MINIREVIEWS

Role of Infectious and Immune Factors in Coronary and Cerebrovascular Arteriosclerosis

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Arteriosclerosis is the main cause of coronary heart and cerebrovascular disease which, in turn, are the most common causes of death in the industrialized world. An acute event in coronary heart or cerebrovascular disease is typically precipitated by thrombosis occurring at the site of arteriosclerotic plaque disruption. Arteriosclerotic plaques consist of a fibrous cap overlying a lipid-rich core. Many cell types are involved in their formation, including platelets, endothelial cells, activated monocytes, macrophages derived from monocytes, and smooth muscle cells. Macrophages and T lymphocytes are critical in the growth and change of plaques through the secretion of cytokines such as gamma interferon (IFN-γ) and interleukin-1 (IL-1), and extracellular matrix-digesting enzymes such as metalloproteinases, which weaken fibrous caps.

The currently accepted hypothesis is that arteriosclerosis develops as a response to injury and that it is primarily a chronic inflammatory condition. Infections have long been postulated to play an important role in the etiology of arteriosclerosis. Two patterns of association have emerged: a link between chronic low-grade inflammation or infection and the slow process of arteriosclerosis and an association between an acute systemic inflammatory response and a transiently increased risk of an acute cardiovascular event.

New interest in the role of infections and inflammatory mechanisms for the pathogenesis of coronary and cerebral artery diseases is based on the results of seroepidemiologic studies, studies about inflammatory mediators and endothelial dysfunction, and recent studies evaluating the role of antibiotic therapy in arteriosclerosis. The present report reviews the current status of knowledge regarding the role of different infectious agents and of inflammatory and immune mediators in the pathogenesis of coronary and cerebral artery disease and discusses the possible implications of these mechanisms with regard to diagnosis and therapy. We do not discuss the complex entity of vasculopathies of different causes, including cardiac allograft vasculopathy, but restrict this review to arteriosclerosis.

BACTERIAL AND VIRAL INFECTIONS AND ARTERIOSCLEROSIS

Infectious pathogens. (i) Helicobacter pylori. H. pylori is a gram-negative spiral bacterium and the main etiological factor in gastritis and peptic ulcer disease. H. pylori infection is postulated to have an effect on clotting mechanisms (47). A further hypothesis suggests that exposure to H. pylori may lead to an increased risk of arteriosclerosis by an autoimmune process (47). Seroepidemiological studies about the role of H. pylori in the development of coronary and cerebrovascular arteriosclerosis yielded controversial results. Several authors found an association (41, 46, 64, 79; M. Jankovic, A. Hirschli, S. Guber, M. Kundi, and W. Lalouschek, Abstr., Cerebrovasc. Dis. 11:106, 2001), whereas others did not (16, 26, 29, 47, 77). Further studies found an association, adequately explained by the much stronger association of H. pylori infection with age, male gender, and social class, which are linked with coronary heart disease (68, 87, 95). In a study of socioeconomically homogeneous men, controlled for age and smoking, limited evidence of association between H. pylori exposure and risk for future myocardial infarction was found (82). A population-based study did not find an association of elevated H. pylori antibodies with ischemic stroke; however, H. pylori infection was associated with strokes caused by small-artery occlusion (39). To explain the contradictory results regarding the association of H. pylori with arteriosclerosis, it was suggested that strains expressing the virulent cytotoxin-associated gene product A (CagA) are more strongly related to coronary heart disease than are other strains (101). A study in late-middle-aged men, however, showed that CagA-positive strains appear to be no more strongly related to the disease than other strains (101).

(ii) Chlamydia pneumoniae. C. pneumoniae is a gram-negative intracellular bacterium. An association of coronary heart disease and infections with C. pneumoniae, an important respiratory pathogen, has been initially found by seroepidemiologic studies (88). The presence of C. pneumoniae has been shown in atheromatous plaques of coronary and carotid arteries (14, 104). An immune reaction to chlamydial Hsp60 has been shown in coronary atheroma tissue, especially in macrophages and foam cells (2). In human carotid artery plaques, C. pneumoniae-reactive T lymphocytes have been identified (71). In an autopsy study, examining coronary artery specimens, it has been found that intracellular infection with C. pneumoniae...
relates to the severity of arteriosclerosis, whereas serum antibody titers did not (25).

Seroepidemiological studies of the role of *C. pneumoniae* in the development of coronary and cerebrovascular arteriosclerosis yielded controversial results. Several authors found an association (15, 26, 77, 85, 91, 93; Jankovic et al., Cerebrovasc. Dis. 11:106), whereas others did not (16, 19, 39, 41, 42, 61, 63, 83, 95, 100). The main explanations for these controversial findings are that the studies were done in different populations, used different criteria for controls, were adjusted for potential confounders to different degrees, and were, therefore, prone to different biases. Furthermore, most of these seroepidemiological studies detected *C. pneumoniae* antibodies by microimmunofluorescence, a method with inherent diagnostic problems (17). Although there are many indicators, no definite proof exists that the presence of *C. pneumoniae* causes either initiation of arteriosclerosis or activation of an arteriosclerotic plaque.

A further possible role of *C. pneumoniae* in the pathogenesis of arteriosclerosis could be that chronic or acute chlamydial infection anywhere in the body activates arteriosclerotic plaques. An association between immunoglobulin A (IgA), but not IgG, antibodies to *C. pneumoniae* and the subsequent risk of death from ischemic heart disease, the risk of ischemic stroke, and the severity of arteriosclerosis of carotid or femoral arteries has been found (23, 64, 96). In patients undergoing carotid endarterectomy, the plaques were investigated for *C. pneumoniae* and the blood was examined for antichlamydial antibodies. Associated with symptomatic disease was a high-level of antichlamydial IgA, whereas no association between *C. pneumoniae* presence in the plaque and symptomatic disease could be detected (52).

Furthermore, there are indications that seropositivity for *C. pneumoniae* might enhance the atherogenic effects of other vascular injuries. A case-control study suggests that the proatherogenic effects of lipoprotein may be enhanced or partly mediated through the formation of circulating immune complexes containing *C. pneumoniae*-specific IgG antibodies (32). The simultaneous presence of high levels of antibodies to *C. pneumoniae* and of antibodies to human Hsp60 have been found to substantially increase the risk for coronary arteriosclerosis (11).

(iii) **Microorganisms associated with periodontitis.** Dental infections appear as cardiovascular risk factors in some cross-sectional studies and in follow-up studies, and the association is independent of the “classic” coronary risk factors (4, 62). It is hypothesized that this association may be due to an underlying inflammatory response trait, which places an individual at high risk for developing both periodontal disease and arteriosclerosis. Furthermore, periodontal disease might provide a biological burden of inflammatory cytokines that promote arteriosclerosis and thromboembolic events. In an in vitro model of thrombosis, platelet aggregation-associated protein has been found to be expressed on *Streptococcus sanguis* and on *Porphyromonas gingivalis*, both of which are involved in periodontitis (38). Further data and prospective studies are needed to assess the role of dental infections in the pathogenesis of arteriosclerosis.

(iv) **CMV and herpes simplex virus.** Cytomegalovirus (CMV) is the largest of the herpesviruses. CMV infection of endothelial cells may increase cellular proliferation and inhibit apoptosis of infected smooth-muscle cells, thereby contributing to an increase in the mass of arteriosclerotic lesions. Furthermore, individuals infected with CMV have impaired endothelium-mediated coronary vasodilator responses (24). Herpes simplex virus types 1 and 2 have been found in human arteriosclerotic lesions (5).

Seroepidemiological studies of the role of CMV and herpes simplex virus types 1 and 2 in the development of coronary and cerebrovascular arteriosclerosis have yielded controversial results. Several authors found an association (10, 26, 73, 85, 93; Jankovic et al., Cerebrovasc. Dis. 11:106), whereas others did not (16, 27, 41, 64, 98). These conflicting data are again explained by methodological causes (17). Furthermore, individual and gender differences in the host response to CMV might account for the disparate results. Two studies in patients undergoing coronary angiography have shown that CMV elicits a subclinical inflammatory response, but only in certain individuals, and that individuals with an inflammatory response appear to be susceptible to the atherogenic effects of CMV, whereas those without such a response appear to be resistant (9, 107). Postulating that sex might have an effect on patterns of inflammatory and immune responses to CMV infection, a study in patients evaluated for coronary artery disease found that, in men, CMV appears to contribute to coronary artery disease risk, insofar as it predisposes to inflammation. In women, other mechanisms, possibly related to the type of immune response generated by the host, appear to be responsible for the proatherogenic effects of CMV (109).

(v) **HAV.** Hypothesizing that intracellular pathogens associated with a persistent antibody response may contribute to arteriosclerosis, the authors of one study selected hepatitis A virus (HAV) as a candidate pathogen for a serologic examination of patients undergoing coronary angiography. In this study, HAV seropositivity was an independent predictor of risk for coronary artery disease and elevated CRP levels (106).

(vi) **HIV.** The common cardiac manifestations in patients with AIDS are pericardial effusion, myocarditis, dilated cardiomyopathy, endocarditis, pulmonary hypertension, malignant neoplasms, and drug-related cardiotoxicity. However, there have been some reports of acute coronary thrombotic events in patients infected with human immunodeficiency virus (HIV). One or more atherosclerotic plaques within carotid arteries were found in a large proportion of middle-aged HIV-positive individuals. The presence of arteriosclerosis within this population was not associated with the use of protease inhibitors but rather with “classic” cardiovascular risk factors such as smoking and hyperlipidemia, which are amenable to interventions (20). Autopsy studies in HIV-positive children and young adults suggest the presence of an underlying arteriopathy. A recent study demonstrated that the HIV envelope protein, gp120, activates human arterial smooth-muscle cells to express tissue factor, the initiator of the coagulation cascade. The activation of smooth muscle cells by gp120 may play an important role in the vascular, thrombotic, and inflammatory responses to HIV infection (89).

**Infectious burden.** Most of the published studies thus far have investigated the association of only one microorganism with arteriosclerosis. The number of different pathogens to which an individual has been exposed, i.e., the “infectious bur-
den”, possibly may have an influence on the development of arteriosclerosis. Only a few studies have investigated the impact of viral and bacterial infectious burdens. One study in 120 post-myocardial infarction patients ≥50 years in age found that, after adjustment for coronary risk factors and socioeconomic status, the combination of seropositivity to both C. pneumoniae and CMV infection was associated with an increased inflammatory response and a markedly increased risk of premature myocardial infarction (30). In 105 patients with transient ischemic attacks or minor stroke, combined seropositivity to C. pneumoniae, H. pylori, and CMV lead to an elevated risk ratio (Jankovic et al., Abstr., Cerebrovasc. Dis. 11:106). In a coronary heart disease primary prevention trial, high levels of antibody to either herpes simplex virus type 1 or C. pneumoniae indicated increased the risk for coronary events independently of the other, and their joint effect was close to additive (85). One prospective study in 1,018 patients with angiographically documented coronary artery disease evaluated the effect of eight infectious pathogens (herpes simplex virus types 1 and 2, CMV, Epstein-Barr virus, Haemophilus influenzae, C. pneumoniae, Mycoplasma pneumoniae, and H. pylori) on the risk for cardiac death. Seropositivities to Epstein-Barr virus, H. pylori, and herpes simplex virus type 2 were independently associated with cardiac death. An increasing number of pathogen burden was significantly predictive of the long-term prognosis. Seropositivities from 0 to 3, 4 to 5, and 6 to 8 were associated with increasing mortalities of 3.7, 7.2, and 12.6%, respectively. Patients seropositive to ≥5 pathogens compared with those seropositive to <4 pathogens had a 5.1 higher risk of future cardiac death (86). Similar results are reported from a further study which included HAV in the spectrum of pathogens (105).

MARKERS AND MEDIATORS OF INFLAMMATION, IMMUNE RESPONSE, AND ARTERIOSCLEROSIS

Acute-phase reactants. One of the markers of inflammation are the acute-phase proteins, which are, regulated by cytokines, produced in the liver. Further markers of inflammation are decreased serum albumin levels and an increased leukocyte count.

(i) CRP. C-reactive protein (CRP), a major acute-phase protein, is a marker of systemic inflammation. CRP induces the expression of cell adhesion molecule in human endothelial cells and thus may play a direct role in promoting the inflammatory component of arteriosclerosis (78). It has been shown that the CRP concentration correlates with the presence and severity of coronary arteriosclerosis and the risk for acute cardiovascular events (36, 48, 55, 69). Levels of CRP in serum were higher in patients with unstable angina than in patients with stable angina (12). During follow-up, however, CRP levels were not predictive for coronary events in patients with acute coronary syndromes (16).

In a cross-sectional study including men, participants with prevalent coronary heart disease had markedly higher fibrinogen levels than participants without coronary heart disease. The associations weakened after further adjustment for central obesity (45). In a study on patients with stable angina pectoris, the level of fibrinogen in serum was an independent predictor of cardiovascular death, nonfatal myocardial infarction, and the risk of revascularization (37). In contrast, in patients with acute coronary syndromes the fibrinogen levels were not predictive for coronary events during follow-up (16).

(ii) Fibrinogen. Plasma fibrinogen is an acute-phase protein and a hemostatic factor. In a cross-sectional study including men, participants with prevalent coronary heart disease had markedly higher fibrinogen levels than participants without coronary heart disease. The associations weakened after further adjustment for central obesity (45). In a prospective study in the general British male population, baseline values of SAA were associated with future risk of coronary heart disease. However, it was not investigated whether this association was independent of possible confounders. No strong associations of SAA with H. pylori seropositivity or C. pneumoniae IgG titers were observed (16, 18). In patients with stable and unstable angina, no associations of SAA concentrations with the risk of coronary events were detected (16, 36). Again, the role of SAA as a risk factor for the development of arteriosclerosis is uncertain, and further studies are needed to determine whether this association is independent of possible confounders.

(iv) Albumin. During inflammation, the concentration of albumin in serum may decrease by up to 20%. Some studies indicate that patients with lower albumin levels have a higher risk for developing arteriosclerosis (18).

(v) Leukocyte count. Elevated leukocyte levels may lead to arteriosclerotic events either by an effect on chronic inflammation or by inducing acute thrombotic events. A relative elevation in leukocyte count was found to be associated with carotid arteriosclerosis, but this relationship differed by race ethnicity, being strongest in Hispanics, intermediate in black non-Hispanics, and not present in white non-Hispanics (22). In a study of patients with stable angina pectoris, the leukocyte count was an independent predictor of cardiovascular death, nonfatal myocardial infarction, and the risk of revascularization (37). A further study, investigating the leukocyte count at baseline and infections. One study in the general British male population found that baseline values of CRP were associated with future risk of coronary heart disease, but no strong associations of CRP with H. pylori seropositivity or C. pneumoniae IgG titers were observed (18). Similar findings were reported in a study on patients with acute coronary syndromes (16). In another prospective follow-up study in patients with coronary heart disease, the combination of CMV seropositivity and elevated CRP levels was identified as a strong, independent predictor of mortality (73). It remains uncertain whether CRP is an independent risk factor for coronary heart and cerebrovascular arteriosclerosis. First, in many studies, the associations weakened after baseline confounding factors were adjusted for (18). Second, no direct evidence exists showing that CRP directly contributes to vascular damage.

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(iii) SAA. Serum amyloid A (SAA) is an acute-phase protein and apolipoprotein. In a cross-sectional study including men, participants with prevalent coronary heart disease had markedly higher SAA levels than did participants without. The associations weakened after further adjustment for central obesity (45). In a prospective study in the general British male population, baseline values of SAA were associated with future risk of coronary heart disease. However, it was not investigated whether this association was independent of possible confounders. No strong associations of SAA with H. pylori seropositivity or C. pneumoniae IgG titers were observed (16, 18). In patients with stable and unstable angina, no associations of SAA concentrations with the risk of coronary events were detected (16, 36). Again, the role of SAA as a risk factor for the development of arteriosclerosis is uncertain, and further studies are needed to determine whether this association is independent of possible confounders.

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the development of coronary heart disease, did not find an association between leukocyte count and development of coronary heart disease (18). The percentage of aggregated leukocytes in patients with unstable angina is significantly increased compared to that of patients with no evidence of active coronary artery disease (54). This aggregation may be mediated by cellular adhesion molecules (CAMs).

**Indicators of activation of the immune system.**

(i) **Lymphocytes.** T lymphocytes are found in large numbers of human atherosclerotic plaques, indicating that immune and inflammatory mechanisms are important factors in the pathogenesis of atherosclerosis. Upon activation by target cells, T-helper lymphocytes (predominantly CD4+ cells) secrete cytokines. In human coronary atherectomy tissue, T-helper (CD4) cells predominate over suppressor (CD8) cells and B lymphocytes (70). In vitro studies have shown that T lymphocytes from patients with unstable angina, but not those from patients with chronic stable angina or normal control subjects, proliferated in response to autologous proteins from coronary plaques and/or to oxidized low-density lipoproteins (13).

Patients with ischemic heart disease show an increase in the level of circulating cytotoxic T lymphocytes compared to normal control subjects (67). Peripheral blood lymphocytes of patients with unstable angina are immunologically activated and produce soluble factors which may allow their interaction with endothelial cells in areas of inflammation. Circulating activated CD4 and CD8 lymphocytes are suggested to be involved in the inflammatory reaction during episodes of unstable angina (76). The numbers of CD4+ and CD3+/DR− cells were higher in patients with unstable angina than in patients with stable angina (12). In unstable angina, the percentage of double-positive CD3+/DR− cells significantly increased at days 7 to 15 and returned to baseline levels at 6 months. The increment of circulating CD3+/DR− cells was inversely related to the admission levels of CRP and was associated with a better outcome (12).

(ii) **Neopterin.** Neopterin is a by-product of activated macrophage metabolism, and the levels of neopterin increase in several systemic inflammatory and infectious diseases. In patients with unstable angina, those with elevated neopterin levels were more likely to be diagnosed with a non-Q-wave acute myocardial infarction and were more likely to have more severe and extensive atherosclerosis than were patients with low neopterin levels (35). During follow-up, however, neopterin levels were not predictive for coronary events in patients with acute coronary syndromes (16).

(iii) **IgA, IgE, IgG, and IgM.** In patients with coronary artery disease, the levels of IgM, but not of IgA and IgG, in serum were higher than those in healthy controls (97). In patients with unstable angina, IgM levels in serum were higher than in stable angina patients (12). In unstable angina, the levels of IgM in serum significantly increased at days 7 to 15 and returned to baseline levels at 6 months (12). Furthermore, in middle-aged men at increased risk of myocardial infarction, hypertriglyceridemia and low levels of high-density lipoprotein cholesterol were associated with an increased risk of a coronary endpoint only if the levels of IgA, IgE, and IgG were also elevated (50).

(iv) **Serum complement.** (a) Total serum complement. Women without coronary artery disease but with atherosclerotic risk factors (diabetes or hypertension) had significantly elevated levels of total complement in serum compared with a control group. The complement levels were highest in women with a combination of diabetes and hypertension (60).

(b) **Complement C3.** The levels of complement C3 in serum in patients with coronary artery disease were higher than those in healthy controls (97). Women without coronary artery disease but with atherosclerotic risk factors (diabetes or hypertension) had significantly elevated levels of complement C3 compared with a control group. These levels were most elevated in women with a combination of diabetes and hypertension (60).

(v) **Circulating immune complexes.** Women without coronary artery disease but with atherosclerotic risk factors (diabetes or hypertension) had significantly elevated levels of circulating immune complexes compared with a control group. These levels were most elevated in women with a combination of diabetes and hypertension (60).

(vi) **Circulating antitissue antibodies.** Women without coronary artery disease but with atherosclerotic risk factors (diabetes or hypertension) had significantly elevated levels of circulating antitissue antibodies compared with a control group. Women with a combination of diabetes and hypertension had the highest levels of antitissue antibodies (60).

**Cytokines.** During inflammation, cytokines are produced by inflammatory cells in the damaged tissue. Cytokines are also produced in fat tissue. Proinflammatory cytokines exert a number of important effects on vascular reactivity. At one end of the spectrum, cytokines such as IL-1 may induce vascular paralysis. At the other end of the spectrum, cytokines such as tumor necrosis factor can induce endothelial dysfunction and impair vascular dilatation (92).

(i) **IFN-γ.** The inflammatory cytokine IFN-γ has been found to be increased in patients with unstable angina, suggesting that it is probably related to myocardial cell damage or to plaque rupture and thrombus formation (66). Furthermore, it has been shown that in patients with unstable angina pectoris, monocytes are activated by IFN-γ (56).

(ii) **MCP-1.** Levels of the inflammatory cytokine monocyte chemotactic protein 1 (MCP-1) have been found to be increased in patients with unstable angina, suggesting that MCP-1 is probably related to myocardial cell damage or to plaque rupture and thrombus formation (66).

(iii) **IL-2.** Variable IL-2 receptor subtype expression occurs in mononuclear leukocytes infiltrating chronic human atheroma (70). An increase in the percentage of IL-2 receptor-positive T lymphocytes was found in culprit lesions of patients with acute coronary syndromes (99). Levels of IL-2 in serum were higher in patients with unstable angina than in patients with stable angina (12). In unstable angina, the levels of IL-2 in serum significantly increased at days 7 to 15 and returned to baseline levels at 6 months (12). The IL-2 level was increased in patients with acute ischemic syndrome and patients with stable angina compared to healthy controls (67).

(iv) **IL-6.** The level of IL-6 was increased in patients with acute ischemic syndrome compared to patients with stable angina and healthy controls (67). During follow-up, however, IL-6 levels were not predictive for coronary events in patients with acute coronary syndromes (16). A further study in pa-
tients with coronary heart disease showed that in patients with elevated IL-6 levels, CMV seropositivity was independently associated with a 3.2-fold increase in risk of future cardiac death, whereas in individuals without IL-6 elevation, a previous CMV infection had no effect on cardiac mortality (9).

**Indicators of autoimmunity.** It has become apparent that the immune system is actively involved in the process that governs the progression of both early and the mature atherosclerotic plaques. Autoimmune mechanisms have been shown to take part in driving an inflammatory state within the atheroma. Among the candidate autoantigens dominating these processes are the Hsps, oxidized low-density lipoproteins, and endothelial cell-bound antigens.

(i) **Antibodies to Hsps.** Hsps protect other proteins from denaturation and are produced after injuries involving heat, infections, mechanical stress, oxidants, and cytokine stimulation. Cells of the arterial wall have been found to produce high levels of Hsp when exposed to these stress factors. However, Hsps bear the risk of autoimmunity because of their high amino acid sequence homology between different species from prokaryotes to humans. “Antigenic mimicry” may be caused by an immunological cross-reaction between microorganisms and autoantigens and thus may play an important role in the process of vascular endothelial injury (65). It has been suggested that the immune response to Hsp60 or Hsp65 may be a link between exposure to microorganisms and increased cardiovascular risk. In a prospective population-based study, antibodies to mycobacterial Hsp65 in serum were correlated with seropositivity to *C. pneumoniae* and *H. pylori* (64).

The antibody titers to mycobacterial Hsp65 or Hsp60 were strongly associated with carotid and coronary arteriosclerosis (8, 102, 103, 108). In a case-control study in patients undergoing coronary angiography, the simultaneous presence of antibodies to *C. pneumoniae* and high levels of antibodies to human Hsp60 were found to be associated with coronary heart disease (11).

(ii) **Antibodies to oxidized low-density lipoprotein.** Oxidation of low-density lipoprotein occurs in the atherosclerotic plaque and induces generation of antibodies to oxidized low-density lipoprotein. These antibodies have been linked to risk for myocardial infarction, especially in patients with diabetes mellitus (57, 80).

(iii) **AECA.** After percutaneous transluminal coronary angioplasty, anti-endothelial cell antibody (AECA)-positive patients have been shown to have a higher rate of restenoses than do AECA-negative patients (28). However, among chest pain patients, those with no coronary artery disease had levels of AECA that did not differ from those in patients with coronary artery disease (31).

(v) **CAMs.** The adhesion and transendothelial migration of leukocytes is thought to be important in the pathogenesis of arteriosclerosis. These processes are mediated largely by CAMs. Upon histological analysis, human atherosclerotic plaques contain many CAMs. CRP induces the expression of CAMs in human endothelial cells (78). Patients with coronary heart disease were shown to have higher soluble vascular cellular adhesion molecule 1 (sVCAM-1) and endothelial selectin levels than do patients with normal coronary arteries (90). In patients presenting with unstable angina and non-Q-wave infarction, the levels of soluble intercellular adhesion molecule 1 (sICAM-1), sVCAM-1, endothelial selectin, and platelet selectin were elevated at presentation, remained elevated for 6 months compared to healthy controls, and decreased over the following 6 months (74, 94). Whether CAMs have a clinical value in determining cardiovascular risk is at present uncertain. One study showed that in the prediction of coronary heart disease sICAM-1 is unlikely to add much predictive information to that provided by more established risk factors (59). A further study, however, showed that platelet selectin levels are elevated among apparently healthy women at risk for future vascular events (81).

**VACCINATION AND ARTERIOSCLEROSIS**

It has been suggested that acute respiratory infection may be a risk factor for myocardial infarction and hypothesized that influenza vaccine might reduce the risk of recurrent myocardial infarction. A case-control study in patients with coronary heart disease found that vaccination against influenza was negatively associated with the development of myocardial infarction during the same influenza season (75). On the other hand, it has been hypothesized that vaccination may promote atherosclerosis by immunostimulation (53). Vaccination, particularly with heat-inactivated bacteria, may contribute to the development of anti-Hsp antibodies (65).

**ENDOTHELIAL DYSFUNCTION, INFECTION, AND INFLAMMATION**

The endothelium plays an important role in regulating vascular blood flow, and it is now apparent that endothelial dysfunction is an important contributor to the pathogenesis of arteriosclerosis. Recently, infection and inflammation have been linked to endothelial dysfunction (6). The mechanisms by which inflammation may induce endothelial dysfunction are not fully understood. Cytokines have been identified to play a role in this process (7, 40). In men with coronary heart disease it has been shown that CRP concentration, CDS lymphocytes expressing ICAM-1, and antibodies to oxidized low-density lipoprotein were determinants of endothelium-dependent vascular dysfunction (92).

**THERAPY ATTEMPTED TO INFLUENCE INFECTIONS, INFLAMMATORY PATHWAYS, AND IMMUNOLOGY**

**Statins.** Large-scale clinical trials demonstrated significant reductions in cardiovascular event rates with statin therapy. The observed benefit of statin therapy, however, was larger in these trials than that expected on the basis of lipid lowering alone. Emerging evidence from both clinical trials and basic science studies suggests that statins have anti-inflammatory properties, which may also lead to clinical efficacy. Statin therapy reduced the level of CRP independently of its effect on lipid levels (1, 45, 49, 84). Thus, statin therapy might be effective in the primary prevention of coronary events among subjects with relatively low lipid levels but with elevated levels of CRP. Again, this hypothesis needs to be verified in large prospective trials, which are currently under way.

**Antibiotics.** If infection is the cause of arteriosclerosis, treatment with antibiotics should theoretically result in preventing some of the clinical manifestations of coronary and cerebrovascular arteriosclerosis. In investigating the relationship be-
between prior antibiotic use and cardiac events, a population-based case-control study found little or no association between use of macrolide or tetracyclin antibiotics during the previous 5 years and the risk of first myocardial infarction (44). Exposures to short courses of antibiotics during the previous 2 years were not associated with a lower risk of ischemic stroke in patients aged 65 years and older (58). Long-term azithromycin treatment did not affect levels of the adhesion molecules in plasma over a period of 6 months (90). In a small study of patients after percutaneous coronary angioplasty with confirmed 

\( H. pylori \) infection, eradication therapy with clarithromycin, amoxicillin, and omeprazole for 1 week significantly attenuated reduction of the coronary artery lumen (51).

Up to now, three treatment trials with antibiotics for secondary prevention of coronary artery disease events in humans have been carried out (see Table 1) (33, 34, 72). To our knowledge, no trials regarding antibiotics for secondary prevention of stroke in humans have been published. A further study, published only as an abstract, in patients with acute coronary syndrome found that a 1-week therapy of either azithromycin plus metronidazole or amoxicillin plus metronidazole lead to a better clinical outcome within 1 year than did a placebo (A. F. M. Stone, M. Mendall, J. C. Kaski, S. Gupta, J. Camm, and T. Northfield, Abstr., J. Am. Coll. Cardiol. 37:349A, 2001). Further trials are under way to investigate the impact of a 3-month (21) or 1-year (43) course of azithromycin on the progression of coronary heart disease in patients with prior myocardial infarction. According to current knowledge, there are no indications to use antibiotics for secondary prevention of arteriosclerosis, except within well-designed randomized clinical trials. Furthermore, prolonged use of antibiotics in a large population may result in antibacterial resistance, which is a major public concern, especially since the relationship between infection and arteriosclerosis is far from proven.

| Parameter | Gupta et al. (33) | Gurfinkel el al. (34) | Muhlestein et al. (72) |
|-----------|------------------|---------------------|---------------------|
| Yr published | 1997 | 1999 | 2000 |
| Agent* | AZITH | ROXIT | AZITH |
| Treatment duration | 6 days | 30 days | 3 mo |
| No. of patients | 80 | 202 | 302 |
| Enrollment status | Stable CAD, IgG > 1:64 | Unstable angina | Stable CAD, IgG > 1:16 |
| Follow-up period (mo) | 18 | 6 | 24 |

Cardiovascular mortality

| % Active | % Placebo | % Active | % Placebo |
|----------|----------|----------|----------|
| NG* | 2.2 | 2.2 | 3.3 |
| Placebo | 5.2 | 5.2 | 2.6 |

Cardiac events*

| % Active | % Placebo | % Active | % Placebo |
|----------|----------|----------|----------|
| 8 | 14.6 | 8 | 16.4 |

a Sum of cardiovascular mortality, nonfatal myocardial infarction, admission for unstable angina pectoris, or unplanned revascularization data.

AZITH, azithromycin; ROXIT, roxithromycin.

CLINICAL UTILITY OF CIRCULATING MARKERS FOR RISK STRATIFICATION IN UNSTABLE ANGINA

In patients with unstable angina, it is important to identify the subgroup of patients who are at the highest risk for adverse events in order to target the most intensive therapy to these patients. Measurement of markers of myocyte necrosis, such as creatine kinase MB, myoglobin, and troponin I, are only partially successful in risk stratification. Recently, it has been shown, that pregnancy-associated plasma protein A (PAPP-A), a potentially proarteriosclerotic metalloproteinase, is present in unstable coronary plaques and that circulating levels are elevated in acute coronary syndromes. Thus, PAPP-A is a new candidate marker of unstable angina and acute myocardial infarction (3).

CONCLUSION

Arteriosclerosis is a multifactorial disease. Traditional risk factors are associated with arteriosclerosis in a significant number of patients. According to current knowledge, there is only a weak association between infection and the precipitation or acute manifestations of arteriosclerosis. The study results are very contradictory, mainly due to methodological causes. Additionally, many studies have included only male patients, thus leaving open the question as to whether the results are applicable to both genders. Further prospective large investigations need to be conducted to study the association of infection with the pathogenesis of arteriosclerosis. If the infectious pathogens associated with arteriosclerosis are clearly demonstrated, large studies can be designed to assess the efficacy of antibiotics in a selected group of patients in whom infection is the major underlying cause. Furthermore, the anti-inflammatory role of other drugs, such as statins, and their benefit in primary and secondary prophylaxis of arteriosclerosis needs to be evaluated. In the meantime, we should concentrate on the “old-fashioned” risk factors for arteriosclerosis, encouraging patients to stop smoking and to treat their elevated blood pressure, blood glucose, and lipid levels.

REFERENCES

1. Albert, M. A., E. Danielson, N. Rifai, and P. M. Ridker for the PRINCE Investigators. 2001. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA 286:64–70.

2. Bauriedel, G., R. André, J. A. Likangu, A. Welz, P. Braun, U. Welsch, and B. Lüderitz. 1999. Persistenz von Chlamydia pneumoniae in koronarem
Epstein, S. E., Y. F. Zhou, and J. Zhu. 1999. Infection and atherosclerosis. Emerging mechanistic paradigms. Circulation 100:20–28. [Online.]
prevalent coronary heart disease—baseline findings of the PAIS project. Atherosclerosis 156:157–166.

46. Kahau, T., P. Lundman, G. Olsson, and M. Wendt. 2000. Greater than normal prevalence of seropositivity for Helicobacter pylori among patients who have suffered myocardial infarction. Coronary Artery Dis. 11:523–526.

47. Kinsella, A., T. Freir, C. T. Baja, K. Bourgeois, and N. Vakil. 1998. A prospective, controlled study of Helicobacter pylori seropositivity in coronary artery disease. Am. J. Gastroenterol. 93:717–720.

48. Koenig, W., M. Sund, M. Fröhlich, H.-G. Fischer, H. Löwel, A. Döring, W. L. Hutchinson, and M. B. Bryns. 1999. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men. Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study. Circulation 99:1940–1949. Publication 99:237–242.

49. Koth, H., K. Dalhoff, J. Rupp, A. Müller, J. Kreuzer, M. Maass, and H. A. Katus. 2000. Hydromethylglycine xanthine A reductase inhibitors modify the inflammatory response of human macrophages and endothelial cells cocultured with Chlamydia pneumoniae. Circulation 101:760–1763.

50. Kovánek, P. T., M. Mänttäri, T. Palosuo, V. Manninen, and K. Aho. 1998. Prediction of myocardial infarction in dyslipidemic men by elevated levels of immunoglobulin classes A, E, and G, but not M. Arch. Intern. Med. 158:143–145.

51. Kowalski, M. P., C. Konturek, P. Pieniazek, A. Karczewska, A. Kluczka, R. Growe, K. Wranich, R. Nasseri, J. Thale, E. G. Hahn, and S. J. Konturek. 2001. Prevalence of Helicobacter pylori infection in coronary artery disease and effect of its eradication on coronary lumen reduction after percutaneous coronary angioplasty. Digest Liver Dis. 33:222–229.

52. LaBiche, R., D. Koziol, T. C. Quinn, C. Gaydos, S. Azhar, G. Ketron, S. Kowalski, M., P. C. Konturek, P. Pieniazek, E. Karczewska, A. Kluczka, R. Kovanen, P. T., M. Mänttäri, W., M. Sund, M. Fröhlich, K. Dalhoff, J. Rupp, A. Mählich, H.-G. Fischer, H. Löwel, A. Döring, W. L. Hutchinson, and M. B. Bryns. 1999. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men. Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study. Circulation 99:1940–1949. Publication 99:237–242.

53. Lamb, D. J., and G. A. Ferns. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

54. Leibovitz, E., Y. Hertz, E. Liberman, S. Sclarovsky, and S. Berliner. 1997. Increased adhesiveness of white blood cells in patients with unstable angina: additional evidence for an involvement of the immune-inflammatory system. Clin. Cardiol. 20:1017–1020.

55. Lindahl, B., H. Toss, A. Siegbahn, C. Gaydos, and L. Wallentin. 2001. Presence of Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

56. Lamb, D. J., and G. A. Ferns. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

57. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

58. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

59. Mänttäri, M., T. Palosuo, V. Manninen, and K. Aho. 1998. Prediction of myocardial infarction in dyslipidemic men by elevated levels of immunoglobulin classes A, E, and G, but not M. Arch. Intern. Med. 158:143–145.

60. Kowalski, M. P., C. Konturek, P. Pieniazek, A. Karczewska, A. Kluczka, R. Growe, K. Wranich, R. Nasseri, J. Thale, E. G. Hahn, and S. J. Konturek. 2001. Prevalence of Helicobacter pylori infection in coronary artery disease and effect of its eradication on coronary lumen reduction after percutaneous coronary angioplasty. Digest Liver Dis. 33:222–229.

61. LaBiche, R., D. Koziol, T. C. Quinn, C. Gaydos, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

62. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

63. Lamb, D. J., and G. A. Ferns. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

64. Leibovitz, E., Y. Hertz, E. Liberman, S. Sclarovsky, and S. Berliner. 1997. Increased adhesiveness of white blood cells in patients with unstable angina: additional evidence for an involvement of the immune-inflammatory system. Clin. Cardiol. 20:1017–1020.

65. Lindahl, B., H. Toss, A. Siegbahn, C. Gaydos, and L. Wallentin. 2001. Presence of Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

66. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

67. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

68. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

69. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Infection, immunisation and atherosclerosis: is there a link? Vaccine 17:559–564.

70. LaBiche, R., D. Koziol, T. C. Quinn, C. Gaydos, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

71. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

72. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.

73. Lopès-Virella, M. F., G. Virella, T. J. Orchard, S. Azhar, G. Ketron, S. Sood, and T. J. DeGraba. 1999. Chlamydia pneumoniae in human asymptomatic and symptomatic carotid atherosclerotic plaque. Stroke 32:855–860.
an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 298:83–86.

89. Schechter, A. D., A. B. Berman, L. Yi, A. Mosoian, C. M. McManus, J. W. Berman, M. E. Klomtn, and M. B. Taubman. 2001. HIV envelope gp120 activates human arterial smooth muscle cells. Proc. Natl. Acad. Sci. USA 98:10142–10147.

90. Semaan, H. B., P. A. Gurbel, J. L. Anderson, J. B. Muhlestein, J. F. Carlquist, B. D. Horne, and V. L. Serebruany. 2000. The effect of chronic azithromycin therapy on soluble endothelium-derived adhesion molecules in patients with coronary artery disease. J. Cardiovasc. Pharmacol. 36:533–537.

91. Sessa, R., M. Di Pietro, I. Santino, M. del Piano, A. Varveri, A. Dagianti, and M. Penco. 1999. Chlamydia pneumoniae infection and atherosclerotic coronary disease. Am. Heart J. 137:1116–1119.

92. Sinisalo, J., J. Paronen, K. J. Mattila, M. Syrjälä, M. Alifthan, T. Palosuo, M. S. Nieminen, and O. Vaarala. 2000. Relation of inflammation to vascular function in patients with coronary heart disease. Atherosclerosis 149:403–411.

93. Siscovick, D. S., S. M. Schwartz, L. Corey, J. T. Grayston, R. Ashley, S. P. Wang, B. M. Psaty, R. P. Tracy, L. H. Kuller, and R. A. Kronmal. 2000. Chlamydia pneumoniae, herpes simplex virus type 1, and cytomegalovirus and incident myocardial infarction and coronary heart disease death in older adults. The Cardiovascular Health Study. Circulation 102:2335–2340.

94. Smith-Norowitz, T. A., J. Shani, W. Weiser, N. Schulhoff, K. Norowitz, E. Lichstein, and F. Mokhtarian. 1999. Lymphocyte activation in angina pectoris. Clin. Immunol. 93:165–175.

95. Stöllberger, C., G. Mölzer, and J. Finsterer. 2001. Seroprevalence of antibodies to microorganisms known to cause arterial and myocardial damage in patients with or without coronary stenosis. Clin. Diagn. Lab. Immunol. 8:997–1002.

96. Strachan, D. P., D. Carrington, M. A. Mendall, L. Ballam, J. Morris, B. K. Butland, P. M. Sweetnam, and P. C. Elwood. 1999. Relation of Chlamydia pneumoniae serology to mortality and incidence of ischaemic heart disease over 13 years in the Caerphilly prospective heart disease study. BMJ 318:1035–1039.

97. Tao, M. 1996. Study on clinical immunity in patients with coronary artery disease. Blood Press. Suppl. 3:63–64.

98. Tirau, A., R. A. Tio, E. Oostenveld, M. C. Harmsen, B. Tirau, P. Den Heijer, S. H. Mönink, M. M. Wilders-Truschnig, and T. H. The. 1999. Humoral immune response to human cytomegalovirus in patients undergoing percutaneous transluminal coronary angioplasty. Clin. Diagn. Lab. Immunol. 6:45–49.

99. van der Wal, A. C., J. J. Pick, O. J. de Boer, K. T. Koch, P. Teeling, C. M. van der Loos, and A. E. Becker. 1998. Recent activation of the plaque immune response in coronary lesions underlying acute coronary syndromes. Heart 80:14–18.

100. Wald, N. J., M. R. Law, J. K. Morris, X. Zhou, Y. Wong, and M. E. Ward. 2000. Chlamydia pneumoniae infection and mortality from ischaemic heart disease: large prospective study. BMJ 321:204–207.

101. Whincup, P., J. J. Danesh, M. Walker, L. Lennon, A. Thomson, P. Appleby, C. Hawkey, and J. Atherton. 2000. Prospective study of potentially virulent strains of Helicobacter pylori and coronary heart disease in middle-aged men. Circulation 102:1647–1652.

102. Xu, Q., G. Schett, H. Perschinka, M. Mayr, G. Egger, F. Oberhollenzer, J. Willeit, S. Kiechl, and G. Wick. 2000. Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. Circulation 102:14–20.

103. Xu, Q., J. Willeit, M. Marosi, R. Kleindienst, F. Oberhollenzer, S. Kiechl, T. Stulnig, G. Luef, and G. Wick. 1993. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. Lancet 341:255–259.

104. Yamashita, K., K. Ouchi, M. Shirai, T. Gondo, T. Nakazawa, and H. Ito. 1998. Distribution of Chlamydia pneumoniae infection in the atherosclerotic carotid artery. Stroke 29:773–778.

105. Zhu, J., F. J. Nieto, R. D. Horne, J. L. Anderson, J. B. Muhlestein, and S. E. Epstein. 2001. Prospective study of pathogen burden and risk of myocardial infarction or death. Circulation 103:45–51.

106. Zhu, J., A. A. Quyyumi, J. E. Norman, R. Costello, G. Csako, and S. E. Epstein. 2000. The possible role of hepatitis A virus in the pathogenesis of atherosclerosis. J. Infect. Dis. 182:1583–1587.

107. Zhu, J., A. A. Quyyumi, J. E. Norman, G. Csako, and S. E. Epstein. 1999. Cytomegalovirus in the pathogenesis of atherosclerosis. The role of inflammation as reflected by elevated C-reactive protein levels. J. Am. Coll. Cardiol. 34:738–1743.

108. Zhu, J., A. A. Quyyumi, D. Rott, G. Csako, H. Wu, J. Halcox, and S. E. Epstein. 2001. Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. Circulation 103:1071–1075.

109. Zhu, J., G. M. Shearer, J. E. Norman, L. A. Pinto, F. M. Marincola, A. Prasad, M. A. Wacławiw, G. Csako, A. A. Quyyumi, and S. E. Epstein. 2000. Host response to cytomegalovirus infection as a determinant of susceptibility to coronary artery disease. Sex-based differences in inflammation and type of immune response. Circulation 102:2491–2496.