here is increasing understanding of the molecular and cellular pathophysiology of the vascular responses to injury that lead to coronary atherosclerosis and the clinical sequelae of the disease, whether exertional angina or acute myocardial infarction (MI). A common thread that links these events is an inflammatory response to injury, and it is increasingly appreciated that the inflammatory process not only initiates these lesions but often dictates their clinical presentation. A fundamental knowledge of these processes is necessary to understand the natural history of the disease processes that affect the patients who present to the catheterization laboratory and, in addition, to understand the consequences of the therapies employed during coronary intervention. This chapter is therefore designed to describe the known pathophysiology of atherosclerosis, and the conversion of stable atherosclerotic plaques to ones that cause acute coronary syndromes (ACS).

ATHEROSCLEROSIS: A RESPONSE TO INJURY

Atherosclerosis is a chronic inflammatory disease initiated and sustained by injury to the vascular wall (1). Largely through extensive epidemiologic studies, several injurious processes have been identified (Table 1-1). These include metabolic conditions such as sustained exposure to low-density lipoprotein (LDL), hyperglycemia associated with diabetes, and hyperhomocysteinemia. However, other factors, including physical (hypertensive changes in shear stress), environmental (tobacco smoke), and possibly infectious (Chlamydia pneumoniae or viral entities) processes, have also been implicated. The common thread of injury to the vessel wall is an inflammatory response that involves a complex and still incompletely understood sequence of interactions between endothelial and smooth muscle cells (SMCs), leukocytes, and platelets. These cells and their secreted growth factors and cytokines combine with lipids and components of the vessel wall to eventually form the mature atherosclerotic plaque. The central role of inflammation in the pathogenesis of atherosclerosis is evidenced by numerous epidemiologic studies that demonstrate a correlation between circulating markers of inflammation (e.g., fibrinogen, C-reactive protein [CRP], serum amyloid protein, and myeloperoxidase) and subsequent risk of coronary events (2,3).

ATHEROSCLEROSIS: PATHOGENESIS

There are several key biologic events involved in atherogenesis: extracellular lipid accumulation, leukocyte recruitment, foam cell formation, neointimal growth as a result of SMC migration and proliferation, extracellular matrix deposition, and vessel remodeling (Fig. 1-1).

Extra- and Intracellular Lipid Accumulation

The key event in the creation of the incipient atherosclerotic lesion is the accumulation of lipoproteins within the intima. These lipoproteins may subsequently be modified by processes such as oxidation and glycation under the conditions of aging or hyperglycemia. The modification of these lipoproteins helps to elicit a cascade of molecular and cellular events, including stimulation of growth factor and cytokine production from endothelial and SMCs. These early events lead to recruitment of leukocytes and eventually to SMC proliferation and migration, all of which act to form the mature atherosclerotic plaque. Of central importance is the understanding that hyperlipidemia is an inflammatory state. The connection between inflammation and hyperlipidemia is evident from the presence of foam cells, the hallmark of the fatty streak, which is the initial lesion of atherosclerosis. The foam cell is a macrophage named so because of the abundance of lipid within the cell. Macrophages bind and internalize modified lipoprotein particles via a number of “scavenger receptors,” including scavenger receptor-A family members, CD36, and macrosialin. Foam cells are able to further modify lipoproteins. In addition, lipoproteins can prove toxic to macrophages, leading to necrotic debris and free cholesterol clefts and ester within the lesion. This necrotic debris, along with the expression of the tissue factor and other molecules, leads to a very prothrombotic environment within the plaque and is a serious threat to local blood flow when there is loss of integrity of the barrier between the plaque and the blood stream.

Leukocyte Recruitment

Leukocytes, especially macrophages, play pivotal roles in atherosclerosis through their release of critical cytokines and growth factors that influence not only atherogenesis but also processes of plaque rupture and thrombosis. The process of leukocyte recruitment, attachment, and migration into the plaque is under the influence of a variety of molecules. As a response to injury, such as the accumulation of lipoproteins, endothelial cells express certain
adhesion molecules such as E-selectin, which interact with ligands on the surface of circulating leukocytes to begin a process of loose association and rolling along the surface of the vessel (4). Subsequent tight binding mediated by the integrin class of adhesion molecules stops the leukocytes prior to the process of diapedesis. Although their pathologic role is uncertain, soluble forms of cell adhesion molecules (CAMs) can be found in plasma. Human studies have demonstrated that plasma levels of intracellular CAMs (ICAM-1) and E-selectin correlate with clinical manifestations of coronary atherosclerosis (5).

Also central to recruitment of leukocytes to areas of vascular injury, chemokines are a group of chemoattractant cytokines produced by a variety of somatic cells, including SMCs, endothelial cells, and leukocytes. One important chemokine of the C-C class, monocyte chemoattractant protein 1 (MCP-1), participates in the recruitment of monocytes in particular (6). Also critical is the C-X-C chemokine, interleukin-8 (IL-8), which participates in the recruitment of leukocytes to areas of vascular injury. IL-8 has been extensively documented in the recruitment of neutrophils (7), and more recent evidence suggests that the murine analogue of IL-8, KC, also plays a critical role in the recruitment of monocytes to injured areas (8).

As lesions mature, there tends to be excess accumulation of leukocytes at the “shoulder” regions of plaques, where the eccentric plaque merges with the more normal architecture of the vessel. This clustering is thought to make these shoulder regions more vulnerable to the consequences of atherosclerosis (9). In addition, it has long been observed that atherosclerotic lesions develop preferentially at areas of bifurcations within the coronary tree. This likely is related to disturbances in flow patterns and resultant areas of flow separation and altered shear stress, leading to preferential areas of upregulation of adhesion molecules and increased leukocyte recruitment (10). In addition, monocytes may contribute to vascular calcification in response to two cytokines: monocyte colony-stimulating factor and receptor activator of NF-κB (RANKL) (11). Emerging evidence indicates that atherosclerotic plaque calcification is positively correlated with vulnerability. Several inflammatory mediators have been shown to modulate arterial calcification, thus increasing the risk of plaque rupture. Among these factors, RANKL/OPG axis might be of particular interest as a promising biomarker of plaque vulnerability in subjects with diffuse coronary calcification (12).

### Innate versus Adaptive Immunity

There are two major branches of the immune system: the innate or nonspecific arm and the adaptive or specific arm (13). There are several key differences that distinguish these two arms. The innate arm relies predominantly on phagocytic cells such as neutrophils and monocyte/macrophages, the cells most classically associated with atherosclerosis. The innate arm is not antigen-dependent and exhibits immediate response to foreign material. On the other hand, the adaptive arm is characterized by a specific antigen-dependent response that has the characteristic of conferring memory against the pathogen. Unlike the immediate response of the innate arm, the adaptive arm involves a lag time between exposure to the pathogen and response. The primary effector cells of this arm are lymphocytes. These two arms work in concert with dendritic cells of the innate immune system, representing a link between innate and adaptive immunity, as they are phagocytic cells, which then present antigens to cells of the adaptive system (14).
Innate Immune Response in Atherosclerosis

The monocyte is thought to be the first leukocyte recruited to the incipient atheroma after encountering complex signals that include soluble factors affecting general monocyte function in circulation. In addition, local factors affect the cells after monocyte adhesion to the endothelium and migration into the tissue. The defining cell of the fatty streak is the foam cell, a macrophage named so because of the abundance of lipid within the cell. Macrophages bind and internalize oxidized lipoprotein particles via a number of “scavenger receptors,” including scavenger receptor-A family members, CD36, and macrophinulin. Foam cells can also further modify lipoproteins, making them more inflammatory. However, lipoproteins can also lead to toxicity of macrophages, leading to cell death and eventually leaving necrotic debris and free cholesterol clefts and ester within the lesion. Two main directions of monocyte-to-macrophage differentiation are recognized: Type 1, induced by inflammatory stimuli such as interferon (IFN)-γ or lipopolysaccharide (LPS), and Type 2, induced by IL-4, IL-13, and other anti-inflammatory cytokines. Type 1 macrophages (M1) produce high amounts of reactive oxygen species and inflammatory cytokines such as TNF (tumor necrosis factor) or IL-1β. Type 2 macrophages (M2) show high expression of scavenger receptors, produce extracellular matrix components and remodeling enzymes, secrete anti-inflammatory cytokines such as IL-1ra, CCL18, and IL-10, and express typical surface markers (15–17). The phenotype of plaque-associated macrophages seems to be mixed, since infiltrating monocytes/macrophages are described to express markers of both M1 (TNF) and M2 (STAB1, CD163) (18). Macrophages amplify the inflammatory response through the secretion of cytokines such as TNF-α and IL-1β (13). Other cells of the innate immune response that have been implicated in atherosclerosis include mast cells, natural killer cells, and neutrophils (19).

Adaptive Immune Response in Atherosclerosis

In contrast to the monocyte, the CD4+ T-cell is the primary cell of the adaptive arm of the immune response present at atherosclerotic sites. It is believed that these T-cells and their secreted cytokines influence progression and vulnerability of plaques (19). As an example, IFN-γ appears to inhibit the growth of SMCs and promote apoptosis (programmed cell death) of these cells, leading to plaque vulnerability. In addition, IFN-γ appears to limit production of structural proteins (collagen and elastins) that SMCs secrete and may lead to a plaque more prone to rupture, as discussed below (20). CD4+ T-cells also express CD40 ligand and their surface, which is subsequently released as a soluble factor. Among its effects, soluble CD40 ligand appears to influence a variety of cell types to produce the highly procoagulant substance called tissue factor. There are subsets of CD4+ T-cells that have been identified by cellular receptors and the typical cytokines released from these cells. CD4+ TH1 T-cells are the predominant type at atherosclerotic lesions and are believed to promote atherosclerosis. CD4+ TH2 T-cells, on the other hand, are likely antiatherogenic. In addition, regulatory T-cells are present, which seem to suppress activation of other T-cells acting as an antiatherogenic mechanism.

Lastly, B-cells can also be identified within atherosclerotic lesions. Antibodies to oxidized LDL can be identified in human and animal models (19), and it is thought that this humoral immune response acts as a protectant against atherosclerosis, possibly by intercepting and neutralizing antigens before they reach sites of atherosclerosis.

There are other evidences to suggest that infection with agents such as C. pneumoniae or perhaps viruses can create antibodies with autoimmune features that promote atherogenesis (21).

SMC Migration, Proliferation, and Extracellular Matrix Deposition

SMCs and their products are responsible for giving structure to the mature atherosclerotic plaque, which is, at first, little more than a collection of lipids and foam cells. Under the influence of growth factors and chemoattractants such as platelet-derived growth factor and thrombin, SMCs migrate out from the media into the neo-intima, where they begin to proliferate. In addition, SMCs produce extracellular matrix constituents, including collagen, proteoglycans, elastin, fibrinogen, fibronectin, and vitronectin. These proteins often account for a substantial volume of the plaque and are important in determining the structural integrity of the fibrous cap. Giachitti et al. have shown evidence that these cells express bone matrix proteins, which has been subsequently corroborated by other investigators (22–24). This highlights the role of vascular smooth muscle cells in vascular calcification. In some patients, an additional process of mineralization of the atherosclerotic plaque will occur with deposition of calcium and osteopontin. Mineralization does not equate to a stable plaque, and has been associated with higher risk, especially in the elderly (25).

Plaque Angiogenesis and Hypoxia

A newly emerging area of interest is the potential role of angiogenesis in plaque growth and in the pathogenesis of atherosclerotic complications. New vasculature, under the influence of angiogenic growth factors such as hypoxia-inducible factor or vascular endothelial growth factor (26), may grow from the vasa vasorum within the adventitia into the plaque. These vessels may be disrupted and cause plaque hemorrhage independent of plaque rupture. The extravasated erythrocytes provide a local depot of cholesterol-rich red cell membrane and of heme, a source of iron, which is a stimulant for oxidative stress, which in turn would promote further growth.

In addition, analogous to tumor growth, these vessels may stimulate plaque growth. There is experimental evidence demonstrating inhibition of plaque growth by angiogenic inhibitors in a mouse model of atherosclerosis (27,28). Furthermore, neo vessel density is higher in nonstenotic segments and stenotic noncalcified plaques than in normal segments or calcified lesions (29).

The concept of hypoxia not only promotes angiogenesis but also contributes to proteolysis through the promotion of matrix metalloproteinases (MMPs), a family of interstitial collagenases that weaken the fibrous cap, and gelatinases capable of catabolizing nonfibrillar collagen, to which endothelial cells adhere (30–32). Proteolysis would ultimately lead to dissolution of the plaque extracellular matrix, remodeling, and plaque vulnerability. Hypoxia promotes the formation of proinflammatory cytokines and leukotrienes, and promotes Akt and β-catenin pathways with subsequent macrophage activation (31,33). In addition, conditions of lipid accumulation in macrophages are amplified with accumulation of triglyceride containing cytosolic lipid droplets and adipose differentiation protein expression, even in the absence of exogenous lipids. The lipid accumulation is a result of increased triglyceride biosynthesis, reduced [beta]-oxidation of fatty acids, and increased expression of stearoyl-coenzyme A desaturase (SCD-1), an important enzyme in the synthesis of fatty acids (33–35).
The Mature Atherosclerotic Plaque

The mature atherosclerotic plaque is therefore composed of several components, including a fibrous cap consisting of SMCs and extracellular matrix proteins overlying a necrotic lipid core consisting of free cholesterol esters, foam cells, other leukocytes such as T-cells, and necrotic debris of dead foam cells (Fig. 1-1). These plaques commonly are eccentric in nature, and there is heterogeneity in terms of the thickness of the cap as well as the distribution of leukocytes, which tend to cluster in shoulder regions. Both of these features have potential import in terms of propensity of plaques to cause ACS.

Vascular Remodeling

Although angiography remains the mainstay of diagnosis in coronary artery disease, its major limitation is that it provides information only on luminal encroachment of lesions, not on architecture of the vessel wall. Use of intravascular ultrasound has provided a much broader understanding of the nature of atherosclerosis by allowing systematic investigation of plaque architecture not only at sites of flow-obstructing lesions but throughout the vessel. Although the interventional cardiologist is most concerned with focal obstructive lesions in proximal portions of the vessel, it is important to realize that it is now recognized that atherosclerosis is almost always universally present throughout the coronary tree. The extent of impingement of the plaque on the lumen is controlled not only by the growth of plaque volume but also by vascular remodeling. Vascular remodeling involves restructuring of cellular or noncellular components of the wall, and can occur under a variety of stimuli (36). For example, under situations of hypertension, muscle mass of the vessel wall can increase in order to normalize wall stress. In atherosclerosis, remodeling may consist of compensatory enlargement of the vessel to preserve luminal area (Fig. 1-2). Central to the process of vascular remodeling are the MMPs, a family of zinc-dependent proteases that have been demonstrated to be upregulated in areas of vessel-wall remodeling and are thought to play a central role also in plaque rupture (37).

CLINICAL SEQUELAE OF ATHEROSCLEROSIS

Coronary artery disease can be conveniently thought of as a spectrum of syndromes from stable angina at one end, associated with exertional angina and relatively benign outcomes, to ST-segment elevation MI at the other, associated with sudden or complete thrombotic occlusion of an epicardial blood vessel and high rates of morbidity and mortality. The intermediate syndromes of unstable angina and non-Q-wave MI exist between these two extremes. However, unstable angina, non-Q-wave MI, and ST-segment elevation MI are collectively termed the ACS because of their similar pathophysiology and worse prognosis in comparison with those of stable angina. This is explained by the fact that complications from atherosclerosis can result from two related but distinct mechanisms: (a) simple luminal narrowing that can lead to an imbalance between supply and demand for blood, typically resulting in stable exertional angina; or (b) rupture of atheromatous plaques, resulting in thrombi of various degrees of occlusion (38). Critical to the understanding of coronary disease is the knowledge that the propensity for thrombotic complications depends on a variety of vascular biologic factors, not the degree of stenosis. Just as important, the atherosclerotic process fundamentally alters the normal vasomotor functions of the endothelium necessary to autoregulate blood flow in accordance with the demands of hemodynamics, a condition termed endothelial dysfunction.

Progressive Lumen Encroachment and Stable Angina

As atherosclerotic lesions grow in size and depending on the extent of compensatory vascular remodeling that occurs, they may gradually encroach upon the lumen of the vessel (Fig. 1-2). As a response to reduction in flow, there is vasodilation of the distal microcirculation to increase in flow. This reduces coronary vascular reserve or the ability of the coronary circulation to increase blood flow in response to demand, which typically leads to exertional angina, a condition that is short in duration and relieved by rest. At what point luminal encroachment causes symptoms depends on many factors, including the severity of the lesion, the demand of the distal cardiac bed, and the oxygen-carrying capacity of the blood stream. However, in general, lesions begin to produce symptoms when stenosis reaches ~60% to 70% of vessel diameter. Modern techniques of interrogating intracoronary hemodynamics with flow and pressure wires have taught the interventional cardiologist that lesions with the same degree of angiographic stenosis may have very different hemodynamic consequences (39). The measure most commonly used is the fractional flow reserve (FFR), which is defined as the ratio of the distal pressure in the coronary artery beyond the lesion divided by the pressure at the ostium of the artery. An FFR of 0.80 has been used to discriminate lesions likely to be ischemia producing. In the FAME study, routine measurement of the FFR was compared with angiography for guiding percutaneous coronary intervention in patients with multivessel coronary artery disease. The use of the FFR in addition to angiography significantly reduced the rate of all major adverse cardiac events at 1 year. A subanalysis of the 1,414 lesions (509 patients) in the FFR-guided arm of the FAME study was performed. Of these, 1,329 lesions were successfully assessed by FFR. Before FFR measurement, these lesions were categorized into 50% to 70% (47% of all lesions), 71% to 90% (39% of all lesions), and 91% to 99% (15% of all lesions) diameter stenosis by visual assessment. In the category 50% to 70% stenosis, 35% were functionally significant (FFR ≤ 0.80) and 65% were not (FFR > 0.80). In the category 71% to 90% stenosis, 80% were functionally significant and 20% were not. In the category of subtotal stenoses, 96% were functionally significant. Of all 509 patients with angiographically defined multivessel disease, only 235 (46%) had functional multivessel disease (≥2 coronary arteries with an FFR ≤ 0.80) (40,41).
Data from the early thrombolytic trials were instrumental in setting the stage for our modern understanding of ACS. As part of the design of these trials, patients presenting with acute MI underwent mandated angiography after randomization to either placebo or thrombolytic therapy. The angiograms revealed an unexpected finding: the majority of lesions responsible for MI had caused <50% stenosis (44). In addition, data from angiographic studies performed in patients before and after infarction show that mild and moderate stenoses may progress to cause MI in a matter of weeks to months. On analysis of four serial angiographic studies, only ~15% of acute MIs were found to arise from lesions that caused >60% stenosis on an antecedent angiogram (45) (Fig. 1-5).

The implication of these data is that the vascular biologic state of the lesion is responsible for its propensity to cause an infarct, not the severity of stenosis. These data should not be misinterpreted to suggest that lesion severity is correlated with danger of infarction. Rather, noncritical lesions represent a larger population than do critical lesions. In addition, as described earlier, compensatory enlargement of the vessel often accompanies atherosclerosis. Therefore, even mildly stenotic lesions may represent large plaques by volume. In summary, regardless of lesion severity, thrombosis resulting from lesion disruption causes the majority of MI.

Plaque rupture and thrombosis and the ACS

The historical view of the conversion of stable to ACS held that atherosclerotic lesions impinged upon the lumen of the vessel until some critical point was reached at which either vasospasm or thrombosis in situ developed to cause infarction. Considerable debate ensued as to whether thrombus found at autopsy was a premortem or postmortem phenomenon despite the fact that James Herrick had published his findings of thrombus as the predominant cause of sudden coronary obstruction in 1912 (42). The pivotal work was performed by DeWood et al., in his landmark 1980 paper (43), where he demonstrated angiographically that ST-segment elevation was associated with occlusion of epicardial vessels (Fig. 1-3) and made the observation that thrombosis was present at the time of infarct. There is now considerable data to confirm this, including autopsy studies (Fig. 1-4) as well as the use of angioscopy, which has revealed the presence of visible thrombus associated with both unstable angina and acute MI.

![Image](image-url)


**FIGURE 1-4** Histologic example of a ruptured plaque with subsequent thrombosis leading to a fatal myocardial infarction. (From: Constantinides P. Plaque hemorrhages, their genesis and their role in supra-plaque thrombosis and atherogenesis. In: Glagov S, Newman WP III, Schaffer SA, eds. *Pathology of the Human Atherosclerotic Plaque.* New York: Springer-Verlag; 1990:393–411, with permission.)

![Image](image-url)

**FIGURE 1-5** Compiled data from four thrombolytic trials showing that the majority of underlying lesions responsible for acute myocardial infarction are less than 50% diameter stenosis. (From: Smith SC. *Circulation.* 1996;93:2205–2211, with permission.)
FIGURE 1-6 Characteristics of stable versus vulnerable plaques. Vulnerable plaques have thinner fibrous caps and larger, more inflammatory-cell-rich lipid cores. (From: Libby P. Circulation. 1995;91:2844–2850, with permission.)

FIGURE 1-7 Thickness of the fibrous cap is a balance between synthesis of extracellular matrix proteins by SMCs and the breakdown of these products by degradative enzymes. These processes are largely under the influence of inflammatory cells. (From: Libby P. Circulation. 1995;91:2844–2850, with permission.)

due to SMC collagen synthesis. Inflammatory cells in atherosclerotic plaques also produce enzymes such as MMPs and cathepsins that are capable of degrading important constituents of the extracellular matrix (i.e., collagens, elastin) (9). Therefore, inflammatory cells can contribute to plaque weakening by decreasing SMC mass, decreasing extracellular matrix content, and increasing extracellular matrix degradation.

How and why plaques rupture when they do is a subject of increasing study. It has long been observed that there is a circadian variation in presentation of MI with a peak in the early morning hours (47). In addition, MI rates are known to be affected by events that produce population-wide stress, as was observed after the Northridge earthquake in Southern California in 1994 (48). These observations have led researchers to suggest that cortisol and adrenaline levels may impact plaque rupture through their effects on systemic hemodynamic parameters. On a smaller scale, biomechanical studies utilizing finite element analysis indicate that rupture sites coincide with highest circumferential biomechanical forces. These are highest at the shoulder regions of plaques (49). Therefore, there is an interesting combination of both biochemical and biophysical characteristics that makes rupture at the shoulder regions of plaques most likely. This is supported by histologic studies of coronary arteries of patients who died secondary to MI.

Although frank plaque rupture is the major antecedent cause of thrombotic complications of the ACS, other processes may also be responsible. Local superficial denudation of endothelial cells (perhaps secondary to apoptosis) may expose the internal elastic membrane representing an important thrombotic substrate. There is some evidence to suggest that these endothelial erosions occur more frequently in women and in diabetic patients. Mechanical injury during percutaneous coronary intervention is also another source of local plaque disruption, which may lead to thrombotic complications.

The final pathway through which either plaque rupture or endothelial denudation leads to alterations in flow is through thrombosis. Exposure of blood to the lipid core is a potent stimulus for thrombus formation, largely on the basis of exposure to tissue factor associated with lipid-laden and necrotic macrophages. There is a balance between procoagulant–anticoagulant and fibrinolytic–antifibrinolytic factors in the blood stream, which likely predetermines the consequence of any given plaque disruption. In the presence of an intact and robust fibrinolytic system, a mural thrombus might undergo rapid lysis, limiting its clinical consequences to unstable angina or non-Q-wave MI. Similarly, patients on antiplatelet agents such as aspirin obviously are protected to some degree. In the presence of prothrombotic factors, such as
elevated levels of fibrinogen or plasminogen activator inhibitor 1 (PAI-1), growth of a thrombus to occlusion may occur more frequently. Nonocclusive mural thrombus may be incorporated into the plaque during the process of healing, providing a mechanism for plaque growth.

There are numerous trials of antiplatelet therapy that corroborate the thrombotic paradigm of the ACS. Trials of lipid-lowering therapy have similarly demonstrated an interesting corroboration of theories of plaque vulnerability. These trials have demonstrated marked reductions in subsequent coronary events associated with lipid lowering, with essentially no change in lesion severity (43). As stated earlier, the hypothesis is that lipids within the plaque provide the critical initiating and sustaining inflammatory stimuli to plaque growth and rupture, and the beneficial actions of "statin" lipid-lowering agents may derive in part from the reduction of inflammation, leading to stabilization of the fibrous cap and reduced thrombogenicity of the inner core. There is increasing evidence to support lipid-lowering therapy as a vital adjunct to acute as well as chronic therapies for patients presenting with ACS.

A more complete understanding of the mechanisms of plaque rupture and the development of novel strategies to stabilize lesions represent a major goal of vascular biologists and clinical cardiologists. Complicating the clinical situation is the fact that many different lesions of varying vulnerable potential may coexist side by side in a vessel or throughout the coronary tree. Considerable interest exists in imaging techniques on both a macroscopic and molecular level to identify plaques most vulnerable to rupture in order to better prognosticate and direct therapy most effectively.

ENDOTHELIAL DYSFUNCTION

In 1986, Ludmer and colleagues reported that in patients with atherosclerosis, a paradoxical reaction occurred when acetylcholine was administered via an intracoronary route (50). Normally, acetylcholine leads to vasodilation of the epicardial coronary arteries; however, it was found that patients with atherosclerosis had vasoconstriction even in territories without significant lumen encroachment. Acetylcholine had previously been identified as working through an endothelial-dependent mechanism (51), and hence the concept of clinical endothelial dysfunction was born.

The endothelium is a monolayer of cells derived from the embryonic mesoderm that form a continuous layer on the intimal surface of the entire cardiovascular system, including the arterioles, veins, and chambers of the heart (endocardium); the capillary walls consist solely of endothelial cells. The endothelial cells have a variety of functions that play important roles in the maintenance of vascular integrity, including the regulation of vascular tone, vascular permeability, vessel wall inflammation, and thromboresistance, through expression of anticoagulants such as heparin sulfate and enzymes that destroy them (52,53). Given these properties, it is important for the endothelium to undergo rapid repair when damaged and for apoptotic cells to be quickly replaced by circulating endothelial progenitor cells, which are also central to angiogenesis throughout our life span (54).

Vascular tone is regulated by numerous factors whose counterbalance maintains normal vascular tone and responds to various physiologic stimuli. Nitric oxide (NO), generated from L-arginine by the action of endothelial NO synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin, diffuses to the vascular SMCs, and activates guanylate cyclase, which results in cGMP-dependent vasodilation (55). The endothelium also mediates the hyperpolarization of vascular SMCs via a NO-independent pathway, which increases K+ conductance and subsequently propagates the depolarization of vascular SMCs, maintaining vascular tone through the production of endothelium-derived hyperpolarizing factors (56). Other vasoconstrictive molecules such as endothelin-1 through activation of ETA receptors lead to vasoconstriction and proliferation. Other peptides such as endothelin 2 and endothelin 3 have been recently discovered (57,58). Endothelin 1 has also been expressed in the active plaque (59,60). Thromboxane A2, serotonin, and angiotensin II also play similar roles. Vascular permeability and cell-to-cell communication are controlled by endothelial proteins such as vascular endothelial cadherin (61). All of these factors act in a complex interactive fashion to maintain vascular tone in a variety of physiologic states.

Atherogenic stimuli activate cell signaling and therefore modulate cellular function in endothelial cells. The interaction between endothelial cells and immune cells is augmented in response to atherogenic stimuli by an upregulated expression of adhesion molecules. Vascular smooth muscle function is also modified through the altered production of vasoactive substances by endothelial cells (62). A dysfunctional endothelium is an early marker of the development of atherosclerotic changes and can also contribute to cardiovascular events (63). The autocrine/paracrine activity of endothelial cells makes it very difficult to investigate endothelial function in clinical research. Vascular reactivity tests represent the most widely used methods of clinical assessment of endothelial function given the limited ability to visualize vessels <500 μm in diameter. The aim of these tests is to activate or block endothelial cell function while measuring changes in vascular tone in selected vascular districts, and is thus a functional assessment of the microcirculation (64). These methods include coronary flow reserve (CFR) and coronary blood flow (CBF). It is also important to note that there are also noninvasive methods for evaluating endothelial dysfunction, although a thorough discussion of these is beyond the scope of this chapter. CFR is defined as the ratio of near maximal to basal myocardial flow in response to maximal hyperemia. It is a combined measure of CBF through both epicardial coronary arteries and the coronary microcirculation. A decrease in CFR could be attributed to both, and thus in the absence of epicardial vessel obstruction, it reflects solely the microcirculation (65,66).

Acetylcholine produces primarily a vasodilator response in patients with normal coronaries. In contrast, in patients with coronary artery disease or endothelial dysfunction, acetylcholine caused dose-dependent vasoconstriction (67). Adenosine acts predominantly on vessels <150 μm in diameter via stimulation of adenosine A2 receptors on the SMCs, and thus changes flow as a function of decreased resistance (68,69). These are two distinct mechanisms of which the former is endothelial dependent and the latter is endothelial independent. A ≥250% increase in CBF above baseline in response to acetylcholine and a CFR ratio of >2.5 in response to adenosine are considered normal (68). An abnormal response to both acetylcholine and adenosine indicates dysfunction in epicardial and resistance vessels involving endothelium-dependent and endothelium-independent mechanisms. An abnormal response to adenosine with normal response to acetylcholine indicates endothelial-independent dysfunction, while an abnormal response to acetylcholine suggests an endothelium-dependent dysfunction (68).

Endothelial dysfunction has been associated with stable angina as well as unstable angina and MI. Marks et al. followed up patients with chest pain/schismic cardiac disease but with normal coronary angiograms over a mean period of 8.5 years and noted a nearly
Multivariable analysis identified a higher FRS (p < 0.019) as a univariate predictor of lower CFR (p = 0.008), with 36% of all events related to ACS (71). An interesting study was conducted at Mayo Clinic by Rubinshtein et al. (72) to evaluate the relation between the Framingham risk score (FRS) and the presence of coronary risk factors to coronary microcirculatory vasodilator function in patients with early coronary atherosclerosis. The authors evaluated 1,063 patients (age: 50 ± 12 years, 676 [64%] females) without significant narrowing (<30%) on coronary angiography who underwent invasive assessment of coronary endothelial function. Coronary blood flow (CBF) in response to the endothelium-dependent vasodilator acetylcholine as well as the microvascular (endothelium-independent) CFR in response to intracoronary adenosine were evaluated. CBF and CFR were analyzed in relation to the FRS and the presence of traditional and novel risk factors. The estimated 10-year risk in this group was 5.4 ± 5.2%. Higher FRS was associated with lower CBF in men (p = 0.008), and was a univariate predictor of lower CFR (p = 0.012) in all patients. Multivariable analysis identified a higher FRS (p < 0.001), female sex (p < 0.001), and a positive family history of coronary disease (p = 0.043) as independent predictors of reduced CFR.

Other associations with endothelium-dependent microvascular dysfunction included age, elevated BMI, diabetes mellitus, impaired glucose metabolism (high plasma glucose level and glycated hemoglobin), hypercholesterolemia, and elevated L-arginine, while high-sensitivity CRP has no association. This was one of the largest studies evaluating the risk of endothelial dysfunction.

The authors concluded that in patients without obstructive coronary disease, a higher FRS was an independent predictor of reduced CFR (72).

The main treatment of endothelial dysfunction is through the modification of risk factors, although the search for targets is the subject of ongoing research. Current management focuses on lifestyle modification and cardiac rehabilitation (73), lipid-lowering agents that could improve dysfunction by their anti-inflammatory and antioxidant properties and their ability to restore vascular NO availability (63), angiotensin-converting enzyme inhibitors and angiotensin renin blockers (74), β-blockers (75), L-arginine (76), ranolazine (77), xanthine derivatives (78), and enhanced external counterpulsation (79). Calcium channel blockers are effective in Prinzmetal’s angina but ineffective in endothelial dysfunction (80). The agents that are used routinely as coronary vasodilators in the cath lab can be classified as endothelial dependent or independent in their mechanisms and are included in Table 1-2.

### TABLE 1-2

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<th>Vasodilators</th>
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<th>Endothelium-Dependent</th>
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<td>Na nitroprusside</td>
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<td>Nicorandil</td>
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<td>Smooth muscle cell relaxers</td>
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<tr>
<td>5-Hydroxytryptamine</td>
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<td>Bradykinine</td>
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### REFERENCES
