JACC Vol. 31, No. 6
May 1998:1217–25

REVIEW ARTICLE

Interactive Role of Infection, Inflammation and Traditional Risk Factors in Atherosclerosis and Coronary Artery Disease

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Although first suggested at the turn of the 20th century, there is a renewed interest in the infectious theory of atherosclerosis. Studies done in many laboratories around the world over the past several years have shown an association between markers of inflammation and coronary atherosclerosis with an exacerbation of the inflammatory process during acute myocardial ischemia, particularly in the early stages of reperfusion. It is also being recognized that the traditional risk factors, such as smoking, dyslipidemia, hypertension and diabetes mellitus, do not explain the presence of coronary atherosclerosis in a large proportion of patients. We believe that in certain genetically susceptible people, infection with very common organisms, such as *Chlamydia pneumoniae* or cytomegalovirus, may lead to a localized infection and a chronic inflammatory reaction. Persistence of infection may relate to the degree of inflammation and severity of atherosclerosis. Early trials with appropriate antibiotic agents in some patients with a recent history of acute myocardial infarction have led to very salutary results. If patients with an infectious basis of atherosclerosis can be identified, a therapy directed at eradication of the offending organism may be appropriate.

(J Am Coll Cardiol 1998;31:1217–25)
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Atherosclerosis, with its major manifestation coronary artery disease (CAD), is the major cause of morbidity and mortality in the West. Although there has been a marked decline in death from CAD, especially in the United States and to a lesser extent in Europe, during the past 20 years, the prevalence of CAD-related morbidity and mortality in Asia, with almost one-half of the world population, is steadily increasing (1,2). Epidemiologic studies have identified several risk factors, such as smoking, diabetes mellitus, hypertension and dyslipidemia, in the pathogenesis of atherosclerosis. The decline in CAD-related mortality in the West has been ascribed to control of these traditional risk factors and utilization of innovative pharmacologic and coronary interventional therapies (3–5). Several new risk factors, such as homocysteinemia, elevated plasma levels of lipoprotein(a) [Lp(a)], excessive iron load in the body, an imbalance between oxidant and antioxidant species and hypercoagulability, are being described in atherosclerosis (6–11). Several genetic markers of atherosclerosis and CAD, such as angiotensin-converting enzyme polymorphism and human leukocyte antigen (HLA)-DR class II genotypes, have also been identified (12–15).

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Manuscript received September 17, 1997; revised manuscript received January 27, 1998, accepted January 28, 1998.

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Experimental and clinical studies, based on the markers of inflammation and inflammatory mediators in plasma, as well as in tissue samples obtained from atherosclerotic tissues, have provided ample evidence for the presence of ongoing inflammation in atherosclerosis (16–22). This has led to the suggestion that CAD may be an inflammatory, autoimmune disease. The precise stimulus for inflammation continues to elude the investigators; however, new data suggest that bacterial, parasitic or viral infection may initiate the inflammatory process (23).

Another major focus of current interest in the pathophysiology of atherosclerosis and its clinical manifestations is the role of the powerful vasodilator species nitric oxide and alterations in its synthesis, release and activity (24).

In this report, we review our current understanding of the interactive role of different factors in the pathogenesis of CAD, with special emphasis on infection leading to inflammation and alterations in several metabolic abnormalities identified in patients with CAD. If the hypotheses proposed herein are proved, new preventive and therapeutic approaches may then be designed.

Inflammation and CAD

Association of inflammation with acute myocardial ischemia has been known for almost 60 years (25). Infiltration of inflammatory cells in and around the infarcted regions soon after the onset of the ischemic process has been thought to represent part of the healing process. However, it has become amply evident that infiltration of inflammatory cells contributes to infarct extension and expansion and inflammation is a key component of myocardial ischemic injury (26).

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0735-1097/98/$19.00
PII S0735-1097(98)00093-X

JACC Vol. 31, No. 6
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Abbreviations and Acronyms

CAD	=	coronary artery disease
CMV	=	cytomegalovirus
HDL-C	=	high density lipoprotein cholesterol
HLA	=	human leukocyte antigen
IFN-gamma	=	interferon-gamma
IL	=	interleukin
LDL-C	=	low density lipoprotein cholesterol
Lp(a)	=	lipoprotein(a)
PCR	=	polymerase chain reaction
TNF-alpha	=	tumor necrosis factor-alpha

Animal studies. Work done in the animal laboratory has demonstrated the accumulation of leukocytes and their subsequent activation in the blood vessels and myocardium soon after reperfusion. The leukocyte infiltration plateaus at about 4 to 10 h (26). Leukocyte-mediated injury occurs as a result of physical obstruction of the microvasculature in the reperfused tissues (18,19) and release of oxygen-free radicals, proteolytic enzymes, especially elastase (27), and arachidonic acid metabolites (28). The initial infiltration of leukocytes occurs in response to chemotactic stimuli, principally complement (29). Work done in several laboratories has defined the steps leading to leukocyte attachment to the activated endothelial cells and infiltration into the subendothelial vascular layers, and thereafter into the reperfused myocardial tissues. This work includes identification and expression of various adhesion molecules on activated endothelial cells (e.g., P-selectin, intercellular adhesion molecules) and their counterligands on leukocytes (26). Similarly, release of several mediators of inflammation, mainly cytokines (e.g., tumor necrosis factor [TNF-alpha] and interleukins [ILs]), has been described during the early stages of myocardial ischemia (30). There is also evidence of infiltration of reperfused tissues with monocytes and T lymphocytes. Activation of these cells results in release of TNF-alpha, macrophage colony-stimulating factor, macrophage chemoattractant protein-1 and other activators of coagulation cascade (31). Collectively, these mediators may facilitate propagation of in situ thrombosis and atherosclerosis, and hence the ischemic process.

These data have led to the concept that ischemia begets more ischemia. Identification of various steps involved in infiltration of ischemic tissues by leukocytes, particularly at the border zone between infarcted and noninfarcted myocardium, has been the basis for use of novel strategies focused on limitation of tissue injury. The therapies tested thus far in the animal models of myocardial ischemia have included interference with the generation of leukocyte chemotactic factors, such as complement depletion with cobra venom toxin (32), or with blockade of adhesion molecules and their counterligands on leukocytes (33,34). Recent studies have also included use of specific inhibitors of different cytokines (35). Other strategies have been designed to deplete neutrophils with antineutrophil antibodies (36), chemicals (37) and neutrophil filters (38) or to inhibit neutrophil function with prostacyclin analogs (39), lipoxygenase inhibitors (37) or mixed cyclooxygenase and lipoxygenase inhibitors (40). The role of oxygen free radicals and elastase with specific inhibitors (41,42) has also been examined. All these strategies have demonstrated reduction in infarct size, albeit to a varying degree, in animal models of myocardial ischemic injury.

Other studies designed to limit inflammation in atherogenesis in experimental models are currently in progress, although the results thus far have not shown a uniform benefit of this strategy.

In animal models of atherosclerosis induced by a high cholesterol diet, the first cells to appear on the surface of endothelium are monocytes, which soon migrate to the subendothelial layers, engulf oxidized cholesterol and transform into macrophages (16). The lipid core of the atherosclerotic region is characterized by the presence of T lymphocytes, a hallmark of the immune process. The activated T lymphocytes inhibit collagen synthesis by secretion of interferon-gamma (IFN-gamma) and thus interfere with the maintenance and repair of the collagenous framework of the plaque's fibrous cap. IFN-gamma secretion facilitates formation, retention and activation of macrophages, which can breakdown both collagen and elastin. Release of cytokines stimulates neovascularization, in situ thrombosis and loss of endothelium's ability to generate nitric oxide (43). Rupture of neovascular channels within the plaque leads to hemorrhage and thrombosis. These processes collectively have been attributed to the evolution of unstable coronary syndromes (31).

Evidence of inflammation in patients with CAD. Inflammation, as evident by a high blood cell count and high erythrocyte sedimentation rate, has been thought to reflect the body's response to tissue injury in patients with acute myocardial infarction. Work from several laboratories in recent years suggests that inflammation is part and parcel of the syndrome of ischemia-reperfusion injury and that the degree of inflammation correlates with the severity and outcome of acute myocardial ischemia.

Studies by Friedman et al. (44) in 1974 and by Kostis et al. (17) and Lowe et al. (45) in the early 1980s suggested that total leukocyte count correlates with the extent and severity of coronary atherosclerosis and initial leukocyte count during acute myocardial infarction independently predicts the frequency of early ventricular fibrillation. Studies from our institution showed an enhanced chemotactic response of neutrophils and an increased leukotriene B_4 generation in patients with stable angina. Patients with unstable angina and acute myocardial infarction showed increased chemotaxis and morphologic evidence of degranulation and increased levels of neutrophil elastase activity in plasma (46,47). De Servi et al. (48) subsequently confirmed the increased neutrophil activity in patients with exercise-induced angina. They suggested that "priming" of neutrophils in stable angina may enhance platelet aggregation and predispose patients to the development of acute coronary syndromes. These authors also documented release of neutrophil elastase during coronary angioplasty in

patients with stable angina (49). Neri Serneri et al. (50) in elegant studies in patients with a variety of CAD patients showed that peripheral blood lymphocytes from patients with unstable angina are also activated and these activated cells induce expression of procoagulant activity of monocytes.

Mazonne et al. (51) described an increased expression of neutrophil and monocyte adhesion molecules in unstable CAD. CD11b/CD18 expression was higher in the coronary sinus blood than in the aortic blood, indicating intracoronary expression of adhesion molecules in unstable coronary syndromes. Similarly, Rab et al. (52) measured monocyte-macrophage Leu-M_3^+ marker and HLA-DR surface antigens in circulating blood and found these to be increased in patients with unstable angina. We (53) measured circulating levels of intercellular adhesion molecule-1 released from activated endothelial cells and L-selectin shed into the blood from activated leukocytes in patients with a variety of CAD syndromes and found elevated levels of circulating intercellular adhesion molecule-1 in all patients with CAD compared with control subjects, with the highest levels in patients with acute ischemic syndromes. In contrast, plasma L-selectin levels were diminished in CAD patients. Stimulation of leukocytes in vitro was demonstrated to be associated with downregulation of L-selectin expression measured by flow cytometry, suggesting that low plasma levels of L-selectin reflect ongoing leukocyte activation in patients with CAD.

Barath et al. (54) and Kaartinen et al. (55) localized TNF-alpha in human atherosclerotic tissues, not only in macrophages and mast cells, but also in the cytoplasm of and attached to the cell membrane of smooth muscle cells and endothelial cells. In the study by Vaddi et al. (21), enhanced secretion of TNF-alpha and IFN-gamma from mononuclear cells of patients with CAD was demonstrated, with a similar increase in release of these cytokines from cultured mononuclear cells of patients with stable or unstable angina. This study demonstrated a three- to fourfold increase in superoxide generation in neutrophils from patients with CAD (vs. control subjects) and suggested that this phenomenon may be a result of continuous stimulation of mononuclear cells by cytokines. The cytokines TNF-alpha and INF-gamma activate endothelial cells and cause expression of adhesion molecules, neovascularization and hemorrhagic necrosis and promote thrombogenesis (43). In classic pathologic studies in patients who died of acute myocardial infarction, van der Wal et al. (56) found that macrophages and T lymphocytes were the dominant cell types at the immediate site of either rupture or superficial erosion in each case. These sites were characterized by abundant expression of HLA-DR antigen on both inflammatory cells and adjacent smooth muscle cells, suggesting an active inflammatory reaction. Taken together, these observations suggest continuous ongoing inflammation in atherosclerosis, which may play a role in the evolution of uncomplicated atheromatous plaque into a complex atheroma. Repeated episodes of ischemia may further enhance the inflammatory process in the blood vessels and myocardial tissues. Acute exacerbation of inflammation may have a role in destabilizing the fibrous cap and enhance the risk of thrombosis.

Two recent clinical studies on the role of inflammation in CAD are particularly noteworthy. Liuzzo et al. (57) reported that the acute phase reactant, C-reactive protein, and serum amyloid A protein were elevated in most patients with unstable angina and recent acute myocardial infarction. Elevated levels of these acute phase reactants at the time of hospital admission were predictors of a poor outcome in patients with unstable angina, and according to the authors, inflammation may be an important component of acute myocardial ischemia. Ridker et al. (58) recently reported on the plasma C-reactive protein levels in participants of the Physicians' Health Study and found that the level of C-reactive protein was an independent significant predictive value in future myocardial infarction and ischemic stroke. Importantly, the use of aspirin was associated with marked reduction in the risk of myocardial infarction in men in the highest quartile, but with a small insignificant reduction in the lowest quartile. These authors suggested that the anti-inflammatory action may be an important mechanism of the beneficial effect of aspirin.

These observations taken together suggest the possibility that atherosclerosis is a chronic inflammatory disease (22) that develops in response to some metabolic, physical, infectious and environmental process. T cells in the atherosclerotic plaque express late-activation antigens and may also be active participants in an active inflammatory response.

Thus, a stage has been set to examine the concept that inflammation and atherosclerosis are closely associated, and that acute ischemic events reflect exacerbation of a chronic inflammatory state. Furthermore, acute myocardial injury as a result of coronary thrombosis and reperfusion may be associated with more inflammation. Clearly inflammation begets more inflammation.

HLA and Atherosclerosis

The HLA class I and class II molecules are cell surface proteins that are essential for defense against infectious organisms. The class I cell surface antigens are found on almost all nucleated cells, and their function is to present intracellular antigens from virus-infected or malignant cells to cytotoxic T cells. The class II antigens present extracellular antigens from bacteria or parasites to T-helper cells, and their expression is restricted to macrophages and B-lymphocytes. Allelic variation at the HLA genes can influence the ability to bind specific antigens, as well as the interaction with a particular T-cell receptor, by the presence of binding pockets specific to each allele (59–61). Also, the stability of the D-dimer of the class II molecule may be changed by allelic polymorphism.

The loci encoding the HLA antigens are among the most polymorphic protein coding genes found in humans, with >180 alleles at the class I loci and >230 alleles at the class II loci. An important practical consequence of the HLA polymorphism is the rejection of transplanted organs, occurring when the HLA matching between the donor and the recipient is not complete.

Apart from being important in transplantation medicine, the HLA antigen confers susceptibility to many diseases. No other genomic region has been found to be associated with an equivalent number of diseases, most of them with autoimmune features, as the HLA region. Studies by Dahlen et al. (14,15) have shown an association of certain inherited HLA-DR class II genotypes with atherosclerosis and diabetes.

Role of Infection in Pathogenesis of Atherosclerosis

It is well known that the traditional risk factors, such as hypercholesterolemia, smoking, hypertension and diabetes, do not explain atherosclerosis in a significant number of patients. Particularly, the high prevalence of CAD in developing countries is associated with a relatively low frequency of smoking and normal or low total cholesterol levels (62). Notably, the decline in CAD-related mortality in the United States began before the awareness of traditional risk factors and the emphasis on their control. The development of novel therapeutic strategies over the past 10 years (e.g., thrombolytic therapy, cholesterol-lowering drugs, antiplatelet drugs and antithrombins and catheter-based coronary interventions) also cannot explain the gradual and steady decline of CAD-related mortality during the past 25 years. The decrease in CAD-related mortality has been much smaller in Europe despite control of risk factors and the use of therapeutic strategies similar to those in the United States. During the same 20- to 25-year period, there appears to be a marked increase in cases of CAD and CAD-related mortality in Asia (2). These observations also suggest the possibility that different etiologies may be involved in atherogenesis in different populations.

An infectious etiology of atherosclerosis has received considerable attention recently (23). This theory was initially formulated in the first two decades of this century (63,64), but did not receive much attention until the late 1970s when Fabricant et al. (65) showed that chickens infected with avian herpesvirus developed vascular lesions similar to those found in human atherosclerosis. Since then a number of infectious agents have been implicated in human atherosclerosis and include *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus and cytomegalovirus (CMV) (66–70), on the basis of finding these infectious agents in the atherosclerotic segments and by positive serology.

***Chlamydia pneumoniae* and atherosclerosis.** *Laboratory evidence. Chlamydia pneumoniae* is a species of *Chlamydia* that causes pneumonia, bronchitis, pharyngitis and sinusitis. It is distinguished from *Chlamydia trachomatis* and *Chlamydia psittaci* on the basis of DNA sequence and electron microscopic morphology of the elementary body. It has been found to have only one immunotype, named *TWAR*, from the laboratory designation of the first two isolates (TW-183 and AR-39). Now the term TWAR is used synonymously with *C. pneumoniae.*

Gaydos et al. (71) and Wyrick and Brunridge (72) have demonstrated that *C. pneumoniae* can replicate and maintain infection in human macrophages, endothelial cells and aortic smooth muscle cells. These cell types show particular susceptibility to infection with *C. pneumoniae* (73). Fryer et al. (74) have shown that *C. pneumoniae* can infect cultured human vascular endothelial cells and stimulate a fourfold increase in the expression of tissue factor and platelet adhesion, thus providing a link between infection with *C. pneumoniae* and procoagulant activity. Moazed et al. (75) have examined the relation of infection with *C. pneumoniae* with atherosclerosis in two different animal models: apolipoprotein E-deficient transgenic mice, which spontaneously develop atherosclerosis, and C57BL/6J mice, which only develop atherosclerosis on an atherogenic diet. After intranasal inoculation in these animal models, *Chlamydia pneumoniae* was persistently detected in atherosclerotic regions of the aorta by polymerase chain reaction (PCR) and immunocytochemistry. Serum antibody levels (elevated IgG titers) were also positive for up to 12 weeks.

Clinical evidence. The association of *C. pneumoniae* infection with human atherosclerosis comes from several observations. IgG antibodies to *C. pneumoniae* are uncommon among children and increase in early adulthood until the prevalence reaches 70% in men and 50% in women (76). The prevalence of positive serologic evidence is ~25% higher in men than in women (77), which is consistent with the higher prevalence of atherosclerosis in men than in women. Saikku et al. (78) provided the first serologic evidence of an association of *C. pneumoniae* with CAD in 1988. They examined serum samples from 40 male patients with acute myocardial infarction, 30 male patients with chronic CAD and 41 control subjects and found that 68% of patients with acute myocardial infarction and 50% of patients with chronic CAD had elevated IgG ($\geq$1/128) or IgA ($\geq$1/32) titers, or both against *C. pneumoniae*. In contrast, only 17% of control subjects had high titers. Since the initial report by this Finnish group, several studies have demonstrated this association in a variety of patients with CAD using seroepidemiologic evidence (15). It is noteworthy that Spodick et al. (79) in 1984 suggested the association of acute respiratory symptoms with the onset of acute myocardial infarction in a prospective investigation of 150 consecutive patients and matched control subjects.

The presence of the *C. pneumoniae* organism has been demonstrated in the atherosclerotic plaque itself using a variety of techniques, such as PCR, electron microscopy, immunostaining and culture of the organism (69,77,80–83). In 1992 Shor et al. (80) detected *C. pneumoniae* in fatty streaks and atheromatous lesions in seven autopsy cases. TWAR-like organisms were found in the lipid-rich core of fibrolipid plaques and intimal smooth muscle cells. In five of seven cases, immunoperoxidase staining was positive for *C. pneumoniae*. There are now several reports on the presence of *C. pneumoniae* in atherosclerotic plaques from coronary arteries, carotid arteries and the thoracic and abdominal aorta. Kuo et al. (69) and Campbell et al. (77) reported finding *C. pneumoniae* in the coronary arteries of young adults (15 to 34 years old) with atherosclerosis. Ong et al. (81) have recently reported the presence of *C. pneumoniae* by PCR and immuno-

staining in 44% of aortas, 55% of iliac arteries and 40% of femoral arteries of patients with atherosclerosis.

Muhlestein et al. (82) recently reported immunofluorescence positivity for *C. pneumoniae* in 79% of 24 coronary atherectomy coronary artery specimens. The presence of *C. pneumoniae* was not related to conventional CAD risk factors, the extent and severity of CAD or the clinical status of the patients. Not all studies have shown a positivity for *C. pneumoniae* in atherosclerotic tissues; for example, Weiss et al. (84) examined 79 coronary atherectomy specimens for *C. pneumoniae* by PCR or electron microscopy and found only one PCR-positive specimen; all remaining 78 specimens were negative.

We recently examined the association of *Chlamydia* with coronary atherosclerosis in 60 autopsy specimens. Eighteen cases had mild and 42 cases severe atherosclerosis. Of 42 cases with severe atherosclerosis, 36 were immunopositive compared with 1 of the 18 with mild atherosclerosis ($p < 0.001$). Lp(a) levels were 190 ± 44 mg/liter in cases with severe atherosclerosis and 61 ± 12 mg/liter in cases with mild atherosclerosis ($p < 0.0005$). In addition, we found that 48% of cases with severe atherosclerosis were positive for HLA-class II genotypes 13 or 17 in cardiac muscle compared with 19% of cases with mild atherosclerosis ($p < 0.05$). The study showed a correlation between severe coronary atherosclerosis and *Chlamydia* in coronary arteries, high Lp(a) levels and certain HLA-DR genotypes. These results are in favor of the hypothesis that coronary atherosclerosis has a genetic and autoimmune component and may be initiated by an intracellular infection (83).

Dahlen et al. (15) found serologic evidence (IgG titers ≥32) for *C. pneumoniae* infection in 93% of patients with CAD and 78% of control subjects ($p < 0.03$). However, an IgG titer ≥1/256 in combination with Lp(a) levels ≥120 mg/liter increased the predictability of CAD ($p < 0.01$) in male patients. Certain HLA-DR class II genotypes in combination with high Lp(a) levels and *C. pneumoniae* titers were present in 14 (48%) of 29 male patients with CAD compared with 1 (4%) of 27 male control subjects ($p < 0.0005$). This study, like ours, showed an important interaction between infection and certain inherited HLA class II genotypes.

The role of *C. pneumoniae* in carotid atherosclerosis has also been evaluated in carotid endarterectomy specimens in the Atherosclerosis Risk in Communities (ARIC) (70) study by immunocytochemistry and PCR. All five fresh specimens and 32 of 56 archival formalin-embedded endarterectomy tissues were positive for *C. pneumoniae*.

Herpes simplex virus, cytomegalovirus and atherosclerosis. Human endothelial cells infected with herpesvirus demonstrate thrombin formation and increased adherence of platelets (85) and granulocytes (86). These observations provide support for the initial observation of Fabricant et al. (65) that chickens infected with herpesvirus developed vascular lesions similar to those found in human atherosclerosis. Span et al. (86) have studied cultured endothelial cells infected with CMV and shown an increased adherence of leukocytes and platelets to these cells. CMV induces major histocompatibility class I antigen expression in human aortic smooth muscle cells (87). Transfection with this virus also causes expression of genes for several cytokines (e.g., IL-6) (88).

CMV DNA has been observed in the arterial walls of patients with CAD (89,90). Infection with this virus has been postulated to be a major risk factor for the rapid development of vasculopathy or transplant atherosclerosis in patients who underwent heart transplantation (91).

Epidemiologic studies (92) have shown that antibodies against CMV are elevated in patients with CAD compared with control subjects.

In a long-term prospective evaluation of a population-based cohort, the ARIC study (70) found a significant correlation between CMV infection and carotid-intimal-medial thickness. The strongest association with CMV infection was that there was a graded relation between the odds of intimal-medial thickening and serum CMV antibody titer that remained significant even after adjustment for conventional risk factors for atherosclerosis.

Which pathogen is associated with atherosclerosis? Thus far, the strongest association of atherosclerosis appears to be with *C. pneumoniae*. The most significant association in most studies appears to be in coronary atherosclerosis. The organism has been found in mature atherosclerosis and in early lesions. An important association has been seen in whites and in temperate latitudes. The overall association between *C. pneumoniae* and coronary atherosclerosis using immunocytochemistry and direct staining techniques appears to be about 70% to 100%. The immunopositivity for *C. pneumoniae* is more prevalent in areas of severe than mild coronary atherosclerosis (83), which raises the possibility of severe or sustained infection or localization and immobilization of the organism leading to severe atherosclerosis. There appears to be a relatively low association of PCR positivity for *C. pneumoniae* with the severity or presence of atherosclerosis. The discrepancy between the PCR and immunostaining methods for the presence of *C. pneumoniae* may relate to the extremely high sensitivity of the PCR methodology. Actually, in our studies on coronary artery specimens, PCR positivity in nonatherosclerotic and minimally atherosclerotic regions was quite high.

Link Among Infection, Inflammation, Thrombosis and Atherosclerosis

Chlamydia organisms, as well as CMV, induce production of several cytokines, including TNF-alpha, IL-1 and IL-2 (88–93). These cytokines have a variety of actions, including stimulation of fibroblasts and smooth muscle cell proliferation. TNF-alpha inhibits the action of lipoprotein lipase (94), leading to altered lipid metabolism, accumulation of serum triglycerides and a decrease in serum high density lipoprotein cholesterol (HDL-C). Lipopolysaccharide, a bacterial component, binds in human serum to both HDL-C and low density lipoprotein cholesterol (LDL-C) (95) and makes LDL-C immunogenic or toxic to endothelial cells.

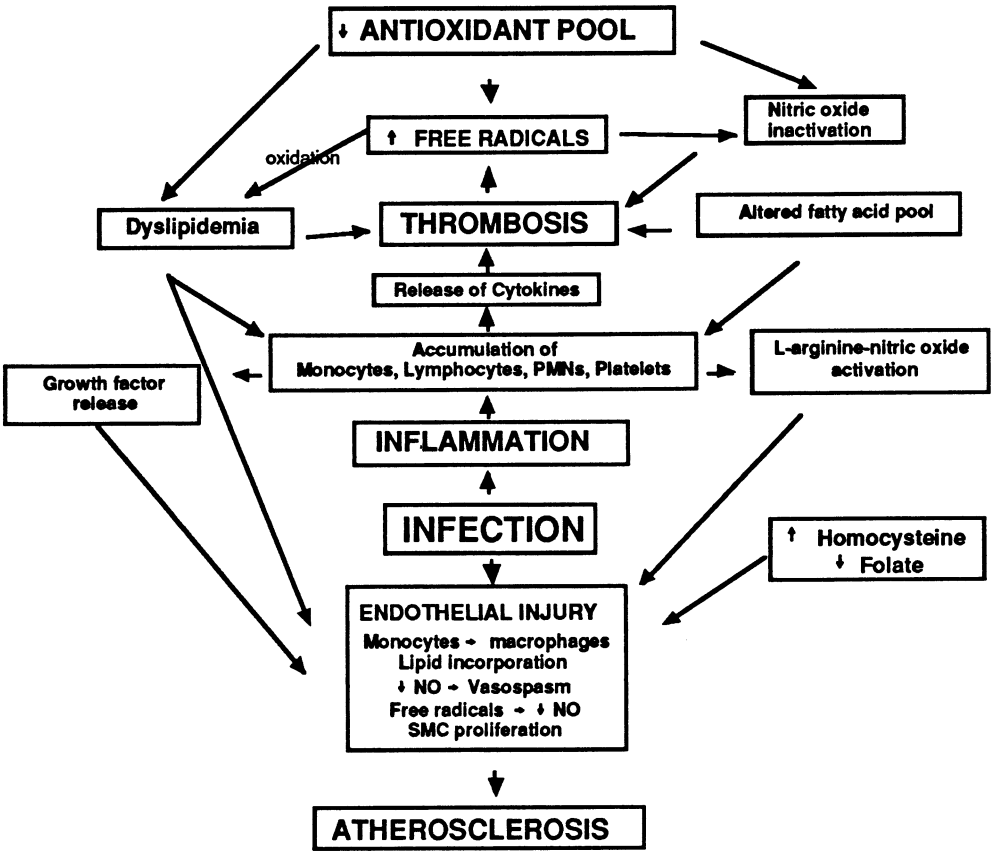

Figure 1. Postulated steps in the pathogenesis of atherosclerosis. In this postulate, infection and inflammation play an important role early in the initiation of endothelial injury. The dysfunctional endothelium permits monocyte deposition and infiltration of lipid-laden macrophages into the subendothelial layers. This is associated with a decrease in constitutive nitric oxide (NO) formation and activity. NO may be rapidly broken down by release of large amounts of free radicals, resulting in focal vasospasm. Dyslipidemia, altered folate metabolism (and resultant increased homocysteine levels) and growth factor release cause smooth muscle cell (SMC) proliferation. Deposition and activation of inflammatory cells lead to release of procoagulant cytokines and thrombosis. Increased release of free radicals and a relative deficiency of endogenous antioxidant pool may oxidize lipids, inactivate NO and enhance thrombosis and prevent thrombolysis. In this postulate, abnormalities in lipid profiles, folate metabolism and other traditional risk factors (e.g., diabetes mellitus and hypertension) play a rather peripheral role and serve to amplify the atherosclerotic process initiated by persistence of infection and inflammation. This postulate is not designed to be operative in all patients with atherosclerosis, but only in those with genetic predisposition. Major abnormalities are listed in **block letters.** PMNs = polymorphonuclear leukocytes.

Cytokines are potent inducers of a neutrophil free radical generation, which may facilitate oxidation of LDL-C, a key event in atherogenesis (9), and attract monocytes and other inflammatory cells to the area of endothelial injury. Coupled with the fibroblast and smooth muscle cell proliferating effects, leukocyte activation may lead to propagation of the atherosclerotic process (16).

Free radicals also stimulate platelet activation and leukocyte chemotaxis and may participate in the formation of thrombus in atherosclerotically narrowed arteries. Cytokines also influence the coagulation cascade by stimulating formation of endogenous tissue plasminogen activator and its fast-acting inhibitor, plasminogen activator inhibitor-1, the overall effect being stimulation of thrombus formation (43). Plasma levels of both tissue-type plasminogen activator and plasminogen activator inhibitor-1 have been shown to be increased in unstable coronary syndromes (10). Cytokines decrease the activity of constitutive nitric oxide synthase (96), a hallmark of atherosclerosis (24), and the loss of release of constitutive nitric oxide may predispose to vasospasm and in situ platelet aggregation and thrombosis.

Bacterial lipopolysaccharide is a potent stimulus for inducible nitric oxide synthase activity (24) leading to the formation of large amounts of nitric oxide, which could cause endothelial dysfunction and disruption followed by deposition of monocytes and platelets on the vessel wall, release of growth factors and migration of smooth muscle cells.

Other well known risk factors, such as smoking, hypertension, homocyteinemia and altered fatty acid pool, may play a variable, but important, role in the development of atherosclerosis in susceptible people and precipitation of an acute ischemic event. It is possible to develop a postulate wherein infection and inflammation play a very important role in atherogenesis in some subjects (Fig. 1).

Potential of Antibiotic Therapy in Atherosclerosis and CAD

Naturally if infection plays an important role in the pathogenesis of chronic CAD and its acute exacerbation, therapy with antibiotics may be a logical choice. Until recently, such therapy would have been inconceivable. On the basis of the laboratory and clinical evidence of *C. pneumoniae* infection in coronary arteries, a small trial with azithromycin was carried out in survivors of acute myocardial infarction in the United Kingdom (97). Among 213 patients with a history of recent myocardial infarction, 59 had undetectable IgG titers against *C. pneumoniae*, 74 had intermediate range titers (between 8 and 32) and 80 had high (>1/64) titers. Patients with high titers were randomized in a double-blind fashion to receive placebo (n = 20) or azithromycin 500 mg daily for 3 days (n = 28) or azithromycin 500 mg daily for 6 days (n = 12). The primary endpoints (cardiovascular death, hospital admission with unstable angina or acute myocardial infarction or need for revascularization) at a mean follow-up period of 18 months were observed in 7% of patients with undetectable initial IgG titers, 15% of patients with intermediate levels and 28% of patients with high IgG titers. Interestingly, the primary endpoints occurred in 8% of patients with high IgG titers who were treated with azithromycin. This dramatic reduction in endpoints in a small number of high risk patients with CAD suggests the potential of antibiotic therapy in preventing acute cardiac events.

Therapy with other antibiotic agents with greater activity toward *C. pneumoniae* is in the planning phases.

Conclusions

Atherosclerosis is a multifaceted disease process with several different well defined risk factors. It is unlikely that a common pathway or pathogenic process can explain this malady, which affects one-third of the world population. We propose that in some genetically susceptible people or populations, a primary or repeated episodes of infection with a common organism (e.g., *C. pneumoniae* or CMV) may result in localization of the organism in arteries that are prone to hemodynamic stress. A deficiency of endogenous protective mechanisms or immune response may prompt persistent growth of the organism in situ and a state of inflammation, platelet activation, vasospasm and thrombosis may ensue. Oxidation of LDL-C and alterations in lipid metabolism may participate secondarily in the process of inflammation. Acute exacerbation of chronically inflamed atherosclerotic tissues may be the basis of rupture of plaque or plaque thrombosis and hemorrhage resulting in the clinical syndromes of unstable angina and acute myocardial infarction. This scenario obviously is not meant or designed to explain atherosclerosis or its manifestations in all people, but it may be quite relevant in many patients. The infectious basis of atherosclerosis may underlie the variable rates of prevalence of CAD in different parts of the world and the rapidly changing patterns of morbidity and mortality from CAD in the United States and elsewhere, which cannot be explained by conventional risk factors. Despite the reported link between atherosclerosis and the presence of *Chlamydia* infection, the cause and effect relation remains to be proved. We believe that large clinical trials of therapy with antibiotics should be conducted to determine whether *C. pneumoniae* plays a critical role in the pathogenesis of atherosclerotic CAD. Meanwhile, large-scale studies need to be done to examine individual susceptibility to *C. pneumoniae* infection and its correlation with atherogenesis.

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