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Cardiovascular disease is the leading cause of mortality in the United States, Europe, a vast majority of Asia, and is likely to be the greatest threat to overall health worldwide. As a major cause of cardiovascular disease, the development of atherosclerosis starts early in childhood. Despite this fact, most individuals are asymptomatic until many decades later. Autopsy studies of coronary arteries from healthy, young American soldiers killed during the Korean conflict revealed surprisingly advanced atherosclerotic lesions. Intimal lesions were discovered in more than 50% of the right coronary arteries of the youngest group (15-19 years of age). More recently, fatty streaks, an early marker of atherosclerosis, have been found in the intima of infants. More advanced atherosclerotic lesions are first identified in the intima of three primary target vessels: the carotid and coronary arteries and the aorta. Although there is significant disparity in the evolution of lesion formation, ischemic coronary disease, stroke, peripheral artery disease, and transient ischemic attacks are among the clinical presentations of matured lesions and ruptured plaques. This chapter reviews the recent literature regarding the biology of atherosclerosis and considers in detail: 1) anatomical structure of the normal and diseased artery, 2) chronic endothelial injury and lipid hypotheses, and 3) the events that contribute to formation of the atherosclerotic lesion. The authors hope that this chapter will serve as a basic tutorial for the understanding of the biology of atherosclerosis, and provide an appreciation for the complexity of this disease by introducing new and exciting research contributions to this area.

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The Vascular Biology of Atherosclerosis

Robert Carter III and Harlan P. Jones

CONTENTS

Introduction

Cardiovascular disease is the leading cause of mortality in the United States, Europe, the vast majority of Asia, and is likely to be the greatest threat to overall health worldwide.[1,2] As a major cause of cardiovascular disease, the development of atherosclerosis starts early in childhood.[3] Despite this fact, most individuals are asymptomatic until many decades later. Autopsy studies of coronary arteries from healthy, young American soldiers killed during

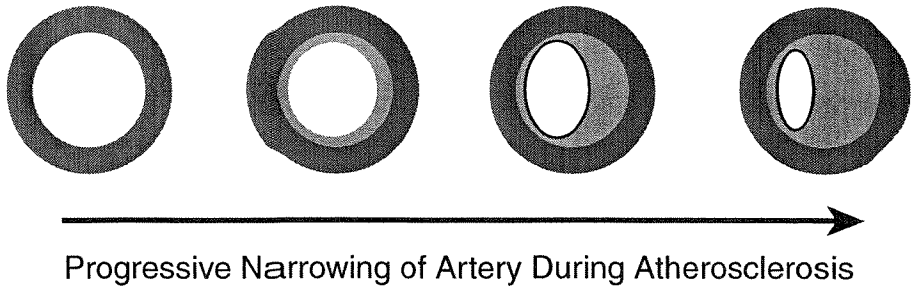

FIGURE 5.1
The progression of atherosclerosis. As the atheroma matures the lumen diameter is reduced which leads to decreased blood flow, thrombosis complications, and unstable plaques. The clinical presentations may be peripheral artery disease, cerebrovascular disease, or ischemic heart disease.

the Korean conflict revealed surprisingly advanced atherosclerotic lesions.[4] Intimal lesions were discovered in more than 50% of the right coronary arteries of the youngest group (15–19 years of age). More recently, fatty streaks, an early marker of atherosclerosis, have been found in the intima of infants.[5] More advanced atherosclerotic lesions are first identified in the intima of three primary target vessels: the carotid and coronary arteries and the aorta.[6,7] Figure 5.1 illustrates the progressive narrowing of the artery during atherosclerosis. Although there is significant disparity in the evolution of lesion formation, ischemic coronary disease, stroke, peripheral artery disease, and transient ischemic attacks are among the clinical presentations of matured lesions and ruptured plaques.[8,9]

Emerging epidemiologic studies[1,10] have shown that elevated low-density lipoprotein (LDL), male gender, increased homocysteine, and ethnicity are among the many risk factors and markers involved in the pathogenesis of atherosclerosis (Table 5.1). In a recent study of 557 first-generation immigrants, it was concluded that acculturation into western societies may also be an independent risk factor for coronary artery disease and atherosclerotic lesion development.[11] Nevertheless, among the consequences of acculturation are stress, dietary patterns, and physical inactivity which also have been identified as major risk factors for atherosclerosis and cardiovascular disease.

This chapter reviews the recent literature regarding the biology of atherosclerosis and considers in detail: (1) anatomical structure of the normal and diseased artery, (2) chronic endothelial injury and lipid hypotheses, and (3) the events that contribute to formation of the atherosclerotic lesion. The authors hope that this chapter will serve as a basic tutorial for the understanding of the biology of atherosclerosis, and provide an appreciation for the complexity of this disease by introducing new and exciting research contributions to this area.

TABLE 5.1

Risk Factors for Atherosclerotic Lesion Formation

Physical inactivity
Smoking
Infectious agents
Family history
Elevated LDL and VLDL
Low levels of HDL
Elevated lipoprotein (a)
Hypertension
Diabetes mellitus
Male gender
Homocysteine
Ethnicity
Obesity
Age

LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein.

Anatomical Structure of the Normal Human Artery

The structure of the normal artery consists of three layers: the intima, the media, and the adventitia (Figure 5.2). The intima, the innermost layer, is composed of an endothelial monolayer lying on the basement membrane with elastic fibers comprised of type IV collagen, laminin, and heparin sulfate proteoglycans.[12] This layer also contains smooth muscle cells (SMCs) embedded in sulfated polysaccharide, hyaluronic acid intimal thickenings.[13]

The endothelium of a normal, healthy artery functions as a non-thrombogenic surface and serves as a selectively permeable barrier, which regulates the transport of solutes across the arterial wall. Importantly, the vascular endothelium is also essential in the regulation of vascular tone, coagulation, and inflammatory responses.[14–16] Changes in shear stress and blood flow lead to phosphorylation of endothelial nitric oxide synthase (eNOS), which generates nitric oxide (NO), which then produces vasodilation.[17] The intima is separated from the media by an internal elastic lamina comprised primarily of the protein polymer elastin.[12]

The tunica media, the middle layer, is primarily comprised of SMCs surrounded by its own basement membrane. The media's basement membrane is anchored within an interstitial matrix composed of type I collagen, fibronectin, dermatan, and chondroitin sulfate proteoglycans.[12,18] This interstitial matrix is intertwined with perforated sheets of elastic fibers.

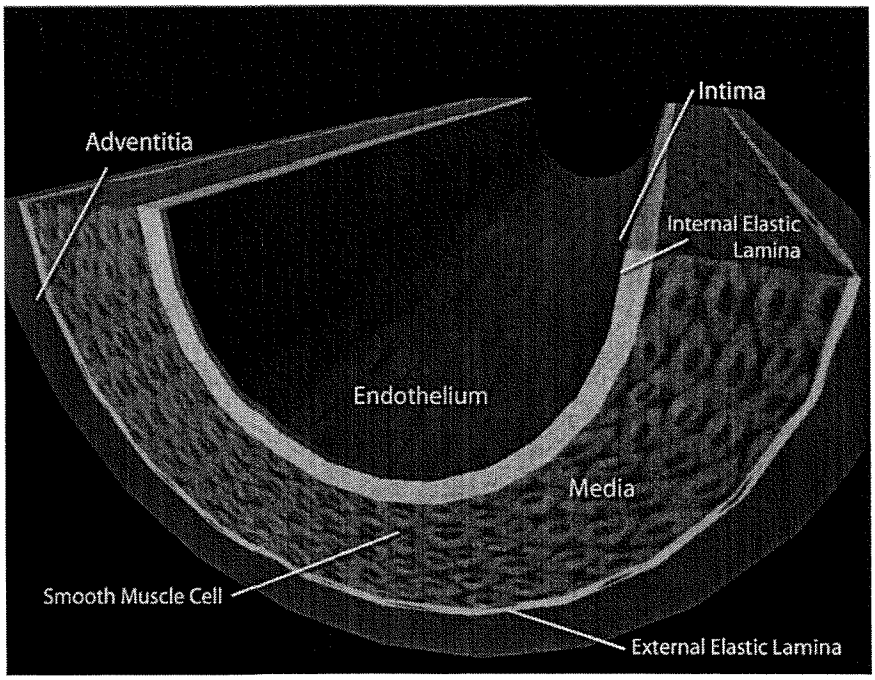

FIGURE 5.2
Anatomical structure of the normal artery. This illustration displays the three distinct layers of the vessel wall: intima, media, and adventitia as well as the endothelium and the external and internal elastic lamina.

The adventitia attaching the vessel to the surrounding tissue is made up of capillaries, fibroblasts, fat cells, proteoglycans, connective tissue, and elastic and collagen bundles. The adventitia is separated from the tunica media by the external elastic lamina.[12] The connective tissue in the adventitia is very compressed where it borders the tunica media, but it changes to loose connective tissue near the periphery of the vessel.[19]

Endothelial Dysfunction

In humans, the normal endothelium has many unique anti-atherosclerotic properties, including vasoregulation of conductive and resistance vessels, monocyte disadhesion, and vessel growth.[14,20] The pathophysiological consequences of disruption of these factors serve as hallmarks of endothelial dysfunction. Endothelial dysfunction as a result of injury leads to compensatory responses that modify the normal physiological characteristics of the endothelium and become the foundation for the disease process.[13]

Endothelial dysfunction is characterized as a systemic, reversible disorder and is associated with an impairment in endothelium-dependent vasodilation and recruitment of inflammatory cells to the vessel wall.[14,21] Potential causes of endothelial dysfunction include hypercholesterolemia, diabetes,[22] smoking,[23] hypertension,[24] and infectious microorganisms[25] such as *Chlamydia pneumoniae*,[26] cytomegaloviral infection, *Helicobacter pylori* infection, and herpes virus infection,[27] many of which are associated with a reduction in availability of vasodilators such as NO, decreased flow-induced vasodilation, and increased endothelium-derived contracting factors.[23] Lipid and cell permeability, lipoprotein oxidation, inflammation, platelet activation, and thrombus formation are all promoted by endothelial dysfunction.[28,29] The paradigm of endothelial dysfunction propagates a proatherogenic milieu that favors atheroma formation.[30]

Ludmer and colleagues, using a selective agonist acetylcholine test, provided the first evidence in humans of impaired endothelium-dependent vasodilation in the presence of atherosclerosis,[31] which is now attributed to a reduced bioavailability of NO.[15,32,33] In large arteries of humans,[23] rabbits,[34] pigs,[35] and monkeys,[36] reduced endothelium-dependent vasodilation due to atherosclerosis and hypercholesterolemia has been reported. However, the sensitivity of injured endothelial cells is not homogeneous for all vasoactive agonists.[37] For example, the responsiveness of the endothelial cells to acetylcholine, substance P, serotonin, and alpha-adrenergic agonists is severely decreased, while the responsiveness to bradykinin and adenosine diphosphate is only mildly attenuated. In contrast, endothelium-independent vasodilation to nitro-containing vasodilators is not altered.[37]

Endothelium dysfunction is also involved in the activation of endothelial-leukocyte adhesion molecules.[38,39] Specifically, P-selectin, E-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule (VCAM-1) are adhesion molecules known to be involved with the recruitment of leukocytes.[40] VCAM-1 plays a role in the binding of both monocytes and leukocytes to endothelial cells. In lesion-prone areas (e.g., endothelial cells exposed to long duration, high shear stress), VCAM-1 is up-regulated and occurs in response to inflammatory cytokines.[38] Increased expression of ICAM-1 on endothelial cells has been detected in both lesion-prone areas as well as on endothelial cells exposed to normal shear stress.[41] In humans, E-selectin is only upregulated on injured endothelial cells and is important in the regulation of adhesive interactions between certain blood cells and the endothelium,[40,42] whereas P-selectin is involved in adhesion of certain leukocytes and platelets to the endothelium.[43–45] The importance of P-selectin during atherosclerosis has also been demonstrated in animal models.[46] For example, P-selectin is expressed on endothelial cells overlying active atherosclerotic plaques, and inactive atherosclerotic plaques lacking in P-selectin expression.[43] Furthermore, animals lacking P-selectin have a decreased tendency to form atherosclerotic plaques.[40]

Several potential mechanisms by which statin therapy, angiotensin receptor blockers, and aspirin might improve endothelial dysfunction have been

suggested, including up-regulation of nitric oxide production, reduction of oxidative stress, and increased adhesion molecule expression.[12,33,47] More recently, the finding that the insulin-sensitizing thiazolidinediones (TZDs), peroxisome proliferator-activated receptor-gamma (transcription factor) agonists have antiproliferative and anti-inflammatory effects has led to the investigation of their possible role in the treatment of endothelial dysfunction and atherosclerotic lesion formation.[12,48]

A Tale of Two Hypotheses: Lipids vs. Endothelium

The chronic endothelial injury and the lipid hypotheses are the two main proliferative mechanisms postulated to explain the underlying pathogenesis of atherosclerosis. These two hypotheses are not mutually exclusive and are closely linked by the culmination of molecular and cellular events. The roles of cell types of the vessel wall in healthy and diseased (atherosclerosis) states are summarized in Table 5.2. Although others[49–53] have postulated alternative hypotheses about the development of atherosclerosis, the chronic endothelium injury hypothesis is the one most widely accepted.

Chronic Endothelial Injury Hypothesis

Based on pathophysiological evidence in animals and humans, Ross and Glomset introduced the endothelial injury hypothesis of atherosclerosis, which initially postulated that endothelial cell uncovering was the initial step in the development of atherosclerosis.[54] However, endothelial dysfunction is presently considered to be the precursor that initiates the atherosclerotic process and is associated with increased lipoprotein accumulation at

TABLE 5.2

Role of Cell Type of the Vessel Wall in Healthy and Diseased (Atherosclerosis) States

Cellular Components	Healthy	Diseased
Endothelial cell	NO production	Loss of NO production
	Vasoreactivity	Paradoxical vasoconstriction
	Anti-adhesive	Leukocyte adhesion
T-cell	Inflammatory signals	Macrophage stimulation
		Cytokine production
Macrophage	Lipid uptake	Cytokine release
		MMP production
Smooth muscle cell	Structural	Intimal migration
	Vasoreactivity	Proliferation

NO, nitric oxide; MMP, matrix metalloproteinases.

the site of injury.[13,29] The response to the chronic endothelial injury hypothesis or "response to injury hypothesis" of atherosclerosis states that the protective, inflammatory response followed by the formation of fibroproliferative response begins as a protective mechanism that with time and continuing insult may become excessive.[55,56] Due to release of chemoattractants and growth regulatory molecules by the altered endothelium,[57] leukocytes,[58] monocytes, and T lymphocytes[59] attach to the endothelial cell surface. The leukocytes migrate to the subendothelial space, between the tiny junctions of the endothelial cells, and aggregate within the intima.[56] The presence of elevated levels of oxidized low-density lipoproteins (oxLDL) is the basis of conversion of monocytes to macrophages, and is a fundamental factor responsible for injury to the vascular wall.[60] Through scavenger cell receptors, macrophages accumulate modified lipid particles and become foam cells. As the process persists, foam cell and lymphocyte accumulation forms the basis for the fatty streak.[61,62] It is believed that fatty streaks frequently form at sites with significant intimal smooth muscle accumulation.[63] More advanced lesions develop as a result of continued cell migration and proliferation[56] which eventually turn into a fibrous plaque.[64] This hypothesis is based on the notion that repeated insult to the endothelium leads to dysfunction, which is followed by a cascade of pathophysiological consequences.

Lipid Hypothesis

In 1913, Nikolai N. Anitschkow demonstrated that cholesterol feeding of rabbits could induce vascular lesions consistent with the characteristics of human atherosclerotic lesions.[65,66] Unknowingly, his research and others established the principles of what is now commonly referred to as the "lipid hypothesis." Through decades of research and much controversy, the "lipid hypothesis" is still believed to be one of the prominent mechanisms contributing to atherosclerosis.[66] Based upon its principles, many discoveries have been made in understanding the pathogenesis of atherosclerosis and the fight against cardiovascular disease.

Although elevated LDL cholesterol is associated with increased risk for cardiovascular disease and the pathogenesis of atherosclerosis, LDL has an essential biological role to transport cholesterol to peripheral tissues.[67] Serum cholesterol is transported by lipoprotein particles that perform important tasks of carrying both dietary and endogenously produced lipids.[68] While the transport of endogenous lipids is mediated by LDL, very low-density lipoproteins (VLDL), and high-density lipoprotein (HDL), the dietary lipids are carried primarily by chylomicrons. For the most part, LDL particles transport the vast majority of serum cholesterol.

The lipid hypothesis postulates that an elevation in LDL levels results in penetration of LDL into the arterial wall, leading to lipid accumulation in SMCs and in macrophages (foam cells).[69] LDL also augments smooth muscle

cell hyperplasia and migration into the subintimal and intimal region in response to growth factors. LDL is modified or oxidized in this environment and is rendered more atherogenic. Small dense LDL cholesterol particles are also more susceptible to modification and oxidation. The modified or oxidized LDL is chemotactic to monocytes, promoting their migration into the intima, their early appearance in the fatty streak,[28] and their transformation and retention in the subintimal compartment as macrophages. Scavenger receptors on the surface of macrophages facilitate the entry of oxidized LDL into these cells, transferring them into lipid-laden macrophages and foam cells. As cell migration and proliferation continues, advanced lesions are formed which leads to plaque formation.

Stages of Atherosclerosis

Initiation of LDL-Mediated Atherogenesis (Lipid Accumulation)

As postulated by the "lipid hypothesis," atherosclerotic lesion development begins with the accumulation of LDL cholesterol levels within the circulation. The studies of Brown and colleagues[70] elucidated that the molecular mechanisms controlling LDL-cholesterol uptake were instrumental in this determination. Under pathologic conditions where LDL levels are elevated, lipid accumulation is noticeable along the lining of the arterial wall termed the tunica lamina (Figure 5.3). The aggregates of lipid particles form intimate associations with epithelia moieties such as proteoglycans and become embedded in the tunica lamina structure (Figure 5.4). In defense, the arterial epithelium fortifies itself with self-protective structural and biochemical mechanisms that maintain a homeostatic environment in the presence of lipid accumulation. The expression of molecules such as heparin sulfate constituents, which provide arterial integrity and blood fluidity and the expression of many antithrombin molecules,[70] are instrumental in protection against atherogenesis.[70] However, under hypercholesterolemic conditions, the protective integrity of the epithelium falls prey to initiation of lesion development.

LDL Oxidative Modification and Fatty Streak Formation

Atherosclerotic lesions present initially in the form of fatty streaks forming along the endothelium of arteries (Figure 5.4). The major contributing event believed to be responsible in fatty streak development is oxidative modifications of the lipid and apolipoprotein B (apo B) components of LDL.[71]

The precise molecular mechanisms responsible for LDL oxidation are largely unknown. Studies have identified several plausible mechanisms supportive of LDL modification. The enzymatic activity of nitric oxide synthase,

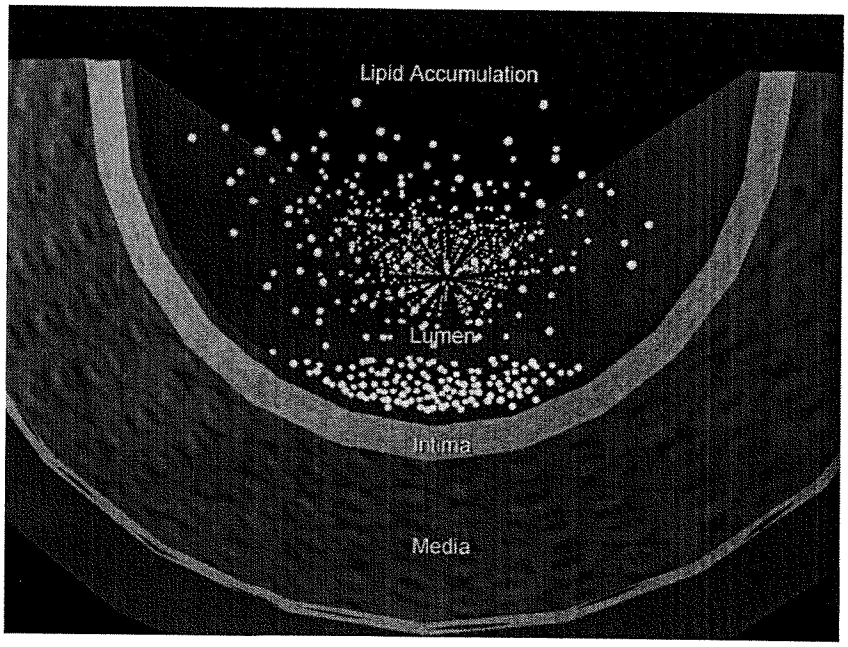

FIGURE 5.3
Initiation of LDL-mediated atherogenesis (lipid accumulation). Atherosclerotic lesion development begins with the accumulation of LDL. Lipid accumulation is noticeable along the lining of the arterial wall.

15-lipoxengenase activity,[72] as well as nitric oxide production by epithelial cells and macrophages[73] have been shown to be capable of LDL modification. Recent findings supporting their proatherogenic role have been documented using gene knockout models.[74–76] Despite formidable evidence that LDL oxidation confers lesion formation, data regarding antioxidant therapy to date have not shown promise.[77] In broad terms, atherosclerosis can be characterized as a chronic inflammatory disease. As such, cellular responses such as cellular adhesion and recruitment during lesion development are central components as in other chronic inflammatory diseases.

The recruitment of monocytes occurs at the sites of lipid accumulation and function in uptake of various lipids and apolipoprotein components produced from oxidative stress and other biochemical breakdown products of LDL (Figure 5.4). Such recruitment is known to be regulated by chemotactic factors[10] as well as being attracted by oxidative-LDL species.[10] Chemokines are small proteins subdivided into three major groups based upon the structural positions of the first two cysteines at the amino terminus of the molecule.[78–80] Chemokines stimulate the migration and activation of cells, especially phagocytic cells and lymphocytes. Most notable is the release of macrophage chemotactic protein 1 (MCP-1) found to be produced locally by endothelial cells[71] and the coordinate expression of chemokine receptor 2 (CCR2), the receptor for MCP-1 by monocytes. In fact, it has been shown

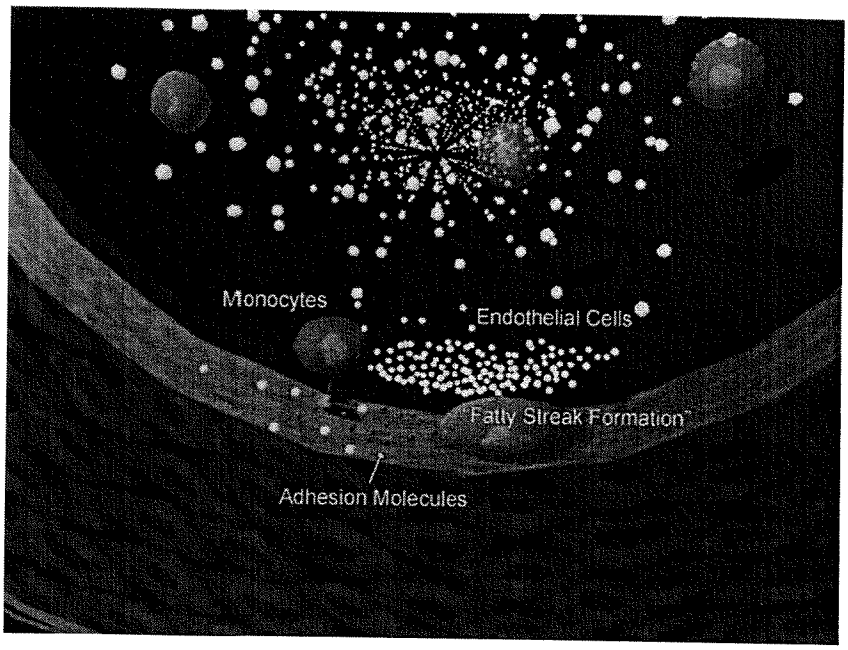

FIGURE 5.4
LDL oxidative modification and monocyte recruitment (fatty streak formation). The initial sign of atherogenic development is the formation of the fatty streak, underlying the endothelium of large arteries. The primary cellular events contributing to the fatty streak formation are the recruitment of monocytes which are converted to macrophages, which uptake LDL. Recruitment of monocytes to lesion probe areas is regulated by adhesion molecules that are expressed on the endothelium cell surface.

that hypercholesterolemia patients exhibit increased MCP-1 production.[81] Furthermore, disruption of MCP-1 and its receptor CCR2 genes was shown to reduce the development of atherosclerosis in mice.[81] Other chemokines such as interleukin-8 (IL-8), RANTES, and IP-10 have also been implicated in monocyte recruitment.[10] Current research in this area offers the potential for therapeutic use in deterring atherogenic processes by impairing leukocyte trafficking.

It has been speculated that macrophage-mediated uptake of modified LDL species may be an initial attempt to dampen the inflammatory environment produced by oxidative LDL species.[10] Ultimately, however, the response and uptake of oxidized LDL species leads to progressive inflammation and atherosclerotic lesions. The uptake of LDL occurs mainly via macrophage LDL receptors or by scavenger receptor-mediated uptake.[10] The mode of LDL uptake is determined by the nature of LDL modification. Studies show that while native LDL is normally endocytosed via specific LDL receptors, highly modified LDL, such as certain apolipoproteins, are not recognizable by the LDL receptors and are relegated to uptake by scavenger receptors. The latter is most associated with macrophage foam cell formation, a topic to be

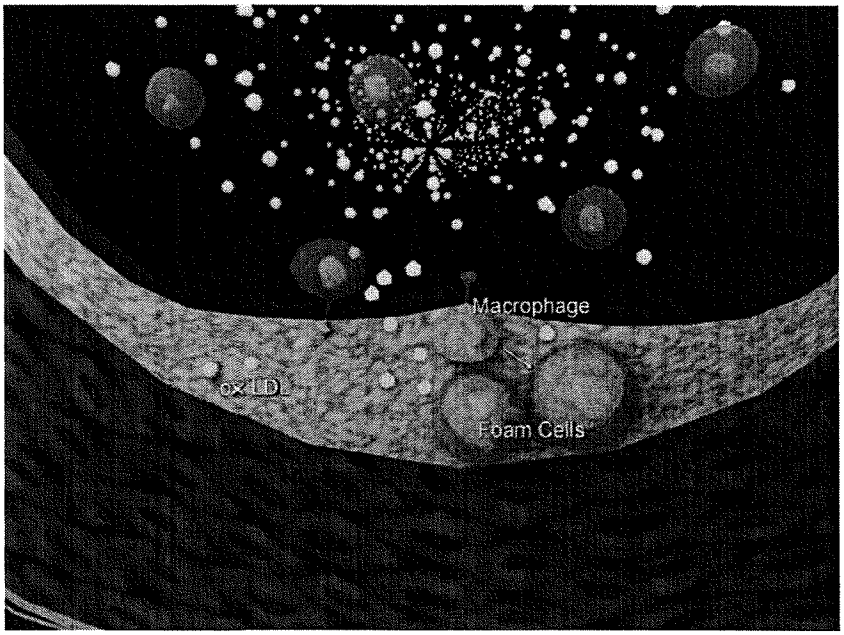

FIGURE 5.5
Foam cell formation (intracellular lipid accumulation) by macrophages. A hallmark of early atherosclerotic lesion development is conversion of the macrophage to foam cells that contain amounts of oxLDL, which is mediated primarily by scavenger receptors.

discussed in a subsequent section of this chapter. As a result of macrophage recruitment and uptake of LDL constituents, fatty streaks form and become what is the initial site of atherosclerotic lesions (Figure 5.5).

Another mechanism responsible for the initiation of atherosclerotic lesions is the increase in adhesion molecules present on endothelial cells. Under normal circumstances, the arterial endothelium is highly resistant toward cellular adhesion. However, studies have shown that hypercholesterolemia induces leukocyte adherence to the endothelium allowing diapedesis between the endothelial cell and entry into the lamina.[10] Several adhesion molecules have been implicated to significantly foster translocation of leukocytes across the endothelium. Vascular cell adhesion molecule-1 (VCAM-1), a member of the immunoglobulin superfamily, is expressed by endothelial cells and regulates the adherence of monocytes and T cells. VCAM-1 has been found to interact with very late antigen-4 (VLA-4) and influence monocyte adherence during the initial stages of atheroma formation.[82] Selectins P and E have also been implicated in monocyte adhesiveness to the endothelium. Quantitative decreases in atherosclerosis were shown in apo E mice lacking their respective genes.[83]

Foam Cell Formation (Intracellular Lipid Accumulation by Macrophages)

As mentioned in a previous section, macrophages play an important role in LDL metabolism by uptake of native LDL cholesterol and modified species of LDL via two major receptor mechanisms, LDL-specific receptor and scavenger receptor endocytosis, respectively. As the accumulation and modification of LDL ensues, macrophages within the subendothelium begin to incorporate large amounts of oxidized LDL species via scavenger receptor uptake, resulting in a phenotype given the term "foam cell" (Figure 5.5). The most notable scavenger receptors identified to date that have been demonstrated to have a significant impact on atherosclerotic development are the scavenger receptor A (SR-A) and the receptors of the cluster differentiation 36 surface molecules (CD36) receptors.[84] In particular, it was shown that in apo E-deficient murine models deficient in SR-A or CD36, gene receptor expression resulted in a significant reduction in lesion formation.[84,85]

As determined by the studies of Brown and colleagues, homeostatic control of cholesterol uptake is under strict mediation through LDL-specific receptor feedback mechanisms regulated by the SREBP transcription factors required for LDL receptor expression.[70] In the presence of elevated membrane-bound cholesterol, inactivation of SREBP occurs, inhibiting LDL receptor expression. In contrast, however, uptake of oxidative LDL species via scavenger receptors, SR-A or CD36 or by macrophage-mediated phagocytosis is not under such regulatory control. Instead, prevention of cholesterol intracellular overload is dependent on mechanisms of active efflux out of the cell. The vast majority of oxidized LDL entering macrophages via the scavenger receptors consists of free cholesterol or esterified cholesterol. There are several fates of native cholesterol metabolism, which include Acyl CoA esterification and the storage of lipid droplets containing cholesterol esters that characterize the phenotype of foam cells. Excretion of excess cholesterol by foam cells is believed to occur through processes that transform cholesterol into a more soluble form through enzymatic modifications.

A major pathway of cholesterol efflux is called the "reverse cholesterol transport" pathway that involves HDL as an acceptor molecule. The HDL-reverse cholesterol transport mechanism received much attention when studies found an inverse relationship between risk for atherosclerosis and HDL content.[86] A genetic basis for HDL-mediated cholesterol transport is shown in patients afflicted with Tangier disease, which is characterized by extremely low levels of HDL and accumulation of cholesterol within macrophages. Mutations in ABCA1, which encodes a member of the ATP binding cassette family of HDL transporters, were found to cause the genetic defect. Although the precise mechanism that is disrupted by this aberration is unclear, studies suggest that mutation in ABC A1 alters cholesterol transport to the HDL acceptor molecules.[87] Under normal conditions, HDL-cholesterol is esterified via lecithin-cholesterol acyltransferase (LCAT) or is directly transported to the liver via SR-B1 binding. Thus, it is clear that macrophages play a paramount role in cholesterol maintenance within its surrounding

environment, but more important is its ability to control the fate of internalized cholesterol for self-preservation.

Immigration of Smooth Muscle Cells

A hallmark of advanced lesion development is the immigration of smooth muscles cells from the arterial wall into the subepithelial space (Figure 5.6). The factors that lead to the mobilization of smooth muscle cells are not well understood, but it is believed to be due to preexisting stimuli. For example, macrophages have been shown to secrete the chemokine platelet-derived growth factor (PDGF), which is a chemoattractant for smooth muscle.[88] In fact, studies have demonstrated PDGF expression to be elevated in individuals with atherosclerosis.[89] Smooth muscle cells found within the atherosclerotic region were found to have distinct characteristics from normal smooth muscle cells. These cells exhibit characteristics of clonal expansion. Studies have demonstrated that the slow but steady proliferation can be attributed

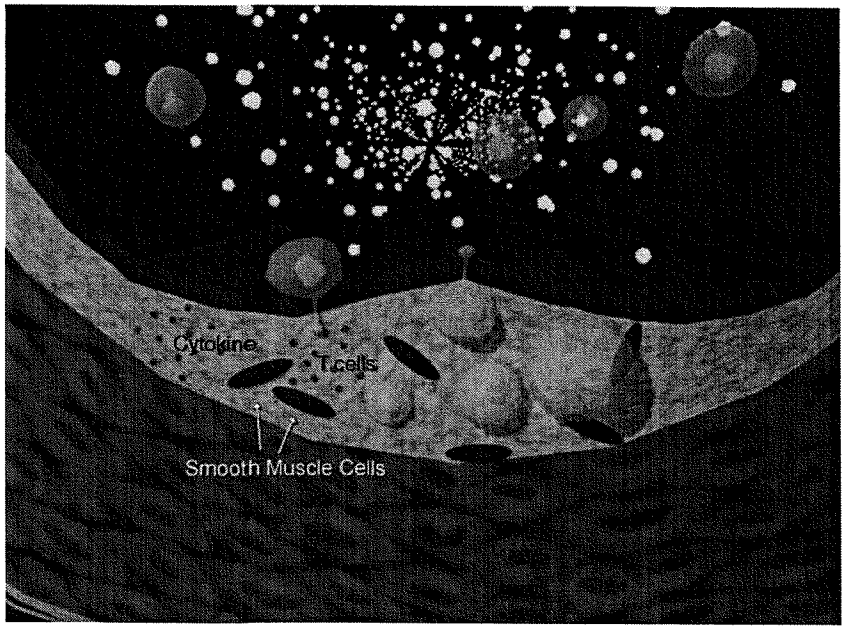

FIGURE 5.6
Immigration of smooth muscle cells and immune responsiveness during atherosclerotic development. A hallmark of advanced lesion development is the immigration of smooth muscle cells from the arterial wall into the subepithelial space, which may also contribute to foam cell development. As with many chronic inflammatory diseases, immune surveillance will ultimately make a significant contribution to the progression and disease outcome. Circulating leukocytes and lymphocytes of mainly T-cells respond to the site of injury.

to a single cell.[90] Smooth muscle cells in developing atheroma also are capable of taking up modified lipoproteins.[89] Not only does their proliferative capacity augment atheroma development, but apoptotic cell death of smooth muscle cells participates in lesion progression. Apoptosis of smooth muscle cells is believed to be associated with the presence of inflammatory cytokines at the lesion site.[91] Thus, smooth muscle cell immigration plays a significant role in progression of atheromas (Figure 5.6). Current research is aimed at developing molecular strategies targeting both proliferative and apoptotic pathways.

Immune Responsiveness during Atherosclerotic Development

With the exception of macrophage activation, lymphocyte activation does not appear to have a major impact on the initial stage of atherosclerotic formation. Studies using RAG-1 recombinase-deficient mice illustrated that the lack of functional B and T lymphocytes had no bearing on atherosclerotic development in the presence of elevated cholesterol.[92] However, as with many chronic inflammatory diseases, immune surveillance will ultimately make a significant contribution to the progression and outcome of disease. Circulating leukocytes and lymphocytes of mainly T lymphocytes respond to endothelial injury. At such stages of lesion development, a multitude of secreted and cell-associated mediators are accessible to lymphocyte recognition. For example, endothelial cell-associated adhesion molecules such as VCAM-1[93] can also increase the avidity for monocytes to enter lesion sites. Also, as previously mentioned, chemokines produced by activated macrophages can attract T-cells to the lesion site. As T-cells begin to accumulate in the surrounding lesion, they become activated and can modulate atherosclerotic development through the release of cytokines (Figure 5.7). Through the release of cytokines, T-cells can elicit both pro-atherogenic and anti-atherogenic responses. Such dichotomy is due to the presence of T subpopulations capable of secreting distinct cytokines that display opposing functionality. These populations of T-cells are commonly referred to as T-helper cells, subdivided into Th1 and Th2 subpopulations.[94] Th1 cells mainly secrete IL-2, interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Th1-associated cytokines mediate pro-inflammatory responses and delayed hypersensitivity responses. On the other hand, the Th2 subpopulation preferentially secretes IL-4, IL-5, IL-6, IL-10 and IL-13.[94] Th2 cells function in anti-inflammatory responses and immune tolerance.

Studies that examined the role of Th1 versus Th2 cytokine responses in the progression of atherosclerosis have shown that T-helper cell cytokine mediation is not as clearly defined along the two divergent functions between Th1 and Th2. In fact, IFN-γ has been shown to suppress scavenger receptor expression and proliferation of smooth muscle cells, suggesting an anti-atherogenic potential.[10] On the other hand IFN-γ is capable of activating macrophages. In studies utilizing apo E-deficient mice that lacked a

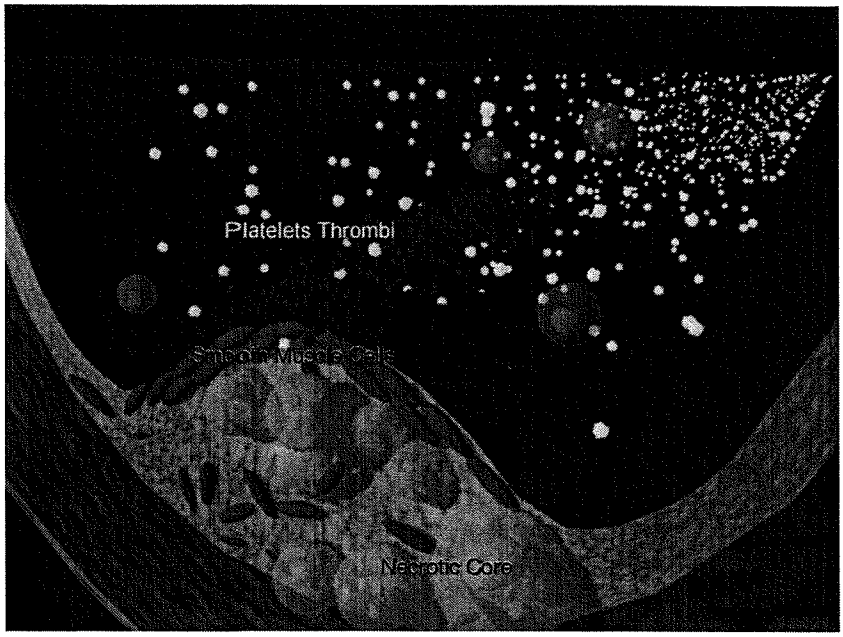

FIGURE 5.7
Plaque formation. Plaques develop from initial fatty streaks that progress into advanced lesions comprised of inflammatory cells, smooth muscle cells, extracellular lipids, and fibrous tissues. Their continued accumulation proliferation and activation within the lesion leads to plaque expansion. Consistent with the earlier events of atherosclerotic lesion development, plaque formation involves the participation of cytokines, chemokines, hydrolytic enzymes, and growth factors in this process. During the advanced stages of plaque formation, lipid moieties, leukocytes and necrotic materials are walled off by a fibrous cap. An accumulation of these cellular constituents and fibrotic tissues leads to further expansion and can lead to ischemic heart disease or stroke, which is due mainly to plaque rupture and thrombosis.

functional IFN-γ receptor, atherosclerosis was decreased as compared to normal mice.[95] The role of Th2 cytokine mediation is also very complex. While IL-4 cytokine production by Th2 cells acts antagonistically toward IFN-γ production, IL-4 has been shown to induce LDL oxidation through induction of 15-LO enzymatic activation.[10] IL-10 production by Th2 cells seems to be the most consistent in opposing pro-atherogenic processes such as macrophage deactivation[96,97] and plaque stability. Thus, the implication of T-cells' activation offers a complex environment in determination of their specific roles in atherosclerotic development and progression.

Plaque Formation

Plaques develop from initial fatty streaks that progress into advanced lesions comprised of inflammatory cells, extracellular lipid, and fibrous tissues (Figure 5.7). Their continued accumulation proliferation and activation within

the lesion leads to plaque expansion. Consistent with the earlier events of atherosclerotic lesion development, plaque formation involves the participation of cytokines, chemokines, hydrolytic enzymes, and growth factors in this process.[98] During the advanced stages of plaque formation, lipid moieties, leukocytes and necrotic materials are walled off by a fibrous cap. An accumulation of these cellular constituents and fibrotic tissues leads to further expansion. At a particular threshold, the compensatory dilation of the artery is overcome by the intrusion of the lesion into the lumen resulting in eventual alterations in blood flow and plaque rupture.

While the initial events of atherogenesis involve mainly the disruption of the endothelia and leukocyte accumulation, the formation of the more advanced plaque includes smooth muscle cells (Figure 5.7). As mentioned previously, smooth muscle cells migrate via chemotactic regulation into the arterial intimal lesion site and become active participants in atheroma development. The smooth muscle cells involved in atheroma exhibit an altered phenotype in comparison to normal arterial tunica media smooth muscle cells. These smooth muscle cells proliferate at a higher rate within atherosclerotic plaques versus normal intimal regions of the aorta.[99] Further justification for the importance of smooth muscle cell proliferation demonstrated that clonal expansion of smooth muscle cells was likely and is the basis for lesion progression.[90] It is still unclear, however, what initiates medial smooth muscle proliferation versus normal smooth muscle cells. It is believed that growth factors in conjunction with additional stimuli promote the proliferative response by smooth muscle cells at the lesion site. For example, vascular smooth muscles cells (VSMCs) in the presence of serum show minimal mitogenic capacity.[100] Other studies substantiate this finding.[100,101] One possibility for the lack of mitogenicity could be due to the presence of suppressive factors. Based upon the evidence of this study it has been postulated that basement membrane constituents such as heparin can suppress smooth cell proliferation.[102,103] Thyberg, Hedin and colleagues also showed that the basement membrane component, laminin, inhibits while the interstitial matrix component, fibronectin, promotes phenotypic modulation of smooth muscle cells.[88,104] In contrast, the metalloproteinases that are induced by inflammatory cytokines[105,106] were found to induce smooth cell proliferation.[107] In addition to smooth muscle proliferation, the apoptosis of smooth muscle cells participates in advanced lesion development. Cell death may be the result of cytokine regulation present within the lesion site.[108] Also, interaction with *fas*-expressing T-cells can lead to cell death.[109] Therefore, understanding the regulation of smooth muscle expansion and depletion with regard to progression of plaque formation will likely have a great impact on the innovation of new therapies to combat atherosclerosis.

A large proportion of the developing atheroma includes connective tissue consisting of extracellular matrix macromolecules. Among the matrix proteins, the class collagens and proteoglycans are commonly associated with plaque development. Matrix proteins are produced by vascular smooth muscle cells and can accumulate within the developing plaque upon stimulation

by transforming growth factor-β and platelet-derived growth factor.[110] Matrix molecules have an important regulatory function. For example, fibronectin and heparan sulfate are found to inhibit cell cycle and cell–matrix interactions and influence chemokine expression by macrophages.[111–114] Matrix accumulation within the intima is under control of matrix metalloproteinases (MMPs).[112] MMPs act in degradation of matrix molecules and therefore control lesion accumulation. Matrix molecules also contribute to the outward growth of the lumina. Thus, the extracellular matrix is a key component in plaque development.

Summary

Cardiovascular disease is the leading cause of mortality in the United States, Europe, and a vast majority of Asia and is likely to be the greatest threat to overall health worldwide. This chapter emphasizes the biological process of atherosclerosis and what is known about the cells and molecules that are associated with the evolution of this multifaceted disease. While evidence suggests that elevated lipids and endothelial dysfunction both play an important role in atherogenesis, more research is needed to determine the molecular and cellular interactions of these factors in promoting the pathogenesis of atherosclerosis. Whereas atherosclerosis has long been an area of significant biomedical, clinical, and epidemiological research emphasis, there is considerable evidence that the quantitative determinants of disease vulnerability must be identified.

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