71

Vascular Remodeling in Health and Disease

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The term *vascular remodeling* is commonly used to define the structural changes in blood vessel geometry that occur in response to long-term physiologic alterations in blood flow or in response to vessel wall injury brought about by trauma or underlying cardiovascular diseases.[1–4] The process of remodeling, which begins as an adaptive response to long-term hemodynamic alterations such as elevated shear stress or increased intravascular pressure, may eventually become maladaptive, leading to impaired vascular function. The vascular endothelium, owing to its location lining the lumen of blood vessels, plays a pivotal role in regulation of all aspects of vascular function and homeostasis.[5] Thus, not surprisingly, endothelial dysfunction has been recognized as the harbinger of all major cardiovascular diseases such as hypertension, atherosclerosis, and diabetes.[6–8] The endothelium elaborates a variety of substances that influence vascular tone and protect the vessel wall against inflammatory cell adhesion, thrombus formation, and vascular cell proliferation.[8–10] Among the primary biologic mediators emanating from the endothelium is nitric oxide (NO) and the arachidonic acid metabolite prostacyclin [prostaglandin I_2 (PGI_2)], which exert powerful vasodilatory, antiadhesive, and antiproliferative effects in the vessel wall. In addition, the endothelium produces a variety of vasoconstrictor and proadhesion molecules such as endothelin-1, angiotensin II (Ang II), and thromboxanes, which counteract the effects of NO and PGI_2. In normal conditions these opposing modulators from the endothelium are in equilibrium, and vessel wall homeostasis is maintained.[5] However, in the presence of sustained pathophysiologic stimuli such as hypertension, hyperlipidemia, and hyperglycemia, the availability of protective moieties such as NO and PGI_2 are reduced, leading to increased vascular tone and enhanced inflammatory cell and platelet adhesion and proliferation of the media smooth muscle, which may increase the occurrence of thrombosis and vascular occlusion.[7–10]

Vascular remodeling is a complex and highly regulated process involving the activation of multiple signaling cascades and downstream transcription factors, which function coordinately to induce the expression of a plethora of genes involved in vascular cell growth, proliferation, apoptosis, migration, and adhesion, and extracellular matrix synthesis and breakdown.[10–15] All components of the vascular wall appear to be involved in the remodeling process,[16–18] and recent evidence suggests that circulating smooth muscle and endothelial progenitor cells originating from the bone marrow may actively participate in vessel remodeling in atherosclerosis and in restenosis associated with revascularization procedures such as angioplasty and bypass vein grafting.[19–25] It is now apparent that oxidative stress is a primary cause of endothelial dysfunction, and reactive oxygen species produced by the vascular cells and infiltrating inflammatory cells play a central role in activating the molecular signals leading to vessel remodeling.

Therapeutic strategies to prevent the negative effects of vessel remodeling have focused on the use of drugs aimed at normalizing blood pressure and circulating cholesterol and glucose levels. Drugs targeting the activity of the renin-angiotensin system [angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers], hepatic hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors (statins), and activators of peroxisome proliferator-activated receptors (fibrates, glitazones) have been shown to improve endothelial function and to inhibit vascular remodeling in hypertensive and hypercholesterolemic patients.[26–29] Surgical strategies using drug-eluting stents releasing cytostatic drugs such as rapamycin have been reported to reduce the rate of restenosis in patients undergoing percutaneous angioplasty and bypass grafting,[30–33] and novel genetic and cell therapy modalities are emerging on the horizon for treatment of vasculoproliferative disease and repair of damaged blood vessels.[34,35]

This chapter reviews the basic mechanisms involved in vascular remodeling in physiologic and pathophysiologic conditions, and the current and emerging therapies for prevention of remodeling in vascular disease.

Vascular Remodeling in Physiologic and Pathologic States

The type and magnitude of structural change that a blood vessel endures during the process of vascular remodeling is determined not only by the type of stimuli impacting those changes but also by the location of the vessels, such that, for example, the type of remodeling in small resistance vessels from hypertensive patients is different from the remodeling taking place in large conduit arteries.[1–3] Furthermore, although changes in hemodynamic forces (pressure, flow) are the primary determinants of remodeling in physiologic and pathologic conditions,[1–3,36–38] a variety of pathophysiologic stimuli such as inflammation,[1,13,14] oxidative stress,[1,8,40] and apoptosis[2,12] contribute to vascular remodeling in vascular disease, leading to endothelial dysfunction and an imbalance in the production of vasoregulatory and growth-promoting and growth-inhibiting factors, resulting in abnormal vascular function and structure. The most common cardiovascular disorders that are characterized by abnormal vascular function and structure are hypertension, atherosclerosis, postangioplasty restenosis, and graft atherosclerosis.[1,2,8,12,13,39,41–43] Although the pathophysiologic processes of these disorders are complex and involve the participation of multiple cell types, biologically active molecules, extracellular matrix modulation, and so forth, a predominant feature of all these diseases is abnormal smooth muscle growth.[41–43]

Vascular Remodeling in Response to Alterations in Blood Flow

The relationship between blood flow and vessel structure was first demonstrated by Langille and O'Donnell.[38] In their seminal study, these authors showed that a reduction in flow for up to 1 month through the common carotid of young rabbits led to a decrease in vessel diameter of approximately 70%. Conversely, an increase in basal blood flow was associated with an increase in vessel diameter. This chronic effect was due to a *structural* rather than a functional modification of the arterial wall. The alterations in vessel diameter brought about by changes in blood flow were associated with alterations in elastin accumulation and medial smooth muscle proliferation, which are deposited in the circumferential direction, where flow reduction led to a marked decrease in elastin and DNA accumulation in the vessel wall.[38] The vascular remodeling response to changes in flow appears to be exclusively mediated by the endothelium, because the flow-induced changes in vessel diameter are fully abolished by removal of the endothelium.[39,44] The endothelial mechanism mediating these structural effects remains undefined. A plausible mechanism is that the endothelium senses alterations in shear stress and induces changes in vessel structure by producing mediators that regulate cell growth, extracellular matrix production, and proteolysis. Although many mediators may be involved in vascular remodeling, experimental evidence suggests that flow stimulates the release of NO, platelet-derived growth factor (PDGF), and transforming growth factor-β_1 (TGF-β_1) (reviewed elsewhere[10,45]). Nitric oxide, in particular, plays an obligatory role in flow-induced vascular remodeling. For example, Tronc and colleagues[46] showed that the increase in vessel diameter associated with increased blood flow in a rabbit arteriovenous fistula model was abolished by chronic treatment with the nitric oxide synthase inhibitor NG-nitro-L-arginine methylester. Similarly, using a rat model of combined unilateral external and internal carotid ligation, Miyashito and colleagues[47] reported flow-induced adaptive remodeling of the contralateral artery with increased flow. Conversely, reduction in blood flow in the common carotid of endothelial nitric oxide synthase (eNOS) knockout mice failed to elicit the expected decrease in vessel diameter, leading instead to a paradoxical increase in wall thickness associated with media hyperplasia, whereas the wild-type mice responded normally with a reduction in internal diameter in response to the decrease in flow triggered by ligation of the ipsilateral external carotid artery.[44] These authors further documented the time course of reduced flow-induced remodeling of the common carotid in mice and reported that the decrease in vessel diameter, although fully reversible 3 days after ligation by perfusion with vasodilators, the reduction in diameter was permanent after 7 days of flow reduction in association with impaired NO production.[48]

There are multiple examples where physiologic alterations in basal blood flow are accompanied by vessel remodeling. For example, epicardial coronary arteries were reported to be enlarged in physically active rats compared with sedentary litter mates,[49–52] and short-term exercise training of dogs produces a significant increase in epicardial coronary artery diameter of their epicardial coronary arteries,[53] presumably to accommodate the required need for increased myocardial blood flow. Similarly, men with physically active occupations have larger than expected coronary arteries.[54,55] A commonly cited example is the case report based on the autopsy of the marathon runner Clarence De Mar, which reported epicardial vessels that were "two or three times the normal size."[56] Mann and colleagues[57] found that vigorously active Masai tribesmen dying of noncardiovascular causes and with no clinical evidence of coronary disease had as much coronary atherosclerosis at autopsy as American men but had patent arterial lumina because of the large size of their epicardial vessels. Rose and associates[54] at autopsy studied the hearts of a group of men and women with and without infarction and found an association between increasing physical activity of occupation and increasing coronary artery diameter. Several cross-sectional[58,59] and exercise-training[60,61] studies have demonstrated that in trained men and women, there is significantly greater hyperemic blood flow in the calf and forearm, even after correction for differences in muscle mass. These results are consistent with a greater capacity for dilatation in the vasculature of trained skeletal muscle unrelated to muscle hypertrophy. The increased blood flow appears to be due to adaptation of the vascular structure, possibly an increase in the caliber or number of resistance arterioles. The beneficial effects of exercise training at least in part may be due to flow-induced changes in vascular structure and reactivity, probably associated with enhanced basal NO production.[60,61] Others have shown that endothelial dependent vasomotor responses are modulated by alterations in blood flow. For example, Miller and colleagues[62] showed that an increase in femoral blood flow for 6 weeks in dogs via arteriovenous fistula led to enhanced endothelial function in the vessel exposed to increased flow. Conversely, a chronic decrease in blood flow

associated with low cardiac output leads to reduced endothelial function in animal models of heart failure.[63,64]

Flow-related changes in vascular remodeling have also been seen in clinical situations. Perhaps the most striking example is seen in patients with arteriovenous fistulas. In these patients, coronary flow is dramatically increased in association with a marked increase in the diameter and tortuosity of the vessel involved in the fistula.[65] Evidence of flow-induced remodeling is also often seen in patients with coronary artery disease. Glagov and associates[66] found that many atherosclerotic vessels with significant atheroma maintained normal lumen diameter via enlargement of the vessel wall. They proposed that as the lumen is narrowed by accumulating atheroma, the resultant increase in shear stress may induce remodeling of the vessel wall akin to flow-induced remodeling.

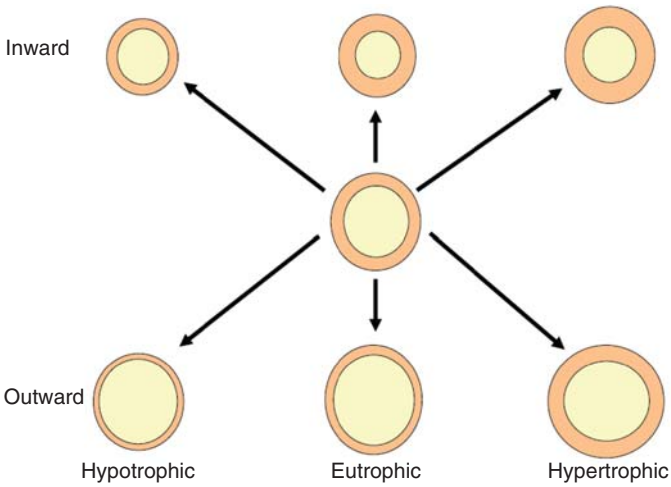

FIGURE 71.1. Potential outcomes of vascular remodeling. Remodeling may be eutrophic, in which case the wall materials are rearranged without a net increase in media cross-sectional area. In hypertrophic remodeling, there is net increase in media cross-sectional area through hypertrophy and hyperplasia of the media vascular smooth muscle cells and possibly fibroblasts and vascular progenitor cells from the adventitia. In hypotrophic remodeling, there is a net decrease in media cross-sectional area through loss of cells via apoptosis. Remodeling may be inward in which there is a reduction in lumen diameter, or outward, in which the lumen diameter is increased. In hypotrophic remodeling the media-lumen ratio is decreased, whereas in hypertrophic remodeling the wall-lumen is increases.

Vascular Remodeling in Hypertension

The predominant hemodynamic alteration in hypertension is increased total peripheral resistance, which is primarily determined by the small terminal arteries (<500μm) and arterioles.[2–4] Several lines of evidence suggest that the increase in vascular resistance in hypertension is due primarily to rarefaction of small vessels and luminal narrowing due to vascular remodeling, with the later alteration predominating in most forms of hypertension.[3,4] In essential hypertension, the resistance vessels undergo adaptive changes that are characterized by reduced lumen and increased media/lumen ratios.[3] This remodeling of vessel geometry serves to normalize wall stress, but also increases basal vascular reactivity, amplifying the vascular response to vasoconstrictors and thereby perpetuating hypertension.[2,4] The remodeling of resistance vessels in essential hypertension appears to be due to rearrangement of the wall material around a smaller lumen, without a net gain in media cross-sectional area or in the size or number of smooth muscle in the media.[2,3] This type of remodeling is known as *eutrophic remodeling* (Fig. 71.1). In other types of hypertension such as in renal hypertension, the remodeling process involves hypertrophy and possibly hyperplasia, as well as extracellular matrix accumulation triggered by Ang II and other growth factors,[67] leading to *hypertrophic remodeling* (Fig. 71.1), which is a process of active growth of the vessel wall that is characterized by increased cross-sectional areas. This type of remodeling is seen in hypertension induced by the administration of deoxycorticosterone acetate and a high-salt diet [deoxycorticosterone acetate (DOCA) salt], and is associated with increase in the steady-state levels of TGF-β_1 and PDGF receptor gene expressions in the aorta.[68,69] However, some investigators have argued that the term *remodeling* should strictly be used to describe structural rearrangement of the vessel wall without any increase in wall cross-sectional area.

A myriad of recent studies have focused on deciphering the molecular mechanisms involved in hypertension-induced vascular remodeling. Although a large percentage of these studies have been carried out in cell culture systems, the emerging picture is that vascular remodeling is regulated by an intricate mechanism involving the synergistic activation of multiple signal transduction pathways by growth factors and hemodynamic forces.[14,15,37,70] It is now clear that reactive oxygen species generated by pro-oxidant enzyme systems such as reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, and uncoupled eNOS play an essential role in activating these signaling pathways and in triggering the sequelae of events that culminate in remodeling of the vessel wall.[8,12,40,70–76] Apoptosis, inflammation, and extracellular matrix remodeling have all been considered key effector elements of vascular remodeling, but their role in hypertension-induced vessel remodeling remains controversial.[2,12,13,77] In principle, apoptosis and inflammation could be triggered by a variety of hypertensive stimuli including growth factors and reactive oxygen species (ROS).[2,10,40,67,72,73] Regarding apoptosis, enhanced rate of apoptosis has been reported in the aorta of DOCA salt and spontaneously hypertensive rats (SHRs) compared to normotensive controls[78,79] (reviewed elsewhere[2]). Furthermore, cultured vascular smooth muscle cells (VSMCs) from hypertensive animals are more prone to apoptosis than cells from normotensive animals.[80] A combination of growth and apoptosis has been suggested to mediate eutrophic remodeling.[2,3,81] However, the rate of apoptosis in resistance arteries of SHRs has been reported to be lower than in normotensive animals,[82] thus raising doubts about the role of apoptosis in this type of vessel remodeling, at least in this genetically hypertensive animal model. The mechanisms initiating apoptosis of VSMCs in hypertension have not been fully elucidated, but appear to involve ROS and growth modulators such as angiotensin and NO.[40,71,72,83]

The role of inflammation in hypertension-mediated remodeling is also controversial. ROS and Ang II, via its

effect on ROS production, activate the transcription factors nuclear factor (NF)-κB and AP-1, which regulates the transcriptional activity of a number of redox sensitive genes involved in the inflammatory response.[13,14,40,73,74] Among these are adhesion molecules such as vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM), cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and chemokines such as monocyte chemoattractant protein-1 (MCP-1), which participate in the recruitment and infiltration of inflammatory cells into the vessel wall.[10,39,43] For example, hypertensive rats harboring the human renin and angiotensinogen transgenes showed increased adhesion molecule expression and avid infiltration of inflammatory cells in the kidney and heart vascular beds,[84] thus suggesting a role of Ang II in triggering inflammatory events in these vascular beds. However, the role of Ang II in promoting inflammation in other resistance vessels during development of hypertension is not known.

In addition to alterations in cell growth, vascular remodeling in hypertension involves changes in the extracellular matrix.[2] Extracellular matrix remodeling in hypertension involves both synthesis and breakdown of matrix components by matrix metalloproteinases (MMPs). Several studies have demonstrated the accumulation of extracellular matrix proteins in hypertensive animal models. Collagen I, III, and fibronectin levels are increased in vessels from SHRs, DOCA salt– and Dal salt–sensitive hypertensive rats,[85–91] and in humans with essential hypertension.[92] The increase in collagen content in the extracellular matrix contributes to the increased stiffness commonly seen in arteries from hypertensive animals. Angiotensin II plays a dominant role in stimulating collagen production by smooth muscle cells via activation of angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors.[93,94] The mechanism involves activation of the mitogen-activated protein kinase/ERK pathway and the participation of autocrine mechanisms mediated by TGF-β and PDGF.[95] The accumulation of extracellular matrix proteins in hypertensive vessels may be partly associated with decreased MMP activity. The activities of several MMPs were reported to be decreased in mesenteric arteries of young SHRs before the onset of hypertension,[96] leading to decreased collagen degradation.

Vascular Remodeling in Atherosclerosis

Atherosclerosis is now widely recognized as an inflammatory disease.[39,97] Inflammatory processes play a pivotal role in atheroma formation, and endothelial dysfunction plays an essential role in the mediating the inflammatory response triggered by hyperlipidemia.[6–8,39,97] The dysfunctional endothelium expresses VCAM and other adhesion molecules, which help recruit leukocytes, thus initiating the process of inflammation of the vessel wall.[12,39] The induction of VCAM is triggered by the inflammatory response mounted by the modified lipoproteins that lodge in the subintimal space. The mechanisms of induction are transcriptional, regulated by the redox-sensitive transcription factor NF-κB, which is activated by upstream signaling mechanisms that are sensitive to ROS and cytokines such as TNF-α. The monocytes adhering to the activated endothelium then migrate into the subintimal space by diapedesis.[13,39,97] The migration of monocytes requires a chemokine gradient that is mediated by MCP-1, produced by activated vascular smooth muscle cells in the media; MCP-1 interacts with its cognate receptor CCR2, which is expressed in the diapedesing monocytes. Once in the subintimal space, the infiltrating monocytes acquire the properties of macrophages and express scavenger receptors (SRA) and CD36, which take up modified lipoprotein particles, becoming foam cells in the process and forming the fatty streak, a hallmark of the developing atherosclerotic lesion.[39,97] The foam cell is a secretory factory, producing a variety of ROS, proinflammatory cytokines, growth factors, and MMPs, which not only aggravate the inflammatory state of the vessel wall but also stimulate the migration and proliferation of smooth muscle cells from the media and possibly fibroblasts and vascular progenitor cells from the adventitia and bone marrow into the subintimal space.[19–21,39,98–100] In the subintimal space, the migrated smooth muscle cells and other vascular progenitors proliferate to form a neointima and a fibrous cap that covers the developing atheroma, preventing it from contact with the blood.[39,43,97] The process of smooth muscle cell migration is aided by digestion of the extracellular matrix by MMPs. However, as the lesion progresses, the proinflammatory state of the atheroma leads to enhanced extracellular matrix degradation and accentuated apoptosis of smooth muscle forming the fibrous cap, leading to thinning of the cap and plaque instability.[39,97,101] The unstable plaque may eventually rupture, exposing the highly thrombogenic subendothelial contents of the atheroma, resulting in thrombus formation and vessel occlusion.[101] It is now known that the plaque undergoes a dynamic process of remodeling associated with matrix turnover and vascular cell proliferation and apoptosis,[102–106] which ultimately determines the stability of the plaque.[101]

Vascular smooth muscle proliferation is a prominent and essential component of atherosclerosis development.[41–43,97] Although avid VSMC proliferation in the plaque leads to stenosis of the vessel, VSMC proliferation is also essential for plaque stability.[2,39,43,97,101,104,105] Thus, new therapies for atherosclerosis should aim at achieving a balance between VSMC proliferation and stabilization of the plaque, such that reduction of lumen stenosis is not achieved at cost of plaque vulnerability. Considering the central role of ROS and inflammation in atheroma formation and in neointimal smooth muscle proliferation, it seems logical that reducing oxidative stress and inflammatory processes should be a primary therapeutic target in atherosclerosis.[97] For example, lipid-lowering drugs such as statins (HMG CoA inhibitors) and fibrates, as well as Ang II antagonists such as ACE inhibitors and angiotensin receptor antagonists exert beneficial effects in treatment of atherosclerosis that are largely unrelated to reduction of lumen stenosis.[97] Instead, these drugs appear to exert pleiotropic effects in the vessel wall by reducing inflammation and oxidative and inhibiting the sequence of pathologic events triggered by these stimuli,[29] including endothelial dysfunction and VSMC proliferation.

Vascular Remodeling in Vasculoprotective Disease

In response to injury induced by interventional procedures such as balloon angioplasty and bypass grafting, a reparative process is activated that may lead to restenosis. Excessive

TABLE 71.1. Phases leading to restenosis

Phase	*Events*	*Duration*
I. Acute injury and release of mediators	Endothelial denudation; interaction of platelets and thrombin with the vessel wall; release of growth factors and cytokines	Minutes to hours
II. Smooth muscle replication and inflammation	A. Activation and replication of medial VSMCs; migration of medial VSMCs to intima	Days to weeks
	B. Replication of intimal VSMCs	Days to months
	C. Leukocyte infiltration and replication	Days to months
III. Vascular remodeling	Modulation of extracellular matrix and shrinkage Remodeling	Weeks to months

VSMCs, vascular smooth muscle cells.

vascular smooth muscle proliferation is the hallmark of vasculoprotective diseases,[33,43,107] leading to neointima formation and stenosis of the vessel or graft. Recent evidence suggests that circulating progenitor cells may also contribute to neointima development.[19,22,24,25] The neointima provides a substrate for the development of atherosclerosis, which represents the terminal step in the pathologic process leading to transplant vasculopathy and in bypass graft failure.[43,108] Given the central role of vascular smooth muscle proliferation in the pathogenesis of these diseases, it is not surprising that current and emerging therapies are focusing on strategies to inhibit cell proliferation. Prominent among these is the use of cytostatic agents to inhibit cell cycle entry, and several advances have been made in this direction with the introduction of drug-eluting stents capable of releasing cytostatic drugs such as rapamycin and paclitaxel. Cytotoxic strategies such as brachiotherapy have found less wide acceptance in clinical application.[109] Molecular therapies aimed at targeting specific cell-cycle regulatory molecules may provide new options for treatment of vasculoproliferative disease in the future, and proof-of-concept experiments have already provided validation of these strategies in animal models of vascular injury (reviewed elsewhere[43]). The recent availability of methods for isolation and manipulation of endothelial progenitor cells and other adult stem cells provides yet another option for vascular repair following injury, and early animal studies provides support for the therapeutic potential of these adult stem cells in vessel repair and tissue engineering (reviewed elsewhere[34]).

The development of restenosis involves cellular and noncellular events that may be arbitrarily divided into three phases (Table 71.1), according to the sequence of events compiled from studies of several animal models and analysis of human histologic, angiographic, and intravascular ultrasound data.

Phase I: Acute Injury and Release of Mediators

Balloon angioplasty causes local vascular injury, including endothelial denudation, rupture of the internal elastic lamina, lysis of some medial VSMCs, and fracture of the atherosclerotic plaque. With further stretching, medial dissection may result in subsequent dilatation of the outer media and adventitia. Initiated by this mechanical injury, phase I is characterized by interaction of platelets and thrombin with the vessel wall (phase IA) and release of numerous biologically active mediators (phase IB). These events occur over minutes to hours after injury.

Phase IA: Activation of Platelets and Thrombin

Exposure of the subintimal layers and collagen to bloodborne elements leads to activation of the hemostatic system with extensive platelet deposition and fibrin formation.[110] Platelet aggregation is mediated by release of adenosine diphosphate, serotonin, thromboxane A_2, fibrinogen, fibronectin, and von Willebrand factor. Platelets make contact with subendothelial layers and other platelets by glycoprotein Ib and IIb/IIIa receptors. Interestingly, the thickness of the deposited platelet layer and the predisposition to thrombus formation are proportional to the amount of subendothelial injury. With severe injury, thrombus formation ultimately may contribute to an organized fibrocellular plug. These initial events begin within minutes after injury, peak 4 to 12 hours later, and are sustained for at least 24 to 48 hours. Thrombin generation (through the intrinsic and extrinsic pathways) can promote platelet aggregation and fibrin production. Thrombin generation by apoptotic VSMCs has also been described.[111] This may be important in light of reports describing significant VSMC apoptosis immediately after balloon injury.[112,113] Thrombin has been shown to stimulate growth factor release, VSMC proliferation, and alterations in extracellular matrix composition.[114,115] Fibrin is also chemotactic for VSMCs in vitro. Therefore, the activation of thrombin and the coagulation cascade are likely to contribute to the development of neointimal hyperplasia.

Phase IB: Release of Growth Factors and Cytokines

A number of vasoconstrictors (e.g., thromboxane and serotonin) and mitogens are released by activated platelets, the most important of which are PDGF, epidermal growth factor, and TGF-β_1.[116] As a chemotactic and mitogenic agent, PDGF is a potent stimulus to VSMC migration and proliferation. A polyclonal antibody to PDGF has been shown to reduce neointima formation in rats. Mechanical injury itself may lead to VSMC proliferation by resulting in denudation of the endothelium, with consequent loss of endothelium-derived growth inhibiting factors such as NO and prostacyclin and release of growth factors from the injured ECs and VSMCs. Fibroblast growth factor appears to be one such mitogen for ECs and VSMCs.[117,118] Reduction in the levels of these important inhibitors of VSMC growth and migration contributes to the initial process of neointima formation.

Phase II: Smooth Muscle Replication and Inflammation

The intermediate phase is characterized by initial activation and replication of medial VSMCs followed by migration of VSMCs from the media to the subintima (phase IIA). This process occurs over a period of time lasting days to weeks, and is followed by VSMC replication, initiating the development of neointimal hyperplasia (phase IIB), which may last weeks to months. Concomitant to these processes is the infiltration of leukocytes brought into the area by cytokines and chemotactic agents as well as adhesion molecules and the proliferation of these inflammatory cells (phase IIC).

Phase IIA: Medial Smooth Muscle Cell Replication and Migration

Approximately 30% of medial VSMCs become activated within the first few days after balloon angioplasty[119] by PDGF (released from platelets, macrophages, injured ECs, and VSMCs), thrombin, fibroblast growth factor (FGF) (from injured ECs and VSMCs), and other factors. These cells increase DNA synthesis, express the "synthetic" phenotype, and begin to replicate.[120,121] With extensive injury, up to 30% of the medial VSMCs may migrate to the subintimal space[122] and replicate, usually beginning in the first few days after angioplasty.[123] Although PDGF appears to be a principal growth factor stimulating cells to migrate, FGF, Ang II, and changes in the extracellular matrix (with expression of proteolytic enzymes) may also participate.

Phase IIB: Replication of Intimal Vascular Smooth Muscle Cells

This phase is characterized by "autoreplication" of VSMCs that have migrated to the intima. In humans, intimal hyperplasia can be detected by the second to third week and appears to plateau by the third to fourth month after coronary angioplasty.[121,123] During this proliferative phase, intimal VSMCs, fibroblasts, and macrophages express autocrine and paracrine growth factors, including PDGF, FGF, insulin-like growth factor-I (IGF-I), TGF-β, and Ang II. These local factors play an important role in stimulating VSMC proliferation.

Phase III: Vascular Remodeling

An active process of extracellular matrix modulation occurs in restenosis. There is evidence of both matrix deposition and degradation. Fibroblast and inflammatory cells contribute to these processes. As the intimal VSMCs lose their capacity to replicate, they also produce large amounts of extracellular matrix proteoglycan. Experimental and clinical studies, especially those using intravascular ultrasound, have suggested that a reduction in vessel caliber resulting from vascular remodeling may play an important role in the narrowing of the restenotic segment.[124] Although metalloproteinase inhibitors have been shown to affect the early response after balloon injury by influencing VSMC cell migration, they have not been shown to affect restenosis.[125]

Role of Progenitor Cells in Atherosclerosis and Vasculoproliferative Disease

In addition to resident VSMC and fibroblasts from the adventitia, recent evidence suggests that circulating smooth muscle progenitor originating in the bone marrow may also contribute to the pathogenesis of vascular remodeling in atherosclerosis and vasculoproliferative disease (reviewed elsewhere[19]). Using mouse models of postangioplasty restenosis, graft vasculopathy, and hyperlipidemia, Sata and colleagues[22] showed that hematopoietic stem cells from the bone marrow can transdifferentiate into smooth muscle cells. Furthermore, these authors reported that the bone-marrow–derived vascular progenitors gave rise to the majority of the smooth muscle cells participating in neointima formation and graft atherosclerosis; however, the relative percentage contribution of these cells to remodeling has been questioned by others[20,21,126] (reviewed elsewhere[19]). Circulating smooth muscle progenitors have also been reported in human peripheral blood after culture of mononuclear cells from the buffy coat in medium containing PDGF-BB.[127] More recently, Caplice and colleagues[23] reported the presence of donor-derived smooth muscle cells in autopsy specimens of atherosclerotic lesions collected from patients who had previously undergone gender mismatched bone marrow transplantation for treatment of leukemia. The authors reported significant recruitment of donor-derived cells to the sites containing atherosclerotic lesions but not in healthy vessels.

In contrast, to these findings, Hu and colleagues[20] did not find any evidence of bone-marrow–derived progenitors in vein graft atherosclerotic lesions. In these studies, the authors isografted segments of the vena cava to the carotid artery between transgenic mice expressing the bacterial β-galactosidase gene (*LacZ*) in vascular smooth muscle cells (*SM-LacZ*) or in all tissues (*ROSA26*) and wild type. The investigators reported the presence of β-galactosidase in all neointimal and atherosclerotic lesions in all of the chimeric vein grafts made between the *LacZ* transgenic mice and the wild type. Further characterization of the cells in the lesion revealed that approximately 60% of the cells originated from the donor vessel. The possibility that the cells in the lesion originated in the bone marrow was excluded by the absence of β-galactosidase positive cells in atherosclerotic lesions of chimeric mice that had undergone lethal irradiation and bone marrow reconstitution with β-galactosidase marked cells.[20] These investigators subsequently reported that the adventitia may be the source of progenitor cells accumulating in the atherosclerotic lesions.[21] The authors found that the adventitia of aortic roots yielded an abundance of cells expressing cell surface stem cell markers such as Sca-1, c-*kit*, CD34, and Flk-1. Furthermore, culture of these cells gave rise to heterogeneous outgrowths that could be directed to specific vascular lineages under defined growth conditions.[21] They further demonstrated that transplantation of the adventitia-derived Sca-1$^+$ cells from ROSA26 mice to the adventitial side of vein grafts in apolipoprotein E (ApoE)-deficient mice resulted in migration of the Sca-1$^+$ cells to the atherosclerotic lesion where they differentiate into smooth muscle cells (SMCs) that populate approximately 30% of the neointima.[21] However, the ontogeny of the adventitial cells is not known. It has not been identified whether these cells

are bona fide resident vascular precursor cells, or whether they represent bone-marrow–derived progenitors that have migrated earlier in development to take up residence in the adventitia. Others have reported that bone marrow progenitors do not contribute significantly to endothelial regeneration in transplant arteriosclerosis.[26] This finding is intriguing, in light of the fact that vascular injury has been reported to be a strong stimulus for recruitment and homing of bone-marrow–derived endothelial progenitor cells. The role of progenitors in the pathogenesis of human vascular disease and in vessel repair has not yet been established.

Molecular Mechanism of Vascular Remodeling

It is now known that the adaptations of the vessel wall to physiologic and pathologic alteration in hemodynamic forces share several mechanisms in common, namely apoptosis and proliferation of vascular cell types, extracellular remodeling, and rearrangement of wall materials to accommodate the new hemodynamic status. However, whereas the adaptations of the vessel wall to physiologic alterations in pressure and flow remain within the realm of homeostasis, the changes that occur in response to abnormal changes in hemodynamic forces in vascular disease become maladaptive in the face of pervasive oxidative and inflammatory stresses, which lead to endothelial dysfunction and dysregulation of apoptosis and growth processes.

Endothelial Dysfunction

Endothelial dysfunction is the earliest event that signals the onset of cardiovascular disease. This alteration in endothelial function is characterized by decreased vasodilation and increased proinflammatory and prothrombotic activity of the endothelial cells (Fig. 71.2). In normal conditions, NO exerts multiple effects that are essential for maintenance of vessel wall homeostasis.[5] These include vasodilation, inhibition of vascular smooth muscle proliferation and migration, and downregulation of inflammatory and adhesion molecules. However, risk factors for cardiovascular disease induce oxidative stress, which plays a major role in endothelial dysfunction.[6,7] Reactive oxygen species accelerate the catabolism of NO and activate redox-sensitive transcription factors such as NF-κB, which upregulate the transcription of various proinflammatory genes, chemokines, adhesion molecules, and prothrombotic factors in the endothelium.[8,11,13,43,72,73,75,76,127] The activated endothelial cells produce excessive amounts of ROS and adhesion molecules, resulting in increased vascular tone, microvascular dysfunction, and enhanced leukocyte adhesion.[73] Thus, reduced NO bioactivity provides a link between oxidative stress, endothelial dysfunction, and the pathogenesis of cardiovascular disease.

Role of Oxidative Stress

Reactive oxygen species play a major role in the initiation and progression of cardiovascular diseases such as hypertension and atherosclerosis.[8,71,72] In the vascular wall, ROS is produced by multiple sources, including the endothelial cells, VSMCs, and infiltrating inflammatory cells.[71] The production of ROS in excess in the vascular wall overwhelms endogenous antioxidant systems, resulting in oxidative stress. Within the vessel wall, the predominant ROS are the free radical superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). $O_2^{\cdot-}$ is produced enzymatically primarily by reduced nicotinamide adenine dinucleotide (NADH) oxidase and to a lesser extent by xanthine oxidase and myeloperoxidase.[71,73,74,103] In addition, in the presence of oxidative stress eNOS becomes uncoupled due to inactivation of its cofactor tetrahydrobiopterin and generates ROS as well, further exacerbating oxidative stress.[8] H_2O_2 is produced by dismutation of $O_2^{\cdot-}$ and can react with transition metals to form the highly reactive hydroxy radical ($^{\cdot}OH$) species.[71] At physiologic concentrations, ROSs play essential roles as signaling molecules[72] and modulate a variety of physiologic functions in the vessel wall. However, when in excess, ROS exerts a series of deleterious effects that lead to impaired vascular function, including decreased NO production, stimulation of vascular cell migration, proliferation and apoptosis, cytokine and adhesion molecule production, inflammatory cell adhesion, and extracellular matrix degradation (reviewed elsewhere[8,71]). All components of the vessel wall are affected by ROS. In the endothelial cells, oxidative stress uncouples NO synthase and stimulates adhesion molecule expression, culminating in endothelial dysfunction, whereas in the media, ROS promotes VSMC hypertrophy, proliferation, migration, and apoptosis.[2,8,12,71,72] These effects appear to be critically dependent on production of ROS by NADPH oxidase.[98] In addition, ROS activates the VSMC to express proinflammatory genes and chemokines, including TNF-α, IL-6, and MCP-1,[71] and ROS stimulates MMP production, which plays an essential role in matrix remodeling in vascular diseases.[71,103] All of these effects of ROS in the cells of the vessel wall are central to the initiation and progression of hypertension, atherosclerosis, and vascular proliferative diseases.[8,71]

Role of Apoptosis

Another central feature of vascular remodeling is apoptosis.[12] Apoptosis is involved in vessel remodeling during development and in response to physiologic changes in blood flow (reviewed elsewhere[37]). In addition, apoptosis has been reported in hypertension, atherosclerosis, and neointima hyperplasia.[12,43,128,129] The role of apoptosis in hypertension-induced vascular remodeling remains controversial (reviewed elsewhere[2]). Some evidence suggests that it may play a role in maintaining constant medial cross-sectional area during eutrophic remodeling of resistance arteries in essential hypertension by counterbalancing VSMC hyperplasia.[2] On the other hand, the rate of apoptosis was found to be reduced in young SHRs, suggesting that the decrease in apoptosis may contribute to the enhanced growth of the resistance vessels in these animals.[82] Apoptosis has also been documented in inflammatory vasculoproliferative disease,[43,128,129] and several studies have reported apoptosis of VSMC in atherectomy specimens from atherosclerotic and restenotic vessels.[128,129]

Apoptosis assumes particular importance in atherosclerosis, where it may contribute to plaque instability and rupture (reviewed elsewhere[12,97]). Vascular smooth muscle cell apoptosis is pronounced in advanced atherosclerotic plaques, particularly in the shoulder regions of the

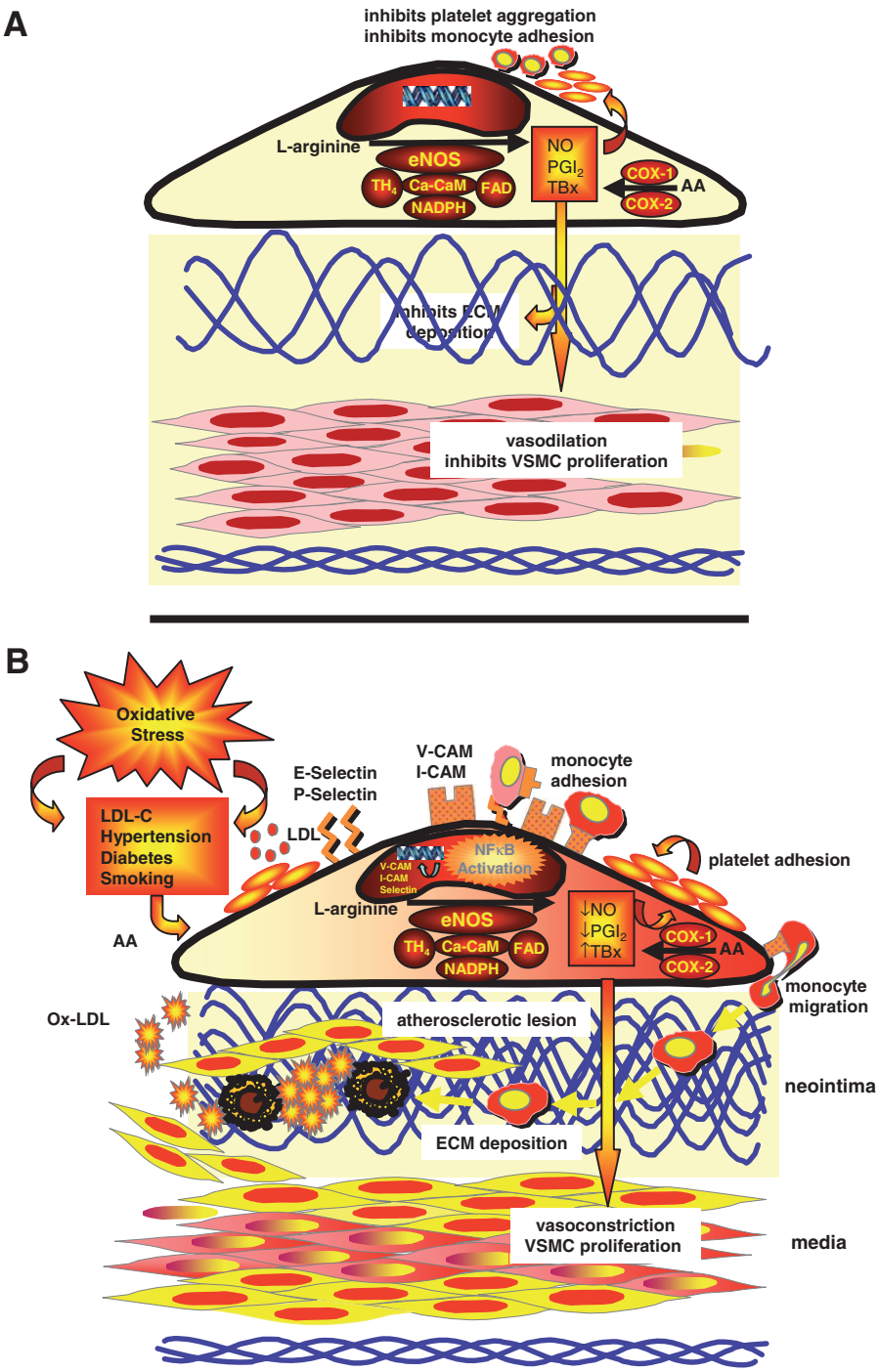

FIGURE 71.2. Pathophysiology of endothelial dysfunction. (A) In normal conditions, the endothelial cell plays a pivotal role in maintaining vessel wall homeostasis by producing a plethora of vasoactive, antiinflammatory, antithrombotic, and cytostatic agents that help maintain vessel tone and protect the vessel wall against inflammatory cell and platelet adhesion, thrombus formation, and vascular cell proliferation. Nitric oxide (NO) released from the terminal guanidine group of L-arginine by endothelial nitric oxide synthase (eNOS), and prostacyclin (PGI_2) derived from arachidonic acid by the action of cyclooxygenase (COX) and prostacyclin synthase play crucial roles in maintenance of endothelial cell homeostasis. (B) When endothelial homeostasis is disturbed by pathologic stresses such as oxidative stress, hyperlipidemia, hypertension, and diabetes, endothelial dysfunction ensues. Nitric oxide production is decreased and the balance between vasodilator and vasoconstrictor moieties such as endothelin and thromboxanes is disrupted, leading to vasoconstriction. The endothelial cell becomes "activated" and synthesizes cell surface adhesion molecules such as selectins and integrins, which increase leukocyte and platelet adhesion and thrombus formation. The loss of growth inhibiting mediators from the endothelium triggers the activation and migration of vascular smooth muscle into the intimal space where they proliferate to form the neointima. In time the infiltration of inflammatory cells into the intimal space and accumulation of oxidized low-density lipoprotein (LDL) results in the formation of the atherosclerotic lesion. CaM, calmodulin; ECM, extracellular matrix; FAD, flavin adenine dinucleotide; ICAM, intercellular adhesion molecule; NADPH, reduced nicotinamide adenine dinucleotide phosphate; TBx, thromboxane; TH4, tetrabiopterin; VCAM, vascular cell adhesion molecule; VSMC, vascular smooth muscle cells.

plaque.[12,97,102,103,105] It is believed that the reduced plaque cellularity associated with VSMC apoptosis is a major contributing factor leading to plaque rupture.[97] In addition to its effects on plaque stability, the apoptosing VSMCs stimulate inflammatory cell infiltration, thus aggravating the proinflammatory microenvironment of the atherosclerotic plaque.[97,130,131]

Endothelial cell apoptosis also appears to contribute to the pathogenesis of atherosclerosis, and increased endothelial cell turnover is seen in areas prone to atherosclerotic lesion development.[102,132–134] The apoptosing endothelial cell becomes highly proadhesive, thus increasing the occurrence of thrombus formation.[132] Avid apoptosis has also been documented in animal models of vascular injury.[43,128,129] In balloon-injured arteries apoptosis occurs in two waves: an early intense wave that occurs within hours of the injury, leading to markedly reduced cellularity of the vessel wall, and a late-onset protracted response occur within days to weeks.[128] It could be argued that in the setting of postangioplasty restenosis, apoptosis may actually be beneficial by providing a mechanism to limit excessive VSMC proliferation. However, the massive loss of medial VSMCs during the first wave of apoptosis may in fact mount an enhanced healing response to counteract the cell loss, thereby exacerbating neointima hyperplasia rather than reducing it. Indeed, as with endothelial cells, the apoptosing VSMCs release a variety of proinflammatory cytokines that could stimulate proliferation.

A variety of physical and humoral factors play a role in regulating apoptosis in the vessel wall. Cell-cell and cell-matrix interaction and shear stress play an essential role in survival of endothelial cells, and loss of cell-cell contact and reduction in shear stress promote endothelial cell apoptosis.[12,102,135] Extracellular matrix protein such as fibronectin, vascular endothelial (VE)-cadherin, and growth factors such as vascular endothelial growth factor (VEGF) and FGF also promote endothelial survival, whereas decreases in shear stress or endothelial growth factor deficiency lead to apoptosis.[12,102,135–137] On the other hand, ROS and proinflammatory cytokines such as TNF-α and IL-6 induce endothelial cell apoptosis.[102] In VSMCs, growth factors such as PDGF-BB, FGF-2, TGF-β_1, and IGF-I and cell-cell and cell-matrix interactions serve as survival signals,[97,102,107] whereas ROS, oxidized low-density lipoprotein (LDL), and proinflammatory cytokines induce apoptosis.[12,97] Interestingly, NO, when produced at high levels by induction of inducible nitric oxide synthase, leads to marked apoptosis. In fact, it is thought that this is the primary mechanism by which proinflammatory cytokines induce VSMC apoptosis in the vessel wall.[12,83,102]

Growth

The vessel wall produces several growth-promoting factors, such as PDGF, basic fibroblast growth factor (bFGF), IGF-I, and IL-1.[138–140] Both the A and the B chains of PDGF are synthesized by the endothelium, VSMCs, and macrophages. All three dimeric isoforms of PDGF (AA, AB, BB) may be produced and secreted in the vessel wall. Production of either the AB or the BB isoform promotes VSMC migration and proliferation and may play a role in some forms of vascular remodeling (Table 71.2).

The cytokine IL-1 is expressed by endothelium and may play a role in vascular remodeling associated with inflammation. Endothelial IL-1 expression is increased by proinflammatory substances, such as endotoxin and TNF. Administration of IL-1 to cultured VSMCs induces proliferation only during prostaglandin synthesis blockade.[141] The IL-1 proliferative response appears to be mediated by the induction of autocrine production of PDGF AA.[142] Studies of cultured ECs suggest that IL-1 inhibits EC proliferation and angiogenesis. Interestingly, increased autocrine expression of IL-1 is associated with EC senescence. This decrease in proliferative capacity can be reversed by antisense oligonucleotides directed against IL-1 production.[143]

The growth factor IGF-I participates in the regulation of EC and VSMC growth. Several reports suggest that it plays an autocrine growth-promoting role in microvascular ECs.[144,145] In VSMCs, IGF-I acts as a growth progression factor for quiescent cells rendered "competent" to proceed through the cell cycle toward DNA synthesis by growth factors such as PDGF and bFGF.[146] In the absence of other growth factors, IGF-I promotes VSMC hypertrophy and matrix production.[147] The growth effects of IGF-I are modulated by IGF binding proteins that may either inhibit or potentiate its activity, depending on experimental conditions.[148]

Basic FGF is a potent autocrine growth factor for ECs and a VSMC mitogen.[149,150] In vitro studies with neutralizing antibodies suggest that bFGF plays a critical role in EC proliferation, cell migration, cell invasion, matrix alterations, and angiogenesis.[140,150] It lacks a classic signal peptide and is primarily intracellular but can be recovered from the extracellular matrix.[140] The peptide contains a nuclear localization sequence that, when deleted by site-directed mutagenesis, abolishes its mitogenic properties. These findings suggest that bFGF may behave as an intracrine growth factor. In addition to these intracellular actions, bFGF is bound to heparin sulfate within the matrix. The matrix acts as a reservoir that binds bFGF released during cell wounding/lysis and may release it in response to proteases, such as heparinitase, released by platelets and leukocytes.[140] Although regulation of its function is still poorly defined, bFGF has profound effects on vascular structure.

Vascular endothelial growth factor is an endothelial-specific growth factor that has no effect on VSMCs, fibroblasts, or other vascular cells. It has been shown to induce EC proliferation, stimulate cell migration,[151,152] and inhibit apoptosis.[153,154] In vivo, VEGF plays a central role in vasculogenesis and angiogenesis in response to tissue hypoxia.[155] Because of the latter effect, VEGF is gaining increasing attention as a therapeutic tool to induce postnatal angiogenesis in ischemic conditions.[156]

TABLE 71.2. Vessel wall-derived growth factors

Growth-promoting substances
Platelet-derived growth factors AA, AB, BB
Acidic and basic fibroblast growth factors
Insulin-like growth factor-I
Vascular endothelium-derived growth factor
Interleukin-1
Angiotensin II*
Endothelin
Growth-inhibitory substances
Prostacyclin
Nitric oxide
Heparan sulfate
TGF-β_1*
Vasoactive substances with growth-regulatory properties
Angiotensin II*
Endothelin
Bradykinin
Nitric oxide
Prostacyclin
Type C natriuretic peptide
Vascular substances with proapoptotic properties
Nitric oxide (VSMC)†
Angiotensin II (EC)†
TGF-β_1 (EC)†
Vascular substances with antlapoptotic properties
Nitric oxide (EC)†
Angiotensin II (VSMC)†
TGF-β_1 (VSMC)†
Insulin-like growth factor-I
Vascular endothelium-derived growth factor

EC, endothelial cell; TGF-β_1, transforming growth factor-β_1; VSMC, vascular smooth muscle cell.

* Bifunctional growth response.

† Cell-specific effect; parentheses denote target cell.

In addition to growth promoters, the vessel wall also produces growth inhibiting factors. Campbell and Campbell[157] and Castellot and colleagues[158] have observed that confluent ECs secrete growth-inhibitory substances that appear to promote the expression of certain characteristics exhibited by the most quiescent, differentiated-appearing VSMC phenotype. Other studies suggest that heparin sulfate produced by ECs inhibits VSMC growth and migration.[159,160] Studies demonstrate that the endothelium VSMC and macrophage also produce TGF-β_1.[161–163] This multifunctional growth factor promotes angiogenesis and inhibits EC proliferation and migration.[162,164,165] Transforming growth factor-β_1 has a bifunctional effect on VSMC growth in that it either inhibits mitogen-induced proliferation or stimulates VSMC proliferation that is mediated by the autocrine production of PDGF AA.[138,139,166] Based on the available data, we would speculate that TGF-β_1 and heparin participate in vascular remodeling. Factors such as TGF-β_1 may be particularly important in structural changes in which the vessel lumen size decreases or blood vessels undergo rarefaction or regression, that is, settings in which cell loss and matrix production are important.

Classic mitogens, such as PDGF and epidermal growth factors, have vasomotor effects, whereas vasoactive substances, such as Ang II and serotonin, can be mitogenic,[167,168] suggesting that vasoactive-agents and growth factors share overlapping signal transduction pathways. In confluent quiescent VSMCs in culture, Ang II induces cellular hypertrophy[169] associated with increased messenger RNA (mRNA) levels of proto-oncogenes c-*fos*, c-*jun*, and c-*myc*, and the autocrine growth factors PDGF A chain, bFGF, and TGF-β_1.[170] These autocrine growth factors may mediate angiotensin-induced hypertrophy.

On the other hand, Ang II–induced TGF-β_1 production is responsible for modulating the mitogenic effect of PDGF and bFGF because blockade of the TGF-β_1 effect by specific antibodies or antisense oligonucleotide resulted in Ang II–induced DNA synthesis and cell proliferation.[170,171] Thus, angiotensin is a bifunctional growth factor able to activate proliferative (PDGF, bFGF) and antiproliferative (TGF-β_1) cellular mechanisms simultaneously. The latter is dependent on the activation of a protein kinase C (PKC)–dependent pathway. In addition to its direct effects on VSMCs, Ang II also can interact with other growth factors in the vessel wall. Angiotensin may potentiate serum-, bFGF-, and PDGF-induced DNA synthesis.[172] These findings suggest that angiotensin may modulate the proliferative response to autocrine/paracrine growth factors. Moreover, alterations in VSMC phenotype may modulate the Ang II–induced growth response by altering the susceptibility to proliferative versus antiproliferative factors.

Similar to the response to Ang II, we and others have shown that endothelin also can induce an increased expression of c-*myc* in association with VSMC proliferation.[173] Growth-promoting effects on VSMCs also have been described for many other vasoconstrictors, for example, norepinephrine, thromboxane, leukotrienes, vasopressin, substance K, and serotonin.[168,174–177] The sympathetic nervous system appears to exert a trophic effect on the vasculature to promote growth and remodeling.[177,178] Removal of this neural input attenuates the structural responses of the vasculature. The growth effects of catecholamines may be mediated by the autocrine production of PDGF AA.[179] The growth effects of vasoactive substances are also associated with effects on VSMC migration. Ang II, serotonin, and norepinephrine have been shown to enhance cell migration as well as stimulate growth.[180,181] In contrast, endogenous vasodilators that activate adenylate cyclase, such as prostacyclin, prostaglandin E_2, and adenosine, or vasodilators that activate guanylate cyclase, such as NO, atrial natriuretic peptide and adrenomedullin, inhibit VSMC growth.[182–186] The effects of vasodilators on VSMC migration are not well defined.

The effect of vasoactive substances on EC growth is not as well characterized. It has been reported that catecholamines, histamine, and adenosine enhance serum-stimulated cell proliferation.[187–189] The growth stimulation of adenosine appears to be potentiated in the setting of hypoxia.[189] Conversely, activation of guanylate cyclase inhibits the proliferation of conduit vessel endothelium, and protein kinase C activation inhibits microvascular endothelial proliferation.[190,191] The effects on endothelial growth usually are associated with other functional changes, such as alterations in cell movement. It has been reported that Ang II, serotonin, norepinephrine, and histamine inhibit EC migration in vitro.[68,69]

Signaling Mechanisms Involved in Remodeling

The mechanism involved in remodeling involves multiple signaling cascades and downstream transcription factors that function coordinately to activate a large number genes involved in regulation of growth, apoptosis, and extracellular matrix metabolism.[2,3,12–15,27,28,40,43,67,71,72,106,136,137] These signaling mechanisms are activated by a variety of stimuli including mechanical stress (shear stress, stretch), ROS, growth factors, and cytokines.[14,40,67,68,71–77,98–100] Stretch of the vessel wall caused by sustained increase in intravascular pressure acts on all layers of the vessel wall, whereas shear stress acts predominantly on the endothelium in response to changes in blood flow.[1,14,45,135] Stretch activates several growth promoting signaling pathways, including focal adhesion kinase (FAK) pathway, the mitogen-activated protein kinase (MAPK) cascade, and the renin-angiotensin system within the vessel wall.[2,13,71,72,75] Integrins at the cell surface are the likely sensors of stretch and shear stress (mechanosensors), and stretch-sensitive nonselective cation channels, and possibly potassium channels, function as transducers, converting the mechanical stimulus into activation of the biochemical pathways that lead to stimulation of growth and remodeling of the vessel wall.[14]

The process of transmission of mechanical stress begins the clustering of $\alpha_5\beta_1$ integrin and gathering of the cytoskeletal proteins talin and vinculin to form focal adhesions and stress fiber that anchor to the cytoskeleton. FAK is recruited to the focal adhesion and becomes tyrosine phosphorylated. The small G protein RhoA is also involved in the formation of focal adhesion and stress fibers by promoting the phosphorylation of FAK and paxillin as well as promoting actin filament polymerization. The increase in mechanical stress activates the tyrosine kinase c Src, which translocates to the focal adhesions, where it phosphorylates FAK, which becomes

activated in the process. The MAPK pathway is the primary signaling mechanisms by which mechanical stress and growth factors regulate expression of the genes involved in growth and remodeling of the vessel wall. All three MAPK pathways (MAPk, JNK, p38) are involved.[14] In endothelial cells MAPK is the predominant cascade activated by increases in shear stress, whereas both ERK and JNK are activated in response to stretch.[14] In addition, Akt has been found to be activated in endothelial cells in response to shear stress, where it stimulates eNOS phosphorylation and activity and promotes endothelial cell survival and migration.[192–194] Platelet-derived growth factor-β appears to play an essential role in activating the MAPK cascade in response to mechanical stress and Ang II, and the nonreceptor tyrosine kinase c-Src plays an essential role in this process.[2,3,14]

Several other G protein and PKC-mediated mechanisms are also involved in activation MAPK pathway in response to mechanical stress. Upon phosphorylation by upstream kinases, the end terminal ERK1/2 kinase translocates to the nucleus, where it phosphorylates multiple transcription factors involved in cell cycle regulation and protein synthsesis.[14] Many of the common growth promoting factors in the vasculature including Ang II and PDGF appear to exert their effects, at least in part, via the MAPK pathway as well.[98,101] However, non-MAPK mechanisms may also be involved. Some factors such as Ang II and proinflammatory also produced ROSs, which serve as intermediate messengers to activate multiple signaling pathways including proximal tyrosine kinases such as c-Src, which appears to play a major role in PDGF receptor and EGF receptor transactivation by Ang II, leading to downstream activation of the low molecular weight guanosine triphosphate (GTP) binding protein, which subsequently activated the MAPK pathway.[71,72,98–101] In addition, ROSs also directly stimulate MAPK, including ERK 1/2, p38 MAPK, JNK/SAPK, and Akt. The activation of these signaling cascades by ROSs leads to the upregulation of multiple redox sensitive genes involved in growth inflammation and extracellular remodeling of the vascular wall. The ROSs may also bypass these signaling cascades to directly regulate the activity of redox sensitive transcription factors such as NF-κB, Egr-1, and AP-1.[72–76]

Therapeutic Strategies for Vascular Remodeling

Drug Therapies

Several of the currently used antihypertensive and cholesterol-lowering drugs have been shown to have beneficial effects in reversing the structural changes associated with vessel remodeling in hypertensive patients and in plaque stabilization in patients with atherosclerosis.[26,27,29,97] Among the most successful drugs in this regard are the ACE inhibitors and angiotensin receptor blockers,[27] peroxisome-proliferator-activated receptor (PPAR) agonists,[28] and HMG CoA inhibitors.[29] Hypertensive patients on long-term ACE inhibitor therapy consistently show improved endothelial function and reduced remodeling in small arteries.[27,195,196] Interestingly, treatment with the beta-blocker atenolol failed to improve endothelial function and vessel remodeling despite similar reduction in blood pressure.[196] Similar results were seen with the angiotensin receptor blocker losartan.[197] The mechanisms involved in improving endothelial function and vessel remodeling after treatment with blockers of the renin-angiotensin system are not known, but likely involve inhibition of the growth-promoting, profibrotic, and proinflammatory actions of Ang II.

Another drug family that has been found to have beneficial effects in vessel remodeling is the peroxisome-proliferator activator receptor agonists belonging to the fibrate and glitazone family (reviewed elsewhere[28]). These drugs exert antiinflammatory, antioxidant, and antiproliferative effects in the vessel wall and are able to antagonize the actions of Ang II in vivo.[28] Treatment with these drugs improves the lipid profile and reduces the concentrations of circulating inflammation markers such as C-reactive protein (CRP) and CD40L in diabetic patients.[198,199] Rosiglitazone has been reported to reduce endothelial activation in nondiabetics with coronary artery disease.[198] Other studies have shown that rosiglitazone treatment significantly improved endothelial function and reduced BP and fasting insulin levels in patients who met the criteria of metabolic syndrome.[200] Thus, these studies suggest that PPAR agonists are useful in reducing atherogenic and inflammatory effects and improving endothelial function in patients with diabetes or coronary artery disease.

Another family of drugs that has been found to have potentially beneficial vasculoprotective effects is the HMG CoA inhibitor family of drugs called statins.[29] It is now recognized that in addition to their cholesterol-lowering effect, these drugs have pleiotropic effects in the vessel wall that may lead to plaque stabilization and reduction of vascular remodeling[201–203] (reviewed elsewhere[29,97]). Some of these effects include improvement of endothelial vasodilation, a phenomenon that is possibly due to the ability of statins to stimulate Akt and phosphorylation of eNOS.[204,205] In addition statins exert antiinflammatory effects.[201] Statins also stimulate endothelial progenitor cell mobilization, and this may accelerate endothelial recovery and decrease restenosis following balloon angioplasty.[206] On the other hand, statins also promote angiogenesis, and this could potentially accelerate plaque development (reviewed elsewhere[207]).

Percutaneous Coronary Revascularization

With the number of percutaneous transluminal coronary angioplasty (PTCA) procedures growing exponentially over the last decade, physicians and researchers were urged to find effective strategies to prevent restenosis. The introduction in the clinical arena of the stent represented a major advancement in the prevention of the occurrence of restenosis (Fig. 71.3). This device can in fact abolish both elastic recoil and vessel remodeling, consequently reducing restenosis of the treated lesion. Nevertheless, in-stent restenosis exists and the incidence may vary from 8% to as high as 80% at 6 months, depending on both anatomic and clinical risk factors (reviewed elsewhere[33]). The main determinant of in-stent restenosis is the neointimal formation due to exaggerated VSMC proliferation.[31,33,104] Mechanical approaches have proved too simplistic to prevent in-stent restenosis. Interfering with molecular cell proliferation is a more effective

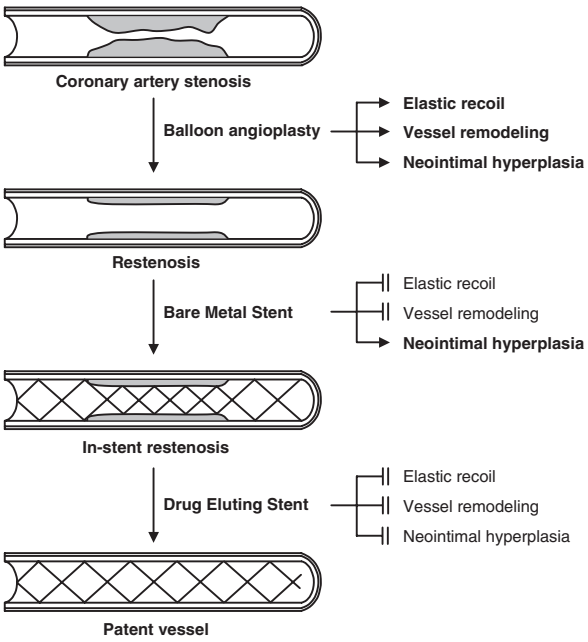

FIGURE 71.3. Evolution of invasive treatment of coronary artery disease. More than 25 years ago percutaneous transluminal coronary angioplasty (PTCA) was introduced in clinical practice. Despite the good results obtained, soon it became evident that renarrowing of the treated vessel occurred in a great percentage of cases. Pathophysiologic studies uncovered the causes of restenosis: thrombus formation, elastic recoil, vessel remodeling, and neointimal hyperplasia. By the mid-1980s, scientists and cardiologists started working on mechanical solutions to prevent restenosis. The result was the introduction of the stent, a metal device that, inserted into the artery after PTCA, can prevent elastic recoil and late vascular remodeling. In 1986 the first stent was inserted into a human coronary artery in Toulouse, France, and in 1994 the first Palmaz-Schatz stent was approved for use in the United States. Despite the fact that stents prevent elastic recoil and vessel remodeling, in-stent restenosis still occurs due to neointimal hyperplasia stimulated by the mechanical insult of the struts of the stent to the vessel wall. This led to the development of strategies to inhibit the proliferation of VSMC. Drug-eluting stents are the successful result of a decade of intense preclinical studies; sirolimus- and paclitaxel-eluting stents are now approved for use in humans and the rate of in-stent restenosis dropped above 10%. The development of new dedicated platforms and the identification of drugs effective also in patients at high risk such as diabetics will further decrease this number and possibly eliminate the problem of restenosis.

manner of limiting neointimal formation. The development of drug-eluting stents (DESs) has been a major breakthrough in this direction, and numerous clinical trials have in fact demonstrated that DESs strikingly reduce the incidence of in-stent restenosis compared with bare metal stents.[30,33] Some drug can be loaded directly onto the metallic surface of the stent.[32,33] However, biomedical engineering progress has facilitated the development of specific coating matrix–containing drugs that are released in situ after stent deployment. Different polymers have been tested so far and many more are currently under investigation.[30,33,109] The selection of a noninflammatory, inert coating has been a challenge only partially resolved. There is a long list of candidate substances for stent coating in continuous expansion. These substances can be categorized as organic, inorganic, biodegradable, nonbiodegradable, or synthetic.[31,32] The ideal antirestenotic agent for local delivery should have antiproliferative effects preserving the vascular healing of the vessel.

Many biologic agents have been proposed as antiproliferative drugs but so far only two DESs have demonstrated clinical efficacy in large randomized trials: sirolimus- (Rapamue; Wyeth-Ayerst Laboratories) and paclitaxel-eluting stents (Taxol; Bristol-Myers Squibb). Sirolimus is a macrolide antibiotic that inhibits VSMC proliferation by binding an intracellular protein receptor, the FKBP12. The sirolimus-FKBP12 complex binds to the mammalian target of sirolimus, a signal transduction protein (mTOR), which is upregulated in human neointimal VSMCs[208] and inhibits its activation. The inhibition of mTOR ultimately prevents cell cycle progression at the G_1-to-S phase transition.[208,209] Sirolimus increases levels of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ that binds and inactivates the cyclin E/cdk2 complex and inhibits cell-cycle progression. In fact, when inactivated, the cyclin E/cdk2 complex cannot phosphorylate the retinoblastoma protein and leads to its dissociation from the transcriptor factor E2F, which is essential to regulate the expression of genes encoding proteins required for the G_1-to-S transition. Sirolimus has other cellular effects that probably contribute to its ability to prevent stent restenosis, for instance through the inhibition of the translational regulators p70S6K and 4E-BP1,[210] and, as direct consequence, pathways involved in protein translation. However, the contribution of inhibition of protein translation to the ability of sirolimus to prevent stent restenosis remains unclear.[211]

The main action of paclitaxel is to shift the cytoskeleton equilibrium toward assembly, with a consequent reduction in vascular cell proliferation, migration, and signal transduction.[212] At all phases of the cell cycle, bundles of disorganized microtubes are formed preventing progression through M-phase.[213]

The sirolimus-eluting stent is composed of a stainless steel stent, the BX Velocity™ stent (Cordis Cypher™), coated with a mixture of polyethylenevinylacetate and polybutylmethacrylate containing 140 μg/mm^2 of rapamycin. The sirolimus stent that is now approved by the Food and Drug Administration (FDA) for use in humans is a slow-release platform (≥28-day drug release). Of note, the levels of sirolimus in the whole blood go below the lower limit of quantification (0.4 ng/mL) 72 hours after stent implantation.[214] The First in Man Study (FIM) testing the sirolimus-eluting stent was initiated in 1999 and yielded extraordinary results, with an angiographic restenosis rate of 0% maintained at 4 years and no increase of side effects compared with a bare metal stent.[215] This pioneering study provides invaluable long-term information on sirolimus-eluting stents and seems to mitigate concerns about a potential risk of late restenosis or late side effects.

The randomized study with the sirolimus-eluting BX Velocity™ balloon-expandable stent (RAVEL) included 238 patients treated for revascularization of single, de novo lesions in native coronary arteries.[216,217] The luminal loss was the primary end point and was significantly lower in the sirolimus stent group (+0.01 mm) than in the standard stent group (0.80 mm, $p < .001$).

The SIRolImUS-coated BX Velocity™ stent in the treatment of patients with de novo coronary artery lesions (SIRIUS) study was a multicenter, randomized, double-blind

study enrolling patients with coronary lesions from 15 to 30 mm in length.[218] Such a cohort represents more closely the clinic reality of everyday practice compared with the RAVEL patients. The primary end point was target vessel failure, including cardiac death, myocardial infarction, or target vessel revascularization at 9 months from the treatment. In the Cypher™-treated group 10.5% of the patients reached the primary end point compared with 19.5% in the control group. Furthermore, the binary in-stent restenosis (2.0%) and the in-lesion restenosis (9.1%) were significantly reduced in the sirolimus group. This large-scale trial confirmed the safety and proved the efficacy of the sirolimus-eluting stent also in a cohort of patients presenting lesions at higher risk to develop restenosis compared with the RAVEL.

More recently, the Canadian Study of the Sirolimus-Eluting Stent in the Treatment of Patients with Long De Novo Lesions in Small Native Coronary Arteries (C-SIRIUS)[219] and the European Sirolimus-Eluting Stents for Treatment of Patients with Long Atherosclerotic Lesions in Small Coronary Arteries (E-SIRIUS)[220] studies confirmed the anti-restenotic effect of sirolimus-eluting stents in longer lesions in small vessels. Another randomized study reported that the use of sirolimus-eluting stents to treat atherosclerotic lesions in small coronary arteries reduces restenosis and may also reduce major adverse cardiac events.[221] However, diabetic patients seem to be less responsive to sirolimus-eluting stents, with higher rate of restenosis developing at 9 months as compared to nondiabetics.[222]

Numerous clinical trials have evaluated paclitaxel-eluting stents to prevent in-stent restenosis. In a first study, TAXUS I, the patients received either a slow-release low-dose polymer-coated NIRx Conformer™ stent (Boston Scientific Corp., Boston, MA) or a bare stent.[223] The restenosis rate was 0% for the drug-eluting stent and 10.3% for the bare stent. The TAXUS II study compared two different polymer configurations, both loaded with a low dose of paclitaxel.[224] Both the slow-release (2.3% vs. 17.9%) and the moderate-release polymer (4.6% vs. 20.2%) reduced the incidence of in-stent restenosis. The TAXUS III trial reported the feasibility of the treatment of in-stent restenosis.[225] TAXUS IV is a large, randomized trial testing the efficacy of paclitaxel-eluting Express™ stent (Boston Scientific Corp.) in patients with de novo lesions. In this trial 1314 patients, who were receiving a stent in a single, de novo coronary artery stenosis were prospectively randomized to the slow-release, polymer-based, paclitaxel-eluting TAXUS stent or an identical-appearing bare-metal Express™ stent (Boston Scientific Corp.).[226] After 9 months the rate of angiographic restenosis was significantly reduced from 26.6% to 7.9% with the paclitaxel-eluting stent. Statistically significant differences were reached also for the target-lesion revascularization required in 3.0% of the patients who received a paclitaxel-eluting stent, as compared with 11.3% of the group treated with a bare-metal stent. The TAXUS IV demonstrated both the safety and the efficacy of polymer-based, paclitaxel-eluting stent. Recent post-hoc analysis of TAXUS IV suggested that also among diabetic patients the rate of restenosis is significantly reduced with the use of a paclitaxel-eluted stent compared with a bare stent, but further prospective trials are needed to confirm these data.[227] Finally, the TAXUS V has enrolled 421 patients to demonstrate a superior or noninferior 9-month target vessel revascularization rate for the TAXUS Express™ paclitaxel-eluting SR stent compared with intracoronary brachytherapy for the treatment of in-stent restenosis. The results of TAXUS V, which are pending, will tell us if such an approach is safe and effective also for the treatment of this difficult subset of patients.

Rapamycin- and paclitaxel-eluting stents are the culmination of years of intense research into stent design and pharmacologic studies to abolish neointimal hyperplasia. In-stent restenosis is not completely abolished yet, but we are getting very close. Despite the fact that DESs have proved to be extremely effective in the prevention of in-stent restenosis, some important issues still remain to be clarified. The possible increased risk of thrombosis due to incomplete reendothelialization after DES is a potential serious problem that has to be taken carefully into consideration when testing new DESs. For example, the high incidence of stent thrombosis observed in the early paclitaxel studies may be due to high drug concentrations or poor stent design. To avoid such problems, it is important to carry out accurate preclinical studies without rushing into potentially unsafe clinical trials. The need for prolonged regimens of antiplatelet therapy must be also considered. The deployment of DESs does not require particular skills compared with bare stent. However, it is becoming evident from intravascular ultrasonography (IVUS) analysis carried out after DES implantation that an accurate stent placement can be very important to successfully reduce neointimal hyperplasia. Incomplete lesion coverage, the gap between stents, and balloon injury beyond the edges of the stent represent the new face of "geographic miss" in the DES era and have been related to treatment failures. Most of these operator-dependent factors occur when treating complicated lesions, often in small and tortuous vessels or at the level of a bifurcation. In the near future the design of dedicated platforms seems to be the major challenge but it won't be an obstacle. We envision in the future DESs that are more flexible and easier to deliver compared to the one actually available. The identification of drugs effective also in diabetics and in patients resistant to sirolimus or paclitaxel will further decrease the rate of in-stent restenosis and possibly abolish it completely.

Gene Therapy for Enhancement of Endothelial Function and Prevention of Restenosis

Despite the relative ease of delivering genes to endothelium, very few studies have considered endothelial-specific transgene expression as a therapeutic modality for cardiovascular disease. This seems paradoxical, considering the prominent role of endothelial dysfunction in cardiovascular disease, and the potential therapeutic value of genetic modulation of endothelial function.[5–7] Genetic strategies to reduce inflammation and vascular smooth muscle proliferation may be useful for prevention of postangioplasty restenosis (reviewed elsewhere[228]).

Endothelial Function

A number of genes could serve as targets for genetic modulation of endothelial function, and therapeutic strategies aimed

at reducing oxidative stress should increase NO bioavailability and should help improve endothelial function in cardiovascular disease. In this regard, endothelium-targeted overexpression of antioxidant enzymes such as superoxide dismutase, catalase, or heme oxygenase may provide a strategy to scavenge excess ROS and reduce tissue injury in conditions associated with high oxidative stress such as hypertension, atherosclerosis, and coronary artery disease.[2,40,229] The overexpression of vasodilatory and antiproliferative genes such as nitric oxide synthase (NOS) and atrial peptides in the endothelium or the inhibition of endothelium-derived vasoconstrictors and growth factors may be effective in the treatment of hypertension and vascular proliferative disease, whereas the expression of antithrombotic, antiadhesion, or antiinflammatory genes may be useful in prevention of plaque rupture, thrombosis, and acute myocardial infarction (MI). The overexpression of NOS in the endothelium by exogenous gene transfer may be particularly suitable for vascular diseases,[230] given the broad vasculoprotective effects of NO.

Neointima Proliferation

Two anti-restenosis gene therapy strategies have been used to inhibit neointimal hyperplasia, using a wide variety of therapeutic targets. Cytostatic strategies involve the inhibition of key proteins regulating cell-cycle progression in order to arrest neointimal cell proliferation.[231–234] We used this strategy to treat jugular veins in vivo with hemagglutinating virus of Japan (HVJ)-liposome complexes containing antisense oligonucleotide against cell-cycle regulators' proliferating cell nuclear antigen (PCNA) and cdc2 kinase in atherosclerotic New Zealand rabbits prior to carotid artery interpositional grafting[233] (Fig. 71.3). The gene therapy led to adaptive remodeling of the graft, successfully inducing medial hypertrophy while inhibiting neointimal hyperplasia, to yield conduits that resemble normal arteries[234] (Fig. 71.4). Subsequent histologic and functional analysis of the treated vein graft showed marked inhibition of graft atherosclerosis, decreased inflammation, and improved endothelial function[234] (Fig. 71.4). More recently, it was reported that the treatment of vein grafts prior to implantation with a decoy deoxyoligonucleotide bearing the consensus binding sequence of E2F-1, a transcriptional factor involved in cell-cycle progression, resulted in prolonged resistance to neointimal hyperplasia and improved the patency of the graft after transplantation.[235] An interesting variant in cytostatic gene therapy involves the targeted expression of the thymidine kinase gene. The gene renders the transduced cells sensitive to antiviral drugs such as ganciclovir, such that treatment with the drugs eradicates the vector targeted cells. This strategy, commonly known as suicide gene therapy, has been used successfully to reduce neointima proliferation in atheromatous iliac arteries from rabbits.[236]

Delivery of antiproliferative genes such as those coding for the nitric oxide synthases offer another approach to achieve inhibition of neointima hyperplasia. All three isoforms of nitric oxide have been shown to exert vasculoprotective and antiproliferative effects after gene transfer (reviewed elsewhere[230]). Endothelial and inducible NOS (iNOS) gene transfer are equally efficacious in reducing neointimal thickening in balloon-injured vessels.[237–240] Local delivery of antioxidant enzymes such as HO-1[241,242] and ecSOD[243,244] by adenovirus has also been shown to inhibit neointima hyperplasia in various animal models of restenosis, possibly due to reduction in inflammation and oxidative stress during the early phase of vascular injury.

Some trials have also been undertaken to evaluate the effect of cell-cycle inhibition on neointima proliferation and vein graft failure. A phase I prospective, randomized, double-blind trial of human saphenous vein graft treatment with E2F decoy (Project in Ex-Vivo Vein Graft Engineering Via Transfection, PREVENT-1) was carried out in high-risk patients suffering from peripheral arterial occlusive disease.[245] Using nondistending pressure to deliver the E2F decoy oligonucleotide ex vivo prior to arterial interpositional grafting, we demonstrated that E2F decoy treatment was safe and feasible. Although the results were preliminary, the study provided evidence that cytostatic gene therapy is feasible for clinical application. More recently the PREVENT II has largely confirmed the finding of the PREVENT I trial. The PREVENT II is a randomized double-blinded, placebo-controlled phase II trial designed to evaluate the effect of E2F decoy treatment on coronary artery bypass graft (CABG) failure in 202 patients (half treated with E2F decoy) undergoing bypass surgery for at least two vessels.[246] The interim results confirmed the feasibility and safety of E2F-1 decoy. Analysis of the secondary end points using quantitative coronary angiography and three-dimensional intravascular ultrasound demonstrated increased patency and adaptive vessel remodeling characterized by reduction in neointimal size and volume in the treated group 1 year after treatment, leading to a 40% reduction in critical stenosis. The results of the PREVENT IV have recently been published.[247] This phase III, multicenter, randomized, double-blind, placebo-controlled trial evaluated the therapeutic efficacy of ex vivo treatment of autologous vein grafts with E2F decoy (Edifoligide) in 3014 patients from 107 sites undergoing CABG. The primary end point evaluated was vein graft failure, defined as death or >75% stenosis in treated vein grafts at 12- to 18-month angiographic follow-up. The results showed that Edifoligide was no more effective than placebo in preventing graft failure 12 to 18 months after CABG surgery,[247] and the authors concluded that a longer follow-up period will be necessary to determine whether treatment with the E2F decoy has delayed beneficial effects to improve the durability of CABG surgery.

Cell-Based Therapy for Vascular Protection and Repair

A developing field in vascular therapeutics is the use of autologous endothelial progenitor cell (EPC) transplantation for repair of damaged vessels and bioengineering of vascular prosthetic grafts and stents.[248–256] Several groups have reported the identification and isolation of EPCs from adult peripheral blood.[257,258] These cells are thought to originate from a common hemangioblast precursor in the bone marrow[257,259] and express endothelial lineage markers such as CD34, Flk-1, VE-cadherin, PECAM-1 (CD31), von Willebrand factor, eNOS, and E-selectin[257–259](reviewed elsewhere[260]) (Fig. 71.5). The cells have high proliferative potential,[257] and under specific

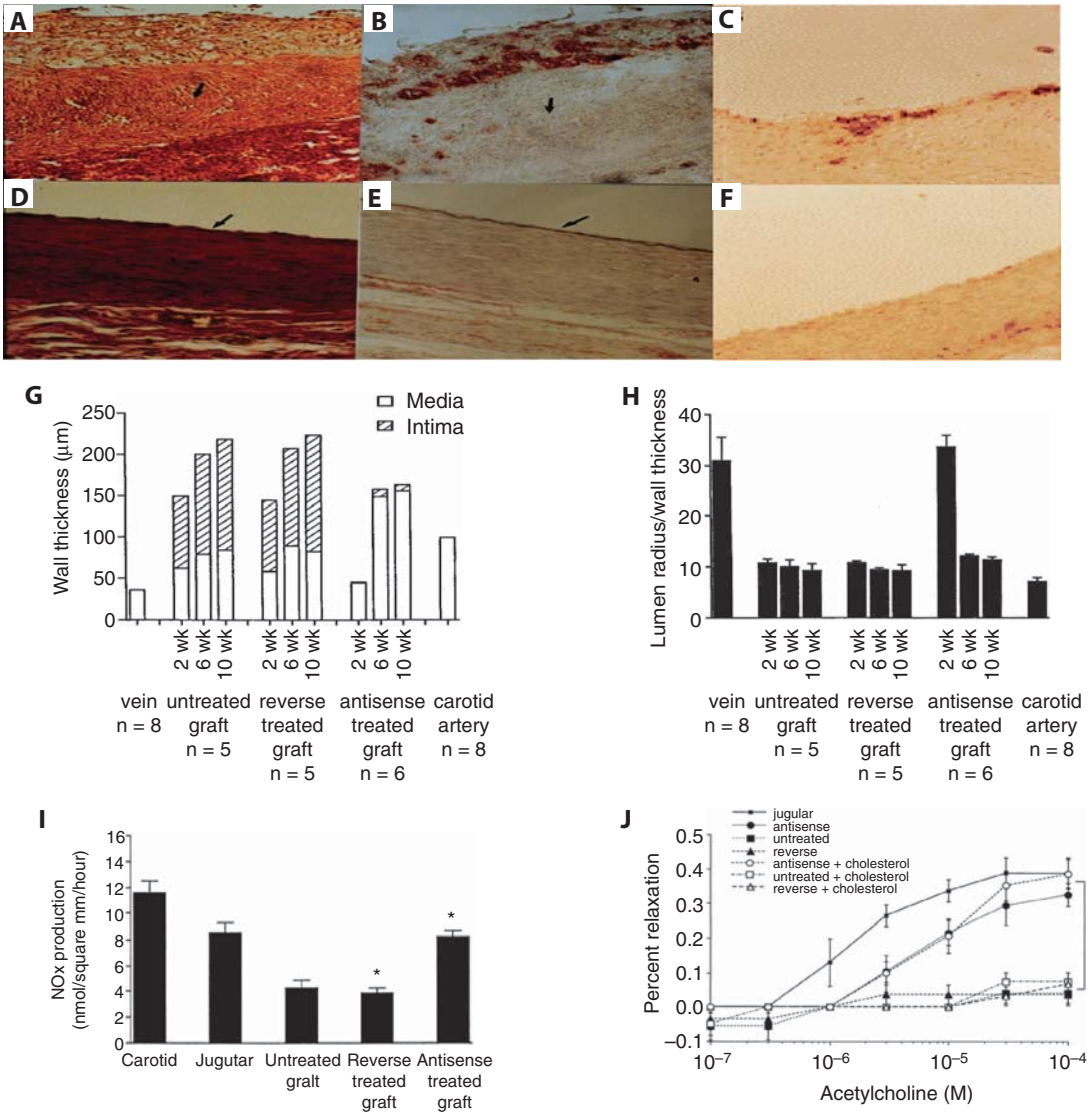

FIGURE 71.4. Intraoperative genetic engineering of atherosclerosis-resistant vein grafts by selective inhibition of cell-cycle regulatory gene cdc2 kinase and proliferating cell nuclear antigen (PCNA) using antisense deoxyoligonucleotides. Jugular vein grafts from hypercholesterolemic rabbits 6 weeks after carotid interpositional grafting showed significant neointima hyperplasia (A), infiltration of foam cells throughout the intima and subendothelial regions (B), and increased vascular cell adhesion molecule-1 (VCAM-1) expression (C). Selective blockade of cdc2 kinase and PCNA in hypercholesterolemic rabbits leads to inhibition of neointimal deposition (D), significantly reduced foam cell accumulation and plaque formation (E), and inhibition of VCAM-1 expression (F). (G,H) Inhibition of cdc2 and PCNA with the antisense oligonucleotides promoted positive remodeling of the vein graft characterized by increased media hypertrophy and reduced neointima hyperplasia to yield fully arterialized conduits. Treatment of the vein grafts with the antisense deoxyoligonucleotide resulted in increased nitric oxide production (I) and improved endothelial function (J).

growth conditions differentiate into mature endothelial cells that can be expanded in culture[257,261–265] (reviewed elsewhere[266]). The relative abundance of circulating EPCs is low in basal conditions.[257,267] However, the number of circulating cells increases severalfold after exogenous stimulation with cytokines such as VEGF and granulocyte-colony stimulating factor (G-CSF)[265,268–271] (reviewed elsewhere[266,267]). The mechanisms governing the mobilization, homing, and differentiation of the EPC in vivo have only recently begun to be uncovered. The cells appear to be recruited predominantly to sites of injury such as ischemic myocardium and damaged blood vessels[262,265,272] (reviewed elsewhere[267]), suggesting that signals emanating from the injury site may play a central role in the mobilization, homing, and differentiation processes. Injured tissues release various cytokines, chemokines, adhesion molecules, and extracellular matrix proteins locally, which may act in concert to mediate these processes[273–275] (reviewed elsewhere[276]). At the site of injury, the

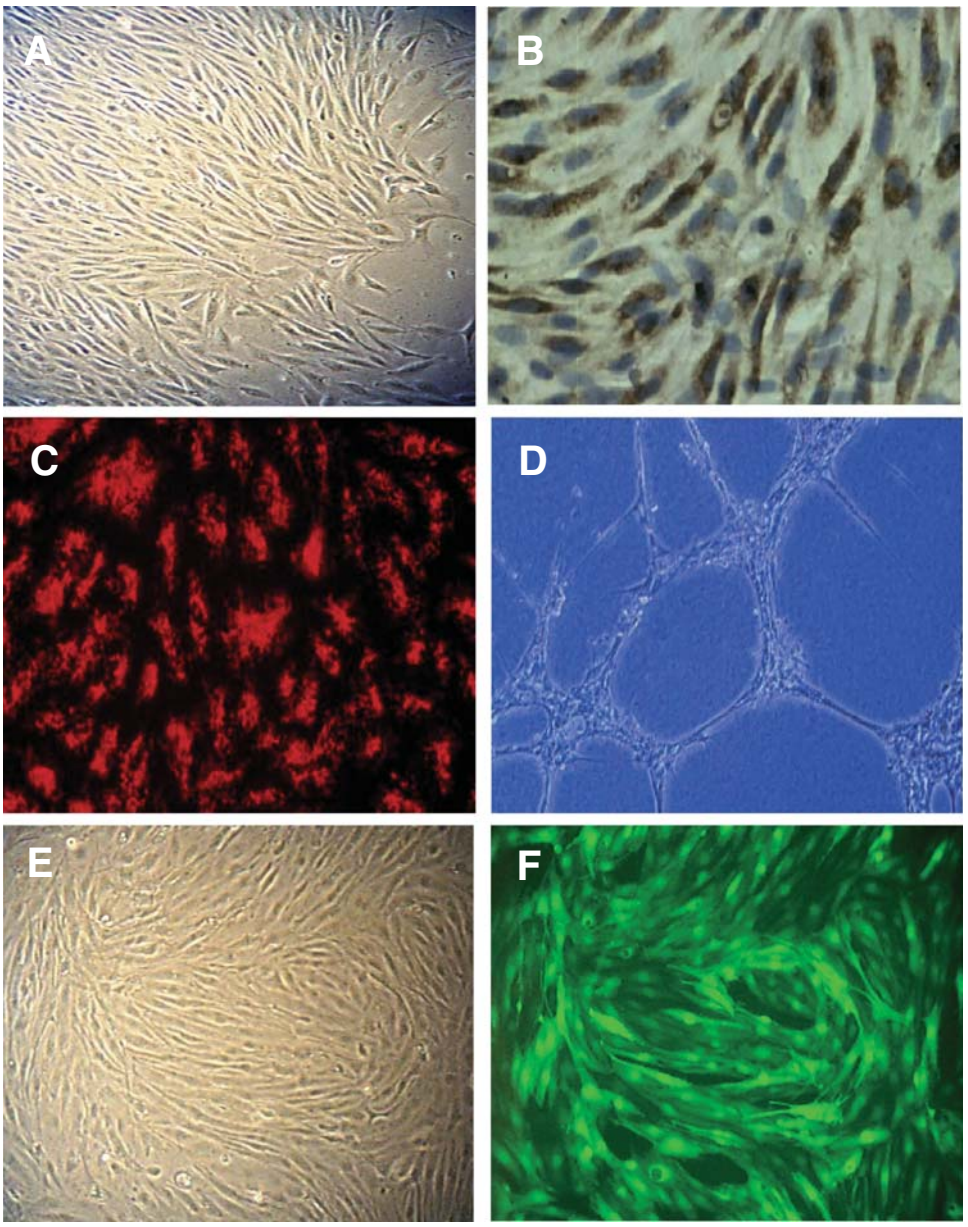

FIGURE 71.5. Characterization and genetic manipulation of circulating endothelial progenitor cells (EPCs). (A) EPC at 2 weeks after initial plating (×200) present the cobblestone morphology typical of endothelial cells. (B) EPC staining positive for cytoplasmic von Willebrand factor (×200). (C) EPC takes up acetylated LDL particles from media (×200). (D) EPC forms vascular-like tubes when plated on matrigel-coated dishes (×40). (E,F) Ex vivo transduction of EPC with pseudotyped retroviral vector expressing GFP. E, white light; F, same field viewed under green fluorescent light (×100). Transduction efficiency in excess of 80% is consistently observed.

production of adhesion molecules may provide a microenvironment for implantation and subsequent proliferation and differentiation of the EPC.

We have recently reported that transplantation of autologous EPC into balloon-injured rabbit carotid arteries leads to nearly complete reendothelialization of the denuded vessels as early as 4 days after cell transplantation (Fig. 71.5).[277] In contrast, little evidence of reendothelialization was seen at this time in the untreated vessels (Fig. 71.6). The rapid endothelial recovery of the treated vessels was followed by marked reduction in neointima hyperplasia, whereas a prominent neointima was present in the untreated vessel 4 weeks after the injury (Fig. 71.7). We showed subsequently that genetic modification with a retroviral vector expressing eNOS potentiates the therapeutic effect of the transplanted cells, presumably by enhancing the vasculoprotective properties of the endothelium (Fig. 71.7).[252] We proposed that the transplantation of autologous EPC expressing vasculoprotective genes at the time of angioplasty may be useful as a strategy to prevent postintervention complications such as thrombosis and restenosis after revascularization procedures.

More recently, we showed that mobilization of EPC in rats by exogenous administration of G-CSF for several days prior to balloon injury of carotid artery leads to accelerated reendothelialization of the denuded vessels.[278] In contrast, little reendothelialization was seen at this time in injured vessels from untreated animals. The vessels from the treated animals showed a decrease in neointima formation, whereas a prominent neointima was present in the vessels from the untreated animals. We believe that this may represent a

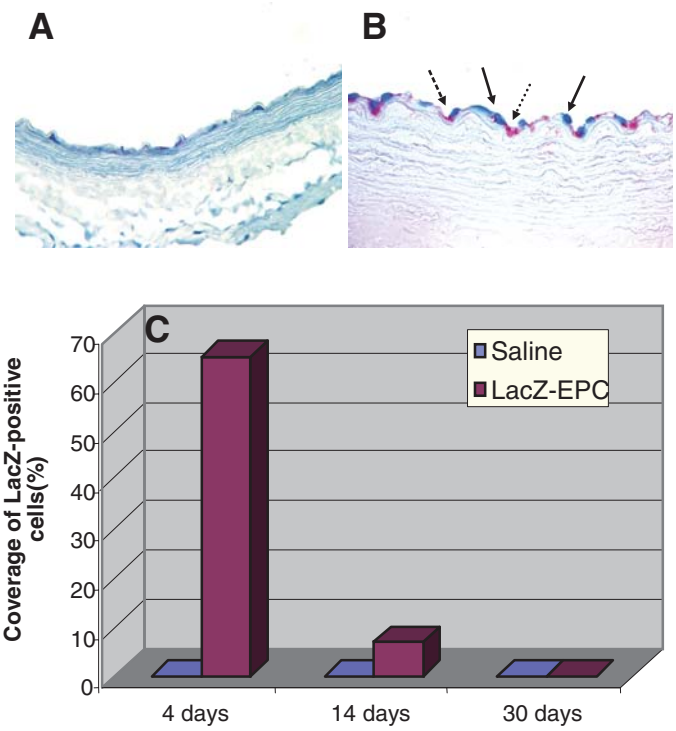

FIGURE 71.6. Transplantation of genetically modified autologous circulating endothelial cells onto denuded carotid artery. (A) Unseeded balloon-denuded vessel. No evidence of reendothelialization was observed in the unseeded vessels. (B) Endothelialization of balloon-denuded rabbit carotid arteries four days after transplantation of autologous LacZ-expressing cell. Almost complete reendothelialization was observed in the seeded vessels, characterized by a continuous layer of cells coexpressing CD31 (broken arrow) and β-galactosidase activity (solid arrows). (C) Percentage of luminal coverage with LacZ-positive cells in denuded vessels. In excess of 70% reendothelialization was seen in the animals treated with endothelial progenitor cells (EPCs) as early as 4 days after transplantation, indicating early endothelial recovery. Time dependent decrease in LacZ positive cells is likely due to cell turnover.

novel noninvasive strategy for prevention of restenosis following balloon angioplasty. The emphasis on pretreatment with this strategy of premobilization of EPC represents a paradigm shift in the treatment of restenosis, focusing on prevention rather than rescue. Presumably the increase in abundance of circulating progenitors provides a substrate for rapid endothelial healing and recover following procedural injury, thereby reducing the negative effects of remodeling. Others have reported evidence that statin therapy[206,279] and estrogen[280] increases the number of PB-EPC and reduces neointima hyperplasia in animal models of arterial injury. Interestingly, Assmus and colleagues[281] showed that statins reduce senescence and stimulate proliferation of PB-EPC by regulating the activity of crucial cell-cycle genes such as the cyclins and cyclin-dependent kinase inhibitors. These findings suggest that the therapeutic potential of endothelial progenitor cells could potentially be harnessed by noninvasive pharmacologic manipulation and used to accelerate the endogenous repair mechanisms for inhibition of neointimal hyperplasia and prevention of restenosis following revascularization procedures. The simplicity and cost-effectiveness of this approach are major advantages compared to the stent and drug therapies currently in use. However, the long-term outcome of these strategies and their safety for use in patients have not been established.

We[277] and several other groups[248–250,254,255,282–290] have also shown the suitability of EPC for the seeding of prosthetic grafts and stents. Seeding of autologous EPC into polytetrafluoroethylene (ePTFE) segments led to rapid endothelialization of the graft segments after carotid interpositional grafting.[277] Furthermore, the cells remained attached to lumen of the graft for at least 4 weeks after transplantation. Using a similar approach, Kaushal et al.[285] showed that seeding of EPC into decellularized porcine iliac vessels implanted as coronary interposition grafts formed a functional endothelial layer and improved vasodilatory function and patency of the grafts, and Dichek et al.[249] reported that retrovirally transduced sheep endothelial cells overexpressing tissue-type plasminogen activator (t-PA) remained attached to stainless steel intravascular stents after balloon inflation in vitro. These authors have subsequently demonstrated that the seeded endothelial cells remain attached to the surface of the stent when exposed to pulsatile flow in vitro.[286] Others have shown that delivery of proangiogenic cytokine VEGF accelerates endothelialization of stents after deployment in balloon-injured arteries.[267,287] Recently, Aoki et al.[288] used CD34 antibody coated to metal stents to capture EPC to the stent. Sixteen patients with de novo coronary artery disease were treated with the bioengineered stents

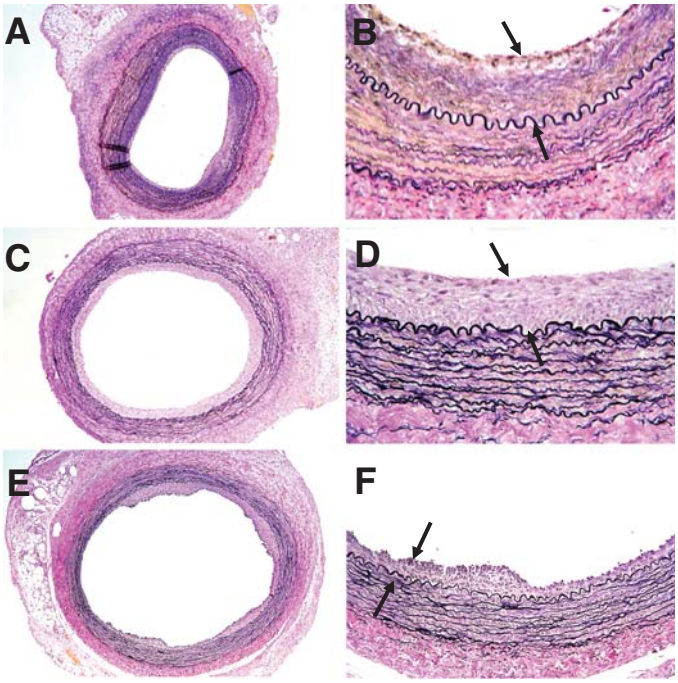

FIGURE 71.7. Inhibition of neointimal proliferation by EPCs in balloon-injured carotid arteries. All sections were stained with Accustain elastic stain. Arrows indicate neointima. (A,B) Saline-injected (untreated) arteries 2 weeks after injury. (C,D) Arteries treated with genetically modified EPC expressing GFP. (E,F) Arteries treated with genetically modified EPC expressing eNOS.

in this single-center, prospective nonrandomized trial (HEALING-FIM study: Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth–First in Man). The authors reported no stent thrombosis, and histologic analysis at 1 month indicated that the percent of luminal stenosis was significantly reduced with the EPC capture stents compared to the stainless steel stents. However, late neointima hyperplasia normally associated with stainless steel stent implantation was not reduced in the EPC capture stents, and the authors attributed this to the instability of the EPC. Nevertheless, this trial established the feasibility and safety of this strategy, and future efforts should focus on improving the stability of the transplanted cells. These findings imply that seeding of stents prior to implantation with genetically modified EPC may be useful for prevention of in-stent restenosis and thrombosis. Mobilization of EPC with cytokines has also been shown to be effective in promoting in vivo endothelialization of prosthetic grafts.

Bhattacharya et al.[289] and Shi et al.[290] reported that mobilization of bone marrow by exogenous G-CSF enhances endothelialization and patency of small-caliber prosthetic grafts implanted as carotid interposition grafts in association with an increase in the number of circulating EPCs, suggesting that the mobilized cells are recruited to the site of grafting to participate in endothelialization of the graft.

Summary

The role the endothelium in the maintenance of vascular homeostasis is firmly established, and endothelial dysfunction is recognized as the precursor of vascular diseases. Vascular remodeling is a pathophysiologic feature of vascular disease, and neointima hyperplasia is the major cause of restenosis and graft atherosclerosis. Despite the remarkable achievements in the medical and surgical treatment of vascular diseases, hypertension and atherosclerosis remain the major causes of morbidity and premature death, and the still relatively high percentage of restenosis and graft failure following surgical revascularization calls for continued development of novel therapeutic strategies to overcome the deficiencies of the current approaches.

Given the role of endothelial injury in restenosis, it appears logical that strategies to enhance endothelial recovery should be considered. In this regard, endothelial progenitor cells may provide a substrate for reendothelialization of damaged vessels, and several preclinical studies have attested to the feasibility and potential offered by these cells. Nevertheless, the successful clinical application of cell therapy for vessel repair will have to overcome several technical and biologic hurdles. For example, the scarcity of circulating progenitor cells makes it difficult to expand sufficient the number of cells without incurring the risk of cell senescence, and cells from patients with cardiovascular disease have various degrees of dysfunction, thus limiting their use as an autologous source for vascular repair in these patients. Also, strategies to improve retention and survival of the transplanted cells need to be developed. Genetic engineering may provide an important strategy to enhance the survival, engraftment, and function of these cells and render them efficient therapeutic vehicles in treatment of vascular disease. Likewise the success of gene therapies for vascular disease has been limited by the poor efficiency of the delivery vector systems. The development of vectors with enhanced tropism for the vessel wall and capable of prolonged gene expression may provide a solution to the efficiency issues associated with vascular gene transfer. As we have learned from the various formulations used in drug-eluting stents for prevention of restenosis, the complexity of the pathologic process involved in restenosis suggests that simultaneous manipulation of multiple targets, such as inflammation, proliferation, apoptosis, and extracellular matrix, may be necessary for effective and sustained therapeutic benefit. Many of these novel strategies are currently being developed and evaluated using animal studies, and we should expect to see some of these forthcoming developments moving into clinical trial and into the clinical arena.

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