Chlamydia pneumoniae and Cardiovascular Disease

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Chlamydia pneumoniae is a ubiquitous pathogen that causes acute respiratory disease. The spectrum of *C. pneumoniae* infection has been extended to atherosclerosis and its clinical manifestations. Seroepidemiologic studies have associated *C. pneumoniae* antibody with coronary artery disease, myocardial infarction, carotid artery disease, and cerebrovascular disease. The association of *C. pneumoniae* with atherosclerosis is corroborated by the presence of the organism in atherosclerotic lesions throughout the arterial tree and the near absence of the organism in healthy arterial tissue. *C. pneumoniae* has also been isolated from coronary and carotid atheromatous plaques. To determine whether chronic infection plays a role in initiation or progression of disease, intervention studies in humans have been initiated, and animal models of *C. pneumoniae* infection have been developed. This review summarizes the evidence for the association and potential role of *C. pneumoniae* in cardiovascular disease.

Chlamydia pneumoniae, a common cause of human respiratory disease, was first isolated from the conjunctiva of a child in Taiwan in 1965 and was established as a major respiratory pathogen in 1983 when it was isolated from the throat of a college student at the University of Washington. *C. pneumoniae* causes approximately 10% of community-acquired pneumonia and 5% of pharyngitis, bronchitis, and sinusitis (1). The clinical symptoms of *C. pneumoniae* pulmonary infections are similar to those caused by other respiratory pathogens, except for a few distinguishing features (1). Subacute onset and pharyngitis are common. Often a biphasic pattern is observed, with pharyngitis resolving before bronchitis or pneumonia develops. Cough is very common and prolonged. Although pneumonia is often relatively mild, recovery is slow, even with antibiotic therapy; and cough and malaise may persist for many weeks.

Considerable knowledge of the epidemiology of *C. pneumoniae* infection has been derived from serologic studies using the *C. pneumoniae*–specific microimmunofluorescence test. *C. pneumoniae* infection is ubiquitous. Virtually everyone is infected at some point in life, and reinfection occurs commonly. Antibodies against *C. pneumoniae* are rare in children under the age of 5, except in developing and tropical countries. Antibody prevalence increases rapidly at ages 5 to 14, reaches 50% at the age of 20, and continues to increase slowly to 70% to 80% at ages 60 to 70 (1).

C. pneumoniae has been associated with other acute and chronic respiratory diseases (e.g., otitis media, chronic obstructive pulmonary disease, pulmonary exacerbation of cystic fibrosis, and asthma) as well as other clinical syndromes (e.g., erythema nodosum, Reiter syndrome, and sarcoidosis [1]). These associations are determined by seroepidemiologic observations, case reports, isolation or direct detection of the organism in specimens, successful response to antichlamydial antibiotics, or a combination of these methods.

The expanding spectrum of *C. pneumoniae* infection has been extended to atherosclerosis and related clinical manifestations such as coronary heart disease, carotid artery stenosis, aortic aneurysm, claudication (occlusion of the arteries of the lower extremities), and stroke. This overview summarizes the studies associating *C. pneumoniae* infection with atherosclerosis and discusses preliminary in vitro and in vivo studies suggesting the plausibility of a causative role.

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Is *C. Pneumoniae* Present in Atherosclerotic Tissues?

Patients with coronary artery disease are significantly more likely than healthy persons to have serologic evidence of past infection with *C. pneumoniae* (2,3). Immune complexes containing *C. pneumoniae* lipopolysaccharide have also been associated with 42- and 98-kDa *C. pneumoniae* species-specific antigens (4). These associations have been confirmed and extended to carotid artery disease and cerebrovascular disease (5,6). Although the high prevalence of *C. pneumoniae* antibodies in the population leaves a very narrow window for demonstrating statistically significant differences between cases and controls, seroepidemiologic studies have shown a consistent association. *C. pneumoniae* antibodies have been associated with coronary heart disease (16 studies) and cerebrovascular disease (2 studies) in 17 of 18 published studies; most had an odds ratio of 2.0 or greater (6). Most were statistically significant, and the risk is independent of other atherosclerosis risk factors (i.e., hypercholesterolemia, cigarette smoking, hypertension, diabetes, and family history). Studies of 2,700 patients and 5,000 controls have demonstrated a serologic association of *C. pneumoniae* antibody and cardiovascular disease (6).

Compelling evidence of the association between *C. pneumoniae* and atherosclerosis has been obtained by polymerase chain reaction (PCR), immunocytochemical (ICC) staining, and electron microscopy, which have detected *C. pneumoniae* in atherosclerotic lesions (Table). Structures found within coronary atheromas were remarkably similar to the pear-shaped elementary body morphologic characteristics described for *C. pneumoniae* (Figure 1) (25). By ICC staining using a *C. pneumoniae*–specific monoclonal antibody (Mab), *C. pneumoniae* was demonstrated within the atherosclerotic lesion in 5 of 7 tissues. By PCR or ICC stain, the organism was detected in 20 of 36 coronary artery tissues from autopsy (7). At the University of Washington, the organism has been detected in coronary, carotid, aortic, femoral, and popliteal atheromas in both early lesions and fibrolipid plaques (7-10,15,18,22,26,27). In all studies using ICC stain, control tissues were stained with control antibody to rule out background staining (Figure 2). The organism was found in tissues of male and female study participants of different ages and ethnic groups. Other investigators have confirmed these findings and have also found the organism in atherosclerotic lesions in iliac arteries and tissues from abdominal aortic aneurysms and

Table. Studies of *Chlamydia pneumoniae* in atherosclerotic tissue

Source of specimens (reference)	Artery	Type of specimen	Atherosclerotic tissue[a] (% positive)
South Africa (7)	Coronary	Autopsy	20/36 (56)
PDAY[b] study (8)	Coronary	Autopsy	8/18 (44)
Univ. Washington (9)	Coronary	Atherectomy	20/38 (53)
Alaskan Natives (10)	Coronary	Autopsy	23/59 (39)
Louisville, Kentucky (11)	Coronary	Vascular surgery	7/12 (58)
Japan (12)	Coronary	Atherectomy	20/29 (69)
Salt Lake City, Utah (13)	Coronary	Atherectomy	71/90 (79)
India (14)	Coronary	Coronary artery bypass	4/40 (10)
California & Univ. Washington (15)	Carotid	Endarterectomy	37/61 (61)
Germany (16)	Carotid	Endarterectomy	7/50 (14)
Canada (17)	Carotid	Endarerectomy	54/76 (71)
Univ. Washington (18)	Aorta	Autopsy	7/21 (33)
Finland (19)	Aorta	Vascular surgery	12/12 (100)
Italy (20)	Aorta	Vascular surgery	26/51 (51)
United Kingdom (21)	Aorta, femoral, iliac	Vascular surgery	15/33 (45)
California (22)	Popliteal femoral	Vascular bypass	10/23 (43)
Finland (23)	Aortic valve	Autopsy	25/46 (54)
Sweden (24)	Aortic Valve	Surgery	19/39 (49)

[a]Number positive by immunocytochemical staining and/or polymerase chain reaction over number tested.
[b]PDAY, pathobiological determinants of atherosclerosis in youth.

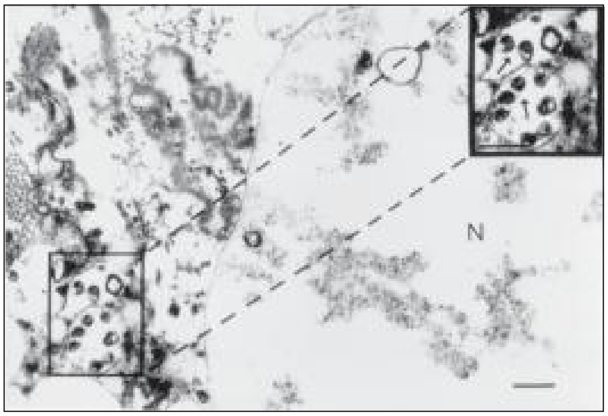

Figure 1. Ultrastructural evidence of *Chlamydia pneumoniae* in coronary atheroma. This transmission electron micrograph demonstrates the presence of endosomes containing *C. pneumoniae* pear-shaped elementary bodies within a foam cell in tissue from coronary artery atheroma. Arrows in inset point to the elementary bodies. Bar=0.5 µm. (Reprinted from Journal of Infectious Diseases (8) with permission from the publisher, University of Chicago Press).

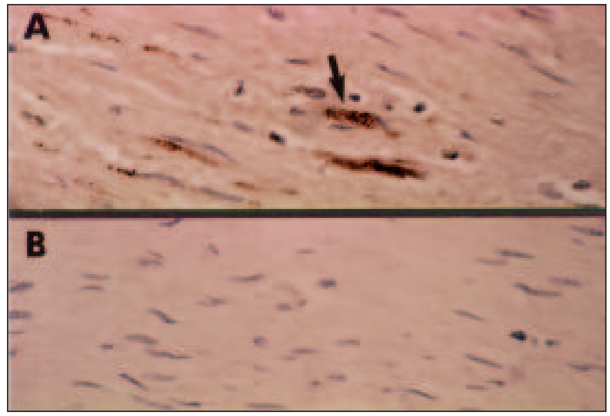

Figure 2. Immunocytochemical staining demonstrating *Chlamydia pneumoniae* in fibrolipid plaque from coronary artery atheroma. Panel A illustrates positive staining of foam cells in the plaque with the *C. pneumoniae*–specific monoclonal antibody TT-401. Panel B shows negative staining of the adjacent section using normal ascites fluid as the control.

aortic valve stenosis (11-14,16,17,19-21,23,24,28). The organism has been found in atherosclerotic lesions in 257 (52%) of 497 tissue specimens (6). In contrast, the organism has been found in only 5% of tissues that appear normal. Remarkably, the detection in diseased arterial tissue compared to normal arterial tissue represents an odds ratio of 10 [95% Confidence Interval (CI) 5-22]. In our studies the organism was not found in cardiovascular tissue that appeared histologically normal (5).

C. pneumoniae has been difficult to isolate from atheromatous tissues, not surprising as isolation from infected tissues in chronic chlamydial infection or after repeated experimental inoculation of animal models is rare. However, the organism is often demonstrated by DNA and antigen detection methods, and a chronic inflammatory response persists. The immunologic characteristics of atherosclerosis are similar to the inflammatory response resulting from chronic infection. In two recent cases, *C. pneumoniae* has been isolated from atheromatous plaques (11,15). The first isolate was obtained during a multicenter study that examined coronary arteries of patients (with or without coronary artery disease) undergoing heart transplants. The organism was found in the coronary artery of 7 of 10 patients with atherosclerosis; it was not found in two patients without evidence of coronary atherosclerosis. *C. pneumoniae* was isolated from the atherosclerotic lesion of one patient with severe coronary artery disease (11).

The second isolate was from carotid atheroma obtained in a study of patients undergoing carotid endarterectomy in Seattle. *C. pneumoniae* was detected in 11 of 16 specimens and was cultured from a patient undergoing elective carotid endarterectomy (15).

In addition to *C. pneumoniae*, other infectious agents including herpes simplex virus (HSV), cytomegalovirus (CMV), and *Helicobacter pylori* have been associated with cardiovascular disease. Only a few studies have concurrently investigated the presence of *C. pneumoniae* and these infectious agents within lesions. In a study of risk factors for atherosclerosis in young persons, 6 of 7 samples with atheromas and 2 of 11 with intimal thickening were positive for *C. pneumoniae*; all 31 unaffected tissues were negative (8). Two specimens from atheromas and two from normal tissues were positive for CMV. Only one coronary atheroma was positive for both CMV and *C. pneumoniae*. In a study investigating the presence of *C. pneumoniae*, CMV, and HSV in atherosclerosis of the carotid artery, *C. pneumoniae* was also detected more frequently (71%) than CMV and HSV (35% and 10%, respectively). Infection with two or three of these agents occurred in 23.7 and 7.9%, respectively, and *C. pneumoniae* and CMV were independently associated with an increased risk for thrombosis (17). In a study addressing the presence of *C. pneumoniae* or *H. pylori* or both in aortic aneurysm, *C. pneumoniae* was found in 26 of 51 patients with plaques (20). Despite the fact that 47 of 51 patients were seropositive for *H. pylori*, this agent was not detected in any atherosclerotic plaques (20). Thus, although *C. pneumoniae* and HSV or CMV may be found in the same lesion, *C. pneumoniae* has been more frequently found as the only infectious agent.

Does *C. pneumoniae* Play a Role in Atherogenesis?

Despite solid evidence that *C. pneumoniae* exists in atherosclerotic lesions, evidence that the presence of the organism is related to disease pathogenesis is circumstantial. Three possibilities can be examined. The organism 1) persists in vascular cells but does not contribute to pathologic abnormality, 2) causes the initial injury and induces the atherosclerotic process, or 3) accelerates the severity or progression of the disease. If the organism is involved, its role must fit within the context of events in atherogenesis. The early events in lesion development include endothelial injury or activation resulting in monocyte/macrophage adherence to the endothelium, migration to the subendothelium, uptake of oxidized low-density lipoproteins transforming them into foam cells, and release of cytokines. These cytokines upregulate endothelial cell adhesion molecules leading to increased leukocyte adhesion. Platelet aggregation at the site of endothelial damage results in the release of platelet-derived growth factor, which stimulates smooth muscle cell proliferation. Dedifferentiated smooth muscle cells secrete collagen, elastin, and proteoglycans leading to the formation of fibrous tissue. The mature fibrolipid plaque consists of a lipid/cholesterol-rich core surrounded by a fibrous cap composed of matrix elements (29).

Human Tissue Studies

To investigate the hypothesis that *C. pneumoniae* is an "innocent bystander," one study explored how frequently the organism was found in tissues from different anatomic sites. Multiple tissues, including coronary artery, lung, liver, spleen, and bone marrow, were obtained at autopsy from 38 patients, half of whom had cardiovascular disease recognized before death. Twenty-one of the patients had *C. pneumoniae* in one or more tissues. Eighteen had the organism in cardiovascular tissue (11 in cardiovascular tissue only) and seven in both cardiovascular and noncardiovascular tissue. Of the remaining three *C. pneumoniae*–positive patients, two had the organism only in lung tissue and one only in the spleen. While this study shows that *C. pneumoniae* could be detected in noncardiovascular tissue, it also shows the organism is much more frequently found in cardiovascular tissue (27).

Another possible explanation for the frequent presence of *C. pneumoniae* in atheroma is that infected lung macrophages disseminate to any granulomatous tissue. Of 33 surgical or autopsy specimens of granuloma from patients with tuberculosis, leprosy, coccidioidomycosis, Crohn disease, Wegner disease, rheumatoid nodule, giant cell tumor, or sarcoidosis, three contained *C. pneumoniae*, all sarcoid skin granulomas (27). Sarcoidosis is a disease of unknown etiology for which a serologic association with *C. pneumoniae* has been reported (30). These studies suggest that *C. pneumoniae*–infected cells are found preferentially within atheromas.

In Vitro Studies

Consistent with observations in animal models and humans, in vitro studies have demonstrated that vascular cells are susceptible to *C. pneumoniae* infection and that *C. pneumoniae* produces productive infection in human macrophages, endothelial cells, and artery smooth muscle cells (31,32), key cellular components in atherosclerosis. In vitro studies have also measured whether infection leads to the production of immunomodulators. The primary host cells are the epithelial cells that line the trachea and nasopharynx. The first in vitro study addressing a potential role of *C. pneumoniae* in atherogenesis found that exposure of human monocyte-derived macrophages to *C. pneumoniae* followed by the addition of low-density lipoprotein resulted in foam cell formation and the accumulation of cholesteryl esters (33). Foam cell formation is an early event in the atherosclerotic process.

Ultrastructural studies have demonstrated *C. pneumoniae* in ciliated bronchial cells in mice, in lung macrophages in mice and rabbits, and in human foam cells (which are macrophages) and smooth muscle cells that take up lipids (7,34,35); by double ICC staining with chlamydia-specific and cell-specific MAbs, *C. pneumoniae* was found in both smooth muscle cells and macrophages within aortic atheroma lesions (18). Similarly, in specimens removed from patients with symptomatic coronary artery disease, *C. pneumoniae* was found within macrophages in atherectomy tissue was also found in tissue removed at atherectomy, *C. pneumoniae* bacteremia, as determined by PCR positivity of buffy coat specimens, was found in 13% of patients with symptomatic coronary atherosclerosis; control specimens were negative (13,53). In mouse models of *C. pneumoniae* infection, *C. pneumoniae* was detected by culture and PCR in peripheral blood mononuclear cells but not in plasma after intranasal inoculation (36).

The ability of *C. pneumoniae* infection to induce production of proinflammatory and procoagulant activities was investigated to determine its putative role in eliciting immune responses consistent with atherosclerotic processes. *C. pneumoniae* infection of human vascular endothelial cells results in production of tissue factor, increased levels of monocyte chemotactic protein-1, and increased platelet adhesion to infected cells (37,38). Infection of endothelial cells also results in expression of adhesion molecules, including E-selectin, intercellular adhesion molecule-1, and vascular adhesion molecule-1, which are important in leukocyte adhesion (38,39). Lastly, infection of macrophages results in the production of proinflammatory cytokines, tumor necrosis factor alpha, interleukin-1 beta, interleukin-6, and interleukin-8 as well as in expression of CD14 molecules (38,40).

Animal Models

Although studies support a potential role for *C. pneumoniae* in atherogenesis, etiology can be established only through animal models or intervention studies. Rabbits and mice are susceptible to *C. pneumoniae* infection and provide well-defined models of atherosclerosis. Respiratory disease in both species is characterized by multifocal interstitial pneumonia. The disease is more severe and longer lasting in mice, and organisms are reisolated more readily from the lungs and aorta.

Rabbit models have been used to determine whether *C. pneumoniae* respiratory infection leads to vessel wall infection and inflammatory changes characteristic of atherosclerosis. In one study, New Zealand White rabbits received a single nasopharyngeal inoculation of *C. pneumoniae* at 1 month of age. Two of ten rabbits demonstrated atherosclerotic changes. The changes were observed 7 and 14 days postinoculation. One rabbit had an accumulation of foam cells in the aortic arch (characteristic of an early lesion) and focal perioaortitis in the abdominal aorta (41). A second rabbit demonstrated spindle cell proliferation of smooth muscles cells in the aorta. Both rabbits had bronchiolitis and pneumonitis (41). In another study, atherosclerotic-li ke changes were found in the aortas of six of nine New Zealand White rabbits 2 to 4 weeks after they received two inoculations of *C. pneumoniae* (42). In both studies, rabbits were fed normal diets, and atherosclerotic-like changes were not observed in any of the controls.

We used two mouse models, C57BL/6J and apolipoprotein E (apoE)-knockout, to test the hypothesis that following upper respiratory tract infection, lung macrophages are infected, disseminate to the aorta, and contribute to atherogenesis. C57BL/6J mice, the background strain of apoE-knockout mice, get atherosclerosis only if fed a diet high in fat and cholesterol. In contrast, apoE-knockout mice get atherosclerotic lesions, with some characteristics of human disease, spontaneously on a regular chow diet in a time- and age-dependent manner. Using C57Bl/6J mice, we demonstrated that after intranasal or intraperitoneal infection, *C. pneumoniae* infects alveolar and peritoneal macrophages, respectively (36). Additionally, the organism was found in blood monocytes and not in plasma cells, which indicates that a cell-associated bacteremia followed acute infection. Passive transfer by intraperitoneal inoculation of alveolar or peritoneal macrophages obtained from mice inoculated intranasally or intraperitoneally with *C. pneumoniae* resulted in dissemination of infection to the lung, thymus, spleen, and abdominal lymph nodes (36).

To determine whether infection disseminates to the aorta and is found within atherosclerotic lesions as in human disease, apoE-knockout mice received single or multiple intranasal inoculations. *C. pneumoniae* was detected for up to 20 weeks postinfection in the aorta and within the lesion in apparent foam cells by ICC staining (43). When the aorta was positive, the percentage of *C. pneumoniae*–positive mice was 33% to 100%. Controls remained negative for *C. pneumoniae* (43). In contrast, 1 (0.8%) of 12 animals of the background strain (C57BL6/J mice) fed a nonatherogenic diet contained the organism in the aorta for up to (but not later than) 2 weeks postinfection. Like studies of human granulomatous tissues and tissues from different anatomic sites (27,43), these studies suggest that the organism has a tropism to atherosclerotic lesions.

Computer-assisted morphometric analysis of lesion size has been used to determine if *C. pneumoniae* infection alters disease progression. Comparisons of 10 infected and 10 uninfected mice at different times following three intranasal inoculations of *C. pneumoniae* suggest that infection significantly augments the progression of lesion size (44). Thus, early studies in rabbit and mouse models indicate that *C. pneumoniae* infection can induce inflammatory changes similar to those of atherogenesis and augment the progression of the atherosclerotic lesion.

Persistent Infection

Although *C. pneumoniae* antigen and DNA are often found in atheromas, isolation is rare. Similarly, in the mouse model of *C. pneumoniae* infection, lungs remain PCR positive and pathologic lesions persist after the organism can no longer be cultured. In the apoE model, the organism can be cultured from the lung and aorta in a few mice for up to 3 weeks postinfection but is detected by PCR and ICC up to 20 weeks postinfection at both sites. Does PCR positivity in the absence of culture positivity reflect persistent infection or undegraded DNA? Two lines of evidence strongly support the presence of viable organisms. Two independent studies using two different strains of mice have demonstrated that lung infection could be reactivated by treatment with cortisone (45,46), specifically after intranasal inoculation, when the organism could no longer be cultured from lungs. Animals were treated with cortisone or saline. With saline tratment, *C. pneumoniae* was not cultured, although animals were frequently PCR positive. In contrast, with cortisone treatment, reactivated infection was demonstrated by culture in 46% and 60% of infected mice. The second line of evidence came from mice acutely infected intranasally with live or UV-inactivated organisms; alveolar macrophages were isolated at various times postinfection. When live organisms were used, the organism could be cultured and detected by PCR up to 7 days postinfection. In contrast, when mice were inoculated with UV-inactivated organisms, the organism could only be detected by PCR immediately after inoculation in isolated macrophages (36). These experiments demonstrated that DNA from dead organisms is rapidly degraded, whereas live organisms survive within macrophages.

Drug efficacy studies of chlamydia in animal models or humans must be interpreted with caution because chlamydial infections can persist after antibiotic therapy. The efficacy of two antibiotic therapies was investigated in the mouse model of pneumonitis. After infection, mice were treated with a single dose of doxycycline each day for 3 days or with a single dose of azithromycin. After either treatment, when the organism could no longer be cultured, the infection appeared to be cleared. However, *C. pneumoniae* DNA could be detected by PCR isolation in 25% or 77% of mouse lungs, depending on the infecting dose. No differences were observed in the lungs of treated and untreated mice (47). These results suggest that the organism can persist after single-dose treatment regimens and that prolonged treatment may be needed.

Intervention Studies

Animal Models

Animal models of *C. pneumoniae* infection and atherosclerosis further define a causative role by determining whether intervention with antimicrobial agents can alter disease progression and by identifying successful treatment regimens. New Zealand White rabbits were fed a diet with 0.25% cholesterol and were inoculated intranasally three times with *C. pneumoniae* (48). Infected rabbits and controls were treated for 7 weeks with azithromycin. Three months after the final inoculation, the maximal intimal thickness (MIT) of the thoracic aortas increased in infected rabbits but not in controls. The MIT of azithromycin-treated rabbits was less than that of untreated infected rabbits and similar to that of controls. However, the organism was detected by immunofluorescence in the aorta of treated rabbits as frequently as it was detected in the aorta of untreated rabbits (48).

Human Studies

Three small pilot studies on the potential use of antibiotics against *C. pneumoniae* have yielded promising results. The first intervention study focused on male patients at least 6 months after myocardial infarction. Titers were arbitrarily classified as seronegative (8), intermediately positive (>8, 32), or highly positive (64). Those who had serum antibodies that persisted 3 months later were treated with one or two courses of azithromycin (500 mg per day orally for 3 days). Treated patients had a fivefold decrease in cardiovascular events and a reduction in immunoglobulin (Ig)G titer (49).

The second study included 202 patients hospitalized with unstable angina or non-Q wave infarctions. The study was randomized with half of the patients receiving roxithromycin (150 mg twice a day for 30 days) and the other half receiving a placebo. Serology was not considered for inclusion or exclusion in the study. After 30 days, a statistically significant decrease in cardiovascular events was observed in treated patients, while those receiving the placebo were treated only when a combined endpoint of severe recurrent angina, acute myocardial infarction, or ischemic death was used (50).

In the third (randomized) study, 88 patients with percutaneous coronary revascularization procedures were treated with azithromycin (500 mg per day for 2 days and 250 mg per day for 28 days) or received placebo (51). After 6 months, patients receiving azithromycin had lower frequencies of both angiographically confirmed restenosis (9% versus 16%) and recurrent angina (40%) than patients receiving placebo (60%). No changes in antibody titers were observed after azithromycin treatment. Despite study limitations (52) and antiinflammatory effects of the antibiotic on atherosclerosis, the results are encouraging and warrant carefully designed larger-scale intervention studies with longer observation times.

Conclusions

A causative role of *C. pneumoniae* infection in cardiovascular disease has not yet been firmly established. However, the high frequency of infection found in human atherosclerotic tissue in comparison to normal tissue, the induction and progression of atherosclerotic-like inflammatory changes in infected animal models of atherosclerosis, and the early results from antichlamydial intervention studies in humans are consistent with a causative role of *C. pneumoniae* in the disease process.

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