Role of Angiogenesis in Cardiovascular Disease
A Critical Appraisal

Rohit Khurana, MD, PhD; Michael Simons, MD; John F. Martin, FRCP; Ian C. Zachary, PhD

Abstract—The role of angiogenesis in atherosclerosis and other cardiovascular diseases has emerged as a major unresolved issue. Angiogenesis has attracted interest from opposite perspectives. Angiogenic cytokine therapy has been widely regarded as an attractive approach both for treating ischemic heart disease and for enhancing arterioprotective functions of the endothelium; conversely, a variety of studies suggest that neovascularization contributes to the growth of atherosclerotic lesions and is a key factor in plaque destabilization leading to rupture. Here, we critically review the evidence supporting a role for angiogenesis and angiogenic factors in atherosclerosis and neointima formation, emphasizing the problems raised by some of the landmark studies and the suitability of animal models of atherosclerosis and neointimal thickening for investigating the role of angiogenesis. Because many of the relevant studies have focused on the role of vascular endothelial growth factor (VEGF), we consider this work in the wider context of VEGF biology and in light of recent experience from clinical trials of VEGF and other angiogenic cytokines for ischemic heart disease. Also discussed are recent findings suggesting that, although angiogenesis may contribute to neointimal growth, it is not required for the initiation of intimal thickening. Our assessment of the evidence leads us to conclude that, although microvessels are a feature of advanced human atherosclerotic plaques, it remains unclear whether angiogenesis either plays a central role in the development of atherosclerosis or is responsible for plaque instability. Furthermore, current evidence from clinical trials of both proangiogenic and antiangiogenic therapies does not suggest that inhibition of angiogenesis is likely to be a viable therapeutic strategy for cardiovascular disease. (Circulation. 2005;112:1813-1824.)

Key Words: angiogenesis ■ atherosclerosis ■ endothelium ■ growth substances

The role of blood vessel formation within diseased blood vessels has become one of the outstanding puzzles in the biology of cardiovascular disease. The generation of blood vessels is a prerequisite for embryonic development and is increasingly recognized to play essential roles in the pathogenesis of diverse chronic human diseases.1–3 In cancer, rheumatoid arthritis, ocular disorders, and other diseases, neovascularization is integral to the disease process, and inhibition of angiogenesis is a major goal of therapeutic drug development. Efforts to develop an antiangiogenic therapeutic approach to cancer have recently culminated in the approval by the US Federal Drug Administration of Avastin, an antibody specific for VEGF (or VEGF-A), the key angiogenic cytokine vascular endothelial growth factor, for the treatment of metastatic colorectal carcinoma.4 In atherosclerosis, however, the role of angiogenesis remains a highly contentious issue, and no consensus exists as to whether angiogenesis either is a key causative factor in the pathogenesis of atherosclerotic plaque formation or is a way to treat coronary heart disease. The controversy surrounding the role of angiogenesis in ischemic heart disease reflects, in part, the complexity of the underlying disease process. A growing body of evidence supports an association between intraplaque angiogenesis with atherosclerotic plaques that cause acute coronary syndromes.5 These vulnerable plaques are more likely to rupture and progress to cause intra-arterial occlusion. In the case of coronary arteries, this sudden and catastrophic restriction of the blood supply to the heart causes an acute coronary syndrome, often resulting in a fatal loss of cardiac function.6 The acute problem in the case of coronary artery disease is therefore vascular insufficiency, but this is the outcome of a complex pathophysiological process in which angiogenesis may itself play a vital, although as-yet undecided, role.

Debate surrounding the pathogenic role of angiogenesis in atherosclerosis has been particularly energetic because a key therapeutic objective has been to use angiogenic cytokines such as VEGF or members of the fibroblast growth factor (FGF) family to stimulate collateral blood vessel formation in the ischemic heart and limb, an approach called therapeutic angiogenesis.7 Although this strategy is supported by an impressive body of preclinical research suggesting that VEGF, FGF-2, and other angiogenic cytokines can promote revascularization in diverse animal models of ischemic car-

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1813
diovascular disease, the data from clinical trials have so far been inconclusive. More problematic still for proponents of therapeutic angiogenesis have been several recent studies suggesting that VEGF and other angiogenic factors can promote atherosclerosis in certain animal models and potentially destabilize coronary plaques by promoting intraplaque angiogenesis.8–10

The origin of newly formed vessels and the pathogenic role of neovascularization are important unresolved issues in our understanding of the mechanisms of plaque formation; furthermore, the lack of consensus concerning the contribution of angiogenesis has serious practical implications because it continues to place a question mark on the use of VEGF and other angiogenic factors to treat ischemic cardiovascular disease. Although more experimental work is clearly needed to address these questions, an overview of the role of angiogenesis in vessel wall disease is timely and will help to illuminate some of the key outstanding problems.

Angiogenesis and Atherosclerosis

Many large human arteries possess a microvasculature in their adventitial layers called the vasa vasorum.11 Normal vasa vasorum originate from coronary artery branch points at regular intervals and run longitudinally along the vessel wall (first-order vasa vasorum). These vessels then separate to form circumferential arches around the main coronary lumen (second-order vasa vasorum). Because diffusion of blood nutrients from the lumen is limited to a distance of \( \approx 100 \, \mu \text{m} \), a primary function of these vessels is thought to be the transport of nutrients to the vessel wall, although other roles are not precluded.

An association between intimal neovascularization and atherosclerosis was first noted by Koester12 in 1876; similar observations were made by Winternitz and coworkers13 in 1938. Patterson14 first suggested in 1938 that rupture of plaque capillaries could trigger intraplaque hemorrhage, leading to coronary thrombosis. It was later found that the intimas of adult human arteries are avascular until they exceed a certain thickness.15 The seminal study of Barger et al16 synthesized many of these earlier observations in the hypothesis that proliferation of the adventitial vasculature of coronary arteries is an active process in the atherosclerotic milieu.

Plaque vessels are found in the neointima, media, and adventitia, but most vessels appear to originate from the adventitia, and the regions of plaques most vulnerable to rupture. Microvessels appear to have a predilection for the shoulder regions of atherosclerotic plaques, whereas a recent study of neovascularization in 269 advanced human atherosclerotic plaques concluded that microvessel formation is strongly correlated with both plaque rupture and the signature features of vulnerable plaques. Thus, an increase in microvessel density occurred in ruptured compared with nonruptured plaques but was also found in the shoulder regions of plaques and was strongly associated with a high degree of macrophage infiltration, intraplaque hemorrhage, and thin-cap lesions.26 In addition to an association between microvessels and vulnerable plaques, several proangiogenic cytokines are expressed in human lesions (Table 1), lending further weight to the argument that neovascularization is an active process in the atherosclerotic milieu.

What conclusions can be drawn from human and large animal studies? Neovascularization is undoubtedly a common, although not invariable, feature of the pathology of atherosclerosis and intimal thickening (Table 2). However, none of these studies demonstrated that vasa vasorum significantly contributes to the disease process. Just as problematic is the notion that neovascularization is a significant factor contributing to plaque instability and rupture. The interpretation of data supporting a role for angiogenesis in atherosclerosis is also subject to a developing understanding of the correlation between the number of vasa vasorum and wall area in hypercholesterolemic porcine coronary arteries. A role for neovascularization in plaque instability has been widely hypothesized, but direct evidence for it is lacking, partly because the critical factors that precipitate plaque rupture remain largely unknown and partly because reliable animal models of plaque rupture analogous to the human situation have not yet been developed. Nevertheless, studies in human lesions suggest that a spatiotemporal relationship exists between microvessels and the regions of plaques most vulnerable to rupture. Microvessels appear to have a predilection for the shoulder regions of atherosclerotic plaques, whereas a recent study of neovascularization in 269 advanced human atherosclerotic plaques concluded that microvessel formation is strongly correlated with both plaque rupture and the signature features of vulnerable plaques. Thus, an increase in microvessel density occurred in ruptured compared with nonruptured plaques but was also found in the shoulder regions of plaques and was strongly associated with a high degree of macrophage infiltration, intraplaque hemorrhage, and thin-cap lesions. In addition to an association between microvessels and vulnerable plaques, several proangiogenic cytokines are expressed in human lesions (Table 1), lending further weight to the argument that neovascularization is an active process in the atherosclerotic milieu.
mechanisms underlying angiogenesis. The αβ3 integrin has been proposed to play a key role in angiogenesis, being upregulated on endothelial cells when they undergo an “angiogenic switch.”27 Thus, increased expression of αβ3 integrin in adventitial and intraplaque microvessels was taken as evidence of the role of angiogenesis in plaque development.28 However, recent data have challenged the original view that αβ3 plays a major role in pathophysiological angiogenesis and suggest that this integrin may in fact suppress neovascularization.29 Because β3 deficiency increases atherosclerosis in the LDL receptor−/− mouse,30 this may nevertheless be consistent with a role of angiogenesis in the development of atherosclerosis.

The strongest experimental evidence that angiogenesis plays a causative role in atherosclerosis has come from studies in the hypercholesterolemic apolipoprotein E−deficient (ApoE−/−) mouse model (see Table 3). Moulton et al9 found that 2 endothelium-specific inhibitors of angiogenesis, endostatin and TNP-470, reduced plaque area in ApoE−/− mice by 85% and 70%, respectively. This study had a major impact because it provided the first direct evidence that angiogenesis was involved in the process of plaque formation. The same laboratory extended its earlier findings by showing that another angiogenesis inhibitor, angiostatin, reduces atherosclerosis in the same mouse model.31 In some respects, however, the work of Moulton et al has raised as many questions as it answered. As subsequent

### TABLE 1. Expression of Angiogenic Factors in Human Atherosclerotic Lesions and Relationship to Intraplaque Angiogenesis

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Localization Within Plaque</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>αβ3</td>
<td>Expression within macrophage foam cells</td>
<td>100</td>
</tr>
<tr>
<td>VEGF/VEGFR</td>
<td>VEGF-A, VEGF-B, VEGFR-1, and VEGFR-2 staining evident within plaque SMCs</td>
<td>45</td>
</tr>
<tr>
<td>FGF2</td>
<td>Secreted by intraplaque mast cells</td>
<td>101</td>
</tr>
<tr>
<td>PD-ECGF</td>
<td>Expression within plaque macrophages and ECs of plaque neovessels, from coronary atherectomy specimens</td>
<td>102</td>
</tr>
<tr>
<td>PAF</td>
<td>Expression correlated with CD68+ monocytes</td>
<td>103</td>
</tr>
<tr>
<td>PDGF-A and-B</td>
<td>Expression correlated with SMCs and macrophages</td>
<td>104</td>
</tr>
<tr>
<td>HGF</td>
<td>Expression correlated with carotid atherosclerotic plaques but not normal arteries</td>
<td>105</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Expression with activated macrophages, T lymphocytes, and SMCs</td>
<td>106</td>
</tr>
<tr>
<td>HB-EGF</td>
<td>Macrophages and SMCs</td>
<td>107</td>
</tr>
<tr>
<td>IL-8</td>
<td>Protein and mRNA present within coronary atherectomy homogenates</td>
<td>57</td>
</tr>
<tr>
<td>tPA, uPA</td>
<td>Intimal SMCs, macrophage-derived foam cells, and plaque neovessels</td>
<td>108</td>
</tr>
</tbody>
</table>

VEGFR indicates VEGF receptor; SMC, smooth muscle cell; PD-ECGF, platelet-derived endothelial cell growth factor; ECs, endothelial cells; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; HGF, hepatocyte growth factor; TGF, transforming growth factor; HB-EGF, heparin-binding epidermal growth factor–like growth factor; t/uPA, tissue/urokinase-type plasminogen activator, EC, endothelial cell; and SMCs, smooth muscle cells.

### TABLE 2. Occurrence of Neovascularization in Models of Intimal Thickening and Atherosclerosis

<table>
<thead>
<tr>
<th>Model</th>
<th>Incidence of Microvessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE−/− mice</td>
<td>Rare or undetectable in early and small (&lt;100 μm thick) lesions; neointimal microvessels in ~30% of large lesions (&gt;250 μm thick)</td>
</tr>
<tr>
<td>LDLR−/− mice</td>
<td>Adventitial neovascularation detected</td>
</tr>
<tr>
<td>Rat carotid PTCA</td>
<td>Adventitial neovascularation</td>
</tr>
<tr>
<td>Rabbit carotid PTCA</td>
<td>Adventitial neovascularation</td>
</tr>
<tr>
<td>Hypercholesteremic rabbit</td>
<td>No neointimal microvessels detected</td>
</tr>
<tr>
<td>Hypercholesterolic rabbit</td>
<td>Changes in adventitial neovascularization</td>
</tr>
<tr>
<td>Rabbit vein–carotid artery graft</td>
<td>No neointimal microvessels detected</td>
</tr>
<tr>
<td>Canine balloon-injured iliofemoral artery</td>
<td>Neointimal microvessels detected</td>
</tr>
<tr>
<td>Canine vein–femoral artery graft</td>
<td>Neointimal neovascularization when lesions are &gt;250 μm thick</td>
</tr>
<tr>
<td>Porcine coronary artery</td>
<td>Neointimal microvessels detected</td>
</tr>
<tr>
<td>Porcine coronary PTCA and stenting models</td>
<td>Adventitial neovascularation</td>
</tr>
<tr>
<td>Hypercholesterolemia monkey</td>
<td>Adventitial neovascularation</td>
</tr>
</tbody>
</table>

LDLR−/− indicates LDL receptor negative; ND, not determined. See text and Tables 3 and 4 for details and references.
The major effects of VEGF in this study were increases in bone marrow–derived CD34+/Flk1+ endothelial progenitor cells (EPCs) and macrophage/monocytes, plaque endothelial cell density, and macrophage infiltration. In the ensuing vigorous debate, some scientists highlighted apparent discrepancies that call into question the mechanism underlying the proatherogenic effect of VEGF. For example, an increase in circulating CD34+/Flk1+ EPCs was not evident until 2 to 3 weeks after VEGF administration, and plaque macrophage content did not increase until week 3, whereas significant increases in plaque area and endothelial density occurred 1 week after VEGF administration. The causal relationships between the observed effects of VEGF in this study are therefore unclear. A proatherosclerotic effect of VEGF administration was also demonstrated in the hypercholesterolemic rabbit model, although it was not related to plaque angiogenesis.8 While arguing that VEGF might cause “potential destabilization” of plaques, the study also failed to provide direct evidence that VEGF caused plaque destabilization or rupture. Another concern with the work of Celletti et al8 is that long-term effects were apparently elicited by a single bolus administration of VEGF protein, yet whether this resulted in significantly increased circulating levels of VEGF likely to produce meaningful long-term biological effects was not examined. Because animal and human pharmacokinetic studies of VEGF protein indicate that it is cleared from the circulation within a few hours after a bolus intravenous administration,33 the biological efficacy of a single dose must be open to question. Interestingly, another study found that an antibody directed against Flk-1, the mouse homologue of KDR, had no effect on atherosclerotic plaque development in ApoE−/− mice.34 Because KDR/Flk-1 is the major receptor mediating angiogenic effects of VEGF, this suggests that angiogenesis, at least driven by the VEGF/KDR
pathway, is not a major contributor to atherosclerosis in the ApoE<sup>−/−</sup> mouse. This model has also been criticized because most of the cholesterol is carried in large ApoB100-containing VLDL and chylomicron remnants, whereas in both human atherosclerosis and in LDL receptor−deficient mice, cholesterol is predominantly associated with smaller ApoB100-containing LDL particles. Interestingly, a recent study has found that systemic delivery of either VEGF-A protein or adenoviruses encoding VEGF A, B, C, and D had no effect on lesion growth or neovascularization in hypercholesterolemic mice deficient in LDL receptor and ApoB48. The VEGF-related factor, placental growth factor (PIGF), is implicated in the promotion of early atherosclerosis in ApoE-deficient mice acting via Flt-1, but its proatherogenic effects are not mediated by increased angiogenesis. Thus, anti–Flt-1 antibodies inhibited early lesion growth in Apo E<sup>−/−</sup> mice without affecting plaque neovascularization, whereas mice doubly deficient in Apo E and PIGF (Apo<sup>−/−</sup>; PIGF<sup>−/−</sup>) exhibited a reduction in the size, number, and macrophage content of specifically early atherosclerotic lesions compared with their ApoE<sup>−/−</sup> littermates but with no effect on either the number of plaque microvessels (undetectable in early ApoE<sup>−/−</sup> lesions) or the growth of advanced lesions.

A more general problem with studies of VEGF-dependent angiogenesis in atherosclerosis is that, because VEGF causes a complex array of other biological responses, including increased vascular permeability, monocyte chemotaxis, vasodilatation, and hypotension, it is very difficult to attribute the effects of VEGF administration to angiogenesis alone. A further strong counterargument to the view that VEGF and other angiogenic cytokines are proatherogenic is that a variety of preclinical and clinical studies do not support this conclusion. In several clinical trials evaluating the safety and efficacy of VEGF gene and protein therapy for ischemic coronary and peripheral arterial disease, VEGF has displayed an excellent safety profile with no evidence that it enhances atherosclerosis or increases the symptoms of disease. In the VEGF in Ischemia for Vascular Angiogenesis (VIVA) placebo-controlled double-blind trial in 178 patients, intracoronary and intravenous infusion of recombinant human VEGF was safe and well tolerated. However, these observations should be tempered by the lack of clinical efficiency of VEGF as used in this trial.

**Is Hypoxia the Stimulus Driving Intraplaque Neovascularization?**

The notion that angiogenesis contributes to the progression of lesion formation remains attractive, partly because it mirrors recent developments in the understanding of the role of hypoxia in regulating tumor angiogenesis. Theoretical arguments suggest that once vessel wall thickness exceeds a critical depth as a result, for example, of intimal thickening induced by hypercholesterolemia or injury, the supply of oxygen and other nutrients to the media and neointima will be restricted by the increased distance either from the lumen or from adventitial vasa vasorum. A study of oxygen profiles in balloon-injured rabbit iliofemoral arteries found that the arterial wall oxygen supply is impaired after injury but is later compensated for by the formation of new adventitial vasa vasorum. When the critical threshold distance of 100 μm between tissues and a capillary (or vessel lumen) is exceeded, a hypoxic environment will form in the interior of the artery, which in turn will provide a stimulus for the accumulation of hypoxia-inducible transcription factors (HIFs) such as HIF-1α that induce expression of VEGF and other angiogenic regulators. Secretion of VEGF stimulates angiogenesis, thereby promoting plaque growth by increasing the oxygen supply to the media and neointima. Consistent with this hypothesis, expression of VEGF, FGF, and HIF-1α has been demonstrated in atherosclerotic lesions (Table 1). It is important to emphasize that, according to this model, angiogenesis does not initiate plaque formation but serves as a permissive factor allowing later plaque growth once a critical arterial thickness has been reached.

Oxidant stress has been reported as an alternative hypoxia-independent pathway to trigger an angiogenic switch and to enhance arterial lesion formation in a transgenic mouse model overexpressing p22phox, a key component of NAD(P)H oxidase. Extensive neointimal vascularization was observed in the transgenic mice compared with wild-type controls, but the correlation between the extent of neovascularization and lesion size was not discussed.

**Are Inflammatory Cells the Drivers or Passengers of Plaque Neovascularization?**

Plaques vulnerable to rupture are commonly composed of a lipid core separated from the vessel lumen by a thin fibrotic cap and contain higher macrophage counts. Monocytes/macrophages release mitogenic, proinflammatory, prothrombotic, and tissue lytic factors that enhance progression of atherothrombotic lesions. Macrophages and vascular smooth muscle cells (VSMCs) also secrete angiogenic factors, several of which are expressed in human atherosclerotic lesions (Table 1). The shoulder regions of these plaques, where the cap joins the remainder of the arterial wall, constitute rupture-prone “hot spots” where fissures and tears are most likely. These shoulder regions usually possess the greatest density of inflammatory cells, which are capable of secreting extracellular matrix-degrading metalloproteinases (MMPs) that weaken the fibrous cap, thereby facilitating rupture of the atherosclerotic plaque. MMPs and their cosecreted tissue inhibitors have been reported to be critical in atherogenesis and in VSMC migration across the basement membrane. Interleukin (IL)-8, also produced by macrophages infiltrating atherosclerotic plaques, has been shown to have angiogenic activity equipotent to that of VEGF and FGF-2. IL-8 was detected almost exclusively in atheroma-plaque macrophages/macrophages, creating a “neovascular” milieu. In a similar fashion, inflammatory cell release of MMP-9 in tumor progression models mobilizes matrix-bound VEGF and thereby initiates the angiogenic switch. The view that inflammatory cells drive angiogenesis in atherosclerotic plaques is supported by the finding that vasa vasorum density in the atherosclerotic vessel wall.
lesions of ApoE−/− mice is highly correlated with the occurrence of foci of inflammatory cells rather than atheroma size.31 The authors also argued that plaque vessels could promote atherosclerosis by providing a conduit for leukocyte entry, thereby facilitating the recruitment of inflammatory cells to the plaque. In support of this argument, microvessels within lipid-rich plaques strongly express adhesion molecules (ICAM-1, VCAM-1, E-selectin, CD40L) that would facilitate transendothelial migration of inflammatory cells into the plaque microenvironment.59,60 According to such a model, angiogenesis is both the result of and an amplifier of inflammation. However, effects of angiogenic cytokines in atherosclerosis-associated inflammation may not be mediated solely by angiogenesis. As mentioned, administration of an inhibitory antibody directed against the VEGF Flt-1 receptor limited growth of atheromatous plaques in ApoE−/− mice through a reduction in the mobilization and infiltration of hematopoietic cells, including monocytes, granulocytes, and progenitor cells, but without any concomitant inhibition of plaque neovascularization, whereas anti-Flk1 antibody had no effect.54

Also germane to this discussion is recent research indicating that inflammatory cells and mediators are key players in the mechanisms mediating both atherogenesis and collateral vessel formation, something that forms a key component in the “Janus phenomenon” in which interventions that enhance collaterogenesis also increase atherosclerosis.61 Macrophages, for example, are central to both of these processes, whereas several factors that enhance collaterogenesis have also been shown to exacerbate atherogenesis. It is questionable how well VEGF and other angiogenic cytokines fit the Janus phenomenon paradigm, however. As discussed, any apparent convergence between the mechanisms underlying collateral artery formation and atherosclerosis must be qualified in the case of VEGF by weaknesses in previous studies of the atherosclerotic effects of this factor, by results of recent studies of VEGFs and their receptors in mouse models of atherosclerosis (Table 3).34,36,37 by the complexity of VEGF biology, and by the substantial body of clinical experience with angiogenic cytokines, which has not yet indicated a significant proatherogenic effect.

Role of Angiogenesis in Neointimal Growth
Increased neovascularization has been observed at sites of intimal hyperplasia in models of arterial stenting.62 angioplasty,25,63 and venous bypass graft failure.54,65 In pig coronary arteries, adventitial neovascularization correlated with vessel stenosis after balloon injury and stent implantation, and VEGF expression was reported after stenting.66 Neointimal angiogenesis was also observed in autologous vein grafts and their anastomoses in the dog femoral artery when the neointima exceeded 250 µm in thickness.67 However, the existence of a more complex relationship between function of the adventitial vasa vasorum and intimal thickness is indicated by studies showing that adventitial damage may itself trigger neointimal formation. Occlusion of the vasa vasorum in the pig femoral artery stimulated intimal hyperplasia, whereas intimal thickening increased on removal of the adventitia from the rabbit carotid artery and regressed concomitantly with adventitial regeneration.68 These findings suggest that the adventitia may exert an inhibitory effect on intimal hyperplasia,69 whereas a decreased blood supply through the adventitial vasa vasorum could trigger atherogenic intimal thickening.70

The oxygen deprivation hypothesis proposes that adventitial neovascularization is an adaptive response to hypoxia resulting from vessel wall thickening. Hypoxia induces multiple biological responses, including the upregulation of several growth factors and cytokines that are implicated in endothelial and VSMC proliferation and migration.43 The role of angiogenesis in the development of a VSMC-rich neointima has been studied in several animal models by the intravascular or periadventitial delivery of either angiogenic growth factors, particularly VEGF and FGF,71 or antiangiogenic agents (Table 4). These studies have not produced a consensual picture of the role of angiogenesis in neointimal formation, largely because the outcomes of such studies vary depending on the nature of the model, species, and type of stimulus. Interpretation of these studies is also confounded by the fact that several of the proangiogenic factors examined are also, like FGF-2, mitogens for VSMCs or, in the case of PDGF-BB, are primarily VSMC mitogens and indirect angiogenic factors.

In balloon-injury or stent-implantation models in which neointimal lesions are induced by endothelial denudation and arterial injury, the rate of reendothelialization is a critical determinant of neointima formation. Several studies in mouse, rat, and rabbit models of balloon denudation have demonstrated neointima-reducing and, in some cases, antithrombogenic effects of VEGF protein or gene delivery resulting from accelerated endothelial regeneration (Table 5).72–74 However, in a porcine coronary balloon injury model, local periadventitial liposome-mediated VEGF-A165 gene transfer had no significant effect on angiogenesis or intimal hyperplasia.75 Other studies of the influence of VEGF on injury-induced intimal hyperplasia have reached different conclusions. Intramuscular administration of VEGF protein increased intimato-media ratios in the balloon-injured rabbit femoral artery,76 whereas VEGF blockade with sFlt1 attenuated neointima formation induced by intraluminal injury in rabbits, mice, and rats.77 The debate on the role of angiogenic factors in restenosis was recently spotlighted by a series of studies appearing together in a single issue of Circulation that reached different conclusions; VEGF was shown either to inhibit or to promote injury-induced intimal thickening.78 In a situation in which neointimal VSMC proliferation is induced by deendothelialization, the ability of VEGF to accelerate endothelial regeneration may outweigh any neointima-increasing effects that might result from VEGF-induced intralesion neovascularization or inflammatory cell infiltration. However, there appears to be a delicate balance between the neointima-reducing and neointima-increasing effects. Often, balloon catheter injury damages not only the endothelium but also the underlying medial VSMC layer. The study of Khurana et al,79 together with previous work,80 indicates that VEGF can also promote VSMC migration, suggesting that where VSMC injury is extensive, the balance may shift in favor of a neointima-increasing effect. At present, there is scanty evidence of the effect of angiogenic cytokines on graft-induced intimal thickening at an anastomosis, although adenoviral VEGF-A transfer was shown to increase intimal thickening in a rat cardiac allograft model, possibly
TABLE 4. Effects of Angiogenic Factors in Animal Models of Intimal Thickening

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Model and Mode of Delivery</th>
<th>Effect on Neointima</th>
<th>Vessel Wall Microvessels</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-A</td>
<td>Intraluminal protein incubation to rat carotid PTCA model</td>
<td>Decrease</td>
<td>ND; accelerated re-endothelialization</td>
<td>74</td>
</tr>
<tr>
<td>FGF1</td>
<td>Intraluminal protein to balloon-injured iliofemoral artery</td>
<td>Increase</td>
<td>Increased adventitial neovessels</td>
<td>79, 86</td>
</tr>
<tr>
<td>FGF2</td>
<td>Intraluminal gene to balloon-injured porcine iliofemoral artery</td>
<td>Increase</td>
<td>Increased adventitial neovessels</td>
<td>79, 86</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>Intraluminal gene to balloon-injured porcine iliofemoral artery</td>
<td>Increase</td>
<td>Increased adventitial neovessels</td>
<td>79, 86</td>
</tr>
<tr>
<td>HGF</td>
<td>Intraluminal protein to rabbit iliac PTCA model</td>
<td>Increase</td>
<td>Increased adventitial neovessels</td>
<td>79, 86</td>
</tr>
<tr>
<td>PR39</td>
<td>Intraluminal protein to rabbit iliac PTCA model</td>
<td>Increase</td>
<td>Increased adventitial neovessels</td>
<td>79, 86</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 3, plus NZW indicates New Zealand White.

secondary to increased intragraft influx of macrophages and neovascularization within the intimal lesions.81

A different approach to this problem has been to examine the effects of angiogenic stimuli in a model in which intima formation is induced by placement of a perivascular Silastic collar around the adventitia of the rabbit carotid artery,62 without damaging the endothelium. Advantages of this model are that it allows the collar to serve as a localized delivery reservoir for the candidate agent and that the luminal endothelium remains structurally and macroscopically intact. Low-efficiency, liposome-mediated periadventitial VEGF-A gene transfer had a neointima-reducing effect in this model associated with a weak adventitial neovascularization stimulus in the collared arteries of hypercholesterolemic rabbits.84 In contrast, a higher-efficiency adenoviral angiogenesis model also suggests that local concentration of angiogenic factors is a crucial determinant of the therapeutic or biological outcome. Apparently contradictory findings obtained with VEGF in different animal models may be reconciled by relating distinct biological effects of VEGF in the cardiovascular system to concentration.88 At low concentrations resulting from low-efficiency gene transduction methods, VEGF elicits a mainly arterioprotective effect and a weak angiogenic response; at higher concentrations produced by high-efficiency adenoviral delivery, the arterioprotective effect of VEGF may be impaired, and the angiogenic effects may predominate; at still higher concentrations, an excessive and pathophysiological neovascular response may prevail and possibly mediate proatherogenic effects such as intimal thickening and accelerated atherosclerosis. Although such a model cannot explain all findings with induced by VEGF and PR39 to the level seen with a collar alone.
TABLE 5. Effects of Antiangiogenic Factors in Animal Models of Intimal Thickening

<table>
<thead>
<tr>
<th>Antiangiogenic Factor</th>
<th>Model and Mode of Delivery</th>
<th>Effect on Neointima</th>
<th>Vessel Wall Microvessels</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>Intraluminal protein to rat aorta PTCA model</td>
<td>Decrease</td>
<td>ND</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>IV protein to hypercholesterolemic rabbit carotid PTCA and stent model</td>
<td>Decrease</td>
<td>ND</td>
<td>123</td>
</tr>
<tr>
<td>TNP-470</td>
<td>SC protein to rat carotid PTCA</td>
<td>Decrease</td>
<td>ND</td>
<td>124</td>
</tr>
<tr>
<td>Anti–MCP-1</td>
<td>IM plasmid to rat and monkey carotid PTCA</td>
<td>Decrease</td>
<td>ND</td>
<td>125</td>
</tr>
<tr>
<td>Soluble TGFβRII</td>
<td>IV protein to rat carotid PTCA</td>
<td>Decrease</td>
<td>ND</td>
<td>126</td>
</tr>
<tr>
<td>Antisense FGF2</td>
<td>Intraluminal Ad to rabbit femoral PTCA</td>
<td>Decrease</td>
<td>ND</td>
<td>127</td>
</tr>
<tr>
<td>PI-88 (anti-FGF2 signaling)</td>
<td>SC protein to rat and rabbit carotid PTCA</td>
<td>Decrease</td>
<td>ND</td>
<td>128</td>
</tr>
<tr>
<td>VEGF-trap</td>
<td>IV Ad to mouse (C57Bl/6) carotid PTCA</td>
<td>Increase ND; delayed endothelialization</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Soluble Flt1 (anti-VEGF)</td>
<td>Intraluminal gene to rabbit/rat/mouse PTCA model</td>
<td>Decrease</td>
<td>ND</td>
<td>77</td>
</tr>
<tr>
<td>Soluble Flt1</td>
<td>IM plasmid to rat chronic eNOS inhibition model</td>
<td>Decreased medial thickening</td>
<td>ND</td>
<td>129</td>
</tr>
<tr>
<td>Soluble Flt1</td>
<td>Periadventitial protein to NZW rabbit collared carotid artery</td>
<td>No effect on basal IM; inhibition of PR39 stimulation Decreased</td>
<td>ND</td>
<td>79</td>
</tr>
<tr>
<td>Soluble Flt1</td>
<td>Periadventitial protein to rat carotid PTCA</td>
<td>No effect on basal IM</td>
<td>Decreased</td>
<td>79</td>
</tr>
</tbody>
</table>

Ad, adenovirus; PTFE, polytetrafluoroethylene; IV, intravenous; IM, intramuscular; SC, subcutaneous; ND, not determined; VEGF-trap, soluble chimeric VEGF receptor; eNOS, endothelial nitric oxide synthase; and NZW, New Zealand White.

VEGF in models of cardiovascular disease, it is consistent with other studies showing a critical role of VEGF concentration in determining its biological effects in vivo.89

Role of Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) have been widely heralded as having immense promise for the treatment of coronary artery disease, largely because of their ability to regenerate endothelial cells after angioplasty and their potential for the revascularization of ischemic tissue.90,91 However, recent findings indicate that both circulating and bone marrow–derived EPCs and stem cells are a major source of lesion-associated VSMCs, endothelial cells, and plaque microvessels in mouse models of transplant atherosclerosis.92–94 In some respects, the contrasting findings with EPCs parallel the controversy surrounding the role of angiogenesis in atherosclerosis. Much, but not all, of the experimental evidence in favor of a role for both angiogenesis and EPC in atherosclerosis has come from mouse models—in the case of EPCs, largely models of allograft atherosclerosis, whereas several studies support the use of both VEGF and EPCs for reendothelialization of balloon-injured arteries or for the seeding of prosthetic grafts and stents (Table 4).85,90 Furthermore, most of the powerful mobilizers of EPCs such as G-CSF are also proinflammatory, mirroring the Janus phenomenon.51 There is currently too little evidence to say whether EPCs either are a major cause of plaque neovascularization in general or play a role in atherogenesis; equally, the lack of clinical trial data precludes statements about the safety or efficacy of EPC therapy for cardiovascular disease. Notwithstanding the findings that EPCs may contribute to atherosclerotic plaque formation, it seems likely that the momentum currently building behind the medical use of EPC and stem cells in general will lead to clinical trials for cardiovascular disease in the foreseeable future.

Conclusions and Perspectives

There is strong evidence that the development of human atherosclerotic plaques is associated with the formation of new microvessels within the plaque, but experimental support for a causative relationship between neovascularization and lesion growth has proved difficult to establish. Similarly, the hypothesis that angiogenesis contributes to plaque destabilization and rupture currently is based on circumstantial evidence and the “guilty by association” argument. It is also important to recognize that there are limitations inherent in using the results of animal studies as pointers to the human situation. Injury-induced neointima formation in animal models is usually nonocclusive and occurs in an otherwise healthy artery, whereas in human restenosis, an occlusive lesion is superimposed on an already severely diseased vessel. Thus, relatively small but significant changes in intimal thickness in animals may not be a very reliable indicator of effects on human disease. Furthermore, a plethora of studies involving the prototypical angiogenic cytokine VEGF have so far failed to produce a consensual view or unifying model for the role of angiogenesis in either neointimal formation or atherosclerosis (Tables 3 and 4). The apparently paradoxical effects of VEGF and other angiogenic regulators probably reflect several factors, among which are the elusive complexities of vessel wall disease; the multifaceted biology of VEGF,
FGF-2, and other endothelial factors; and the dual role of the endothelium as both guardian of vascular integrity and the essential component of new vessels. Despite the apparent conundrum posed by VEGF, the model proposed in which specifically adventitial neovascularization can promote later neointimal growth without initiating it (the Figure), together with an emphasis on the importance of differences in local VEGF concentration, may be a step toward resolving some of the remaining problems.

The role of angiogenesis in atherosclerosis is likely to be both more complex and dependent on the stage of the disease process. Studies in ApoE−/− and other mouse models of atherosclerosis indicate that neovascularization is either absent from or rare in early lesions but occurs more frequently in larger, advanced plaques. This is consonant with the data from human studies obtained largely from advanced and end-stage lesions. Although these findings are seemingly consistent with the oxygen deprivation theory of plaque angiogenesis, an alternative and perhaps more attractive hypothesis is that lesion neovascularization is driven by the inflammatory milieu of the late plaque. In either case, the evidence indicates that neovascularization is unlikely to be a prerequisite for early plaque growth in small rodent models. Furthermore, recent studies of VEGF and related factors do not indicate that atherosclerosis in ApoE−/− and LDL receptor-deficient mouse models is enhanced by a strong angiogenic stimulus.36,37 In the early stages of human atherosclerosis, adventitial neovascularization may be necessary for significant intimal thickening but not for accumulation of cholesterol-laden macrophages in “fatty streaks.” Additional large animal studies of angiogenesis inhibitors and promoters are clearly required to clarify the role of neovascularization in models of vessel wall disease that mimic the human situation more closely.

The role of angiogenesis in destabilization and rupture of atherosclerotic lesions remains an even more thorny and refractory problem, but one to which recent shifts in thinking about the underlying causes of plaque instability may bring a fresh perspective. It has been argued that VSMC-rich lesions are stable because of their high cellular content, whereas relatively acellular lesions, with a higher degree of calcification, fibrosis, and lipids, are more prone to fracture and rupture.95 Therefore, it could be argued that, by enriching the supply of nutrients to the plaque core, plaque neovascularization may increase plaque cellularity and thereby act as an underlying cause of plaque stabilization. Atheromas develop microvascular channels as a result of neovascularization; these new vessels are both fragile and prone to hemorrhage. Intraplaque deposition of fibrin, fibrin-split products, and hemosiderin provides evidence of intraplaque hemorrhage.96 Thrombosis in situ leads to generation of thrombin, which potently triggers the release of PDGF, further stimulating the migration and proliferation of VSMCs. Activated platelets also elaborate transforming growth factor-β, the most potent stimulus known for interstitial collagen synthesis by VSMCs.97 Hence, plaque neovascularization and subsequent silent microvascular hemorrhage could plausibly participate in a virtuous cycle of growth spurts that might contribute as much to plaque stability as to instability.

It remains to ask whether inhibition of angiogenesis could be a therapeutic target in atherosclerotic disease. The available evidence suggests that, although antiangiogenic therapies may potentially have some effect on the growth of atherosclerotic and neointimal lesions, particularly in vein graft stenosis and restenosis after angioplasty, any benefit is likely to be nullified by the harmful effects of inhibiting endothelial function and regeneration. This is also supported by work from a number of laboratories indicating that VEGF exerts protective effects on the arterial endothelium.83–85 Recent evidence from clinical trials of the VEGF inhibitory antibody Avastin or bevacizumab for cancer indicates that up to 5% of all patients treated with Avastin may have an increased risk of thromboembolism, including cerebrovascular events, myocardial infarction, and deep vein thrombosis.98,99 If borne out by further work, these findings suggest that endogenous VEGF may play an arterioprotective role in the adult human vasculature. The multiplicity of the biological roles of VEGF and the importance of endothelial integrity for vascular function are both strong arguments currently militating against an antiangiogenic approach to the treatment of cardiovascular disease. The prospects for proangiogenic therapy for ischemic heart disease appear better but still require unambiguous support from clinical studies.

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References


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