A link between infectious agents and atherosclerosis has been postulated for decades. This review describes the epidemiological and biological evidence linking cytomegalovirus and *Chlamydia pneumoniae* to atherosclerotic disease. Case-control studies and histologic evidence from atheromatous specimens support an association between atherosclerosis and infection with these two microorganisms, and small interventional trials appear to confirm the link with *C. pneumoniae*, but these findings require confirmation in larger studies. A lack of clinically relevant animal models has hampered investigations regarding biological mechanisms, particularly for *C. pneumoniae*.

The link between certain infectious agents and chronic diseases that afflict the elderly (e.g., *Treponema pallidum* and dementia) has been well established for decades, but the link between others (e.g., *Helicobacter pylori* and peptic ulcer disease) has only recently been confirmed. Infectious etiologies are currently being investigated with regard to several chronic diseases, including rheumatoid arthritis, inflammatory bowel disease, certain types of cancer, and even psychiatric disorders. The focus of this review is the link between cytomegalovirus (CMV) and *Chlamydia pneumoniae* to atherosclerosis.

### Establishing Causality

When infectious disease physicians discuss causality, they often think of the Henle-Koch postulates, used to establish the causal relationship of *Mycobacterium tuberculosis* and many other agents with specific diseases [1]. These postulates state that (1) the organism is always present with the disease; (2) the organism is not found with any other disease or as a colonizer without disease; and (3) after culture outside the body, the organism reproduces disease in a susceptible host. Fulfilling the Henle-Koch postulates establishes the organism as the agent of disease and is very useful when linking an organism to acute disease. However, chronic diseases rarely have an agent; rather, a causal relationship is supported by mounting epidemiological and biological data until causality has been established.

The criteria used to establish causality for infectious organisms in chronic diseases have evolved over time [1] and include the (1) strength of the association; (2) consistency of the association; (3) specificity of the association; (4) existence of a dose-response relationship; (5) temporal correctness of the association; and (6) biological plausibility (i.e., supporting basic mechanism data). For example, most physicians would readily identify cholesterol as a cause of atherosclerosis, a concept supported by tremendous epidemiological and basic science data. However, cholesterol is not the agent of atherosclerosis but participates with other factors in its development. Thus, causes of chronic diseases are termed risk factors, and when microorganisms that are risk factors for chronic disease are identified, the Henle-Koch postulates may not be fulfilled. Thus, the broader criteria outlined above should be used to establish causality.

### CMV and Atherosclerosis

CMV is a ubiquitous virus, infecting ~60%–70% of adults in the United States, and is well documented to establish chronic infection. The link between CMV and atherosclerosis grew out of biological plausibility. In the late 1970s, French investigators published evidence that a similar herpesvirus, Marek’s disease virus (MDV), could cause atherosclerotic lesions in chickens [2]. Subsequent investigations identified MDV in arterial smooth-muscle cells of atherosclerotic plaques and established cholesterol as a cofactor [3, 4]. Most striking, immunization with a related turkey herpesvirus could prevent the disease [4]. Additional mechanisms linking CMV to atherosclerosis include enhanced scavenger receptor expression (and subsequent uptake of oxidized low-density lipoprotein) by arterial smooth-muscle cells related to expression of a CMV immediate-early antigen (IE), IE-72 [5], and proliferation of arterial smooth-muscle cells correlated with a second CMV IE (IE-84) [6]. IE-84 may inhibit p53-mediated braking of the cell cycle in smooth-muscle cells [6]. The hypothesized mechanisms linking CMV to atherosclerosis are summarized in figure 1 [7].

The epidemiological evidence linking CMV to atherosclerosis is not as strong. CMV seropositivity has been linked to athero-
These investigators initially reported an overall OR of 1.57 (95% CI, 1.03–2.40), linking CMV seropositivity to atherosclerosis. However, after adjustment for other atherosclerosis risk factors, the OR fell to a nonsignificant 1.36 (95% CI, 0.81–2.28) [9]. Thus, the strength of the association was quite modest, but specificity was suggested by the lack of an association for atherosclerosis and antibodies to herpes simplex virus type 1 or 2. In a subsequent report of the same data [10], the OR, after adjustment for other atherosclerosis risk factors, demonstrated a dose response (OR: 1.0 for CMV titer of <4, 1.5 [95% CI, 0.8–2.9] for CMV titer of 4–20, and 5.3 [95% CI, 1.5–18.0] for CMV titer of ≥21). Subjects in the ARIC Study report had donated the blood that was tested for CMV antibodies in 1974 and were examined by ultrasonography in 1987–1992, thus establishing that CMV seropositivity predated atherosclerotic disease (temporal correctness of association).

The most compelling epidemiological evidence for a role of CMV in atherosclerosis comes from restenosis data following coronary atherectomy. Zhou et al. [11] reported that 22 (43%) of 49 CMV-seropositive subjects developed restenosis (as measured by angiography repeated at 6 months), vs. only 2 (8%) of 26 CMV-seronegative subjects (OR, 9.00 [95% CI, 1.91–42.38]; \( P = .002 \)). Furthermore, CMV seropositivity was a stronger predictor of restenosis than was diabetes (OR, 1.16; \( P = 1.0 \)), hypertension (OR, 1.96; \( P = .18 \)), smoking (OR, 0.57; \( P = .34 \)), male gender (OR, 2.44; \( P = .19 \)), or presence of unstable angina at the time of atherectomy (OR, 0.85; \( P = .78 \)). A dose-response relation linking increasing CMV antibody titer with increasing risk of restenosis was found, similar to ARIC Study data. No association was found with seropositivity for hepatitis A virus (i.e., specificity).

Doubt about CMV’s role in atherosclerosis has been cast by pathological studies that demonstrated CMV nucleic acids and proteins in atherosclerotic plaques but also frequently in non-atherosclerotic arterial tissue (overall weighted OR for finding CMV in atherosclerotic vs. nonatherosclerotic tissue, 1.4 [95% CI, 1.0–1.9]) [8]. However, these studies have also revealed significant methodological differences (PCR vs. dot-blot or other detection methods), calling into question their overall validity.

**C. pneumoniae and Atherosclerosis**

In contrast to CMV, the link between *C. pneumoniae* and atherosclerosis was initiated and continuously strengthened by epidemiological data, and the biological/mechanistic evidence is much weaker. In the late 1980s, researchers in Finland noted an approximately twofold higher risk of IgG and/or IgA antibodies to *C. pneumoniae* in patients with myocardial infarction or angina than in similar control subjects [12]. Since then, no less than 17 other studies have confirmed an association between coronary events or prevalent atherosclerotic disease and seropositivity for *C. pneumoniae*, with ORs of 1.5–10 (average OR, ~2.5) (reviewed in [8]). Only one study showed no associ-
ation, and the 95% confidence interval for the OR includes 1.0 in only four of the positive studies.

Thus, the consistency of the association is excellent, and the strength is similar to that for other well-established atherosclerotic risk factors. Many of the studies used coronary events as the measure of association, and thus it is not clear whether *C. pneumoniae* is linked to the atherosclerotic process itself or to plaque instability and consequent events. Potential shortcomings of these data center around the definition of seropositivity. Many of the studies utilized different assays and levels of antibody to define a seropositive subject. In some studies, seropositivity was even defined post-hoc. There may also be a significant bias against publishing negative data, thus slanting the preponderance of evidence toward an association.

Further epidemiological evidence suggests that the association of *C. pneumoniae* with atherosclerotic tissue is more specific than for CMV. Histologic or genetic analysis of atherosclerotic tissue has been performed and in six of seven studies demonstrated *C. pneumoniae* in some atherosclerotic lesions (reviewed in [13]). Immunofluorescence staining of atherectomy specimens identified *C. pneumoniae* in up to 79% of atherosclerotic lesions, vs. only 4% of nonatherosclerotic tissue specimens [14]. *C. pneumoniae* has even been isolated from the arterial lesions of at least one patient with coronary disease [15] and another with cerebrovascular disease [16]. These data add to the strength and specificity of the association between *C. pneumoniae* and atherosclerosis.

However, like seropositivity data, histologic and PCR data have been collected with nonstandardized methods. For example, for the culture-positive patient described by Ramirez et al. [15], only 1 of 2 laboratories performing immunostaining and only 3 of 4 laboratories performing PCR found the arterial sample from that patient to be positive; of the 9 specimens in that study that were culture-negative, 6 were positive by either immunostaining or PCR. Furthermore, inhibitors of the PCR assay may be present in atheromatous material [13]. Thus, there may be significant false-positives and/or false-negatives when PCR or immunohistochemistry is utilized.

Temporal sequence of *C. pneumoniae* infection predating atherosclerosis (or at least a coronary event) is supported by the time delay of several weeks to generate antibody to *C. pneumoniae* after infection; thus, all studies of seropositivity support the theory that *C. pneumoniae* infection predates the clinical coronary event but not necessarily atherosclerosis itself. However, the ARIC Study investigators have provided evidence of *C. pneumoniae* seropositivity predating prevalent atherosclerosis, as measured again by B-mode ultrasonography of the carotid arteries [17]. In that study, atherosclerosis was defined by a carotid artery intimal-medial thickness in the >90th percentile, and *C. pneumoniae* seropositivity was defined by a serum antibody titer of ≥1:8. After adjustment for age, hypertension, diabetes mellitus, low- and high-density lipoprotein cholesterol levels, and educational level, the OR for *C. pneumoniae* seropositivity for subjects with atherosclerosis was 2.0 (95% CI, 1.19–3.35), thus establishing a temporal relation for prevalent disease.

Biological plausibility is the weakest link for establishment of *C. pneumoniae* as a cause of atherosclerosis. Studies have been profoundly hindered by a lack of appropriate animal models [18]. One study utilizing rabbits that were inoculated intranasally with *C. pneumoniae* and fed a cholesterol-enhanced diet demonstrated accelerated atherosclerosis in these rabbits vs. its development in uninfected animals [19]. Furthermore, treatment of infected animals with azithromycin prevented this acceleration. However, chlamydial antigen was detected in only two of nine infected rabbits [19]. In vitro, chlamydial replication can be altered under the influence of specific cytokines to form an aberrant or persistent infection [13]. Until mechanistic data are more sound, the question as to whether *C. pneumoniae* is a cause of atherosclerosis or is an innocent bystander will remain [20].

### Interventional Studies in Humans

Of all epidemiological data, randomized, blinded intervention trials provide the highest level of evidence of a cause-effect association. CMV infection may not need to proceed to lytic stages of replication to cause disease, on the basis of mechanistic data regarding IE-72 and IE-84. Thus, antiviral therapy may not prevent atherosclerotic lesions, and the most effective therapies for CMV are intravenous preparations with marked toxicity and cost. No interventional trials with use of anti-CMV therapy have been performed among patients with atherosclerosis. In contrast, there are low-cost, well-tolerated oral therapies for *C. pneumoniae* infection, and two small preliminary studies have recently been reported [21, 22].

One of these studies, the ROXIS pilot study [21], randomly assigned 212 men with recent evidence of coronary syndromes to receive roxithromycin, a macrolide with good activity against *C. pneumoniae*, or placebo. The major outcomes measured were severe, recurrent ischemia, acute myocardial infarction (MI), death, or combinations of these. The study found a significant difference between the groups when the combined endpoint of recurrent angina plus acute MI plus death was assessed (nine events with placebo vs. one event with roxithromycin; *P* = .04). However, there are many flaws in this study. *C. pneumoniae* antibody status was not determined prior to study enrollment, but was subsequently shown to be roughly equal in the placebo and roxithromycin groups (49% and 47%, respectively). There were many dropouts in both groups due to revascularization procedures (e.g., coronary artery bypass grafting [CABG]), which the authors did not include as an endpoint. If CABG had been included as an endpoint, the number of events in the placebo group would have been 22, vs. 17 in the roxithromycin group.

The second interventional study was methodologically more sound [22] but also of limited size/power. In that study, *C. pneumoniae*—seropositive men with recent MI were random-
ized to receive placebo or azithromycin for 3–6 days. The outcomes measured included death, unstable angina/recurrent MI, or revascularization (either angioplasty or CABG). Five events occurred among 21 patients (25%) in the placebo group, vs. only 3 among 40 patients (8%) in the azithromycin group; the relative risk was 0.2 (P = .03) for azithromycin recipients. The event rate in the azithromycin group was similar to that seen in a C. pneumoniae–seronegative untreated cohort (7% event rate) and significantly lower than in the untreated C. pneumoniae–serointermediate group (serum antibody titer, 1:8–1:32, 15% event rate) and C. pneumoniae–seropositive but nonrandomized group (antibody titer, >1:32; 30% event rate) in the same study. This study provides strong evidence to justify a larger, randomized trial of antibiotic therapy for secondary prevention of coronary events in C. pneumoniae–seropositive subjects.

Even if macrolide antibiotics decrease the risk of subsequent coronary events, it is not clear that the effect is due to the anticlamydial activity of these drugs. Macrolides, including roxithromycin, have potent antiinflammatory effects [23, 24] that may account for their efficacy in secondary prevention of coronary events. Furthermore, treatment may not be adequate to eradicate the organism, lower titers of antibodies to Chlamydia, or affect other coronary disease risk factors [25].

Conclusion

A link between infectious agents and atherosclerosis has been postulated for decades. Epidemiological and biological data suggest that CMV and C. pneumoniae may play a role in atherosclerosis itself, acute coronary events (e.g., unstable angina or MI), or restenosis following coronary atherectomy. Lack of animal models has hampered investigations of mechanisms, particularly with regard to C. pneumoniae. Early interventional trials of therapy aimed against C. pneumoniae suggest that antimicrobial treatment may be useful for secondary prevention of coronary events. This concept is currently under investigation in a large, randomized trial.

References