Vascular Biology
The Past 50 Years

R. Wayne Alexander, MD, PhD; Victor J. Dzau, MD

It is an interesting endeavor to review progress in a field that did not exist 50 years ago in the present context of the term “vascular biology.” Certainly, in 1950, there was basic understanding of the role of arteries and veins in cardiovascular physiology and of capillaries in gas and nutrient transport. Furthermore, there was considerable clinical knowledge about the role of atherosclerosis in ischemia and infarction of the heart and other organs and of hypertension in inducing microvascular damage and organ failure, particularly in the kidney. “Vascular biology,” which connotes the study of the biology of the constituent cells of the normal and diseased vascular wall, first gained some currency in the 1970s in defining this new field of study, which has enjoyed explosive growth in the past 25 years. Thus, the state of knowledge in 1950 must be placed in the modern context inferentially.

Vascular Biology, 1950
The vascular smooth muscle cell was appreciated for its role in controlling vascular tone in the resistance arteries. Nitroglycerin had been known for decades to relieve angina pectoris, and Osler had speculated about the role of vasospasm in precipitating ischemia and infarction. It was presumed, although not generally specifically stated, that hypercontractility of a particular coronary artery segment was being invoked, inferentially indicting the vascular smooth muscle cells. The endothelium was known to be a nonthrombogenic surface, although little was understood of the underlying molecular mechanisms. Conversely, acute cardiovascular ischemic events, such as stroke and myocardial infarction, were known to be associated frequently with localized arterial thrombus formation. As alluded to above, investigators knew that hypertension damaged blood vessels but did not know the specific mechanisms involved. Finally, investigators knew that atherosclerosis itself involved inflammatory cell and lipid accumulation in the arterial wall, although their mechanisms of entry were not known. These facts are summarized in the Table.

Vascular Biology, 2000
Endothelium and Endothelial Dysfunction
A central role in the pathogenesis of atherosclerosis for the endothelial cells that line the arterial wall was posited in the 1970s, when it was discovered that their removal by mechanical means dramatically enhanced the ability of a high-lipid diet to induce the disease in animal models.1 These observations led to the original response-to-injury hypothesis of the pathogenesis of atherosclerosis.1 Subsequent observations in humans and animal models in the late 1970s, however, usually showed that the endothelium overlying atherosclerotic lesions was morphologically intact. To rationalize these apparently inconsistent observations, Gimbrone proposed the concept of endothelial dysfunction that acknowledged the central role of the normal endothelium in protecting against the development of atherosclerosis while positing that its cellular functions were abnormal in this setting. “Activation” of endothelial cell inflammatory responses by cytokine stimulation in in vitro studies became something of a surrogate for gaining molecular insights into potential mechanisms for endothelial dysfunction in vivo.

Endothelium-Derived Relaxing Factor/Nitric Oxide
Arguably, the most momentous changes that have occurred in the field of vascular biology in the past 50 years have been the discovery and elucidation of the endocrine/paracrine roles of the endothelium. Initial insights into the endothelium as a modulator of the hormonal milieu resulted from the observation that generation of angiotensin II from angiotensin I was dependent on angiotensin-converting enzyme (ACE) located at the endothelial surface. Hormonally regulated release by endothelial cells of vasoactive prostaglandins was described in the mid-1970s,2 and prostacyclin (PGI₂), which was both a vasodilator and a platelet anti-aggregatory agent, was subsequently described as an endothelial product.3 The most seminal event in this area, however, occurred in 1980. Robert Furchgott, who was later to win the Nobel Prize, described the phenomenon of endothelium-dependent vasorelaxation.4 In simple but elegant experiments, he and his colleagues showed, in in vitro experiments in organ baths, that preconstricted arterial rings would relax in response to muscarinic cholinerergic agonists only if endothelial cells were present. Removing the endothelium by any means abolished the vasorelaxation, which was mediated by an undefined endothelium-derived substance that was named endothelium-derived relaxing factor (EDRF). EDRF subsequently was
shown to be, in large part, nitric oxide (NO), which diffuses to the underlying vascular smooth muscle and stimulates the second-messenger cGMP to cause relaxation. Many, if not most, vasodilator stimuli, such as flow and multiple G-protein–coupled receptors, including those for serotonin and muscarinic cholinergic agonists, act through this indirect, endothelium-dependent mechanism.

**Vasospasm and Endothelial Dysfunction**

Attilio Maseri and his colleagues demonstrated coronary vasospasm angiographically in patients with unstable coronary syndromes in 1978 and initiated a period of intense interest in vasomotor abnormalities across the spectrum of coronary artery disease. Thus, in the early 1980s, there was a confluence of ideas—endothelial dysfunction as a cause of atherosclerosis, the endothelium as a major determinant of vasomotor tone, and coronary artery spasm as an important element in ischemic syndromes—that led to the development of major new insights into vascular biology at both the basic and clinical levels. The issue was whether the endothelial abnormality (dysfunction) that was associated with the development of coronary atherosclerosis also involved the endothelium-dependent vasodilator mechanism described by Furchgott. Furthermore, such an abnormality might contribute to coronary vasospasm, which could be evaluated clinically. The results of a study evaluating these possibilities were published in 1986. The endothelium-dependent vasodilator acetylcholine, a muscarinic cholinergic agonist, was infused selectively into the arteries of patients with stable angina pectoris who were undergoing PTCA. Acetylcholine predictably dilated angiographically normal segments but produced a paradoxical vasoconstriction in segments with either severe stenoses or minimal angiographic disease. Stimulation of the sympathetic nervous system with cold pressor testing elicited similar results. These observations provided some of the initial evidence for abnormalities in endothelial function in coronary artery disease and contributed to the development of vascular biology as a clinically important science.

Abnormalities of endothelial function, as reflected in disordered vasomotor control, have been demonstrated both in large arteries and in the microvasculature in multiple cardiovascular diseases in addition to atherosclerosis and including systemic and pulmonary hypertension and congestive heart disease. Many of the risk factors associated with cardiovascular disease, including diabetes mellitus, smoking, dyslipidemias, and low-estrogen states, are also associated with endothelial dysfunction. These associations suggested that there might be common mechanisms through which endothelial function is being perturbed, as will be discussed below. In addition, improvement in disordered vasomotor tone control after therapeutic interventions was quickly perceived to be a potential measure of clinical efficacy. This issue will also be discussed subsequently.

**Inflammation, Atherosclerosis, and Endothelial Dysfunction**

Abnormalities in endothelial vasodilator function, although they are likely to be clinically important, are really a surrogate for the more important question of how this dysfunction relates to the pathogenesis of atherosclerosis. As noted above, the fundamental inflammatory nature of atherosclerosis has been known in principle, but not really appreciated, for decades. A number of publications from the mid-1980s onward have emphasized the importance of inflammation in the disease process as reviewed by Munro and Cotran. Postmortem analysis of infarct-related lesions in coronary arteries showed a localized inflammatory response manifested by intense accumulations of mononuclear cells. The mechanisms by which these inflammatory cells are attracted into the arterial wall became a major focus of research. Considerable progress had been made from in vitro studies in defining leukocyte adhesion molecules that appeared on the endothelial surface after cytokine stimulation, and reagents (antibodies) were developed for binding and identifying these inducible proteins. This technology was then applied to the study of arteries of rabbits after several days of cholesterol feeding. Monocyte adhesion was observed at branch points in areas of low or disturbed flow that are known to be sites of predilection for the development of atherosclerotic lesions. In some cases, the mononuclear cell had entered into the cell wall and was located just beneath the overlying endothelial cell. In either instance, there was evidence of the expression on the endothelial cell surface of a new protein that was found to be the rabbit equivalent of human vascular cell adhesion molecule-1 (VCAM-1), which, through interaction with its counterligand VLA-4, causes adhesion of monocytes and T cells to endothelium. Thus, VCAM-1 became the prototype for what is undoubtedly a set of molecules that is stimulated by a hyperlipidemic milieu and that is involved in recruiting mononuclear cells into atherosclerotic lesions. These data raised the question, in the specific context of VCAM-1, of the identity of the intracellular signaling pathways in endothelial cells that stimulate the expression of adhesion and chemoattractant molecules.

**Oxidation in Vascular Regulation**

One of the major developments in vascular biology over the past 15 to 18 years has been the understanding of the importance of oxidation mechanisms in mediating physiological and especially pathophysiological responses in blood vessels. The seminal observations came initially from the Steinberg group in San Diego and subsequently from the Fogelman group in Los Angeles. The original observation was that cultured mononuclear cells would not take up freshly isolated LDL to form lipid-laden foam cells (the characteristic cell of the atherosclerotic lesion) but that exposure of the
LDL to cultured endothelial cells modified the lipoprotein so that it was taken up by the monocytes/macrophages to form foam cells. The modification of the LDL that permitted recognition and uptake was an oxidative one, and oxidized LDL was found to have protein biological, proinflammatory activities itself. Thus, an extracellular oxidation mechanism is thought to be centrally important in the cell biology of atherosclerosis. The UCLA group has made a compelling case for the role of minimally oxidized LDL as an initial proinflammatory stimulus that differs in a number of fundamental aspects from more extensively oxidized LDL. The primary point to be made here is that oxidative stress is a fundamental feature of atherosclerosis.

Extrapolating from the points about endothelial dysfunction made earlier, it is apparent that the vasomotor control abnormalities seen in atherosclerosis are occurring in the presence of oxidative stress, ie, the production of excessive amounts of reactive oxygen species (ROSs). These facts were important because, in a larger context, they suggested a molecular mechanism for endothelial dysfunction. It had been known earlier that NO, which itself is a radical, is degraded and inactivated by oxygen free radicals, the ultimate source of many ROSs. A series of papers by Harrison and colleagues in the early and mid 1990s provided compelling evidence that both hypertension and atherosclerosis are associated with enhanced production of oxygen free radicals as directly measured and at a time when NO production is continuing so endothelium-dependent relaxation is impaired. Inferentially, NO is being degraded and inactivated.

The demonstration of enhanced ROS production in settings known to be associated with inflammatory responses in arteries (atherosclerosis and hypertension) raised the possibility that oxygen-derived radicals might be involved in the intracellular signaling events controlling VCAM-1 gene expression. In fact, the gene for endothelial VCAM-1 is regulated by a reduction/oxidation (redox)-dependent activation of the transcription factor NF-κB. A number of intracellularly active antioxidants inhibit VCAM-1 expression in vitro in cultured endothelial cells and in hypercholesterolemic animal models. In these models, fatty streak and foam cell formation can be inhibited even in the presence of very high plasma cholesterol levels, raising the possibility that certain antioxidants may inhibit atherosclerosis by mechanisms other than the inhibition of the oxidation of LDL.

NO itself is a powerful antioxidant and is generated from the amino acid arginine by one of several isoforms of the enzyme NO synthase (NOS). Normal synthesis of NO by the amino acid arginine by one of several isoforms of the enzyme NO synthase (NOS). Normal synthesis of NO by the endothelium-dependent relaxation is impaired. Inferentially, NO is being degraded and inactivated.

The demonstration of enhanced ROS production in settings known to be associated with inflammatory responses in arteries (atherosclerosis and hypertension) raised the possibility that oxygen-derived radicals might be involved in the intracellular signaling events controlling VCAM-1 gene expression. In fact, the gene for endothelial VCAM-1 is regulated by a reduction/oxidation (redox)-dependent activation of the transcription factor NF-κB. A number of intracellularly active antioxidants inhibit VCAM-1 expression in vitro in cultured endothelial cells and in hypercholesterolemic animal models. In these models, fatty streak and foam cell formation can be inhibited even in the presence of very high plasma cholesterol levels, raising the possibility that certain antioxidants may inhibit atherosclerosis by mechanisms other than the inhibition of the oxidation of LDL.

NO itself is a powerful antioxidant and is generated from the amino acid arginine by one of several isoforms of the enzyme NO synthase (NOS). Normal synthesis of NO by the endothelium-dependent relaxation is impaired. Inferentially, NO is being degraded and inactivated.

The demonstration of enhanced ROS production in settings known to be associated with inflammatory responses in arteries (atherosclerosis and hypertension) raised the possibility that oxygen-derived radicals might be involved in the intracellular signaling events controlling VCAM-1 gene expression. In fact, the gene for endothelial VCAM-1 is regulated by a reduction/oxidation (redox)-dependent activation of the transcription factor NF-κB. A number of intracellularly active antioxidants inhibit VCAM-1 expression in vitro in cultured endothelial cells and in hypercholesterolemic animal models. In these models, fatty streak and foam cell formation can be inhibited even in the presence of very high plasma cholesterol levels, raising the possibility that certain antioxidants may inhibit atherosclerosis by mechanisms other than the inhibition of the oxidation of LDL.

Angiotensin II

The peptide angiotensin II was identified by the 1950s as a major pressor activator derived ultimately from the kidney. The physiology of the renin-angiotensin system was well characterized, and its role in renovascular hypertension was defined by the 1960s and 1970s. The requirement for conversion of the precursor angiotensin I to angiotensin II by a peptidase activity to generate a pressor effect was understood. The physiological importance of this activity and that of the enzyme that was named ACE became known with the discovery in the 1960s of inhibitory peptides derived from a South American toad that blocked the pressor effects of angiotensin I. The development of oral ACE inhibitors and the demonstration of their wide clinical efficacy were intellectual triumphs of the past half-century.

The physiology and cell and molecular biology of the renin-angiotensin system in general and of angiotensin II in particular were extensively characterized between 1975 and 2000. The first 15 years of that time, angiotensin II was generally viewed primarily as a uniquely potent and important vasopressor. In retrospect, the first suggestion that angiotensin II might have a broader role in vascular biology was the observation by Laragh and his colleagues in 1972 that patients with high-renin (and thus high–angiotensin II) hypertension had a higher cardiovascular mortality rate than did those with low-renin hypertension.

When and how the group convincingly confirmed their earlier observations in 1992, the same year that 2 major trials studying the effects of ACE inhibitors on the development of congestive heart failure and ventricular remodeling after acute myocardial infarction, SAVE and SOLVD, also reported a decrease in recurrent cardiac ischemic events. These studies raised the possibility, although it was not fully articulated at the time, that angiotensin II might have direct atherogenic, ie, vascular proinflammatory and proliferative, actions. In the early 1990s, however, there was no or little experimental context in which the findings could be understood at a mechanistic level. This situation changed rapidly over the rest of the decade.

Angiotensin II was found to induce oxidative stress on vascular cells in culture and in arteries in animal models. Infusion of the peptide into rats produced hypertension that was associated with endothelial dysfunction in much the same fashion as did hypercholesterolemia, as discussed earlier. The model of oxidative stress-induced vascular proinflammatory responses described previously would predict that hypertension produced experimentally by angiotensin II would be associated with arterial infiltration by mononuclear leukocytes. In fact, this was precisely what was observed.
Thus, angiotensin II is a potent proinflammatory molecule for blood vessels. It is also a potent growth factor for vascular smooth muscle and a major mediator of vascular growth and development.

Thus, the early clinical trials that inferred a major role for angiotensin II in the pathogenesis of atherosclerosis and acute cardiovascular events can now be understood, to an important extent, mechanistically in terms of the underlying vascular biology.

Vascular Smooth Muscle Cells and Vascular Remodeling

In 1950, the vascular smooth muscle was thought to have the sole function of controlling vascular tone through contraction or relaxation. Only a few vasoactive substances were known at the time: norepinephrine, epinephrine, and angiotensin. By the year 2000, a myriad of molecules with vasoactive properties had been discovered. The endogenous vasoconstrictors include endothelin, leukotriene, serotonin, thrombosome, etc; the vasodilators include NO, natriuretic peptide, prostaglandin E, and prostacyclin, to name a few. The paradigm of 1950 was that the contractile tone was under neuroendocrine control. We now realize that many of the vasoactive substances are synthesized locally within the vessel wall, thereby exerting autocrine and paracrine effects. The receptors and intracellular signaling pathways mediating the actions of these vasoactive substances have, in large part, been characterized, as noted above. With the introduction of molecular biological techniques, most of the genes encoding the vasoactive peptides, the processing enzymes, and the receptors have been cloned and genetically studied. As a result, our understanding of systemic, regional, and local control of vasomotor tone is much improved, and the pathophysiological role of the imbalance of these factors in cardiovascular diseases such as hypertension, angina, and congestive heart failure has been elucidated. An important outcome of these advances is the discovery of drugs that target these vasoactive substances, their actions, their synthesis, and cellular pathways that have yielded effective therapy for these disorders. These included ACE inhibitors, as discussed earlier; angiotensin (AT1) receptor antagonists; calcium channel blockers; α- and β-blockers; and more recently, leukotriene antagonists and possibly endothelin antagonists in the near future, to name a few. For these accomplishments and others in related areas, the Nobel Prize in Medicine and Physiology was awarded in 1982 to Bergstrom, Samuelsson, and Vane for their discoveries of prostanooids; in 1988 to Black, Elion, and Hitchings for synthesis of β-blockers; and in 1998 to Furchgott, Ignaro, and Murad for their discoveries of NO and its function.

In addition to contractile function, the vascular smooth muscle has been shown to be a pleiotropic cell capable of phenotypic changes associated with the synthesis of many biologically active molecules that mediate cell growth, death, and migration, as well as matrix modulation and inflammation. These actions of vascular smooth muscle play important roles in physiological vascular functions, such as vascular remodeling, and in pathological disorders, such as atherosclerosis, restenosis, transplant vasculopathy, and other vascular diseases. A cadre of endogenous biological mediators regulating smooth muscle phenotype and function has been identified; none of these were known to exist 50 years ago. These molecules, commonly synthesized in the vessel wall, include growth factors, proapoptotic factors, matrix glycoproteins, metalloproteinases, cytokines, chemokines, and adhesion molecules.

The ability of the blood vessel to adapt to and accommodate long- and short-term changes in flow is a critical function in cardiovascular homeostasis. In addition to the short-term changes in vasomotor tone in response to alterations in shear stress mediated by release of vasoactive substances, eg, NO, by the endothelium, long-term adaptive responses to sustained alterations in physiological or pathophysiological conditions are dependent primarily on changes of vascular structure. Earlier, Glagov proposed that atherosclerotic arteries could remodel structurally to maintain an adequate lumen. This concept was derived from careful morphological analysis of human atherosclerotic arteries. It is now known that active remodeling of the blood vessel involves cell growth or apoptosis, extracellular matrix expansion or contraction, and activation or inhibition of specific proteolytic enzymes or glycosidases. This remodeling response is usually a long-term adaptive process occurring in response to chronic changes in hemodynamic conditions. Abnormal or pathological remodeling in conditions such as hypertensive vascular hypertrophy, atherosclerosis, bypass graft disease, restenosis, and transplant vasculopathy involves inappropriate cellular and extracellular changes leading to narrowing or occlusion of the lumen. Thus, much progress has been made in the past 5 decades in the understanding of the fundamental importance of vascular remodeling in cardiovascular homeostasis and of the complex biological processes mediating physiological and pathological remodeling.

Angiogenesis

An area of vascular biology that has potentially significant clinical impact is angiogenesis. The generation of new blood vessels is central to the pathogenesis of certain disease processes, such as diabetic retinopathy, tumorigenesis, and chronic tissue ischemia. Indeed, 5 decades ago, the pathologists were well aware of the existence of excess collateral vessels in the myocardium associated with advanced coronary artery disease. Subsequently, angiographic techniques documented the importance of collaterals in ischemic heart disease. 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. During the past 2 decades, the cellular process of angiogenesis has been elucidated and families of molecules that mediate angiogenesis have been identified, as well as their receptors and intracellular signaling pathways. The emerging field of therapeutic angiogenesis holds promise for the treatment of ischemic disorders through increased angiogenesis and for conditions of pathological angiogenesis (through the inhibition of angiogenesis) such as neoplasm and diabetic retinopathy.

Impact of Vascular Biology on Clinical Medicine

The most important contribution of vascular biology is in the treatment of cardiovascular diseases. During the past 50
years, we have witnessed major transformation in the diagnosis and therapy of coronary artery disease, hypertension, and congestive heart failure. As we gain understanding of the mechanisms regulating vascular tone, hemostasis, thrombosis, and inflammation and of the pathobiology of acute ischemic syndromes, plaque pathology, and pathological remodeling, the new knowledge has been applied to the clinical arena. Indeed, diagnostic tools such as angiography, intravascular ultrasound, angiography, and nuclear imaging and MRI were developed in parallel with the information derived from vascular biology research. The expanded knowledge has also resulted in the development of effective therapeutics such as drugs, devices, and surgery. The future holds much promise for the development of novel therapies such as small molecule, antisense oligonucleotides, transcriptional factor decoys, protein therapy, gene therapy, cell-based therapy, and gene, cell, or tissue engineering. The interface of genetic or molecular technologies with devices or surgery is particularly exciting. It is important to note that proofs of concept in several areas have already been demonstrated by a number of laboratories.

Vascular biology successfully brought together basic scientists and clinical investigators. It stimulated interactions and collaborations among researchers from multiple disciplines and developed new training opportunities. From these activities emerged the discipline of vascular medicine. This trend has been fostered by the American Heart Association, the American College of Cardiology, and the National Institutes of Health. In the 1990s, the National Heart, Lung, and Blood Institute initiated a request for applications for Program Projects to develop centers of Vascular Biology and Medicine, as well as Academic Awards in Systemic and Pulmonary Vascular Disease. The American College of Cardiology formed the vascular disease committee to examine training and practice in this area. The American Heart Association, recognizing the importance of this field, developed an inter council vascular biology working group that eventually merged with the Council of Arteriosclerosis and the Thrombosis Council to form a new Council of Arteriosclerosis, Thrombosis, and Vascular Biology in 1996. Thus, from modest beginnings, vascular biology has grown and matured over the past 50 years to be a major factor in cardiovascular science and medicine.

References

Key Words: vasculature ■ endothelium ■ atherosclerosis
Vascular Biology: The Past 50 Years
R. Wayne Alexander and Victor J. Dzau

doi: 10.1161/01.CIR.102.suppl_4.IV-112

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/102/suppl_4/Iv-112

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

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

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