CHAPTER 6

Pathogenesis of Atherosclerosis

Jay W. Heinecke, Alan Daugherty

Atherosclerosis, a disorder affecting the intima of medium- to large-sized muscular arteries, is the leading cause of death in Western industrialized society. Its clinical manifestations include myocardial infarction, stroke, and gangrene of the lower extremities. Recent studies have shown that atherosclerosis is a reversible process, emphasizing the importance of controlling risk factors for vascular disease. In this chapter the pathology of atherosclerosis and clinically relevant hypotheses regarding potential atherogenic mechanisms are discussed.

THE NORMAL ARTERY

The wall of the normal artery is composed of three concentric layers (Figure 6–1A) termed the intima, the media, and the adventitia.[1,2] The innermost region, the *intima,* consists of a monolayer of endothelial cells lining the lumen of the artery. The only cell type found in the normal intima is the endothelial cell. The intermediate region, the *media,* is composed entirely of smooth muscle cells and extracellular matrix material. The media is the major structural element of muscular arteries. The outermost region of the artery wall, the *adventitia,* is a loosely woven connective tissue that contains small nutrient vessels called the vasa vasora. The intima is separated from the media by a sheet of well-developed connective tissue, the internal elastic lamina. The boundary between the media and the adventitia is a less distinct layer of connective tissue, the external elastic lamina.

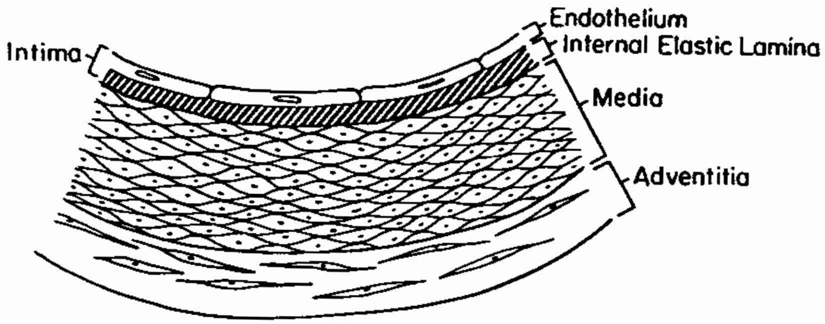
MORPHOLOGICAL EVENTS IN ATHEROGENESIS

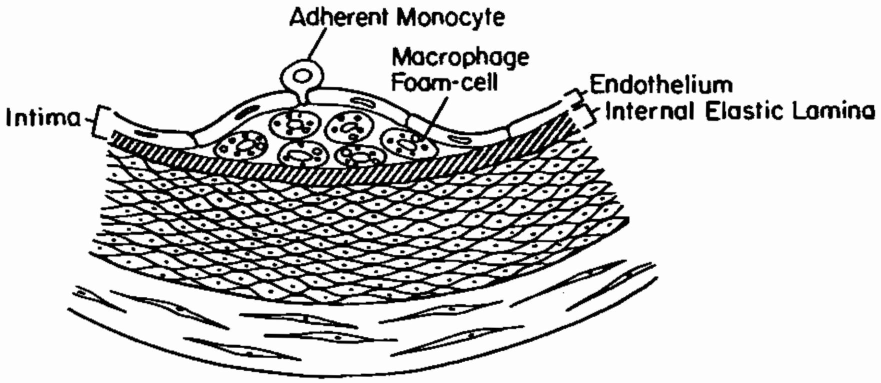
The Fatty Streak

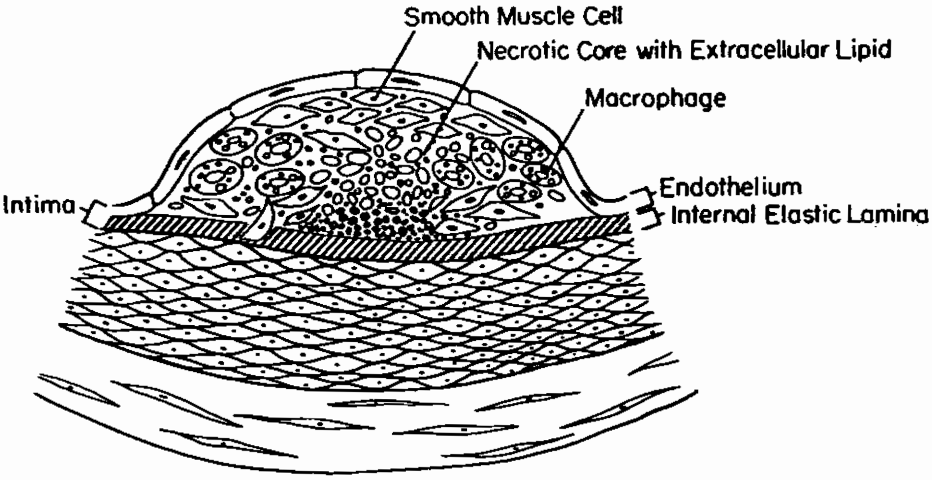
Human atherosclerosis begins early in childhood[1–3] with the appearance of slightly elevated, lipid-rich fatty streaks (Figure 6–1B). This lesion is characterized pathologically by the presence of cholesteryl ester-laden foam cells in the intima beneath an intact endothelial cell layer.[4] Immunohistochemical studies have shown that the majority of foam cells are tissue macrophages derived from circulating monocytes.[5,6] Also present are T lymphocytes, which may represent an immune component in the disease process.[6] Fatty streaks first appear in the aorta, but they may later be found in any of the medium- to large-sized muscular arteries.[3,7] The lesions are nonobstructive and are not associated with clinical manifestations of cardiovascular disease. Fatty streaks are apparently the precursors to more advanced atherosclerotic lesions but they do not invariably progress. Indeed, investigations in animals have revealed that fatty streak formation is a fully reversible process.[8] Cross-cultural studies further support this conclusion because fatty streak formation is found in the aortae of middle-aged adults in all populations, but subsequent clinically significant atherosclerosis is restricted to a subset of these societies.[9]

The Fibrous Plaque

The hallmark of the fibrous plaque (fibroproliferative) is the appearance of smooth muscle cells together with macrophages in the intima of the artery wall (Figure 6–1C). The classic lesion is eccentrically located and protrudes into one side of the arterial lumen.[1–3,6–8,10] Fibrous plaque formation begins in young adulthood in regions of the vasculature prone to fatty streak formation, such as arterial branch points and the coronary arteries.[1,2,7,8] Cerebrovascular disease develops later in life. Fibrous plaque formation should be distinguished from the intimal thickening that occurs during normal aging[1]: this process also involves the appearance of smooth muscle cells in the intima, but widening of the intima occurs symmetrically and is not associated with







the presence of numerous foam cells. In the early stages of fibrous plaque formation, cholesteryl ester is present intracellularly in foam cells which are derived from both macrophages and smooth muscle cells.[2,6,8] Crystals of free cholesterol appear extracellularly as the fibrous plaque matures.[2,6,8] The fully developed fibroproliferative lesion exhibits a necrotic core, rich in extracellular lipid, that is covered by a fibrous cap predominantly composed of smooth muscle cells and connective tissue.[2,3,6,8,10] The shoulder region of the plaque contains abundant numbers of macrophages as well as smaller numbers of T lymphocytes.[6]

Fibrous plaques are clinically significant lesions that impair blood flow by at least two different mechanisms.[11,12] First, the fibroproliferative response of the artery wall mechanically occludes the arterial lumen and progressively restricts blood flow. Second, the fibrous plaque often serves as a nidus for thrombosis

Figure 6–1. Pathological events in atherosclerosis. (**A**) The normal artery. The medium- to large-sized muscular artery is composed of three layers: the intima, the media, and the adventitia. A monolayer of endothelial cells and its underlying connective tissue, the internal elastic lamina, constitute the intima. The media, composed of a concentric layer of smooth muscle cells and connective tissue, is the major structural component of muscular arteries. The adventitia is a loosely woven connective tissue that surrounds the media. (**B**) The fatty streak. The earliest lesion of atherosclerosis is the fatty streak. The hallmark of the fatty streak is the presence of lipid-laden foam cells beneath an intact endothelium. Most foam cells are tissue macrophages derived from circulating monocytes, but recent studies indicate T lymphocytes are also present in significant numbers. A key event in fatty streak formation may be the appearance of chemoattractant molecules for monocytes on the surface of endothelial cells. Fatty streaks do not significantly obstruct blood flow and are clinically silent. Experimental studies in animals and pathological studies in humans suggest that the fatty streak is the precursor of advanced atherosclerotic lesions. (**C**) The fibrous plaque. This lesion is characterized by the appearance of smooth muscle cells and extracellular free cholesterol crystals in the intima. Fibrous plaques protrude into the arterial lumen and often restrict blood flow: they are also prone to fissuring and rupture, which may precipitate acute arterial thrombosis and occlusion. Continued accumulation of smooth muscle cells, monocyte-derived macrophages, and extracellular lipid, together with progressive calcification and formation of a necrotic core ultimately result in the formation of the advanced atherosclerotic lesion.

and acute obstruction of the arterial lumen. Clinical trials have shown that occlusive disease is reversible in patients with hypercholesterolemia who are treated with aggressive lipid-lowering therapy, indicating that the atherosclerotic process is dynamic and can be influenced by alterations in risk factors.[13–15] Moreover, the incidence of acute myocardial events is reduced in the treated patients,[14] suggesting that thrombosis is also inhibited, perhaps by alterations in the lipid composition of lesions.

The Complicated Lesion

The complicated (advanced) atherosclerotic lesion is characterized by endothelial denudation, a large necrotic core with numerous extracellular cholesterol crystals, and extensive calcification.[2,3,8,10] Loss of the endothelial layer renders the advanced lesion prone to thrombosis and acute arterial occlusion.[11,12] Disruption of complex lesions by flow-induced shear stress, mechanical trauma, or acute hemorrhage into the necrotic core commonly triggers clot formation and may rarely lead to shedding of cholesterol emboli.[11,12] Secondary changes in the media underlying the advanced lesion are common and result in weakening of the arterial wall, which may progress to aneurysm formation.

CELLULAR INTERACTIONS IN ATHEROSCLEROSIS

Endothelium

The endothelial cells lining the lumen of the arterial wall play a critical role in vascular biology. Substances released by endothelial cells, including endothelin and nitric oxide, regulate vascular tone by controlling the contractile state of the underlying arterial smooth muscle cells.[16] The endothelium also presents a nonthrombotic surface to circulating platelets and clotting factors[2,12,17] and actively secretes small molecules like prostacyclin and nitric oxide, which actively inhibit platelet aggregation.[12,16] Loss of these antithrombotic effects by damaged endothelium may be of central importance in thrombus formation during atherogenesis. Endothelial dysfunction is one of the earliest cellular events in atherogenesis and is likely to be induced by a wide variety of insults, including hypercholesterolemia, hypertension, and cigarette smoking.[2] Hypercholesterolemia induces arterial endothelial cells to express VCAM-1,[18] a monocyte adhesion protein, and oxidized low-density lipoprotein (LDL) causes cultured endothelial cells to produce monocyte chemotactic protein 1.[19,20] Both of these molecules are likely to be important in mediating the recruitment of monocytes from the circulation into the intima. Endothelial cells form a highly impermeable barrier, which prevents the unregulated egress of blood components into the arterial wall.[21] One of the first events observed in regions of the vasculature of hypercholesterolemic animals prone to lesion formation is greatly enhanced transudation of plasma LDL into the subintimal space.[22] Low-density lipoprotein in the artery wall may convert macrophages into foam cells and promote further endothelial dysfunction.[2,23]

Monocyte-Derived Macrophages

Tissue macrophages are abundant in all stages of atherosclerotic lesion formation. Cholesteryl ester-laden macrophages are the predominant cell type in the fatty streak and represent a substantial fraction of the cells found in fibrous plaques and complex lesions.[1,4–6,10,23,24] Several biochemical pathways convert macrophages into foam cells in vitro. A cell surface protein on macrophages, the scavenger receptor, is one well-characterized pathway for the delivery of cholesterol.[23,24] Because the activity of the receptor is not regulated by cellular sterol stores, macrophages exposed to lipoproteins that bind to the scavenger receptor rapidly accumulate cholesteryl ester. Macrophages are also able to ingest particulate matter such as microorganisms by phagocytosis.[25] Phagocytosis of aggregated lipoproteins induces macrophage foam cell formation in vitro, suggesting that lipid-rich particulate material might stimulate tissue macrophages to accumulate cholesteryl ester. Lipoproteins complexed with arterial wall proteoglycans or digested with phospholipase C are taken up by this pathway.[26,27]

Monocytes and macrophages produce an extraordinary number of secretory products with potential roles in atherogenesis.[28–30] Cytokines and chemoattractant factors generated by phagocytes may be a major stimulus for the recruitment of smooth muscle cells into the intima.[29] The subsequent proliferation of smooth muscle cells, a key factor in the expansion of the fibrous plaque, may also be promoted by macrophage-derived growth factor.[29] Proteases released by phagocytes may predispose fibrous plaques and advanced lesions to rupture.[31] Plaque disruption exposes thrombotic material and releases potent procoagulants from the necrotic core, promoting acute thrombosis and tissue infarction.[11,12] Activated monocytes and macrophages employ a membrane-associated NADPH oxidase to synthesize superoxide, the one electron reduced form of molecular oxygen.[32,33] Superoxide breaks down to form hydrogen peroxide: in the presence of redox catalysts, hydrogen peroxide and superoxide can generate the hydroxyl radical.[34] Superoxide also reacts with nitric oxide to form another potent oxidant, peroxynitrite.[35] Reactive oxygen species generated by cells may oxidatively damage lipids and proteins in the artery wall.[36] One important target for oxidation may be LDL. Oxidized LDL is toxic to cultured endothelial cells and binds to the scavenger receptor on macrophages, producing foam cells in vitro.

Smooth Muscle Cells

Smooth muscle cells are the pathological hallmark of the fibrous plaque and the predominant cell type in the complex lesion.[2,6,10,29,37] The fibroproliferative response presumably involves the migration of smooth muscle cells into the intima and their subsequent entry into the cell cycle.[2,29,37,38] All of the cells found in the atherosclerotic lesion, including endothelial cells, monocytes, macrophages, and T lymphocytes, as well as smooth muscle cells themselves, can secrete growth factors that cause smooth muscle cells to proliferate.[2,29,30] Cytokines released by macrophages, such as interleukin-1 and tumor necrosis factor, induce the gene expression and protein synthesis of platelet-derived growth factor (PDGF) by endothelial cells.[29,39] Platelet-derived growth factor is a potent chemoattractant and mitogen for smooth muscle cells and may play a critical role in the fibroproliferative response of the injured artery wall.[29,37,39,40] The entry of intimal smooth muscle cells into the cell cycle may thus involve the release of a multitude of different growth factors and cytokines. It is noteworthy that PDGF, basic fibroblast growth factor, interleukin-1, and tumor necrosis factor are present in atherosclerotic lesions but are absent in normal arterial tissue.[29]

The matrix of extracellular connective tissue secreted by smooth muscle cells also makes an important contribution to the fibroproliferative response and lesion expansion.[2,8,10,29,30] It has been proposed that arterial smooth muscle cells exhibit two phenotypes termed *contractile* and *synthetic*.[41,42] Cultured smooth muscle cells in the contractile state demonstrate abundant myofilaments and respond to vasoactive factors, such as nitric oxide and endothelin. Cultured smooth muscle cells in the synthetic state exhibit large numbers of ribosomes and a profuse endoplasmic reticulum; this phenotype favors the production of large quantities of extracellular matrix. Conversion of smooth muscle cells from the contractile to the synthetic state may promote the secretion of connective tissue and impair the response of the vasculature to vasoactive agents. Growth factors may also be of critical regulatory importance in the production of the extracellular matrix.[29,30,41,42] For example, transforming growth factor β is a potent agonist for connective tissue synthesis by arterial smooth muscle cells.[43]

Lipid-laden smooth muscle cells are often observed in atherosclerotic lesions,[6,8,29,30] but the pathways that might lead to cholesteryl ester accumulation by this cell type are poorly understood. One mechanism may involve the uptake of lipids released by macrophage foam cells.[44] Scavenger receptors, which recognize modified forms of lipoproteins, are also present in small numbers on the cell surface of cultured smooth muscle cells.[45] Activation of the smooth muscle cells with certain agonists, such as the tumor promoter, phorbol ester, dramatically increases the expression of scavenger receptors. If similar activation events take place in vivo, the uptake of modified lipoproteins might also be involved in the formation of lipid-laden smooth muscle cells.

T Lymphocytes

Fatty streaks, fibrous plaques, and advanced atherosclerotic lesions all exhibit significant numbers of T lymphocytes, suggesting that cellular immune mechanisms play a role in atherosclerosis.[6,46,47] T cells are predominantly localized in the shoulder region and fibrous cap of atherosclerotic lesions. Both helper/inducer ($CD4^+$) and cytotoxic/suppressor ($CD8^+$) T lymphocytes are present, but few B lymphocytes and neutrophils are detectable.[46–48] Many of the T lymphocytes and smooth muscle cells in atherosclerotic plaques express class II (HLA-DR and HLA-DQ) antigens,[48] suggesting the cells are in an immunologically activated state. T cells and smooth muscle cells in normal arterial wall tissue do not exhibit class II molecules.[48] These observations raise the possibility that antigen presentation and delayed type hypersensitivity reactions are taking place in atherosclerosis.

Immune mechanisms are also likely to be involved in the arterial disease observed in transplanted human hearts undergoing rejection. Lymphocytes and macrophages are found in extraordinary numbers in such arteries.[49,50] The underlying pathology, however, is quite different from that of atherosclerosis. Vascular disease in cardiac transplantation is characterized by extensive intimal smooth muscle cell proliferation involving the entire circumference of the artery.[49] Foam cells are uncommon in these lesions. In contrast, the classic atherosclerotic lesion is eccentric, and cholesteryl ester-laden foam cells are abundant. The expression of class II antigens also differs: the endothelial cells in rejected arteries, but not in common atherosclerosis, strongly express HLA-DR molecules.[50] The difference in the pathological appearance of the two disorders suggests that the underlying immunological processes are distinct.

THEORIES OF ATHEROGENESIS

A number of hypotheses have been advanced to explain certain of the morphological and biochemical events in atherosclerosis. Three theories, which appear to account for many of the clinically relevant aspects of vascular disease, are reviewed.

Response to Injury

The hypothesis that endothelial injury is the trigger for atherosclerosis was first proposed by Ross and Glomset.[2,29,51] Originally, it was suggested that the initial vascular insult was frank endothelial denudation.[51] Subsequent studies in animals and humans have shown that the endothelium remains intact until late in the evolution of the complicated lesion, raising the possibility that more subtle forms of endothelial dysfunction initiate the atherogenic response.[12,29] Manifestations of injury may include the expression of monocyte chemoattractant and adhesion proteins by endothelial cells, which may be of critical importance in recruiting monocytes into the intima during lesion formation.[18–20,29,30] Increased transudation of lipoproteins into the intima may be another reflection of endothelial cell injury.[21,22,29] Endothelial cells, monocytes, macrophages, and smooth muscle cells oxidize LDL.[52] Minimally oxidized LDL induces the synthesis of monocyte chemotactic protein 1 by endothelial cells,[19,20] and extensively oxidized LDL is cytotoxic.[52] Lesion formation may thus involve a destructive cycle of endothelial dysfunction, expression of monocyte adhesion and chemoattractant molecules, and enhanced entry of monocytes and LDL into the artery wall, followed by the conversion of LDL to an atherogenic form, producing further injury of the endothelium.

Numerous factors in addition to hypercholesterolemia have been suggested to mediate endothelial injury, including cigarette smoke, hyperglycemia, and shear stress related to flow disturbance or hypertension (Figure 6–2).[2,29] A well-characterized insult results from the amino acid homocysteine,[2,53–55] which can exist in either the reduced (homocysteine) or disulfide (homocystine) state. Patients with the genetic disorder homocystinuria, who generally suffer from a deficiency of the enzyme cystathionine synthase, have massive elevations in plasma homocystine levels and frequently suffer from premature coronary artery disease.[53] In these patients, thrombosis in both the venous and arterial system is common, suggesting that homocysteine or another intermediate involved in homocysteine metabolism might damage the endothelium.[53,54] Nonhuman primates continuously infused with homocysteine develop endothelial denudation, accelerated platelet turnover, and vascular thrombosis, strongly suggesting that the amino acid itself is the toxic agent.[55] The underlying mechanism for injury may involve the generation of oxygen-, carbon-, or sulfur-centered free radicals, alterations in the intracellular redox status, or platelet activation. Clinical studies indicate that elevated levels of plasma homocystine are an independent risk factor for atherosclerosis even in subjects who are not suffering from the homozygous form of homocystinuria.[56–58]

One important implication of the injury hypothesis is that the progression of atherosclerosis depends on continual endothelial dysfunction.[2,29] This suggests that the removal of the factors mediating endothelial injury might halt or reverse the atherosclerotic process. Alternatively, the pathological events induced by endothelial injury might be targets for therapeutic intervention. Consonant with this idea, a polyclonal antibody to PDGF blocked smooth muscle cell proliferation in an experimental model for angioplasty-induced vascular disease.[40] Similarly, antisense oligonucleotides to the protooncogene *c-myb,* which interrupt smooth muscle proliferation in cultured smooth muscle cells, have been shown to suppress smooth muscle cell accumulation in injured carotid arteries in rats.[59]

Lipoprotein Oxidation

Clinical, genetic, and epidemiological studies have convincingly shown that hypercholesterolemia is a ma-

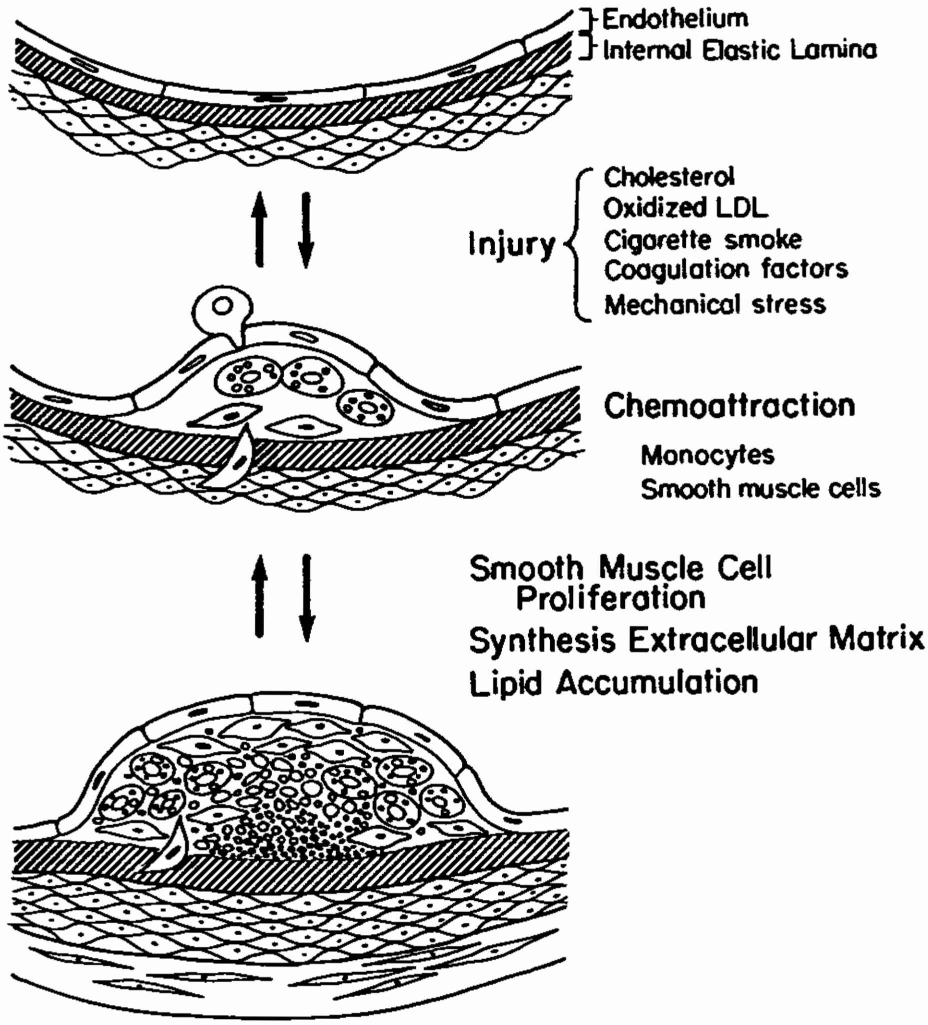


Figure 6–2. The response to injury hypothesis. This hypothesis proposes that subtle forms of endothelial dysfunction, perhaps induced by hypercholesterolemia, hypertension or other forms of vascular insult, triggers the atherogenic response. Among the earliest events are increased infiltration of circulating monocytes and lipoproteins into the intima. Subsequently, smooth muscle cells migrate into the lesion and begin to proliferate, converting the fatty streak into the clinically significant fibrous plaque. Secretion of growth factors by macrophages, endothelial cells, and smooth muscle cells may be especially important in this stage of the disease process. Lipid accumulation and synthesis of extracellular matrix, perhaps in response to cytokines such as transforming growth factor β, further contribute to lesion expansion. This hypothesis makes the important prediction that atherosclerotic lesions are potentially reversible if the source of endothelial injury is removed or ameliorated.

jor risk factor for atherosclerosis.[60,61] Elevated levels of LDL, the major carrier of blood cholesterol, are an even better predictor of atherosclerotic risk.[60,61] A key biochemical feature of atherosclerosis is the accumulation of cholesterol and cholesteryl ester in arterial wall macrophages.[4,5,24] It is thus paradoxical that high concentrations of LDL fail to convert cultured macrophages into foam cells, suggesting that native LDL is not involved in this process.[23,52] In contrast, when the major protein of LDL is chemically altered by acetylation of lysine residues, the modified lipoprotein becomes recognized by the scavenger receptor on macrophages.[23] Acetylated LDL rapidly causes macrophages to assume the morphological and biochemical features of foam cells.[23,24] This discovery led to the proposal that LDL must be modified to participate in atherogenesis.[23] Endothelial cells, smooth muscle cells, and monocyte-derived macrophages alter LDL to a form that is internalized via the macrophage scavenger receptor, suggesting that cells of the arterial wall might convert LDL to an atherogenic particle.[52,62] LDL modification by cultured cells requires iron or copper, is inhibited by metal chelators and antioxidants, and involves lipid peroxidation.[63–65] These results indicate that the underlying mechanism for LDL modification in vitro involves oxidative damage. In addition to promoting the accumulation of cholesteryl esters by macrophages, oxidized LDL has been shown to exert a multitude of potentially atherogenic effects on cultured endothelial cells, including cytotoxicity, induction of monocyte chemoattractant protein(s) and factors, and expression of prothrombotic molecules.[66]

Several lines of investigation support the hypothesis that oxidized LDL plays a role in vascular disease. Monoclonal and polyclonal antibodies that recognize specific protein-bound products of lipid peroxidation are located along with LDL in atherosclerotic lesions.[67,68] Lipoproteins with properties suggestive of oxidative modification have been isolated from human and animal atherosclerotic tissue.[69,70] Several chemically unrelated antioxidants that are potent inhibitors of LDL oxidation in vitro[71,72] slow the progression of atherosclerosis in animal models for hypercholesterolemia.[72–74] Recent epidemiological studies suggest that the dietary intake of vitamin E, a lipid-soluble molecule with antioxidant properties, is inversely associated with the risk for coronary artery disease.[75] These results collectively indicate that lipoprotein oxidation may be important in the progression of atherosclerosis by several different mechanisms (Figure 6–3).

The physiologically relevant pathways for lipoprotein oxidation have yet to be identified, but in vitro studies have suggested a number of potential mechanisms. Reactive oxygen species damage lipids and proteins by reactions that often require metal ions.[34,76] Phagocytic white blood cells employ two such species, superoxide and hydrogen peroxide, to destroy invading pathogens.[32,33] Monocytes and monocyte-derived macrophages are prominent components of atherosclerotic lesions. Monocytes activated with phorbol ester and opsonized zymosan oxidize LDL in vitro by hydrogen peroxide and superoxide-dependent reactions.[77,78] Reactive oxygen species may also play a role in LDL oxidation by nonphagocytic cells. Cultured endothelial cells and smooth muscle cells generate extracellular superoxide,[36,79,80] and superoxide dismutase, a scavenger of superoxide, inhibits LDL oxidation.[36,79,80] Superoxide production and LDL modification by smooth muscle cells are both L-cystine-dependent.[79] Thiols are known to autoxidize with the

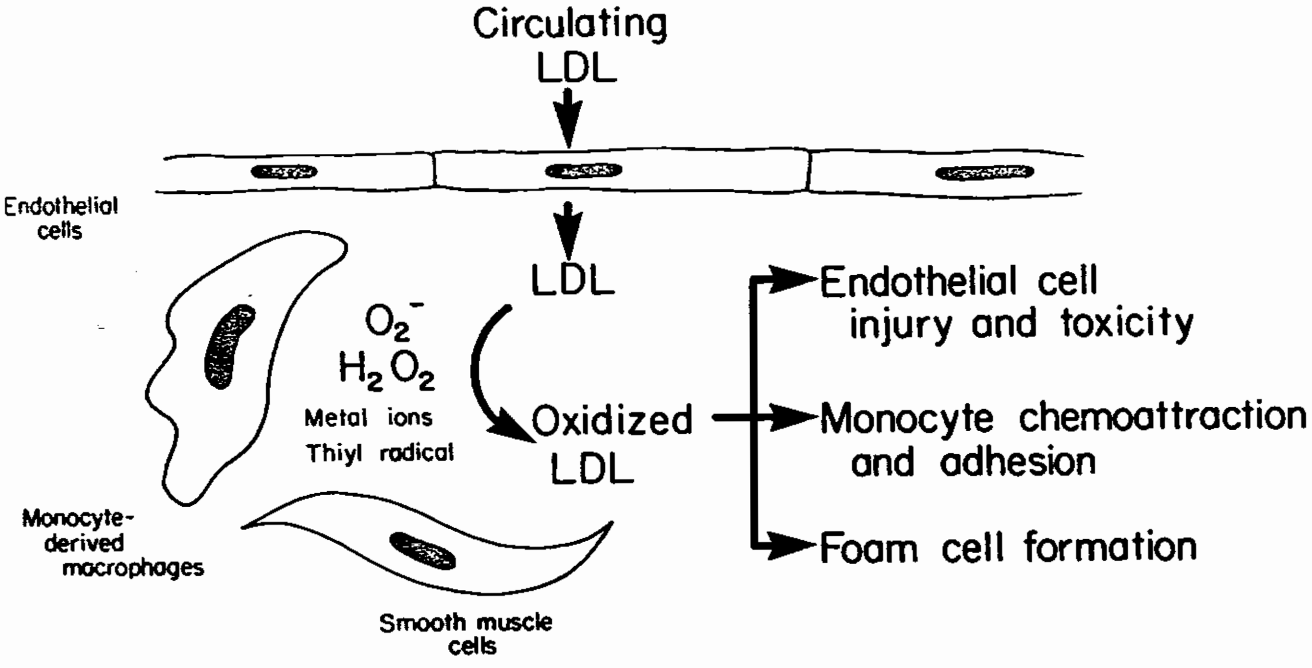


Figure 6–3. Oxidized lipoproteins and atherogenesis. Hypercholesterolemia or other forms of endothelial cell injury may cause LDL to enter the artery wall at an increased rate in regions of the artery wall prone to lesion formation. The oxidation hypothesis proposes that LDL in the intima is then converted into an atherogenic particle by reaction pathways that may involve cell-generated reactive oxygen species, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), as well as metal ions and thiyl radical. Oxidatively modified LDL has been shown to initiate a number of events in vitro that may be important in lesion formation and progression. Thus, minimally oxidized LDL induces the expression of monocyte chemoattractant molecules on endothelial cells, which may be important in recruiting circulating monocytes into the intima. Extensively oxidized LDL converts macrophages into lipid-laden foam cells, is cytotoxic, and may mediate other forms of cellular injury. The oxidation hypothesis suggests that the initiation or progression of atherosclerosis might be retarded by interventions designed to block the free radical damage of lipoproteins.

production of superoxide, hydrogen peroxide, and thiyl radical.[81–83] Because all of these reactive intermediates initiate lipid peroxidation under certain conditions, these observations raise the possibility that cultured cells oxidize LDL by secreting thiols. Indeed, endothelial cells and macrophages have been shown to generate extracellular thiol by an L-cystine-dependent pathway,[84] and thiols oxidize LDL in the absence of cells.[85,86] It is noteworthy that elevated plasma levels of homocysteine are strongly associated with an increased risk of coronary artery disease and vascular thrombosis.[53–59] A number of other potent oxidants, including hydroxyl radical[87] and peroxynitrite derived from nitric oxide,[88] have recently been shown to mediate LDL oxidation in vitro.

The oxidation hypothesis suggests that LDL is necessary but not sufficient for atherogenesis.[52] By understanding the links between lipoprotein oxidation and vascular disease, it may become possible to interrupt the atherogenic process itself. A particularly exciting possibility is that naturally occurring dietary antioxidants, like vitamin E, beta-carotene, and ascorbic acid, may be useful in the prevention of atherosclerotic vascular disease.[52,75,89]

Plaque Disruption and Thrombosis

Clot formation and fibrotic organization of the thrombus were proposed to participate in the progression of atherosclerosis by von Rokitansky over 100 years ago.[90] Pathological studies of patients who have died of noncardiac disease reveal that fissures with overlying fibrin–platelet thrombi are common in atherosclerotic lesions.[91,92] This suggests that repeated rounds of fissuring, thrombus formation, and fibrosis contribute to plaque formation.[11,12,91–94] Indeed, the morphology of complicated atherosclerotic lesions often shows organized thrombus incorporated into the plaque.[94] The release of PDGF and other growth factors by activated platelets might augment lesion expansion by stimulating smooth muscle cell proliferation and the synthesis of extracellular connective tissue.[12,29]

Angiographic and pathological studies[94–96] have shown that ulceration of complex lesions and plaque disruption precipitate acute coronary artery events (Figure 6–4). It has only recently been appreciated that less severe lesions may make an important contribution to sudden death, acute myocardial infarction, and unstable angina.[11,12,91–96] Sequential angiographic investigations show that the majority of coronary artery lesions that progress rapidly are small (less than 50% stenosis). Moreover, small to modest-sized lesions (less than 70% stenosis) appear to account for most cases of acute myocardial infarctions.[97,98] These observations do not imply that patients with severe coronary artery stenosis have a better prognosis than those with less advanced lesions, because moderate lesions are more frequent in patients with advanced lesions, and severe lesions are more likely to progress to complete obstruction. Because small to modest-sized lesions are far more common than advanced lesions, however, lesions of intermediate size present the greatest absolute risk for thrombosis. In addition, because smaller lesions have a minimal effect on coronary perfusion, there is little development of collateral circulation, and the risk of acute infarction is much greater when thrombosis occurs.[11,12,96,99] These observations have lead to the proposal that unstable angina is more commonly due to progressive obstruction of a high-grade lesion with well-developed collateral circulation.[11,12,96,99,100] In contrast, acute myocardial infarction and sudden death generally involve vascular lesions of intermediate size.[11,12,96,99,100] Macrophage-rich plaques may be especially prone to disruption, perhaps due to the decreased mechanical integrity of the fibrous cap.[12,100] Plaque disruption and formation of a platelet–fibrin thrombus occurs when increased shear force subjects these lesions to stress that exceeds the strength of the fibrous cap (see Figure 6–4). In autopsy studies, tearing of the tissue between the fibrous cap and normal artery is common, and hemorrhage into the lesion is often observed.[91,93]

Aggressive treatment to lower serum lipid concentrations in patients with known coronary artery disease has recently been shown to reduce dramatically the incidence of acute coronary events[14] as well as to prevent the progression of vascular disease.[13–15] The reduction in cardiovascular morbidity and mortality is greater than can be accounted for by the statistically significant but modest decrease in the extent of coronary artery disease. Perhaps the marked decline in vascular events reflects alterations in the lipid composition or content of atherosclerotic lesions. It is known that such lipids, which may be released when plaques are disrupted, are intensely thrombotic.[11,12,100] Alternatively, the susceptibility of plaques to rupture may have been decreased by removal of lipid from the necrotic core, by changes in the cellular and extracellular makeup of the fibrous cap, or by decreased flow disturbance and shear stress.[11,12,100]

These observations may have considerable implications for the therapy of atherosclerosis. Athough much emphasis has been placed on the role of lowering lipid levels in the primary prevention of atherosclerosis,[101] it is clear that it is even more important to treat hypercholesterolemia in patients with known vascular disease.[13–15,101] Indeed, many would argue that lipid levels should be lowered in all patients with coronary artery disease regardless of their absolute level of cholesterol. Another important issue is the role of antithrombotic therapy in the treatment of vascular dis-

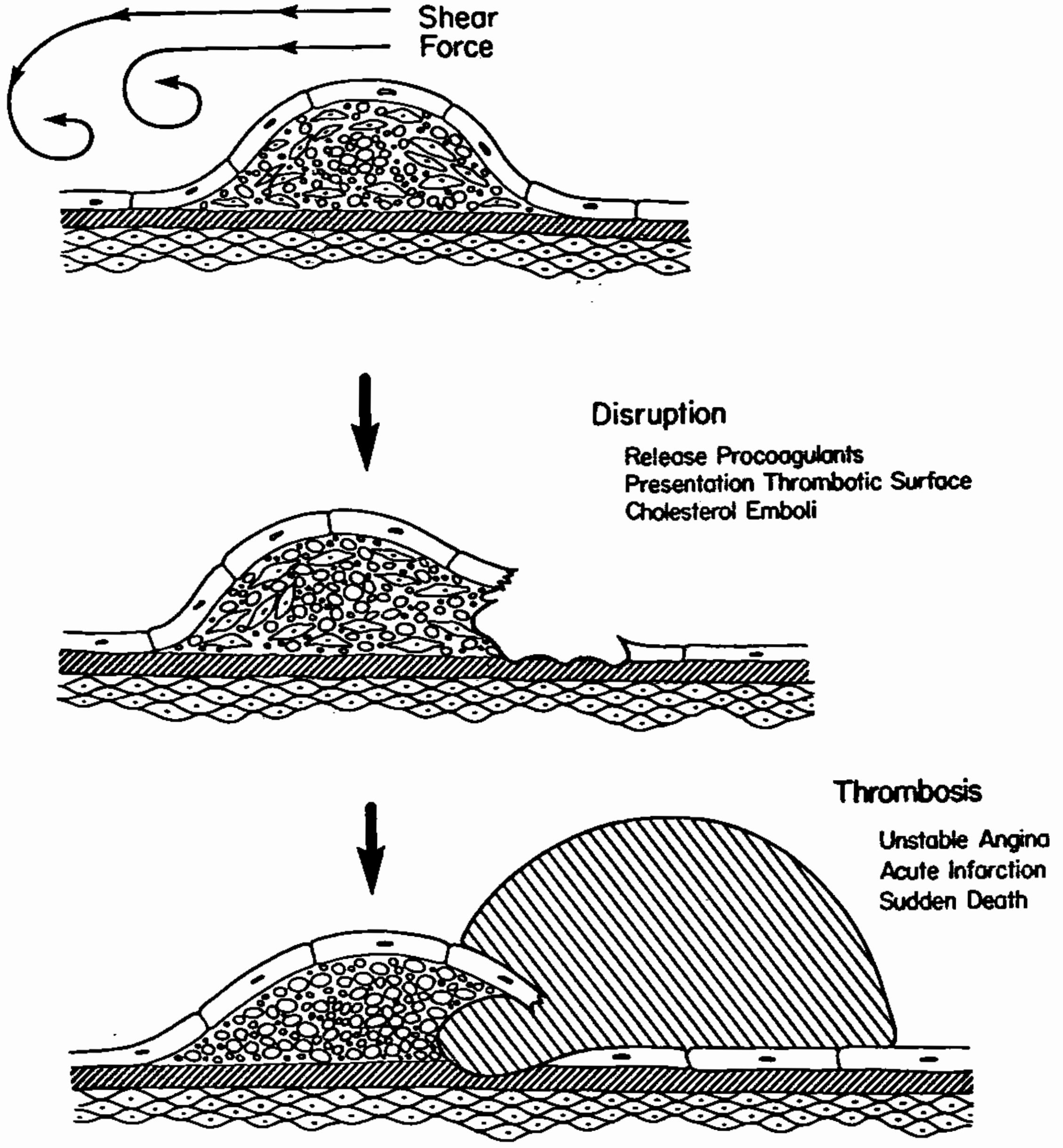


Figure 6–4. Plaque disruption and thrombosis. Pathological studies reveal that fissuring and rupture of atherosclerotic lesions are the cause of acute arterial obstruction in many patients. Shear stress induced by arterial flow induces tension that is most prominent in the upstream shoulder region of the atherosclerotic plaque. Because of decreased mechanical strength in lesions rich in lipids or macrophages, this region may be especially prone to fissuring. Rupture of fibrous plaques probably accounts for the majority of acute events in coronary artery disease. Complicated atherosclerotic lesions that are heavily calcified and fibrotic may be less susceptible to disruption. Occlusion caused by lesion expansion or acute thrombosis secondary to endothelial denudation may be more important in the progression of advanced lesions.

ease. Aspirin is known to be effective in the primary prevention of acute myocardial infarction, to decrease the risk of cardiac events after coronary artery bypass surgery, and to prevent stroke after a transient ischemic attack.[11,12,100] Aspirin interferes with thrombosis by blocking the synthesis of platelet thromboxane A_2, a potent platelet-aggregating agent: in contrast, endothelial generation of the vasodilator, prostacyclin, remains intact.[12] Other pathways for platelet aggregation and clot formation still function in patients treated with aspirin. Combination therapy designed to block additional components of the coagulation cascade may thus offer promise in the prevention of both the acute and chronic complications of atherosclerosis.[12]

FUTURE DIRECTIONS

The oxidation hypothesis has raised the possibility that therapies designed to inhibit free radical damage to lipids and proteins may offer a mechanism for retarding the progression of vascular disease.[52] Another attractive target for intervention may be growth factors that stimulate smooth muscle proliferation and the secretion of connective tissue matrix.[29] The notion that many coronary events are caused by acute thrombosis of moderately stenotic lesions has important implications for use of antithrombotic agents in the primary and secondary prevention of atherosclerosis.[11,12,100] It is reasonable to believe, therefore, that further progress in our understanding of the molecular mechanisms involved in vascular disease may ultimately provide the means of preventing atherosclerosis and its devastating clinical sequelae.

REFERENCES

1. Stary HC, Blankenhorn DH, Chandler B, et al. A definition of the intima of human arteries and its atherosclerosis-prone regions. *Arterio Thromb.* 1992; 12:120–134.
2. Ross, R. The pathogenesis of atherosclerosis—An update. *N Engl J Med.* 1986; 314:488–500.
3. Geer JC, McGill HC, Strong JP. The fine structure of human atherosclerotic lesions. *Am J Pathol.* 1968; 33:263–287.
4. Shio H, Farquhar MG, DeDuve C. Lysosomes of the arterial wall. *Am J Pathol.* 1974; 767:1–16.
5. Gerrity RG. The role of the monocyte in atherogenesis. *Am J Pathol.* 1981; 103:181–190.
6. Gown AM, Tsukada T, Ross R. Human atherosclerosis. *Am J Pathol.* 1986; 125:191–207.
7. McGill HC, Geer JC, Strong JP. *Natural History of Human Atherosclerotic Lesions.* Academic Press, New York, NY, p 39–65, 1963.
8. Small DM. Progression and regression of atherosclerotic lesions. *Arteriosclerosis.* 1988; 8:103–129.
9. McGill HC Jr. The geographic pathology of atherosclerosis. *Lab Invest.* 1968; 18:463–653.
10. World Health Organization: Classification of atherosclerotic lesions. *WHO Tech Rep Ser.* 1985; 143:1–20.
11. Epstein SE, Quyyuni AA, Bonow RO. Sudden cardiac death without warning. *N Engl J Med.* 1989; 5:320–324.
12. Fuster V, Badimon L, Badimon JJ, et al. The pathogenesis of coronary artery disease and the acute coronary syndromes. *N Engl J Med.* 1992; 4:242–250.
13. Blankenhorn DH, Nessim SA, Johnson RL, et al. Beneficial effects of combined-colestipolniacin therapy on coronary atherosclerosis and coronary venous bypass grafts. *JAMA.* 1987; 257:3233–3240.
14. Brown G, Albers JS, Fisher LD, et al. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. *N Engl J Med.* 1990; 19:1289–1298.
15. Kane JP, Malloy MJ, Ports TA, et al. Regression of coronary atherosclerosis during treatment of familial hypercholesterolemia with combined drug regimens. *JAMA.* 1990; 264:3007–3012.
16. Gryglewski RJ, Botting RM, Vane JR. Mediators produced by the endothelial cell. *Hypertension.* 1988; 12:530–548.
17. Thorgersson G, Robertson AL. The vascular endothelium-pathobiological significance. *Am J Pathol.* 1978; 93:804–848.
18. Cybulski MI, Gimbrone Jr MA. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* 1991; 251:788–791.
19. Berliner JA, Territo MC, Sevanian A, et al. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. *J Clin Invest.* 1990; 85:1260–1266.
20. Cushing SD, Berliner JA, Valente AJ, et al. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci USA.* 1990; 87:5134–5138.
21. Smith EB. The relationship between plasma and tissue lipids in human atherosclerosis. *Adv Lipid Res.* 1974; 12:1–49.
22. Schwenke DC, Carew TE. Quantification in vivo of increased LDL content and rate of LDL degradation in normal rabbit aorta occurring at sites susceptible to early atherosclerotic lesions. *Circ Res.* 1988; 62:699–710.
23. Goldstein JL, Ho YK, Basu SK, et al. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein producing massive cholesterol deposition. *Proc Natl Acad Sci USA.* 1979; 76:333–337.
24. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. *Ann Rev Biochem.* 1983; 52:223–261.
25. Silverstein SC, Steinman RM, Cohn ZA. Endocytosis. *Ann Rev Biochem.* 1977; 46:669–772.
26. Falcone DJ, Mated N, Shio H, et al. Lipoprotein-heparin-fibronectin-denatured collagen complexes enhance cholesteryl ester accumulation in macrophages. *J Cell Biol.* 1984; 99:1266–1274.
27. Suits AG, Chait A, Aviram M, et al. Phagocytosis of aggregated lipoprotein by macrophages: Low density lipoprotein receptor-dependent foam cell formation. *Proc Natl Acad Sci USA.* 1986; 86:2713–2717.
28. Adams DO, Hamilton TA. The cell biology of macrophage activation. *Ann Rev Immunol.* 1984; 2:283–318.
29. Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature.* 1993; 362:801–809.
30. Schwartz CJ, Valente AJ, Sprague EA, et al. The pathogenesis of atherosclerosis. *Clin Cardiol.* 1991; 14(suppl. I):I-1–I-16.
31. Henney A, Wakeley P, Davies M. Localization of stromelysin gene expression in atherosclerotic plaques by

in situ hybridization. *Proc Natl Acad Sci USA.* 1991; 88:8154–8158.

32. Babior, BM. Oxygen-dependent microbial killing by phagocytes. *New Engl J Med.* 1978; 298:659–663.
33. Segal AW. The electron transport chain of the microbicidal oxidase of phagocytic cells and its involvement in the molecular pathology of chronic granulomatous disease. *J Clin Invest.* 1989; 83:1785–1793.
34. Cohen G. The Fenton reaction In: *CRC Handbook of Methods for Oxygen Radical Research.* Boca Raton, Fl: CRC Press Inc. 1985; pp 55–64.
35. Beckman JS, Beckman TW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA.* 1990; 87:1620–1624.
36. Heinecke JW, Baker L, Rosen H, et al. Superoxide-mediated modification of low density lipoprotein by arterial smooth muscle cells. *J Clin Invest.* 1986; 77:757–761.
37. Dilley RJ, McGeachie JK, Prendergast FJ. A review of the proliferative behavior, morphology, and phenotypes of vascular smooth muscle. *Atherosclerosis.* 1987; 63:99–107.
38. Clowes AW, Clowes MM, Fingerle J, et al. Regulation of smooth muscle cell growth in injured arteries. *J Cardiovasc Pharmacol.* 1989; 14(suppl 6):S12–S15.
39. Libby P, Warner SJC, Salomon RN, et al. Production of platelet-derived growth factor-like mitogen by smooth muscle cells from human atheroma. *N Engl J Med.* 1988; 318:1493–1498.
40. Ferns GA, Raines EW, Sprugel KH, et al. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. *Science* 1991; 253:1129–1132.
41. Campbell GR, Chamley-Campbell, JH. The cellular pathobiology of atherosclerosis. *Pathology.* 1981; 13:423–440.
42. Chamley-Campbell J, Campbell GR, Ross R. The smooth muscle cell in culture. *Physiol Rev.* 1979; 59:1–61.
43. Sporn MB, Roberts AB, Wakefield LM, et al. Transforming growth factor β. *J Cell Biol.* 1987; 105:1039–1045.
44. Woflbauer G, Glick JM, Minor LK, et al. Development of the smooth muscle foam cell: Uptake of macrophage lipid inclusions. *Proc Natl Acad Sci.* 1986; 83:7760–7764.
45. Pitas RE. Expression of the acetyl low density lipoprotein receptor by rabbit fibroblasts and smooth muscle cells. *J Biol Chem.* 1990; 265:12722–12727.
46. Emorson EE, Robertson AL. T lymphocytes in aortic and coronary intimas. *Am J. Pathol.* 1988; 130:369–376.
47. Jonasson L, Holm J, Skalls O, et al. Regional accumulation of T cells, macrophages, and smooth muscle cells in human atherosclerotic plaque. *Arteriosclerosis.* 1986; 6:131–138.
48. Hansson GK, Holm J, Jonasson L. Detection of activated T lymphocytes in human atherosclerotic plaque. *Am J Pathol.* 1989; 135:169–175.
49. Billingham ME. Cardiac transplant atherosclerosis. *Transplant Proc.* 1987; 19:19–25.
50. Saloman RN, Hughes CCW, Schoen FJ, et al. Human coronary transplantation-associated artheriosclerosis. *Am J Pathol.* 1991; 138:791–798.
51. Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell. *Science.* 1973; 180:1332–1339.
52. Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. *New Engl J Med.* 1989; 320:915–924.
53. Mudd SH, Levy HL, Skovby F. *The Metabolic Basis of Inherited Disease.* McGraw-Hill, Inc. New York, NY, 1989. pp 693–743.
54. Harker LA, Slichter SJ, Scott CR, et al. Homocystinemia. *New Engl J Med.* 1974; 291:537–543.
55. Harker LA, Ross R, Slichter SJ, et al. Homocystine-induced artheriosclerosis. *J Clin Invest.* 1976; 58:731–741.
56. Brattstrom LE, Hardebo JE, Hultberg BL. Moderate homocysteinemia—A possible risk factor for arteriosclerotic cerebrovascular disease. *Stroke.* 1984; 6:1012–1016.
57. Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: An independent risk factor for vascular disease. *New Engl J Med.* 1991; 324:1149–1155.
58. Boers GHJ, Smals AGH, Trijbels FJM, et al. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *N Engl J Med.* 1985; 313:709–715.
59. Simons M, Edelman ER, Dekeyser J, et al. Antisense *c-myb* oligonucleotides inhibit intimal arteial smooth muscle cell accumulation in vivo. *Nature.* 1992; 359:67–70.
60. Kannel WB, Castelli WP, Gordon T, et al. Serum cholesterol, lipoproteins, and the risk of coronary heart disease: The Framingham study. *Ann Intern Med.* 1971, 74:1–12.
61. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science.* 1986; 232:34–47.
62. Henriksen T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: Recognition by receptors for acetylated low density lipoproteins. *Proc Natl Acad Sci USA.* 1981; 78:6499–6503.
63. Heinecke JW, Rosen H, Chait A. Iron and copper promote modification of low density lipoprotein by human arterial smooth muscle cells in culture. *J Clin Invest.* 1984; 74:1890–1894.
64. Steinbrecher VP, Parthasarathy S, Leake DS, et al. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci USA.* 1984; 81:3883–3887.
65. Morel DW, DiCorletto PE, Chisolm GM. Endothelial and smooth muscle cells alter low density lipoprotein in vitro by free radical oxidation. *Arteriosclerosis.* 1984; 4:357–364.
66. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest.* 1991; 88:1785–1792.

67. Haberland ME, Fong D, Cheng L. Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science.* 1988; 24:215–218.
68. Rosenfeld ME, Palinski W, Yla-Herttuala S, et al. Distribution of oxidation specific lipid–protein adducts and apolipoprotein B in atherosclerotic lesions of varying severity from WHHL rabbits. *Arteriosclerosis.* 1990; 10:336–349.
69. Daugherty A, Zweifel BS, Sobel BE, et al. Isolation of low density lipoprotein from atherosclerotic vascular tissue of Watanabe heritable hyperlipidemic rabbits. *Arteriosclerosis.* 1988; 8:768–777.
70. Yla-Herttula 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; 84:1086–1095.
71. Parthasarathy S, Young SG, Witztum JL, et al. Probucol inhibits oxidative modification of low density lipoprotein. *J Clin Invest.* 1986; 77:641–644.
72. Sparrow CP, Doebber TW, Olszewski MS, et al. Low density lipoprotein is protected from oxidation and the progression of atherosclerosis is slowed in cholesterol-fed rabbits by the antioxidant *N, N'*-diphenyl-phenylenediamine. *J Clin Invest.* 1992; 89:1885–1891.
73. Kita T, Najano 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 USA.* 1987; 84:5928–5931.
74. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypercholesterolemic 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 USA.* 1987; 84:7725–7729.
75. Steinberg D. Antioxidant vitamins and coronary heart disease. *New Engl J Med.* 1993; 328:1487–1489.
76. Stadtman ER. Protein oxidation and aging. *Science.* 1992; 257:1220–1224.
77. Cathcart MK, Morel DW, Chisolm GM. Monocytes and neutrophils oxidize low density lipoprotein, making it cytotoxic. *J Leukocyte Biol.* 1985; 38:341–350.
78. Hiramatsu K, Rosen H, Heinecke JW, et al. Superoxide initiates oxidation of low density lipoprotein by human monocytes. *Arteriosclerosis.* 1987; 7:55–60.
79. Heinecke JW, Suzuki L, Chait A. The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J Biol Chem.* 1987; 262:10098–10103.
80. Steinbrecher VP. Role of superoxide in endothelial-cell modification of low-density lipoprotein. *Biochim Biophys Acta.* 1988; 959:20–30.
81. Packer JE. *The Radiation Chemistry of Thiols.* John Wiley & Sons, New York, NY, 1974. pp 481–517.
82. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest.* 1986; 77:1370–1376.
83. Schoneich C, Dillinger V, Bruchhausen FV, et al. Oxidation of polyunsaturated fatty acids and lipids through thiyl and sulfonyl radicals: Reaction kinetics, and influence of oxygen and structure of thiyl radicals. *Arch Biochem Biophys.* 1992; 292:456–476.
84. Sparrow CP, Olszewski J. Cellular oxidation of low density lipoprotein is caused by thiol production in media containing transition metal ions. *J Lipid Res.* 1993; 34:1219–1228.
85. Parthasarathy S. Oxidation of low-density lipoprotein by thiol compounds leads to its recognition by the acetyl-LDL receptor. *Biochim Biophys Acta.* 1987; 917:337–340.
86. Heinecke JW, Kawamura M, Suzuki L, et al. Oxidation of low density lipoprotein by thiols: Superoxide-dependent and -independent mechanisms. *J Lipid Res.* 1993; 34:2051–2061.
87. Bedwell S, Dean RT, Jessup W. The action of defined oxygen-centered radicals on human low-density lipoprotein. *Biochem J.* 1989; 262:707–712.
88. Graham A, Hogg N, Kalyanaraman B, et al. Peroxynitrite modification of low-density lipoprotein leads to recognition by the macrophage scavenger receptor. *FEBS Lett.* 1993; 330:2:181–185.
89. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci USA.* 1988; 85:9748–9752.
90. von Rokitansky C; Day GE, trans. *A Manual of Pathological Anatomy, IV.* London 1852. p 261.
91. Davies MJ, Thomas A. Thrombosis and acute coronary artery lesions in sudden cardiac ischemic death. *N Engl J Med.* 1984; 310:1137–1140.
92. Davies MJ, Woolf N, Rowles PM, et al. Morphology of the endothelium over atherosclerotic plaques in human coronary arteries. *Br Heart J.* 1988; 60:459–464.
93. Davies MJ, Bland MJ, Hangartner WR, et al. Factors influencing the presence or absence of acute coronary thrombosis in sudden ischemic death. *Eur Heart J.* 1989; 10:203–208.
94. Ridolfi RL, Hutchins GM. The relationship between coronary artery lesions and myocardial infarcts: Ulceration of atherosclerotic plaques precipitating coronary thrombosis. *Am Heart J.* 1977; 93:468–486.
95. Levin DC, Fallon JT. Significance of the angiographic morphology of localized coronary stenosis: Histopathological correlations. *Circulation.* 1982; 66:310–316.
96. Ambrose JA, Winters SL, Stern A. Angiographic morphology and the pathogenesis of unstable angina. *J Am Coll Cardiol.* 1985; 5:609–616.
97. Little WC, Constatinescu MS, Applegate RJ, et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease. *Circulation.* 1988; 78:1157–1166.
98. Ambrose JA, Tannenbaum MA, Alexopoulos D, et al. Angiographic progression of coronary artery disease and the development of myocardial infarction. *J Am Coll Cardiol.* 1988; 12:56–62.

99. Rentrop KP, Thorton JC, Felt F, et al. Determinants and protective potential of coronary arterial collaterals as assessed by an angioplasty model. *Am J Cardiol.* 1988; 61:677–684.

100. MacIsaac AI, Thomas JD, Topol EJ. Toward the quiescent coronary plaque. *J Am Coll Cardiol.* 1993; 22:1228–1241.

101. Summary of the second report of the National Cholesterol Education Panel on detection, evaluation, and treatment of high blood cholesterol in adults: Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. *JAMA.* 1992; 269:3015–3023.