Review Article

Mechanisms of Disease

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

ATHEROSCLEROSIS — AN INFLAMMATORY DISEASE

RUSSELL ROSS, PH.D.

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.

From the Department of Pathology, University of Washington School of Medicine, Box 357470, Seattle, WA 98195-7470, where reprint requests should be addressed to Dr. Ross (e-mail: rross@u.washington.edu).
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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
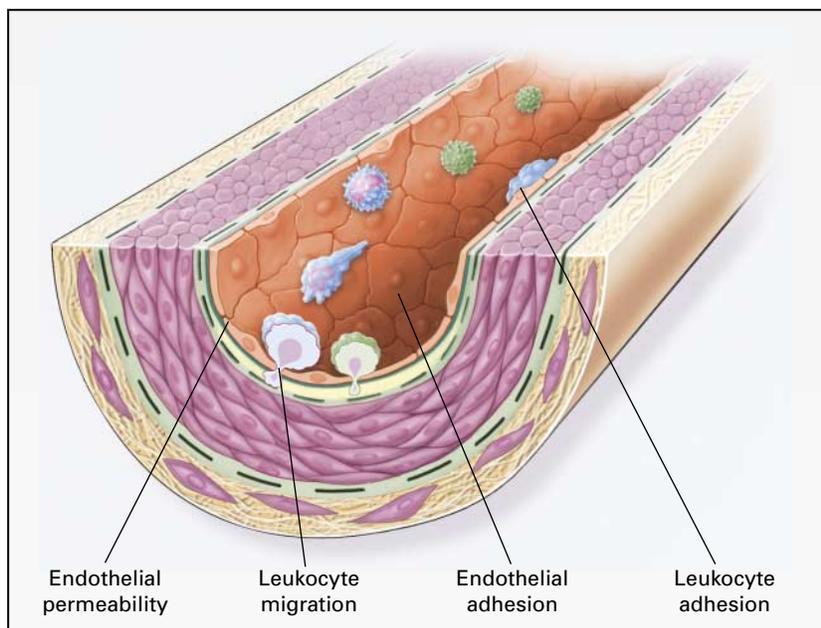
Figure 1. Endothelial Dysfunction in Atherosclerosis.

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
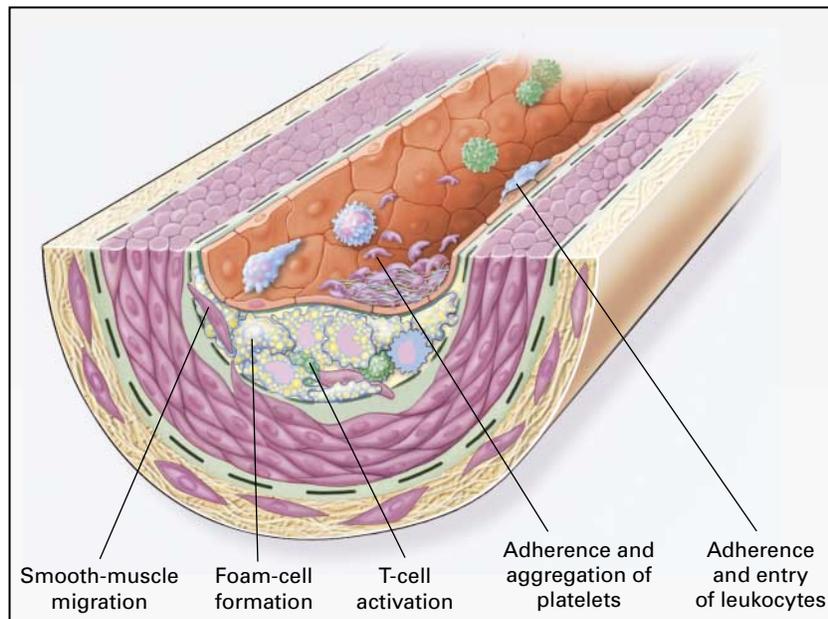
Figure 2. Fatty-Streak Formation in Atherosclerosis.

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 in-flammatory 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-
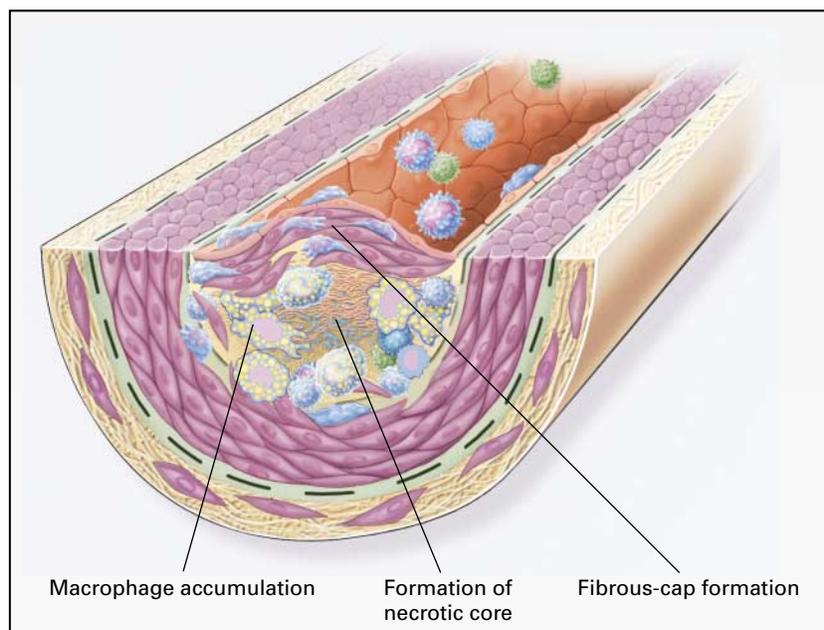
Figure 3. Formation of an Advanced, Complicated Lesion of Atherosclerosis.

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.

Labels in figure: Macrophage accumulation · Formation of necrotic core · Fibrous-cap formation

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-
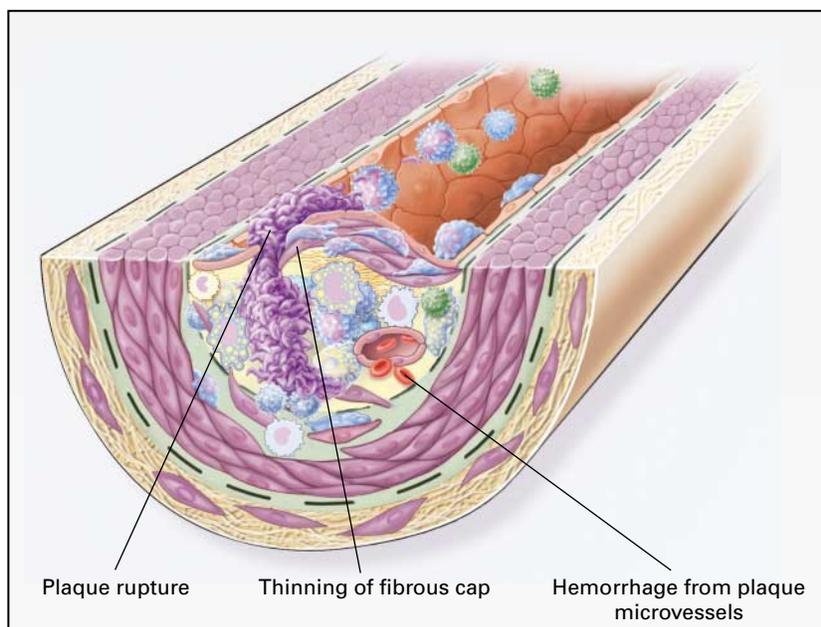
Figure 4. Unstable Fibrous Plaques in Atherosclerosis.

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 metallo-proteinases 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, diminish-

es 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 MACRO-PHAGES	LYMPHO-CYTES	GRANU-LOCYTES	CONNECTIVE-TISSUE CELLS	EXTRACELLULAR MATRIX	PATHOGENETIC MECHANISMS	STUDIES
Atherosclerosis	+	+	−	Smooth-muscle cells	Collagen types I, III, and IV, elastin, fibro-nectin, proteoglycan	Endothelial-cell injury and dys-function; fibrous cap; new matrix formation and degra-dation; 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] Antho-ny et al.[112]
Rheumatoid arthritis	+	+	+/−	Synovial fibroblasts	Collagen types I and III, fibronectin, pro-teoglycan	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; de-crease in glomerular filtra-tion; 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 ex-tensive 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; peri-ductal inflammation; intersti-tial 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 throm-bosis may be responsible for as many as 50 percent of cases of acute coronary syndromes and myocar-dial 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 deriva-tion of the smooth-muscle cells that make up the ar-teries in different regions. Smooth-muscle cells have different embryonic origins, depending on the seg-ment of the arterial system involved. In some verte-brates, smooth-muscle cells in the upper portion of the thoracic aorta are derived from a neuroectoder-mal 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 dif-ferent lineages suggests that smooth muscle in dif-ferent parts of the arterial tree may respond differ-

ently 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 dif-ferent 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 col-lagen, whereas in the lesions of atherosclerosis it con-sists 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 non-fibrillar, 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.

REFERENCES

1. National Cholesterol Education Program. Second report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). Bethesda, Md.: National Heart, Lung, and Blood Institute, 1993. (NIH publication no. 93-3095.)
2. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4 444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383-9.
3. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301-7.
4. Breslow JL. Cardiovascular disease burden increases, NIH funding decreases. Nat Med 1997;3:600-1.
5. Braunwald E. Shattuck Lecture — cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med 1997;337:1360-9.
6. Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. Science 1973;180:1332-9.
7. *Idem.* The pathogenesis of atherosclerosis. N Engl J Med 1976;295: 369-77, 420-5.
8. Ross R. The pathogenesis of atherosclerosis — an update. N Engl J Med 1986;314:488-500.
9. *Idem.* The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-9.
10. *Idem.* Atherosclerosis: a defense mechanism gone awry. Am J Pathol 1993;143:987-1002.
11. Napoli C, D'Armiento FP, Mancini FP, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia: intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J Clin Invest 1997;100:2680-90.
12. Stary HC, Chandler AB, Glagov S, et al. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1994;89:2462-78.
13. Simionescu N, Vasile E, Lupu F, Popescu G, Simionescu M. Prelesional events in atherogenesis: accumulation of extracellular cholesterol-rich liposomes in the arterial intima and cardiac valves of the hyperlipidemic rabbit. Am J Pathol 1986;123:109-25.
14. Ross R. Atherosclerosis — a problem of the biology of arterial wall cells and their interactions with blood components. Arteriosclerosis 1981;1: 293-311.
15. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. N Engl J Med 1987;316:1371-5.
16. Stary HC. The histological classification of atherosclerotic lesions in human coronary arteries. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:463-74.
17. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis 1986;6:131-8.
18. van der Wal AC, Das PK, Bentz van de Berg D, van der Loos CM, Becker AE. Atherosclerotic lesions in humans: in situ immunophenotypic analysis suggesting an immune mediated response. Lab Invest 1989;61:166-70.
19. Libby P, Ross R. Cytokines and growth regulatory molecules. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:585-94.

20. Raines EW, Rosenfeld ME, Ross R. The role of macrophages. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:539-55.

21. Falk E, Shah PK, Fuster V. Pathogenesis of plaque disruption. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 2. Philadelphia: Lippincott-Raven, 1996:492-510.

22. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997;272:20963-6.

23. Khoo JC, Miller E, McLoughlin P, Steinberg D. Enhanced macrophage uptake of low density lipoprotein after self-aggregation. Arteriosclerosis 1988;8:348-58.

24. Khoo JC, Miller E, Pio F, Steinberg D, Witztum JL. Monoclonal antibodies against LDL further enhance macrophage uptake of LDL aggregates. Arterioscler Thromb 1992;12:1258-66.

25. Navab M, Berliner JA, Watson AD, et al. The Yin and Yang of oxidation in the development of the fatty streak: a review based on the 1994 George Lyman Duff Memorial Lecture. Arterioscler Thromb Vasc Biol 1996;16:831-42.

26. Morel DW, Hessler JR, Chisholm GM. Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipid. J Lipid Res 1983;24:1070-6.

27. Griendling KK, Alexander RW. Oxidative stress and cardiovascular disease. Circulation 1997;96:3264-5.

28. Han J, Hajjar DP, Febbraio M, Nicholson AC. Native and modified low density lipoproteins increase the functional expression of the macrophage class B scavenger receptor, CD36. J Biol Chem 1997;272:21654-9.

29. Diaz MN, Frei B, Vita JA, Keaney JF Jr. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997;337:408-16.

30. Falcone DJ, McCaffrey TA, Vergilio JA. Stimulation of macrophage urokinase expression by polyanions is protein kinase C-dependent and requires protein and RNA synthesis. J Biol Chem 1991;266:22726-32.

31. Nunes GL, Robinson K, Kalynych A, King SB III, Sgoutas DS, Berk BC. Vitamins C and E inhibit O_2^- production in the pig coronary artery. Circulation 1997;96:3593-601.

32. Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci U S A 1987;84:2995-8.

33. Rajavashisth TB, Andalibi A, Territo MC, et al. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. Nature 1990;344:254-7.

34. Leonard EJ, Yoshimura T. Human monocyte chemoattractant protein-1 (MCP-1). Immunol Today 1990;11:97-101.

35. Stopeck AT, Nicholson AC, Mancini FP, Hajjar DP. Cytokine regulation of low density lipoprotein receptor gene transcription in HepG2 cells. J Biol Chem 1993;268:17489-94.

36. Hajjar DP, Haberland ME. Lipoprotein trafficking in vascular cells: molecular Trojan horses and cellular saboteurs. J Biol Chem 1997;272:22975-8.

37. Geng Y-J, Libby P. Evidence for apoptosis in advanced human atheroma: colocalization with interleukin-1 beta-converting enzyme. Am J Pathol 1995;147:251-66.

38. Palkama T. Induction of interleukin-1 production by ligands binding to the scavenger receptor in human monocytes and the THP-1 cell line. Immunology 1991;74:432-8.

39. Palkama T, Matikainen S, Hurme M. Tyrosine kinase activity is involved in the protein kinase C induced expression of interleukin-1 beta gene in monocytic cells. FEBS Lett 1993;319:100-4.

40. Ylä-Herttuala S, Palinski W, Rosenfeld ME, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 1989;84:1086-95.

41. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci U S A 1987;84:7725-9.

42. Kita T, Nagano Y, Yokode M, et al. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc Natl Acad Sci U S A 1987;84:5928-31.

43. Sasahara M, Raines EW, Chait A, et al. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol. I. Is the extent of atherosclerosis related to resistance of LDL to oxidation? J Clin Invest 1994;94:155-64.

44. Chang MY, Sasahara M, Chait A, Raines EW, Ross R. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol. II. Cellular composition and proliferation. Arterioscler Thromb Vasc Biol 1995;15:1631-40.

45. Fruebis J, Gonzalez V, Silvestre M, Palinski W. Effect of probucol treatment on gene expression of VCAM-1, MCP-1, and M-CSF in the aortic wall of LDL receptor-deficient rabbits during early atherogenesis. Arterioscler Thromb Vasc Biol 1997;17:1289-302.

46. Reaven PD, Khouw A, Beltz WF, Parthasarathy S, Witztum JL. Effect of dietary antioxidant combinations in humans: protection of LDL by vitamin E but not by beta-carotene. Arterioscler Thromb 1993;13:590-600.

47. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993;328:1450-6.

48. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 1993;328:1444-9.

49. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study. Lancet 1996;347:781-6.

50. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334:1145-9.

51. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150-5.

52. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111-28.

53. Mudd SH, Skovby F, Levy HL, et al. The natural history of homocystinuria due to cystathionine β-synthase deficiency. Am J Hum Genet 1985;37:1-31.

54. Nehler MR, Taylor LM Jr, Porter JM. Homocysteinemia as a risk factor for atherosclerosis: a review. Cardiovasc Surg 1997;6:559-67.

55. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337:230-6.

56. Malinow MR. Plasma homocyst(e)ine and arterial occlusive diseases: a mini-review. Clin Chem 1995;41:173-6.

57. Harker LA, Ross R, Slichter SJ, Scott CR. Homocystine-induced arteriosclerosis: the role of endothelial cell injury and platelet response in its genesis. J Clin Invest 1976;58:731-41.

58. Hajjar KA. Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. J Clin Invest 1993;91:2873-9.

59. Majors A, Ehrhart LA, Pezacka EH. Homocysteine as a risk factor for vascular disease: enhanced collagen production and accumulation by smooth muscle cells. Arterioscler Thromb Vasc Biol 1997;17:2074-81.

60. Upchurch GR Jr, Welch GN, Fabian AJ, et al. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. J Biol Chem 1997;272:17012-7.

61. Verhoef P, Stampfer MJ. Prospective studies of homocysteine and cardiovascular disease. Nutr Rev 1995;53:283-8.

62. Omenn GS, Beresford SAA, Motulsky AG. Preventing coronary heart disease: B vitamins and homocysteine. Circulation 1998;97:421-4.

63. Chobanian AV, Dzau VJ. Renin angiotensin system and atherosclerotic vascular disease. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:237-42.

64. Gibbons GH, Pratt RE, Dzau VJ. Vascular smooth muscle cell hypertrophy vs. hyperplasia: autocrine transforming growth factor-beta 1 expression determines growth response to angiotensin II. J Clin Invest 1992;90:456-61.

65. Lacy F, O'Connor DT, Schmid-Schönbein GW. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. J Hypertens 1998;16:291-303.

66. Swei A, Lacy F, DeLano FA, Schmid-Schönbein GW. Oxidative stress in the Dahl hypertensive rat. Hypertension 1997;30:1628-33.

67. Vanhoutte PM, Boulanger CM. Endothelium-dependent responses in hypertension. Hypertens Res 1995;18:87-98.

68. Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. Circulation 1997;96:4095-103.

69. Hendrix MG, Salimans MM, van Boven CP, Bruggeman CA. High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. Am J Pathol 1990;136:23-8.

70. Jackson LA, Campbell LA, Schmidt RA, et al. Specificity of detection of Chlamydia pneumoniae in cardiovascular atheroma: evaluation of the innocent bystander hypothesis. Am J Pathol 1997;150:1785-90.

71. Thom DH, Wang SP, Grayston JT, et al. Chlamydia pneumoniae strain TWAR antibody and angiographically demonstrated coronary artery disease. Arterioscler Thromb 1991;11:547-51.

72. Melnick JL, Adam E, Debakey ME. Cytomegalovirus and atherosclerosis. Eur Heart J 1993;14:Suppl K:30-8.

73. Gupta S, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camm AJ. Elevated Chlamydia pneumoniae antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. Circulation 1997;96:404-7.

74. Hajjar DP, Fabricant CG, Minick CR, Fabricant J. Virus-induced atherosclerosis: herpesvirus infection alters aortic cholesterol metabolism and accumulation. Am J Pathol 1986;122:62-70.

75. Nicholson AC, Hajjar DP. Herpesviruses in atherosclerosis and thrombosis: etiologic agents or ubiquitous bystanders? Arterioscler Thromb Vasc Biol 1998;18:339-48.

76. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? Lancet 1997;350:430-6.

77. Gotlieb AI, Langille BL. The role of rheology in atherosclerotic coronary artery disease. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:595-606.

78. Springer TA, Cybulsky MI. Traffic signals on endothelium for leukocytes in health, inflammation, and atherosclerosis. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:511-38.

79. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med 1993;178:449-60.

80. Giachelli CM, Lombardi D, Johnson RJ, Murry CE, Almeida M. Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli in vivo. Am J Pathol 1998;152:353-8.

81. Nagel T, Resnick N, Atkinson WJ, Dewey CF Jr, Gimbrone MA Jr. Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. J Clin Invest 1994;94:885-91.

82. Resnick N, Collins T, Atkinson W, Bonthron DT, Dewey CF Jr, Gimbrone MA Jr. Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear-stress-responsive element. Proc Natl Acad Sci U S A 1993;90:4591-5.

83. Lin MC, Almus-Jacobs F, Chen HH, et al. Shear stress induction of the tissue factor gene. J Clin Invest 1997;99:737-44.

84. Mondy JS, Lindner V, Miyashiro JK, Berk BC, Dean RH, Geary RL. Platelet-derived growth factor ligand and receptor expression in response to altered blood flow in vivo. Circ Res 1997;81:320-7.

85. McMillan DE. Blood flow and the localization of atherosclerotic plaques. Stroke 1985;16:582-7.

86. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. Arterioscler Thromb Vasc Biol 1998;18:842-51.

87. Boring L, Gosling J, Chensue SW, et al. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. J Clin Invest 1997;100:2552-61.

88. Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. J Clin Invest 1998;101:353-63.

89. Hynes RO, Wagner DD. Genetic manipulation of vascular adhesion molecules in mice. J Clin Invest 1996;98:2193-5.

90. Herren B, Raines EW, Ross R. Expression of a disintegrin-like protein in cultured human vascular cells and in vivo. FASEB J 1997;11:173-80.

91. Black RA, Rauch CT, Kozlosky CJ, et al. A metalloproteinase disintegrin that releases tumor-necrosis factor-α from cells. Nature 1997;385:729-33.

92. Moss ML, Jin S-LC, Milla ME, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour necrosis factor-α. Nature 1997;385:733-6.

93. De Caterina R, Basta G, Lazzerini G, et al. Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:2646-54.

94. Hwang S-J, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 1997;96:4219-25.

95. Rosenfeld ME, Ross R. Macrophage and smooth muscle cell proliferation in atherosclerotic lesions of WHHL and comparably hypercholesterolemic fat-fed rabbits. Arteriosclerosis 1990;10:680-7.

96. Hansson GK, Libby P. The role of the lymphocyte. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:557-68.

97. Hansson GK, Jonasson L, Seifert PS, Stemme S. Immune mechanisms in atherosclerosis. Arteriosclerosis 1989;9:567-78.

98. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci U S A 1995;92:3893-7.

99. Folcik VA, Aamir R, Cathcart MK. Cytokine modulation of LDL oxidation by activated human monocytes. Arterioscler Thromb Vasc Biol 1997;17:1954-61.

100. Wick G, Romen M, Amberger A, et al. Atherosclerosis, autoimmunity, and vascular-associated lymphoid tissue. FASEB J 1997;11:1199-207.

101. Hollenbaugh D, Mischel-Petty N, Edwards CP, et al. Expression of functional CD40 by vascular endothelial cells. J Exp Med 1995;182:33-40.

102. Mach F, Schönbeck U, Bonnefoy J-Y, Pober JS, Libby P. Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor. Circulation 1997;96:396-9.

103. Schönbeck U, Mach F, Sukhova GK, et al. Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture? Circ Res 1997;81:448-54.

104. Schönbeck U, Mach F, Bonnefoy J-Y, Loppnow H, Flad H-D, Libby P. Ligation of CD40 activates interleukin 1β-converting enzyme (caspase-1) activity in vascular smooth muscle and endothelial cells and promotes elaboration of active interleukin 1β. J Biol Chem 1997;272:19569-74.

105. Mach F, Schönbeck U, Sukhova GK, Atkinson E, Libby P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. Nature 1998;394:200-3.

106. Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: evidence for a GPIIbIIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), $\alpha_v\beta_3$ integrin, and GPIbα. J Exp Med 1998;187:329-39.

107. Davies MJ. A macro and micro view of coronary vascular insult in ischemic heart disease. Circulation 1990;82:Suppl II:II-38–II-46.

108. Badimon JJ, Meyer B, Feigen LP, et al. Thrombosis triggered by severe arterial lesions is inhibited by oral administration of a glycoprotein IIb/IIIa antagonist. Eur J Clin Invest 1997;27:568-74.

109. Libby P, Hansson GK. Involvement of the immune system in human atherogenesis: current knowledge and unanswered questions. Lab Invest 1991;64:5-15.

110. Ross R, Fuster V. The pathogenesis of atherosclerosis. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven, 1996:441-60.

111. Maher JJ. Hepatic fibrosis caused by alcohol. Semin Liver Dis 1990;10:66-74.

112. Anthony PP, Ishak KG, Nayak NC, Poulsen HE, Scheuer PJ, Sobin LH. The morphology of cirrhosis: definition, nomenclature, and classification. Bull World Health Organization 1977;55:521-40.

113. Sewell KL, Trentham DE. Pathogenesis of rheumatoid arthritis. Lancet 1993;341:283-6.

114. Harris ED Jr. Rheumatoid arthritis: pathophysiology and implications for therapy. N Engl J Med 1990;322:1277-89.

115. Johnson RJ. What mediates progressive glomerulosclerosis? The glomerular endothelium comes of age. Am J Pathol 1997;151:1179-81.

116. Magil AB, Cohen AH. Monocytes and focal glomerulosclerosis. Lab Invest 1989;61:404-9

117. Kuhn C III, Boldt J, King TE Jr, Crouch E, Vartio T, McDonald JA. An immunohistochemical study of architectural remodeling and connective tissue synthesis in pulmonary fibrosis. Am Rev Respir Dis 1989;140:1693-703.

118. Lukacs NW, Ward PA. Inflammatory mediators, cytokines, and adhesion molecules in pulmonary inflammation and injury. Adv Immunol 1996;62:257-304.

119. Brody AR, Soler P, Basset F, Haschek WM, Witschi H. Epithelial-mesenchymal associations of cells in human pulmonary fibrosis and in BHT-oxygen-induced fibrosis in mice. Exp Lung Res 1981;2:207-20.

120. Sarles H, Bernard JP, Johnson C. Pathogenesis and epidemiology of chronic pancreatitis. Annu Rev Med 1989;40:453-68.

121. DiMagno EP, Layer P, Clain JE. Chronic pancreatitis. In: Go VLW, DiMagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA, eds. The pancreas: biology, pathobiology, and disease. 2nd ed. New York: Raven Press, 1993:665-706.

122. Tormey VJ, Faul J, Leonard C, Burke CM, Dilmec A, Poulter LW. T-cell cytokines may control the balance of functionally distinct macrophage populations. Immunology 1997;90:463-9.

123. Garlanda C, Dejana E. Heterogeneity of endothelial cells: specific markers. Arterioscler Thromb Vasc Biol 1997;17:1193-202.

124. Fuster V. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. Circulation 1994;90:2126-46.

125. Lee RT, Libby P. The unstable atheroma. Arterioscler Thromb Vasc Biol 1997;17:1859-67.

126. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493-503.

127. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. N Engl J Med 1995;332:635-41.

128. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973-9.

129. Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina: European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Lancet 1997;349:462-6.

130. Toss H, Lindahl B, Siegbahn A, Wallentin L. Prognostic influence of increased fibrinogen and C-reactive protein levels in unstable coronary artery disease. Circulation 1997;96:4204-10.

131. Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in 'active' coronary artery disease. Am J Cardiol 1990;65:168-72.

132. Levenson J, Giral P, Razavian M, Gariepy J, Simon A. Fibrinogen and silent atherosclerosis in subjects with cardiovascular risk factors. Arterioscler Thromb Vasc Biol 1995;15:1263-8.

133. Topouzis S, Majesky MW. Smooth muscle lineage diversity in the chick embryo: two types of aortic smooth muscle cell differ in growth and receptor-mediated transcriptional responses to transforming growth factor-β. Dev Biol 1996;178:430-45.

134. Frid MG, Aldashev AA, Dempsey EC, Stenmark KR. Smooth muscle cells isolated from discrete compartments of the mature vascular media exhibit unique phenotypes and distinct growth capabilities. Circ Res 1997; 81:940-52.

135. Chamley-Campbell JH, Campbell GR, Ross R. Phenotype-dependent response of cultured aortic smooth muscle to serum mitogens. J Cell Biol 1981;89:379-83.

136. Babaev VR, Bobryshev YV, Stenina OV, Tararak EM, Gabbiani G. Heterogeneity of smooth muscle cells in atheromatous plaque of human aorta. Am J Pathol 1990;136:1031-42.

137. Koyama H, Raines EW, Bornfeldt KE, Roberts JM, Ross R. Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of Cdk2 inhibitors. Cell 1996;87:1069-78.

138. Assoian RK, Marcantonio EE. The extracellular matrix as a cell cycle control element in atherosclerosis and restenosis. J Clin Invest 1996;98: 2436-9.

139. Mercurius KO, Morla AO. Inhibition of vascular smooth muscle cell growth by inhibition of fibronectin matrix assembly. Circ Res 1998;82: 548-56.

140. Wesley RB II, Meng X, Godin D, Galis ZS. Extracellular matrix modulates macrophage functions characteristic to atheroma: collagen type I enhances acquisition of resident macrophage traits by human peripheral blood monocytes in vitro. Arterioscler Thromb Vasc Biol 1998;18:432-40.

141. Smith RE, Hogaboam CM, Strieter RM, Lukacs NW, Kunkel SL. Cell-to-cell and cell-to-matrix interactions mediate chemokine expression: an important component of the inflammatory lesion. J Leukoc Biol 1997;62: 612-9.

142. Shalon D, Smith SJ, Brown PO. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. Genome Res 1996;6:639-45.

143. DeRisi J, Penland L, Brown PO, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 1996;14:457-60.

144. Ramsay G. DNA chips: state-of-the-art. Nat Biotechnol 1998;16:40-4.

145. Chien KR. Genes and physiology: molecular physiology in genetically engineered animals. J Clin Invest 1996;97:901-9.

146. Plump AS, Smith JD, Hayek T, et al. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 1992;71:343-53.

147. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 1994;14:133-40.

148. Qiao J-H, Tripathi J, Mishra NK, et al. Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteopetrotic mice. Am J Pathol 1997;150:1687-99.

149. de Villiers WJS, Smith JD, Miyata M, Dansky HM, Darley E, Gordon S. Macrophage phenotype in mice deficient in both macrophage-colony-stimulating factor (op) and apolipoprotein E. Arterioscler Thromb Vasc Biol 1998;18:631-40.

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