Role of Infection as a Risk Factor for Atherosclerosis, Myocardial Infarction, and Stroke

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An increasing body of evidence has linked infections to atherosclerosis and thrombosis. Herpesviruses cause atherosclerosis in experimental animals. Herpesviruses can also be detected in atherosclerotic lesions in humans. Cytomegalovirus may play a role in arteriosclerosis in transplanted hearts, and this virus, together with tumor suppressor protein p53, can be found in restenosis lesions following angioplasty. *Chlamydia pneumoniae* and dental infections are associated with coronary heart disease in cross-sectional and longitudinal studies, and preceding respiratory infections are associated with ischemic stroke. Infections may favor formation of atherosclerosis and thrombosis by elevation of blood levels of fibrinogen, leukocytes, clotting factor, and cytokines and by alteration of the metabolism and functions of endothelial cells and monocyte macrophages. Low-grade infections may also be one of the causes of the inflammatory reaction observed in atherosclerotic lesions and acute ischemic symptoms, reflected in elevated levels of C-reactive protein. These observations warrant further studies in this field.

The development of atherosclerosis in arterial walls, with complications such as chronic coronary heart disease (CHD), acute myocardial infarction (AMI), and ischemic stroke, has remained one of the leading causes of death and morbidity in Western industrialized countries. The classic risk factors for these disease entities do not explain all their clinical and epidemiological features. An increasing body of evidence suggests that infections also play a role, an idea proposed by Sir William Osler in the beginning of this century [1]. Inflammation, one of the causes of which may be low-grade infections, has been shown to be associated with atherosclerosis and acute coronary syndromes [2].

It should be emphasized that this ''infection hypothesis'' regarding cardiovascular diseases does not argue against the role of the classic coronary risk factors. Infections may in fact act through or in concert with the same elements as the classic risk factors, e.g., serum lipoproteins or thrombus formation.

The atherosclerotic process may begin in childhood, and as discussed later, it is possible that childhood infections are involved in this process; thus, infections and atherosclerosis may be tightly linked throughout human life.

This article reviews the recent evidence linking infections to various forms of atherosclerosis and its complications, with emphasis on the most recent findings in humans. Pure inflammatory reactions (quite arbitrarily defined) are not described in detail, and some immunologic phenomena observed in patients with atherosclerosis-related diseases, like the presence of anticardiolipin antibodies, are considered beyond the scope of this review.

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Atherosclerosis

Atherosclerosis is the common background behind acute myocardial infarction, unstable angina, and ischemic stroke. Therefore, it is important to elucidate the relation of infections to atherosclerosis; indeed, many different viral and bacterial infections have been linked with the disease.

Viruses

Herpesviruses. The observation in the late 1970s that a herpes-type DNA virus of chickens (Marek's disease virus) causes atherosclerosis closely resembling that seen in humans meant a new emergence of the infection hypothesis regarding CHD. The atherosclerotic lesions developed also in normocholesterolemic virus-infected chickens; a cholesterol-rich diet changed the lesions from proliferative to fatty and fatty-proliferative. The virus could be found in the arterial walls by immunofluorescence techniques [3]. Cultured chicken aortic smooth-muscle cells infected with this virus were also shown to contain higher amounts of cholesterol and other lipids than noninfected cells [4]. A twofold increase in cholesteryl ester synthetic activity and a significant reduction in lysosomal and cytoplasmic cholesteryl ester hydrolytic activity were observed in postmortem analysis of aortic wall specimens [4, 5]. What's more, Marek's disease virus–induced atherosclerosis could be

prevented by administering herpesvirus from turkeys as a vaccine before the infection [6].

Span et al. [7] examined the effect of cytomegalovirus (CMV) on the development of atherosclerosis in rats. Increased adhesion of leukocytes to aortic intima was observed in CMV-infected normocholesterolemic rats, which also developed atherosclerotic lesions resembling those found in noninfected hypercholesterolemic rats. In hypercholesterolemic rats, CMV infection further increased the proportion of endothelial cells containing lipids, as compared with the proportion in hypercholesterolemic noninfected rats. These observations strongly suggest that herpesviruses can promote atherosclerosis in experimental animals.

These studies have been extended to humans. Herpesviruses are present in atherosclerotic arteries, as shown by Gyorkey et al. [8] in a study of patients with atherosclerosis undergoing cardiovascular surgery; by Benditt et al. [9] in a study of patients undergoing coronary bypass surgery; by Yamashiroya et al. [10] in a study of the coronary arteries of young trauma victims; and by Melnick et al. [11], who found the viruses in cultures of aortic cells and in the carotid arteries of 132 patients undergoing blood vessel surgery. Raza-Ahmad et al. [12] found histologic evidence of inflammation in 61% of coronary artery biopsy specimens taken during bypass grafting, and 45% of the samples were positive for herpes simplex virus type 2 antigens and 1% for herpes simplex virus type 1 antigens. What's more, there was a significant association between recent occurrence of cold sores, positivity for herpes simplex virus type 2, and inflammatory changes in the biopsy specimens.

The amount of herpesviruses has been correlated with the severity of the atherosclerotic process in the arterial tree. CMV nucleic acids could be demonstrated by PCR in 90% of samples taken from the abdominal aorta or femoral artery of CMV-seropositive patients undergoing vascular surgery, as compared with 53% of samples taken at autopsy (performed within 12 hours after death) from CMV-seropositive individuals with atherosclerosis of maximal grade I [13]. However, there seems to be no difference in the occurrence of CMV in atherosclerotic and uninvolved aortic segments within the arterial trees of the subjects studied; CMV was detected in up to 90% of the samples [14, 15].

A significantly higher prevalence of CMV antibodies and higher titers were observed among patients undergoing vascular surgery for atherosclerosis than among hyperlipidemic controls matched for age, race, and socioeconomic background [16]. It should be noted that this study is one of few comparing patients with atherosclerotic vessel disease with controls matched also for presence of hyperlipidemia. Sorlie et al. [17] analyzed antibodies to CMV and herpes simplex type 1 and 2 viruses in 340 matched case-control pairs. Cases had thickened carotid artery walls, as noted in ultrasonographic examinations. However, no statistically significant association was observed between carotid atherosclerosis and any of the viruses in multivariate analyses.

The contribution of infections to atherosclerosis and restenosis was recently reviewed by Epstein et al. [18]. The above studies clearly indicate that CMV and other herpesviruses can be found in the arterial walls, which present a site of latency for these viruses. As stated by Hendrix et al. [13], one interpretation of these findings is that herpesviruses may be involved in the pathogenesis of atherosclerosis but that end-stage atherosclerosis may no longer be influenced by them. However, because of inflammation in the blood vessel wall, they could expose the individual to thrombotic complications.

Restenosis of the dilated vessel may occur in up to 50% of patients after coronary angioplasty. Recently, Speir et al. [19] found that 38% of restenosis lesions examined contained tumor suppressor protein p53 and that this correlated with the presence of CMV. Smooth-muscle cells from the lesion areas expressed CMV protein IE84 and contained high amounts of p53. Furthermore, CMV infection of cultured human smooth-muscle cells enhanced p53 accumulation, which in turn had a temporal association with IE84 expression. These results were given strong support by the observations of Zhou et al. [20]. In their prospective study, restenosis after coronary atherectomy occurred in 43% of CMV-antibody positive patients, as compared with only 8% of CMV-negative patients, a highly significant difference. It seems likely that CMV, via IE84-mediated inhibition of p53 function, is involved in the development of coronary restenosis.

The major factor limiting long-term survival after cardiac transplantation is the arteriosclerotic process taking place in the arteries of the transplanted heart, a phenomenon considered to represent chronic rejection. Practically all patients surviving for >1 year have vascular changes in the cardiac allograft at autopsy [21]. Morphologically, these changes consist of perivascular inflammation, focal myocytic necrosis, and concentric generalized intimal thickening [22].

Nucleic acids of CMV (together with those of herpes simplex virus and Epstein-Barr virus) can be found with in situ hybridization in coronary arteries of transplanted hearts [23, 24]. CMV infection has been associated with chronic rejection and arteriosclerosis in cardiac allografts in several [25–27] but not all clinical studies [28–30].

The carefully conducted study by Koskinen et al. [27] strongly suggests that CMV has a role in cardiac allograft arteriosclerosis. These investigators carried out a detailed analysis of consecutive endomyocardial biopsy specimens and coronary angiograms, taken regularly over 4 postoperative years from 53 heart transplant recipients. For the first 2 postoperative years, CMV infection was significantly associated with endothelial cell accumulation and thickened intima; the endothelial cell response subsided after this, but the intimal thickness continued to increase. In coronary angiography, CMV infection–associated acceleration of coronary narrowing was marked for 2–3 postoperative years, after which it subsided.

Still another form of cardiac allograft vasculopathy, distinct from the slowly progressing arteriosclerosis described above in that it leads to nonfunctioning of grafts within 1 year, has been reported by Paavonen et al. [31]. These investigators observed a strong inflammatory reaction with lymphocyte accumulation taking place in the subendothelial space in the intima, a phenomenon they referred to as endothelialitis. This reaction was also associated with CMV infection, and the investigators concluded that CMV may play a role in this form of arterial injury as well.

The immunosuppressed state of organ transplant recipients may of course make way for herpesviruses, and this should be taken into consideration when one interprets these results.

HIV. Paton et al. [32] described a series of eight HIV-positive males (five of whom were classified as having AIDS) whose autopsies revealed marked excentric atherosclerotic changes (up to 80%–90% obstruction) in the coronary arteries. These changes were disproportionate for the patients' young age (23–32 years) and lack of family history of coronary artery disease and other coronary risk factors (except for smoking by some of the individuals). The excentric type of lesions was different from the concentric type observed after cardiac transplantation. The authors concluded that viral infection, either by HIV or coexisting herpesviruses, had probably contributed to the development of the coronary lesions.

Bacteria

Bacterial infections and intimal thickenings in the coronary arteries of infants. Intimal thickenings, composed mainly of smooth-muscle cell proliferations, can be observed in the coronary arteries even in infancy. These intimal thickenings are related to the well-known risk factors of CHD, such as male sex, place of birth, and family history of CHD [33]. It was noted as early as the 1940s that particularly thick intima layers in the coronary arteries could be found in the context of pneumonia [34].

This finding has been confirmed and examined in more detail by Pesonen et al. [35] and Kaprio et al. [33]. Their studies showed that preceding infections, mostly of bacterial origin, are associated with intimal thickening and an increased degree of luminal narrowing, even after adjustment for potential confounders such as age, gender, and family history of CHD. As the intimal thickenings are suspected to be precursors of future atherosclerotic lesions, these observations link infections with CHD from a very early age.

Kawasaki disease is presumed to have an infectious etiology. Occurring mostly in children aged $\leq$2 years, it carries sequelae into adulthood. The details of this disease are beyond the scope of this review, but it is common knowledge that some of the patients develop CHD at a young age [36, 37].

Chlamydia pneumoniae. Seropositivity for *Chlamydia pneumoniae* was significantly more common among patients with AMI and chronic CHD than among random controls matched for age group, sex, and locality [38]. The association could not be explained by the classic coronary risk factors. Since this original observation, the association between *C. pneumoniae* and cardiovascular diseases has been confirmed and extended in several studies.

Individuals with CHD verified clinically with angiography or ultrasonography carry serological markers of chronic *C. pneumoniae* infection significantly more often than do matched random controls. These markers include more prevalent and higher titers of *C. pneumoniae* antibodies [39–43] (positive serology is associated with a 2 to 7 fold risk of CHD) and more prevalent circulating immune complexes containing *C. pneumoniae* [44]. The latter study indicated that many patients with atherosclerosis seem to have chlamydial components in their blood circulation.

In the study of Thom et al. [40], the association between CHD and *C. pneumoniae* seemed to be limited to smokers. Furthermore, when individuals with normal coronary angiograms were used as the control group, the association was no longer statistically significant. However, the association between positive *C. pneumoniae* serology and CHD remained significant even after adjustment for the effect of smoking and other potential confounders in all other studies cited above.

Dahlén et al. [43] found that the combination of male sex, HLA (human leukocyte antigen) DR II genotype 13a or 17, elevated Lp(a) levels ($>$120 mg/L), and IgG titers of $>$256 (positive *C. pneumoniae* serology) was very strongly associated with CHD (this combination was observed in 14 of 29 patients with CHD and in 1 of 27 controls). These findings suggest that genetic susceptibility and an infectious factor are involved in *C. pneumoniae*–associated atherosclerosis, a scenario resembling that occurring in the pathogenesis of reactive arthritis.

In 1992, *C. pneumoniae* was detected in coronary arterial fatty streaks and atheromatous plaques with use of electron microscopy and/or immunoperoxidase staining [45]. Kuo et al. found *C. pneumoniae* in coronary artery atheromas by means of immunocytochemistry, PCR, and electron microscopy; some 40%–55% of the samples studied were positive by at least one of the techniques [46]. The investigators extended their studies by showing *C. pneumoniae* in fatty streaks and fibrous plaques, where the organism was found both in macrophages and in smooth-muscle cells [47]. In the material of Campbell et al. [48], *C. pneumoniae* was found in 20 of 38 specimens from atherosclerotic vessels, and there was a trend toward the occurrence of *C. pneumoniae* more frequently in restenosis (following coronary angioplasty) lesions than in native lesions. In another study *C. pneumoniae* was observed in 5 of 5 carotid artery endarterectomy specimens with atherosclerotic changes, and it was not found in any of the 13 normal specimens [49].

Furthermore, the same investigators have demonstrated that *C. pneumoniae* cannot be detected in nonatherosclerotic areas

of the arteries of young (15–34 years old) individuals [50]. This finding was confirmed by Muhlestein et al., who found evidence of *C. pneumoniae* in 79% of atherosclerotic coronary tissue specimens but in only 4% of nonatherosclerotic specimens [51]. On the other hand, Weiss et al. [52] found *C. pneumoniae* in only one of 58 atherosclerotic coronary specimens they investigated.

Blasi et al. found *C. pneumoniae* in aortic aneurysm samples from 26 of 51 subjects undergoing abdominal aortic surgery [53]. This observation has been confirmed by Juvonen et al. [54], who found chlamydial lipopolysaccharide (LPS) and antigens in abdominal aortic aneurysms but not in control samples from healthy aortic tissue.

Of note, in the (quite small) materials studied, the presence of *C. pneumoniae* in atherosclerotic vessels has not been associated with positive *C. pneumoniae* serology [48]. On the contrary, in the study of Puolakkainen et al. [55], the microbe was found more often in the vessels in patients with low titers of IgG to *C. pneumoniae.* In this study, reactivities with 42-kD and 52-kD proteins were associated with the microbe's presence in the vessel wall. Serology therefore poorly reflects the *C. pneumoniae*–associated arterial disease status. The above results should be taken into consideration when further studies in this field are planned.

In 1995 *C. pneumoniae* was finally isolated from a coronary atherosclerotic lesion [56]. It is interesting that preliminary findings suggest that a positive *C. pneumoniae* serology is also associated with hypertension [57].

Thus, several lines of evidence link *C. pneumoniae* to atherosclerosis. The studies cited above, however, cannot definitively answer whether the microbe really is a causative organism in the atherosclerotic process or whether it is just a bystander that locates in diseased segments of the arterial tree.

Dental Infections

Caries and periodontal disease are the most common dental diseases. Gram-positive bacteria, especially *Streptococcus mutans,* are involved with the etiology of caries [58], whereas gram-negative bacteria, including *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans,* and *Prevotella intermedia,* are involved with that of periodontitis [59]. The host's reaction to bacterial agents plays an important role in the destructive process in periodontitis [60]. The course of caries and periodontitis is usually chronic, but occasionally acute complications occur, such as endodontal infections and periodontal abscesses as well as various odontogenic infections in the mandible, maxilla, and soft tissues in the neck area.

The prevalence of periodontitis increases with the age of the subjects studied; prevalence rates have varied between studies. Burt concludes in his review that the prevalence is quite small [61]. However, in a detailed survey carried out in Finland in the late 1970s [62], periodontal tissues were healthy in only 4% of subjects, and the prevalences of moderately severe and severe periodontitis were 50% and 20%, respectively.

Mattila et al. [63] studied the relation of dental infections to coronary atherosclerosis in 100 patients with proven CHD by relating a score based on pantomographic findings (the sum of the number of periapical lesions, lesions caused by tertiary caries, vertical bone pockets, and furcation and pericoronitis lesions), assessed blindly by a single dentist, to the extent of coronary atheromatosis. This was scored on the basis of coronary angiographs, also assessed blindly by a single radiologist. The pantomography index was significantly higher among those with advanced coronary atherosclerosis, and this association remained significant in multivariate analysis taking into account the effect of age, serum lipids, smoking, hypertension, social class, and other confounders [63].

It could be argued that atherosclerosis is the *cause* and not the consequence of dental infections; individuals with coronary atherosclerosis could also have compromised arterial circulation in the periodontium. The recent study of Adams et al. [64], however, strongly argues against this explanation. The investigators performed ultrasonographic examination of the carotid arteries in 350 consecutive patients with angiographically verified coronary atherosclerosis. Intima-media thickness in the carotid arteries was correlated with the extent of coronary atherosclerosis, but this correlation, although statistically significant, was considered clinically unimportant ($r = 0.29$; $r^2 < 0.1$). Thus, it is unlikely that there would be any strong correlation between the coronary and gingival arteries either. Rather, the finding suggests that patients with CHD do not necessarily have severe atherosclerotic changes in the arteries in the region of the head.

Thus, dental infections represent another type of chronic bacterial infection that is quite strongly associated with atherosclerosis.

Acute Myocardial Infarction and Unstable Angina Pectoris

These acute coronary events are characterized by thrombus formation in a diseased coronary artery segment. Both viral and bacterial infections have been observed in conjunction with these entities as well.

Viruses

It is likely that some of the cases diagnosed as acute myocardial infarctions may in reality be focal myocarditis [65–67]. Individuals with acute myocardial infarction sometimes have evidence of recent viral infections, especially with coxsackie B virus. Controlled studies in this field have yielded conflicting results [68–75]. The epidemic nature of viral diseases, the very large number of infective agents, and the great variation in antibody response between individuals make this topic difficult

to investigate in clinical studies. The relatively small sample sizes indicate the possibility of a type II error as well.

As CMV and inflammation have been implicated in the pathogenesis of coronary artery disease, Kol et al. [76] investigated the role of CMV replication in atherosclerotic plaque instability. They examined coronary atherectomy specimens from 20 patients with unstable angina pectoris. However, none of the specimens tested showed a positive signal for markers of early replication, thus arguing against a role for CMV replication in unstable angina pectoris.

A preceding ''influenza-like illness,'' defined by symptoms of upper respiratory tract infection (i.e., nasal congestion, rhinorrhea, sore throat, head cold, or cough, with or without fever), was significantly associated with acute myocardial infarction in three studies specifically designed to study the phenomenon [35, 75, 77]. Lack of association has been noted in other investigations originally not designed to study this particular phenomenon [67, 68]. This syndrome occurred 1–5 weeks before the infarction in ~20%–30% of patients (a 2.5-fold prevalence in comparison with that among random controls or patients with chronic CHD in a ''cold'' phase). So far, no association has been found between this syndrome and a positive viral or bacterial serology or dental infections. It is noteworthy, however, that the above symptoms again refer to inflammation, known to occur in acute cardiovascular events.

On the other hand, influenza epidemics are associated with increased numbers of deaths due to CHD, especially in younger age groups [78]. This could be explained by the fact that levels of fibrinogen and factor VIII clotting activity are higher in winter, causing a ''hypercoagulabile'' state that in turn is strongly correlated with an elevated neutrophil count, elevated C-reactive protein (CRP) level, and self-reported cough [79].

In accordance with the above, Jousilahti et al. [80] recently published a report on a 13-year follow-up of >19,000 randomly selected individuals from eastern Finland (individuals with a history of CHD or stroke were removed from the cohort). The investigators defined chronic respiratory infection as occurrence of symptoms of chronic bronchitis. In their large amount of carefully analyzed data, symptoms of chronic respiratory infection predicted coronary death with a risk ratio of ~1.5. Adjusting the result for smoking, cholesterol level, and systolic blood pressure decreased the risk somewhat, but it remained statistically significant (as did the risk for developing CHD).

Chlamydia pneumoniae

As mentioned before, *C. pneumoniae* seropositivity was significantly more common among patients with AMI and chronic CHD than among their matched random controls. Two-thirds of these patients with AMI showed seroconversion in EIA with LPS antigen, core-deficient Re-LPS, purified from *Salmonella minnesota* mutant (as compared with only one patient of the other groups combined). This Re-LPS shares with chlamydial LPS a major carbohydrate antigen (alpha-2-4 linked keto deoxyoctonate [KDO]-disaccharide). Chlamydial LPS has an additional epitope, alpha-2-8 linked KDO-disaccharide. A positive reaction was observed only in assays containing this latter epitope. No reaction was observed, for example, with LPS of *Yersinia enterocolitica* or with serum from patients known to have other types of circulating immune complexes [81, 82].

C. pneumoniae–specific immune complexes were detected significantly more often in patients with AMI (57%) than in random controls (12%) and patient controls with diseases characterized by circulating immune complexes (10%) [82]. Immediately after the AMI, most of the circulating *C. pneumoniae*–specific immune complexes were detectable with the IgM-capture EIA method. On the contrary, LPS capture detected most immune complexes 1 month later. This shift in the composition of these immune complexes was not observed in the samples taken from the random controls.

These results suggested that after AMI, the immune complexes were formed in chlamydial LPS excess, which then converted into IgM antibody excess. The reason for and significance of this interesting finding are not known.

Cook et al. [83] measured IgG, IgM, and IgA *C. pneumoniae* antibodies in 1,874 patients admitted to a hospital in Birmingham, England over a 2-year period. Serological markers of both acute and chronic *C. pneumoniae* infection were significantly more common among patients admitted with unstable angina, AMI, or ischemic stroke than in patients admitted with other conditions. It is noteworthy that the 2-year-long recruitment period in this study excludes the possibility that a rapid start or cessation of a *C. peumoniae*-associated epidemic would have influenced the differences between the groups. Furthermore, the results were obtained by comparing the patients with hospital controls, among whom factors that might increase the prevalence of chlamydial infections, like smoking and alcohol abuse, are probably more common than among random controls.

Positive *C. peumoniae* serology is associated with cardiovascular disease in follow-up studies as well. In the Helsinki Heart Study, after adjustment for the effect of the ''classic'' coronary risk factors (age, smoking, systolic blood pressure, the ratio of high-density lipoprotein [HDL] to total cholesterol), the presence of *C. pneumoniae*–specific immune complexes and/or an elevated IgA antibody titer 3–6 months before clinical signs of CHD was associated with a >2-fold risk of developing CHD [84]. As all individuals included in that study had to be free from CHD at the time of enrollment, the conclusions exclude the possibility that *C. pneumoniae* antibodies or immune complexes would represent only a false-positive result, due to infarcted myocardium, in the assays used. Preliminary results also suggest that a positive *C. pneumoniae* serology and an elevated level of CRP, when occurring in conjunction in patients with unstable angina, accurately predict future ischemic events [85].

In a 7-year follow-up of diabetic and nondiabetic subjects in two areas in Finland, Miettinen et al. [86] observed that

C. pneumoniae seropositivity was associated with future CHD events in nondiabetic subjects in eastern Finland. However, no association could be observed in diabetics and in individuals in western Finland. The reasons for this kind of finding were unknown, but the authors speculated that diabetes is so strong a risk factor that it could have masked the effect of weaker risk factors.

As smoking exposes individuals to respiratory infections and cardiovascular diseases, smoking is potentially an important confounder in the link between *C. pneumoniae* and cardiovascular disease. In most studies, smoking has not been associated with a positive *C. pneumoniae* serology or, at the least, the *C. pneumoniae*–CHD association has remained significant in multivariate analyses controlling for the effect of smoking. In a study of Thom et al. [40] comparing patients with angiographically verified CHD with matched controls, a *C. pneumoniae*–CHD association could be observed only among ''ever smokers.''

Two studies have specifically addressed the relation of positive *C. pneumoniae* serology and smoking. Hahn and Golubjatnikov [87] investigated 365 outpatients with respiratory illness. Current smokers were significantly more likely than nonsmokers to have a *C. pneumoniae* antibody titer of $\geq$1:128, and this titer category was positively correlated with current smoking.

Karvonen et al. [88] analyzed the relation of smoking to *C. pneumoniae* seropositivity in a large number of individuals (2,346). Overall, ''ever smokers'' had a 50% greater risk of *C. pneumoniae* IgG seropositivity. Ex-smokers and current smokers had little difference in this respect, suggesting that ''ever smokers'' should be handled as a single group in future studies.

Thus, in large series of individuals, smoking seems to increase the risk of developing chronic *C. pneumoniae* infection. It should be noted, however, that in the study of Hahn and Golubjatnikov [87], the individuals studied were younger (mean age, 34 years) than in most clinical studies on *C. pneumoniae* and CHD. In addition, the individuals were recruited from among patients with an ongoing respiratory illness (whereas random or healthy controls were used in most other studies). Looking at the odds ratios for *C. pneumoniae* seropositivity according to age groups in the study of Karvonen et al. [88], one can see that the findings in their study were more evident among ex-smokers and younger individuals, especially those younger than 36 years. Thus, the association between smoking and *C. pneumoniae* may have been at least partly due to the fact that smoking exposes individuals to a chronic *C. pneumoniae* infection at a younger age.

More important, even if smoking does expose individuals to chronic *C. pneumoniae* infection, this does not exclude the possibility that the microbe is involved in the pathogenesis of coronary artery disease. As Hahn and Golubjatnikov [87] themselves pointed out, chronic *C. pneumoniae* infection could have this effect, irrespective of the possibility that smoking has exposed the individual to the infection.

In the above-mentioned study of Karvonen et al. [88], *C. pneumoniae* seropositivity was more common among males than females, and this could not be explained by the more common smoking among males. This is in keeping with the male-female ratio of the prevalence of CHD. On the other hand, the prevalence of *C. pneumoniae* seropositivity was higher in southwestern than eastern Finland in their second study, a finding not in keeping with the higher prevalence of CHD in eastern Finland [89].

Dental Infections

Dental infections were significantly associated with CHD in a case-control study of 100 patients with AMI and 102 random controls matched for locality, sex, and age range [90]. In this study, the severity of dental infections was graded by dentists using two scores. One score (''TDI'') was based on nonblinded, systematic, clinicoradiological scoring of the severity of caries (0–3), periodontitis (0–3), periapical lesions (0–3), and pericoronitis (0–1) in the participants. The other comprised blind assessment of pantomographs, as described earlier.

The prevalence of edentulousness was higher in the AMI and chronic CHD groups than among the random controls. Multivariate analyses suggested that the association was independent of the effect of smoking, social class, and other potential confounders. The number of missing teeth, which may be regarded as an indirect indicator of dental infection experience, was related to the prevalence of CHD among 1,384 men aged 45–64 years living in different regions of Finland. In a multivariate analysis controlling for the effect of smoking, hypertension, educational level, and age, the number of missing teeth was significantly associated with CHD [91].

DeStefano et al. [92] have published the largest study in this field: a 14-year follow-up of 9,760 individuals who were free of CHD at enrollment and had undergone a dental examination at the beginning of the follow-up. Dental infections were quantitated by the number of decayed teeth, periodontal disease classification, and a periodontal index; an oral hygiene index was employed to indicate the degree of dental health. The results showed that among all subjects studied, after careful adjustment for several potential confounders, periodontitis was associated with a 25% increase in CHD risk. Among men younger than 50 years (most of the individuals in the above-mentioned studies), this excess risk was 70%. However, the oral hygiene index was also associated with excess CHD risk and total mortality, and edentulousness was associated with excess total mortality.

The mechanism linking edentulousness with CHD in several studies remains speculative. Likewise, despite the large amount of data, long follow-up, and excellent statistics in the study by DeStefano et al., the truly independent role of dental infections in the development of CHD remains unclear.

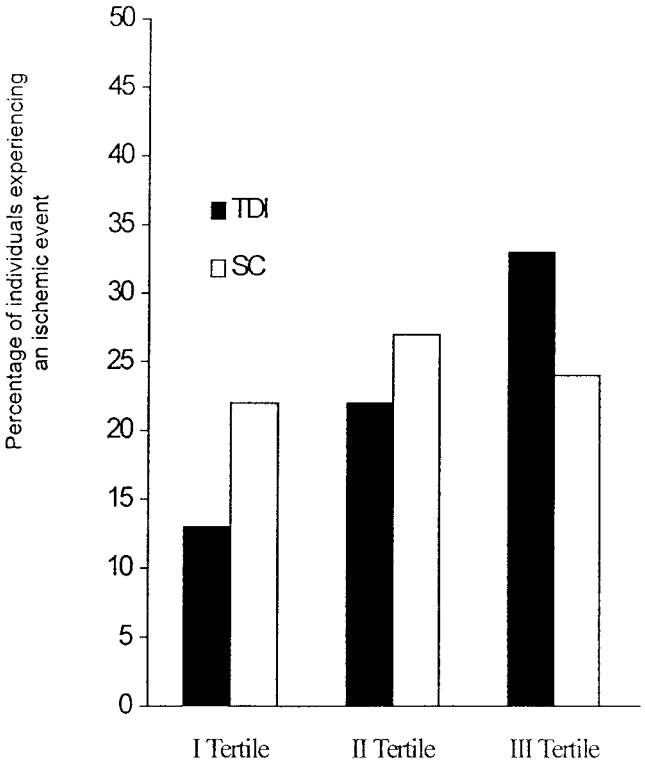

Figure 1. Percentage of individuals experiencing an ischemic event during follow-up in the three tertiles of Total Dental Index (TDI) and Social Class (SC). Test for linear trend: TDI, $P = .01$; SC, $P =$ not significant.

The patients with CHD in the two Finnish studies described [63, 75] have been followed for subsequent ischemic events. Among the 214 patients enrolled, 52 met the end-point criteria (fatal and nonfatal AMI, episodes of unstable angina) during the 7 years of follow-up. The severity of dental infections (i.e., the above-described ''TDI'') was significantly associated with the risk of ischemic events both in univariate analysis and in the Cox model, accounting for the effect of age, enrollment smoking status, social class, blood lipids, and other confounders [93].

As can be seen in figure 1 (based on the results of Mattila et al. [93], previously unpublished), the risk of an ischemic event increased from the lowest to the highest TDI tertile in a completely linear fashion, whereas no relation was observed between the event risk and social class.

Thus, the severity of dental infections seems to predict future CHD events in individuals with and without CHD at enrollment. It is clear that in these kinds of studies there is a risk of bias caused by lifestyle-related factors, such as diet and health care practices. A causal relationship is also possible, however, and further studies are indicated in this field.

Helicobacter pylori

Patel et al. [42] compared men with electrocardiographic evidence of CHD with men whose electrocardiograms were normal, with regard to *Helicobacter pylori* and *C. pneumoniae* seropositivity. Serological evidence of these infections was associated with odds ratios of 3.8 and 3.1, respectively, for CHD after adjustment for various confounders. Martîn-de-Argila et al. [94] published their findings with regard to 101 CHD patients and 68 healthy controls of similar age and socioeconomic status. The *H. pylori* seropositivity rate was 84% among the patients with CHD vs. 58% among the controls, a highly significant difference. Likewise, in the material published by Morgando et al. [95], *H. pylori* was again associated with CHD, especially among those younger than 60 years of age.

However, Murray et al. [96] could not associate *H. pylori* seropositivity with CHD, high blood pressure, plasma viscosity, total cholesterol level, and social class for randomly selected subjects aged 25–64 years (1,182 men and 1,198 women). A weak negative association was found between *H. pylori* and fibrinogen and between *H. pylori* and HDL in women. A lack of association between *H. pylori* and CHD was reported also by Sandifer et al. [97] and Delaney et al. [98]. Furthermore, the only prospective study on *H. pylori* seropositivity and the risk of CHD in elderly individuals also failed to show any association between the infection and CHD [99].

Like *C. pneumoniae, H. pylori* was associated with hypertension and body mass index in another study, of 162 individuals [100].

Enterobacterial Infections

Enterobacterial common antigen is an antigenic component of all Enterobacteriaceae organisms, except defective mutants. It is not present in other gram-positive or gram-negative organisms [101]. Among the patients with AMI in the aforementioned study by Mattila et al. [75], 15 (38%) had at least fourfold increases in the level of enterobacterial common antigen antibodies; in comparison, one subject in the population control group and none in the chronic CHD group had a diagnostic rise in antibody level. Significant elevations in levels of IgG, IgA, and IgM anticardiolipin antibodies were also observed in the AMI group, and these two reactions were closely associated. The etiology and significance of these findings are unknown.

Cerebral Infarction

The infections observed in patients with cerebral infarction have mostly been of bacterial origin. It has long been known that infective endocarditis is complicated by cerebral infarction in up to 15% of cases [102]. Another well-known association has been that with *Mycoplasma pneumoniae* [103]. Observational studies have shown that some 5%–10% of patients with bacteremic infections but without endocarditis or CNS infection develop stroke within a few weeks after the onset of bacteremia [102, 104]. Uza reported that bacteriuria was almost six

times more common among patients with ischemic cerebrovascular disease than among age-matched controls [105].

In the case-control study of Syrjänen et al., febrile infection was associated with cerebral infarction with a relative risk of 9 (CI, 2.2–80), and the most common preceding infections were upper respiratory infections [106]. These associations remained significant in multivariate analyses controlling for the effect of other risk factors of cerebral infarction. The investigators additionally showed that young (<50 years old) male patients with ischemic stroke had significantly more severe dental infections than did the controls [107].

The association between recent infections and stroke was confirmed by Grau et al. [108], who compared 197 patients who had ischemic strokes with 197 random controls matched for age, sex, and area of residence. A preceding infection carried an increased risk for stroke, with an odds ratio of 4.5 (95% CI, 2.1–9.7); likewise, a febrile illness in the patient's history was associated with cerebral infarction, with an odds ratio of 7 (95% CI, 2.5–20). Again, the preceding infections in the stroke patients were upper respiratory infections of bacterial origin, and adjusting for the effect of potential confounders did not diminish the above associations.

Cook et al. [83] investigated the association between *C. pneumoniae* and various arterial thrombotic diseases, including ischemic cerebral syndromes. Serological markers of both acute and chronic *C. pneumoniae* infection were significantly more common in these patients than in hospital controls, and the magnitude of these differences was even bigger than that between patients with CHD and controls.

This finding was confirmed by Wimmer et al. [109], who showed that positive *C. pneumoniae* IgA antibody levels and *C. pneumoniae* immunocomplexes were associated with ischemic cerebral attacks after adjustment for age, sex, hypertension, and migraines, with an odds ratio of 2.2.

Infection, Atherosclerosis, and Infarction: Possible Mechanisms of Action

The idea of infection as a risk factor for myocardial infarction may at first seem strange. However, there are several mechanisms that could mediate the effect of infections on both atherogenesis and thrombogenesis. For example, infections can evoke cytokine production; cytokines in turn probably play an important role in the pathogenesis of atherosclerosis [110].

Viruses

At the least, herpes simplex virus type 1, adenovirus type 7, the measles and mumps viruses, poliovirus type 1, echovirus type 9, and parainfluenza type 3 viruses are capable of infecting human endothelial cells in culture [111]. Infection with herpes simplex virus type 1, poliovirus, and adenoviruses has been shown to increase granulocyte adherence to human endothelial cells [112]. Adherent granulocytes can release toxic products (especially oxygen radicals) capable of damaging the endothelium after endothelial exposure to immune complexes, endotoxin, or activated complement [113–115]. Immune complexes in turn induce production of tissue factor, the initiator of the extrinsic coagulation pathway, in human endothelial cells [116]. Infection of human endothelial cells with herpes simplex virus type 1 leads to induction of immune complex receptors [117], enhanced thrombin generation and platelet binding [118], and decreased endothelial-cell plasminogen activator inhibitor [119]. Herpes simplex virus type 1 does not require productive infection to induce tissue factor in human umbilical vein endothelial cells [120]. Furthermore, herpesviruses can make the vascular endothelium more prone to thrombus formation by causing loss of surface heparans and thrombomodulin, a decrease in prostacyclin synthesis, and generation and release of tissue factor [121].

In human arterial smooth-muscle cells, this virus leads to accumulation of saturated cholesteryl esters and triglycerides (partly due to decreased cholesteryl ester hydrolysis) and a reduced capacity to produce prostacyclin [5]. Besides herpes simplex virus, CMV, at the least, is capable of replicating in human arterial smooth-muscle cells [122].

Bacteria

Many studies of the interactions between bacterial products and host tissue have involved LPS, a structural component of gram-negative bacteria. Although less thoroughly studied, components of gram-positive bacteria have similar properties.

LPS influences endothelial cell function, even without induction of denuding endothelial damage. It enhances the synthesis of IL-1 in human monocytes and endothelial cells and of TNF in human endothelial cells. These substances induce procoagulant activity in human vascular endothelium and are known to increase the adherence of polymorphonuclear leukocytes and monocytes to it [123–126]. LPS stimulates endothelial production of platelet activating factor [127] and induces release of tissue factor [128, 129], plasminogen activator inhibitor [130, 131], and von Willebrand factor antigen [132] from human endothelial cells. Furthermore, LPS enhances production of the factor stimulating growth of granulocytes and macrophages and the colony stimulating activity in cultured human endothelial cells [133].

C. pneumoniae is able to multiply and cause a persistent infection in human endothelial cells [134]. It also induces expression of endothelial-leukocyte, intracellular, and vascular cell adhesion molecules in human endothelial cells [135].

LPS has several effects on monocyte-macrophages. LPS of *Escherichia coli* remains within macrophages [136]. LPS stimulates secretion of type-beta transforming growth factor in human monocytes [137] and stimulates secretion of growth factors for smooth muscle cells and fibroblasts and the expression

of c-sis proto-oncogene coding for one of the platelet-derived growth-factor chains in human monocytes [138–140], together with induction of IL-2 receptors on monocytes. IL-2 then augments generation of reactive oxidative intermediates and their cytotoxic activity in monocytes [141].

Monocyte adhesion to blood vessel endothelium and lipid metabolism of monocyte-macrophages are important factors in atherogenesis. As mentioned above, LPS enhances monocyte adherence to endothelium via stimulating the production of IL-1 and TNF in human monocytes and endothelial cells [142, 143] and, at least in animal preparates, by causing increased endothelial-cell turnover. The latter is also among the factors increasing monocyte adherence [144]. Monocytes are also involved in the indirect activation of endothelium by endotoxin, which takes place during the neutralization process of LPS. LPS activates monocytes via cell membrane CD14, leading to secretion of TNF and IL-1, which in turn activate endothelium [145].

Besides this, LPS induces secretion of factors chemotactic to monocytes in human arterial smooth muscle cells and macrophages [146]. Repeated exposure of monocytes to endotoxin augments the subsequent release of inflammatory mediators from monocytes [147]. This can be observed clinically, as shown by Schäfer et al. [148]. They showed that patients with liver diseases had elevated plasma endotoxin levels and that monocytes of these patients released increased amounts of bioactive TNF, as compared with such activity in healthy controls.

C. pneumoniae can grow inside human peripheral blood mononuclear cells, and it induces a cytokine response (with TNF, IL-1, IL-6, and IFN) in these cells [135]. Unstimulated and LPS-stimulated monocytes from patients with periodontitis, at least those who develop periodontitis at a relatively young age, release higher concentrations of IL-1 than do those from subjects with few or no dental infections [149].

An elevated granulocyte count is an independent risk factor for CHD, and leukocyte count correlates with the extent of angiographically assessed severity of coronary atherosclerosis [150]. As mentioned above, LPS significantly increases adherence of neutrophils to human endothelial cells. LPS activates granulocytes to increase the release of oxygen metabolites. Activated granulocytes in turn can damage endothelial cells via lysosomal enzymes, oxygen radicals, elastase, and other toxic substances in vitro [114, 115, 151–154].

Hydrogen peroxide stimulates the synthesis of platelet activating factor in endothelium and induces endothelial cell–dependent neutrophil adhesion [155]. LPS directly enhances production of platelet activating factor in neutrophils [156]. On the other hand, platelet activating factor enhances neutrophil responses, e.g., elastase release and aggregation, to various stimuli [157].

Besides its direct effects, LPS also activates granulocytes by stimulating monocytes to excrete granulocyte-activating mediators [158] and platelet-derived growth factor, also known to promote granulocyte activation [159].

In vivo investigations have shown that hydrogen peroxide production by neutrophils in patients with bacterial infection is increased [160], as is neutrophil elastase production in healthy volunteers given minute amounts of LPS [161]. Stimulated peripheral neutrophils of adult patients with periodontitis have been reported to have a >2-fold higher release of free oxygen radicals in comparison with those of individuals without periodontitis [162, 163]. Individuals with periodontal disease also have significantly elevated levels of fibrinogen and leukocytes, and these differences were not explained by factors like smoking or social class in the study of Kweider et al. [164].

The fate of LPS that invades the human body is linked with lipoproteins. All major lipoprotein classes in the blood bind LPS in direct proportion to their cholesterol concentration, and low-density lipoprotein (LDL) cholesterol binds most LPS in human plasma [165]. Lipoprotein-binding protein is also involved in the processing of LPS, by catalyzing the movement of LPS from endotoxin micelles to soluble CD14 (sCD14) and to plasma lipoproteins [166]. The LPS-sCD14 complexes can directly activate endothelium.

Injection of LPS into experimental animals leads to a hyperlipidemic response, which is considered a host-defense mechanism against this compound [167, 168]. The magnitude of the increase in cytokine production produced by endotoxin is greater if hypolipidemia is induced in the experimental animals [169].

In the studies of Morel et al. [169], neither LPS nor LDL cholesterol alone showed cytotoxicity to relatively quiescent human endothelial cells. However, LPS induced endothelial cells to produce free radicals, which oxidize LDL cholesterol. Oxidized LPS–LDL cholesterol complex then could enter the endothelial cells via the scavenger receptor, and this led to clearly diminished survival of endothelial cells. The toxicity could be inhibited by blockers of acetyl-LDL cholesterol scavenger receptor.

Brand et al. [170] showed that oxidized LDL cholesterol alone did not induce tissue factor expression in monocytes but it significantly enhanced tissue factor expression induced by LPS. LPS–LDL cholesterol complex can enter macrophages via the apolipoprotein B/E receptor [165]. After this, the complex affects the expression of scavenger receptor activity during monocyte differentiation in vitro [165] and activates monocytes to oxidize LDL cholesterol, making it toxic to other cells [171].

LPS causes increased cholesteryl ester synthesis and accumulation in human macrophages [172]. Analogous with the fact that HDL cholesterol (unlike LDL cholesterol) complexed with LPS is not toxic to human endothelial cells, HDL cholesterol inhibits the LPS-mediated increase in oxidative metabolism and lysozyme release of granulocytes [173].

Clinical studies have shown that both viral and bacterial infections cause significant changes in serum lipoprotein levels. In the study of Sammalkorpi et al. [174], a reduction in HDL

cholesterol and LDL cholesterol levels and in the ratio of HDL cholesterol to LDL cholesterol was observed. The changes were related to the severity of the infection.

The above-mentioned observations link bacterial compounds and infections to the levels of HDL and LDL cholesterol and the HDL/LDL cholesterol ratio, powerful predictors of CHD.

Microbial compounds may also favor thrombus formation. *Staphylococcus aureus* has been shown to be as strong a stimulus for release reaction of human platelets as collagen, thrombin, and adrenalin [175]. Furthermore, *Fusobacterium necrophorum* and *Streptococcus pyogenes* have been shown to aggregate human platelets in vitro [176, 177]. *Streptococcus sanguis,* ubiquitous in dental plaque, can aggregate human platelets [178]. Miragliotta et al. [179] showed that *H. pylori* has tissue-factor-like procoagulant activity that could activate the extrinsic pathway of blood coagulation.

LPS can induce monocytes to aggregate platelets [180] and to produce factor VII activity in the presence of lymphocytes [181]. LPS induces generation of fast-acting inhibitor of plasminogen activator from human monocytes and endothelial cells. This has been demonstrated in experimental animals, in human preparates in vitro, and in patients with septicemia. The phenomenon can be induced both by LPS itself and via the action of IL-1 [130, 131, 182].

Furthermore, LPS can activate Hageman factor, suggesting that LPS can initiate the intrinsic pathway of coagulation [183]. The profound effects of LPS were clearly shown by Suffredini et al. [161], who gave minute amounts (4 ng/kg) of LPS to normal subjects. LPS induced a fourfold rise in the level of von Willebrand factor antigen and a clear ''procoagulant state,'' characterized by an increase in plasminogen-activator inhibitor activity and diminished t-PA (tissue-type plasminogen activator) activity.

A procoagulant state, which may persist weeks after both viral and bacterial infections, has also been observed in clinical studies. This has been shown by measuring β-thromboglobulin, spontaneous aggregation of platelets, and platelet responsiveness to various stimuli [184, 185]. Likewise, elevations in fibrinogen levels after both viral and bacterial infections were observed in the 1970s [186]. Patients with severe dental infections seem to have elevated levels of von Willebrand factor antigen [187]; IgG, IgM, and IgD; and circulating immune complexes [188]. Ameriso et al. [189] explored the potential mechanisms mediating the association between infections and cerebral infarction. They compared cerebral infarction patients with ($n = 17$) and without ($n = 33$) a preceding infection, with regard to immunohematologic characteristics. Patients with a preceding infection had significantly higher levels of fibrin D–dimer and fibrinogen (studied within 2 days) and enhanced cardiolipin immunoreactivity of the IgG isotype. Grau et al. [190] further extended their studies by a detailed clinical and biochemical evaluation of infection-associated stroke. In their data on 159 stroke patients without infection and 38 patients with infection, they observed more severe neurological deficits on admission, more frequent cortical infarcts in the middle cerebral artery territory, more frequent cardioembolic strokes, and a smaller prevalence of extracranial artery stenoses.

Heat-shock proteins (hsp's) are a family of proteins of which expression is increased by various stress factors such as high temperature, mechanical stress, and infections. They show highly homologous sequences between different species, from bacteria to man. The hsp65 family has been linked with certain autoimmune diseases because they may evoke autoimmune responses by cross-reaction between hsp's and the host [191]. Arteriosclerosis can be induced in normocholesterolemic rabbits by immunization with hsp65, which is expressed in high concentrations in human atherosclerotic lesions.

Anti-hsp65 antibodies are associated with carotid atherosclerosis in humans, independent of the classic risk factors [192]. It has been postulated that atherosclerosis develops primarily as an autoimmune response aggravated by factors such as hypercholesterolemia [192].

The origin of hsp's in individuals with atherosclerosis is not known. It is of interest that periodontal pathogens and *C. pneumoniae* possess hsp:n with high sequence identity with human hsp [193–196]. Antibodies to the 64-kD protein of *A. actinomycetemcomitans* cross-react in turn with 65-kD proteins of *Haemophilus influenzae* and *E. coli* [194]. Thus, hsp-mediated reactions could be one mechanism linking infection and development of atherosclerosis and thrombosis.

As mentioned earlier, an increasing body of evidence has linked inflammation to CHD. Chronic low-grade infections like those with *C. pneumoniae* and *H. pylori* may well be one of the factors behind this inflammation, which can be detected and measured by an accurate measurement of CRP [2]. In a cross-sectional study of 1,484 patients with angina pectoris, Juhan-Vague et al. [197] showed that plasma insulin levels increased independently of other risk factors with age, body mass index, triglyceride levels, plasminogen activator inhibitor-1, and markers of inflammation, such as CRP.

Mendall et al. [198] investigated the factors determining CRP concentration within the so-called normal range in a random sample of 388 men. *C. pneumoniae* or *H. pylori* seropositivity and an infectious symptom (production of phlegm for 3 months) were among the determinants of CRP levels. Patel et al. [199] observed mutually adjusted differences of 0.43 g/L (95% CI, 0.12–0.75) and 0.52 g/L (95% CI, 0.15–0.9) in fibrinogen levels between persons seropositive for *H. pylori* and *C. pneumoniae* and those who were seronegative; these differences corresponded to 65% and 80% of the standard deviation for fibrinogen, respectively. The investigators have also shown that *C. pneumoniae* and *H. pylori* seropositivity are associated with raised fibrinogen and malondialdehyde concentrations and leukocyte counts [42].

Conclusions

The idea of infection as a risk factor for atherosclerosis and related diseases is an old one, and infection should not be taken

as a ''competing view'' for the classic coronary risk factors. ''New'' evidence linking infection to cardiovascular diseases has accumulated since the early 1970s and includes several different infectious agents causing quite different infectious processes: persistent or latent infections like those caused by herpesviruses or *C. pneumoniae;* low-grade bacterial infections like dental infections and *H. pylori* gastritis; and acute, strongly symptomatic infections like bacteremic infections and common upper respiratory infections.

The mechanisms of action probably also vary, ranging from subtle, cytokine-mediated alterations in blood vessel endothelial function to a marked temporary shift of the blood coagulation mechanism to a thrombogenic direction. Likewise, the consequences of these infections vary in different individuals; a marked ''procoagulant state'' caused by acute infections probably favors thrombus formation in those individuals whose coronary or cerebral arteries are affected by an atherosclerotic process impairing the endothelial functions. On the other hand, *C. pneumoniae*–linked chronic arterial disease seems to be related to the host's HLA status, a circumstance resembling that occurring in the development of reactive arthritis. Infections may also act in concert with the classic coronary risk factors.

Synergy between infection and cholesterol was evident in the studies by Minick et al. [3] and Span et al. [7]. This may well be the case in humans as well. In their prospective 2-year study of 3,043 patients with angina pectoris, Thompson et al. [200] showed that the cholesterol-related relative risk of coronary events did not increase at all in those post-AMI patients who belonged to the lowest CRP tertile, while the risk increased exponentially in individuals in the highest tertile.

Some of the hypotheses generated by the results described earlier have already received support from preliminary results of studies with humans. Gurfinkel et al. reported that the combination of positive *C. pneumoniae* serology and elevated CRP level predicted extremely well future ischemic events in patients with unstable angina, suggesting that the microbe may really be behind the inflammatory reaction observed in these patients [85]. Holme et al. reported a high correlation between *H. pylori,* coronary atherosclerosis, and antibodies to mycobacterial hsp65, again suggesting that it is the infection that causes the autoimmune reaction observed in individuals with atherosclerosis [201].

All in all, as yet the data linking infections to CHD do not prove causality. However, the findings obtained do indicate an urgent need for further studies in this field.

Addendum

Since preparation of this article, reports of two randomized, double-blind studies showing that antibiotic treatment reduces ischemic events have been published [202, 203]. Although the studies were relatively small, they strongly support a causative role for infections in the pathogenesis of CHD.

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This stamp was issued by the Dominican Republic on 2 June 1979 to publicize the Dominican Cardiology Institute. It illustrates a thrombosed atherosclerotic artery overlying a heart with an occluded coronary artery and an acute myocardial infarct. (From the medical philately collection of Dr. J. N. Shanberge, University of Michigan.)