Review Article

Mechanisms of Disease

FRANKLIN H. EPSTEIN, M.D., *Editor*

ATHEROSCLEROSIS — AN INFLAMMATORY DISEASE

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ATHEROSCLEROSIS is an inflammatory disease. Because high plasma concentrations of cholesterol, in particular those of low-density lipoprotein (LDL) cholesterol, are one of the principal risk factors for atherosclerosis,[1] the process of atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall; however, it is much more than that. Despite changes in lifestyle and the use of new pharmacologic approaches to lower plasma cholesterol concentrations,[2,3] cardiovascular disease continues to be the principal cause of death in the United States, Europe, and much of Asia.[4,5] In fact, the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease.[6-10]

The lesions of atherosclerosis occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain, or extremities, resulting in infarction. They may be present throughout a person's lifetime. In fact, the earliest type of lesion, the so-called fatty streak, which is common in infants and young children,[11] is a pure inflammatory lesion, consisting only of monocyte-derived macrophages and T lymphocytes.[12] In persons with hypercholesterolemia, the influx of these cells is preceded by the extracellular deposition of amorphous and membranous lipids.[11,13] By asking questions about arterial inflammation, we may be able to gain insight into the process of atherogenesis.

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FACTORS THAT INDUCE AND PROMOTE INFLAMMATION OR ATHEROGENESIS

Numerous pathophysiologic observations in humans and animals led to the formulation of the response-to-injury hypothesis of atherosclerosis, which initially proposed that endothelial denudation was the first step in atherosclerosis.[6] The most recent version of this hypothesis emphasizes endothelial dysfunction rather than denudation. Whichever process is at work, each characteristic lesion of atherosclerosis represents a different stage in a chronic inflammatory process in the artery; if unabated and excessive, this process will result in an advanced, complicated lesion. Possible causes of endothelial dysfunction leading to atherosclerosis include elevated and modified LDL; free radicals caused by cigarette smoking, hypertension, and diabetes mellitus; genetic alterations; elevated plasma homocysteine concentrations; infectious microorganisms such as herpesviruses or *Chlamydia pneumoniae*; and combinations of these or other factors. Regardless of the cause of endothelial dysfunction, atherosclerosis is a highly characteristic response of particular arteries.[6-9,14]

The endothelial dysfunction that results from the injury leads to compensatory responses that alter the normal homeostatic properties of the endothelium. Thus, the different forms of injury increase the adhesiveness of the endothelium with respect to leukocytes or platelets, as well as its permeability. The injury also induces the endothelium to have procoagulant instead of anticoagulant properties and to form vasoactive molecules, cytokines, and growth factors. If the inflammatory response does not effectively neutralize or remove the offending agents, it can continue indefinitely. In doing so, the inflammatory response stimulates migration and proliferation of smooth-muscle cells that become intermixed with the area of inflammation to form an intermediate lesion. If these responses continue unabated, they can thicken the artery wall, which compensates by gradual dilation, so that up to a point, the lumen remains unaltered,[15] a phenomenon termed "remodeling." As for the inflammatory cells, granulocytes are rarely present during any phase of atherogenesis.[16] Instead, the response is mediated by monocyte-derived macrophages and specific subtypes of T lymphocytes at every stage of the disease.[17,18]

Continued inflammation results in increased numbers of macrophages and lymphocytes, which both emigrate from the blood and multiply within the lesion. Activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines, and

growth factors,[19,20] which can induce further damage and eventually lead to focal necrosis.[21] Thus, cycles of accumulation of mononuclear cells, migration and proliferation of smooth-muscle cells, and formation of fibrous tissue lead to further enlargement and restructuring of the lesion, so that it becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue — a so-called advanced, complicated lesion. At some point, the artery can no longer compensate by dilation; the lesion may then intrude into the lumen and alter the flow of blood.

Hypercholesterolemia and Modified Lipids and Lipoproteins

LDL, which may be modified by oxidation, glycation (in diabetes), aggregation, association with proteoglycans, or incorporation into immune complexes,[22-25] is a major cause of injury to the endothelium and underlying smooth muscle.[25-27] When LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalized by macrophages by means of the scavenger receptors on the surfaces of these cells.[22,24-28] The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters, resulting in the formation of foam cells. The degree to which LDL is modified can vary greatly.[25,27,29] Once modified and taken up by macrophages, LDL activates the foam cells. Removal and sequestration of modified LDL are important parts of the initial, protective role of the macrophage in the inflammatory response[28-30] and minimize the effects of modified LDL on endothelial and smooth-muscle cells. Antioxidants such as vitamin E can also reduce free-radical formation by modified LDL.[31] In addition to its ability to injure these cells,[25,27] modified LDL is chemotactic for other monocytes and can up-regulate the expression of genes for macrophage colony-stimulating factor[32,33] and monocyte chemotactic protein[34] derived from endothelial cells. Thus, it may help expand the inflammatory response by stimulating the replication of monocyte-derived macrophages and the entry of new monocytes into lesions.

The inflammatory response itself can have a profound effect on lipoprotein movement within the artery. Specifically, mediators of inflammation such as tumor necrosis factor α, interleukin-1, and macrophage colony-stimulating factor increase binding of LDL to endothelium and smooth muscle and increase the transcription of the LDL-receptor gene.[35,36] After binding to scavenger receptors in vitro, modified LDL initiates a series of intracellular events[36] that include the induction of urokinase[30] and inflammatory cytokines such as interleukin-1.[37-39] Thus, a vicious circle of inflammation, modification of lipoproteins, and further inflammation can be maintained in the artery by the presence of these lipids.

Oxidized LDL is present in lesions of atherosclerosis in humans.[40] In animals with hypercholesterolemia, antioxidants can reduce the size of lesions,[25,41-44] and they reduce fatty streaks in nonhuman primates.[44] The latter observation suggests that the antioxidants have an antiinflammatory effect, perhaps by preventing the up-regulation of adhesion molecules for monocytes.[45] Antioxidants increase the resistance of human LDL to oxidation ex vivo[46] in proportion to the vitamin E content of the plasma. Vitamin E intake is inversely correlated with the incidence of myocardial infarction, and vitamin E supplementation reduced coronary events in a preliminary clinical trial.[47-49] In contrast, other antioxidants, such as beta carotene, have no benefit.[46,50,51]

Homocysteine

High plasma homocysteine concentrations were initially thought to be associated with advanced atherosclerosis on the basis of autopsy findings in patients with homozygous defects in enzymes necessary for homocysteine metabolism, such as cystathionine beta-synthase or methylenetetrahydrofolate reductase.[52-56] In patients with such defects, severe atherosclerosis develops in childhood, and many have their first myocardial infarction by the age of 20 years.[55,56] Homocysteine is toxic to endothelium[57] and is prothrombotic,[58] and it increases collagen production[59] and decreases the availability of nitric oxide.[60]

Plasma homocysteine concentrations are slightly elevated in many patients who have no enzymatic defects in homocysteine metabolism.[61] These patients have an increased risk of symptomatic atherosclerosis of the coronary, peripheral, and cerebral arteries.[61] Treatment with folic acid can return their plasma homocysteine concentrations to normal. Trials are under way to determine whether folic acid will prevent the progression or possibly even induce the regression of atherosclerotic lesions.[62]

Hypertension

Concentrations of angiotensin II, the principal product of the renin–angiotensin system, are often elevated in patients with hypertension; angiotensin II is a potent vasoconstrictor. In addition to causing hypertension, it can contribute to atherogenesis by stimulating the growth of smooth muscle.[63] Angiotensin II binds to specific receptors on smooth muscle, resulting in the activation of phospholipase C, which can lead to increases in intracellular calcium concentrations and in smooth-muscle contraction,[63] increased protein synthesis, and smooth-muscle hypertrophy.[64] It also increases smooth-muscle lipoxygenase activity, which can increase inflammation and the oxidation of LDL. Hypertension also has proinflammatory actions, increasing the formation of hydrogen peroxide and free radicals such as superoxide anion and hydroxyl radicals in plasma.[27,65,66] These substances reduce the formation of nitric oxide by the

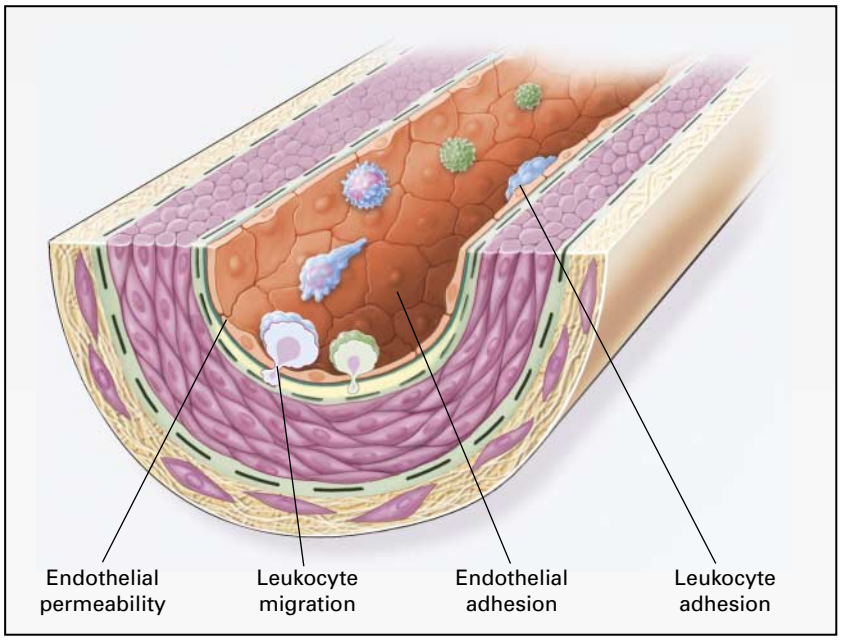

Figure 1. Endothelial Dysfunction in Atherosclerosis.

The earliest changes that precede the formation of lesions of atherosclerosis take place in the endothelium. These changes include increased endothelial permeability to lipoproteins and other plasma constituents, which is mediated by nitric oxide, prostacyclin, platelet-derived growth factor, angiotensin II, and endothelin; up-regulation of leukocyte adhesion molecules, including L-selectin, integrins, and platelet–endothelial-cell adhesion molecule 1, and the up-regulation of endothelial adhesion molecules, which include E-selectin, P-selectin, intercellular adhesion molecule 1, and vascular-cell adhesion molecule 1; and migration of leukocytes into the artery wall, which is mediated by oxidized low-density lipoprotein, monocyte chemotactic protein 1, interleukin-8, platelet-derived growth factor, macrophage colony-stimulating factor, and osteopontin.

endothelium,[67] increase leukocyte adhesion,[66] and increase peripheral resistance. Thus, free-radical formation mediates some of the effects of both hypertension and hypercholesterolemia.

Infection

Several reports have shown a correlation between the incidence of atherosclerosis and the presence of at least two types of infectious microorganisms, herpesviruses and *C. pneumoniae*.[68-70] Both organisms have been identified in atheromatous lesions in coronary arteries and in other organs obtained at autopsy.[69,70] Increased titers of antibodies[71] to these organisms have been used as a predictor of further adverse events in patients who have had a myocardial infarction.[72,73] Nonetheless, there is no direct evidence that these organisms can cause the lesions of atherosclerosis.[68,74,75] Although these organisms are ubiquitous in many tissues and organs, the fact that lesions cannot be induced experimentally in animals by injection of the organisms leaves their role as etiologic agents in question. It is nevertheless possible that infection, combined with other factors, may be responsible for the genesis of the lesions of atherosclerosis in some patients.[68,76]

THE NATURE OF THE INFLAMMATORY RESPONSE

Interactions among Endothelial Cells, Monocytes, and T Cells

Specific arterial sites, such as branches, bifurcations, and curvatures, cause characteristic alterations in the flow of blood, including decreased shear stress and increased turbulence.[77] At these sites, specific molecules form on the endothelium that are responsible for the adherence, migration, and accumulation of monocytes and T cells. Such adhesion molecules, which act as receptors for glycoconjugates and integrins present on monocytes and T cells, include several selectins, intercellular adhesion molecules, and vascular-cell adhesion molecules.[78] Molecules associated with the migration of leukocytes across the endothelium, such as platelet–endothelial-cell adhesion molecules,[79] act in conjunction with chemoattractant molecules generated by the endothelium, smooth muscle, and monocytes — such as monocyte chemotactic protein 1, osteopontin,[80] and modified LDL — to attract monocytes and T cells into the artery (Fig. 1).[33]

The nature of the flow — that is, whether shear stress or turbulence is high or low — appears to be

important in determining whether lesions occur at these vascular sites. Changes in flow alter the expression of genes that have elements in their promoter regions that respond to shear stress. For example, the genes for intercellular adhesion molecule 1,[81] platelet-derived growth factor B chain,[82] and tissue factor[83] in endothelial cells have these elements, and their expression is increased by reduced shear stress.[84] Thus, alterations in blood flow appear to be critical in determining which arterial sites are prone to have lesions.[77,85,86] Rolling and adherence of monocytes and T cells occur at these sites as a result of the up-regulation of adhesion molecules on both the endothelium and the leukocytes.

Chemokines may be responsible for the chemotaxis and accumulation of macrophages in fatty streaks (Fig. 2).[87,88] Activation of monocytes and T cells leads to up-regulation of receptors on their surfaces, such as the mucin-like molecules that bind selectins, integrins that bind adhesion molecules of the immunoglobulin superfamily, and receptors that bind chemoattractant molecules.[78] These ligand–receptor interactions further activate mononuclear cells, induce cell proliferation, and help define and localize the inflammatory response at the sites of lesions (Fig. 1).

In genetically modified mice that are deficient in apolipoprotein E (and have hypercholesterolemia), intercellular adhesion molecule 1 is constitutively increased at lesion-prone sites.[86] In fact, it is present on the surface of the endothelium at these sites in normal mice and is increased in mice with apolipoprotein E deficiency. In contrast, vascular-cell adhesion molecule 1 is absent in normal mice but is present at the same sites as intercellular adhesion molecule 1 in mice with apolipoprotein E deficiency.[86] Thus, adherence of monocytes and T cells may occur after an increase in one or more of the adhesion molecules, which may act in concert with chemotactic molecules such as monocyte chemotactic protein 1, interleukin-8, or modified LDL. Would interference with only one of the several adhesion molecules be sufficient to decrease inflammation and thus slow or counteract the process of atherogenesis? In mice that are completely deficient in intercellular adhesion molecule 1, P-selectin, CD18, or combinations of these molecules, lipid

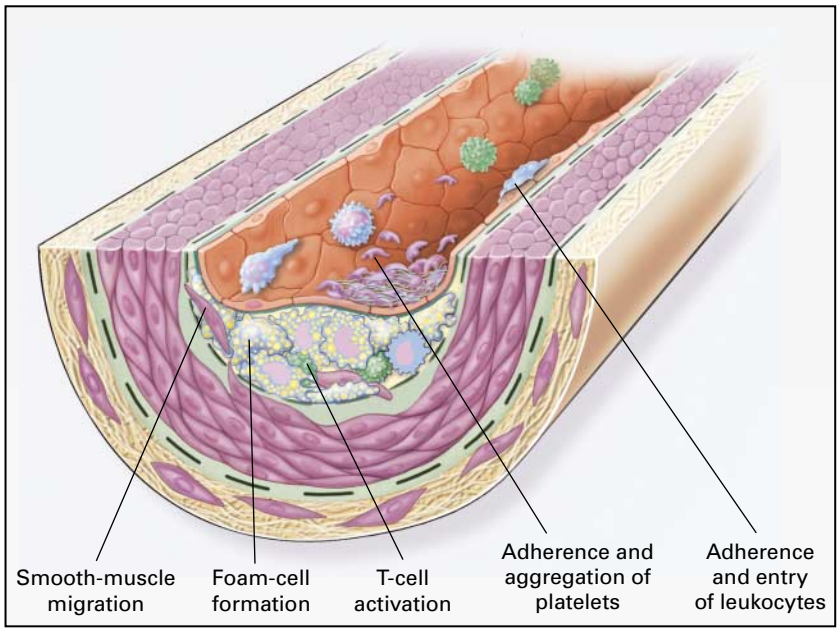

Figure 2. Fatty-Streak Formation in Atherosclerosis.

Fatty streaks initially consist of lipid-laden monocytes and macrophages (foam cells) together with T lymphocytes. Later they are joined by various numbers of smooth-muscle cells. The steps involved in this process include smooth-muscle migration, which is stimulated by platelet-derived growth factor, fibroblast growth factor 2, and transforming growth factor β; T-cell activation, which is mediated by tumor necrosis factor α, interleukin-2, and granulocyte–macrophage colony-stimulating factor; foam-cell formation, which is mediated by oxidized low-density lipoprotein, macrophage colony-stimulating factor, tumor necrosis factor α, and interleukin-1; and platelet adherence and aggregation, which are stimulated by integrins, P-selectin, fibrin, thromboxane A_2, tissue factor, and the factors described in Figure 1 as responsible for the adherence and migration of leukocytes.

feeding leads to smaller lesions of atherosclerosis.[89] Comparison of the relative roles of these molecules in inflammation in the arteries and the microvasculature may provide clues to the relative feasibility of modifying the inflammatory process at these sites, and thus of modifying atherosclerosis.

A recently discovered class of molecules, the disintegrins, sometimes called metalloproteinase-like, disintegrin-like, cysteine-rich proteins (MDCs), has been identified in endothelium, smooth muscle, and macrophages.[90] These transmembrane proteins, which appear to be involved in cell–cell interactions,[90] contain a metalloproteinase sequence in their extracellular segment that permits them to activate molecules such as tumor necrosis factor α.[91,92] They are not found in normal arteries, but one of them, MDC15, is present in lesions of atherosclerosis.[90] Adhesion molecules such as L-selectin can be cleaved from the surface of leukocytes by a metalloproteinase (L-selectin sheddase), which suggests that in situations of chronic inflammation it may be possible to measure the "shed" molecules, such as the different adhesion molecules, in plasma, as markers of a sustained inflammatory response.[93,94] Disintegrins may participate in these shedding processes. If shedding occurs, it may be detectable in different types of inflammatory responses. Increased plasma concentrations of shed molecules might then be used to identify patients at risk for atherosclerosis or other inflammatory diseases.

Monocytes and Immunity

The ubiquitous monocyte, the precursor of macrophages in all tissues, is present in every phase of atherogenesis. Monocyte-derived macrophages are scavenging and antigen-presenting cells, and they secrete cytokines, chemokines, growth-regulating molecules, and metalloproteinases and other hydrolytic enzymes. The continuing entry, survival, and replication of mononuclear cells in lesions depend in part on factors such as macrophage colony-stimulating factor and granulocyte–macrophage colony-stimulating factor for monocytes and interleukin-2 for lymphocytes. Continued exposure to macrophage colony-stimulating factor permits macrophages to survive in vitro and possibly to multiply within the lesions. In con-

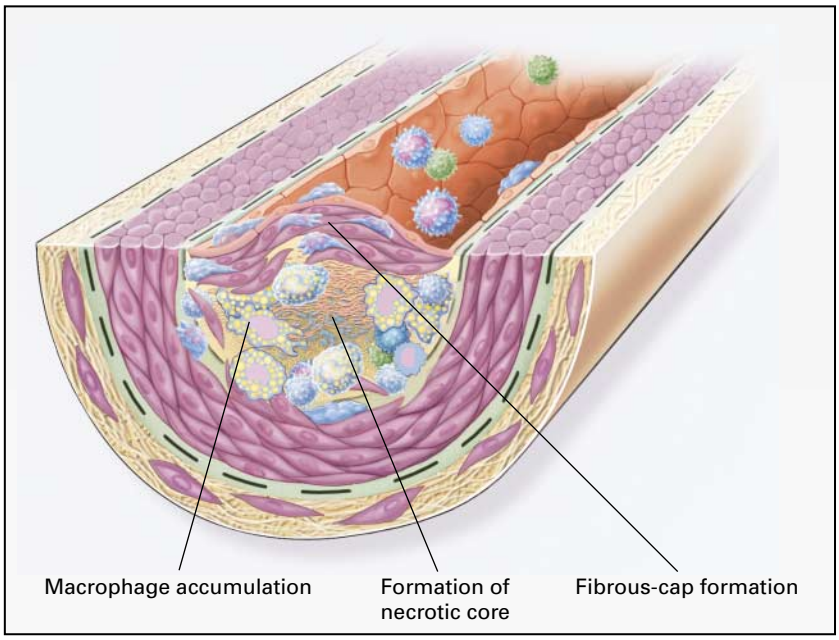

Figure 3. Formation of an Advanced, Complicated Lesion of Atherosclerosis.

As fatty streaks progress to intermediate and advanced lesions, they tend to form a fibrous cap that walls off the lesion from the lumen. This represents a type of healing or fibrous response to the injury. The fibrous cap covers a mixture of leukocytes, lipid, and debris, which may form a necrotic core. These lesions expand at their shoulders by means of continued leukocyte adhesion and entry caused by the same factors as those listed in Figures 1 and 2. The principal factors associated with macrophage accumulation include macrophage colony-stimulating factor, monocyte chemotactic protein 1, and oxidized low-density lipoprotein. The necrotic core represents the results of apoptosis and necrosis, increased proteolytic activity, and lipid accumulation. The fibrous cap forms as a result of increased activity of platelet-derived growth factor, transforming growth factor β, interleukin-1, tumor necrosis factor α, and osteopontin and of decreased connective-tissue degradation.

trast, inflammatory cytokines such as interferon-γ activate macrophages and under certain circumstances induce them to undergo programmed cell death (apoptosis). If this occurs in vivo, macrophages may become involved in the necrotic cores characteristic of advanced, complicated lesions (Fig. 3).

Initially, the only cells thought to proliferate during expansion of atherosclerotic lesions were smooth-muscle cells. However, replication of monocyte-derived macrophages and T cells is probably of equal importance.[95] The ability of macrophages to produce cytokines (such as tumor necrosis factor α, interleukin-1, and transforming growth factor β), proteolytic enzymes (particularly metalloproteinases), and growth factors (such as platelet-derived growth factor and insulin-like growth factor I) may be critical in the role of these cells in the damage and repair that ensue as the lesions progress (Fig. 2).

Activated macrophages express class II histocompatibility antigens such as HLA-DR that allow them to present antigens to T lymphocytes.[20] Thus, it is not surprising that cell-mediated immune responses may be involved in atherogenesis, since both CD4 and CD8 T cells are present in the lesions at all stages of the process.[96,97] T cells are activated when they bind antigen processed and presented by macrophages. T-cell activation results in the secretion of cytokines, including interferon-γ and tumor necrosis factor α and β, that amplify the inflammatory response.[97] Smooth-muscle cells from the lesions also have class II HLA molecules on their surfaces, presumably induced by interferon-γ, and can also present antigens to T cells.[97] One possible antigen may be oxidized LDL,[98] which can be produced by macrophages.[99] Heat-shock protein 60 may also contribute to autoimmunity. This and other heat-shock proteins perform several functions, including the assembly, intracellular transport, and breakdown of proteins and the prevention of protein denaturation. These proteins may be elevated on endothelial cells and participate in immune responses.[100]

An immunoregulatory molecule, CD40 ligand,[101] can be expressed by macrophages, T cells, endothelium, and smooth muscle in atherosclerotic lesions in vivo, and its receptor, CD40, is expressed on the same cells. Both are up-regulated in lesions of atherosclerosis, providing further evidence of immune activation in the lesions.[102,103] Furthermore, CD40 ligand induces the release of interleukin-1β by vascular cells, potentially enhancing the inflammatory response.[104] Inhibition of CD40 with blocking antibodies reduces lesion formation in apolipoprotein E–deficient mice.[105]

Platelets

Platelet adhesion and mural thrombosis are ubiquitous in the initiation and generation of the lesions of atherosclerosis in animals and humans (Fig. 2).[9] Platelets can adhere to dysfunctional endothelium, exposed collagen, and macrophages. When activated, platelets release their granules, which contain cytokines and growth factors that, together with thrombin, may contribute to the migration and proliferation of smooth-muscle cells and monocytes.[106] Activation of platelets leads to the formation of free arachidonic acid, which can be transformed into prostaglandins such as thromboxane A_2, one of the most potent vasoconstricting and platelet-aggregating substances known, or into leukotrienes, which can amplify the inflammatory response.

Plaque rupture and thrombosis are notable complications of advanced lesions that lead to unstable coronary syndromes or myocardial infarction (Fig. 4).[9,21,107] Platelets are important in maintaining vascular integrity in the absence of injury and protecting against spontaneous hemorrhage. Activated platelets can accumulate on the walls of arteries and recruit additional platelets into an expanding thrombus. An important component of the platelets is the glycoprotein IIb/IIIa receptor, which belongs to the integrin superfamily of adhesion-molecule receptors and appears on the surface of platelets during platelet activation and thrombus formation. These receptors serve an important hemostatic function, and antagonists to them prevent thrombus formation in patients who have had a myocardial infarction.[108]

ATHEROSCLEROSIS IN RELATION TO OTHER CHRONIC INFLAMMATORY DISEASES

The cellular interactions in atherogenesis are fundamentally no different from those in chronic inflammatory–fibroproliferative diseases such as cirrhosis, rheumatoid arthritis, glomerulosclerosis, pulmonary fibrosis, and chronic pancreatitis (Table 1). In the examples in Table 1, the response of each particular tissue or organ depends on its characteristic cells and architecture, its blood and lymph supply, and the nature of the offending agents. Thus, the cellular responses in the arteries (atherosclerosis), liver (cirrhosis), joints (rheumatoid arthritis), kidneys (glomerulosclerosis), lungs (pulmonary fibrosis), and pancreas (pancreatitis) are similar yet are characteristic of each tissue or organ.

Inflammatory Response

Does the inflammatory response in arteries differ from that in other tissues? Granulocytes are rare in atherosclerosis, and among the other disorders in Table 1, they are present only in rheumatoid arthritis and pulmonary fibrosis. In the case of arthritis, although the early response begins with granulocytes, they are found primarily within the joint cavity. Macrophages and lymphocytes predominate in the synovium, leading to erosion of cartilage and bone, which is replaced by fibrous tissue (pannus). In pulmonary fi-

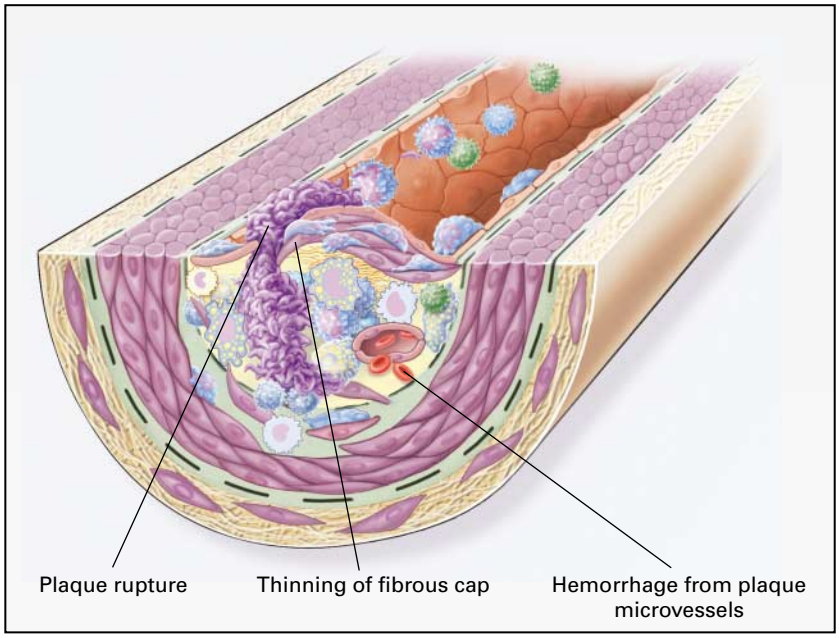

Figure 4. Unstable Fibrous Plaques in Atherosclerosis.

Rupture of the fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis and usually occurs at sites of thinning of the fibrous cap that covers the advanced lesion. Thinning of the fibrous cap is apparently due to the continuing influx and activation of macrophages, which release metalloproteinases and other proteolytic enzymes at these sites. These enzymes cause degradation of the matrix, which can lead to hemorrhage from the vasa vasorum or from the lumen of the artery and can result in thrombus formation and occlusion of the artery.

brosis, granulocytes initially appear in the alveolar spaces; however, the lung parenchyma, where fibrosis ultimately occurs, is infiltrated by macrophages and lymphocytes. Thus, there are parallels between atherosclerosis and these other inflammatory diseases.

Are there particular aspects of the chronic inflammatory response in atherosclerosis that can be used to advantage? At least three different types of macrophages, each regulated by different T-cell cytokines (interferon-γ, interleukin-2, interleukin-4, and interleukin-10) have been identified.[122] These differences raise the question whether there are subgroups of monocytes that "home" to a specific tissue or organ. Are there differences in arterial endothelium and microvascular endothelium such that different types of monocytes are attracted to each, and could one take advantage of such differences?[123] One might try to use such differences to modify the inflammatory response so as to emphasize its protective rather than its destructive characteristics.

If the injurious agent or agents are not removed or nullified by the inflammatory response and the inflammation progresses, the response changes from a protective to an injurious response. Such constant or repetitive injury can stimulate each tissue to repair or wall off the damage by means of a fibroproliferative response, which, when excessive, diminishes the functional capacity of the tissue or organ and becomes part of the disease process (Table 1).

Instability and Rupture of Plaque

Chronic inflammatory responses are often associated with specific types of injurious or granuloma-inducing agents. In most patients myocardial infarctions occur as a result of erosion or uneven thinning and rupture of the fibrous cap, often at the shoulders of the lesion where macrophages enter, accumulate, and are activated and where apoptosis may occur.[124,125] Degradation of the fibrous cap may result from elaboration of metalloproteinases such as collagenases, elastases, and stromelysins (Fig. 4).[126] Activated T cells may stimulate metalloproteinase production by macrophages in the lesions, which promotes plaque instability and further implicates an immune response.[103] These changes may also be accompanied by the production of tissue-factor procoagulant and other hemostatic factors,[102,127] further increasing the possibility of thrombosis.

Stable advanced lesions usually have uniformly dense fibrous caps. The potentially dangerous lesions are often nonocclusive and thus difficult to diagnose by angiography, yet at autopsy active inflammation is evident in the accumulation of macrophages at sites of plaque rupture.[107] Macrophage accumulation may

TABLE 1. CHARACTERISTICS OF ATHEROSCLEROSIS AND OTHER CHRONIC INFLAMMATORY DISEASES.*

DISEASE	MONOCYTES AND MACROPHAGES	LYMPHOCYTES	GRANULOCYTES	CONNECTIVE-TISSUE CELLS	EXTRACELLULAR MATRIX	PATHOGENETIC MECHANISMS	STUDIES
Atherosclerosis	+	+	−	Smooth-muscle cells	Collagen types I, III, and IV, elastin, fibronectin, proteoglycan	Endothelial-cell injury and dysfunction; fibrous cap; new matrix formation and degradation; necrotic core	Ross,[9] Libby and Hansson,[109] Ross and Fuster[110]
Cirrhosis	+	+	−	Fibroblasts, Ito cells	Collagen types I and III	Parenchymal-cell injury; new matrix and scarring replacing necrotic parenchyma	Maher,[111] Anthony et al.[112]
Rheumatoid arthritis	+	+	+/−	Synovial fibroblasts	Collagen types I and III, fibronectin, proteoglycan	Synovial-cell injury; erosion of cartilage; new matrix scarring (pannus)	Sewell and Trentham,[113] Harris [114]
Glomerulosclerosis	+	+	−	Mesangial cells	Collagen types I and IV, fibronectin	Epithelial- and endothelial-cell injury and dysfunction; decrease in glomerular filtration; new matrix formation	Johnson,[115] Magil and Cohen[116]
Pulmonary fibrosis	+	+	+/−	Smooth-muscle cells, fibroblasts	Collagen types III and IV, fibronectin	Inflammatory exudate in alveoli and bronchi, organized by extensive matrix deposition and scarring	Kuhn et al.,[117] Lukacs and Ward,[118] Brody et al.[119]
Chronic pancreatitis	+	+	−	Fibroblasts	Collagen, fibronectin, proteoglycan	Epithelial (ductal) injury; periductal inflammation; interstitial fat necrosis; new matrix formation	Sarles et al.,[120] DiMagno et al.[121]

*Plus signs denote the presence of a cell type, and minus signs its absence.

be associated with increased plasma concentrations of both fibrinogen and C-reactive protein,[128-130] two markers of inflammation thought to be early signs of atherosclerosis.[128,131,132] Plaque rupture and thrombosis may be responsible for as many as 50 percent of cases of acute coronary syndromes and myocardial infarction.[21]

NEW PERSPECTIVES ON THE FORMATION AND PROGRESSION OF LESIONS

Smooth Muscle

To understand the factors that are important in the proliferative and migratory responses that lead to differences in the organization and enlargement of the lesions in different parts of the arterial tree, it may be helpful to understand the embryonic derivation of the smooth-muscle cells that make up the arteries in different regions. Smooth-muscle cells have different embryonic origins, depending on the segment of the arterial system involved. In some vertebrates, smooth-muscle cells in the upper portion of the thoracic aorta are derived from a neuroectodermal source, whereas those in the abdominal aorta are derived from a mesenchymal source.[133] Although likely, this has not been confirmed in humans. The smooth-muscle cells of coronary arteries appear to originate from a third precursor population in the intracardiac mesenchyme. The existence of these different lineages suggests that smooth muscle in different parts of the arterial tree may respond differently to the stimuli that generate atherosclerotic lesions at each of these sites. To complicate matters further, smooth-muscle cells within the media of large arteries may be heterogeneous, with different proliferative and matrix-producing capabilities.[134]

These differences in the origin of smooth-muscle cells raise questions about whether these cells, on the basis of their lineage, respond differently to different cytokines, mitogens, chemotactic factors, or extracellular matrixes.[135-137] Is there selection of a particular lineage based on the cells' responses to these different substances? Does cell lineage help to explain why lesions in peripheral arteries differ from those in the carotid and coronary arteries?

The Role of the Matrix

Smooth-muscle cells in the media of arteries, as well as in lesions, are surrounded by different types of connective tissue. In the media of arteries, the matrix consists largely of type I and III fibrillar collagen, whereas in the lesions of atherosclerosis it consists largely of proteoglycan, intermixed with loosely scattered collagen fibrils.

When cultured human arterial smooth-muscle cells are plated on collagen in fibrillar form, the collagen inhibits cell proliferation by up-regulating specific inhibitors of the cell cycle.[137] In vivo degradation of the collagen by collagenase, or migration away from this inhibitory environment, may allow the smooth-muscle cells to respond to mitogenic stimuli and rep-

licate, as they do when they are cultured on nonfibrillar, monomeric collagen. Other matrix molecules, such as fibronectin and heparan sulfate, may be involved, because they can also inhibit the cell cycle, and cell–matrix interactions can lead to the expression of chemokines by macrophages.[138-140] If these interactions were to occur in arteries, they could profoundly influence the inflammatory and fibroproliferative response.[141] Thus, the matrix that surrounds the cells is not neutral and may determine whether they remain quiescent or multiply in response to growth factors.

CONCLUSIONS

Cells may express different constellations of genes and therefore vary phenotypically, depending on their environment. New techniques have been developed to identify DNA that should yield a vast amount of information about which genes are expressed and in what patterns, information that should help decipher the complex nature of atherogenesis.[142-144] Because atherosclerosis is a multigenic disease, understanding patterns of gene expression may help to explain differences in susceptibility to agents that cause disease. Furthermore, the patterns of gene expression may vary in lesions from different persons and at different sites and may provide clues regarding genetic differences in susceptibility as well as response to therapy.

Advances in molecular genetics have made it possible to remove or insert genes and to determine the roles of their products in disease.[145] Numerous animal models that are useful in studying the genetics of atherogenesis have been produced, such as apolipoprotein E–deficient mice.[146,147] In the absence of apolipoprotein E, lipoprotein remnants are not carried to the liver, where they are normally metabolized, and the mice become hypercholesterolemic and lesions of atherosclerosis develop that are similar to those in humans. To explore the role of monocytes and platelets and of platelet-derived growth factor in atherogenesis, studies are under way in which apolipoprotein E–deficient mice have been made chimeric for a deficiency of platelet-derived growth factor in circulating monocytes and platelets.

Studies in transgenic mice have revealed that Lp(a) lipoprotein, cholesterol ester transfer protein, apolipoprotein A (the principal apoprotein of high-density lipoprotein), and other molecules have little effect on atherogenesis, whereas macrophage colony-stimulating factor appears to be important in the regulation of the numbers of monocytes and macrophages and in lesion formation.[148,149]

Thus, although hypercholesterolemia is important in approximately 50 percent of patients with cardiovascular disease,[5] other factors need to be taken into consideration. Atherosclerosis is clearly an inflammatory disease and does not result simply from the accumulation of lipids. If we can selectively modify the harmful components of inflammation in the arteries and leave the protective aspects intact, we may create new avenues for the diagnosis and management of disease in the 50 percent of patients with cardiovascular disease who do not have hypercholesterolemia.

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