Placental Growth Factor Promotes Atherosclerotic Intimal Thickening and Macrophage Accumulation

Rohit Khurana, MD*; Lieve Moons, PhD*; Shahida Shafi, PhD; Aernout Luttun, PhD; Désiré Collen, MD, PhD; John F. Martin, FRCP; Peter Carmeliet, MD, PhD*; Ian C. Zachary, PhD*

Background—Placental growth factor (PIGF) has been implicated in the pathophysiological angiogenesis and monocyte recruitment that underlie chronic inflammatory disease, but its role in atherosclerosis has not been examined. We investigated the effects of exogenous PIGF, delivered by adenoviral gene transfer, on atherogenic intimal thickening and macrophage accumulation induced by collar placement around the rabbit carotid artery and examined the effects of PIGF deficiency on atherosclerosis in apolipoprotein E–deficient (apoE<sup>−/−</sup>) mice.

Methods and Results—Periadventitial transfer of PIGF2-encoding adenoviruses significantly increased intimal thickening, macrophage accumulation, endothelial vascular cell adhesion molecule-1 expression, and adventitial neovascularization in the collared arteries of hypercholesterolemic rabbits and increased the intima-to-media ratio in rabbits fed a normal diet. Neointimal macrophages were associated with increased expression of the PIGF receptor Flt-1. The size and macrophage content of early atherosclerotic lesions were reduced in mice deficient in both apoE and PIGF compared with apoE-deficient mice.

Conclusions—Local adenoviral PIGF2 delivery promotes atherogenic neointima formation in hypercholesterolemic rabbits, and PIGF is required for macrophage infiltration in early atherosclerotic lesions in apoE<sup>−/−</sup> mice. These findings support a novel role for PIGF in the pathogenesis of atherosclerotic disease. (Circulation. 2005;111:2828-2836.)

Key Words: angiogenesis ■ atherosclerosis ■ cell adhesion molecules ■ endothelium ■ monocytes

Vascular endothelial growth factor (VEGF or VEGF-A) is essential for endothelial cell (EC) differentiation and angiogenesis during embryogenesis and plays a major role in neovascularization in diverse human diseases.1,2 Two protein tyrosine kinase receptors for VEGF, VEGFR-2/KDR/Flk-1 and VEGFR-1/Flt-1, are essential for embryonic vascular development, but signal transduction and biological responses in endothelia are mediated primarily via KDR.1,3 Flt-1 is believed to function during embryogenesis as a negative regulator of KDR-mediated actions, in part by acting as a “decoy” receptor and in part through direct modulation of KDR activity.1,4 However, a growing body of evidence indicates that the VEGF homologue, placental growth factor (PIGF), stimulates pathophysiological angiogenesis and other biological responses in the postembryonic state by signaling through its receptor Flt-1 on ECs.5 Loss of PIGF impairs collateral artery growth in mouse limbs and neovascularization in tumors and ischemic retinas, whereas exogenous PIGF delivery stimulates angiogenesis and collateral growth in ischemic hearts and limbs.5–7 Inhibition of Flt-1 suppresses pathophysiological angiogenesis; in addition, Flt-1 mediates PIGF-induced monocyte chemotaxis,9 and rescue of impaired ischemia-driven arteriogenesis in the rabbit hind limb or in PIGF<sup>−/−</sup> mice is effected by infusion of exogenous PIGF and prevented by ablation of monocytes,10 which indicates that some biological effects of PIGF in vivo are mediated by acting on Flt-1<sup>+</sup> monocytes. Flt-1 has been implicated in atherogenesis because anti-Flt-1 blocking antibody reduced atherosclerotic plaque growth in apolipoprotein (apo) E<sup>−/−</sup> mice,6 but the involvement and mechanisms of action of its ligand PIGF in atherosclerosis or associated cellular processes remains to be determined.

In the present study, the role of PIGF in atherogenic lesion formation was investigated by 2 distinct approaches. First, we examined the effects of local periadventitial transfer of PIGF-encoding adenoviruses on intimal thickening and macrophage accumulation in the rabbit collar model. In this model, neointimal lesions are induced by periadventitial placement of an inert silicone collar around the carotid artery and examined the effects of PIGF deficiency on atherosclerosis in apolipoprotein E–deficient (apoE<sup>−/−</sup>) mice.
providing the means of local gene delivery.11–16 Second, we evaluated the effects of loss of the PlGF gene on the growth and macrophage content of atherosclerotic lesions in apoE-deficient mice. The results show that adenoviral PlGF expression increases intimal thickening, neointimal macrophage accumulation, and adventitial neovascularization in hypercholesterolemic rabbits and demonstrate a requirement of PlGF for macrophage accumulation and plaque growth in early atherosclerotic lesions in apoE−/− mice. These findings support the conclusion that PlGF promotes atherogenic intimal thickening and macrophage infiltration and identify a novel role for PlGF in the pathogenesis of early atherosclerosis.

### Methods

**Adenoviruses**

Adenoviral constructs (E1-, E3-deleted) encoding either LacZ (Ad.LacZ) or mouse PlGF2 (Ad.PlGF2) were produced as described previously.11–12 Adenoviruses were desalted with G50 Sephadex columns (Boehringer Mannheim) immediately before their use in animal studies.

**Collar Placement and Gene Transfer**

All experiments were conducted in accordance with the animal care guidelines and Ethics Committee of University College London and the United Kingdom Home Office Animals (Scientific Procedures) Act of 1986. Twenty-six New Zealand White male rabbits (weight 2.5 to 3.2 kg) were fed a normal diet supplemented with 1.5% cholesterol and 2.5 to 3.2 kg) were fed a normal diet supplemented with 1.5% cholesterol without added cholesterol. Placement of a biologically inert silastic collar (Ark Therapeutics Ltd) around the right carotid artery was performed by omission of the primary antibody.

**PlGF2 and β-actin were, respectively, 5′-GGA GGG3′/H11032 and 5′-ATA GAG GGT AGG TAC CAG CAG 3′/H11032, respectively.**

**Immunohistochemistry**

The following antibodies were used: mouse IgG1 to rabbit vascular cell adhesion molecule-1 (VCAM-1; Rb1/9; gift of MI Cybulsky) at a 1:100 dilution in frozen sections; macrophage-specific RAM-11 (1:50, Dako); mouse anti-human CD31 (1:500; Dako); VSMC-specific α-actin antibody (1:150; Dako); rat anti-mouse PlGF monoclonal antibody (Pharmingen Inc; 10 μg/ml); and Fit-1 anti-mouse monoclonal antibody (Santa Cruz Inc; 1:50). Primary antibodies were diluted in Tris-buffered saline, pH 7.2. Staining was performed on deparaffinized or frozen sections as described previously and visualized with a Vectastain Elite ABC Kit (Vector Laboratories). For PlGF staining only, a tyramide amplification step (Perkin Elmer Inc) was performed before secondary antibody incubation. The 5-μm frozen sections were fixed for 10 minutes in acetone at −20°C and then air dried. Negative controls for all immunostainings were performed by omission of the primary antibody.

**Morphometry and Image Analysis**

Images of sections at ×5 and ×40 were acquired with a high-resolution color camera (Zeiss microscope, Jenoptik Camera) and analyzed with automated image-analysis software (Image J, National Institutes of Health). Intimae were defined as the regions between luminal endothelium and the internal elastic lamina. The media was defined as the area between the internal and external elastic laminae and analyzed blindly. Ratios of intimai to media areas were determined in serial sections cut at 500-μm intervals and stained with hematoxylin and eosin; intimai-media values were averaged and expressed as mean±SE. Neovascularization was quantified by counting the numbers of CD31-positive vessels. CD31 staining was regarded as positive if a single cell or a vessel with a lumen was present, and numbers of CD31-positive cells and vessels were expressed per square millimeter of total adventitial area. Total RAM-11–positive macrophages were counted in the intimai and expressed per square millimeter. VCAM-1 immunostaining was quantified on high-resolution (1300×1030 pixel) images with OpenLab 3.14 software (Improvement Ltd) and expressed as the number of pixels representing endothelial VCAM-1 immunostaining as a percentage of the total endothelial pixel count.

**apoE−/− and apoE−/−:PlGF2−/− Mice**

apoE−/− mice were intercrossed with previously generated PlGF2−/− mice,6 which yielded apoE−/−:PlGF2−/− offspring. Breeding of these compound heterozygous mice generated apoE−/−:PlGF2−/− mice and their apoE−/−:PlGF2−/− littermates. Mice (all female) were maintained on regular chow for 5 weeks, after which they were fed a cholesterol-rich diet for an additional 10 or 25 weeks.9 Mice were anesthetized (60 mg/kg pentobarbital; Nembutal, Abbott Laboratories) and perfused with saline followed by 1% phosphate-buffered paraformaldehyde. Hearts were harvested and postfixed in the same fixative.
for another 3 hours, dehydrated, and embedded in paraffin. Seventymicrometer cross sections of the aortic origin and the descending aortas (thoracic and abdominal region) were cut, and cross-sectional plaque area measurements were performed as described previously.6,17 Macrophage content was analyzed on sections stained with a rat anti-mouse macrophage-specific Mac-3 antibody (Pharmingen). Morphometric analyses on Mac-3–stained sections were performed with a Zeiss Axioiplan2 microscope (Zeiss), a 3 CCD video camera (DXC-930P; Sony), and KS300 software. Plaque macrophage content is expressed as the ratio of the area positive for Mac3 staining to total plaque area. Plaque microvessels were analyzed after staining of cross sections of the aortic root for endothelium-specific markers, von Willebrand factor (Dako) and CD31 (Pharmingen), as described previously.6 T lymphocytes were stained with a rabbit anti-CD3 antibody (Laboratory Vision Corporation).

Statistical Analysis

Differences in serum cholesterol levels and morphometric differences between different treatment groups in rabbits were evaluated by ANOVA and Bonferroni correction (SPSS). Data obtained from mouse knockout models, expressed as mean ±SE, were analyzed with the unpaired Student t test and the Mann-Whitney U test, which yielded similar results. Data were considered statistically significant at P<0.05.

Results

Periadventitial PlGF2 Adenoviral Gene Delivery in Collared Carotid Arteries

Collar placement around the carotid arteries of rabbits fed a normal diet for 14 days induces the formation of neointimal lesions composed of VSMCs with no detectable involvement of macrophages.11,12 In contrast, collaring of the carotid artery in rabbits fed a 1.5% cholesterol diet generates neointimas comprising VSMC with the involvement of Ram-11–stained macrophages (results not shown), very similar to previous findings.12 Blood LDL cholesterol and total cholesterol increased to 10 and 14 mmol/L, respectively, after 7 days on the high-cholesterol diet and 20 and 26 mmol/L after 21 days. The contralateral sham-operated carotid arteries in the cholesterol-fed rabbits displayed no evidence of either intimal thickening or increased Ram-11 immunostaining (results not shown). Immunostaining of ECs with CD31 antibody indicated that neither collaring nor hypercholesterolemia caused significant discontinuities in the endothelium, which remained essentially intact (results not shown). Staining for β-galactosidase in arteries transduced with a control LacZ gene revealed abundant strongly stained cells in the adventitia consistent with a high efficiency of gene transfer (≈5% to 10%) and in agreement with previous findings (Figure 1A).12,18

Expression of the PlGF2 transgene after periadventitial delivery of Ad.PIGF2 to carotid arteries was examined by RT-PCR and immunohistochemistry. An amplicon of predicted size, 270 bp, corresponding to PlGF2 was detected only in RNA prepared from collared arteries transduced with Ad.PIGF2 (Figure 1B). Expression of β-actin was very similar in RNA prepared from all transduced arteries. No PIGF2 transgene expression was detected in Ad.LacZ-transduced arteries, segments of the transduced carotid arteries distal to the collared arterial region, contralateral noncollared control arteries, or other nontargeted tissues, including lung, liver, kidney, and testis (results not shown). These results indicate that the perivascular collar was effective in targeting transgene expression specifically to the collared region of the artery. Immunostaining of sections of transduced arteries with a specific antibody to mouse PIGF2 showed strong expression of PIGF in the adventitia and additional expression in the neointima and endothelium (Figure 1C), which confirmed the RNA analysis.

Ad.PIGF-2 Increases Macrophage Accumulation and Flt-1 Expression

Ad.PIGF2 delivery to collared carotid arteries in cholesterol-fed rabbits significantly increased intimal thickening (Table 1; Figure 2A), and it significantly enhanced neointima formation in the collared carotid arteries of rabbits fed a normal low-cholesterol diet (Figure 2B). Immunostaining with Ram-11 showed that PIGF2 increased neointimal macro-
phage accumulation in the collared arteries of hypercholesterolemic rabbits (Figure 3A). Compared with either Ad.LacZ-transduced arteries or nontransfected collared arteries, periadventitial Ad.PlGF2 delivery significantly increased the neointimal density of Ram-11–positive cells (Figure 3B). Ad.PlGF2 also increased the number of adventitial macrophages in collared hypercholesterolemic rabbits from 13.5 \pm 1.5/mm² to 22.5 \pm 3.6/mm² (Table 1). Although macrophages did not accumulate in the neointimas of collared arteries in rabbits fed a normal diet, consistent with previous findings, a significant increase in adventitial macrophage staining was detected in Ad.PlGF2-transduced arteries compared with Ad.LacZ (Table 1).

Expression of the PlGF receptor Flt-1 was also examined in collared arteries. In Ad.PIGF2-transduced arteries of hypercholesterolemic rabbits, Flt-1 expression was readily detected in neointimal cells and in areas of the endothelium and adventitia, and it was increased in Ad.PIGF2-transduced vessels compared with Ad.LacZ-expressing arteries (Figure 3C). Ram-11 staining of serial sections showed close association between some Flt-1–positive regions and Ram-11–positive staining in the neointima, which suggests that lesion macrophages expressed Flt-1.

**Ad.PIGF2-2 Induces Neovascularization and Endothelial Activation**

Recent findings have discovered a role for PlGF in pathophysiological angiogenesis in vivo. It was therefore examined whether local Ad.PIGF2 delivery increased neovascularization in collared carotid arteries. Immunostaining of CD31 revealed the presence of new adventitial vessels in the Ad.PIGF2-transduced arteries of rabbits fed a high-cholesterol diet (Figure 4A) or normal diet (results not shown). Quantification of the adventitial density of CD31-positive vessels indicated that neovascularization was increased in the Ad.PIGF2-transduced arteries of rabbits regardless of dietary regimen (Figure 4B). In both experimental groups, CD31-positive cells and vessels were not detected in the media, and apart from luminal staining of the endothelium, they were also absent from neointimal lesions.

---

**Table 1. Effects of Ad.PIGF2 Gene Delivery on Lesion Formation in Collared Rabbit Carotid Arteries**

<table>
<thead>
<tr>
<th></th>
<th>1.5 % Cholesterol Diet</th>
<th>Normal Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad.LacZ (n=10)</td>
<td>Ad.PIGF2 (n=10)</td>
</tr>
<tr>
<td>Intima/media</td>
<td>0.15\pm0.02</td>
<td>0.28\pm0.01‡</td>
</tr>
<tr>
<td>Macrophages/mm²</td>
<td>246\pm30</td>
<td>470\pm66*</td>
</tr>
<tr>
<td></td>
<td>neointima</td>
<td>13.5\pm1.5</td>
</tr>
<tr>
<td>Macrophages/mm²</td>
<td>6.7\pm2.3</td>
<td>28.7\pm2.3§</td>
</tr>
<tr>
<td>adventitia</td>
<td>24.0\pm1.6</td>
<td>47.8\pm4.4*</td>
</tr>
<tr>
<td>% Endothelial VCAM1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|                      | Values represent mean \pm SE for carotid arteries infected with Ad.LacZ or Ad.PIGF2 in rabbits fed either 1.5% cholesterol or normal diet. Neointimal macrophages and endothelial VCAM1 staining were not detected (ND) in rabbits on a normal diet. Other experimental details are provided in Methods and figure legends. **P<0.05, †P<0.01, ‡P<0.001, and §P<0.0001 for Ad.PIGF2 vs Ad.LacZ.**
Upregulation of the EC adhesion molecule, VCAM-1, is thought to play a central role in mediating increased monocyte adhesion to the endothelium and transendothelial monocyte migration leading to neointimal accumulation of macrophages. Lumenal endothelial expression of VCAM-1 was increased in collared arteries from cholesterol-fed rabbits compared with either the sham-operated contralateral control arteries in the same animals or collared arteries from normocholesterolemic rabbits (results not shown). In hypercholesterolemic rabbits, Ad.PlGF2-transduced collared arteries displayed increased endothelial VCAM-1 staining compared with Ad.LacZ (Figure 5). Quantification of VCAM-1 immunostaining showed that the total percentage of the endothelium positive for VCAM-1 immunostaining was significantly elevated ($P<0.001$) in PlGF2-transduced arteries (Figure 5).

Deficiency of PlGF Reduces Early Atherosclerotic Lesion Development in apoE$^{-/-}$ Mice

The results presented so far indicated that local adenoviral PlGF2 expression induced atherogenic processes that included intimal thickening, macrophage recruitment, and endothelial VCAM-1 expression in the hypercholesterolemic rabbit. To investigate whether endogenous PlGF played a role in neointimal macrophage accumulation in atherosclerosis, plaque formation at the aortic root was analyzed in mice lacking both PlGF and apoE (apoE$^{-/-}$:PlGF$^{-/-}$) and compared with single apoE-deficient littermates (apoE$^{-/-}$:PlGF$^{-/-}$). PlGF deficiency caused a significant reduction in size ($P=0.034$) and macrophage content ($P=0.007$) of early atherosclerotic plaques in apoE$^{-/-}$ mice after 10 weeks on a high cholesterol diet compared with mice deficient only in apoE (Figure 6; Table 2). After 25 weeks of diet, the mean plaque area was reduced in apoE$^{-/-}$:PlGF$^{-/-}$ mice compared with apoE$^{-/-}$ mice, but the effect was not statistically significant (Table 2). In addition, a reduction in plaque macrophages was not observed after 25 weeks on a high-fat diet. At both time points, macrophage distribution within the athero-
sclerotic lesions was similar in both genotypes. Macrophages penetrated throughout the entire early lesions (Figures 6e and 6f), whereas they were more confined to the surface of plaques in advanced lesions (Figures 6i and 6j). Semiquantitative analysis revealed no significant differences in the macrophage distribution between apoE^−/−:PlGF^+/+ and apoE^−/−:PlGF^−/− mice in early or late lesions (Table 2). Analysis of plaques in the descending thoracic and abdominal aorta showed that cross-sectional plaque areas were not significantly reduced in apoE^−/−:PlGF^+/+ compared with apoE^−/−:PlGF^−/− mice (17.73±5.63 and 20.19±3.31 μm^2, respectively; n=10 for each genotype; P=NS). However, PlGF-deficient mice developed significantly fewer lesions in the descending aorta compared with their apoE^−/−:PlGF^+/+ littermates; plaque numbers were 1.5±0.3 and 3.5±0.6 for apoE^−/−:PlGF^+/+ and apoE^−/−:PlGF^−/− mice, respectively (n=10 for each genotype; P<0.05). Plaque number and size in the descending aorta were similar in both genotypes after 25 weeks.

In agreement with a possible effect of PlGF on macrophage recruitment and as a potential mechanism for reduced early lesion growth, the total leukocyte count in peripheral blood was slightly reduced in the apoE^−/−:PlGF^+/+ mice with early lesions (2.97±0.26×10^7/μL in apoE^−/−:PlGF^+/+ mice versus 2.23±0.16×10^7/μL in apoE^−/−:PlGF^−/− mice; n=20; P<0.05). No difference in leukocyte counts was detected in mice with advanced complex lesions (after 25 weeks of diet), with values of 3.33±0.70×10^7/μL in apoE^−/−:PlGF^+/+ mice versus 3.09±0.34×10^7/μL in apoE^−/−:PlGF^−/− mice (n=8; P=NS). Unlike macrophages, accumulation of CD3-positive T lymphocytes in early atherosclerotic lesions was very low but similar in both genotypes (percent of CD3-positive area/total plaque area: 0.09±0.01 in apoE^−/−:PlGF^+/+ mice versus 0.08±0.01 in apoE^−/−:PlGF^−/− mice; n=5; P=NS). In addition, no significant differences in CD3-positive lymphocyte content or distribution were detected in advanced lesions (percent of CD3-positive area/total plaque area: 1.21±0.64 in apoE^−/−:PlGF^+/+ mice versus 0.13±0.55 in apoE^−/−:PlGF^−/− mice; n=5; P=NS). Consistent with our previous findings, plaque microvessels detected by staining for the endothelial markers von Willebrand factor and CD31 were not found in the early lesions of either apoE^−/−:PlGF^+/+ or apoE^−/−:PlGF^−/− mice, which suggests that plaque neovascularization does not have a major role in the early stages of plaque growth. In advanced lesions, the numbers of vessels were similar in the advanced lesions of both types of mice (number of plaque vessels per mouse: 2.0±0.4 in apoE^−/−:PlGF^+/+ mice versus 1.9±0.6 in apoE^−/−:PlGF^−/− mice; n=8; P=NS).

**Discussion**

This study shows that targeted periadventitial delivery of an adenoviral vector encoding PlGF2 increased a spectrum of atherogenic processes in the collared carotid arteries of cholesterol-fed rabbits, including arterial intimal thickening, endothelial VCAM-1 expression, and macrophage accumulation. Furthermore, the reduction in the size, number, and macrophage content of plaques in mice doubly deficient in PlGF and apoE supports the conclusion that endogenous PlGF plays an important role in recruitment of macrophages and plaque growth in early atherosclerosis. These findings are consonant with previous work showing that inhibitory Flt-1 antibody reduced plaque growth and macrophage infiltration particularly in early and intermediate lesions of apo E^−/− mice.6 The less significant effect of PlGF deficiency on plaque growth and macrophage content in more advanced lesions may be due to a more important role of other inflammatory factors in the promotion of monocyte/macrophage migration or to a more dominant role for deposition of extracellular matrix (ie, collagen) in plaque growth during the later stages of atherosclerosis. These findings demonstrate that local PlGF delivery can directly promote atherogenic intimal thickening and intimal macrophage recruitment and indicate that PlGF production is required for macrophage migration into early atherosclerotic lesions.

The biological roles of both PlGF and its specific receptor, Flt-1, have remained enigmatic because Flt-1 does not have a clearly defined signaling function, and during embryonic development, it is thought to act primarily as a regulator of VEGF-A functions mediated via KDR. However, recent findings have revealed a role for PlGF in postembryonic angiogenesis in diverse pathophysiological settings. Importantly, Flt-1, unlike Flk-1, is expressed on cells of the myeloid lineage,5,22 and there is increasing evidence that PlGF-induced mobilization of Flt-1–expressing bone marrow–derived hematopoietic cells and their progenitors is a key mediator of the biological effects of the PlGF-Flt-1 axis.8 Impaired pathophysiological angiogenesis in PlGF^−/− mice can be rescued by transplantation of wild-type bone marrow, and inhibitory anti-Flt-1 antibodies block neovascularization in ischemia, tumor growth, and arthritis in part because of...
reduced mobilization of bone marrow–derived myeloid progenitors. PlGF also stimulates hematopoiesis after bone marrow irradiation by recruiting Flt-1–positive, bone marrow–derived stem cells. Furthermore, Ram-11–positive macrophages in collared arteries also expressed Flt-1, which suggests that intra-arterial PlGF acted by directly inducing macrophage influx in Ad.PlGF2-transduced arteries. The cytokine-inducible adhesion molecule VCAM-1 is upregulated in the arterial endothelium at atherosclerosis-prone sites in hypercholesterolemic rabbits and mice, and is essential for early atherosclerotic lesion formation in LDL receptor–deficient mice.

The mechanism by which PlGF2 increases plaque area is likely to contribute to atherosclerosis, and partly mediates the infiltration of macrophages into early lesions in the apoE2−/− mouse. The findings presented here also indicate that local PlGF overexpression in the rabbit carotid artery directly stimulates atherogenesis by increasing recruitment of macrophages from the circulation. Because PlGF2 transgene expression was detected only in the collared region of the rabbit carotid artery and was absent from other tissues, it is highly likely that the effects of PlGF in this model are not attributable to increased systemic PlGF leading to increased mobilization of bone marrow–derived stem cells. Furthermore, Ram-11–positive macrophages in collared arteries also expressed Flt-1, which suggests that intra-arterial PlGF acted by directly inducing Fli-1 expression and migration of Flt-1–expressing monocytes into lesions.

Increased endothelial expression of VCAM-1 is likely to be an important mediator of the increased neointimal macrophage influx in Ad.PlGF2-transduced arteries. The cytokine-inducible adhesion molecule VCAM-1 is upregulated in the arterial endothelium at atherosclerosis-prone sites in hypercholesterolemic rabbits and mice, and is essential for early atherosclerotic lesion formation in LDL receptor–deficient mice.

The mechanism by which PlGF2 increases...
endothelial VCAM-1 could involve either direct Flt-1-mediated upregulation of VCAM-1 expression or an indirect pathway mediated by inflammatory cytokines. Consistent with the latter possibility, PI GF treatment of human monocytes increased mRNA expression of tumor necrosis factor-α and interleukin-1β, both strong inducers of endothelial VCAM-1 expression.25

Periadventitial transfer of Ad.PIGF2 also markedly increased adventitial neovascularization in collared rabbit arteries. This finding further supports recent reports that PI GF is a key angiogenic mediator in diverse models of adult pathophysiological angiogenesis. However, because neovascularization was not a feature of early lesions in either apoE−/− or apoE−/−:PIGF−/− mice, and PI GF deficiency did not alter the numbers of plaque microvessels detected in advanced lesions, atherogenic effects of PI GF appear to be largely independent of angiogenesis, at least in the apoE−/− mouse model. This conclusion is consistent with previous findings showing that an inhibitory anti-Flt-1 antibody reduced atherosclerosis in apoE−/− mice without inhibiting plaque neovascularization.6 The role of angiogenesis as a mediator of the neointima-promoting effects of PI GF2 in VSMC hyperplasia induced by clinically relevant interventions such as balloon injury or vein grafting is not known, however, and requires further investigation.

In rabbits fed a normal diet, Ad.PIGF2 enhanced intimal thickening without increasing neointimal macrophage accumulation, which indicates that PI GF2 also has a neointima-increasing effect that does not result from intimal macrophage recruitment. Because Flt-1 is expressed in plaque VSMCs in vivo6,26,27 this effect could be mediated via PI GF-induced VSMC proliferation or migration, a possibility that warrants further study. The increased adventitial macrophage staining observed in Ad.PIGF2-transduced arteries in normocholesterolemic animals does suggest, however, that PI GF2 promotes monocyte chemotaxis to the adventitia in the absence of high blood cholesterol. The significance of monocyte recruitment to the adventitia for the neointima-increasing effects of PI GF2 is unclear, but this effect could contribute to the adventitial neovascular response to PI GF2.

The present study supports the emergent view that in adult animals, PI GF is a functional cytokine, particularly in pathophysiological settings, able to stimulate angiogenesis and atherogenic migration of monocytes/macrophages into the arterial wall. The finding that PI GF and its receptor, Flt-1, have a potential role in the pathogenesis of atherosclerotic disease has clinical implications. Therapeutic strategies targeting either PI GF or Flt-1 may be an attractive and novel approach to the inhibition of inflammatory macrophage infiltration underlying early atherosclerotic plaque formation. This notion is supported by previous findings that administration of anti-Flt-1 antibody has an atheroprotective effect in atherosclerosis-prone apoE−/− deficient mice by reducing early atherosclerotic plaque growth and plaque macrophage infiltration.6 The finding that functional loss of PI GF and Flt-1 selectively inhibits the early stages of atherosclerosis without reducing late lesion development may not militate against a therapeutic role for PI GF-induced arteriogenesis6,10 in isch-
dependent and independent phases of intimal hyperplasia. 


Placental Growth Factor Promotes Atherosclerotic Intimal Thickening and Macrophage Accumulation
Rohit Khurana, Lieve Moons, Shahida Shafi, Aernout Luttun, Désiré Collen, John F. Martin, Peter Carmeliet and Ian C. Zachary

_Circulation_. 2005;111:2828-2836; originally published online May 23, 2005;
doi: 10.1161/CIRCULATIONAHA.104.495887

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/111/21/2828

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

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

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/