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CHLAMYDIAL INFECTIONS OF THE CARDIOVASCULAR SYSTEM

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1. ABSTRACT

This paper presents a review on cardiovascular diseases which can be caused by chlamydial infection with the emphasis in the recent development in association between *Chlamydia pneumoniae* and cardiovascular disease. The review includes seroepidemiologic observations; the discovery of *C. pneumoniae* in atheromatous plaques; *in vivo* studies using animal models indicating that *C. pneumoniae* is a co-risk factor of hyperlipidemia for atherosclerosis; *in vitro* studies demonstrating putative mechanisms by which *C. pneumoniae* could contribute to the immunopathology of atherosclerosis; and early promising antibiotic intervention studies.

2. INTRODUCTION

Chlamydiae are obligate intracellular Gram-negative bacteria (1). These organisms have a unique life cycle and replicate within the membrane-bound phagosomes (inclusions). Two human species, *Chlamydia trachomatis* (1) and *Chlamydia pneumoniae* (2), cause distinct disease syndromes. *C. trachomatis* causes ocular and genital infections, *C. pneumoniae* the upper and lower respiratory infections. *Chlamydia psittaci* (1) and *Chlamydia percorum* (3) causes infection of avian species and lower mammals. Infection in animals are usually systemic. Humans may be infected from sick animals. However, the virulence of animal species for humans varies. Avian strains are much more virulent to humans than mammalian strains. In humans, infection from exposure to sick birds are usually in the form of severe pneumonia or fever-of-unknown etiology. Infection from mammalian strains are rare. Chlamydiae are susceptible to macrolides, tetracyclines, quinolones, and rifampin groups of antibiotics. However, the antibiotics of choices are macrolides and tetracyclines.

A characteristic of chlamydial infection is chronic or persistent infection (4). The chronic inflammatory response has been considered a major factor in the development of tissue damage leading to functional impairments of the involved organs, such as blindness and fallopian tube obstruction and infertility in *C. trachomatis* ocular and genital infection, respectively (5). No analogous chronic conditions have been identified with *C. pneumoniae* infection. However, evidence accumulated during the past decade has indicated that atherosclerosis may be a manifestation of chronic or persistent *C.*

Table 1. Summary of results for serologic studies on association between *Chlamydia pneumoniae* antibody and cardiovascular disease

Study (ref.)	Disease[a]	No. of cases/ controls	Antibody titer cutoff[b]	Odds ratio (95% CI)[c] or % positive
Finland (9)	CHD	70/41	IgG ≥ 32	3.8 (1.4 - 10.7)
			IgA ≥ 32	6.5 (1.9 – 24.4), adj
Finland (18)	CHD	44/44	LPS – IC (+/-)	57% vs 12%, p<0.001
Finland (19)	CHD	46/46	IC (+/-)	41% vs 15% p<0.01
USA (20)	CHD	461/95	IgG ≥ 16	1.5 (1.0 – 2.6), adj.
			IgG ≥ 64	2.0 (1.0 – 4.0), adj
USA (21)	CHD	171/120	IgG ≥ 8	2.6 (1.4 – 4.8), adj.
USA (22)	CHD	238/231	IgG ≥ 16	1.1 (0.8 – 1.5)
Finland (23)	CHD	140/103	IgA ≥ 64	2.7 (1.1 – 6.5)
			IC (+/-)	2.1 (1.1 – 3.9)
UK (24)	CHD	210/103	IgG ≥ 64	2.3 (1.1 – 4.8)
			IgA ≥ 64	7.4 (1.7 – 33.1)
Italy (25)	CHD	178/50	IgG ≥ 32	60% vs 38%
			IgA ≥ 16	44% vs 22%
				p=0.02, both
USA (27)	Carotid Stenosis	326/326	IgG ≥ 8	2.0 (1.2 – 3.4), adj.
Denmark (28)	AAA expansion	139	IgA ≥ 20	48% vs 20% p<0.05
Canada (29)	AAA	81/56	IgG ≥ 256	9.6 (0.7 – 12.5), adj.
Germany (30)	Stroke	58/52	IgA ≥ 16	1.7 (1.1 – 2.7)
Sweden (31)	Stroke	130/164	IgA or	8.6 (1.1 – 68.8)
	CHD		IgG ≥ 32	2.7 (1.0 – 7.0)

a. CHD: coronary heart disease; AAA: abdominal aortic aneurysm, expansion: associated with AAA expansion. b. Titers in reciprocal 2-fold dilutions; IC (+/-): immune complex against *C. pneumoniae* antigens, ICC present (+) or absent (-); LPS: chlamydial-specific lipopolysaccharide. c. CI: confidence intervals; adj: adjusted for age and sex, and also for hypertension and cigarette smoking in some studies.

pneumoniae infection of atheromatous plaques (6). These findings prompted the Infectious Disease Society of America to convene a symposium to discuss this topic in 1999 (7). In this chapter, the cardiovascular diseases that can be caused by chlamydial infection will be reviewed. The main focus, however, will be directed to the recent developments in the association of *C. pneumoniae* and atherosclerosis.

To avoid overwhelming the article with references, only key articles of original and significant work will be provided. Reports on contradictory findings will be quoted for the purpose of discussion. The readers can obtain additional references from review articles provided in this article and from the internet sources.

3. CHLAMYDIAL INFECTION AND ACUTE CARDIAC DISEASE

Although *C. trachomatis* and the avian species of *C. psittaci* have been associated with acute cardiac diseases such as myocarditis, endocarditis, and pericarditis in humans--these are sporadic cases. An extensive review on these acute cardiac diseases was reported in 1992 by Odeh and Oliven (8). Only *C. pneumoniae* has been associated with chronic cardiovascular diseases.

4. *C. PNEUMONIAE* INFECTION AND ATHEROSCLEROSIS

4.1. History

The association of *C. pneumoniae* and coronary heart disease (CHD) was first demonstrated by serologic evidence by Saikku *et al*. in 1988 (9). The presence of the organism in atheromatous tissue of the coronary artery was first demonstrated by electron microscopy (EM) and immunohistochemistry (IHS) by Shor *et al.* in 1992 (10). The first *C. pneumoniae* isolate was obtained by Ramirez *et al.* in 1996 (11) from the coronary artery of a bypass patient with coronary atherosclerosis. To address whether *C. pneumoniae* may play a pathogenic role in atherosclerosis, experimental animal models have been employed. For example, using a rabbit model, Muhlestein *et al.* demonstrated that *C. pneumoniae* was a co-risk factor with hyperlipidemia for atherosclerosis in 1998 (12). In addition, similar observations were made in a mouse model by Moazed *et al.* in 1999 (13). Following these publications the research on *C. pneumoniae* and atherogenesis has increased rapidly over the next decade in various areas. These areas have included seroepidemiology, detection of *C. pneumoniae* in atherosclerotic lesions in the artery, use of animal models for studying the causative role and pathogenic mechanisms of *C. pneumoniae* in atherosclerosis (14-16). In addition, *in vitro* studies have been employed using vascular wall cells to elucidate the cellular and molecular mechanisms of *C. pneumoniae* infection in atherogenesis (17).

4.2. Seroepidemiology

Since the original report by Saikku in 1988 showing an association of antibodies against *C. pneumoniae* and chronic CHD (9,18,19), which was later confirmed by Thom *et al.* (20,21), over 50 papers have appeared in the literature that have investigated the association of anti-*C. pneumoniae* antibody and CHD. Except for a few negative studies (22), the vast majority of the studies demonstrated a positive association. The average odds-ratio in these studies is in general 2.0 or greater and the risk is independent of other risk factors of atherosclerosis, i.e. hypercholesterolemia, cigarette smoking, hypertension, diabetes, and family history (20,21,23-25) (Table 1). The association of *C. pneumoniae* antibody and CHD was made despite the relatively high prevalence of antibody in the general population (26). Fifty

percent of middle-age and 70% to 80% of older adults have antibody against *C. pneumoniae*. The seroepidemiologic studies have also demonstrated an association of antibody against *C. pneumoniae* and carotid artery stenosis (27), aortic aneurysm (28,29), and cerebrovascular disease (30,31). The early reports on the positive association of *C. pneumoniae* antibodies and CHD raised the question as to whether the organism is present in the atheromatous lesions. This lead to the next stage of studies to determine if the organism could be detected in atheromatous lesions.

4.3. Detection of the organism in atheromatous lesions

There have been over 45 publications demonstrating *C. pneumoniae* organisms in atheromatous tissues by IHC, PCR, EM and isolation following the original reports by Shor *et al.* in 1992 (10) and Kuo *et al*. in 1993 (32).

4.4. Detection of Antigen and DNA and organism isolation

Overall, *C. pneumoniae* was detected by IHC, PCR, or both in atheromatous tissues from 50% of subjects but was rarely found (1%) in normal vascular tissues (33,34). The most commonly used antibodies for IHC staining are monoclonal antibodies against *C. pneumoniae* species-specific antigens and Chlamydia-genus specific lipopolysaccharide antigen (33). Only a few papers reported failure in detecting *C. pneumoniae* in atheromatous tissues (35-37). Finding the organism in lesions but not in normal arteries may suggest that the organism is involved in the disease process. The lesions in which *C. pneumoniae* were detected were obtained from the coronary artery, including coronary bypass of the saphenous vein (38), carotid, pulmonary, iliac, femoral, and popliteal arteries and the aorta (33) from patients with coronary artery stenosis (angina and myocardial infarction) (10,32) and carotid artery stenosis (39), stenosis of the arteries of the lower extremities (claudication) (40) and aortic aneurysm (29,41-43). *C. pneumoniae* was also detected in nonrheumatotic aortic stenotic valves (44-46). However, this finding has been disputed (47).

The youngest age at which *C. pneumoniae* was detected in the artery was 15 years (48). In the Pathological Determination of Atherosclerosis in Youth study, a higher detection rate was found in subjects 25-34 years old (7 (26%) of 27 subjects) than in those 15-24 years old (1 (5%) of 22 subjects), suggesting a pathogenic role of *C. pneumoniae* in the atherosclerotic process (49).

Variability in detection rates has been noted among investigators, arteries, and methods used for detection (33). These rates have been reported to range from 0% to 100%. One reason for this variability is the localized nature of *C. pneumoniae* in lesions and the fact that most studies examined only one or a few 4 um sections with IHC and 8 um sections with PCR. In general, detection rates were higher with IHC than PCR whether the same tissues or different tissues were tested. This discrepancy has been attributed to the presence of tissue inhibitors for PCR. A lack of correlation between IHC and PCR has also been noted. In our studies in which specimens were positive by both methods, only 25%-50% of specimens were positive by both tests (33). The lack of correlation has also been noted between detection and serology (32,50-53). Finally, there has been no correlation shown between the presence of the organism and the severity of atherosclerosis or clinical diagnosis, such as acute myocardial infarction, thrombosis or ulceration of atheroma, and rupture of aneurysm (33).

The organism has been isolated from atheromatous tissue of the coronary and carotid arteries by several independent investigators (11,39,52). However, isolation has been the most difficult to achieve and the most important step toward determining a causal relationship between *C. pneumoniae* and atherogenesis and fulfillment of Koch's postulate.

4.5. Detection of the organism in peripheral blood mononuclear cells

In attempting to identify a marker for laboratory diagnosis of *C. pneumoniae* infection in coronary disease patients and for assessment of the outcome of antibiotic therapy, PCR has been used for direct detection of *C. pneumoniae* in circulating monocytes. The detection rates ranged from 8.8% to 59% in patients with coronary artery disease including unstable angina and myocardial infarction (54-56) and abdominal aortic aneurysm (57) patients. Similar detection rates ranging from 8.9% to 46% (54,58,59) have also been shown in healthy blood donors. The presence of the organism in circulating mononuclear cells may indicate a mechanism by which *C. pneumoniae* disseminate systemically from the lungs to other organs (60).

5. CHLAMYDIAL INFECTION OF THE HEART VALVES

As mentioned previously, *C. pneumoniae* has been detected in the sclerotic aortic valves of non-rheumatic origin (44-46). Finding of the organism in the aortic valves raised the possibility that *C. pneumoniae* could also infect other cardiac valves. The fact that the organism has been found only in the aortic valves may indicate that it is an extension of the infection of the aorta.

6. HISTOPATHOLOGIC FINDINGS IN ATHEROSCLEROTIC LESIONS POSITIVE FOR *C. PNEUMONIAE*

No distinguishable differences in histopathology were observed between *C. pneumoniae*-positive and *C. pneumoniae*-negative atheromatous lesions under light microscopy in tissue sections stained with hematoxylin and eosin (6). Utilizing IHC, the organism has been detected in early (fatty streaks) and advanced (atheromatous plaques) lesions, but rarely in histologically normal-looking arteries. The organism is often located in foam cells within the plaque and in macrophages infiltrating the intima, media and adventitia. By double-labeling or co-localization staining, the organism has been located in macrophages and the smooth muscle cells (61), but is rarely found in endothelial cells (62). EM demonstrates that the organisms

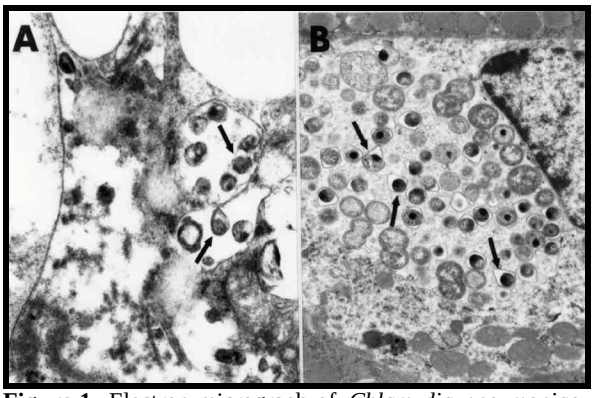

Figure 1. Electron micrograph of *Chlamydia pneumoniae* in a foam cell in the coronary artery atheroma (A) and in an interstitial macrophage of the lung from a mouse inoculated intranasally with *C. pneumoniae* (B). Note the typical pear-shaped morphology of the elementary body of *C. pneumoniae* (arrows); 9,300x magnification.

are found within cytoplasmic vacuoles of foam cells (32) (Figure 1).

7. PATHOGENESIS OF CHLAMYDIA INFECTIONS

Infection with *C. trachomatis* and *C. pneumoniae* induces chronic inflammatory reactions, consisting of macrophages and lymphocytes, at the site of infection (6). Atherosclerosis has been regarded as a chronic inflammatory reaction with lipid accumulation. Both *in vivo* and *in vitro* experiments have shown that *C. pneumoniae* infection induces pro-inflammatory cytokines. Infection of macrophages results in the production of TNF-alpha, interleukin-1beta (IL-1beta), IL-8 (for review see (17)). In the atheromatous lesion, *C. pneumoniae* infection of macrophages may induce foam cell formation by chlamydial LPS (63) and oxidation of low density lipoprotein (a key lipid in foam cells) by chlamydial heat shock protein-60 (hsp-60) (64). Chlamydial hsp-60 can activate macrophage TNF-alpha and expression of matrix metaloproteinase (65), which may cause plaque destabilization.

How *C. pneumoniae* establishes persistent infection in arterial cells has been investigated *in vitro*. It has been found that the interaction of monocytes with endothelial (66) and smooth muscle (67) cells promotes the growth of *C. pneumoniae* in these cells. However, different mechanisms seem to be involved in the growth promotion in different cell types. In endothelial cells, the growth is enhanced by the insulin-like growth factor-2 (IGF-2) secreted by monocytes (68), while a direct cell-to-cell contact is required for the growth promotion in smooth muscle cells (67).

8. ANIMAL MODELS OF PNEUMONIA AND ATHEROSCLEROSIS

The most commonly used animals for studying the pathogenesis of *C. pneumoniae* induced pneumonitis and atherosclerosis are mice. Rabbits have also been used for studying atherosclerosis. Using the genetically induced hyperlipidemic animals (apoE-knockout mice) (13,69) and diet-induced hyperlipidemic animals (LDL receptor knockout mice (70), C57BL/6J mice (71), and New Zealand white rabbits (12,72)) in these studies have shown that intranasal inoculation with *C. pneumoniae* accelerate plaque development in these hyperlipidemic animals. However, others have not observed an exacerbation (73). In the absence of hyperlipidemia infection induces inflammatory reactions in the artery, but no definitive atherosclerotic lesions have been induced (74,75). In general, the findings from these animal experiments indicate that *C. pneumoniae* is a co-risk factor of hyperlipidemia for atherosclerosis.

9. THERAPY

The most commonly used antibiotics for treating chlamydial infections are tetracyclines, macrolides, and azalides (26). These drugs have been used to determine whether treatment would ameliorate the effect of *C. pneumoniae* infection on atherosclerosis in animals and decrease clinical events in patients with coronary artery disease.

Treatment studies in animal models have yielded variable results. Muhlestein *et al.* showed that a 7-week course of azithromycin started immediately following the inoculation was effective in preventing the enhancing effects of *C. pneumoniae* on atherosclerotic lesion development in diet-induced hyperlipidemic rabbits (12). A similar effect was obtained by Fong *et al.* (76). However, in Fong's study the beneficial effects were observed with the early, but not late treatment with azithromycin. In the apoE-deficient mouse model, a two-week treatment with azithromycin had no effect on *C. pneumoniae* accelerated lesion development (77).

There have been four small scale therapeutic trials using roxithromycin, clarithromycin and azithromycin for secondary prevention of acute coronary events (78-81). The sample sizes range from 80 to 300 patients with the duration of treatment ranging from one or two courses of a 3 day treatment (78) to the longest of 3 months (81). Follow-ups ranged from 30 days to 1 year and 6 months. Three of these four studies showed statistically significant beneficial effects ($p=0.03$). The one study which did not show a reduction in the outcome of acute cardiac events involved 302 patients who were given azithromycin (500 mg per day for 3 days followed by 500 mg per week for 3 months) and examined at 3 and 6 months (81).

Two large scale multi-center double-blind clinical trials of azithromycin on CHD are being conducted in North America. These two studies were the WIZARD (Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders (82) and ACES (Zithromax and Coronary Events Study) (83). The enrollment criteria were post myocardial infarction for both studies. Additional criteria were *C. pneumoniae* antibody titers of 1:16 or greater in the WIZARD study and 50% stenosis in the ACES study. The dosage was 600 mg azithromycin once a

week for 3 months in the WIZARD study and for 12 months in the ACES study. Of the 3,300 patients in the WIZARD study, half were treated with azithromycin and half with placebo. Patients were followed for 1 year and 6 months. In the ACES study, half of the 4,000 patients were treated with azithromycin and half with placebo. The endpoints in both studies were CHD events. The WIZARD study has been completed in the year 2001 and the data are being analyzed. The expected date of completion for the ACES study is December 2003.

10. CONCLUSION

The most significant development in chlamydial infection and cardiovascular biology has been the observation of an association between chlamydial infection and cardiovascular disease, the discovery of *C. pneumoniae* in atheromatous plaques, *in vivo* experimental studies in animals indicating that *C. pneumoniae* is a co-risk factor of hyperlipidemia for atherogenesis, and *in vitro* studies demonstrating putative mechanisms by which *C. pneumoniae* could contribute to the immunopathology of atherosclerosis. Early promising pilot intervention studies have paved the way for large scale controlled clinical trials. Future studies in molecular and cellular mechanisms of the pathogenic role of *C. pneumoniae*-related atherosclerosis will define the role of *C. pneumoniae* in atherosclerosis. This will lead to the development of new preventive or intervention measures.

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