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Reviews

Chronic Infection in the Etiology of Atherosclerosis—the Case for *Chlamydia pneumoniae*

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Summary: Established cardiovascular risk factors do not fully explain the variations in the prevalence and severity of coronary heart disease. Recent evidence suggests that common chronic infections may contribute, either by direct or indirect mechanisms, to the etiology and/or progression of coronary atherosclerosis. Of the candidate infectious agents implicated, *Chlamydia pneumoniae* has emerged as the most likely pathogen to have a causal role. Evidence for this is based on seroepidemiologic, pathologic, and laboratory-based evidence, in addition to recent small-scale antibiotic intervention studies. Concerted efforts are now focused on the design of large prospective trials with antibiotics active against *C. pneumoniae* in the secondary prevention of coronary heart disease.

Key words: *Chlamydia pneumoniae*, atherosclerosis, coronary heart disease

Introduction

It is important to identify the cardiovascular risk factors which explain the occurrence or predict the severity of coronary heart disease (CHD). Not infrequently, however, one encounters a patient with severe CHD who is a nonsmoker, normotensive, nondiabetic, with a normal lipid profile and a blameless family history and lifestyle. In fact, established atherogenic risk factors probably account for no more than half of the known variations in pathogenesis, prevalence, and severity of CHD.[1] This deficiency in our knowledge has prompted research into other potential and hitherto unrecognized influences in atherogenesis. Recently, there has been increasing interest in the possible role of common chronic infections in the development of atherosclerotic disease. This review discusses the infectious agents that have been implicated with atherosclerosis and, in particular, focus on the organism for which evidence is strongest—*C. pneumoniae.*

Chronic Infection and Mechanisms of Atherogenesis

The "response to injury" model of atherosclerosis states that infection may trigger and aggravate endothelial damage.[2] There are also several other direct and indirect mechanisms, by which infections might initiate or perpetuate atherosclerosis. (Table I). Endotoxin secreted by bacteria triggers vascular damage in rabbit[3] and pig[4] models of atherosclerosis, and has been shown to bind to lipoproteins in the circulation, some of which are then avidly taken up by macrophages. Infection and activation of macrophages or smooth muscle cells may lead to production of cytokines, major histocompatibility complex upregulation, and synthesis of acute phase proteins such as fibrinogen and C-reactive protein—processes which would perpetuate an inflammatory response.[5] Infections may also directly affect cholesterol metabolism and lipid oxidation,[6] thereby leading to a more atherogenic lipid profile.

Infection may lead to a hypercoaguable state, via increased tissue factor expression, following the activation of monocytes.[7] This may increase the risk of local or distal thrombosis. Infection of endothelial cells may also alter thrombin generation on the cell surface, increase platelet accumulation, or reduce prostacyclin secretion.[8]

Candidate Pathogens in Atherogenesis

Herpesviruses

Studies in the late 1970s demonstrated that infecting chickens with Marek's disease virus (an avian herpesvirus) lead to

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TABLE I Possible mechanisms by which infections may contribute to atherosclerosis

Endothelial cell damage by bacterial lipopolysaccharide
Lipoprotein disturbances and endotoxin-lipid complex formation
Monocyte activation and triggering of cytokine production
Increased synthesis of acute phase proteins and inflammatory markers
Enhanced activity of hemostatic and procoagulant mediators
Heat shock protein expression and immune cross reactivity
Other–electrolyte disturbances, glucose intolerance, platelet activation

aggressive lipid deposition within coronary arterial walls,[9] forming lesions that resembled human fibro-fatty atherosclerotic plaques. Prior vaccination of the animals reduced the occurrence of such lesions.[10]

In humans, cytomegalovirus (CMV) antigens (but not infectious virus) have been found in carotid endarterectomy specimens,[11] and CMV DNA has been identified in coronary atherectomy specimens from arteries that had restenosed after previous coronary angioplasty.[12] In a prospective study, Zhou *et al.* found that patients with previous CMV infection had a high independent risk of restenosis after coronary angioplasty—43% in those with CMV IgG antibodies versus 8% in seronegative patients.[13] These investigators proposed that angioplasty-induced vessel wall injury might reactivate latent CMV infection and enhance the smooth muscle cell proliferation typical of restenosis. By contrast, in a nested case-control study involving patients with asymptomatic carotid artery thickening and controls without such disease, Sorlie *et al.* found only a weak independent association between CMV seropositivity and atherosclerotic disease.[14] Recently, Kol *et al.* could find no expression of CMV gene mRNA (an early marker of viral replication and hence active infection) in atherectomy specimens from 40 patients with ischemic heart disease[15]—further evidence against the a causative role for locally active CMV infection in atherogenesis.

Cytomegalovirus infection is much more clearly linked with accelerated allograft vasculopathy in transplanted hearts. Such atherosclerosis usually comprises diffuse smooth muscle cell proliferation and collagen accumulation; atheromatous plaques are uncommon.[16] Among consecutive heart transplant recipients in Stanford, Grattan showed that, compared with CMV-negative patients, CMV-positive individuals were at higher risk of developing early and fatal atherosclerosis (actuarial mortality rates 10 versus 30%, respectively).[17] These observations have been confirmed by other investigators.[18]

Winter Respiratory Infections

The incidence of myocardial infarction may be 20–40% higher during winter and spring than in summer and autumn,[19] possibly due to seasonal respiratory infections.[20] Influenza epidemics have been associated with increased cardiovascular mortality in winter.[21] Evidence of direct involvement of influenza in acute coronary syndromes is, however, lacking.

In a prospective hospital-based case-control study, Spodick *et al.* found that in the 2 weeks preceding admission common cold or flu-like symptoms had occurred in 42 of 150 (28%) patients with myocardial infarction and 23 of 150 (15%) controls without cardiorespiratory disease (odds ratio 2.2).[22] A prospective study in the United Kingdom found increased fibrinogen levels in winter (compared with other seasons)[23]that correlated with elevated neutrophil counts, coryzal, and cough symptoms. The researchers suggested that mild respiratory infections might have activated acute phase reactants, and hence caused the raised fibrinogen levels.

Helicobacter pylori Infection

Helicobacter pylori is a bacterial infection, mainly acquired in childhood, that colonizes the stomach and causes peptic ulcer disease and type B gastritis.[24] Interest in a possible association between *H. pylori* and CHD originated from earlier observations of associations between peptic ulceration and CHD.[25] In case-control studies, seropositivity to *H. pylori*, correlated significantly with both angiographically diagnosed CHD and myocardial infarction.[26,27] Furthermore, Patel *et al.* also showed an association between *H. pylori* and a number of serum cardiovascular risk markers such as leukocyte count, Factor VIIa, C-reactive protein, and fibrinogen.[28] The investigators postulated that the association with markers of cell activation and inflammation indicated how common infections like *H. pylori* might contribute to atherogenesis. However, two subsequent studies have failed to show any such relationship between coagulation and inflammatory markers with *H. pylori* infection.[29,30] Furthermore, a large prospective study recently showed that an association between *H. pylori* infection and increased risk of myocardial infarction and stroke was substantially confounded by the relationships between the infection, social class, and other cardiovascular risk factors.[31] Upon considering prevalence data from developed nations, other observers have even suggested a negative association between seropositivity to *H. pylori* and deaths from CHD.[32]

There are no data documenting the direct involvement of *H. pylori* in the pathogenesis of atherosclerotic plaques. A recent study examining aortic plaque tissue from patients undergoing abdominal aortic aneurysm surgery found no evidence of *H. pylori* presence using polymerase chain reaction techniques, notwithstanding a high seroprevalence observed in the cohort (47 of 51 patients, 92%).[33]

Chronic Dental Sepsis

Dental infections have also been proposed as a risk factor for coronary events, but prospective data are lacking. In a large cross-sectional survey of nearly 1,400 Finnish men, there was a two-fold risk correlation for a history of CHD in persons who had lost over half of their teeth compared with those with more teeth.[34] This association was independent of age, height,

and educational status. Possible mechanisms include direct endothelial damage caused by gram-negative bacteria or indirect increases in fibrinogen, C-reactive protein, and hemostatic markers caused by dental sepsis. However, any link between dental hygiene and CHD may simply reflect health habits in general rather than a causal relationship between dental sepsis and atherosclerosis.

The evidence presented above suggests that none of the infectious agents discussed is clearly implicated in atherogenesis. Available data are patchy, inconsistent, and in some instances conflicting. The positive association between CMV and restenosis has largely come from a single-center experience. The proposed link between dental sepsis and CHD is based on case-control studies with scant prospective evidence. The original finding suggesting correlation between *H. pylori* seropositivity and CHD has now been superseded by larger studies taking account of confounding factors. In contrast to the position with other infective organisms, there is ample evidence to suggest *C. pneumoniae* probably has a causative role in atherosclerosis and CHD development. This evidence is discussed below.

The Role of *Chlamydia pneumoniae* in Atherogenesis

The Organism

C. pneumoniae, first recognized as a distinct chlamydia species in 1989,[35] is an obligate, intracellular, respiratory pathogen. Most acute infections are subclinical or cause only benign "flu-like" symptoms; rarely is the infection life-threatening.[36] The prevalence of antibodies to *C. pneumoniae* increases with age and is about 50% in middle-aged adults throughout the world.[36, 37] The primary significance of *C. pneumoniae* in human disease is its ability to cause chronic infections. The infection has been associated with several chronic inflammatory conditions, in particular atherosclerosis and CHD.[38–42]

C. pneumoniae Antibodies and Coronary Heart Disease

In 1988, a Finnish case-control study demonstrated that patients with recent myocardial infarction had significantly elevated IgG and IgA antibody titers against *C. pneumoniae* compared with controls.[42] Most patients (68%) also had an antibody response against an epitope of *Chlamydia lipopolysaccharide*, an antibody present in only 3% of controls. The investigators suggested that acute myocardial infarction might be associated with an acute exacerbation of chronic *C. pneumoniae* infection. The association between elevated antibody titers against *C. pneumoniae* and atherosclerosis has been confirmed by American[41, 43, 44] and further European studies, including three investigations conducted in the United Kingdom.[28, 40, 45, 46]

In a nested case-control study using sera collected prospectively in the Helsinki Heart Study,[47] elevated IgA titers against *C. pneumoniae* and the presence of immune complexes containing *C. pneumoniae* lipopolysaccharide antigen were associated with an increased risk of developing a cardiac event 3–6 months after serological examination (odds ratio 2.3, 95% confidence index 1.3–5.2), independent of age, hypertension, and smoking habit. Patients with persistent high titers in the study (suggesting chronic infection) were more likely to suffer a cardiac event.

In Seattle, investigators have shown that in patients undergoing coronary angiography, those who had an elevated IgG titer against *C. pneumoniae* (a marker of previous or chronic infection) had a two-fold increased risk of having angiographically detectable coronary artery disease,[44] an association which weakened when smoking status was entered into the model. Smokers have lower levels of circulating immunoglobulins than nonsmokers,[48] consistent with their increased susceptibility to respiratory infections. Hahn and Golubjatnikov showed a small but significant association between smoking and seroconversion to *C. pneumoniae*,[49] and suggested that *C. pneumoniae* respiratory infection in such patients might account for any supposed link between smoking and CHD. However, other investigators have shown that, even after controlling for cigarette smoking (and other traditional risk factors), *C. pneumoniae* remains as a independent risk factor for CHD.[40, 47]

Recently, investigations in our institution have shown an association between seropositivity to *C. pneumoniae* and prevalent CHD in a community cross-sectional survey,[40] thereby corroborating the findings of Finnish and American investigators. In particular, there was a strong correlation between an elevated IgG titer of $\geq 1/64$ and CHD that was independent of conventional risk factors, age, and social class. In the same study it was shown that IgA and IgG *C. pneumoniae* titers of a group of men (age 45–65 years) with angiographic coronary artery disease were significantly higher than those in controls with normal electrocardiographs and without angina. An extension of this primary investigation showed that elevated levels of serum cardiovascular risk factors (fibrinogen, Factor VIIa, leukocyte count, and C-reactive protein) were associated with an elevated *C. pneumoniae* antibody titer, suggesting that deleterious effects of infection in atherosclerosis may be mediated by activation of inflammatory and procoagulant markers.[28] In two other recent studies,[50, 51] a significant proportion of patients admitted with acute myocardial infarction or unstable angina had serological evidence of chronic or previous *C. pneumoniae* infection. The studies provide further evidence of an association between serological markers of infection and acute ischemic events.

Elevated *C. pneumoniae* antibody titer is also linked to atherosclerosis in noncardiac arteries. In a case-control study, Melnick *et al.* reported that 73% of adults with asymptomatic carotid artery thickening (on ultrasonography) had raised levels of IgG antibodies to *C. pneumoniae* infection compared with 63% of controls.[43] After adjustment for blood pressure, diabetes mellitus, cigarette smoking, low-density lipoprotein cholesterol, and education, there was still a two-fold risk for carotid atherosclerosis in those with elevated *C. pneumoniae* titers.

The serological studies can be criticized with regard to the controls used, the borderline statistical significances sometimes found, and *C. pneumoniae* titer cut-offs arbitrarily selected to indicate seropositivity. Whether an elevated antibody titer is a reliable indicator of underlying *C. pneumoniae* infection or simply a reflection of antigenic cross reactivity also remains unclear. Evidence for a causal association is also limited by the wide variation in antibody responses to this organism secondary to differences in infective dose, mode of infection, or previous exposure.

C. pneumoniae Identified within Atheromatous Plaque Tissue

Clearer evidence for an association between *C. pneumoniae* and atherosclerosis has come from tissue examination of plaque material. In one study, *C. pneumoniae* was detected by both the polymerase chain reaction and immunohistochemical analysis of coronary atheroma from autopsy specimens (Fig. 1).[38] Of 36 subjects dying of noncardiac causes, the organism was detected in 20 (56%). *C. pneumoniae* was identified only within sites of tissue damage and not found in normal tissue adjacent to the sclerotic lesions, nor in normal coronary arteries from 11 control patients.[38] Recently, similar diagnostic methods have detected *C. pneumoniae* in atherectomy specimens from patients with angina[52] and in atheromatous arteries of patients with other vascular diseases.[53, 54] Muhlestein *et al.* tested for the presence of *C. pneumoniae* in both arterial specimens from 90 patients who underwent coronary atherectomy for symptomatic angina,[55] and 24 control specimens from subjects without atherosclerosis (either normal coronary arteries from patients dying of noncardiac causes, or diffusely diseased coronary artery tissue from patients with chronic transplant rejection at autopsy or repeat heart transplantation). Tissue specimens from patients with CHD were more frequently positive for *C. pneumoniae* than those from to controls (79 vs. 4%, $p < 0.001$).

Laboratory Evidence

The presence of *C. pneumoniae* within coronary atheroma implies that the organism must be conveyed from the lung to allow infection of the cellular components of coronary arteries. Monocytes/macrophages probably play an important role in perpetuating chronic chlamydial infections and may act as vehicles for dissemination of the organism. Circumstantial evidence of such dissemination and of local infection at the site of the coronary artery has been provided by Gaydos *et al.*[56] They showed that strains of *C. pneumoniae* could replicate within a macrophage cell line, human aortic-derived endothelial cells, and smooth muscle cells. Whether such growth of the organism actually occurs in vivo in these cell types has not

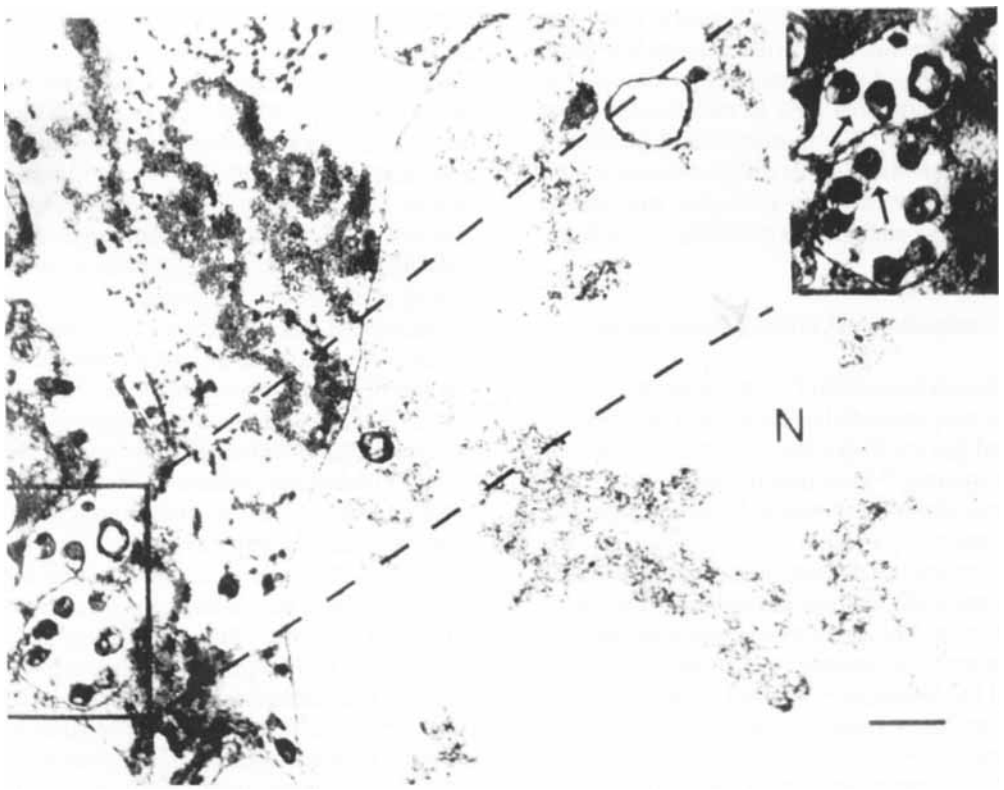

FIG. 1 Transmission electron micrograph of endosomes in foam cell with elementary bodies of *Chlamydia pneumoniae*. N = nucleus. Arrows in inset point to elementary bodies. Bar = 0.5 μm. Reproduced from Ref. No. 38 with permission of University of Chicago Press.

been established. The recent isolation and growth of viable *C. pneumoniae* from atherosclerotic tissue of a patient with severe CHD is intriguing, but not yet substantiated.[57]

Some investigators have attempted to create an animal model to investigate the possibility of a direct causal relationship between *C. pneumoniae* infection and atherogenesis. Laitinen and Saikku intranasally inoculated New Zealand White rabbits with *C. pneumoniae* organisms, followed by reinoculation 3 weeks later.[58] They observed that two of four rabbits developed early signs of fibrous plaques in the aorta, and 5 weeks following primary infection three of five animals developed plaques in the ascending aortas. These positive cases also demonstrated positivity against genus-specific *C. pneumoniae* antibodies. A series of control animals had no signs of atherosclerosis.

C. pneumoniae and Macrophage Activation in Atherogenesis

How *C. pneumoniae* enters atheromatous plaques and whether it has a direct causal role in the atherosclerotic process is not clear. Following pulmonary infection, *C. pneumoniae* may spread systemically, carried within monocytes and macrophages. The consequences of *C. pneumoniae* uptake by macrophages and the mechanisms of damage at the site of the coronary artery are not known. There are, however, several possibilities (Fig. 2). The organism may simply reside in the macrophage without causing any harmful effects, and any association may be purely coincidental. Alternatively, chronic macrophage infection may contribute to local inflammation and development of atheromatous plaques. This process may be analogous to the pathogenesis of trachoma, where the closely related *C. trachomatis* causes blindness as a result of fibrosis that follows conjunctival infiltration by macrophages and lymphocytes.[59] Fibrosis may develop in some individuals many years after the original infection, possibly as a result of hypersensitivity rather than as direct effects of the organism itself. *C. pneumoniae* infection may induce a chronic immune activation (mediated by cytokines such as IL-6, and TNF-alpha[60]) that contributes to direct chronic endothelial cell damage or stimulates the synthesis of acute phase proteins such as fibrinogen[61] and C-reactive protein.[62] Of interest, a 57 kDa chlamydial heat shock protein has been identified[63] that has close homology with mycobacterial heat shock protein (which is linked with atherosclerosis[64]). Thus, immune cross reactivity could occur. Finally, since *C. pneumoniae* titers show a weak correlation with important procoagulants such as plasma fibrinogen and Factor VIIa concentration,[28] chronic infection may produce a hypercoaguable state with increased risk of coronary thrombosis. This could be mediated either by monocyte-derived procoagulants such as tissue factor,[7] by circulating immune complexes, or via monocyte-derived cytokines.

Antichlamydial Therapy and Coronary Heart Disease: Pilot Studies

Preliminary results from the first pilot study of antimicrobial therapy in CHD have been presented recently.[65] It provides further evidence for the involvement of *C pneumoniae* in CHD.

Sixty male survivors of myocardial infarction with elevated antibodies to *C. pneumoniae* were randomized to receive the azalide antibiotic, azithromycin—a potent antichlamydial agent—or placebo. Those given the antibiotic had a significant fall in markers of monocyte activation and inflammation (including total leukocyte and monocyte count, monocyte integrins CD11b and CD11c, and fibrinogen) compared with the placebo group. Furthermore, a greater proportion of patients in the azithromycin-treated group had a fall in *C. pneumoniae*

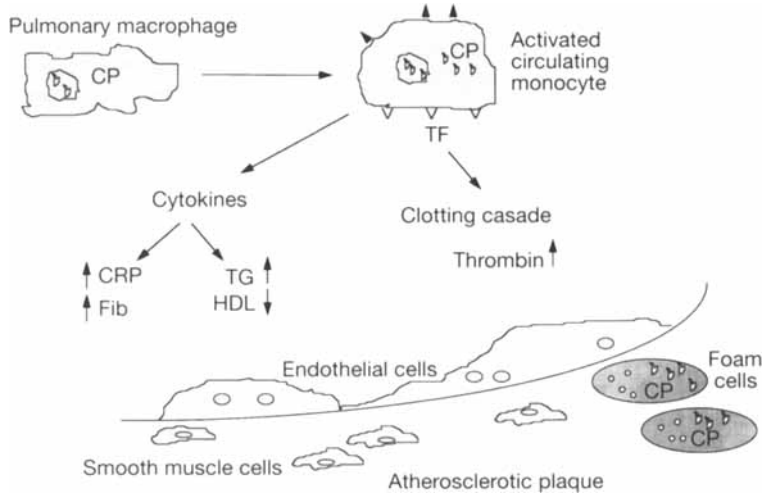

FIG. 2 Possible mechanisms by which *Chlamydia pneumoniae* may act in atherosclerosis (modified from Ref. No. 68, with permission): CP = *Chlamydia pneumoniae*, TF = tissue factor, CRP = C-reactive protein, Fib = fibrinogen, TG = triglycerides, HDL = high-density lipoprotein cholesterol.

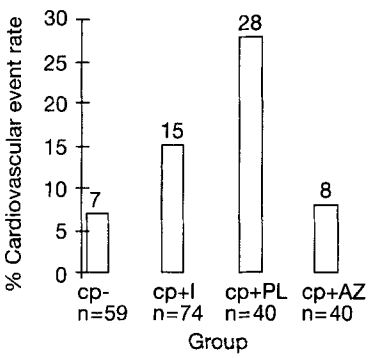

FIG. 3 Frequency of cardiovascular events in 213 postmyocardial infarction male patients during 18 month follow-up. cp−seronegative *C. pneumoniae* titer group, cp+I intermediate *C. pneumoniae* titer group, cp+PL high *C. pneumoniae* titer group given placebo, cp+AZ high *C. pneumoniae* titer group given azithromycin.

antibodies than occurred in the placebo group. The findings support the hypothesis that *C. pneumoniae* may be linked with atherosclerosis via the upregulation of cell activation markers.

In an opportunistic study, the clinical outcome of 213 postmyocardial infarction patients was investigated[66] to check whether there was any relationship between *C. pneumoniae* antibody titer (negative, intermediate, and high) and adverse cardivascular event rate (cardiovascular death, nonfatal myocardial infarction, admission with unstable angina, urgent coronary revascularization) over a follow-up period of 18 months. Whether patients given azithromycin had a more favorable clinical outcome was also investigated.

Increasing *C. pneumoniae* antibody titer correlated significantly with the risk of developing an adverse cardiovascular event, but the frequency of such events was attenuated in the group receiving azithromycin (Fig. 3). Even after controlling for confounding factors there was still a four-fold risk for further events in the high-titer patients receiving placebo compared with the seronegative group [odds ratio 4.2 (95% confidence interval 1.2–15.5, p = 0.03)]. For the high-titer group receiving antibiotic therapy, the adjusted odds ratio was 0.9 (0.2–4.6, p = NS), the same as that in the seronegative group. The results show a potential benefit from antibiotic therapy used as a secondary preventative measure in selected patients post myocardial infarction.

Preliminary results from a study by Gurfinkel *et al.* in Argentina has shown a significant reduction in the incidence of cardiovascular events in patients with acute coronary syndromes receiving the antibiotic roxithromycin compared with patients given placebo.[67]

The exciting findings of these early antibiotic studies have prompted the design of large prospective trials of eradication of *C. pneumoniae* in patients with CHD.

Conclusion

C. pneumoniae appears to be a plausible candidate for the initiation or modulation of atherogenesis. The organism might be acting with or independently of other cardiac risk factors. Atherosclerosis may turn out to be a chronic inflammatory condition with a treatable infective cause (analogous with *Helicobacter pylori* infection in peptic ulceration). Definitive evidence of *C. pneumoniae's* causative role in atherosclerosis will only derive from large prospective antibiotic eradication (and eventually vaccination) studies.[68] *C. pneumoniae* infection is common and treatable. If the large-scale eradication trials currently being designed show conclusive long-term clinical benefit, antibiotics may prove valuable in combating the present "epidemic" of coronary artery disease. The potential implications for public health world-wide are obvious.

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References

1. Solberg LA, Enger SC, Hjermann I, Helgeland A, Holme I, Leren P, Strong JP: Risk factors for coronary and cerebral atherosclerosis in the Oslo Study. In *Atherosclerosis* V (Eds. Gotto AM, Smith LC, Allen B), p. 57–62. New York, N.Y.: Springer-Verlag, 1980
2. Ross R: The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* 1993;362:801–809
3. Reidy MA, Bowyer DE: Distortion of endothelial repair. The effect of hypercholesterolaemia on regulation of aortic endothelium following injury by endotoxin. A scanning microscopy study. *Atherosclerosis* 1978;29:459–466
4. Pesonen E, Kaprio E, Rapola J, Soveri T, Oksanen H: Endothelial cell damage in piglet coronary artery after administration of *E.coli* endotoxin. A scanning and transmission electron microscopic study. *Atherosclerosis* 1981;40:65–73
5. Hansson GK: Immune mechanisms in atherosclerosis. *Atherosclerosis* 1989;9:567–568
6. Hajjar DP, Falcone DJ, Fabricant CG, Fabricant J: Altered cholesteryl ester cycle is associated with lipid accumulation in herpesvirus-infected arterial smooth muscle cells. *J Biol Chem* 1985;260: 6124–6128
7. Leatham EW, Bath PM, Tooze JA, Camm AJ: Increased monocyte tissue factor expression in coronary disease. *Br Heart J* 1995;73: 10–13
8. Visser MR, Tracey PB, Vercellotti GM, Goodman JL, White JG, Jacob HS: Enhanced thrombin generation and platelet binding on herpes simplex virus-infected endothelium. *Proc Natl Acad Sci USA* 1988;85:8227–8230
9. Fabricant CG, Fabricant J, Litrenta MM, Minick CR: Virus-induced atherosclerosis. *J Exp Med* 1978;148:335–340
10. Fabricant CG, Fabricant J, Minick CR, Litrenta MM: Herpesvirus-induced atherosclerosis in chickens. *Fed Proc* 1983;42:2467–2469
11. Melnick JL, Petrie BL, Dreesman GR, Burek J, McCollum CH, DeBakey ME: Cytomegalovirus antigen within human arterial smooth muscle cells. *Lancet* 1983;2:644–647
12. Speir E, Modali R, Huang ES, Leon MB, Shawl F, Finkel T, Epstein SE: Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. *Science* 1994;265:391–394
13. Zhou YF, Leon MB, Waclawiw MA, Popma JJ, Yu ZX, Finkel T, Epstein SE: Association between prior cytomegalovirus infection and the risk of restenosis after coronary atherectomy. *N Engl J Med* 1996;335:624–630

14. Sorlie PD, Adam E, Melnick JL, Folsom A, Skelton T, Chambless LE, Barnes R, Melnick JL: Cytomegalovirus/herpesvirus and carotid atherosclerosis: The ARIC study. *J Med Virol* 1994;42:33–37
15. Kol A, Sperti G, Shani J, Schulhoff N, van de Greet W, Landini MP, Laplace M, Maseri A, Crea F: Cytomegalovirus replication is not a cause of instability in unstable angina. *Circulation* 1995;91: 1910–1913
16. Johnson DE, Gao SZ, Schroeder JS, Hunt S, Alderman EL, Stinson E: The spectrum of coronary artery pathologic findings in human cardiac allografts. *J Heart Transplant* 1989;8:349–359
17. Grattan MT: Accelerated graft atherosclerosis following cardiac transplantation: Clinical perspectives. *Clin Cardiol* 1991;14(suppl II):16–20
18. McDonald K, Rector TS, Braunlin EA, Kubo SH, Olivari MT: Association of coronary artery disease in cardiac transplant recipients with cytomegalovirus infection. *Am J Cardiol* 1989;64: 359–362
19. Marchant B, Ranjadayalan K, Stevenson R, Wilkinson P, Timmis AD: Circadian and seasonal factors in the pathogenesis of acute myocardial infarction: The influence of environmental temperature. *Br Heart J* 1993;69:385–387
20. Enquselassie F, Dobson AJ, Alexander HM, Steele PL: Seasons, temperature and coronary disease. *Int J Epidemiol* 1993;22: 632–636
21. Housworth J, Langmuir AD: Excess mortality from epidemic influenza, 1957-1966. *Am J Epidemiol* 1974;100:40–48
22. Spodick DH, Flessas AP, Johnson MM: Association of acute respiratory symptoms with onset of acute myocardial infarction: Prospective investigation of 150 consecutive patients and matched controls. *Am J Cardiol* 1984;53:481–482
23. Woodhouse PR, Khaw KT, Plummer M, Foley A, Meade TW: Seasonal variations of plasma fibrinogen and factor VIIa activity in the elderly: Winter infections and deaths from cardiovascular disease. *Lancet* 1994;343:435–439
24. Mendall MA, Goggin P, Molineaux N, Levy J, Toosy T, Strachan DP, Northfield TC: Association between childhood living conditions and *Helicobacter* seropositivity in adult life. *Lancet* 1992;i: 896–897
25. Langman M, Cooke A: Gastric and duodenal ulcer and their associated diseases. *Lancet* 1976;i:680–683
26. Mendall MA, Goggin PM, Molineaux N, Strachan DP, Camm AJ, Northfield TC: Relation of *Helicobacter pylori* infection and coronary heart disease. *Br Heart J* 1994;71:437–439
27. Martin-de-Argila C, Boixeda D, Canton R, Gisbert J, Fuertes A: High seroprevalence of *Helicobacter pylori* infection in coronary heart disease (letter). *Lancet* 1995;346:310
28. Patel P, Mendall MA, Carrington D, Strachan DP, Leatham EW, Molineaux N, Levy J, Blakeston C, Seymour CA, Camm AJ, Northfield TC: Association of *Helicobacter pylori* and *Chlamydia pneumoniae* infections with coronary heart disease and cardiovascular risk factors. *Br Med J* 1995;311:711–714
29. Parente F, Maconi G, Imbesi V, Sangaletti O, Poggio M, Rossi E, Piergiorgio D, Porro GB: *Helicobacter pylori* infection and coagulation in healthy people. *Br Med J* 1997;314:1318–1319
30. Murray LJ, Bamford KB, O'Reilly DP, McCrum EE, Evans AE: *Helicobacter pylori* infection: Relation with cardiovascular risk factors, ischaemic heart disease, and social class. *Br Heart J* 1995; 74:497–501
31. Whincup PH, Mendall MA, Perry IJ, Strachan DP, Walker M: Prospective relations between *Helicobacter pylori* infection, coronary heart disease and stroke in middle aged men. *Heart* 1996;75: 568–572
32. Sandifer QD, Vuilo S, Crompton G: Association of *Helicobacter pylori* infection with coronary heart disease: Association may not be causal (letter). *Br Med J* 1996;312:251
33. Blasi F, Denti F, Erba M, Cosentini R, Raccanelli R, Rinaldi A, Fagetti L, Esposito G, Ruberti U, Allegra L: Detection of *Chlamydia pneumoniae* but not *Helicobacter pylori* in atherosclerotic plaques of aortic aneurysms. *J Clin Microbiol* 1996;34:2766–2769
34. Paunio K, Impivaara O, Tickso J, Maki J: Missing teeth and ischaemic heart disease in men aged 45–64 years. *Eur Heart J* 1993; 14(suppl K):S54–S56
35. Grayston JT, Kuo C-C, Campbell LA, Wang SP: *Chlamydia pneumoniae* sp. nov. for *Chlamydia*, strain TWAR. *Int J Syst Bacteriol* 1989;39:88–90
36. Saikku P: The epidemiology and significance of *Chlamydia pneumoniae*. *J Infect* 1992;1:27–34
37. Grayston JT, Wang S-P, Kuo C-C, Campbell LA: Current knowledge on *Chlamydia pneumoniae*, strain TWAR, an important cause of pneumonia and other acute respiratory diseases. *Eur J Clin Microbiol Infect Dis* 1989;8:191–202
38. Kuo C-C, Shor A, Campbell LA, Fukushi H, Patton DL, Grayston JT: Demonstration of *Chlamydia pneumoniae* in atherosclerotic lesions of coronary arteries. *J Infect Dis* 1993;167:841–849
39. Leinonen M, Linnanmaki E, Mattila K, Nieminan MS, Valtonen V, Leirisalo-Repo M, Saikku P: Circulating immune complexes containing chlamydial lipopolysaccharide in acute myocardial infarction. *Microbiol Pathog* 1990;9:67–73
40. Mendall MA, Carrington D, Strachan DP, Patel P, Molineaux N, Levy J, Toosey T, Camm AJ, Northfield TC: *Chlamydia pneumoniae*: Risk factors for seropositivity and association with coronary heart disease: *J Infect* 1995;30:121–128
41. Thom DH, Grayston JT, Siscovick DS, Wang SP, Weiss NS, Daling JR: Association of prior infection with *Chlamydia pneumoniae* and angiographically demonstrated coronary artery disease. *J Am Med Assoc* 1992;268:68–72
42. Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, Huttenen JK, Valtonen V: Serological evidence of an association of a novel chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 1988;2: 983–986
43. Melnick SL, Shahar E, Folsom AR, Grayston JT, Sorlie PD, Wang SP, Szklo M: Past infection by *Chlamydia pneumoniae* strain TWAR and asymptomatic carotid atherosclerosis. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Am J Med* 1993;95:499–504
44. Thom DH, Wang S-P, Grayston JT, Siscovick DS, Stewart DK, Kronmal RA, Weiss NS: *Chlamydia pneumoniae* strain TWAR antibody and angiographically demonstrated coronary artery disease. *Arterioscler Thromb* 1991;11:547–551
45. Haidl S, Juul-Möller S, Israelsson B, Persson K: Ischaemic heart disease and antibodies to *Chlamydia pneumoniae* (TWAR) (abstr). *Proc Eur Soc Chlam Res* 1992;2:174
46. Cook PJ, Lip GY, Zarifis J, Honeybourne D, Wise R, Beevers DG: Is *Chlamydia pneumoniae* infection associated with acute cardiac ischaemic syndromes? (abstr). *J Am Coll Cardiol* 1996;324(suppl A):807–812
47. Saikku P, Leinonen M, Tenkanen L, Linnanmaki E, Ekman MR, Manninen V, Mantarri M, Frick MH: Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Intern Med* 1992;116:273–278
48. Holt PG: Immune and inflammatory function in smokers. *Thorax* 1987;42:241–249
49. Hahn DL, Golubjatnikov R: Smoking is a potential confounder of the *Chlamydia pneumoniae*–coronary artery disease association. *Arterioscler Thromb* 1992;12:255–260
50. Cook PJ, Honeybourne D, Lip GY, Beevers DG, Wise R: *Chlamydia pneumoniae* and acute arterial thrombotic disease. *Circulation* 1995;92:3148–3149
51. Aceti A, Mazzacurati G, Amendolea M, Pennica A, Zechini B, Trappolini M, Puletti M: Relation of C reactive protein to cardiovascular risk factors: *Helicobacter pylori* and *Chlamydia pneumoniae* infections may account for most acute coronary syndromes. *Br Med J* 1996;313:428–429
52. Campbell LA, O'Brien ER, Cappuccio AL, Kuo CC, Wang SP, Stewart D, Patton DL, Cummings P K, Grayston JT: Detection of *Chlamydia pneumoniae* TWAR in human coronary atherectomy tissues. *J Infect Dis* 1995;172:585–588

53. Ong G, Thomas BJ, Mansfield AO, Davidson BR, Taylor Robinson D: Detection and widespread distribution of *Chlamydia pneumoniae* in the vascular system and its possible implications. *J Clin Path* 1996;49:102–106
54. Grayston JT, Kuo C-C, Coulson AS, Campbell LA, Lawrence RD, Lee MJ, Strandness ED, Wang SP: *Chlamydia pneumoniae* (TWAR) in atherosclerosis of the carotid artery. *Circulation* 1995;92:3397–3400
55. Muhlestein JB, Hammond EH, Carlquist JF, Radicke E, Thomson MJ, Karagounis LA, Woods ML, Anderson JL: Increased incidence of *Chlamydia* species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. *J Am Coll Cardiol* 1996;27:1555–1561
56. Gaydos CA, Summersgill JT, Sahney NN, Ramirez JA, Quinn TC: Replication of *Chlamydia pneumoniae* in vitro in human macrophages, endothelial cells, and aortic artery smooth muscle cells. *Infect Immunol* 1996;64:1614–1620
57. Ramirez JA: Isolation of *Chlamydia pneumoniae* from the coronary artery of a patient with coronary atherosclerosis. The *Chlamydia pneumoniae* Atherosclerosis Study Group. *Ann Intern Med* 1996;125:979–982
58. Laitinen K, Saikku P: *Chlamydia pneumoniae* induced atherosclerosis in rabbits (abstr). *Proceedings of the International Symposium of the Immunopathogenesis of Chlamydial Infections* 1996;4:196
59. Holland MJ, Bailey RL, Hayes LJ, Whittle HC, Mabey DC: Conjunctival scarring in trachoma is associated with depressed cell-mediated immune responses to *chlamydial* antigens. *J Infect Dis* 1993; 168:1528–1531
60. Kaukoranta-Tolvanen SS, Teppo AM, Laitinen K, Saikku P, Linnavuori K, Leinonen M: Growth of *Chlamydia pneumoniae* in cultured human peripheral blood mononuclear cells and induction of a cytokine response. *Microbiol Pathog* 1996;21:215–221
61. Patel P, Carrington D, Strachan DP, Goggin P, Leatham EW, Northfield TC, Mendall MA: Fibrinogen: A link between chronic infection and coronary heart disease. *Lancet* 1994;343:1634–1635
62. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC: C reactive protein and its relation to cardiovascular risk factors: A population based cross sectional study. *Br Med J* 1996;312:1061–1065
63. Morrison RP, Belland RJ, Lyng K, Caldwell HD: Chlamydial disease pathogenesis. The 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J Exp Med* 1989;170:1271–1283
64. Xu Q, Willeit J, Marosi M, Kleindienst R, Oberhollenzer F, Kiechl S, Stulnig T, Luef G, Wick G: Association of serum antibodies to heat shock protein 65 with carotid atherosclerosis. *Lancet* 1993; 341:255–259
65. Gupta S, Leatham EW, Carrington D, Mendall MA, Ireson N, Tooze J, Bevan D, Camm AJ, Kaski JC: The effect of azithromycin in post myocardial infarction patients with elevated *Chlamydia pneumoniae* antibody titres (abstr). *J Am Coll Cardiol* 1997; 755:209
66. Gupta S, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camm AJ: Elevated *Chlamydia penumoniae* antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. *Circulation* 1997;96:404–407
67. Gurfinkel E, Bozovich G, Darococa A, Beek E, Mautner B: Randomized trial of roxithromycin in non-Q-wave coronary syndromes: ROXIS pilot study. *Lancet* 1997;350:404–407
68. Gupta S, Camm AJ. *Chlamydia pneumoniae* and coronary heart disease. *Br Med J* 1997;314:1778–1779