INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 22, no. 1, 9-14 (2009)

EDITORIAL

CHLAMYDIA PNEUMONIAE AND ATHEROSCLEROSIS: CURRENT STATE AND FUTURE PROSPECTIVES

R. SESSA, M. NICOLETTI[1], M. DI PIETRO, G. SCHIAVONI, I. SANTINO, C. ZAGAGLIA, M. DEL PIANO and P. CIPRIANI

Department of Public Health Sciences, "Sapienza" University, Rome;
[1]Department of Biomedical Sciences, "G. d'Annunzio" University, Chieti, Italy

Received February 16, 2008 – Accepted July 23, 2008

***Chlamydia pneumoniae*, an intracellular bacterial pathogen, is known as a leading cause of human respiratory tract infections worldwide. Over the last decade, several reports in the literature have suggested that infection with *C. pneumoniae* may contribute to the pathogenesis of atherosclerosis. In order to play a causative role in chronic disease, *C. pneumoniae* would need to persist within infected tissue for extended periods of time, thereby stimulating a chronic inflammatory response. *C. pneumoniae* has been shown to disseminate systemically from the lungs through infected peripheral blood mononuclear cells and to localize in arteries where it may infect endothelial cells, vascular smooth muscle cells, monocytes/macrophages and promote inflammatory atherogenous process. The involvement of *C. pneumoniae* in atherosclerosis was investigated by seroepidemiological and pathological studies, *in vivo* and *in vitro* studies, and in clinical antibiotic treatment trials. This review will provide an update on the role of *C. pneumoniae* in atherosclerosis focusing on the recent insights and suggesting areas for future research.**

Atherosclerosis is a major health problem in developed countries, contributing to death in more than 50% of patients affected (1). Atherosclerosis is widely regarded as a chronic inflammatory process in the artery vessel wall which is characterized by endothelial injury, accumulation of monocytic cells, and increased secretion of cytokines indicating an active local inflammatory response.

A number of infectious agents have been implicated in atherosclerosis, such as *Helicobacter pylori* and Cytomegalovirus, but *Chlamydia pneumoniae* (*C. pneumoniae*) is the sole viable pathogen detected in atherosclerotic plaque arteries (2).

C. pneumoniae, a common cause of respiratory infections, is presumed to play a role in the pathogenesis of atherosclerosis for its ability to systemically disseminate via macrophages, monocytes and lymphocytes migrating into vessel walls or into vessels of pre-existing atheromatous lesions (3-4).

C. pneumoniae, an intracellular obligate pathogen, has a unique developmental cycle with two alternating functional and morphological forms: the elementary body (EB) and the reticulate body (RB). The EB is the metabolically inert, infectious form of the organism that is capable of transient extracellular survival. The RB is the

Key words: C. pneumoniae, diagnosis, atherosclerosis

Mailing address: Iolanda Santino, MD,
Department of Public Health Sciences,
"Sapienza" University,
P.le Aldo Moro, 5
00185 Rome, Italy
Tel: ++39 6 49914622 Fax: ++39 6 49914634
e-mail: iolanda.santino@uniroma1.it

0394-6320 (2009)
Copyright © by BIOLIFE, s.a.s.
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.
Unauthorized reproduction may result in financial and other penalties

intracellular replicative form of the microorganism but is not infectious (5). The developmental cycle of *C. pneumoniae* can be inhibited by treatment with certain antibiotics, with cytokines such as IFN-γ or by nutrient depletion resulting in atypical chlamydial forms (Fig. 1). These forms may persist for a long time inside the host-cell and remain viable in a culture-negative state. Persistent forms of *C. pneumoniae* are inherently more suited to evade the host immune response and are more difficult to eradicate with antibiotics (6). This could lead to extended survival of *C. pneumoniae* inside epithelial cells and monocytes, which in combination with the elimination of protective cells may promote the development of chronic inflammatory diseases.

This review will provide an update on the role of *C. pneumoniae* in atherosclerosis, focusing on the recent insights that have been obtained in seroepidemiological and pathological studies, *in vivo* and *in vitro* studies, and in clinical antibiotic treatment trials.

Seroepidemiological studies

Exposure to *C. pneumoniae* is extremely common and infections occur repeatedly among most people. Population prevalence studies demonstrate that infections are not restricted geographically and that re-infections occur frequently.

The first suggestion that *C. pneumoniae* may be associated with atherosclerotic cardiovascular diseases was proposed in 1988 by Saikku et al. (7). They showed that patients with acute myocardial infarction and chronic heart diseases had anti-*C. pneumoniae* antibodies more frequently (68%) than population controls (17%).

Since then, several studies (cross-sectional, case-control, retrospective) have confirmed the association between serological evidence of *C. pneumoniae* infection and atherosclerotic cardiovascular diseases (2, 8). In contrast, large-scale prospective studies, unlike cross-sectional studies that are not able to determine a causal relationship between *C. pneumoniae* and atherosclerotic cardiovascular diseases, have failed to demonstrate such an association (9).

The majority of seroepidemiological studies used the microimmunofluorescence (MIF) test that is considered by the Center for Disease Control and Prevention to be the only currently acceptable serological test. It is considered to be the reference standard for serodiagnosis, despite the significant limitations such as poor specificity and reproducibility.

The poor specificity of MIF may result from serological cross-reactivity with other chlamydial species, as well as M*ycoplasma, Bartonella,* and *Yersinia* species. In addition, drawbacks of MIF include lack of standardization of reagents, technical complexity, and subjective end points, all of which result in significant intra- and inter-laboratory variation of test performance (10).

Lastly, the interpretation of serologic results is complicated since a large part of the population has pre-existing IgG antibodies from previous exposure(s) and, hence, it is difficult to identify differences in seropositivity among patients and controls.

Pathological studies

Further evidence that *C. pneumoniae* might play a role in atherosclerosis comes from studies in which the microorganism has been identified in atherosclerotic lesions by immunohistochemistry (IHC), electron microscopy (EM), polymerase chain reaction (PCR) and cell culture (CC) (2).

In 1992, Shor et al. (11) were the first to detect *C. pneumoniae* by EM and IHC in atherosclerotic coronary plaques. About 100 studies were published between 1992 and 2007 on direct detection of *C. pneumoniae* in atherosclerotic lesions.

The detection rates of *C. pneumoniae* in atherosclerotic lesions vary significantly among studies; this variability may be attributed to the lack of a standardized method. However, data collected from studies show that *C. pneumoniae* is more common in atherosclerotic plaques (28%) than in healthy vessels (7%) (2, 9, 12-13).

A total of 716 (53%) of 1,357 atherosclerotic lesions were positive by IHC. EM gave positive results in 60 (45%) of 132 specimens. The percentage of positive by PCR was significantly less: 1,240 (26%) of 4,817 specimens, whereas CC yielded a prevalence of only 5%. The lowest positivity frequency obtained with CC may be due to difficulty in growing *C. pneumoniae* in cell culture, especially from atherosclerotic tissue since this microorganism

is present in a non-cultivable, persistent state (10).

Data collected from studies evaluating the presence of *C. pneumoniae* DNA in PBMC strongly suggested *C. pneumoniae* DNA in PBMC as marker of chronic infection (14-17). Even more important are the studies showing the presence of *C. pneumoniae* DNA concurrently in PBMC (35%) and atherosclerotic lesion (32%) obtained from the same patient. Pooled data suggested that detection of *C. pneumoniae* DNA in PBMC may be a rapid alternative approach to identify subjects carrying chlamydial DNA in the vascular wall (14-19).

In vivo studies

The first animal models tested involved genetically-induced hyperlipidaemic mice, diet-induced hyperlipidaemic mice and cholesterol fed New Zealand white rabbits. In these models, repeated *C. pneumoniae* infection has been shown to accelerate plaque development suggesting that *C. pneumoniae* is a co-risk factor with hyperlipidaemia and that the atherogenic effects of *C. pneumoniae* infection are contingent on the vascular response to hyperlipidaemia (9). Furthermore, in both animal models, *C. pneumoniae* was identified in foam cells or at sites of inflammation.

In contrast, infection of normolipidaemic mice and rabbits with *C. pneumoniae* did not induce plaque development but transient inflammatory reactions in the aorta (20). Importantly, atherosclerotic plaques did not develop in mice after infection with *Mycoplasma pneumoniae*, *H. pylori* and *Chlamydia trachomatis,* showing that the atherogenic effect is species specific to *C. pneumoniae* (21).

Mouse models have also provided detailed information about dissemination of *C. pneumoniae* to vasculature and to multiple organs. Indeed, the presence of *C. pneumoniae,* after intranasal inoculation, in lungs, spleen and buffy coats provided the first experimental evidence that *C. pneumoniae* is able to disseminate via the blood stream throughout the body (20).

Further studies have tested the effect of antibiotic treatment in modifying experimental atherogenesis in *Chlamydia*-infected mice and rabbits. They reported that azithromycin prevented accelerated atherosclerosis in hyperlipidemic rabbits infected with *C. pneumoniae* but did not appear to eradicate the microorganism as demonstrated by ICC and PCR. Furthermore, *in vivo* infection models have also demonstrated the evidence of persistent chlamydial infection. In mice infected intranasally with *C. pneumoniae*, the microorganism was not cultured from lung tissue but it was detected using PCR. Particularly convincing evidence of chlamydial persistent infection comes from studies that described reactivation of persistent infection in infected mice (20).

In vitro studies

In vitro studies have shown that *C. pneumoniae* has various pro-atherosclerotic effects on atheroma-associated cells types (21). Infection of monocytes with *C. pneumoniae* increases adherence of infected monocytes to endothelial cells and promotes low density lipoprotein oxidation, resulting in accelerated uptake of cholesterol by macrophages and subsequent foam cell formation. Furthermore, the multiplication of *C. pneumoniae* inside monocytes or macrophages triggers the production of pro-inflammatory cytokines, such as interleukin (IL)-1 α, IL-6, tumor-necrosis factor (TNF)-α, monocytes chemoattract protein-1, macrophage inflammatory protein 1α and IL-12, that may promote lesion progression and IL-10 that may prevent apoptosis to perpetuate inflammation. Chlamydial heat shock protein 60 (cHSP60) has also been observed to activate macrophages, stimulating TNF-α and matrix metalloproteinase expression, which may contribute to plaque weakening and subsequent rupture.

Infection of endothelial cells with *C. pneumoniae* upregulates expression of adhesion molecules (endothelial-leukocyte adhesion molecule-1, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), chemokines (monocyte chemoattractant protein-1) and cytokines (IL-1, IL-8, TNF-α) resulting in pro-atherosclerotic process such as enhanced adherence and migration of leukocytes. *C. pneumoniae* infection of endothelial cells can also trigger smooth muscle cell (SMC) proliferation through induction of endogenous Hsp60 and platelet-derived growth factor (PDGF) which is a strong mitogen.

There is also evidence that *C. pneumoniae* induces endothelial procoagulant activity through IL-8, IL-6 and plasminogen activator inhibitor-1 which would

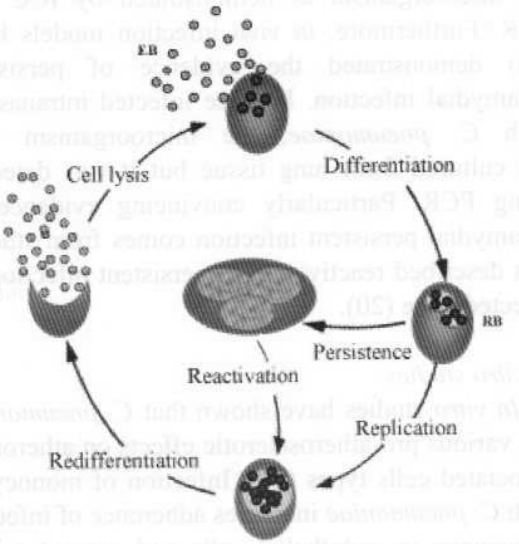

Fig. 1. *The developmental cycle of C. pneumoniae. The cycle can be inhibited by treatment with antibiotics, cytokines such as IFN-γ, or by nutrient depletion resulting in persistent forms.*

further contribute to the atherosclerotic process. Infection of SMC with *C. pneumoniae* induces the production of IL-6, basic fibroblast growth factor (bFGF) and matrix metalloproteinases, which may contribute to plaque destabilization.

Although *in vitro* data point to atherosclerotic effects of *C. pneumoniae*, most of this evidence still has to be confirmed *in vivo*.

Clinical antibiotic treatment trials

Antibiotic treatment trials for the prevention of cardiovascular events in animal models of atherosclerosis have produced results that were so encouraging as to inspire treatment trials in patients.

Numerous clinical trials have examined whether treatment of *C. pneumoniae* is beneficial in the secondary prevention of events in patients with atherosclerotic cardiovascular diseases, but the results have been inconclusive (9). The discordant results may be due to the small number of patients, inadequate dosing and duration of treatment, length of observation, and patient population selected. In addition, different serology criteria were used to define seropositivity to *C. pneumoniae* in the patients enrolled. Lastly, the reduction in inflammatory markers reported in several studies may be secondary to inflammatory activity of macrolides and tetracyclines (2).

Three large randomized trials, WIZARD, PROVE-IT and ACES, were carried out which involved a large number of patients, much longer antibiotic treatments and more extended follow-up, although they varied in the type of patients recruited and in the antibiotic regimen used. None of these three trials showed a reduction of the occurrence of cardiovascular events (9).

Recently, a meta-analysis of all published clinical trials that evaluated the effect of anti-chlamydial antibiotic treatment on the outcomes of patients with coronary artery diseases did not support any benefit of antibiotic therapy in reducing mortality or cardiovascular events in such patients (22).

However, the failure to improve clinical outcomes by treatment with anti-chlamydial antibiotics does not exclude *C. pneumoniae* infection as a potential etiology of acute coronary events. The negative results may be related to the pathobiology of *C. pneumoniae* infection. Indeed the infectious, extracellular form of the microorganism is not susceptible to antibiotics and may remain viable in the organism for weeks or months. Furthermore, the intracellular replicating form that is susceptible to antibiotics can switch to the persistent form that is not susceptible to antibiotics.

Conclusions and future prospectives

This review provides the current state of knowledge of *C. pneumoniae*-associated atherosclerosis, critically evaluating the available data of different fields of research.

Although seroepidemiological studies have initially helped to identify an intriguing and potentially important association between *C. pneumoniae* infection and atherosclerosis, they show significant limitations. Indeed, neither the serologic procedures nor the criteria to define a past or chronic persistent infection with *C. pneumoniae* are standardized. Lastly, serological tests do not appear suitable for predicting vascular infection since there is poor correlation between anti-chlamydial antibodies and the presence of *C. pneumoniae* within atherosclerotic plaques.

Pathological studies have shown that *C.*

pneumoniae is more frequently detected in atherosclerotic plaques than in healthy vessels although a wide variability in *C. pneumoniae* detection exists as a result of a lack of standardization of used methods.

In vitro and *in vivo* studies have provided the evidence that *C. pneumoniae* disseminates systemically from the lungs through PBMC and infects cells of the vascular wall involved in the atherogenic process. It has also been demonstrated that *C. pneumoniae* is able to induce inflammation and initiate or promote lesion development in animal models.

Clinical antibiotic treatment trials do not confirm a causal relationship between *C. pneumoniae* and atherosclerosis since they have failed to demonstrate any effect of anti-chlamydial antibiotic treatment on cardiovascular events.

Clearly, there is an important issue raised by the studies on the association of *C. pneumoniae* with atherosclerosis: the lack of well-standardized and adequately validated diagnostic test for detecting *C. pneumoniae*. This problem is made more difficult for the presence of *C. pneumoniae* persistent forms which are not consistently detectable by currently used methods. This diagnostic limitation had important implications on results of all studies concerning the involvement of *C. pneumoniae* in atherosclerosis and mainly on antibiotic trial data that would have finally helped to establish a causal link between *C. pneumoniae* infection and atherosclerosis.

At present, the role of *C. pneumoniae* in atherosclerosis is still controversial. The recent application of real-time PCR for *C. pneumoniae* DNA detection in PBMC may be useful for assessing the infection status. Therefore the measurement of chlamydial load in PBMC will be helpful in providing an understanding of pathogenesis of the infection, degree of infectivity, and risk of developing sequelae. It will be important for current and future investigators to perform the monitoring of chlamydial load in PBMC since this would provide a logical basis for clinical antibiotic treatment trials.

Investigations on molecular mechanism involving persistence and active forms of *C. pneumoniae* are in progress. A better understanding of the ability of *C. pneumoniae* to transform between these two stages, will clarify the pathogenetic mechanism that link *C. pneumoniae* to development of atherosclerosis.

REFERENCES

1. Siscovick DS, Schwartz SM, Caps M, Wang SP, Grayston JT. *Chlamydia pneumoniae* and atherosclerotic risk in populations: the role of seroepidemiology. J Infect Dis 2000; 181:S417-20.
2. Boman J, Hammerschlag MR. *Chlamydia pneumoniae* and atherosclerosis: critical assessment of diagnostic methods and relevance to treatment studies. Clin Microbiol Rev 2002; 15:1-20.
3. Moazed TC, Kuo CC, Grayston JT, Campbell LA. Evidence of systemic dissemination of *Chlamydia pneumoniae* via macrophages in the mouse. J Infect Dis 1998; 177:1322-5.
4. Yamaguchi H, Haranaga S, Widen R, Friedman H, Yamamoto Y. *Chlamydia pneumoniae* infection induces differentiation of monocytes into macrophages. Infect Immun 2002; 70:2392-8.
5. Hogan RJ, Mathews SA, Mukhopadhyay S, Summersgill JT, Timms P. Chlamydial persistence: beyond the biphasic paradigm. Infect Immun 2004; 72:1843-55.
6. Gieffers J, Fullgraf H, Jahn J, Klinger M, Dalhoff K, Katus A, Solbach W, Maass M. *Chlamydia pneumoniae* infection in circulating human monocytes is refractory to antibiotic treatment. Circulation 2001; 103:351-6.
7. Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, Huttunen JK, Valtonen V. Serological evidence of an association of novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 1988; ii:983-6.
8. Sessa R, Di Pietro M, Santino I, del Piano M, Varveri A, Dagianti A, Penco M. *Chlamydia pneumoniae* infection and atherosclerotic coronary disease. Am Heart J 1999; 137:1116-9.
9. Watson C, Alp NJ. Role of *Chlamydia pneumoniae* in atherosclerosis. Clin Sci 2008; 114:509-531.
10. Dowell SF, Peeling RW, Boman J, et al. Standardizing *Chlamydia pneumoniae* assays recommendations from the Center for Disease Control and Prevention (USA) and the Laboratory Center for Disease Control (Canada). Clin Infect Dis 2001; 33:492-503.

11. Shor A, Kuo CC, Patton DL. Detection of *Chlamydia pneumoniae* in coronary arterial fatty streaks and atheromatous plaques. S Afr Med J 1992; 82:158-61.
12. Cochrane M, Pospischil A, Walker P, Gibbs H, Timms P. Distribution of *Chlamydia pneumoniae* DNA in atherosclerotic carotid arteries: significance for sampling procedures. J Clin Microbiol 2003; 41: 1454-7.
13. Sessa R, Di Pietro M, Schiavoni G, et al. Detection of *Chlamydia pneumoniae* in atherosclerotic coronary arteries. Int J Immunopathol Pharmacol 2004; 17: 301-06.
14. Sessa R, Di Pietro M, Schiavoni G, et al. *Chlamydia pneumoniae* DNA in patients with symptomatic carotid atherosclerotic disease. J Vasc Surg 2003; 37: 1027-31.
15. Blasi F, Boman J, Esposito G, et al. *Chlamydia pneumoniae* DNA detection in peripheral blood mononuclear cells is predictive of vascular infection. J Infect Dis 1999; 180:2074-6.
16. Prager M, Türel Z, Speidl WS, et al. *Chlamydia pneumoniae* in carotid artery atherosclerosis: a comparison of its presence in atherosclerotic plaque, healthy vessels, and circulating leukocytes from the same individuals. Stroke 2002; 33:2756-61.
17. Cochrane M, Pospischil A, Walker P, Gibbs H, Timms P. Discordant detection of *Chlamydia pneumoniae* in patients with carotid artery disease using polymerase chain reaction, immunofluorescence microscopy and serological methods. Pathology 2005; 37:69-75.
18. Sessa R, Di Pietro M, Schiavoni G, et al. *Chlamydia pneumoniae* in asymptomatic carotid atherosclerosis. Int J Immunopathol Pharmacol 2006; 19:111-8.
19. Sessa R, Di Pietro M, Schiavoni G, et al. Measurement of *Chlamydia pneumoniae* bacterial load in peripheral blood mononuclear cells may be helpful to assess the state of chlamydial infection in patients with carotid atherosclerotic disease. Atherosclerosis 2007; 195:224-30.
20. de Kruif MD, van Gorp EC, Keller TT, Ossewaarde JM, ten Cate H. *Chlamydia pneumoniae* infections in mouse models: relevance for atherosclerosis research. Cardiovasc Res 2005; 65:317-27.
21. Kalayoglu MV, Libby P, Byrne GI. *Chlamydia pneumoniae* as an emerging risk factor in cardiovascular disease. JAMA 2002; 288:2724-31.
22. Andraws R, Berger JS, Brown DL. Effects of antibiotic therapy on outcomes of patients with coronary artery disease. A meta-analysis of randomized control trials. JAMA 2005; 293:2641-7.