Emerging relations between infectious diseases and coronary artery disease and atherosclerosis

Review

Synthèse

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This article has been peer reviewed.

CMAJ 2000;163(1):49-56

Abstract

CARDIOVASCULAR DISEASE IS THE LEADING CAUSE OF DEATH in developed countries. The cause is multifactorial. A substantial proportion of patients with coronary artery disease (CAD) do not have traditional risk factors. Infectious diseases may play a role in these cases, or they may intensify the effect of other risk factors. The association of CAD and *Chlamydia pneumoniae* infection is firmly established, but causality is yet to be proven. The link with other infectious agents or conditions, such as cytomegalovirus, herpes simplex virus, *Helicobacter pylori* and periodontitis, is more controversial. Cytomegalovirus infection is more strongly linked than native CAD to coronary artery restenosis after angioplasty and to accelerated CAD after cardiac transplantation. However, new data on this topic are appearing in the literature almost every month. The potential for novel therapeutic management of cardiovascular disease and stroke is great if infection is proven to cause or accelerate CAD or atherosclerosis. However, physicians should not "jump the gun" and start using antibiotic therapy prematurely for CAD. The results of large randomized clinical trials in progress will help establish causality and the benefits of antimicrobial therapy in CAD.

The leading cause of death in developed countries is related to cardiovascular and cerebrovascular diseases. The main underlying pathological process of these 2 diseases is atherosclerosis. To appreciate the possible role of infectious diseases, it is necessary to understand the pathogenesis of atherosclerosis.

Atherosclerosis of the major arteries is present universally in young adults at autopsy and appears to start early in childhood. The basic mechanism — a mild chronic inflammatory reaction to injury — was first proposed well over a century ago by Von Rokitansky[1] and Virchow[2] and was recently reviewed by Ross.[3] In humans, no single factor can account for all the causes of coronary artery disease (CAD), and a substantial proportion of patients have none of the traditional risk factors, such as hypertension, smoking, obesity, hypercholesterolemia or genetic predisposition. Inflammatory markers in the blood that have been associated or correlated with risk of cardiovascular disease include highly sensitive C-reactive protein, fibrinogen, serum amyloid and interleukins, tumour necrosis factor-α (TNF-α), interleukin-6, and vascular and cellular fibrinogen adhesion molecules.[4]

The association or link between CAD (and, indirectly, atherosclerosis) and infectious diseases is based on 3 main sets of evidence: epidemiological, pathological and microbiological. The evidence to support causality includes in vitro data indicating biologic plausibility, and data from animal models and clinical trials. The conditions or infectious agents most frequently studied are *Chlamydia pneumoniae*, cytomegalovirus (CMV), herpes simplex virus (HSV), *Helicobacter pylori* and periodontitis.

In this article I review the various infections under the subheadings of epidemiological evidence, pathological–microbiological evidence, biological plausibility, animal models and clinical evidence. A summary of the strength of the evidence is given in Table 1.

Postulated mechanisms

There are several possible ways in which infectious agents may induce or accelerate atherosclerosis (Fig. 1). These include

- direct invasion of the vessel wall causing an inflammatory response, which in turn leads to a local increase in lymphocytes and macrophages and production of cytokines and tissue growth factors;
- local release of endotoxin (lipopolysaccharide), which may increase cholesterol ester uptake by macrophages to form foam cells;
- molecular mimicry of microbial heat shock protein-60, with human heat shock protein inducing an autoimmune reaction;
- indirect systemic effect of remote infections causing systemic release of lipopolysaccharide, causing damage to the endothelium, systemic increase in cytokines, with activation of inflammatory markers, and stimulation of procoagulants, leading to thrombosis and acute ischemia; and
- induction of changes in lipoproteins by cytokines, which indirectly predisposes patients to atherosclerosis. For instance, a secondary increase in low-density lipoprotein (LDL) levels and a decrease in high-density lipoprotein (HDL) levels may be induced, resulting in pre-atherosclerotic conditions.

Lipid metabolism is regulated extensively during the host response to infection. Lipids represent part of the host defence, with lipoproteins scavenging for infectious particles such as endotoxin. These events are mediated by cytokines, such as TNF-α, interleukin-1, interleukin-6 and the interferons. Cytokines can decrease lipoprotein lipase activity and triglyceride clearance and increase very-low-density lipoprotein (VLDL) levels.[5]

Viruses

Epidemiological evidence

The link between infections and atherosclerosis is not a new concept; it has existed for over a century.[6–8] Renewed interest has occurred since the 1970s.[9,10] Of the 18 epidemiological studies of CMV antibodies and cardiovascular disease reviewed by Danesh and colleagues,[11] relatively few were related to native CAD. More than 1200 of the 1600 cases were related to coronary restenosis after atherectomy or the development of lesions on transplanted hearts. Thus, although CMV infections are associated with a greater risk of accelerated atherosclerosis following heart transplantation[12] and restenosis following coronary atherectomy,[13,14] the data are less compelling for native CAD. In a recent report of 900 consecutive patients undergoing angiography (but not transplantation), CMV seropositivity was not identified as a significant risk factor for the presence of more than 50% blockage in any coronary artery.[15] Similarly, in 3 recent case–control studies, neither CMV nor HSV seropositivity was associated with CAD, stroke or carotid artery disease.[16–18]

Pathological–microbiological evidence

Microorganisms may directly infect arterial intima. The resulting injury and inflammatory response induces or accelerates atherosclerosis. Finding organisms in atherosclerotic plaques is another method of establishing association but does not prove causality, because the organisms may be "innocent bystanders" trapped in the damaged vessel wall.

Several investigators have searched for CMV (predominantly) or HSV particles in atheromatous arteries and arteries from healthy control subjects using immunohistochemical stains, electron microscopy, in situ hybridization or polymerase chain reaction (PCR) to detect genetic material. In general, the rate of detection with immunohistochemistry or in situ hybridization has been about 10% to 16%,[19–24] with no virus being observed in the control arteries. However, with PCR the overall rate of detection of CMV was 57% in diseased vessels and 37% in control vessels.[11] Melnick and associates[25] detected CMV DNA in 90% of 47 atherosclerotic tissue specimens and 93% of 13 uninvolved aortas. Together, these studies indicate that CMV commonly causes latent infection of the arterial wall, but the presence of the virus is not specific for atherosclerosis. No studies have reported on the culture of CMV or HSV from atheromas in humans.

Table 1: Strength of the evidence associating infections with coronary artery disease and atherosclerosis*

Condition or infectious agent	Epidemiological evidence	Pathological evidence	Biological plausibility	Evidence from animal models	Clinical evidence
Cytomegalovirus or herpes simplex virus	+	+	++	+ to ++	0
Restenosis after angioplasty	++				
Chlamydia pneumoniae	++	+++	+++	++	+
Helicobacter pylori	+	0	+	0	0
Periodontitis	+	±	+ to ++	±	0

*0 = no evidence, + = weak evidence, ++ = moderate evidence, +++ = strong evidence, ± = unpublished data presented at meetings (but not reviewed in this article).

Biological plausibility

There is experimental evidence that human CMV can infect human coronary smooth muscle cells and initiate viral replication.[26] Vascular cells generate reactive oxygen species in response to stress, and this may lead to increased transcription of atherosclerosis-related cellular and viral genes and to reactivation of latent CMV infection. Interestingly, ASA can directly and indirectly attenuate this augmented gene transcription.[26] CMV binds to and inactivates p53, a tumour-inhibiting protein indirectly involved in DNA repair. Infection of smooth muscle cells by CMV that inactivates p53 is associated with cellular proliferation that can lead to coronary restenosis after angioplasty.[27] HSV-1 can also infect human endothelial cells, enhance thrombosis and platelet binding, and cause generation and release of tissue factors.[28] In human arterial smooth muscle cells, HSV leads to accumulation of saturated cholesterol esters and triglycerides, partly because of decreased cholesterol ester hydrolysis.[22]

Animal models

Animal models of viral-induced atherosclerosis have been limited to investigation of Marek's disease virus (an avian herpesvirus) and CMV. In normocholesterolemic chickens Marek's disease virus induced atherosclerotic lesions, with increased cholesterol ester in aortic smooth muscle cells.[29] Furthermore, the virus-induced atherosclerosis was prevented by a vaccine prepared from turkey herpesvirus. In rats CMV induced vascular injury of the aortic intima resembling lesions found in uninfected hypercholes-

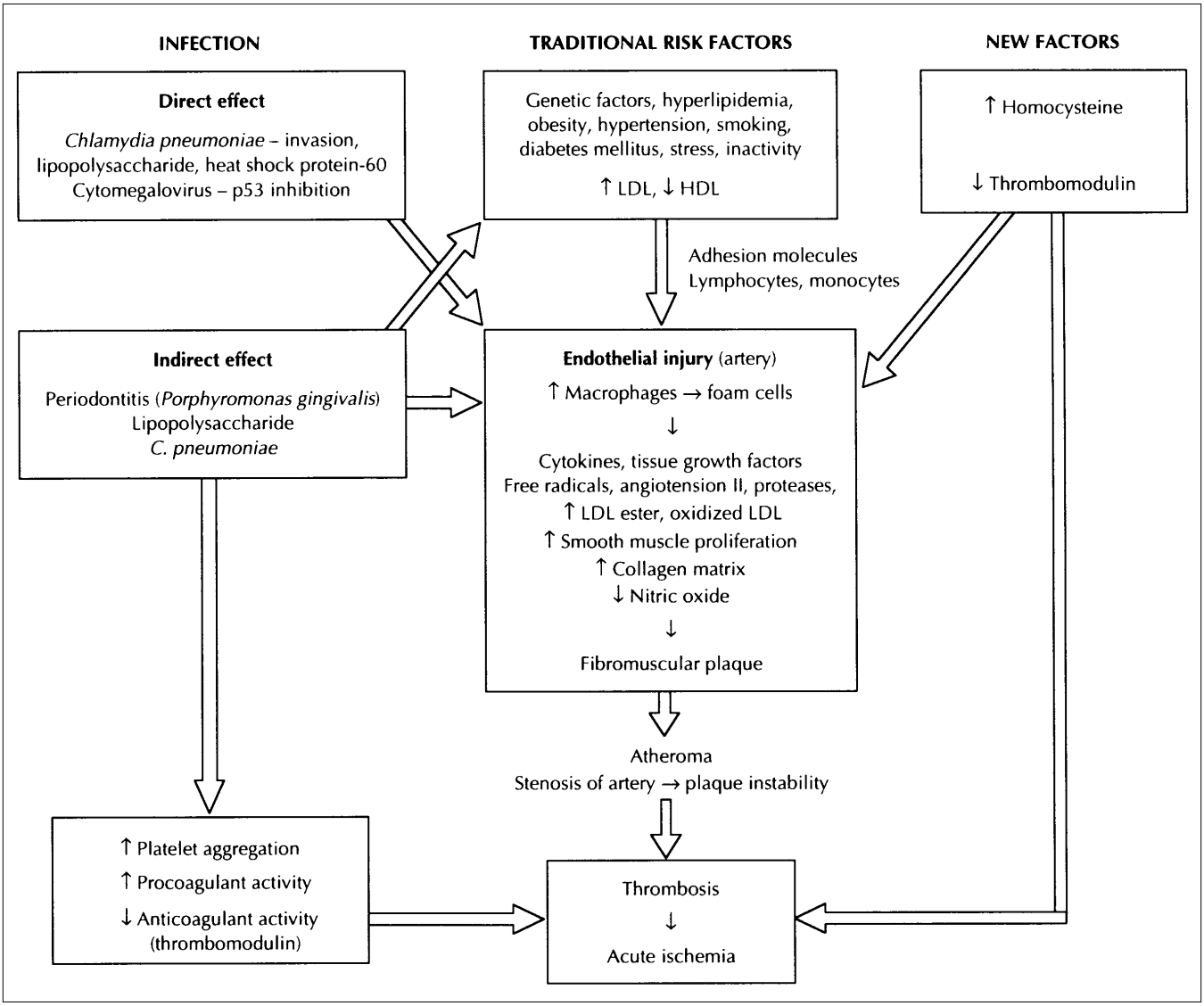

Fig. 1: Potential mechanisms of induction of atherosclerosis and coronary artery disease by infectious diseases. LDL = low-density lipoprotein, HDL = high-density lipoprotein, ↑ = increased, ↓ = decreased.

terolemic rats.[30] In addition, CMV may enhance the uptake of lipids in endothelial cells in rats fed a cholesterol-enriched diet. There is also experimental evidence of CMV inducing accelerated allograft arteriosclerosis in a rat model[31] and of prevention of these changes by ganciclovir prophylaxis.[32] There are no published human intervention trials to assess CMV infection and cardiovascular disease.

Chlamydia pneumoniae

Epidemiological evidence

C. pneumoniae, a recently discovered intracellular bacterium of the Chlamydiaceae family, is a common respiratory pathogen causing community-acquired pneumonia, bronchitis, sinusitis and upper respiratory tract symptoms. The infection is frequently mild and clinically unapparent, and its prevalence in the population with increasing age[33] almost mirrors the prevalence and extent of atherosclerosis.[34] *C. pneumoniae* infection is rare before 5 years of age, but by 20 years 50% of people have antibodies, and by 65 years more than 80% have been infected.[33]

Most of the 18 epidemiological studies of *C. pneumoniae* antibodies and CAD or cerebrovascular disease reviewed by Danesh and colleagues[11] showed a 2-fold or larger odds ratio. Potential shortcomings of these data are the different end points used to define seropositivity and the subjectivity of interpretation of the microimmunofluorescence assay. Although some of the studies were small and may have had statistical biases for subgroups, in general the total of 2700 cases supported the existence of an association between *C. pneumoniae* and CAD.

Since the review by Danesh and colleagues,[11] there have been several relatively small case–control series that have shown the same association.[35–39] However, 3 recent studies warrant further mention. In a relatively large case–control study, the presence of antibodies to *C. pneumoniae* was found to be associated with stroke or transient cerebral ischemia.[40] A large prospective longitudinal study over 13 years showed independent correlation of IgA antibodies, but not IgG antibodies, to *C. pneumoniae* with excess mortality from CAD.[41] A smaller prospective study over 12 years showed no correlation between *C. pneumoniae* IgG seropositivity and future myocardial infarction or C-reactive protein; there were no tests for IgA.[42] Chronic *C. pneumoniae* infection itself may be associated with a serum lipid profile that predisposes to atherosclerosis.[43]

Pathological–microbiological evidence

C. pneumoniae has been detected in various atheromatous vessels (coronary, carotid and aortic aneurysms, and femoral and popliteal arteries) but not often in healthy arteries. Immunohistochemical methods appear to be the most sensitive for detection, with electron microscopy, in situ hybridization and PCR being relatively insensitive. The insensitivity of PCR may be due to tissue inhibitors or to the small size of tissue specimens from atherectomy. There are over 20 reports from various areas of the world on *C. pneumoniae* in atheromatous tissues.[44] Rates of detection with immunocytochemistry range from 40% to 100% but were 54% on average in the Seattle experience.[44] The detection rates with PCR vary from 0% to 60%;[45–49] in all 3 studies with negative results, PCR was used.[44] *C. pneumoniae* antigen has been detected in early lesions (fatty streaks) in children at autopsy and in more mature lesions of older adults. The organism may be present with CMV and HSV in the same atheromatous tissue[50] and may predispose to acute thrombus formation on existing plaques.

C. pneumoniae is difficult to culture from atheromatous tissue and has been recovered on only a few occasions. It has been grown from a single carotid endarterectomy specimen,[51] a coronary artery from a cardiac transplant recipient[52] and, more successfully, from 16% of coronary atherectomy tissue specimens.[53] The current theory for the difficulty in culturing the organism from atheromas is that it is residing in a latent, persistent state with low metabolic activity associated with "unculturable forms" of *Chlamydia*.

Biological plausibility

In vitro studies have shown that *C. pneumoniae* is able to infect and reproduce in human smooth muscle cells, coronary artery endothelial cells and macrophages.[54] Furthermore, infection of human endothelial cells can induce proliferation of smooth muscle cells through a soluble factor derived from endothelial cells.[55] The organism can also induce macrophage foam cell formation, with the lipopolysaccharide stimulating uptake and accumulation of cholesterol ester.[56] It has recently been shown that *C. pneumoniae* heat shock protein-60 localizes in human atheroma and regulates macrophage TNF-α and matrix metalloproteinase expression.[57] In addition, chlamydial heat shock protein-60 can activate human vascular endothelium, smooth muscle cells and macrophages[58] and can stimulate cellular oxidation of LDL in vitro.[59]

Animal models

C. pneumoniae infection of the respiratory tract in normocholesterolemic rabbits induced vascular damage of the aorta resembling early changes of atherosclerosis.[60,61] These changes were not produced by sham infection or by *Mycoplasma pneumoniae*, which produces similar lung disease. The histopathological features of *C. pneumoniae*-induced aortic lesions closely resembled early changes produced by a diet enriched with low amounts of cholesterol (0.15% cholesterol by weight of chow) in the rabbit, which resulted in serum cholesterol levels (4.1 mmol/L) similar to that recommended for humans.[62] *C. pneumoniae* infection was also

found to accelerate the development of atherosclerosis in rabbits fed a cholesterol-enriched diet, and treatment with azithromycin partially reversed this effect.[63] In the murine model *C. pneumoniae* was detected in the atherosclerotic aorta in cholesterol-fed or apolipoprotein E-deficient mice[64] but did not induce vascular changes by itself. In mice with LDL-receptor deficiency and apolipoprotein E deficiency, *C. pneumoniae* was found to exacerbate hypercholesterolemia-induced atherosclerosis.[65,66]

Clinical evidence

Two large retrospective studies of the prophylactic effect of antibiotics in preventing future myocardial infarctions have been published. In a case–control study involving 3315 patients with first-time acute myocardial infarction (and free of clinical conditions related to increased risk), the case subjects were significantly less likely than the 13 139 matched control subjects to have used tetracycline or quinolones 3 years earlier;[67] this difference indicates a possible protective value of prior use of certain antibiotics against myocardial infarction. Jackson and collaborators[68] reported a lack of association between first myocardial infarction and prior use of erythromycin, tetracycline or doxycycline in 1796 case subjects and 4882 matched control subjects. However, their study was smaller and did not exclude patients with other known risk factors for myocardial infarction; thus, it may have lacked power in showing a protective effect of antibiotics.

Preliminary trials of antibiotic therapy for secondary prevention of cardiovascular events suggested a benefit of newer macrolides after acute myocardial infarction or in unstable angina.[69,70] However, in the larger randomized study, the reduction in secondary events observed at 1 month after treatment was lost at 6 months' follow-up.[71] In another relatively small randomized study involving patients with CAD (previous myocardial infarction, coronary artery bypass surgery or more than 50% stenosis of one or more major coronary arteries) who were seropositive for *C. pneumoniae*, no reduction in secondary cardiovascular events was present 6 months after a 3-month regimen with azithromycin;[72] however, there was reduction of a global rank sum score of 4 inflammatory markers (C-reactive protein, interleukin-1, interleukin-6 and TNF-α) in the treated group.

Helicobacter pylori

Epidemiological evidence

H. pylori, the cause of peptic ulcer disease, has been associated with CAD and cerebrovascular disease in some studies but not in others.[11] Most of the studies showing a positive association were small and did not adjust for potential confounders.[11] Twenty larger studies with proper control groups tended to show no or weaker associations.[11] A similar trend has been seen with subsequent work. Relatively small case–control series or cross-sectional studies have tended to show a positive association of *H. pylori* seropositivity with CAD or cerebrovascular disease,[73–75] but larger prospective studies have failed to show any significant association.[35,76–78]

Pathological–microbiological evidence

There is no evidence of systemic invasion of *H. pylori* beyond the intestinal mucosa. Only 2 groups have searched for *H. pylori* DNA by means of PCR in atheromatous tissue specimens. Blasi and coworkers[79] did not detect *H. pylori* DNA in 50 patients with abdominal aortic aneurysms, but *C. pneumoniae* DNA was present in about half of the patients. In a study by Danesh and colleagues,[80] only 1 of 39 atheromatous specimens of the carotid artery was positive for *H. pylori* DNA; however, the possibility of contamination could not be excluded.

Biological plausibility

There are few data to support the theory that *H. pylori* plays a role in the pathogenesis of cardiovascular disease or atherosclerosis. Hypothetical mechanisms include stimulation of an autoimmune reaction to human endogenous heat shock protein-60 by *H. pylori* heat shock protein, and an indirect effect of systemic inflammatory mediators stimulated by local mucosal inflammation, which may affect homeostasis. A small study[73] suggesting that people who are seropositive for *H. pylori* have an increased concentration of inflammatory or procoagulant markers has not been confirmed by larger studies.[76,81] In a study involving 84 patients with CAD and antibodies to *H. pylori* or *C. pneumoniae*, or both, the 43 treated patients had significantly lower fibrinogen levels at 6 months than the untreated control subjects.[82] However, these preliminary data need to be confirmed by larger trials. The reduction in fibrinogen may have been related primarily to the effect of clarithromycin on *C. pneumoniae* infection.

Animal models and clinical evidence

There are no published studies indicating that *H. pylori* causes or exacerbates atherosclerosis in an animal model. There are no clinical intervention trials of *H. pylori* infection and subsequent cardiovascular end points.

Periodontal disease

Epidemiological evidence

Periodontitis is a bacteria-induced chronic inflammatory disease that is an important cause of tooth loss in adults. Its prevalence increases with increasing age, and the disease is quite common in middle-aged and older people. Two stud-

ies have shown a relation between periodontal disease and cardiovascular disease.[83,84] It has also been shown that tooth loss may be associated with an increased risk of CAD and stroke.[85,86] Most of these studies were relatively small case–control series, mainly hospital based, or involved subjects in long-term care facilities. In a large, prospective study 9760 subjects free of CAD at enrolment had a dental examination at the beginning of the study and follow-up over 14 years.[87] After adjustment for potential confounders, periodontitis was associated with an increase of 25% in CAD risk and an increase of up to 70% among men less than 50 years of age. In 2 recent reviews of this topic in the dental literature, conclusions varied somewhat.[88,89] There does appear to be increasing evidence of a relation between dental health and CAD, especially in men aged 40 to 50 years.[88] However, available evidence suggests that further studies are needed to rule out that confounding is a possible explanation for the relation between tooth loss and CAD, and that the role of diet needs more study.[89]

Pathological–microbiological evidence

There are no detailed studies in peer-reviewed journals of direct pathological involvement of atherosclerotic plaques with oral bacteria implicated in dental infection or periodontitis, such as *Porphyromonas gingivalis*. Indirect pathological evidence includes increased markers of inflammation that have been associated with CAD, such as fibrinogen.[90] Increased activity of procoagulants, such as factor VIII,[91] has been reported with periodontitis and poor dental state in a relatively small study.[91]

Biological plausibility

Interactions of *P. gingivalis* with the host immune system are believed to be the basis for the low-grade destructive inflammatory response characteristic of periodontitis. However, there may be systemic spread of the organism. In vitro, *P. gingivalis* can invade bovine aortic and heart endothelial cells as well as human umbilical vein endothelial cells.[92]

Periodontal disease is a candidate infectious disease that can predispose to vascular disease given the abundance of gram-negative species involved, the local production of lipopolysaccharide with detectable levels of proinflammatory cytokines, the involvement of inflammatory cells, the association of periodontal disease with increased peripheral fibrinogen and leukocyte counts,[90] and the chronicity of the disease. *Streptococcus sanguis*, a supragingival plaque organism, can increase platelet aggregation,[93] and *P. gingivalis*, which expresses the platelet-aggregation-associated protein (PAAP), may increase the risk of acute thrombosis. *P. gingivalis* can also stimulate the coagulation cascade by activation of factor X.[94] In a rabbit model, infusion of PAAP-positive *S. sanguis* resulted in acute electrocardiographic changes indicative of ischemia that are not seen with the PAAP-negative strain.[95]

Animal models and clinical evidence

There are no published studies showing that oral pathogens induce or exacerbate pathologically demonstrable atherosclerosis in an animal model. There are no intervention trials of periodontal disease and subsequent cardiovascular end points.

Conclusion

The association of *C. pneumoniae* infection and cardiovascular disease is well established by numerous seroepidemiological and pathological studies. The link between CMV infection and coronary artery restenosis after angioplasty and accelerated coronary atherosclerosis after cardiac transplantation is fairly firm, but the association with native CAD is weak. The association of *H. pylori* infection and cardiovascular disease is weak and controversial, and the link with periodontal disease is suggestive but not established. There is accumulating evidence to suggest a causal relation between *C. pneumoniae* infection and atherosclerosis, but this is not well established. Infections may indirectly accelerate or enhance atherosclerosis in the presence of other risk factors by several mechanisms, or they may induce atherosclerosis through local infection, inflammation or autoimmune reaction.

Future studies of *C. pneumoniae* infection and cardiovascular disease should focus on proving causality. This requires larger clinical trials and further animal models. More large, population-based, prospective epidemiological studies are needed to prove the association of CMV infection, periodontal disease and mixed infection with cardiovascular disease.

Large randomized intervention trials are in progress to determine the value of antibiotic therapy in preventing secondary cardiovascular events associated with *C. pneumoniae* infection, and these will be of value in determining causality. The current method of identifying previous exposure to *C. pneumoniae* by serologic testing is not sufficiently predictive of the presence of the organism in atheromatous plaques. The selection of patients to receive antibiotic therapy (if confirmed to be effective in clinical trials) needs to be addressed and refined. PCR detection of *C. pneumoniae* in peripheral blood mononuclear cells is a promising investigative tool for identifying patients with the organism in the arterial wall. Until these issues are resolved, the empiric use of antibiotics for cardiovascular disease is not recommended or warranted.

I am grateful to Ms. Dawn Bajhan for her assistance in preparing the manuscript, and to Dr. James B. Mahony for reviewing an earlier version of the manuscript.

Competing interests: None declared.

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