Review Article

Association between *Chlamydia pneumoniae* and atherosclerotic lesions

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Summary

There can no longer be any doubt that viable *Chlamydia pneumoniae* organisms are present in atherosclerotic lesions. Indeed, the endovascular presence of *C. pneumoniae* in coronary artery disease (CAD) is common. The fact that this lesion, which is the major cause of stroke, coronary heart disease (CHD), peripheral vascular disease and aortic aneurysm, can no longer be regarded as sterile has prompted a good deal of study and speculation. Atherosclerotic lesions have been studied in detail, but until recently histological descriptions of the lesion have not included *C. pneumoniae* organisms. Reviews and analysis of the literature confirm the association between *C. pneumoniae* and atherosclerotic lesions and CHD. The possibility that *C. pneumoniae* plays a causal or contributory role in the development of atherosclerotic lesions has been debated. It is of major importance as there is already evidence that antibiotic therapy may be of clinical benefit in patients with CHD. Large clinical trials using antichlamydial agents have been embarked upon which may provide further evidence of a causal role for *C. pneumoniae*. The underlying mechanism of how *C. pneumoniae* contributes to lesions and the effect of antibiotic therapy on lesions remain unknown. The association between *C. pneumoniae* and atherosclerosis is reviewed. Particular attention is paid to the lesion itself and the presence of *C. pneumoniae*. Potential areas of study that may contribute to this rapidly expanding area of research is explored.

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Chlamydia pneumoniae is one of four species of *Chlamydia*. Chlamydiae are Gram-negative bacteria that have, as part of their growth cycle, an obligate intracellular existance. The growth cycle takes from 48 to 72 hours. Small, infectious elementary bodies, about 300 nm in diameter, attach to and are taken into cells where they develop into larger reticulate bodies. By a process of binary fission, the reticulate bodies divide to produce new elementary bodies that are released from the cell.[1] *C. pneumoniae* was first described in 1986.[2] It is a common cause of a spectrum of upper and lower respiratory tract diseases in humans and is estimated to be responsible for at least 10% of community-acquired pneumonias.[3] In addition to its association with pneumonia, where the radiological features seen are those of an atypical pneumonia, *C. pneumoniae* has also been associated with acute bronchitis, rhinitis, otitis media, chronic obstructive airways disease, chronic pharyngitis and even lung cancer.[4]

Sero-epidemiology

An association between antibodies to *C. pneumoniae* and coronary heart disease (CHD) was first made in Finland.[5] Over 24 sero-epidemiological studies have been carried out to examine the association between *C. pneumoniae* and CHD. In general they have been case-controlled studies that examined immunoglobulin levels, as a marker for *C.*

pneumoniae infection and clinical parameters or outcomes associated with CHD. Using ultrasonography it has been shown that there is an association between atherosclerosis of the carotid arteries and immunoglobulin G (IgG) antibody titres to *C. pneumoniae*.[6] Review of the evidence from sero-epidemiological studies is in overall support of an association between *C. pneumoniae* and CHD.[7-10] The serological methods employed, however, lack uniformity,[11] and while titres of IgG or immunoglobulin A (IgA) to *C. pneumoniae* represent exposure to the organism at some time in the past, they do not necessarily represent an ongoing chronic infection.[10] Other difficulties with sero-epidemiological studies include the fact that age-matched control populations used in studies of coronary artery disease (CAD) are usually in the sixth decade or more, where the incidence of seropositivity to *C. pneumoniae* is nearly 70%.[12] Recent studies have shown serological evidence for an association between *C. pneumoniae* and atherosclerosis of vessels other than the coronary arteries.[13-15] As *C. pneumoniae* occurs in atherosclerotic lesions of all the major arteries, where it presumably contributes to the total antibody production, the relationship between *C. pneumoniae* antibodies and CHD is difficult to comprehend.[16] There is some direct evidence of the association, with *C. pneumoniae* being detected in a lesion of the coronary artery that caused a fatal myocardial infarction in a 39-year-old patient.[17]

Presence in lesion

In 1992 a study using transmission electron microscopy to examine atherosclerotic coronary arteries in South African goldminers first interpreted some of the lipid vesicles, which are inherent components of the lesion, as *C. pneumoniae* organisms.[18] These were subsequently confirmed as *C. pneumoniae* in a collaborative study.[19] While the finding of *C. pneumoniae* organisms in atherosclerotic lesions was unexpected,[20] it has been confirmed in studies conducted around the world using a variety of independent techniques and arterial tissue from several anatomical sites.[10] Analysis of such studies has shown that the association of *C. pneumoniae* with atheromatous tissue is 59% and only 3% with control arterial tissue.[1]

Although polymerase chain reaction (PCR) has been the most common method used to detect *C. pneumoniae* in arteries, it does not appear to give the most consistent results and has been associated with detection rates in atherosclerotic lesions from 0%[21-23] to 100%.[24] The standardisation of the PCR technique has been called for.[11,25,26]

Other organisms

C. pneumoniae is not the only organism to be associated with atherosclerotic plaques or CHD. An association has been claimed for cytomegalovirus, herpes simplex virus, Epstein-Barr virus, human herpes virus 6, *Helicobacter pylori, Porphyromonas gingivalis,* and *Streptococcus sanguis*.[27-29] The evidence for the association with *C. pneumoniae,* however, appears to be the strongest,[9,10,30-32] and of all the candidate organisms *C. pneumoniae* appears most likely to be involved in atherogenesis.[33] Cytomegalovirus has been detected by immunocytochemistry and *in situ* hybridisation techniques in arterial tissue; however, it does not appear to be specific to atherosclerotic lesions.[34] Current opinion does not support a significant role for *H. pylori* in atherogenesis.[32,33] A meta-analysis of 18 serological studies[35] suggests that the involvement of *H. pylori* in CAD appears to be weak.[1] Furthermore, *H. pylori* has never been detected in atherosclerotic lesions, which tends to rule out the possibility of direct involvement.[36]

Distribution in arterial tissue

The presence of *C. pneumoniae* has been demonstrated in lesions in a range of arteries[37] including coronary,[18,19] femoral, iliac, carotid, cerebral, pulmonary[34] and renal[1] arteries. It has also been detected in the aorta[34] and abdominal aortic aneurysms,[36,38-41] although one study failed to detect the organism in symptomatic abdominal aortic aneurysms.[23] The presence of *C. pneumoniae* does not appear to be confined to arterial lesions and it has been detected in stenotic aortic valves.[42] There is also evidence from PCR techniques that it may be present in some veins[1,37] and cardiac muscle.[1,17]

Viability

In addition to its presence in atherosclerotic arterial tissue, *C. pneumoniae* appears to be viable. It has been cultured from atherosclerotic coronary[43,44] and carotid arteries.[45] It has also been cultured from 16% of occluded coronary artery venous grafts.[46] Viability in atherosclerotic plaques of carotid arteries has also been demonstrated using the reverse transcriptase PCR.[47] Indeed, the endovascular presence of viable *C. pneumoniae* in CAD is common.[44] *C. pneumoniae* is the only bacterium or virus that has been cultured from or shown to be viable in atherosclerotic lesions.

Possible role

Controversy currently revolves around the possible role of the bacterium in initiating or contributing to atherosclerosis. Although much has been written on the subject, there is more speculation than fact concerning the role of *C. pneumoniae*.[48] Opinion varies from an innocent bystander to a vicious assassin.[1,49-51] While some studies conclude that *C. pneumoniae* is not an important factor in atherosclerosis,[52] the lesion has been interpreted as a chlamydial granuloma of arteries — a chlamydioma.[53] The problem has been likened to the old 'chicken or egg' conundrum.[37] Koch's postulates are often cited for determining if an organism causes a specific disease.[54] These postulates are largely impractical because of ethical considerations. Criteria to help determine whether an association is causal have been examined for *C. pneumoniae* and atherosclerosis and many of the criteria are fulfilled.[34]

Antibiotic therapy studies

In order to test the hypothesis that *C. pneumoniae* causes or contributes to atherosclerosis, clinical trials to eradicate the organism have been proposed.[1,34,55,56] If *C. pneumoniae* causes or contributes to the lesion, then eradication using antichlamydial agents should have a beneficial effect. Such trials have been initiated, mainly using macrolide antibiotics. The results from some small trials have been reported.[57-61] They suggest that antibiotics may confer some benefit in the treatment of patients with CHD. These findings should be followed up with larger trials.[62] While these initial results are encouraging and appear to lend support to the hypothesis that *C. pneumoniae* contributes to atherosclerosis, many questions remain unanswered. It has been postulated that the benefit derived by the patient might be ascribed to the anti-inflammatory properties of the antibiotics rather than their antichlamydial properties.[11] Issues such as the optimal type of antibiotic, optimal dose and duration of therapy need clarification along with questions about antibiotic resistance if the use of antibiotic therapy for the treatment of atherosclerosis becomes widespread.[63] Despite the objections that have been raised concerning the use of antibiotics for the treatment of atherosclerosis, the enormous potential benefits, such as a new, effective, non-surgical treatment for CHD justify persevering with eradication trials.[55] Several large trials are in progress and results are expected to be available in the next 2 years.[55,63,64]

In addition to intervention studies, there has been some evidence to suggest that individuals who suffer acute myocardial infarction are less likely than age-matched controls to have used tetracycline antibiotics or quinolones in the past 3 years.[65] Such studies, which retrospectively examine the possibility that antibiotic treatment has an effect on *C. pneumoniae* which in turn has a bearing on the incidence of myocardial infarction, have been criticised.[66]

Studies using animal models suggest that vascular lesions can be induced by *C. pneumoniae*.[67] *C. pneumoniae* appears to accelerate the development of atherosclerotic lesions,[68] while antibiotic administration can prevent it.[69]

Examination of the lesion

Atherosclerotic lesions have been well described.[70-72] The presence of lipid in the lesion has added to the strength of the intensely studied association between CHD and lipids. Atherosclerotic vascular disease, however, remains the largest cause of morbidity and mortality in the Western world. There is an increasing awareness of the role of inflammation in atherosclerotic disease, which is reviving interest in the possible role of infectious agents.[33,70,73] This new impetus should revive interest in the atherosclerotic lesion itself. To date, only a few histopathological studies have included a description of *C. pneumoniae* in the lesion.[48,74,75] Some information is available from studies that are mainly concerned with the presence or absence of *C. pneumoniae* through the use of immunohystochemistry, transmission electron microscopy and *in situ* hybridisation. *C. pneumoniae* appears to be present in all stages of development of the lesion.[34,48,76] Its presence in the earliest detectable lesions has been used as an argument against the suggestion that *C. pneumoniae* is a secondary invader of existing lesions.[34] Apart from one report of weakly positive staining using immunohistochemistry,[77] *C. pneumoniae* has not been demonstrated in human arterial endothelium *in vivo*. Using *in situ* hybridisation, staining for *C. pneumoniae* has been reported in the intimal layer of an atherosclerotic coronary artery.[43] Immunohistochemical double labelling techniques have localised chlamydial antigens to smooth muscle cells, macrophages and unidentified mononuclear cells within the intima of atherosclerotic arteries.[33,77-80] These observations have been supported by transmission electron microscopic studies in atherosclerotic lesions that are positive for *C. pneumoniae* by PCR or immunohistochemical techniques.[17-19, 34,53,75]

The morphology of the elementary body of *C. pneumoniae* has been confirmed in a human atherosclerotic lesion using immunogold labelling and transmission electron microscopy.[53] Foam cells, derived from smooth muscle cells and macrophages, have also been shown to contain *C. pneumoniae*.[34,53,74,75] The presence of *C. pneumoniae* in smooth muscle cells within atherosclerotic lesions has been associated with cellular damage, in the form of vacuolation with a concomitant loss of intracellular myofilaments and an accumulation of intracellular lipid.[17-19,34,48,75] Fragmented, smooth muscle cells have been associated with the presence of *C. pneumoniae* and these fragments, along with their accompanying organisms, may be engulfed by macrophages.[48,53] This is one mechanism to explain the presence of *C. pneumoniae* in macrophages.[48] The fragmentation of infected smooth muscle cells may also account for the presence of *C. pneumoniae* in extracellular matrix.[53,75] The consensus of the available, limited, histopathological evidence is that *C. pneumoniae* is present in smooth muscle cells, macrophages and foam cells. It is also found in the extracellular matrix in association with cellular damage. Lesions containing *C. pneumoniae* do not show the presence of organisms or pathological changes in the endothelium.[53] Using light microscopy, lesions that are positive for *C. pneumoniae* are seen to contain inclusion bodies with a finely granular sand-like appearance in smooth muscle cells and macrophages. This inclusion material has a golden colour with haematoxylin and eosin staining, appears brown with Masson's trichrome method, pale blue with Giemsa, and grey with the periodic acid Schiff method.[53]

Relevant questions

The existence of *C. pneumoniae* in arteries is well documented. Greater ingenuity is now required to determine the importance of this fact.[1] Studies of the atherosclerotic lesion may help to elucidate several important questions. It is not known by what route *C. pneumoniae* reaches arterial tissue; via the endothelium or *vaso vasorum*, or how it is transported. We do not know what triggers the inflammatory response seen in atherosclerotic lesions and it is not known what happens to a lesion following treatment with antichlamydial agents. Infection of the respiratory tract by

C. pneumoniae is common[3] and it has been postulated that following an infection, macrophages from the lung transport the organism to arteries.[49,78,81] The macrophages in arterial lesions, however, do not possess the pigment characteristic of lung macrophages.[48,53] It is possible that the presence of *C. pneumoniae* in macrophages within atherosclerotic lesions may be, at least partly, the result of ingestion of organisms included with fragmenting smooth muscle cells.[48,53] It has been suggested that circulating, bone marrow-derived monocytes, not macrophages, enter the arterial wall.[82,83] Evidence for this is largely based on animal studies and it is usually represented schematically.[82,84]

It is not known what stimulates the initial inflammatory response. At one time it was thought to be triggered by injury to endothelial cells.[85] Evidence for this endothelial damage has not been confirmed in studies of human tissue *in vivo* and the current concept involves physiological or metabolic damage to the endothelium.[70] Studies of very early lesions describe the earliest pathological changes as occurring in the intima, not the endothelium. These changes take the form of non-fatty, focal areas of oedema.[86,87] More extensive and detailed studies of the earliest lesions may help to elucidate the initial phase of atherogenesis.

In the light of ongoing clinical antibiotic intervention trials, of immediate and practical importance is the question of what happens to a lesion rendered sterile by antibiotic therapy? As many of the symptoms of atherosclerotic vascular diseases are due to the space occupied by the lesion, the fate of a treated lesion would appear to have pertinence. Is the natural history of the lesion arrested? Does it resolve? It would be difficult to imagine an advanced,[72] complicated lesion that has calcified resolving. In order to understand the mechanism whereby antibiotic therapy confers benefit in atherosclerotic vascular disease, examination of the lesion itself may provide valuable information.

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