Editorial Response: The Role of Chlamydia pneumoniae in Atherosclerosis

The current state of knowledge regarding the role of Chlamydia pneumoniae in atherosclerosis is that an association between C. pneumoniae and atherosclerosis has been demonstrated, but it is unproven whether C. pneumoniae plays a role in the pathogenesis of atherosclerosis or its complications.

In 1988, workers in Finland found C. pneumoniae antibody more frequently in persons with coronary artery disease than in controls. Similar results were found by a large number of other investigators throughout the world, while some failed to find the association [1]. These seroepidemiological studies were useful primarily in alerting us to the possibility of an association of C. pneumoniae with atherosclerosis and the diseases that it causes.

See article by Jantos et al. on pages 988–92.

The crucial findings in demonstrating that there is an association between C. pneumoniae and atherosclerosis are from studies of atherosclerotic tissue. The presence of C. pneumoniae in atherosclerotic lesions has been demonstrated repeatedly by four different techniques: electron microscopy, isolation of the organism, PCR, and immunocytochemistry.

There are now over 20 reports from many areas of the world of studies in which the organism has been found by one or more of these techniques in atheroma of coronary, carotid, and femoral/popliteal arteries and the aorta. The reports have shown a wide variation in the frequency of finding the organism in atherosclerotic lesions. While some of the variation may be due to the source of the specimens, the nature of the tests used and the expertise of the investigators are the most important. Those investigators who used immunocytochemistry or direct staining techniques found from ~40% to 100% of the lesions to be positive for C. pneumoniae. Investigators using PCR found only about zero to 60% to be positive. The article by Jantos et al. reports results at the lower end of this frequency [2].

In our nine reported tissue studies of a total of 362 persons from diverse populations, we used both PCR and immunocytochemistry. The organisms were found in lesions in young persons as well as in extensive disease in older persons. Overall, 54% of the developed lesions were positive for C. pneumoniae. The organism was not found in normal artery specimens. The frequency of positives was invariably greater with use of immunocytochemistry. For example, in our study of atherectomy specimens, immunocytochemistry detected C. pneumoniae in 55% of the specimens, vs. 32% by PCR [3].

The reason for the relative insensitivity of PCR is not completely understood but most likely involves tissue inhibitors of the reaction that are not eliminated by standard methods. Jantos et al. state that this is unlikely in their study because of the use of nested PCR and internal controls and the spiking of specimens with target DNA. Unfortunately, there is a lack of detail in the Materials and Methods section concerning the amount of target DNA, the internal controls, and the results that rule out inhibition. Problems with PCR inhibition have been seen in studies of tissue specimen unrelated to C. pneumoniae. However, as Jantos et al. point out, the discrepant PCR results underscore the need for standardization of these assays for C. pneumoniae detection. The methods used for extraction of DNA from clinical specimens should also be standardized. To this end, Dr. Jens Boman in Umeå, Sweden, has spearheaded a multilaboratory study for evaluation of differing PCR protocols.

The condition of the specimens can also affect the frequency with which the organism is found. For example, atherectomy specimens are not ideal. They often provide only small amounts of tissue that may include considerable debris. One laboratory that originally reported a very low percentage of PCR-positive atherectomy specimens [4] has recently reported a series of 17 carotid endarterectomy specimens studied by both PCR and immunocytochemistry. While only 2 were positive by PCR, for 15 of 17 there was some evidence of C. pneumoniae in the lesion, as revealed by immunocytochemistry with monoclonal antibodies [5].

Jantos and colleagues propose that the immunocytochemical reactions may not be true-positives but due to nonspecific reactivity with components of the atherosclerotic tissue. This explanation is highly unlikely for a number of reasons. Adjacent tissue sections are stained with an irrelevant antibody, such as normal mouse ascitic fluid, to specifically rule out that staining is due to a nonspecific reaction. Monoclonal antibodies, both Chlamydia genus-specific and C. pneumoniae-specific, are used. In our studies, none of the tissues that stained positive with either the genus- or the species-specific antibody reacted with a Chlamydia trachomatis-specific antibody. Positive staining of “histologically normal” arterial tissue with chlamydial antibodies is rare.

When different segments of a single atherosclerotic plaque are examined, it is not uncommon that some segments are positive and some negative [6]. The immunocytochemical observations are usually based on a single 4-μm-thick section. If a series of sections are made, some may be positive and some negative. Even the high percentage of atherosclerotic lesions positive by immunocytochemistry may be an underestimation, because of the small sample size examined.
Isolation of *C. pneumoniae* from an atheroma has been difficult, and until recently only two groups had reported single isolates, one from coronary and one from carotid artery lesions. Maass et al. isolated *C. pneumoniae* from 11 of 50 specimens of coronary artery or bypass vessels from open-heart surgery [7].

In situ hybridization so far has not been a useful technique with *C. pneumoniae*. This organism does not have a plasmid that has provided a method for an adequate signal to be obtained with *Trachomatis*.

When we state that the association of *C. pneumoniae* and atherosclerosis has been proven beyond a reasonable doubt, it is important to say what we mean by the word association. The dictionary says it is “the state of being associated.” It carries no implications about whether one of the associated items is causing the other. The fact that *C. pneumoniae* and atherosclerosis are associated does not mean that *C. pneumoniae* causes atherosclerosis. The data overall suggest that despite the fact that relatively insensitive techniques are used and very small pieces of tissue are examined, the organism is found with considerable frequency in the diseased tissue and not in normal artery tissue. Whether the true incidence of *C. pneumoniae* in atherosclerotic plaques is closer to 100% than the 8% found by Jantos et al. is largely moot. The field has moved beyond a question of whether there is an association between *C. pneumoniae* and atherosclerosis.

Further studies either of seroepidemiology or of the frequency of the organism in different tissue specimens, as determined by various techniques, will not help us answer the most important question: Does *C. pneumoniae* play a causative role in the pathogenesis of atherosclerosis or its disease complications? We believe that investigators’ energy should be devoted to this important question. Two methods are now being used: one is animal models in rabbits and mice, and the other is human secondary-prevention trials with antibiotics. To date, in both mouse and rabbit models, there is evidence that infection contributes to the development and progression of atherosclerosis [8–10].

While animal model studies in primates and primary prevention trials would be valuable, we know of none that are in progress. Demonstrating the organism’s role in the pathogenesis of atherosclerosis will not be easy and will not come from a single experiment. This is a question of great importance because of the possibility of treatment or prevention of the number one cause of death.

**References**