

EDITORIAL

***Chlamydia pneumoniae* pathogenesis**

Clinical manifestations of *Chlamydia pneumoniae* infection

Chlamydia pneumoniae is a human respiratory pathogen that causes acute respiratory disease and accounts for 5–10% of the cases of community-acquired pneumonia, bronchitis and sinusitis. *C. pneumoniae* has been associated with other acute and chronic respiratory diseases such as otitis media and chronic obstructive pulmonary disease [1]. There have also been reports associating *C. pneumoniae* with asthma, reactive airway disease, Reiter's syndrome and sarcoidosis [1]. The clinical spectrum of *C. pneumoniae* infection has been extended to atherosclerosis [2, 3], a major cause of cardiovascular disease and death in the Western world.

Association of *C. pneumoniae* with atherosclerosis

Three types of evidence support an association of *C. pneumoniae* with atherosclerosis including sero-epidemiological studies, direct detection of bacterial DNA or antigen, or both, in atherosclerotic lesions and isolation of the organism from atheromatous tissue [1, 2]. Following the initial report of Saikku *et al.* demonstrating an association of *C. pneumoniae* antibody with myocardial infarction and coronary heart disease [4], there have been more than 50 studies demonstrating a sero-epidemiological association between *C. pneumoniae* and cardiovascular disease. The strongest evidence for an association of *C. pneumoniae* with atherosclerosis has been the demonstration of *C. pneumoniae* by PCR, immunocytochemical staining and electron microscopy in atherosclerotic lesions and culture of the organism from atheromata [5, 6]. Within the atherosclerotic lesion, the organism has been detected in foam cells derived from macrophages and smooth muscle cells, a hallmark of early lesion formation, and in endothelial cells [5, 6]. Although the percentage of atherosclerotic lesions in which the organism has been found covers a wide range and correlation between different detection methods has not been good [5, 6], more than 45 peer-reviewed publications have confirmed the initial report of Shor *et al.* [7] demonstrating the organism in human atherosclerotic lesions. In a series of studies performed in our laboratories, the organism was detected in 148 (54%)

of 272 atherosclerotic tissues tested and was not found in any of the 52 normal arteries tested [5]. Moreover, *C. pneumoniae* has not been detected in other granulomas, with the exception of sarcoid tissue, and has been found more frequently in atherosclerotic lesions than in other tissues from the same person, suggesting that the organism has a tropism for atheromas [2, 3]. These cumulative observational studies leave no question that *C. pneumoniae* is present in atherosclerotic lesions. Thus, it is doubtful that any further detection studies would provide any additional insight on the association of this organism with cardiovascular disease. Rigorous research efforts should now be focused on whether *C. pneumoniae* plays a role in atherosclerosis and on defining the pathogenic mechanisms by which *C. pneumoniae* infection could contribute to atherogenesis. This brief overview summarises the in-vitro and in-vivo studies that support a role for *C. pneumoniae* infection.

Epidemiology

If *C. pneumoniae* infection contributes to atherosclerotic lesion development or progression, the incidence and prevalence of infection is of considerable significance. Seroepidemiological studies on the age-specific incidence of acute infection have shown that virtually everyone becomes infected between the ages of 5 and 14 at an incidence of 6–9% per year [1]. Antibody against *C. pneumoniae* is rare in children under the age of 5 except in underdeveloped and tropical countries. The antibody prevalence reaches 50% at the age of 20 years and continues to increase slowly to 70–80% in older persons [2], suggesting that re-infection is common.

Atherosclerosis

To elucidate a plausible role by which infection could contribute to atherosclerotic processes, it is important to consider those events occurring in this progressive disease. Atherosclerosis is a chronic inflammatory disease [8]. Early events in lesion development include endothelial injury or activation that can be triggered by multiple factors. This activation results in monocyte/macrophage adherence to the endothelium and migration into the sub-endothelium (intima). In the intima,

monocytes/macrophages take up modified forms of lipoproteins that are trapped in the arterial matrix. This uptake of oxidised low-density lipoproteins transforms them into foam cells, the hallmark of early atherosclerotic lesions. Many of the foam cells within the lesion are activated to release cytokines that in turn further up-regulate endothelial cell adhesion molecules leading to increased leucocyte adhesion. Smooth muscle cells migrate from the media into the intima, proliferate and synthesise connective tissue forming a fibrous matrix. The mature fibrolipid plaque consists of a fibrous cap comprised of smooth muscle cells and extracellular matrix that encapsulates an acellular lipid-rich necrotic core derived from the death of foam cells.

Animal models and *C. pneumoniae*

Both normocholesterolaemic and hypercholesterolaemic mouse and rabbit models have been used to determine whether *C. pneumoniae* infection induces atherosclerotic changes or exacerbates atherosclerosis, respectively. In our laboratory, we have focused on mouse models to investigate the effects of *C. pneumoniae* respiratory tract infection on atherosclerosis. C57Bl/6J mice fed a normal chow diet do not develop atherosclerosis. However, C57Bl/6 mice are prone to fatty streak lesion formation in the aortic sinus when they are fed diets containing saturated fat and cholesterol [9]. ApoE^{-/-} mice (on a C57Bl/6J background) have a marked hypercholesterolaemia due to a deficiency in clearance of cholesterol and develop extensive and spontaneous atherosclerosis in a time-dependent manner ranging from early foam cell lesions to more advanced lesions [10]. Intranasal inoculation of C57Bl/6J mice with *C. pneumoniae* results in infection of lung macrophages and peripheral blood mononuclear cells (PBMC) [11]. In man, *C. pneumoniae* has been detected in peripheral blood mononuclear cells [12] and T lymphocytes [13], suggesting that these cells serve as the vehicle for dissemination of the organism to the site of endothelial activation. In apoE^{-/-} mice, respiratory infection results in dissemination of the organisms to the aorta and establishment of persistent infection in atherosclerotic lesions [11]. In apoE^{-/-} mice fed a normal chow diet and C57Bl/6J mice fed a high fat and high cholesterol diet, repeated *C. pneumoniae* infection accelerates atherosclerotic lesion development [11, 14]. Analogous results of *C. pneumoniae* infection have been reported in low-density lipoprotein receptor knockout mice (LDLR^{-/-}) mice and New Zealand White rabbits fed a high fat and high cholesterol diet [15, 16].

In rabbits on a normal diet, *C. pneumoniae* infection induced atherosclerotic-like changes [17, 18]. Although inflammatory infiltrates and intimal thickening were observed, foam cell lesions did not develop in the C57Bl/6J mice nor in LDLR^{-/-} mice fed a normal

chow diet [11, 15]. Interestingly, infection exacerbates aortic sinus lesions if C57Bl/6J mice are fed a high fat and high cholesterol diet at the time of infection, but not if infection precedes administration of such a diet (unpublished data). These results suggest that *C. pneumoniae* is a co-risk factor with hyperlipidaemia and that the atherogenic effects of *C. pneumoniae* infection are dependent on the presence of hyperlipidaemia [11, 14, 15]. Significantly, in hyperlipidaemia, there is an abundance of lipoproteins, which are thought to be oxidised within the artery wall, resulting in foam cell formation and apoptotic events associated with atherosclerosis. As discussed below, *C. pneumoniae* infection can induce foam cell formation *in vitro*, suggesting one mechanism by which *C. pneumoniae* infection could accelerate atherosclerotic lesion progression.

In-vitro studies

In-vitro studies have provided insights into potential that *C. pneumoniae* plays in atherosclerosis [5, 19, 20]. Consistent with in-vivo findings in animal models and man, *C. pneumoniae* infects human macrophages, endothelial cells, arterial smooth muscle cells, peripheral blood lymphocytes and purified T lymphocytes in cell culture [5, 20]. *C. pneumoniae* infection of vascular cells induces the production of pro-inflammatory and procoagulant activities that are consistent with the atherosclerotic processes. Infection of human vascular endothelial cells results in the production of tissue factor, increased levels of monocyte chemoattractant protein-1, increased platelet adhesion to infected cells, and expression of adhesion molecules that support leucocyte adhesion and diapedesis [5, 19, 20]. Infection of macrophages results in the production of pro-inflammatory cytokines including TNF- α , IL-1 β , IL-6 and IL-8 that are likely to exacerbate the chronic inflammatory processes in atherosclerosis [5, 19, 20]. Importantly, *C. pneumoniae* has been shown to induce human mononuclear phagocytes to form foam cells by chlamydial LPS and low-density lipoprotein oxidation by chlamydial hsp60 [21]. Somewhat surprisingly, lipid loading of macrophages *in vitro* with oxidised LDL or acetylated LDL renders them less susceptible than untreated macrophages or macrophages exposed to native LDL (which does not result in foam cell formation) to infection with *C. pneumoniae* [22]. However, in foam cells in which *C. pneumoniae* infection was established, the burst size was equivalent to that of infected macrophages. Importantly, foam cell formation does not inhibit *C. pneumoniae* induction of pro-inflammatory cytokine production. These results suggest that *C. pneumoniae* can contribute to the inflammatory process without requiring a widespread infection. Chlamydial hsp60 also activates macrophage expression of matrix metalloproteinases that may weaken atherosclerotic plaques and make them susceptible to rupture [23].

C. pneumoniae infection of monocytes has also been shown to enhance monocyte adhesion to endothelial and smooth muscle cells [24]. Co-culture experiments of macrophages and endothelial cells to simulate *in vitro* an influx of *C. pneumoniae* bearing monocytes/macrophages to the site of endothelial activation have also shown that *C. pneumoniae* infection can be transmitted from monocytes to endothelial cells [25]. Moreover, infection of endothelial cells was enhanced by a soluble factor released from macrophages, which was identified as insulin-like growth factor-2 [26]. Similar co-culture experiments of infected macrophages with smooth muscle cells have shown that an alternative mechanism in which cell contact is critical for enhancing susceptibility of smooth muscle cells to *C. pneumoniae* infection (unpublished data). *C. pneumoniae* infection of human endothelial cells induces expression of soluble factors that induce smooth muscle cell proliferation [27]. Cumulatively, these *in vitro* studies suggest that *C. pneumoniae* can affect the expression of cellular pathways, which ultimately contribute to atherosclerosis.

Conclusions

In vitro and *in vivo* studies support a contributory role of *C. pneumoniae* infection in atherosclerosis through augmentation of inflammatory processes, signalling pathways and oxidative stress. Future studies in animal models should focus on delineating those pathogenic mechanisms by which *C. pneumoniae* infection affects atherosclerotic lesion development. However, the burden of proof ultimately lies in the outcome of ongoing large-scale multi-centre clinical trials investigating the effect of antimicrobial intervention on coronary artery disease.

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