) or 4% paraformaldehyde in PBS (pH 7.4) at 4°C for 3 h (PDGF-ß staining). After being washed in PBS, paraformaldehyde-fixed mesenteries were incubated in trypsin-EDTA at 37°C for 5 min. Methanol-fixed mesenteries did not require proteolytic pretreatment. Tissues were incubated overnight in rabbit polyclonal antibodies to PDGF-ß (sc-432, Santa Cruz Biotechnology) at a 1:500 concentration and PDGF-ß (sc-7878, Santa Cruz) at a 1:100 concentration in 2% BSA and 5% normal goat serum (NGS) in PBS. After being washed in PBS, secondary biotinylated goat anti-rabbit IgG antibodies (Jackson Immunoresearch) were applied for 1 h at room temperature at a concentration of 1:500 in 2% BSA and 5% NGS in PBS. CY3-conjugated streptavidin (Jackson Immunoresearch) was then applied at 1:1,000 concentration in 2% BSA for 1 h. Slides were washed and then incubated in FITC-conjugated mouse monoclonal antibody to SM-α-actin (Sigma) at a concentration of 1:100 in 2% BSA in PBS or mouse monoclonal antibody to the rat endothelial-specific marker OX-43 (Sero-tec) at a concentration of 1:100 in 2% BSA in PBS. Secondary antibodies for OX-43 labeling were CY2-conjugated goat anti-mouse IgG (Jackson Immunoresearch). OX-43 has been used previously to verify that Tie2 is an endothelial-specific marker in rats (26), and in separate experiments, we have shown that OX-43 colocalizes with Tie2 and flk1 in the endothelium of mesenteric microvessels (data not shown). Negative control slides for PDGF-B and PDGFR-β were generated by removing the primary antibodies from the initial incubation and replacing the primary antibodies with the same concentration of rabbit IgG. Coverslips were applied to the specimens, and the whole mount networks were observed with a Bio-Rad MicroRadiance confocal scanner attached to a Nikon TE-300 inverted microscope.

**PDGF-B and PDGFR-β staining.** PDGF-B and PDGFR-β expression was assayed in young adult rats (10–11 wk old) because, at this age, mesenteric microvessel density is ~50% of that in adult rats (16–20 wk), indicating that these networks are still developing (5). Animals were anesthetized with an intramuscular injection of ketamine (80 mg/kg body wt) and xylazine (8 mg/kg body wt) and then euthanized by an overdose of anesthesia. The mesentery was exposed, and the mesenteric vein was cannulated in a retrograde direction. Heparinized PBS (pH 7.4) was then infused through the catheter to remove blood from the microvessels. Mesenteric windows were dissected free, dried as whole mounts on a gelatin-coated slide, and fixed in either 100% MeOH at −20°C for 30 min (PDGF-ß staining) or 4% paraformaldehyde in PBS (pH 7.4) at 4°C for 3 h (PDGF-ß staining). After being washed in PBS, paraformaldehyde-fixed mesenteries were incubated in trypsin-EDTA at 37°C for 5 min. Methanol-fixed mesenteries did not require proteolytic pretreatment. Tissues were incubated overnight in rabbit polyclonal antibodies to PDGF-ß (sc-432, Santa Cruz Biotechnology) at a 1:500 concentration and PDGF-B (sc-7878, Santa Cruz) at a 1:100 concentration in 2% BSA and 5% normal goat serum (NGS) in PBS. After being washed in PBS, secondary biotinylated goat anti-rabbit IgG antibodies (Jackson Immunoresearch) were applied for 1 h at room temperature at a concentration of 1:500 in 2% BSA and 5% NGS in PBS. CY3-conjugated streptavidin (Jackson Immunoresearch) was then applied at 1:1,000 concentration in 2% BSA for 1 h. Slides were washed and then incubated in FITC-conjugated mouse monoclonal antibody to SM-α-actin (Sigma) at a concentration of 1:100 in 2% BSA in PBS or mouse monoclonal antibody to the rat endothelial-specific marker OX-43 (Sero-tec) at a concentration of 1:100 in 2% BSA in PBS. Secondary antibodies for OX-43 labeling were CY2-conjugated goat anti-mouse IgG (Jackson Immunoresearch). OX-43 has been used previously to verify that Tie2 is an endothelial-specific marker in rats (26), and in separate experiments, we have shown that OX-43 colocalizes with Tie2 and flk1 in the endothelium of mesenteric microvessels (data not shown). Negative control slides for PDGF-B and PDGFR-β were generated by removing the primary antibodies from the initial incubation and replacing the primary antibodies with the same concentration of rabbit IgG. Coverslips were applied to the specimens, and the whole mount networks were observed with a Bio-Rad MicroRadiance confocal scanner attached to a Nikon TE-300 inverted microscope.
blasts to a point source application of PDGF in mesenteric connective tissue. To ensure that the initial distribution of PKH26-labeled fibroblasts was similar for each treatment group, cells were counted within the entire FOV and within the 50- and 100-μm radius circles of the micropipette at the start of each experiment. Table 1 indicates that the average number of labeled cells within each area was similar between groups.

Figure 1 illustrates the change in number of cells within 50 μm of the pipette tip with time compared with the initial number of PKH26-labeled cells (see Table 1). The PDGF-AA treatment group had only a slight and insignificant increase in cells. The PDGF-BB group exhibited an increase in cell number after 30 min and a steady increase in the number of cells at later times. The increase in cell numbers was significantly different than control at 165 and 180 min after the start of infusion.

Figure 2 indicates the change in number of PKH26-labeled cells within 100 μm of the pipette tip for each of the treatment groups. The data were similar to that for the 50-μm distances, but the trend of an increase in cell numbers for the PDGF-BB group was more dramatic in this case. Cell numbers increased steadily until ~105 min after the start of the infusion, and the number of cells remained relatively constant for later times. The change in the number of cells was significantly different than that for control for the PDGF-BB group at times after 75 min, and the control and PDGF-AA groups exhibited no significant differences with time.
Figure 3 is composed of representative images for each of the treatment and control groups at the start of the infusion (A, D, and G), at 90 min (B, E, and H), and at 180 min (C, F, and I) after the start of the infusion. The transilluminated images were superimposed on the red fluorescent PKH26 cell images, and circles of radius 50 and 100 μm centered on the tip of the pipette were drawn and used for counting. Figure 3, J and K, contains higher magnification images of one of the PDGF-BB treatment mesenteries and clearly shows the positive change in number of red PKH26-labeled cells within 50 μm of the pipette tip with time.

Figures 4 and 5 demonstrate that PKH26-labeled fibroblasts become embedded in the mesenteric tissue after injection and do not attach to the upper or lower mesothelial surfaces. Figure 4 is a representative confocal montage acquired through the complete thickness of a region of mesentery containing PKH26-labeled fibroblasts. In the center of the tissue, but not at the upper and lower surfaces, PKH26 fluorescence is intense, and the PKH26-labeled cells are clearly focused. Figure 5 is a histogram quantifying the position of the PKH26-labeled fibroblasts through the depth of the tissue. A total of 348 PKH26-labeled fibroblasts were examined in three separate specimens. The distance of each cell from the closest surface was normalized to total tissue thickness, yielding a histogram in which a value of 0.0 represents the tissue surfaces and a value of 0.5 represents the tissue midplane. PKH26-labeled fibroblasts were evenly distributed throughout a normalized tissue depth of 0.25–0.5, with <3% of the PKH26-labeled fibroblasts seen at or near the upper and lower tissue surfaces (i.e., depth of 0.0–0.1).

Fig. 4. Confocal montage of PKH26-labeled fibroblasts within a mesenteric window. The number in the lower right-hand corner of each image denotes the depth into the tissue (in μm) at which the image was acquired. Note that PKH26-positive fibroblasts are bright and focused within the tissue but absent from the upper and lower surfaces at 0 and 15 μm, respectively. Each image is 168 × 168 μm.
denoting the presence of an arteriole. In Fig. 6 cell expression of PDGF-B is clearly evident. An additional observation of surrounding SM cells, capillary endothelial colocalization of PDGF-B is somewhat difficult to discern, though positive for PDGF-B, as shown in Fig. 6A. While endothelial colocalization of PDGF-B is somewhat difficult to discern in the arterioles and venules due to the presence of surrounding SM cells, capillary endothelial cell expression of PDGF-B is clearly evident. An additional region is labeled for SM α-actin in Fig. 6C, denoting the presence of an arteriole. In Fig. 6D, PDGF-B expression is seen in capillaries, a small arteriole, and a small venule, demonstrating that PDGF-B is expressed by microvessels without SM cells or SM α-actin positive pericytes. Figure 6, E and F, depicts an arteriole at higher magnification expressing SM α-actin and PDGF-B, respectively. SM expression of PDGF-B is clearly evidenced here because the pattern of PDGF-B labeling in Fig. 6C matches the corresponding SM α-actin expression in Fig. 6E. To ensure that the CY3 (red) fluorescent signal attributed to PDGF-B staining in the SM and endothelium was not being caused by bleed-through from SM α-actin/FITC or OX-43/CY2 fluorescence, we examined separate specimens that were labeled with only FITC or CY2 using the CY3 filter settings. These specimens exhibited no detectable bleed-through from the green spectrum into the red spectrum. Figure 6, G and H, denotes negative staining controls.

Figure 7 illustrates the expression of PDGFR-β in whole mount mesenteric microvascular networks by confocal microscopy. Figure 7, A and C, shows regions of mesenteric network labeled for SM α-actin. In Fig. 7B, PDGFR-β expression is visible in a venule, an arteriole, and the interstitium. At higher magnification (Fig. 7D), it is evident that PDGFR-β is expressed by SM cells in the arterioles and fibroblasts in the interstitium. PDGFR-β expression in each cell type is indicated by the absence of nuclear staining in Fig. 7D. While expression of PDGFR-β appears to be greater in the microvessels than in the interstitium in Fig. 7, A and C, it is important to note that the interstitium contains only a single layer of fibroblasts, whereas the microvessels contain multiple layers. Thus it is possible that the enhanced signal in the microvessels is a summation of fluorescence from multiple cells and the expression of PDGFR-β in the fibroblasts is no less than in the endothelium and SM cells. In addition to the SM and interstitial fibroblast nuclei shown in Fig. 7D, an endothelial cell nucleus is also depicted, suggesting that endothelial cells may express this receptor. Endothelial cell expression of PDGFR-β is then verified in Fig. 7, E–H, where it is shown to be colocalized with the endothelial cell specific marker OX-43. Figure 7, I and J, denotes negative staining controls.

**DISCUSSION**

The purpose of this study was to investigate the potential role of PDGF in mediating perivascular cell recruitment during microvessel assembly in vivo by determining whether a concentration gradient of PDGF elicits the migration of fibroblasts through native mesenteric tissue and by describing the spatial expression patterns of PDGF-B and its receptor PDGFR-β in mesenteric microvascular networks that continue to develop into adulthood. The results indicate that, at 30 ng/ml, PDGF-BB but not PDGF-AA is chemotactic for fibroblasts embedded in native mesenteric tissue. Additionally, we found that PDGF-B is expressed by endothelial cells and SM cells in microvessels and PDGFR-β is expressed by SM cells, endothelial cells, and interstitial fibroblasts. Because PDGF-BB elicits migration of interstitial fibroblasts in the mesentery and PDGF-B and PDGFR-β are spatially expressed in a pattern that is indicative of paracrine signaling from the microvessels to the fibroblasts in the extravascular space, these results are consistent with a role for PDGF-B and its receptor in mediating the recruitment of perivascular cells to microvessels during normal development from postnatal stages to adulthood.

**PDGF and fibroblast migration.** Cell migration is a coordinated cycle of several processes, including lamellipodial extension, formation of lamellipodium-substratum attachments, cytoskeletal contraction, and release of cell-substratum attachments. In the current study, fibroblast migration was observed on a native matrix as it exists in vivo. This is important because recent studies suggest that a complex interplay exists between growth factors, their receptors, extracellular matrix proteins, and adhesion molecule expression and...
clustering. In particular, synergies exist between PDGF, PDGF receptors, and the adhesion molecules that modulate cellular migration (4, 15, 16, 27). Moreover, the ability of cells to migrate in response to PDGF is dependent on integrin-mediated cell-substratum attachments (2, 10, 13, 20, 27). Given this information and the uncertainty in attempting to characterize the extracellular matrix composition of a given tissue, it is difficult to extrapolate the results of in vitro cell migration studies to the in vivo setting. Here, although the extracellular matrix components are unknown, we are essentially recapitulating the in vivo process. Thus we conclude that a sufficient concentration gradient of PDGF-BB in mesenteric tissue will elicit fibroblast migration.

Our data and observations from the chemotaxis assays illustrate two other crucial points. First, the migration of fibroblasts occurred only in subpopulations of labeled fibroblasts. We counted an increase of ~8 cells within 100 μm of the pipette tip when there were ~40 PKH26-labeled fibroblasts on average within the FOV. Thus some cells did not exhibit directed motion, suggesting a heterogeneity of PDGF-BB responsiveness. This heterogeneity may be due to the fact that the primary explant technique selects cells that migrate out of the tissue and are predisposed to enhanced motility. Furthermore, it is inevitable that the tissues were stretched to varying degrees. Because any slight stretching of the mesentery affects permeability and extracellular matrix organization, this may be a source of heterogeneity. Second, the data from the fibroblast migration experiments indicate that PDGF-BB but not PDGF-AA elicits the fibroblast migration on a native mesenteric matrix. PDGF-BB has been well established as a chemoattractant for fibroblasts in vitro, but the
chemoattractant potential of PDGF-AA remains controversial. It has been found that PDGF-AA and PDGF-BB elicit similar chemotaxis responses in lung fibroblasts (17); however, corneal fibroblasts are significantly more responsive to PDGF-BB than PDGF-AA (11, 12). Our data clearly agree with the latter investigation. While we believe our data are conclusive evidence that PDGF-BB is chemotactic for mesenteric fibroblasts in vivo, we cannot extend this conclusion to all fibroblast subpopulations and tissues because differences clearly exist. Furthermore, our study is limited to a single source concentration.
of growth factor. It is possible that, at different source concentrations, PDGF-AA elicits fibroblast migration on this matrix.

**PDGF signaling and microvessel assembly.** In the context of vascular development, PDGF-B and PDGFR-β expression have been well studied in the embryo. However, relatively little is known of the expression patterns of these molecules during microvessel development during maturation from postnatal stages to adulthood. Furthermore, while it has been shown that exogenous application of PDGF-B elicits angiogenesis in vivo (19) and disrupts pericyte-endothelial interactions (1), the role of endogenously produced PDGF-B in these processes in the adult is poorly defined. To better understand the potential role of PDGF signaling in microvascular development, we examined PDGF-B and PDGFR-β protein expression in growing mesenteric microvascular networks of young adult rats (10–11 wk). At this age, an approximate doubling in total mesenteric microvessel density remains before full adulthood (16–18 wk) (5); thus these networks were studied in an actively growing stage.

PDGF-B protein expression patterns in these networks are generally consistent with previous observations made in the embryonic vasculature. Here, PDGF-B protein was localized to all microvessels, with endothelial cells, SM cells, and (to a lesser extent) interstitial fibroblasts showing immunopositivity for PDGF-B. PDGF-B expression in SM cells appeared to be greater than in endothelial cells, with arteriolar SM exhibiting intense expression. It is, however, important to note that this enhancement may have been due to the greater number of cell layers in vessels containing SM. During embryonic vascular development, PDGF-B is expressed by capillary and arteriolar endothelial cells, but venous endothelial cells lack PDGF-B (6, 14). Postnatal PDGF-B expression is limited to short capillary sprouts in many tissues (6). Our observations also indicate that PDGF-B is expressed by capillary sprouts (data not shown). However, in contrast to our observations, PDGF-B is not located in mature capillaries in late embryogenesis or early postnatal development, and nonendothelial PDGF-B expression has not been detected (6).

PDGFR-β protein was localized in all microvessels in mesenteric networks, with endothelial and SM cells both exhibiting strong immunopositive responses. We also observed intense staining for PDGFR-β in the fibroblasts in the interstitium and immediately surrounding microvessels. Similarly, in the small vessels of the embryonic vasculature, PDGFR-β is expressed in pericytes and mesenchyme immediately surrounding the endothelium (14, 22). SM expression of PDGFR-β is also evident during these developmental stages (6, 14). Endothelial PDGFR-β expression in small embryonic vessels is, however, controversial, because claims that endothelial cells express PDGFR-β (8, 22) have been disputed (14). PDGFR-β is required for endothelial cell participation in wound healing angiogenesis (4), and our observations support the hypothesis that microvascular endothelial cells express PDGFR-β.

The spatial patterns of PDGF-B and PDGFR-β protein expression provide insight into how PDGF signaling may occur. First, because SM and endothelial cells express both PDGF-B and PDGFR-β, autocrine signaling is possible. Autocrine signaling by PDGF-B has been proposed as a means by which endothelial cell populations are expanded during early stages of placentangiosis (8). To our knowledge, PDGF-B expression by SM cells during embryonic or adult microvascular development has not been observed, and autocrine SM PDGF signaling has not been proposed as a mechanism of microvessel growth. While autocrine SM PDGF-B signaling may be proposed here as a means of enlarging developing microvessels via SM hyperplasia, lineage studies are needed to explore this hypothesis.

Second, endothelial PDGF-B and SM and interstitial fibroblast PDGFR-β expression support a paracrine signaling hypothesis. In the embryo, paracrine PDGF signaling from the endothelium to the pericytes and SM appears to play a role in angiogenic sprouting and vessel enlargement (6). However, because PDGFR-β-positive cells are still found adjacent to developing vessels in PDGF-B-deficient mice, a PDGF-B-independent induction of PDGFR-β cells from the mesenchyme has been proposed (6). In contrast, we observed PDGFR-β expression well beyond the mesenteric microvessels and throughout the tissue, indicating that the fibroblasts and SM cells immediately surrounding these vessels are not unique in their potential ability to respond to PDGF-B. When coupled with the fact that a concentration gradient of PDGF-B will elicit fibroblast migration in this tissue, the PDGFR-β expression pattern raises the possibility that long range (i.e., greater than one cell diameter) paracrine signaling with PDGF-B can occur in these networks. Moreover, it has been shown that, during wounding angiogenesis, PDGFR-β is required for fibroblast participation (4). This indicates that, in contrast to embryonic development, induction of PDGFR-β-positive mesenchymal cells likely requires PDGF-B in the adult. Ultimately, the answers to these questions and a clear definition of the role of PDGF signaling in microvascular development during normal maturation will require inducible cell-specific gene expression or gene-targeting models for PDGF and its receptors in the future. At present, our results indicate that PDGF-BB is able to recruit fibroblasts in mesenteric connective tissue and that, during normal maturation, PDGF-B and PDGFR-β are expressed in a pattern that is consistent with a role for PDGF in mediating the microvascular development process during normal maturation.

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