Vascular Biology and Medicine in the 1990s: Scope, Concepts, Potentials, and Perspectives

Victor J. Dzau, MD; Gary H. Gibbons, MD; John P. Cooke, MD; and Nowa Omoigui, MD

A vascular disease is the leading cause of mortality and morbidity in the United States. Coronary artery disease, cerebrovascular disorders, pulmonary venous thromboembolism, and systemic and pulmonary hypertension account for one third of the national annual mortality. Traditionally, the practice of cardiology has emphasized the diagnosis and treatment of the sequelae of these disorders. This approach has been exemplified by major advances in cardiovascular surgery, interventional catheter techniques, and heart-lung transplantations, to name a few. Recently, major conceptual shifts have occurred in our approach to vascular disease. Increasingly, we recognize the importance of preventive cardiology and the need to understand the fundamental mechanisms mediating these disorders. Specifically, there is an explosion of new information on the complex and intricate processes that maintain homeostasis of the vessel wall and the pathobiological events that result in vascular diseases. We believe that the future in the management of vascular disease will depend on the development of novel diagnostic and therapeutic strategies based on our growing knowledge of vascular biology. This article is intended to review the latest developments in vascular biology and provide our perspectives on the future of the emerging discipline of vascular medicine.

The concept of vascular biology and medicine has evolved dramatically from William Harvey's original description of the circulation in which the vasculature was conceived as a passive conduit transporting blood to and from vital organs. It is now recognized that the vasculature is a complex organ capable of sensing its environment, transducing signals to the cells within the vasculature or to the surrounding tissue, and synthesizing local mediators that promote functional or structural responses. A constant feature of these interactions is the delicate balance between countervailing mechanisms. Vascular cells produce vasoconstrictors as well as vasodilators, procoagulants as well as anticoagulants, proinflammatory mediators as well as anti-inflammatory agents, and growth stimulators as well as inhibitors. Disturbances in this delicate balance may play a role in the development of vascular pathology.

For example, a common feature of many forms of vascular diseases is that endothelial function is impaired. Metabolic, mechanical, and immunologic injury to the endothelium may disturb the homeostatic balance within the vasculature by inducing endothelial dysfunction. Given its role as a regulator of vascular tone, structure, hemostasis, and the inflammatory response, any dysfunction of the endothelium may have profound effects on the state of the vasculature. In addition to the endothelium, other cells within the vasculature may also participate in the regulation of vascular function and structure. In particular, smooth muscle cells and infiltrating macrophages release a variety of mediators that modulate vessel tone, hemostatic balance, cell growth, extracellular matrix production, and inflammation. Further elucidation of these cellular interactions and the autocrine and paracrine effects of the mediators released by cells within the vessel wall should provide novel insights into the understanding and treatment of vascular disease.

Advances in vascular biology have depended on the contributions of investigators from multiple disciplines (e.g., cell biology, pathology, cardiology, hematology, molecular genetics, hypertension, lipid metabolism, etc.). Thus, the investigators in vascular research face the difficult challenges of synthesizing and analyzing a broad and diversified body of data and of applying the knowledge of vascular biology to the understanding of the pathogenesis of vascular disease and the development of novel therapeutic strategies for the future.

In order to review systematically this broad topic of vascular biology and medicine, this article is organized into four major sections. The first focuses on the biology of the vessel wall, with an initial review of the autocrine and paracrine functions of the blood vessels, with particular emphasis on the endothelium. These include the sensory functions of the endothelium as well as the mediators involved in the homeostatic control of vascular tone, hemostasis, and vascular structure. This section also examines cell-cell interactions at the endothelium-blood interface. In addition, it includes discussions regarding the mechanisms of incorporation of lipoproteins, the organization of the extracellular matrix within the vessel wall, and how abnormalities of these interactions may lead to pathological states in humans. In the second section, we will review the pathobiological mechanisms of vascular diseases.
better understanding of these pathological conditions has resulted from studies of animal models. Accordingly, specific models of vascular diseases ranging from balloon injury to transgenic animals will be examined, with emphasis on the relevance to human disease in the second section. Next, we focus on new perspectives and future directions in vascular medicine and therapy based on the advances in vascular biology. Treatment modalities range from endovascular reconstruction to antibody-targeted drug therapy to gene therapy as components of emerging strategies in the 1990s. Finally, we discuss the importance of applying the biology of blood vessels to clinical practice and the need for training physicians and scientists in this emerging frontier of vascular biology and medicine.

**Biology of the Vessel Wall**

**Autocrine-Paracrine Mechanisms of Vascular Responses**

Let us first examine the mechanisms by which the blood vessel detects changes in the circulation and regulates its function and structure. A key element in the vessel wall responsible for these functions is the endothelium. The endothelium is strategically located to serve as a sensory tissue assessing hemodynamic conditions such as blood flow and pressure as well as ambient oxygen status. In addition, the endothelium participates in the modulation of flow and pressure by its response to circulating vasoactive and other humoral substances as well as its own production of biological mediators.

The capacity to sense conditions within the local environment of the blood vessel is mediated via specific receptor-activated cellular events. Vasoactive substances such as bradykinin, inflammatory mediators such as platelet activating factor, and factors involved in hemostasis such as thrombin all evoke changes in phosphoinositid metabolism and increases in intracellular calcium within endothelial cells by this mechanism. In addition, these mediators also activate ion channels within the endothelium by receptor-coupled mechanisms. These second messenger systems in turn induce cellular events that enable endothelial cells to regulate tone, inflammation, and hemostasis. In this review, for the purpose of focus, we will limit the discussion to the capacity of the vessel to sense and respond to hemodynamic stimuli.

It has been a long-standing clinical observation that atheromatous lesions develop in areas of disturbed blood flow, particularly at branches and bifurcations within the circulation. In vivo studies suggest that flow disturbances have profound effects on endothelial cell function and influence the infiltration of monocytes during the development of vascular lesions. Moreover, turbulent flow conditions are associated with increased vascular cell proliferation. Thus, hemodynamic forces undoubtedly play an important role in stimulating vascular remodeling and the development of lesions. The question is this: How does the endothelial cell sense the hemodynamic changes?

Studies of cultured endothelial cells exposed to flow in vitro have documented a variety of functional alterations that occur over periods of seconds, minutes, and hours. Shear stress, or the tractive force exerted by blood flow, appears to activate an endothelial potassium channel. In a manner analogous to ligand–receptor binding events, shear stress activates phosphoinositid metabolism, which promotes a modest increase in intracellular calcium and activation of protein kinase C. The increase in intracellular calcium is potentiated by the presence of vasoactive substances that promote increased intracellular calcium, e.g., ATP.

In response to the hemodynamic and humoral changes, the vessel wall (especially the endothelium) synthesizes and secretes biologically active substances that secondarily control its tone and structure. Thus, increased flow or shear stress is associated with increased production of endothelium-derived relaxing factor (EDRF) and prostaglandins as well as decreased endothelin mRNA levels and reduced secretion of the peptide. Over a period of minutes to hours, there is cell realignment in the direction of flow, increased pinocytosis, and increased low density lipoprotein (LDL) receptor expression. In addition, shear stress modulates the thrombogenicity of the endothelium as well as the composition of the extracellular matrix by inducing the expression of tissue plasminogen activator activity and inhibiting fibronectin synthesis. Recent in vitro model studies suggest that the long-term structural adaptation to change in flow may be mediated by the induction of autocrine/paracrine growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β). Indeed, preliminary studies suggest that shear stress induces endothelial cell gene transcription of PDGF by via novel cis and trans regulatory elements. These studies suggest that flow-induced changes in endothelial cell–derived growth factors may modulate vascular structure and pathological lesion formation. We speculate that the nature of the flow stimulus (i.e., laminar versus turbulent) may be an important determinant of the balance of growth stimulatory versus growth inhibitory factors expressed by the endothelium. These molecular mechanisms may be responsible for the development of atherosclerotic lesions at areas of flow disturbance.

Although not as well characterized, endothelial cells also respond to the effect of pressure or stretch. This may be transduced via a recently described stretch-activated channel on the endothelial cell surface. Similar to the response to shear stress, mechanical stretch appears to modulate the cytoskeleton, activate the production of vasoactive substances, and induce factors that modulate the extracellular matrix. These in vitro data are in accord with studies in vivo that hemodynamic stimuli may have profound effects on vascular homeostasis. Clearly, the capacity to sense hemodynamic stimuli and modulate accordingly vessel tone, hemostasis, or vascular cell growth has profound implications in the development of vascular disease.

As mentioned above, the responses of the vessel wall to alterations in the environment include the production or activation of vasoactive substances, factors involved in hemostasis, and adhesive molecules and growth factors. The remainder of this section will examine these autocrine-paracrine mechanisms of vascular regulation beginning with a discussion of vasoactive substances. Although the endothelium participates in the degradation of vasoactive substances such as bradykinin and the
generation of agents such as angiotensin (ang) II, much recent focus has been on endothelium-derived substances, particularly EDRF.\textsuperscript{36,37} It is now appreciated that there are several endothelium-derived vasoactive substances including vasodilators such as prostaglandins, EDRF (nitric oxide or a nitrosodervative that releases nitric oxide), and endothelium-derived hyperpolarizing factor (a labile arachidonic acid metabolite).\textsuperscript{38} In addition, vasoconstrictors such as endothelin and endothelium-derived contracting factors, e.g., superoxide anions and thromboxane A\textsubscript{2}, are produced by the endothelium.\textsuperscript{39,40}

The production of EDRF is not only stimulated by shear stress but also by a variety of mediators such as acetylcholine, vasopressin, ADP, serotonin, bradykinin, and thrombin. It is noteworthy that some of these mediators appear to activate production via pertussis toxin-sensitive pathways (e.g., thrombin), whereas others do not (e.g., bradykinin).\textsuperscript{42} Indeed, the impairment of endothelium-dependent vasodilation that accompanies atherogenesis may be related to dysfunction of endothelial cell pertussis toxin-sensitive G-protein coupling.\textsuperscript{42} Conversely, the reduced endothelium-dependent vasodilation observed in hypercholesterolemia may be due to reduced activity or synthesis of EDRF.\textsuperscript{43} EDRF is an endogenous nitrosodvasodilator that appears to be derived from l-arginine metabolism, has a half-life measured in seconds, and induces vasodilation by activating guanylate cyclase.\textsuperscript{36,37,44,45} Studies conducted with inhibitors of EDRF\textsuperscript{46} suggest that it contributes significantly to the control of basal vascular tone in animal models as well as in humans. It not only serves as a local vasodilator but also modulates hemostasis by inhibiting platelet aggregation and adhesion. Its effect on platelet function is synergistic with prostacyclin.\textsuperscript{36}

Although vasoactive substances released by platelet aggregates (i.e., serotonin and ADP) normally induce vasodilation, these factors promote vasoconstriction in the setting of a diseased vessel with depressed EDRF production.\textsuperscript{47} There is evidence to suggest that leukocytes may play a role in promoting endothelial dysfunction.\textsuperscript{48} Indeed, a decrease in EDRF release has been described in a variety of vascular disease models such as hypertension, balloon injury, reperfusion injury, transplant arteriosclerosis, and atherosclerosis.\textsuperscript{49} Although the cause of this impairment is unclear, it appears to be a marker of endothelial dysfunction. Based on the effects of EDRF on platelet function, it is conceivable that this abnormality of endothelial function may play a significant role in promoting vasoconstriction and thrombosis in disease states. For many years, clinicians have recognized the linkage between vascular disease, vasoconstriction, and thrombosis in the course of treating acute ischemic syndromes. These observations suggest that the endothelium of diseased vessels has a diminished capacity to produce factor(s) that inhibit vasoconstriction and platelet aggregation. EDRF may serve as a useful paradigm in the further study of the mechanisms of acute ischemic syndromes.

Recent insights into the regulation of hemostasis have also clarified a novel mechanism for the linkage between vascular disease and thrombosis. In this context, it is important to recognize that the endothelium serves as a vital platform for the regulation of coagulation and fibrinolysis. Within the endothelial microenvironment there is a complex interplay of coagulation factors, thrombomodulin, thrombin, protein C, heparin antithrombin III, plasminogen, plasminogen activators, and plasminogen activator inhibitors (PAIs). The presence of specific binding sites on the endothelial surface appears to play an important role in these enzymatic cascades.\textsuperscript{50} For example, the binding of plasminogen to the endothelium enhances the catalytic activation of the molecule 10-fold by promoting conversion to the N-terminal lysine-plasminogen form.\textsuperscript{51} Similarly, the binding of tissue plasminogen activator to its surface receptor protects it from the inhibitory effect of PAI.\textsuperscript{52}

Another intriguing molecule that binds to the endothelium is lipoprotein (a) (Lp(a)). Although the physiological function of this protein has yet to be defined, one region of the Lp(a) molecule shows structural homology to LDL apolipoprotein B-100 and is linked to another region that contains multiple kringle domains that are highly homologous to the structure of plasminogen. Indeed, Lp(a) competes with plasminogen for binding to the endothelial cell receptor. As a result, Lp(a) may shift the balance of hemostasis toward thrombosis by decreasing plasmin generation by the endothelium.\textsuperscript{53} Clinical studies have described an association between plasma Lp(a) levels and thrombotic events in acute ischemic syndromes such as myocardial infarction. In addition, Lp(a) immunoreactivity has been described in the intima of atherosclerotic vessels as well as in the neointima of saphenous vein bypass grafts but not in normal vessels.\textsuperscript{54} Hence, increased expression of Lp(a) at sites of vascular disease may diminish the intrinsic fibrinolytic capacity of the vessel and thereby promote thrombosis in patients with atherosclerosis. It is hoped that further elucidation of the mechanisms linking chronic vascular disease with acute ischemic syndromes will facilitate the development of new therapeutic strategies.

Interestingly, many of the factors produced by the endothelium to modulate hemostasis also participate in the modulation of vascular structure. Glysosaminoglycans such as heparan sulfate contribute to the anticoagulant properties of the endothelial surface yet also inhibit vascular smooth muscle growth and migration.\textsuperscript{55,56} Similarly, the synthesis of the serine protease, plasmin, not only results in thrombolysis but also contributes to restructuring the extracellular matrix.\textsuperscript{57} Urokinase plasminogen activator appears to play a role in the associated localized proteolysis that occurs with cell migration.\textsuperscript{58–60} Plasmin also activates the latent collagenase produced by the endothelium and directly participates in the proteolysis of other matrix proteins.\textsuperscript{57}

The regulation of proteolysis within the vessel wall modulates vascular cell growth and migration in addition to its effects on the extracellular matrix. In vitro studies suggest a linkage between cell growth and migration induced by growth factors and the activation of proteolytic enzymes. For example, basic fibroblast growth factor (bFGF) stimulates endothelial cell migration and proliferation in association with the activation of plasminogen activator synthesis.\textsuperscript{61} Blockade of plasmin generation in vitro inhibits the effect of bFGF on cell migration.\textsuperscript{62} In addition, plasmin converts the multifunctional peptide TGF-β\textsubscript{1} from the secreted latent form to its biologically active form.\textsuperscript{63} Biologically active TGF-β\textsubscript{1} in turn has profound effects on matrix produc-

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ular, TGF-β appears to antagonize the endothelial cell migration and proliferation induced by bFGF.\textsuperscript{65} The inhibition of migration appears to be associated with the induction of PAI by TGF-β.\textsuperscript{66} Hence, bFGF and TGF-β regulate plasmin generation by the induction of plasminogen activator and PAI, respectively. These two autocrine growth factors apparently modulate endothelial cell migration by this same mechanism. Also of interest is the observation that very low density lipoprotein (VLDL) can induce PAI-1 from endothelium.\textsuperscript{67} It is anticipated that further research in this area will define the importance of these systems in vivo. These experiments in vitro suggest that the regulation of proteolytic activity by vascular cells is an important determinant of vascular structure. One could speculate that these proteolytic systems may play a role in the migration of vascular smooth muscle cells (VSMCs) into the intimal space, the repair of a denuded endothelial surface, or changes in luminal dimensions that occur in various vascular diseases.

In vitro observations have established a linkage between growth factors, cell migration, and reconstruction of the extracellular matrix. Indeed, the development of vascular disease is associated with abnormalities of cell migration and growth control within an altered extracellular matrix. It is postulated that the vessel remodels itself in response to vascular injury. The question is this: What are the principal mediators that control cell migration, growth, and extracellular matrix in response to vascular injury?

In any vascular response, one observes a net balance between the effects of stimulators of cell migration/proliferation and inhibitors of these same phenomena. In addition, each cell type within the vessel wall appears to have the capacity to produce factors that influence cell migration and proliferation in an autocrine or paracrine fashion. VSMCs and the endothelium produce a variety of growth regulatory substances such as PDGF, bFGF, insulin-like growth factor 1 (IGF-1), TGF-β\textsubscript{1}, colony stimulating factor 1, interleukin-1 (IL-1), modified LDL, prostaglandins, and EDRF. In addition, bloodborne elements such as platelets and monocyte/macrophages also contain or synthesize thrombin, platelet-derived endothelial cell growth factor (PD-ECGF), epidermal growth factor, TGF-α, TGF-β\textsubscript{1}, bFGF, PDGF, modified LDL, IL-1, heparin-binding epidermal growth factor (HB-EGF), and tissue necrosis factor (TNF). The relative contribution of these various growth and chemotactic factors on the vascular response varies according to the nature of the stimulus and cell–cell interactions within the vessel wall.\textsuperscript{68-72} The vascular response is also influenced by the specific actions on different cell types by the various growth regulatory substances. For example, PD-ECGF will affect the proliferation of arterial endothelial cells but not smooth muscle cells.\textsuperscript{73} Moreover, growth regulatory substances may have different effects on various cell types within the blood vessel. The multifunctional peptide TGF-β\textsubscript{1} stimulates smooth muscle cell and monocyte migration but inhibits endothelial cell migration.\textsuperscript{65,76-79} It also appears that TGF-β\textsubscript{1} can either inhibit or stimulate vascular smooth muscle growth, depending on the experimental conditions.\textsuperscript{76,77} The stimulatory effect of TGF-β\textsubscript{1} appears to be mediated by induction of autocrine PDGF-AA production by the smooth muscle cells, whereas the inhibitory effect is a direct response to TGF-β.\textsuperscript{73,79} Furthermore, TGF-β\textsubscript{1} induces a downregulation of the receptor for the PDGF-AA homodimer. Hence, the net growth response to TGF-β\textsubscript{1} may depend on the concentration of the peptide and the relative abundance of receptors for the PDGF-AA homodimer.\textsuperscript{73}

Elucidation of the pathogenesis of these lesions not only depends on defining the interactions among growth factors and their receptors but also defining the relative proportion of different cell types within the lesion. Recent immunohistochemical studies suggest that inflammatory cells are present at early stages of the vascular lesions of hypertension as well as vascular injury.\textsuperscript{80,81} These findings imply that the actual differences in the pathogenesis of hypertensive vascular disease and that characterized by an obvious inflammatory response such as transplant arteriosclerosis may be more quantitative than qualitative.\textsuperscript{81} Each of these clinical disease subtypes may actually be part of the same spectrum of vascular disease involving similar pathophysiological mechanisms.

Advances in molecular biology have enabled investigators to progress beyond morphological description of lesion composition to a description of the mediators involved in the pathogenesis of the lesion. Analysis of growth regulatory peptide expression in the vascular lesions of hyperlipidemic models demonstrates the expressions of these genes in several cell types within the lesion including endothelial cells, smooth muscle cells, and macrophages. There is increased gene expression of the PDGF β-type receptor, the oncogene c-myc (which encodes colony-stimulating factor 1), and TGF-β\textsubscript{1}. Approximately 20% of macrophages expressed the PDGF B chain, but lipid-laden macrophages and smooth muscle cells were negative for this peptide.\textsuperscript{82} Proliferating cell nuclear antigen immunoreactivity, a marker of cell proliferation, was detected in smooth muscle cells that also expressed the PDGF A chain within vascular lesions.\textsuperscript{83} These findings are consistent with the notion that these lesions evolve as a result of the interaction of several cell types and multiple growth regulatory substances. Further definition of the mediators and modulators of vascular cell growth in vascular disease is one of the major tasks in vascular biology and medicine in the 1990s.

The Blood Vessel as an Interface Between Circulating and Tissue Factors

Given the multidimensional capabilities of the vasculature and the strategic location of the vessel as the interface between blood and the tissues, the blood vessel plays an important role in first-line defense against factors that may induce tissue damage. The response to these injurious agents is often complex and may lead to pathology. As discussed above, the evolution of atherosclerotic vascular disease involves many processes such as the infiltration of leukocytes into the vessel wall, alterations in lipid metabolism, cell migration through the extracellular matrix, and eventually, thrombosis caused by platelet aggregation at the site of stenosis. Each of these components appears to involve the interaction of specific ligands and cell surface receptors categorized as adhesion molecules. The expression of these molecules may be an important aspect
of the pathological process. In this section, we will examine the role of adhesive molecules governing cell migration and the effect of these cell–cell interactions on lipoprotein metabolism.

The endothelium as the interface between the blood stream and the vessel wall is ideally situated to function as a gatekeeper regulating the traffic of cell movement from the circulation to sites within tissues. It has become increasingly apparent that the endothelial lining is a complex and heterogeneous structure. The adherence of cells onto the endothelial surface and the transmigration through the vessel wall is dependent on specific interactions that involve cell surface receptors and matrix proteins. The process of cell localization involves a complex interplay between the molecules that govern cell adherence and local cytokines and chemotactic factors that modulate binding site availability and direct cell migration.

The adhesive molecules involved in endothelial cell interactions are composed of three basic families: the immunoglobulin superfamily, which includes the antigen-specific receptors of T and B lymphocytes; the selectins, which are integral to the interaction of lymphocytes and neutrophils with the endothelium; and the integrin family, which is important in platelet adhesion and cell migration.

The integrins are dimeric cell surface glycoproteins consisting of an α-subunit noncovalently bound to a highly disulfide cross-linked β-subunit. The integrin family is composed of subfamilies defined by a common β-subunit. The specificity for individual binding sites arises from the association of the common β-subunit with different α-subunits within each family. The members of the integrin family include cytoadhesins such as the platelet glycoprotein GPIIb/IIIa, leukocyte membrane proteins including LFA-1 (CD11/CD18, αLβ2), Mac 1 (αMβ2), p150/95 (αXβ2), as well as matrix protein receptors for collagen, fibronectin, laminin, and vitronectin. An early event in vascular injury after endothelial denudation involves the attachment and adhesion of platelets to the subendothelial matrix and eventual thrombus formation. The attachment of platelets to the subendothelial matrix involves a multimeric interaction with von Willebrand factor, the nonintegrin glycoprotein Ib complex, as well as the integrin GPIIb/IIIa with fibrinogen and vitronectin. The multivalent nature of the binding provides a so-called “zipperlike” attachment. Although the adhesive molecules within the matrix are always present, the accessibility of the GPIIb/IIIa complex is modulated by platelet activation. Peptides based on the sequence arg-gly-asp (RGD), present as a motif in several adhesive proteins, are capable of blocking platelet adhesion both in vitro and in vivo. These peptides may be of clinical use in the management of acute ischemic syndromes.

The relevance of these proteins to clinical medicine is also illustrated by patients with Glanzmann's thrombasthenia who have dysfunctional platelets based on abnormalities related to the GPIIb/IIIa complex. A variant of this disorder involves a point mutation in a region associated with divalent cation binding in close proximity to the ligand binding site, an observation that underscores the importance of divalent cations in the binding of adhesive molecules in general as well as the role of IIb/IIIa in platelet function in humans.

The control of cell adhesion not only regulates hemostasis but is also critically important in modulating the inflammatory response. Inflammatory chemotactic factors such as platelet-activating factor, leukotriene B4, complement C5a, and formyl-methionyl-leucyl-phenylalanine (FMLP) stimulate inflammatory cell binding to endothelial cells via altered expression of the CD11/CD18 integrins present on the surface of activated leukocytes. CD11/CD18-independent transmigration mechanisms have also been described. Conversely, the cytokines IL-1 and TNF increase leukocyte-endothelial binding by enhancing expression of ICAM-1 (intercellular cell adhesion molecule) and ELAM-1 (endothelial leukocyte adhesion molecule) on the endothelial surface. ELAM-1 is a member of the selectin family that is characterized by a calcium-dependent lectin region and an epidermal growth factor motif. It is transiently expressed in endothelial cells 2–8 hours after IL-1 stimulation and mediates a neutrophil adhesion pathway independent of ICAMs and leukocyte integrins.

The increase in ICAM-1 expression on endothelial cells activated by cytokines is more sustained than ELAM. ICAM-1 and the recently identified ICAM-2 are members of the immunoglobulin superfamily of adhesive molecules and serve as counterreceptors for the integrin LFA-1 on the lymphocyte cell surface. Unlike ICAM-1, ICAM-2 is expressed constitutively on endothelial cells and is not upregulated in response to cytokines. In accord with in vitro observations, the administration of IL-1 in vivo and several inflammatory conditions are associated with the induction of ICAM-1 and ELAM expression and leukocyte infiltration in animal models and in humans.

Upon activation with IL-1, TNF, or endotoxin, the endothelium also secretes a leukocyte adhesion inhibitor recently identified as interleukin-8 (IL-8). Indeed, IL-8 administered intravenously decreases skin inflammation induced by FMLP in vivo. Hence, the endothelium expresses proinflammatory as well as anti-inflammatory mediators that modulate leukocyte adhesion.

Endothelial cells also modulate the entrance and egress of lymphocytes via specific receptor interactions. Lymphocytes in the blood stream enter lymph nodes by binding to specialized “high” endothelial cells. A member of the selectin family, Mel-14, (1-selectin) functions as a peripheral lymph node lymphocyte “homing” receptor. These specialized homing receptors on lymphocytes bind to ligands selectively expressed on the specialized high endothelium termed “addressins.” The interaction of these adhesive molecules may facilitate selective recirculation of cells to the secondary lymphoid organ where a specific antigen was first encountered.

Multiple receptors on human monocytes are involved in adhesion to endothelial cells. Since monocyte adhesion and infiltration into the vessel wall appears to be a central process in atherosclerosis, it would be logical to question whether the early development of this disease (as induced by hypercholesterolemia) is associated with the expression of an adhesive molecule on endothelial cells that specifically promotes monocyte adhesion. Indeed, recent data suggest that such a process may take place. In these experiments, endothelial cells were activated with endotoxin, and antibodies...
were developed that blocked the increase in adhesion of a monocyte cell line in vitro. Immunohistochemical studies with this antibody documented staining of a monocyte adhesive protein in hypercholesterolemic rabbits on endothelial cells overlying foamy macrophages and in the LDL receptor–deficient Watanabe rabbit during the development of foam lesions. These results suggest that an “athero-ELAM” related to VCAM-1 (vascular cell adhesion molecule) may be an endothelial cell mediator and/or marker for early atherogenesis and may be potentially involved in the development of the initial fatty streak lesion.99

Clinical studies have clearly established the link between elevated LDL cholesterol levels and the development of atherosclerotic lesions.98 Once inflammatory cells have invaded the vessel wall, their function is modulated by the local milieu created by abnormalities in lipid metabolism. The monocyte that invades the vascular wall may be transformed into a foamy macrophage within a fatty streak lesion. This transformation can occur as the macrophage takes up modified (acylated or oxidized) LDL. Oxidatively modified LDL may be generated by incubation with endothelial cells, smooth muscle cells, or macrophages in vitro. The mechanism of oxidative modification in vivo is not known with certainty. It may involve the release of superoxide anions from the cells or by the transfer of oxidized lipid peroxides to LDL. The oxidation of LDL may be linked to the activity of the lipoperoxidase pathway. In situ hybridization studies show that 15-lipoxygenase mRNA and protein are present at high levels in macrophage-rich lesions of the Watanabe rabbit.100 It is speculated that inhibition of the oxidation of LDL may be synergistic with or at least additive with measures to reduce plasma levels of LDL in the prevention of atherosclerotic vascular disease.101

**Pathobiology of Vascular Diseases**

Research in vascular biology has improved our understanding of the pathophysiology of vascular diseases. It has been demonstrated that disturbances in vascular homeostasis can result in pathological conditions such as hypertension, thrombosis, atherosclerosis, and restenosis. Advances in these areas have depended on research using cell and molecular techniques, pathological studies, clinical investigations, and animal experimentation. The application of the knowledge of vascular biology to medicine is enhanced by the development of animal models of clinical diseases that allow investigators to examine specific hypotheses, elucidate the pathophysiology of disease processes, and test the potential efficacies of therapeutic modalities. Animal models of atherosclerosis have been studied for many years, and the pathobiology of this disease has been reviewed in the preceding section as well as in other publications.6,7 Although many forms of vascular diseases exist, this article will emphasize the use of molecular and cell biological technology in the studies of four major vascular disorders: myointimal hyperplasia, transplant arteriosclerosis, hypertension, and angiogenesis. In addition, the utility of the transgenic approach to the study of vascular disease will also be discussed. The following section is organized according to these specific areas.

**Myointimal Hyperplasia**

It has been postulated that the lesion of atherosclerosis develops in response to vascular injury. However, the precise nature of the vascular injury remains poorly defined. A large body of evidence has demonstrated that mechanical injury to the vessel wall induced by a balloon catheter stimulates the proliferation and migration of VSMCs into the subintimal space. This process of myointimal proliferation is characteristic of at least one component of the pathogenesis of atherosclerosis and therefore serves as a useful model of vascular disease.

Ironically, mechanical vascular injury is the principle underlying the current use of balloon angioplasty: Percutaneous balloon angioplasty mechanically initiates a pathological remodeling process within the vessel wall that produces luminal patency. However, the resultant myointimal hyperplasia induced in response to vascular injury produces clinically significant restenosis in over 30% of cases within 6–9 months.102

The loss of endothelium and platelet adherence that accompanies balloon injury were considered to be important initial events triggering the proliferative response. It has been postulated that growth factors released by platelets after injury are principal factors in the development of myointimal hyperplasia. However, recent studies showed that brief periods of endothelial denudation and platelet adherence did not result in smooth muscle proliferation or intimal lesions.103 Moreover, thrombocytopenic animals responded to balloon injury with a substantial increase in smooth muscle proliferation within the media.104 However, these animals did not develop intimal lesions despite the proliferative response. Indeed, a recent study demonstrated that the administration of PDGF antibody attenuated the development of myointimal hyperplasia by inhibiting primarily the migration of smooth muscle cells.105 These observations suggest that platelet factors such as PDGF play a primary role in stimulating smooth muscle cell movement into the subintimal space.

The mechanical deformation of balloon angioplasty also promotes the release of bFGF within the vessel wall.106 Vascular injury induces a cycle of cell death and cell proliferation that could result in the release of bFGF that may act as potent mitogenic and migratory stimulus for medial VSMCs. Furthermore, bFGF could also be deposited within the extracellular matrix in vivo as described in the vitro systems.108 It is conceivable that platelets and leukocyte-derived heparinases secreted after angioplasty promote the release of bFGF from this extracellular matrix reservoir to induce VSMC proliferation and migration. In fact, recent studies have shown that blockade of bFGF with neutralizing antibodies inhibits the initial wave of VSMC DNA synthesis after angioplasty in the rat model.106 Thus, it appears that autocrine bFGF and PDGF-BB released by platelets are principal mediators of VSMC DNA synthesis and migration, respectively, after balloon injury.

An additional source of mitogens is the proliferating vascular cells themselves. In response to vascular injury, there is a rapid induction of PDGF A chain within the medial layer of the vessel wall. After the initial induction within the media, PDGF A chain mRNA expression becomes localized to the neointimal cells undergo-
ing cell proliferation along the vessel lumen.\textsuperscript{108} Although it is clear that PDGF-AA plays an important role in the growth-promoting effects of ang II, IL-1, and TGF-\(\beta_1\) in vitro,\textsuperscript{71,109--111} it remains unclear whether PDGF-AA is an important mitogen in vivo. Indeed, the recent studies involving PDGF-BB infusion and neutralizing antibodies against PDGF suggest a predominant effect on cell migration rather than proliferation.\textsuperscript{105,112} It is postulated that PDGF may behave as an intracrine factor capable of inducing cell proliferation via an intracellular mechanism unaffected by exogenous antibodies.\textsuperscript{113} Alternatively, we speculate that the increased PDGF A-chain mRNA expression may be a marker of cell activation and/or phenotypic modulation of neointimal cells rather than an essential mediator of cell proliferation.

The induction of autocrine TGF-\(\beta_1\) expression after vascular injury follows a time course similar to PDGF A chain in the rat carotid model.\textsuperscript{114} Recently, we have described increased expression of TGF-\(\beta_1\) in RNA within restenotic lesions after angioplasty in humans.\textsuperscript{115} Although our laboratory and others have documented increased TGF-\(\beta_1\) expression in restenotic vessels, it is important to note that TGF-\(\beta_1\) is normally secreted in a latent, biologically inactive form.\textsuperscript{69} The studies reported to date have not documented whether TGF-\(\beta_1\) expressed after angioplasty is biologically active. It is intriguing that vascular injury is associated with increased plasmin activity\textsuperscript{68} and that this protease converts latent TGF-\(\beta_1\) to its active form. Hence, it is conceivable that active TGF-\(\beta_1\) is formed in vivo and that this multifunctional peptide modulates vascular cell migration, proliferation, and extracellular matrix composition during the pathogenesis of restenosis.

In addition to the classic peptide growth factors described above, it has become increasingly clear that vasoactive substances are important mediators of long-term changes in vascular structure. Ang II, norepinephrine, endothelin, and other vasoconstrictors can all stimulate VSMC growth.\textsuperscript{71,116,117} Cell culture studies have documented that ang II--induced VSMC growth is mediated by the induction of autocrine PDGF-AA, bFGF, and TGF-\(\beta_1\).\textsuperscript{118} In vivo, the infusion of ang II has been shown to potentiate myointimal lesion formation.\textsuperscript{119} Moreover, our laboratory has recently described a vascular renin--angiotensin system that is activated (especially angiotensin converting enzyme [ACE]) within the vessel wall in response to balloon injury.\textsuperscript{120} Hence, there is increased capacity to generate ang II locally within the vessel wall after vascular injury. Indeed, blockade of local generation of ang II with ACE inhibition or ang II receptor antagonists prevent myointimal proliferation in rodent models of balloon injury.\textsuperscript{120,121} However, recent preliminary clinical data from the Mercator Trial assessing the effect of ACE inhibition on restenosis after angioplasty in humans have apparently failed to confirm these animal model studies.\textsuperscript{122} The disparity between these findings may be related to a number of factors including species differences, adequacy of ACE inhibition in clinical studies, complexity of the response to balloon angioplasty of an atherosclerotic vessel compared with a normal vessel, etc.

It is important to emphasize that most animal models of restenosis fail to accurately simulate the complexity of balloon angioplasty in humans. For example, there is evidence that subintimal VSMCs are phenotypically distinct from normal medial VSMCs and therefore respond to growth regulatory factors differently.\textsuperscript{69} Moreover, the vessel dissection caused by angioplasty creates a unique environment in which the atherosclerotic plaque containing macrophages, lymphocytes, and modified VSMCs is juxtaposed with activated platelets, thrombus, and mechanically injured medial VSMCs. Within this rich cellular milieu, it is conceivable that in addition to the growth factors described above, other factors such as IGF-1,\textsuperscript{123} IL-1, TNF, vascular endothelial growth factor, HB-EGF, serotonin, endothelin, and thrombin\textsuperscript{124} may play a role in the pathogenesis of neointimal lesion formation after angioplasty.

Given the complexity of this pathological process combined with the difficulty of avoiding adverse effects of treatment, it seems unlikely that a “magic bullet” will be discovered that selectively blocks a single mediator to prevent restenosis. Further research will be necessary to identify a circumscribed set of final common pathways that are essential for the pathogenesis of lesion formation. A multipronged therapeutic strategy directed at specific cellular pathways necessary for cell adhesion, proliferation, migration, and matrix production may be necessary to prevent restenosis. Effective preventive strategies developed for restenosis after angioplasty may also be useful in improving the efficacy of other revascularization techniques such as stents, atherectomy, and vein grafts.

**Cardiac Transplant Arteriosclerosis**

Although advancements in immunosuppression therapy have improved the early survival of recipients of heart transplants, the long-term success of the graft is limited by the development of transplant arteriosclerosis.\textsuperscript{125} The morphological appearance of transplant arteriosclerosis is distinct from classic atherosclerosis lesions in that it is mostly diffuse and concentric without significant lipid accumulations.\textsuperscript{126} This form of vascular disease, characterized by myointimal hyperplasia, is particularly interesting because it follows an accelerated course and occurs in vessels that were previously “normal.” This “complication” of transplant technology provides a unique opportunity to study the roles of immune injury, endothelial cell dysfunction, and vascular proliferation in the pathogenesis of vascular lesions in humans.

The factors involved in the pathogenesis of these lesions remain to be defined. Possible contributory factors include the immunosuppressive agents themselves, viral infections (e.g., cytomegalovirus\textsuperscript{127}), as well as the hyperlipidemia and hypertension that are prevalent among these patients. It is postulated that whereas classic rejection involves cytotoxic T cells that result in target cell death, graft arteriosclerosis may represent a delayed type of hypersensitivity reaction mediated by helper T cells (CD4+).\textsuperscript{127}

Initiation of the cellular immune response requires recognition of histocompatibility antigens, specifically class II HLA in the case of human CD4+ lymphocytes. Helper T cell populations expand in response to class II antigens, which define the cells as “foreign.” Experiments performed in vitro have demonstrated that products of activated T cells such as gamma interferon and other cytokines induce increased class II HLA expres-
sion in endothelial cells and VSMCs. Locally released cytokines promote the expression of leukocyte adhesion molecules and factors that promote inflammatory cell infiltration. Locally activated monocytes/macrophages in turn produce cytokines and growth factors that may modulate VSMC growth and migration. Based on in vitro observations, one can postulate that the presentation of foreign antigens by vascular cells may elicit a cascade of events that promote the process of inflammatory cell infiltration and myointimal proliferation that characterize transplant arteriosclerosis. Indeed, examination of pathological specimens obtained from transplanted hearts revealed the expression of the class II antigen HLA-DR on the endothelium in transplant arteriosclerosis but not on the endothelium of atherosclerotic vessels. The intimal thickening within the allograft vessels also contained CD4+ and CD8+ T lymphocytes as well as macrophages and smooth muscle cells identified by selective monoclonal antibodies. In contrast to typical human atheroma, many T cells were localized in a ring beneath the luminal endothelial cells.

These findings indicate that the interaction between foreign vascular cells within the allograft and host leukocytes plays an important role in the development of transplant arteriosclerosis. In addition, the contribution of humoral rejection has received increasing attention in recent years. Advancements in our understanding of this pathological process will not only have profound effects on the treatment of patients with end-stage heart disease but will also provide insight into the role of inflammatory cells and cytokines in the pathogenesis of other vascular lesions.

**Blood Vessels in Hypertension**

Another area of major clinical significance is hypertension. Hypertension may result in vascular damage and end-organ complications. The hemodynamic alterations in hypertension initiate adaptive changes in the conduit and resistance vessels that are characterized by medial smooth muscle hypertrophy or hyperplasia, increased extracellular matrix, reduced compliance, and increased resistance. This adaptive remodeling response normalizes the wall stress and confers an increase in vascular reactivity. These vascular changes may in themselves contribute to the amplification of vasoconstriction, perpetuation of hypertension, and promotion of development of vascular complications such as atherosclerosis. Recent research has focused on these vessel wall changes and the cellular and molecular mechanisms of vascular hypertrophy in hypertension. A series of interesting experiments on this subject have been performed using several models of hypertension. Hypertension induced by the administration of deoxycorticosterone and a high salt diet (DOC-salt) results in a fourfold increase in the steady-state levels of TGF-β1 gene expression in the aorta. Changes in the gene expression of growth factor receptors such as the PDGF receptor have recently been recognized. It has been shown that PDGF β-receptor mRNA levels are increased severalfold in the aortas in DOC-salt-treated rats, spontaneously hypertensive rats, and aging animals.

In addition to alterations in cell growth, vascular remodeling in hypertension involves changes in the extracellular matrix. The onset of hypertension induced by either DOC-salt or ang II infusion resulted in a severalfold increase in fibronectin mRNA levels, which reverted to basal levels after correction of the hypertension. Pulse chase experiments and Western blot analysis also demonstrated increased secretion of fibronectin into the extracellular matrix of hypertensive vessels. Based on the finding that fibronectin influences VSMC growth in vitro, these observations suggest that the modification of matrix composition associated with the hypertensive state may influence the vascular cell growth response to increases in blood pressure.

It is well established that hypertension enhances the development of atherosclerosis in humans. In the presence of hypercholesterolemia, the vascular complications of hypertension are markedly potentiated. Animal models of concomitant hypertension and hypercholesterolemia may be particularly useful for the studies of the pathophysiological mechanisms for accelerated vascular disease. Indeed, the production of renovascular hypertension in the hyperlipidemic animal models results in an increase in atherosclerotic lesions that are directly attributable to elevations in blood pressure. Thus, changes in the extracellular matrix, autocrine/paracrine growth factor expression, and growth factor receptor expression that accompany the vascular response to hypertension may also influence the response to other forms of vascular injury such as hyperlipidemia. These findings provide an understanding of the potential molecular mechanisms that are responsible for the long-standing clinical observations that the interaction of risk factors promote the development of vascular disease. Indeed, single risk factor intervention may be therapeutically inadequate in this setting.

**Models of Angiogenesis in Vascular Diseases**

The generation of new blood vessels is central to the pathogenesis of certain disease processes such as diabetic retinopathy and tumorogenesis. In addition, neovascularization occurs within atherosclerotic plaques and may play a role in plaque rupture. Moreover, new vessels are formed in response to vascular occlusion to provide collateral flow as well as after myocardial infarction as part of the wound healing response.

The generation of new vessels is a complex process involving both cell proliferation and alterations in the state of differentiation of the endothelium. The slow sustained release of acidic fibroblast growth factor (aFGF) from a synthetic biopolymer matrix in vivo elicits an exuberant angiogenic response involving the chemotaxis of microvessel endothelial cells. Both aFGF and bFGF are angiogenic factors that are members of the heparin-binding fibroblast growth factor (HB-FGF) gene family. One of the unique features of these two HB-FGF prototypes is the lack of a signal sequence for secretion of the peptide. Recent mutagenesis studies with aFGF suggest that a nuclear translocation sequence plays an important functional role for the biological activity of the growth factor and imply that it may function as an intracellular polypeptide.

It is intriguing that IL-1α, a distant member of the fibroblast growth factor family, also lacks a signal sequence. In contrast to fibroblast growth factor, IL-1 inhibits endothelial cell proliferation. Interestingly, IL-1 induces the gene expression of cyclooxygenase in
association with this antiproliferative effect. In addition, the constitutive expressions of both IL-1 and cyclooxygenase mRNAs increase with the number of passages until the cells achieve a senescent state. Indeed, blockade of IL-1 expression with antisense IL-1 oligonucleotides promotes the extension of the cell life span.143

These provocative observations suggest a role for autocrine or "intrinsic" growth factors in the cellular transitions between states of proliferation, quiescence/differentiation, and senescence. It seems likely that the lack of a signal sequence is an important regulatory mechanism that limits the local effects of these potent growth factors, thereby maintaining tissue homeostasis. Understanding the mechanisms regulating the transitions between states of differentiation from proliferation to senescence is a fundamentally important biological problem that has important pathophysiological implications.

**Transgenic Approach to Studies of Vascular Disease**

The utilization of transgenic technology is an important approach to develop genetic models of vascular disease and examine the molecular mechanisms of pathological conditions in the future. It is now well established that foreign DNA molecules can be stably introduced into the germ line of experimental animals. This can be accomplished by direct microinjection of the DNA into the pronucleus of a fertilized egg, followed by reimplantation of the microinjected embryos into a suitable foster host. A fraction of the pups derived from the microinjected embryos will have incorporated the foreign DNA into their genome, and in most instances the newly acquired sequences will be passed on to subsequent generations in a mendelian fashion. Alternatively, the foreign DNA can be introduced via methodologies that rely on either the retroviral infection of embryos or by transfection of pluripotent embryonic stem cells. Each of these approaches can generate experimental "transgenic" animals that differ from the wild type by the addition or deletion of a single, defined gene. It is anticipated that the use of transgenic technology will facilitate the assessment of the complex interactions of genes and their products involved in cardiovascular homeostasis. Through the use of transgenics, individual components of these regulatory cascades can be altered so that cause and effect can be established.144

Another application of this technology involves the generation of cell lines from rare or intractable cell types. Typically, fusion genes are constructed such that expression of the oncogene is regulated by the promoter of the gene of interest and targeted to the cell type of interest. Expression of the oncogene is therefore tissue and gene specific, which in turn results in the targeted tumor formation from which cell lines can be derived.144,145

For example, fusion of the atrial natriuretic factor (ANF) gene promoter to the oncogene SV40 T antigen resulted in the generation of mice with a fourfold increase in basal plasma ANF levels.146 The significant reduction in baseline blood pressure observed in the transgenic animals is an intriguing observation that implies that ANF may play a more important role in the long-term regulation of blood pressure than previously believed.147 Consistent with the importance of ANF in fluid homeostasis, the administration of a volume load by intravenous infusion induced a potentiated natriuresis in the transgenic animals without a change in glomerular filtration rate.148 Moreover, it was possible to isolate atrial cells from animals that proliferate in vitro and yet retain certain phenotypic properties such as ANF granules, myosin filaments, and T-tubules.145

In addition to ANF, a number of transgenic models of cardiovascular diseases have been reported. These include models of hypertension, altered lipid metabolism, thrombosis, and vascular and cardiac hypertrophy.144,146-152 It is not difficult to predict that this technology will be very fruitful in the advancement of vascular biology and drug development in the 1990s.

**Vascular Diagnosis and Therapy in the 1990s**

Many of the exciting advancements in the area of vascular biology and medicine have been discussed in this review. It is evident that this field encompasses multiple disciplines and broad research activities. A major task for the 1990s is to integrate these activities under the common goal of vascular medicine. It is hoped that as we gain greater understanding of the mechanisms regulating vascular tone, hemostasis, lipoprotein metabolism, inflammation, and structure with the vessel wall, we will be able to apply our knowledge to the diagnosis and treatment of vascular disease.

Advancements in technology have expanded the capacity to diagnose vascular disease. Innovations such as angiography153 have helped to clarify the pathophysiology of acute ischemic syndromes by providing an intraluminal view of the blood vessel. It is anticipated that this technique will be a useful adjunct to other endovascular procedures such as laser angioplasty and atherectomy. Similarly, intravascular ultrasound154 shows promise as a tool that will complement angiography and angiography by providing a view of the blood vessel wall beyond the lumen. Intravascular ultrasound provides the capability of early detection of nonobstructive atherosclerosis and may be useful for the evaluation of treatment modalities to prevent or regress atherosclerosis and to detect and longitudinally follow early arteriosclerosis (e.g., in heart transplants) and other vascular lesions. Future investigations using this device will characterize the morphology of the vessel wall and will undoubtedly provide novel insights into the process of vascular remodeling in humans.

In addition to these diagnostic tools, technological innovations will also expand the therapeutic armamentarium in the fight against vascular disease. It is conceivable that the progress in the treatment of vascular disease such as that produced by balloon angioplasty may be eclipsed by the emergence of new generations of endovascular procedures.155 These include the use of laser technology and atherectomy catheters to remove atheromatous plaques as well as mechanical stents that physically remodel the diseased vessel wall. Given the ubiquitous problem of restenosis, it is anticipated that mechanical endovascular reconstruction will be coupled to adjunctive therapies that reduce the risk of restenosis.

Agents are being developed that inhibit thrombosis and/or myointimal proliferation. These agents may be incorporated into the vessel matrix or secreted by endovascular drug-releasing polymers seated within the lumen or delivered by intraluminal perfusion. For example, antisense oligonucleotides (short segments of
DNA) can be synthesized that selectively bind to the mRNA of the targeted gene and block the synthesis of the protein. This strategy can be used to selectively inhibit the expression of growth factors, adhesion molecules, and cell cycle regulatory genes necessary for cell migration/proliferation. Indeed, this strategy has recently been used to inhibit myointimal lesion formation in the rat balloon injury model.

In this context, the explosive growth in vascular biology has provided the foundation to formulate therapeutic strategies that include inhibition of thrombus formation, cellular migration, and proliferation. Research on the biology of restenosis and the application of modern biochemical and biotechnological methodologies should become important companions to the current and future interventional modalities in the therapy of patients with vascular disease.

Advancements in vascular biology may also translate into improved treatment of vascular disease by improving the efficacy of prosthetic vascular grafts. Thus far, vascular substitutes function adequately in large vessels under high flow conditions but are prone to acute thrombosis when used in smaller vessels such as the coronary circulation. Prosthetic grafts also develop anastomotic myointimal hyperplasia and luminal occlusion. This latter response appears to be a remodeling response to injury analogous to balloon injury models. The challenge is to construct a conduit with a nonthrombogenic surface and the capacity to inhibit myointimal proliferation. Future developments may entail genetically engineered endothelial cells or endovascular drug delivery from the prosthesis.

Unfortunately, improvements in medical therapy for vascular disease and its complications have lagged considerably behind the development of revascularization procedures. However, recent developments suggest that we are on the threshold of major advancements in the pharmacotherapy of vascular disease. In the 1980s, thrombolytic therapy of acute ischemic syndromes emerged with the use of genetically engineered plasminogen activators. To minimize bleeding complications, similar gene splicing techniques have been used to construct chimeric molecules that combine plasminogen activators with the binding region of fibrin-specific antibodies and thereby enhance drug specificity. As we gain greater insight into the role of endogenously produced molecules in the maintenance of vascular homeostasis, the technology of molecular biology should enable us to use these agents in the treatment of vascular disease.

Among the most exciting developments in cardiovascular medicine involves the use of somatic gene transfer technology. Retroviruses can be used as packages of DNA capable of binding to the cell surface, infecting the cell, and integrating its genetic sequences into the host chromosomal DNA without cytotoxicity. Recombinant retroviruses are engineered that insert the gene of interest into the host genome but lack the capacity to replicate. Several fundamental strategies are used to administer gene replacement therapy. The ex vivo approach involves harvesting cells from the tissue of interest, transfecting the gene of interest with recombinant retrovirus, selecting cells that effectively incorporate the gene, and transplanting the genetically modified cells back into the affected animals. Indeed, recent reports have documented that genetically engineered endothelial cells can be successfully seeded on either denuded regions of the vessel wall after angioplasty or on prosthetic grafts or stents and maintain expression of the gene of interest for extended periods of time. Alternatively, genes have been transferred directly into vessel wall in vivo using retroviral vectors or DNA complexed with liposomes. Gene transfer efficiency may be augmented by targeting methods using cell type-specific receptors or viral protein coats that may allow delivery of virus or genetic materials that influence gene expression directly to the tissue of interest in vivo. In addition to local therapy, systemic replacement can also be achieved, e.g., LDL receptor gene transfer into the liver of Watanabe rabbits that are genetically deficient of LDL receptors. Successful reduction in LDL cholesterol has been achieved with this approach. Recently, genetically engineered myoblasts injected into skeletal muscle have been shown to be another method for systemic hormone production.

Although technical limitations such as achieving optimal viral titers, transfection efficiency, stability of expression, and targeted gene transfer require further investigation, it is clear that this form of therapy and antisense oligonucleotide strategies will have an important impact on cardiovascular medicine in the 1990s and beyond.

Perspective in Research and Training

To develop the full potential of vascular biology and medicine, there is a strong need for close interactions and collaborations between the basic scientists and clinical investigators. Future advances and discoveries in vascular biology and medicine depend on a productive and synergistic relation between these disciplines. To address this need, the National Heart, Lung, and Blood Institute (NHLBI) has recently initiated a request for applications for program projects to develop centers of vascular biology and medicine designed to stimulate the transfer of basic insights in vascular biology into clinical practice. In addition, the NHLBI has initiated a multicenter pilot study to evaluate treatment and prevention strategies for cardiovascular complications in patients with peripheral arterial disease. The American Heart Association (AHA) recently developed an intercouncil vascular biology working group to increase multidisciplinary interactions and collaborations in research and educational programs among the AHA councils. In addition to developing integrated research programs, a generation of clinicians and scientists must be developed who are cognizant of the biology and pathobiology of the vasculature and are knowledgeable in the advances in vascular research. To the cardiologists, knowledge of vascular biology and medicine is essential to their practice. The principle of primary prevention is to prevent or minimize the vessel wall processes that result in vascular diseases such as atherosclerosis. The development of thrombolytic therapy is based on the knowledge of the coagulation cascade and fibrinolytic mechanisms. Interventional procedures such as angioplasty activate vessel wall processes that determine luminal patency or restenosis. Furthermore, cardiologists are increasingly treating patients with peripheral vascular diseases. Therefore, cardiologists must expand their interest and
knowledge to include vascular biology as well as the overlapping clinical disciplines of lipidology, thrombosis, and hypertension.

The necessity for cardiovascular training programs to give greater emphasis to training in vascular medicine and biology has become apparent. More recently, the leadership of these organizations has recognized the need to create the opportunity for cardiologists to receive training in vascular medicine. In 1988, the strategic planning committee of the American College of Cardiology (ACC) responded to the trends in vascular biology and medicine by advising the formation of a vascular disease committee. This committee is now in the process of developing recommendations on issues of training and practice in this area. Concurrently, the AHA and the National Institutes of Health (NIH) have pursued initiatives in vascular medicine. Both have recognized the need for integrated research and training programs. The NIH has galvanized great interest with several recent programs, most notably the Academic Award in Systemic and Pulmonary Vascular Disease. The purpose of these awards is to foster educational and clinical programs, which will integrate physicians from diverse disciplines who have skills in various facets of vascular disease (hypertension, coronary artery disease, thrombosis, and lipid and glucose metabolism). This multidisciplinary approach will provide integrated care for the patient with vascular disease and improve upon our current fragmented approach. The integration of these disciplines of medicine will not only provide superior patient care but will also provide an enriched environment for training in vascular medicine. The program guidelines promulgated by the NHLBI define vascular medicine as that clinical discipline which has as its objectives “the clinical characterization of all vascular diseases (arterial, venous, lymphatic, cerebral, coronary, pulmonary, aortic, renal, and peripheral), the pathogeneses of these diseases (including atherosclerosis, lipid metabolic disorders, systemic and pulmonary hypertension, lymphedema, thrombosis, vasculitis, and vasospastic disorders), as well as the diagnostic, therapeutic, and preventive approaches to these diseases.”

In 1991, vascular medicine programs at Stanford and Harvard universities were funded through this mechanism, and plans are to provide NHLBI sponsorship for additional programs in the near future.

These integrative research and training efforts should result in the development of a new generation of clinicians and scientists with expertise in the principles and applications of vascular medicine. Physicians trained in vascular biology and medicine may pursue academic careers in vascular research and by so doing will facilitate the clinical application of new concepts in vascular pathophysiology. Others will focus on improving education in vascular medicine at the medical school and postgraduate levels. Practitioners trained in these programs will provide expert consultation to their medical colleagues and will complement the work of radiologists, surgeons, and others interested in vascular diseases. The advantages that will accrue to our patients from this enlightened and integrated care and from the advances in vascular therapeutic research provide compelling rationale for moving ahead with these programs.

Recognition of the clinical and scientific potential of vascular medicine and biology has led to new programs at many medical centers, including our own. This trend, which has been fostered by the NIH, AHA, and ACC, promises to provide new discoveries, to generate new concepts, and to find novel solutions for vascular diseases in this decade.

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V J Dzau, G H Gibbons, J P Cooke and N Omoigui

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