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Pathogens and atherosclerosis: Update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis

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Summary

It is currently unclear what causes the chronic inflammation within atherosclerotic plaques. One emerging paradigm suggests that infection with bacteria and/or viruses can contribute to the pathogenesis of atherosclerosis either via direct infection of vascular cells or via the indirect effects of cytokines or acute phase proteins induced by infection at non-vascular sites. This paradigm has been supported by multiple epidemiological studies that have established positive associations between the risk of cardiovascular disease morbidity and mortality and markers of infection. It has also been supported by experimental studies showing an acceleration of the development of atherosclerosis following infection of hyperlipidaemic animal models. There are now a large number of different infectious agents that have been linked with an increased risk of cardiovascular disease. These include: *Chlamydia pneumoniae, Porphyromonas gingivalis, Helicobacter pylori*, influenza A virus, hepatitis C virus, cytomegalovirus, and human immunodeficiency virus. However, there are significant differences in the strength of the data supporting their association with cardiovascular disease pathogenesis. In some cases, the infectious agents are found within the plaques and viable organisms can be isolated suggesting a direct effect. In other cases, the association is entirely based on biomarkers. In the following review, we evaluate the strength of the data for individual or groups of pathogens with regard to atherosclerosis pathogenesis and their potential contribution by direct or indirect mechanisms and discuss whether the established associations are supportive of the infectious disease paradigm. We also discuss the failure of antibiotic trials and the question of persistent infection.

Keywords

Atherosclerosis, bacterial infection, inflammation, infectious diseases

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Introduction

It is now widely accepted that the initiation and progression of atherosclerotic lesions involves a chronic inflammatory response. Classical hallmarks of chronic inflammation are an inability to resolve the inflammation leading to tissue damage and fibrosis. Both tissue damage and fibrosis are prominent components of advanced atherosclerotic lesions. However, it is still unclear what the continuous source of the inflammation is. Based on experimental models, the leading paradigm suggests that lipid deposition and modification is the primary source of the inflammation. However, starting with the observation that the animal herpes virus that causes Marek's disease stimulated the development of atherosclerosis in chickens (1), and the discovery of *Chlamydia pneumoniae* (*C. pneumoniae*) within human atherosclerotic plaques (2, 3), a second paradigm has emerged that suggests that infectious agents may also contribute to the inflammation in the plaques. This has been supported by multiple epidemiological studies that have established positive associations between the risk of cardiovascular disease (CVD) morbidity and mortality and markers of infection. It has also been supported by experimental studies showing an acceleration of the development of atherosclerosis following infection of hyperlipidemic animal models.

There are now a large number of different infectious agents that have been linked with an increased risk of cardiovascular disease (▶ Table 1). These include: *C. pneumoniae, Porphyromonas gingivalis (P. gingivalis), Helicobacter pylori (H. pylori)*, influenza A virus, hepatitis C virus (HCV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV). However, there are significant differences in the strength of the data supporting their association with CVD. In some cases, the infectious agents are found within the plaques and viable organisms can be isolated suggesting a direct effect. In other cases, the association is entirely based on biomarkers, and in the case of HIV the increased risk appears to be the consequence of anti-retroviral therapy (4). In the following review, we evaluate the strength of the data for individual or groups of pathogens and discuss whether the established associations are truly supportive of the infectious disease paradigm. We also address the issue of failed antibiotic trials and persistent infection.

Direct versus indirect mechanisms

An infectious organism can contribute to the inflammation within the blood vessel by directly infecting vascular cells and activating an innate immune response. In order to demonstrate a direct effect, there should be evidence of the presence of the agent within the atherosclerotic plaque but not within normal blood vessels. Better yet is evidence that the organism has infected vascular cells and that viable organisms can be isolated from the plaque suggesting active infection. There should also be an acceleration of atherosclerosis following infection in experimental models. An infectious organism can also contribute by inducing inflammation at a non-vascular site such as the lungs in the case of *C. pneumoniae* and the oral cavity in the case of *P. gingivalis*. The cytokines and other se-creted factors that enter the systemic circulation from either the non-vascular site of inflammation or from an associated acute phase response by the liver are thought to be taken up from the circulation and add to the chronic inflammation that is already on-going within the plaque. In order to demonstrate an indirect effect, there should be measureable increases in plasma cytokines and other acute phase proteins following infection at the non-vascular site and an acceleration of atherosclerosis in experimental models. Optimally, knock out or antagonism of one or more of these pro-inflammatory factors or their receptors in the animal models should prevent or reduce the acceleration of lesion development induced by the infection. Another possible mechanism for an indirect effect is cross-reactivity or molecular mimicry between bacterial and self-antigens such as heat shock proteins or oxidised low-

Table 1: Summary of data in support of direct and indirect mechanisms for the pathogenesis of atherosclerosis.

Infectious agents with reported association to CVD	*Found in human plaque	Found in normal artery or vein	Viable organism isolated from plaque	Increased cytokines and acute phase proteins	Increased lesions in animal models
Bacteria					
Chlamydia pneumoniae	+		+	+	+
Mycoplasma pneumoniae	+	+			
Porphyromonas gingivalis	+		-	+	+
Porphyromonas nigrescens	+		-		
Treponema denticola	+				
Campylobacter rectus					
Aggregatibacter actinomycetemcomitans	+				+
Prevotella intermedia	+		-		
Tannerella forsythia	+				
Fusobacterium nucleatum	+				
Streptococcus sanguis	+				
Streptococcus mutans	+				-
Helicobacter pylori	+			+	+
Enterobacter hormaechei	+		+		
Borrelia burgdorferi					
Viruses					
Influenza viruses				+	+
Cytomegalovirus	+	+		+	+
Hepatitis C virus	+				
Hepatitis B virus				-	
Hepatitis A virus				+	
Human immunodeficiency virus	+				
Epstein Barr virus	+			+	
Herpes simplex viruses	+	+			
Respiratory syncytial virus					
Enteroviruses (echovirus, cocksackie viruses)	+				
Parvovirus	+				
*DNA, RNA, or protein.					

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density lipoprotein (oxLDL) (5, 6). Although treatment with or induction of antibodies against heat shock proteins and oxLDL have effects on the development of atherosclerosis in mice (6, 7), convincing direct evidence for this mechanism is currently lacking for most of the CVD associated pathogens.

There is an emerging concept that no one organism is responsible for the effects of infection on atherosclerosis pathogenesis but that it is the aggregate effects of infection with multiple organisms. This has been called the "infectious burden" or "pathogen burden" (8, 9). There is strong evidence in support of this concept. For example, in one study over 75% of CAD patients had been exposed to at least three of five pathogens tested, and the increasing pathogen burden was significantly associated with increasing CAD risk, even after adjustment for traditional CAD risk factors (10). Thus, ultimately the contribution of infectious organisms to atherosclerosis pathogenesis is likely to involve simultaneous direct and indirect mechanisms involving multiple organisms.

Pathogens associated with CVD

Chlamydia pneumoniae

C. pneumoniae is a gram negative bacterium that is one of the most frequent causes of recurrent low-grade respiratory infection. It is an obligate intracellular pathogen that infects both epithelial cells and macrophages within the lungs and may be disseminated to sites outside of the lungs by infected monocytes and macrophages (11, 12). By far, the largest volume of research demonstrating both an association and cause and effect with regard to atherosclerosis has been conducted with *C. pneumoniae.* However, like all of the organisms discussed here, there have also been studies that have not shown an association or cause and effect. Nevertheless, the vast majority of studies to date on *C. pneumoniae* have been positive. Furthermore, it may be the only pathogen that fulfils all of the requirements listed above for demonstrating both a direct and indirect contribution to atherosclerosis. As noted, it was the first infectious organism to be found within cells of human atherosclerotic plaques but rarely within normal arterial cells (2). It is also one of the few agents where viable organisms have been isolated from plaques (13, 14), and *C. pneumoniae*-reactive T cells have also been isolated from human plaques (15, 16). There have been a large number of studies in experimental models showing an acceleration of atherosclerotic lesion development following respiratory infection with *C. pneumoniae* (17–28), although a few studies have also reported no induction of lesion development (29, 30). The acceleration of lesions is abrogated in the absence of the p55 tumour necrosis factor (TNF)-alpha receptor or IL-17A in mice (31, 32). Furthermore, we and others have recently reported that inoculation of C57Bl/6 or apo E-/- mice leads to measureable increases in plasma or aortic cytokines and acute phase proteins (32–35). We have also shown that infection with *C. pneumoniae* reduces the anti-inflammatory properties of high-density lipoprotein (HDL) and that respiratory infection is associated with an increased frequency of intra-plaque haemorrhage in the innominate artery of older chow-fed apo E-/- mice (33). It has also been shown that knockout of toll-like receptors 2 and 4 and MyD88 attenuates the *C. pneumoniae* accelerated development of lesions in apo E -/- mice and reduces foam cell formation (34, 36). There is also evidence that *C. pneumonia* may contribute via molecular mimicry between bacterial and self-antigens such as heat shock proteins, as T cells reactive to both human HSP60 and *C. pneumoniae* 60-kDa HSP have been isolated from human plaques (16), and autoantibody responses against mouse Hsp60 were reported following infection of mice with *C. pneumoniae* (28).

Periodontal organisms

Periodontal disease (PD) is most often defined as inflammation of the periodontium, the tissue that surrounds and supports the teeth. The gingival plaque associated with PD is colonised by a large number of both gram-positive and gram-negative bacteria (e.g. *P. gingivalis, Treponema denticola, Campylobacter rectus, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Streptococcus sanguis, Streptococcus mutans*) many of which have been associated with CVD (37). There is extensive variability in the strength of the associations in part due to variations used in the markers of PD. These include: salivary flow, self reported or documented periodontal disease, number of teeth, oral organisms, antibodies to oral organisms, total dental index, plaque scores, probing depth, attachment loss, and bone level (38).

A number of periodontal organisms have been found in human atherosclerotic lesions by immunocytochemistry and PCR. These include; *P gingivalis, S. sanguis, F. nucleatum, T. forsythia, P. intermedia, A. actinomycetemcomitans, and B. forsythus* (39–42). *S. mutans* DNA has also been found in heart valves as well as atherosclerotic plaques (43, 44). In contrast, there was no DNA for A. *actinomycetemcomitans, P. gingivalis, T. denticola* found in carotid, coronary or femoral lesions (45) and despite the presence of *P. intermedia* , *P. nigrescens* and *P. gingivalis* DNA in specimens of atherosclerotic plaque from carotid or femoral arteries, none of the positive samples yielded growth of any of the oral bacteria (46).

There is evidence that PD leads to increases in systemic cytokines and acute-phase proteins and thus could have an indirect effect on atherosclerosis (47). For example, C-reactive protein (CRP) levels were elevated two-fold in patients with either PD or CVD as compared to healthy matched controls and three-fold in subjects with both PD and CVD. Increases in serum amyloid A (SAA) and alpha(1)-anti-chymotrypsin were also noted (48). Standard treatment for PD for 12 months reduced plasma haptoglobin, interleukin (IL)-18 and interferon-gamma levels (49). Systemic infection of apo E-/- or apo E-/+ mice with *P. gingivalis* has also been reported to increase plasma IL-6 and SAA (50, 51). Prior treatment of the mice with doxycycline or metronidazole reduced the plasma SAA and cytokines (52, 53).

Infection of heterozygous and homozygous apo E knockout mice on both normal and high-fat diets with *P. gingivalis* led to an

increase in lesion size that was associated with increases in macrophages, T cells, cytokines and lipid within the plaques (52, 54–58). In several cases, *P. gingivalis* DNA was found within the aorta and other tissues in mice following both systemic and oral infection (50, 59). Prior immunisation of apo E-/- mice with either heat killed *P. gingivalis* or the 40-kDa outer membrane protein of *P. gingivalis* has been reported to inhibit the accelerated development of lesions with subsequent challenge with the live organism (55, 58, 60). In contrast, there have been conflicting reports on the acceleration of lesions following IV administration of *A. actinomycetemcomitans* to apo E-/- mice (61, 62). The interpretation of most of these reports is complicated by the fact that accelerated lesion development was in response to systemic administration of large doses of the live organism or purified LPS either via intravenous, intraperitoneal, or subcutaneous routes rather than via normal oral infection (52, 54–56, 60–62). However, a couple of studies have recently reported that oral infection also accelerates lesion development (57, 58).

In keeping with studies of *C. pneumoniae*, *P. gingivalis* has also been reported to accelerate lesion development in part via a TLR-2 mediated mechanism (57) and to increase foam cell formation in infected macrophages (63). LDL cholesterol and triglycerides are elevated while HDL cholesterol is lower in those with periodontitis (64), and HDL cholesterol levels have been reported to decrease following infection of male apo E-/+ mice with *P. ginvivalis* (52). Human HSP60 has been detected in lesions in apo-E-/- mice following intraperitoneal immunisations with *P. gingivalis*. Lesion development was correlated with anti-GroEL antibody levels, supporting the potential involvement of molecular mimicry between GroEL and hHSP60 (54). Despite the failure to isolate viable bacteria from human plaques, the data are supportive of periodontal organisms having both direct and indirect effects on the development of atherosclerosis.

Helicobacter pylori

H. pylori infection is a common cause of gastritis and is another pathogen that has been widely cited as a possible contributor to CVD pathogenesis. However, the strength of the association between markers of *H. pylori* infection and CVD is mixed as a similar number of positive and negative studies have been reported. *H. pylori* DNA has been found in atherosclerotic plaques (65–71), but there have not been any reports of successful isolation of viable organisms from the plaques. *H. pylori* infection did not stimulate release of inflammatory factors in one reported study (72). In contrast, eradication of *H. pylori* infection has been reported to reduce systemic cytokines and to significantly attenuate the reduction in coronary artery lumen area in coronary artery disease patients after percutaneous transluminal coronary angioplasty (PTCA) (69) and to reduce the number of secondary coronary events (73). A statistically significant reduction in CRP and improvement in event free survival has also been reported with combined treatment regimens for both *C. pneumoniae* and *H. pylori*

(74). In another study, eradication had no effect on acute-phase response markers (75). It is controversial as to whether *H. pylori* infection accelerates atherosclerotic lesions in C57Bl/6, LDL-R-/-, or apo E-/+ x LDLR-/+ mice as there have been both positive and negative reports (76–78). In one of these studies, *H. pylori* infection was associated with an elevation of a Th1-immune response against *H. pylori* heat shock protein 60 (Hp-HSP60). Subcutaneous immunisation with Hp-HSP60 or treatment with antibiotics significantly reduced the atherosclerosis (79). In all of the experimental studies with *H. pylori*, the normal pattern of gastric infection was employed. Overall, the strength of the data in support of the direct or indirect paradigms for *H. pylori* is modest, especially in contrast to what has been reported for *C. pneumoniae* and *P. gingivalis*.

Other bacteria: *Mycoplasma pneumoniae, Enterobacter hormaechei, Borrelia burgdorferi, Streptococcus pneumoniae*

M. pneumoniae and *E. hormaechei* DNA have both been found in human atherosclerotic plaques (71, 80, 81). However, *M. pneumoniae* was also found in non-atherosclerotic saphenous veins and in plasma leukocytes suggesting a lack of a direct effect on disease pathogenesis. In contrast, viable *E. hormaechei* was isolated from human femoral lesions and thus could contribute directly to the chronic inflammation within the plaques (81). A positive association between antibody titres for *B. burgdorferi*, the cause of Lyme disease, and carotid intimal-medial thickness has recently been reported (82). In an experimental study, immunisation of LDLR-/- mice with *S. pneumoniae* induced production of oxLDL-specific IgM and a persistent expansion of oxLDL-specific T15 IgM-secreting B cells but reduced the development of atherosclerotic lesions suggesting that molecular mimicry between epitopes of oxLDL and *S. pneumoniae* are protective of atherosclerosis (6).

Cytomegalovirus (CMV)

CMV is a herpes virus that has a high frequency of infection in the general population. Active CMV infection has been associated with accelerated heart transplant vasculopathy and transplant rejection (83, 84). Over the past 20 years, there have been comparable numbers of reports of positive and negative associations with CVD morbidity, but a recent report has shown for the first time an association between CVD mortality and CMV antibody titres (85). There have been a large number of reports of the presence of CMV in human plaques (70, 71, 86, 87–99) as well as a number of failed attempts to document CMV in human lesions (100–107). CMV has also been found in non-atherosclerotic blood vessels from cases and matched controls (86, 91).

There have been a number of experimental studies of the effects of murine CMV infection on atherosclerosis in apo E-/- mice (19,

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108–111). In all cases, lesion development was accelerated and in one case was associated with an influx of T lymphocytes (111). There were also reports on increases in plasma cytokines such as interferon-gamma and TNF-alpha (19, 108, 112) suggesting that an indirect mechanism may be responsible. This possibility has been further supported by a report of an increase in blood pressure in CMV-infected C57Bl/6 mice that was independent of hypercholesterolaemia. The increased blood pressure was associated with increased serum cytokines and angiotensin II and an increase in renin expression by CMV-infected mouse kidney and endothelial cells *in vitro* (113). CMV infection has also been reported to increase the neointimal response to carotid injury in rats and was associated with increases in plasma IL-2 and IL-4 but not with interferon-gamma or TNF-alpha. The combined data suggests that infection with CMV is more likely to have an indirect effect on atherosclerosis pathogenesis than a direct effect.

Hepatitis A, B and C viruses

Although there have been positive associations reported between antibody titres or viral antigens with the presence, severity or mortality from CVD for all of the hepatitis viruses (114–118), many recent studies report no association. In fact, the serum concentrations of glucose, total cholesterol and LDL cholesterol were significantly lower in anti-HCV-positive than anti-HCV-negative haemodialysis patients (119), and hepatitis B surface antigen seropositivity was associated with a decreased risk of ischemic stroke and myocardial infarction but an increased risk of haemorrhagic stroke (120). On the other hand, like with CMV, donor HCV seropositivity is an independent risk factor for increased mortality and for the development of accelerated allograft vasculopathy after cardiac transplantation (121). This suggests that latent virus infection could re-emerge following immuno-suppression and directly contribute to transplant vasculopathy. In addition, positive-strand HCV RNA was detected in carotid plaque tissues from anti-HCV antibody-positive patients but was not detected in anti-HCV antibody-negative patients (122, 123). HBV infection was negatively correlated with plasma CRP levels (124) while the presence of anti-HAV IgG was associated with higher levels of TNF-alpha and sVCAM-1 (118). Finally, in a study of the effects of treatment with an HAV vaccine on atherosclerosis in cholesterol-fed mice, there were no significant differences found in lesion area between the groups (125). Thus, overall the strength of the data for either a direct or indirect contribution to atherosclerosis pathogenesis is quite modest for the hepatitis viruses.

Influenza viruses

Influenza infection is associated with acute coronary syndrome and induction of fatal myocardial infarctions (126). Case-control and cohort studies have shown that the influenza vaccine has a marked protective effect against primary and secondary cardiovascular events, while other studies have shown no effect (127–131). Also, there have been both positive and negative reports of an association between seropositivity for influenza A and B antibodies and coronary artery disease (132, 133) and as yet, no reports of the presence of influenza viral DNA or protein within human atherosclerotic plaques. Short-term studies of the effects of influenza A infection on cellular influx into the arteries of apo E-/- and LDLR-/- mice have reported increases in both macrophages and lymphocytes suggesting that influenza A infection accelerates the development of early atherosclerosis (134, 135). The influx of inflammatory cells was associated with the presence of the influenza virus DNA in the aorta (134, 136) and with increases in plasma cytokines such as IL-6 (135). Influenza A infection in mice has also been reported to induce the loss of the anti-inflammatory properties of HDL (137). Despite the protective effects of the flu vaccine, the data supporting a direct effect of influenza viruses on atherosclerosis is weak. However, the reported increases in plasma cytokines and the loss of the protective effects of HDL in mice suggest that influenza infection may have an indirect effect on atherosclerosis pathogenesis.

Human immunodeficiency virus (HIV)

In simple comparisons between infected and non-infected individuals, several studies have reported a positive association between infection with HIV and the extent of atherosclerosis in the carotid or coronary arteries (138–141). However, the results of a recent meta-analysis as well as a number of independent studies have questioned this association (142–144). Furthermore, as noted, anti-retroviral therapy has been shown to have independent effects on lesion development in several experimental studies (4, 145) and high activity anti-retroviral therapy (HAART) is associated with lipodystrophy, central adiposity, hyperlipidaemia, insulin resistance, and endothelial dysfunction (146–149), all recognised risk factors for CVD. In a number of studies, the positive association between the extent of atherosclerosis and HIV infection was due to or greater when HAART was administered or when there was lipodystrophy and hyperlipidaemia (150–152). However, there have been reports of HIV-induced hypertension and aortic stiffness that are independent of HAART (153, 154), and a recent study has shown that treatment of porcine arterial rings with the HIV Nef protein (an HIV accessory protein) inhibits vasodilation in part, by reducing eNOS expression and increasing superoxide production (155). This suggests that direct HIV infection of endothelial cells could contribute to atherosclerosis by causing endothelial dysfunction. However, there has only been a single report of the presence of HIV infected smooth muscle cells in human plaques (156). *In vitro*, HIV infection of macrophages facilitates foam cell formation by inhibiting ABCA1-mediated cholesterol efflux (157). Evaluations of atherosclerotic plaques from HIV-infected individuals at autopsy revealed extensive lipid deposition and calcification (158), highly accelerated advanced dis-

ease in younger adults (159, 160) and extensive inflammatory infiltration reminiscent of arteritis (161). Thus, given the limited data with regard to the presence of HIV in the plaques and the effects of HAART on CVD risk factors, the likelihood is that HIV has predominantly indirect effects on lesion pathogenesis.

Other viruses: Herpes simplex viruses, Epstein Barr virus, Enteroviruses, Respiratory syncytial virus, Parvovirus

Like many of the infectious agents already discussed, there is mixed data with regard to the strength of association between biomarkers of infection with these viruses and the extent of atherosclerosis. For example, the titres of serum antibodies to HSV-1 and/or HSV-2 have been both positively and negatively associated with CVD in several studies (104, 105, 118, 162–167). Similarly, there have been reports of HSV DNA in human plaques (71, 94, 99, 101, 104, 105, 168, 169) as well as several reports where there was no evidence for the presence of HSV in the plaques (87, 93, 170). HSV DNA has also been found at low frequency in veins and in normal internal mammary arteries (94). HSV-2 seropositivity has been associated with essential hypertension (171). Infection of apo E-/- mice with murine gamma-herpesvirus-68 but not HSV-1 accelerated the formation of lesions 24 weeks following infection and only gamma-herpesvirus-68 DNA could be found in the plaques (172, 173).

A negative association between markers of infection with the Epstein Barr virus and CVD or endothelial dysfunction has been reported (162, 174) despite the fact that several studies have reported the presence of EBV DNA in human plaques (87, 94) or increases in acute phase proteins (174). Other studies have reported no evidence for EBV in human plaques (93). In contrast, there have been positive associations reported for enteroviruses (166), the respiratory syncytial virus (175), and parvovirus b19 (176). Enterovirus (echovirus 9 and coxsackie viruses B1 and B3) DNA has been reported within human plaques (93), and parvovirus DNA was reported only within endothelial cells of plaques (176). Thus, with the possible exception of the enteroviruses, the overall data is rather weak for a role for these viruses in the pathogenesis of atherosclerosis.

Persistent infections and antibiotic trials

The evidence associating various infectious agents as risk factors for cardiovascular disease led to clinical trials to determine the effect of intervention through treatment with appropriate antibiotics (e.g. C. pneumoniae and H. pylori) or prevention through vaccination (Influenza). Initially, several early small clinical trials were undertaken to determine whether treatment with macrolides (azithomycin, roxithromycin and clarithromycin), which have anti-chlamydial activity, had a beneficial effect in secondary pre-

vention of coronary heart disease. Three of eight studies demonstrated a positive reduction in events, one demonstrated a positive result at one month, but not at six months, and four of the studies were negative. As pilot studies, these studies did not have sufficient power and had short durations of treatment (177).

There have been four large clinical trials (WIZARD, ACES, CLARICOR and PROVE IT-TIMI), each enrolling over 4,000 patients focused on stable coronary artery disease (178–181). None of these studies demonstrated any long term benefit of antibiotic intervention in patients with established cardiovascular disease. Although the WIZARD study found a 33% reduction in the incidence of death and myocardial infarction at six weeks, the benefits were not sustained (178). Because of the short-term treatment with azithromycin, a critical question was whether a longer duration of antibiotic treatment would sustain benefits. The ACES study answered this question as it provided a much longer treatment regimen (60 mg azithromycin per week for one year) and follow-up time (46.8 months) with no reduction in cardiovascular events. In addition, in the PROVE IT-TIMI trial, long-term treatment of patients who had been hospitalised for acute coronary syndrome had no benefits. While these well-designed large-scale studies clearly demonstrated that anti-chlamydial antibiotic treatment was not effective in the patients with advanced coronary heart disease, the negative results led some to conclude that this proved that C. pneumoniae was not a contributing factor in atherosclerosis (182) and dampened interest in infectious agents as risk factors for cardiovascular disease (183). As cautioned before the results of the ACES and PROVE IT-TIMI studies were known, if the results were positive, further evidence would be provided for a role of C. pneumoniae in pathogenesis, but would not prove a causality; however, negative results in secondary prevention trials would not rule out a pathogenic role (184).

There are several factors that must be given critical consideration in interpretation of the failed trials that suggest that the role of infection should not be dismissed. First, the difficulty in treating chronic chlamydial infections is well documented for both C. trachomatis and C. psittaci (175, 176, 185). These difficulties are due to the developmental cycle of Chlamydia in which the infectious, but metabolically inactive form (the elementary body), is not susceptible to antibiotics, and the intracellular replicating form (the reticulate body) can establish persistence. In the persistent state, the developmental cycle is arrested and the organisms are not susceptible to antibiotics. Moreover, various antibiotics can induce chlamydial persistence in cell culture (185). In a continuous cell culture model of C. pneumoniae infection, in which aberrant forms characteristic of persistence have been described, prolonged treatment with azithromycin, clarithromycin, or levafloxacin failed to eliminate infection (186). Interestingly, Gieffer et al. demonstrated that persistent C. pneumoniae infection of monocytes was refractory to antibiotic treatment both in vitro and in vivo (187). Unfortunately, there is no clearly defined diagnostic marker of persistent infection.

Second, the subjects in the large scale trials had advanced atherosclerosis. The lack of an effect of antibiotics treatment in such patients is analogous to the lack of benefits observed by anti-

biotic treatment in individuals with "end-stage disease" in which chronic upper genital tract infection or eye infection has resulted in chronic inflammation leading to the fibrosis and scarring observed in tubal factor infertility and trachoma, respectively. Whether antibiotics would have beneficial effect in treatment of patients with early atherosclerosis (such as has been shown in most experimental studies) is unknown and such studies would be difficult to design. An additional consideration is that the events being measured in these patients with advanced disease were likely due to destabilisation and plaque rupture rather than augmentation of occlusive disease (183). Lastly, it is also possible that antibiotic treatment might be ineffective due to pathogen burden as viruses or other bacteria may not be susceptible to the antibiotics used, allowing these agents to still contribute to atherosclerotic processes (183). In considering the large body of evidence that infectious agents can contribute to atherogenesis, rather than concluding that the failure of these secondary prevention trials negates the infectious hypothesis, research should be invigorated in elucidating those mechanisms by which viral and bacterial pathogens contribute to atherosclerotic processes, individually and in concert, individual variability in host responses, interplay of infectious agents with other well-defined risk factors, and the contribution of genetic factors.

Conclusions

In this review, we have listed and evaluated the strength of the evidence in support of both direct and indirect mechanisms for the contribution of a large number of infectious agents to the pathogenesis of atherosclerosis. This included evidence for the presence of the organism in human atherosclerotic plaques, whether viable organisms could be isolated from the plaques, induction of systemic cytokines and acute-phase proteins and whether infection of experimental models accelerated the development of atherosclerotic lesions. Overall, the data suggests that *C. pneumoniae* and some of the periodontal organisms such as *P. gingivalis* could contribute by both direct and indirect mechanisms. The strength of the evidence for most of the other pathogens associated with CVD is weaker and suggests that an indirect mechanism is the most likely explanation for the positive association with CVD. We also conclude that the failure of the antibiotic trials should not lead to the dismissal of the potential role of pathogens in the pathogenesis of atherosclerosis because there are a number of considerations such as lack of susceptibility to antibiotic treatment due to persistent infection in the plaque and pathogen burden that would require simultaneous treatment with multiple antibiotics and antiviral agents.

Conflict of interest
None declared.

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