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ATHEROSCLEROSIS AS AN INFLAMMATORY DISEASE: IMPLICATIONS FOR THERAPY

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1. ABSTRACT

Cardiovascular disease remains a leading cause of mortality in the United States despite the use of new pharmacologic therapy, lifestyle modifications, and different coronary interventions. Atherosclerosis represents a wide variety of pathologic lesions with different clinical impacts. In this review, we address the current understanding of the pathophysiological mechanisms underlying the development of atherosclerosis. We define atherosclerosis as a multifactorial process representing a series of molecular and cellular mechanisms and involving multiple interactions between lipid metabolism, monocyte activation, endothelial cells, cytokines and/or other intracellular metabolic pathways. We also imply that control of atherosclerosis could be achieved through therapeutic interventions at different sites of the

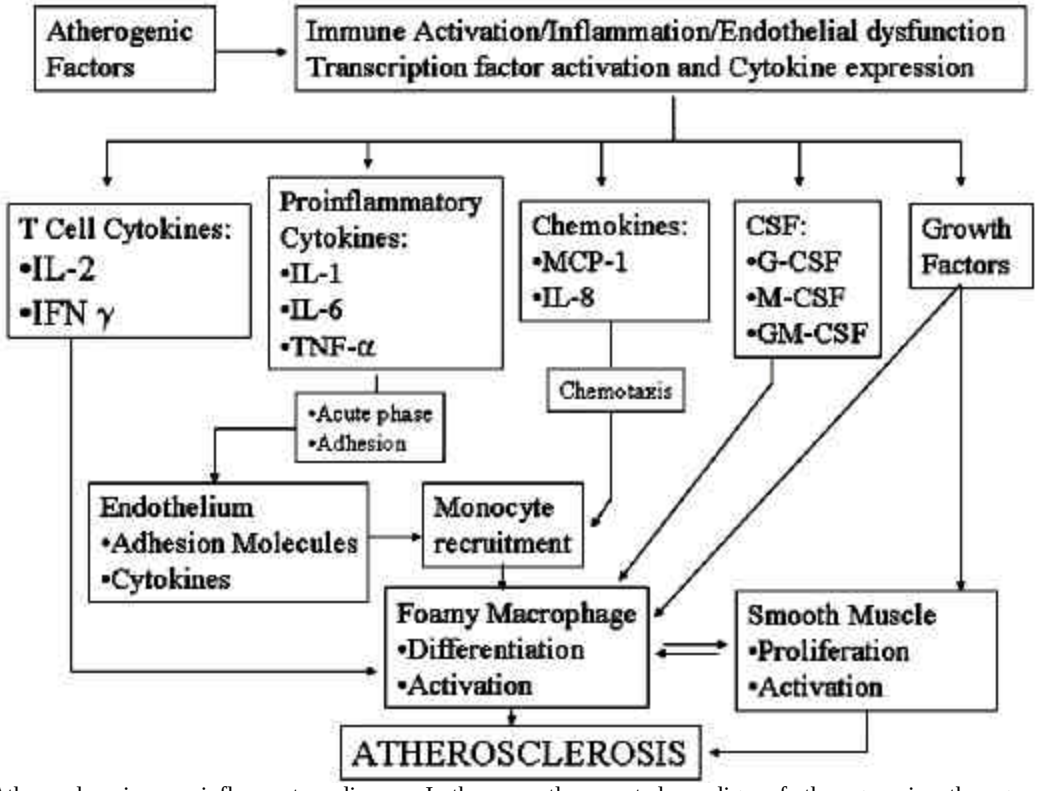

Figure 1. Atherosclerosis as an inflammatory disease. In the currently accepted paradigm of atherogenesis, atherogenetic factors such as modified LDL, tobacco smoking, hemodynamic stress, elevated homocysteine levels, and infections with Chlamydia and Cytomegalovirus, lead to endothelial dysfunction followed by the expression of various cell adhesion molecules, cytokines, and colony stimulating factors (CSF). These cytokines/factors recruit monocytes to become foam cells, activate smooth muscle proliferation, and thus, lead to atherosclerosis.

inflammatory process. Therapeutic targets could include cytokine pathways, growth factors, transcription factors, defective genes and other intracellular metabolic pathways.

2. INTRODUCTION

Atherosclerotic cardiovascular disease has a great social and economic impact worldwide. Almost fifty percent of all deaths in the developed countries and twenty-five percent of all deaths in the developing countries are attributable to cardiovascular disease. Recently, the American Heart Association estimated that in the United States more than 12 million people have coronary artery disease and that there are 650,000 new myocardial infarctions and 450,000 recurrent myocardial infarctions each year. By the year 2020, it is expected that coronary artery disease will be the world's number one cause of death followed by infectious disease.

The American Heart Association formulated a histological classification of atherosclerosis (1). The initial or type I lesion consists of intimal thickening with aberrant smooth muscle cells and isolated macrophages. Type II lesion or fatty streak consists of intracellular accumulation of lipids with foam cell formation. Fatty streaks then progress to intermediate or type III lesions, which consist of continued accumulation of lipids with small pools of extracellular lipids. The extracellular lipid pools then coalesce into a lipid core with a thin fibrous cap forming a type IV lesion or atheroma. With smooth muscle proliferation and collagen deposition, the atheroma becomes a type V lesion or fibroatheroma. Type VI or a complicated lesion will result when surface defects and subsequent hemorrhage or thrombus occur in the fibroatheroma (1).

The term atheroma was first used to describe an arterial plaque with a yellow core. Atheroma was thereafter considered an inert collection of cholesterol, calcium, and fibrous tissue; however, atherosclerosis is much more than accumulation of lipids within arteries. Presently, there is strong evidence defining atherosclerosis as an inflammatory/reparative disease. In fact, it is a slow inflammatory process that starts at a relatively young age, and the fatty streak lesion that is common in infants and young children is an example of inflammatory lesion involving T-lymphocytes and monocyte-derived macrophages (figure 1). Inflammation at affected sites continues throughout life and involves cytokines, and other growth factors and immune cell mediators. These factors act locally in an autocrine/paracrine manner regulating cell migration,

Table 1. The roles of selected cells in inflammation and atherogenesis

Cell Types	Role in Inflammation and Atherogenesis
Endothelial Cells	• Production of cytokines (IL-1[1], IL-5, IL-6, IL-8, IL-11, IL-15, G-CSF, M-CSF, GM-CSF, MCP-1, RANTES, and GRO-alpha) • Expression of selectins and adhesion molecules (ICAM-1 and VCAM-1)
T-lymphocytes	• Production of cytokines (IL-3, IFN-gamma, TNF-alpha, and VEGF) • Induction of smooth muscle cell migration and proliferation
Monocytes/Macrophages	• Formation of macrophage-derived foam cells • Production of coagulation factors and metalloproteinases • Contribution to atheroma formation and progression
Neutrophils	• Production of cytokines • Release of elastase • Production of O_2-derived free radicals
Mast Cells	• Expression of pro-inflammatory cytokines and chemokines (IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, MCP-1, 2, and 3, MIP-1alpha, RANTES, TGF-beta, VEGF, PDGF-A and GM-CSF) • Activation of metalloproteinases • Promotion of foam cell formation • Modulation of coagulation cascade • Stimulation of proliferation in other cells
Platelets	• Production of cytokines and growth factors (PDGF) • Expression of glycoprotein IIb/IIIa receptors • Adhesion to injured endothelium • Induction of mural thrombosis

1 IL-1, interleukin-1; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte/macrophage-colony stimulating factor; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated upon activation normal T-cell expressed and secreted; GRO-alpha? growth-related oncogene-alpha; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; IFN-gamma, interferon gamma; TNF-alpha? tumor necrosis factor-alpha; VEGF, vascular endothelial cell growth factor; MIP-1alpha? macrophage inflammatory protein-1 alpha? TGF-beta, transforming growth factor-beta; PDGF-A, platelet-derived growth factor-A.

proliferation, and extracellular matrix production, and contribute to the development of large obstructive plaques. In this review, we will focus on the current understanding of the pathogenic mechanisms underlying the development of atherosclerotic lesions. We also imply that atherosclerosis is in fact an inflammatory/reparative disease and that future therapy would intervene at different sites of this immune process.

3. EVIDENCE OF INFLAMMATORY ACTIVATION IN ATHEROSCLEROSIS

Studies of the different components of the atheromatous plaque have demonstrated the inflammatory/reparative nature of atherosclerosis. Endothelial cells, T-cells, platelets, macrophage/foam cells, mast cells, myofibroblasts as well as several cell adhesion molecules can all participate in atheromatous inflammation (table 1).

Endothelial cells were initially thought to have a passive role in vascular physiology, forming an inert barrier between the vessel wall and luminal contents. Recent studies, however, have demonstrated a potent inflammatory role for endothelial cells. They have been shown to express interleukin-1 (IL-1), IL-5, IL-6, IL-8, IL-11, IL-15, several colony stimulating factors (CSF) including granulocyte-CSF (G-CSF), macrophage CSF (M-CSF), GM-CSF, and chemokines, such as monocyte chemoattractant protein-1 (MCP-1), regulated upon activation normal T-cell expressed and secreted (RANTES), and growth related oncogene proteins-alpha (GRO-alpha) (2).

IL-1 upregulates intracellular adhesion molecule expression by endothelial cells and thereby stimulates leukocyte adhesion to endothelial cells (3). IL-3, which is expressed by activated T-lymphocytes located in early and advanced atherosclerotic plaque, was found to activate smooth muscle cell migration and proliferation as well as vascular endothelial growth factor (VEGF) production, therefore sustaining the atherosclerotic process (4). IL-8, a potent chemotactic factor for neutrophils, stimulates respiratory burst in and degranulation of neutrophils, and enhances neutrophil adhesion to endothelial cells. It also increases vascular permeability independent of PMNs, via a mechanism involving protein synthesis (5). IL-6 is known to promote vascular smooth muscle proliferation (6). IL-15 has pro-inflammatory and T-cell chemotactic properties and promotes T-cells recruitment to sites of inflammation (7). It has been shown that IL-15 is upregulated in human atherosclerotic lesions and has a role in T-cell activation during atherogenesis (7). One study showed that plaque-derived T-cells have a high proliferative response to IL-15 *in vitro*, and that IL-15 mRNA and protein expression was almost unique to fibrolipid and lipid-rich plaques (7). Therefore, IL-15 provides an alternative pathway for T-cell activation in

atherosclerosis (7). In addition, IL-15 is known to induce angiogenesis, which is of great significance in the development of advanced atherosclerotic lesions (8). All these are reviewed in some detail in reference 6.

Endothelial cells also express adhesion molecules such as selectins (e.g. E and P selectin) and integrin ligands (e.g. ICAM-1, VCAM-1). A marked increase in the expression of adhesion molecules ICAM-1 and E-Selectin on endothelial cells adjacent to subendothelial infiltrates of T-lymphocytes and macrophages has been seen within plaques of human coronary artery and abdominal aorta (9). Regulation of these factors is at the gene expression level, and involves the nulcear factor (NF)-kappaB pathway (see below, Section 5). Protection for endothelial cells may be provided by high-density lipoprotein (HDL), which can prevent the upregulation of adhesion molecule expression and also can increase expression of prostacyclin, a vascular wall relaxant (10).

Monocytes as a precursor for macrophages in tissues have a crucial role in every phase of atherogenesis. Monocyte derived macrophages are as important as smooth muscle cells during progression of atherosclerotic lesions (11). They form a prominent component in atheromas as well as lymphocytes, especially memory T-cells (12). The role of monocyte derived macrophages was explored by mutation in the M-CSF gene, or disruption of the scavenger receptor A gene in mice models (13). Decreased blood peripheral monocyte number or monocyte deficiency in uptake of modified low-density lipoprotein (LDL) particles results in a reduction in the size of atherosclerotic lesions compared with controls suggesting a crucial role for monocyte derived macrophages in atherosclerotic lesion formation (14). Cellular interactions between leukocytes and the endothelium also play a critical role in atherosclerosis and acute coronary syndromes. For instance, when monocytes and endothelial cells are activated via direct cell-cell interactions, both types of cells express several biologically active molecules like adhesion molecules, cytokines, coagulation and fibrinolytic factors, metalloproteinases, and vasoactive substances which play an important role in atherogenesis (15).

T-cells are activated when they bind antigen that is processed and presented by macrophages. Smooth muscle cells from the lesions have class II major histocompatibility complex (MHC) molecules on their surfaces probably induced by interferon-gamma (IFN-gamma) and can present antigens to T-cells. T-cell activation results in the secretion of cytokines including IFN-gamma and tumor necrosis factor-alpha and -beta (TNF-alpha and -beta), which amplify the inflammatory process (16). Total lymphocyte deficiency has also been investigated in atherosclerosis by crossing apo E and rag-I (a gene essential for normal lymphocyte development) knockout mice. Rag-I/apo E knockout mice demonstrated a 40% reduction in atherosclerotic lesion formation as compared with apo E knockout control mice (17).

Platelet adhesion and mural thrombosis are ubiquitous in the lesions of atherosclerosis. Platelets can adhere to dysfunctional endothelium, exposed collagen, and macrophages. Upon activation, platelets release their granules containing cytokines and growth factors, which contribute to migration and proliferation of smooth muscle cells and monocytes (18). Activation of platelets also leads to the formation of free arachidonic acid, which can be transformed into prostaglandins, such as thromboxane A2, a very potent vasoconstrictor and platelet aggregating substance. An important component of platelets is the glycoprotein IIb/IIIa receptor which appears on the surface of platelets during platelet activation and thrombus formation. They serve an important hemostatic function, and antagonists to them are used in patients with acute coronary events to prevent thrombus formation (19).

Recent data suggests that mast cells may also be important in the pathogenesis of atherosclerosis (review in 20, 21). They are present in increased numbers at sites of plaque rupture in patients with coronary artery disease. By secretion of pro-inflammatory cytokines and chemokines, mast cells can assist in recruitment of monocytes and lymphocytes into vascular tissue thereby propagating the inflammatory response. Mast cells can interact with other accessory cells, including endothelial cells, macrophages, lymphocytes and smooth muscle cells in the vascular wall (20,21). Mast cells activate metalloproteinases produced by macrophages, which cleave matrix proteins and may destabilize atheromatous plaque. Mast cells assist in foam cell formation by promoting plasma lipoprotein uptake by vascular smooth muscle cells and macrophages. Mast cell-derived cytokines and growth factors as well as tryptase can stimulate proliferation of endothelial cells, vascular smooth muscle cells, and fibroblasts. Mast cell tryptase via interfering with coagulation may slow thrombus formation at sites of plaque rupture (20,21). Therefore, by virtue of their presence, mast cells can modulate atherogenesis and coronary artery disease.

Cell adhesion molecules have also been increasingly recognized in atherosclerosis and cardiovascular disease; however, the value of these molecules as inflammatory markers of myocardial injury is unclear. At the site of myocardial injury, several adhesion molecules are expressed on activated endothelium, specifically ICAM-1 and E-selectin. ICAM-1 plays a role in leucocyte migration out of the circulation into the myocardium, and this will lead to injury via release of toxic metabolites (22). Recent studies suggested an association between levels of some cell adhesion molecules and the early initiation of acute coronary syndrome (22). High levels of IL-6 and C-reactive protein were found in patients presenting with unstable angina (22). A recent study showed higher soluble ICAM-1 (sICAM-1), IL-6, and C-reactive

Table 2. Factors that cause endothelium injury

Factors	Possible Mechanisms of Effects
Modified LDL	• Involved in foam cell production • Chemotactic for Monocytes • Upregulates M-CSF expression
Tobacco Smoke	• Activates leukocytes • Increases free- radical and Elastase production by Neutrophils • Increases monocyte adherence to endothelium • Induces platelet activation factor production • Increases VW factor level
Homocysteine	• Acts as a pro-thrombotic and is toxic to endothelium • Increases collagen production
Infectious agents, such as *Chlamydia pneumonia*, Cytomegalovirus, *H. pylori*, Hepatitis A virus, and HSV	• Accelerates foam cell formation • Increases endothelial susceptibility to oxidized LDL toxicity • Decreases tissue plasminogen activator production • Increases tissue factor production (a pro-coagulant) • Decreases fibrinolysis • Increases VW factor in endothelial cells • Increases LP(a) production • Plays a role in post-angioplasty restenosis (by inhibiting smooth muscle cell p53 apoptotic activity) • Increases circulating C-reactive protein level
Hemodynamic Factors	• Increases vasoconstrictors synthesis (e.g. Endothelin) • Activates NF-kappaB • Promotes endothelial cell apoptosis

protein levels in patients presenting in the early phase of acute coronary syndrome. It was also noted that sICAM-1 level did not change acutely, but could represent a potential risk factor for the acute coronary syndrome (22). Other factors can affect the levels of adhesion molecules and chemokines in plasma; for instance, hyperlipoproteinemia is involved in the development of atherosclerosis via a change in the expression of adhesion molecules on endothelial and smooth muscle cells (23). One study evaluated the role of sICAM-1, sVCAM-1 and MCP-1 in patients with hyperlipoproteinemia (HLP) IIa and IIb. This study showed no correlation between the plasma levels of these molecules and lipid levels in HLP-IIa and IIb if these parameters were taken separately, however, when both HLP were combined in one group, sICAM-1 and MCP-1 levels correlated with triglycerides and LDL levels respectively (23).

4. ENDOTHELIAL DYSFUNCTION AND THE IMMUNE RESPONSE TO ENDOTHELIAL INJURY

Endothelial dysfunction is probably the key initial step in atherogenesis. Possible causes of endothelial dysfunction include elevated and modified LDL, free radicals caused by cigarette smoking, hypertension, diabetes mellitus, elevated plasma homocysteine concentration, infectious microorganisms such as herpes virus or chlamydia pneumoniae, and combinations of these or other factors (table 2). These factors increase the permeability of the endothelium and its adhesiveness to leukocytes or platelets. The injury also activates the endothelium to express several cytokines, growth factors, and other vasoactive molecules, and induces the endothelium to have procoagulant instead of anticoagulant properties (24). Another important factor is blood flow pattern. It is well known that different patterns of flow have different effects on the activity of several endothelial pro-atherogenic factors. For instance, laminar flow affects the endothelium through different mechanisms: increasing the activity of enzymes that produce antioxidants (e.g. superoxide dismutase), decreasing vasoconstrictor synthesis (e.g. endothelin), maintaining NF-kappaB (a pro-inflammatory factor) in an inactive state, and inhibiting endothelial cell apoptosis (24).

Dyslipidemia is one of the risk factors that can cause an inflammatory insult to the vascular tissue (25). In fact, there is an objective and positive relation between total cholesterol level and coronary artery disease mortality in more than 350,000 men screened for multiple risk factor intervention trial (MRFIT) (25). Current understanding of the mechanism by which elevated LDL promotes atherogenesis is related to oxidative modification of these particles within the artery wall, in turn promoting formation of macrophage derived foam cells and providing a stimulus for inflammation. Removal and sequestration of modified LDL are important parts of the initial protective role of macrophages in the inflammatory response and minimize the effects of modified LDL on endothelial and smooth muscle cells (26, 27). Modified LDL is chemotactic for other monocytes, and can up regulate the expression of genes for M-CSF (28, 29) and

monocyte chemotactic protein derived from endothelial cells (30). Thus, it promotes an inflammatory response by stimulating the replication of monocyte-derived macrophages and the entry of new monocytes into the lesion. It has been shown that high density lipoprotein (HDL) could be anti-inflammatory in the sense that it can prevent or inactivate the LDL-derived phospholipids (31). In addition, paraoxonase (PON) is an enzyme that was shown to prevent the formation of, and also to inactivate, LDL-derived oxidized phospholipids (31). Adding PON to the HDL of mice overexpressing apoA-II was found to convert HDL from pro-inflammatory to anti-inflammatory state suggesting that the PON activity is contributing to the anti-inflammatory activity of HDL (1). Thus, HDL is a lipoprotein which is anti-inflammatory in the basal state and pro-inflammatory in the acute response.

Another modifiable risk factor is tobacco smoke, which is known to have deleterious cardiovascular effects. Smoking leads to an overall increase in white blood cell count, primarily because of an increase in the number of peripheral blood neutrophils (32). Also, smoking as few as two cigarettes in succession leads to activation of leukocytes (33). Activated neutrophils produce increased amounts of oxygen-derived free radicals or elastase, which may damage the endothelium and vessel wall, respectively, and contribute to the development of atherosclerosis (34, 35). Tobacco smoke also stimulates a significant increase in monocytes (36) and results in a dose-dependent increase in monocyte adherence to the endothelium (37). Tobacco smoke exposure also appears to activate platelets by several mechanisms including production of platelet-activating factor and increased levels of circulating von Willebrand factor (38). A consequence of platelet activation and chronic tobacco use is the release of platelet-derived growth factor (PDGF), which results in smooth muscle cell proliferation, one of the initial steps in the development of atherosclerosis (38).

Nicotine from inhaled smoke also activates the sympathetic nervous system and leads to the release of catecholamines. This sympathetic response activates hormone-sensitive lipase to increase the amount of free fatty acids (FFAs) in the blood. The liver removes these FFAs from blood and secretes them into the circulation within very low-density lipoprotein (VLDL). This process results in higher than normal levels of VLDL and, ultimately, higher levels of LDL (39). Tobacco smoke results in a decrease in circulating HDL in a dose-dependent manner (40, 41). Cigarette smoke also generates an abundance of free radicals that, when inhaled, can initiate lipid oxidation. Low-density lipoprotein in smokers circulates in the blood longer than in nonsmokers and is therefore at greater risk for oxidative modification (42). These modifications result in impaired uptake of LDL by its hepatic receptor, and enhanced uptake by macrophage scavenger receptors (43). More specifically, alterations in Apo-B100 contribute to foam cell formation because of a more rapid and unregulated uptake of LDL by macrophages (44). There is some evidence to suggest that tobacco smoke may also modify HDL. One *in vitro* study showed that HDL exposed to cigarette smoke is less effective at stimulating cholesterol efflux from foam cells (45). Modification and oxidation of lipoproteins in the blood of smokers therefore may be an additional mechanism by which smoking contributes to atherogenesis.

Injury to the vascular tissue was also attributable to several mutations in the enzymes involved in homocysteine accumulation. These mutations were found to correlate with thrombosis and in some studies coronary risk. Although thrombosis and atherosclerosis seem intimately linked, direct evidence of an atherogenic effect of hyperhomocysteinemia remains weak. Homocysteine is known to be prothrombotic (46), toxic to the endothelium (47, 48), and to increase collagen production (49). Plasma levels of homocysteine can vary with diet, and a diet rich in folic acid can decrease homocysteine levels.

Infection, as another possible cause of atherosclerosis, was first suggested at the beginning of the twentieth century. Several reports showed a correlation between certain infectious organisms and coronary artery disease. Microorganisms that have been implicated are Cytomegalovirus (CMV), Cocksackie B virus, *Chlamydia pneumoniae*, and *Helicobacter pylori*. In the Helsinki heart study, Saikku *et al.* found that patients who were seropositive for *C. pneumoniae* at baseline were 2.6 times more likely to experience cardiovascular disease during the study than those who were seronegative (50). A number of potential causative mechanisms whereby *C. pneumoniae* might initiate or accelerate the progression of atherosclerosis have been described. *C. pneumoniae* is capable of infecting endothelial cells as well as macrophages (51). In atherosclerotic plaque, although not in all cases, *C. pneumoniae* has been found to be present within the foamy macrophages. Activated foamy macrophages appear to play a critical role in the pathogenesis of unstable atherosclerotic plaques. Kalayoglu and Byrne have reported that infection with *C. pneumoniae* significantly accelerates the development of foam cells and permits this transformation to occur at much lower levels of LDL in the culture medium (52). It might be possible that infection with *C. pneumoniae* enhances the susceptibility of the vessel wall to toxic damage from oxidized LDL. In other cell culture studies infection of smooth muscle cells significantly enhanced their procoagulant state through a decrease in the production of tissue plasminogen activator and an increase in the production of tissue factor, a potent procoagulant (53). It might be possible that infection of endothelial cells with *C. pneumoniae* can stimulate the local coagulation system of the vessel wall and increase the potential for coronary thrombosis. Several studies postulated that *C. pneumoniae* could be involved in the early stages of atherosclerosis, and that antibiotic therapy would be of little value in advanced stages.

CMV has also been implicated in playing a role in the pathogenesis of atherosclerosis. Adam *et al*. noted that seropositivity to cytomegalovirus is significantly higher in patients with known coronary atherosclerosis, as evidenced by the need for coronary bypass surgery, than in a control group with 5 years documentation of the absence of symptomatic atherosclerosis (54). Several investigations have documented that cytomegalovirus infection can result in reduced fibrinolysis with an increase in the production of Lp(a), promoting the development of atherosclerosis (55). Also, cytomegalovirus has been found to alter the von Willebrand factor content in endothelial cells, which may enhance the procoagulant activity of the coronary artery (56). Speir and Epstein demonstrated that CMV infection could be found in restenosed human coronary arteries post angioplasty. This role of CMV in post-angioplasty restenosis is correlated to its ability to inhibit the smooth muscle cell apoptotic p53 activity (57). It was also demonstrated that individuals infected with several pathogens such as CMV, *H. pylori*, Hepatitis A, and Herpes simplex virus, have elevated levels of inflammatory markers such as C-reactive protein (57). Thus, infection with these pathogens could constitute a real risk for coronary artery disease through an atherosclerotic inflammatory process (57).

5. TRANSCRIPTION FACTORS AND REGULATION OF INFLAMMATION

NF-kappaB is a redox sensitive transcription factor regulating a battery of inflammatory genes. Proinflammatory cytokines produced by macrophages, T-cells, and other cells exert their action on target cells by activating NF-kappaB (58-60). This leads to or enhances expression of several genes, including those encoding several cytokines (e.g. IL-6 and IL-12), leukocyte adhesion molecules (e.g. ICAM-1, VCAM-1 and E-Selectin), matrix metalloproteinases, cyclooxygenase-2, and inducible nitric oxide (NO) synthase. NF-kappaB activation in turn induces the expression of pro-inflammatory cytokines in a positive feed back loop (7).

NF-kappaB may play a role in atherogenesis by transducing pathogenic stimulation to expression of genes that promote recruitment and activation of inflammatory cells in the plaque. NF-kappaB-regulated inflammatory mediators such as cytokines and adhesion molecules have been detected in atherosclerotic lesions. Activated NF-kappaB has been identified in macrophages, smooth muscle cells, and endothelial cells in human atherosclerotic plaques, but not in healthy vessels (61, 62). Activation of NF-kappaB in these endothelial cells, smooth muscle cells, and monocyte-derived macrophages may be induced by factors such as reactive oxygen species involved in LDL cholesterol oxidation and components of microorganisms such as *C. pneumoniae* (63). In addition, mice deficient in NF-kappaB signaling exhibit reduced fatty streak formation when fed a fatty diet (64).

6. PIVOTAL ROLE OF CD40/CD40L INTERACTIONS IN ATHEROSCLEROSIS

CD40-CD40L receptor ligand interaction plays a central role in antigen presentation, immunological reaction, and in T-cell and macrophage activation (65). In one study, normal human intima did not contain CD40 or CD40L immunoreactivity; however, the presence of CD40 and CD40L was noted in T-cells (CD3+ cells), macrophages (CD68+ cells), and smooth muscle cells (HHF 35+ cells) from type II lesions (fatty streaks) to advanced type VI lesions (complicated plaques). There was no correlation between lesion type and CD40-CD40L expression, but positive lesions had dense infiltration of macrophages, macrophage-derived foam cells, and T-cells (66). Blocking CD40 signaling either by antibody treatment against CD40 ligand or by using CD40 ligand-knock out mice showed a substantial reduction in atherosclerotic lesion formation (67, 68). Recent data from animal models suggests that disruption of CD40-CD154 pathway may even reverse established lesions (69). Furthermore, anti-CD40L antibody treatment in apo E -/- mice induced a stable plaque phenotype, presumably because anti-CD40L antibody led to a pronounced increase in collagen content, vascular smooth muscle cell/myofibroblast content, and fibrous cap thickness, and to a decrease in lipid core and macrophage content (70).

7. ROLE OF SPECIFIC CHEMOKINES IN ATHEROSCLEROSIS

Chemokines are relatively small, secreted basic proteins (8-10 kd) that are subdivided into four families based on the relative position of their cysteine residues in the amino acid backbone (CC, CXC, C, CXXXC) (71). The largest class includes the CC chemokines, whose prototype is monocyte chemoattractant protein-1 (MCP-1). The second largest class, the CXC chemokines, can be further subdivided depending on the presence or absence of amino acid motif ELR (Glu-Leu-Arg) in their amino terminus. IL-8 and interferon inducible protein 10 kDa (IP 10) are the prototypes of the ELRCXC and non-ELRCXC branches, respectively (71). Only one C chemokine, lymphotactin, and one CXXXC chemokine, fractalkine, have yet been described (71). CC chemokines are potent chemoattractants for monocytes and lymphocytes, ELRCXC for Neutrophils, and non-ELRCXC for lymphocytes. Lymphotactin attracts T lymphocytes, whereas fractalkine attracts monocytes and T-lymphocytes (71).

Both in animal models and human specimens, chemokine expression is associated with atherosclerotic lesion development. MCP-1 has been detected on the luminal endothelium of early atherosclerotic lesions, and is expressed mainly by macrophages in more advanced plaques (72) and also by smooth muscle cells (73). Endothelial cells have been shown to express MCP-1 in response to various stimuli and this could contribute to recruitment of monocytes to vascular wall

Table 3. Selected effects of traditional cardiac drugs on inflammation and atherogenesis

Drugs	Effects on Inflammation and Atherogenesis
Cyclooxygenase inhibitors (salicylates and ibuprofen)	• Decrease circulating MCP-1, IL-6, and CRP levels • Inhibit NF-kappaB activation • Inhibit VCAM-1 expression
HMG-CoA reductase inhibitors (statins)	• Inhibit TNF-alpha and P-Selectin • Decrease MCP-1, GM-CSF, and MIP-1alpha production • Inhibit NF-kappaB activation • Inhibit matrix degrading enzyme metalloproteinases
ACE inhibitors and angiotensin receptor antagonists	• Inhibit NF-kappaB activation • Inhibit IL-8, MCP-1, and PDGF expression • Decrease P-Selectin and MCP-1 expression
Beta Adrenergic antagonists	• Attenuation of cytokine and sIL-2R production • Augmentation of VEGF expression • Inhibition of PDGF-mediated PDGF receptor kinase expression

(74). Infiltrated and activated macrophages could then express MCP-1 resulting in continued influx of monocytes and further expression of MCP-1. MCP-1 has been shown to be an important factor in the development of medial thickening as well as monocyte recruitment (75). It was also noted that the deletion of the CCR2 gene (MCP-1 receptor) partially inhibits the development of atherosclerotic lesion in apoprotein-E deficient mice, which suggests that the MCP-1/CCR2 pathway plays an important role in the development of atherosclerosis (75). Several other chemokines including MCP-4, RANTES, pulmonary and activation regulated chemokines, EBI-1-ligand chemokine, and IL-8 are increased in human atherosclerotic lesions. Compared with normal vessels, the expression of chemokines is highest in the area bordering the necrotic lipid core, close to where the fibrous cap has been shown to rupture in acute coronary syndromes (76).

Chemokine expression is induced by a host of stimuli including oxidized lipids, direct vascular injury, cytokines such as TNF-alpha, IL-1beta, and interferon-gamma, and growth factors such as platelet-derived growth factor (PDGF). Homocysteine treatment of human aortic smooth muscle cells leads to increases in expression of MCP-1 and IL-8 secretion suggesting that homocysteine may increase monocyte recruitment into developing atherosclerotic lesions by upregulating chemokine expression in vascular smooth muscle cells (76). The expression of MCP-1 is increased in the arterial wall in response to balloon injury and may contribute to the restenotic process (58). Both MCP-1 and IL-8 have been observed to be elevated in patients with acute coronary syndromes. Elevated levels of MCP-1, macrophage inflammatory protein-1 alpha (MIP-1alpha), and RANTES were also reported in patients with congestive heart failure, and levels were inversely correlated with left ventricular ejection fraction (77). This may solely reflect the cytokine milieu in heart failure or in fact may represent a pathogenic role in this condition. Data from transgenic, hypercholesterolemic mice models show that mice lacking MCP-1 receptor or the MCP-1 ligand are less susceptible to atherosclerosis and have fewer monocytes in vascular lesions (59). A recent study showed that eotaxin, which is a chemokine initially described as a chemotactic factor for eosinophils, and its receptor CCR3, are over expressed in human atheroma, but not in normal vessels (60). Eotaxin was predominant in smooth muscle cells, and CCR3 was found primarily in macrophage-rich regions. Further investigations are needed to study the effect of deletion and/or inhibition of the Eotaxin/CCR3 receptor pathway on atherogenesis and plaque stability (60). All these data point towards a prominent role for the cytokine-chemokine pathway in atherosclerosis.

8. IMPLICATIONS FOR THERAPY: ASPIRIN, STATINS, ANGIOTENSINOGEN CONVERTING ENZYME INHIBITORS, AND CYTOKINE ANTAGONISTS

As summarized above, inflammation is now believed to be a basic pathological mechanism in the development and maturation of atherosclerosis (6). This idea implies that the control of atherosclerosis could be achieved through therapeutic interventions at different sites of the inflammatory process. Anti-atherogenesis therapy would therefore target growth factors, cytokine pathways, transcription factors, defective genes or other intracellular metabolic pathways. In addition, many cardiovascular drugs including angiotensin converting enzyme (ACE) inhibitors, hydroxy-methyl-glutaryl-converting enzyme A (HMG-CoA) reductase inhibitors, beta-blockers, and salicylates can modulate expression of several proatherogenic cytokines (table 3) (78).

The anti-inflammatory effects of HMG CoA reductase inhibitors are fascinating. The HMG CoA reductase inhibitors, e.g. pravastatin, cerivastatin, and atorvastatin, exert their beneficial effect not only by LDL cholesterol reduction but also through an anti-

inflammatory process. In one study, treatment of heart transplant recipients with pravastatin had a beneficial effect on two inflammatory markers, TNF-alpha and P-selectin. Atorvastatin therapy, on the other hand, decreased the serum levels of several monocyte-related inflammatory markers, such as MCP-1, GM-CSF, and MIP-1alpha in patients with hypercholesterolemia (79). Atorvastatin was recently shown to inhibit neointimal inflammation, MCP-1 expression, and NF-kappaB activation in a rabbit model of atherosclerosis (80). Cerivastatin, another HMG CoA inhibitor, also inhibits the production of the matrix-degrading enzyme metalloproteinases by vascular smooth muscle cells, which are themselves stimulated by the inflammatory mediators IL-1alpha and PDGF-beta (79).

ACE inhibitors also have several anti-inflammatory effects and modulate the cytokine axis. In one study, it was demonstrated that quinapril, an ACE inhibitor drug, inhibits NF-kappaB activation and expression of IL-8, MCP-1 and PDGF in vascular tissues in a rabbit model of accelerated atherosclerosis (81). Another study reported a regression of atherosclerotic vascular changes in a rat model of induced atherosclerosis by the administration of either an ACE inhibitor, or an angiotensin II receptor antagonist (82). Angiotensin II is well known to increase NF-kappaB activity *in vitro* as well as *in vivo* models; therefore, ACE inhibitors or angiotensin II receptor blockers exert their anti-inflammatory effects through a decrease in NF-kappaB activity and proatherogenic chemokine expression (83). Chen *et. al.* studied the beneficial effect of losartan, an angiotensin II receptor blocker, in hypercholesterolemic rabbit models. They demonstrated that losartan administration diminished P- Selectin and MCP-1 expression as well as intimal proliferation. This study showed the potential beneficial effect of losartan and other angiotensin II antagonists in atherosclerosis modulation (84).

Cyclooxygenase (COX) inhibitors, e.g. aspirin and ibuprofen, are also found to have antiatherogenic effect. Aspirin decreases serum levels of MCP-1, IL-6, as well as CRP after six weeks of therapy (85). Aspirin and other non-steroidal anti-inflammatory drugs, such as ibuprofen, also inhibit NF-kappaB activation, which plays a crucial role in atherosclerosis as already mentioned above. In addition, ibuprofen has a beneficial anti-inflammatory effect and contributes to plaque stability by inhibiting expression of VCAM-1 and adhesion of monocytes to endothelial cells (86).

Beta-Blockers attenuate cytokine production in leukocytes and mononuclear cells by altering beta-receptor mediated elevation of intracellular cyclic adenosine monophosphate (cAMP) levels (Unpublished data, Dr Daniel Dube). Matsumura *et. al.* explored the effects of carvedilol on TNF-alpha and IL-6 production and found a significant reduction in IL-6 production but not that of TNF-alpha in nine patients with cardiomyopathy (87). Ohtsuka and coworkers extended these observations, comparing the effects of metoprolol and carvedilol in patients with idiopathic dilated cardiomyopathy (IDCM) (88). In their study, both agents reduced TNF-alpha, however only carvedilol reduced IL-6 levels. The reduction in IL-6 levels correlated with improved left ventricular function (88). In contrast, the Metoprolol Controlled-Release Randomized Intervention Trial in Heart Failure study (MERIT-HF) investigated the effect of metoprolol on TNF-alpha, IL-6, IL-8, and soluble interleukin-2 receptor (sIL-2R) levels and showed a modest and transient reduction in sIL-2R levels after three months (89). In another study, Ohtsuka *et. al*. evaluated the effects of beta-blockers on inflammatory and anti-inflammatory cytokines in patients with dilated cardiomyopathy (DCM) (90). They observed a positive correlation between sTNF-RII and IL-10 levels. Furthermore, the TNF-alpha/IL-10 ratio correlated with serum epinephrine levels. The use of beta-blockers reduced the sTNF-RII, IL-10, TNF-alpha, and TGF-beta levels (90). In congestive heart failure (CHF) subjects, carvedilol augmented VEGF expression, raising the prospect that pro-angiogenic effects of VEGF may mitigate the deleterious effects of vascular occlusion in atherosclerosis (91). Carvedilol also inhibits PDGF-mediated PDGF receptor kinase expression and dampens mitogenesis in cardiac fibroblasts *in vitro* (92). In corroboration of these findings, investigators have shown that in salt-sensitive hypertensive rats, betaxolol inhibited PDGF and TGF-beta1 gene expression (93).

9. CONCLUSIONS

Atherosclerosis is an immune-mediated inflammatory disease (6). It is, therefore, essential to consider novel immunomodulatory therapies to prevent and/or to treat this multi-factorial inflammatory/reparative process. Therapeutic strategies would target several cytokines, chemokines, adhesion molecules, and transcription factors. Ongoing studies have focused their anti-inflammatory approaches to the use of intravenous immunoglobulin (94), chemokine receptor antagonists, chemokine inhibitors, TNF-alpha antagonists, recombinant cytokines (IL-10), and cytokine antagonists such as IL-1 receptor antagonist (95). The results of these preliminary interventions do not allow any firm recommendation to be made. Further research and clinical trials are nevertheless required before a definitive anti-inflammatory strategy can be used therapeutically in coronary disease.

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