Collaborative Multidisciplinary Workshop Report: What Questions Regarding the Role of *Chlamydia pneumoniae* in Atherosclerosis and Cardiovascular Disease Need to Be Addressed Utilizing Animal Models?

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In general, the Animal Model Workshop Committee concluded that animal models are crucial to investigations of the role of *Chlamydia pneumoniae* in atherosclerosis and cardiovascular disease. In vivo animal experiments are essential in studies to establish causality between *C. pneumoniae* and atherosclerosis; however, it is unlikely that an animal model will fulfill all the criteria of Koch’s postulate, which was developed for an acute infectious disease with a single etiology, because atherosclerosis is a chronic disease with multiple etiologic factors. Furthermore, animal models are necessary to discern the possible mechanisms by which *C. pneumoniae* may induce, accelerate, or complicate the processes of atherosclerosis and cardiovascular disease. Experiments are necessary to complement in vitro and human studies in establishing biological plausibility. The establishment of animal models should be a priority to provide guidelines for clinical trials with respect to appropriate interventions and prevention (e.g., guidelines for the most appropriate antimicrobial agent or combination of agents, dose and duration of therapy, and development of a possible vaccine).

**Ideal Animal Model**

There is no perfect or ideal animal model that would mimic all parameters of human atherosclerosis and cardiovascular disease. In the animal kingdom, nonhuman primates are closest to humans, but atherosclerotic changes are not exactly the same in nonhuman and human primates. Moreover, there are disadvantages to using a primate model, primary among which are the cost, the lack of availability to many investigators, and the public’s opposition to use of primates in experiments. Although it was felt by the committee that a primate model would provide useful information, it was not considered absolutely necessary for establishing causality. Although rabbits and mice have been the main animals used to investigate the relationship between *C. pneumoniae* and atherosclerosis, preliminary data on the mini-pig model were presented at this meeting. Each animal model has its own advantages and disadvantages, which will not be addressed in this communication.

Atherosclerotic changes in mini-pigs closely resemble those in humans, and coronary artery disease can be studied in the animal; therefore, the model should be useful even though the size of the mini-pig (30 kg) is still a limiting factor for relatively large treatment studies. Recent recognition of a new *Chlamydia* species that is endemic in pigs (*Chlamydia suis*, swine biovar of *Chlamydia trachomatis*) may pose a problem; however, it also could be an advantage in that this species could be studied in swine as a surrogate for *C. pneumoniae* or as a control infection. New noninvasive techniques to detect coronary artery disease or atherosclerosis, such as B-mode Doppler ultrasound and magnetic resonance imaging, could be applied to these mini-pigs and allow smaller sample sizes.

Members of the committee concluded that multiple animal models are needed to answer different questions, and if several different animals can demonstrate atherosclerotic changes with *C. pneumoniae* infection, then we would be closer to establishing causality.

**Specific Observations and Recommendations**

On the basis of their discussions, committee members made the following observations and recommendations regarding the use of animal models in the study of the role of *C. pneumoniae* in atherosclerosis and cardiovascular disease:

1. One of the limitations of the animal studies reported so far is the inability to recover viable *C. pneumoniae* from aorta or atheromatous tissues in chronic infection models (but it can
be recovered in the aorta during acute infection in the mouse model), although the organism can be demonstrated by immunohistochemical stains and polymerase chain reaction. Studies should be done to address this issue, even though it is recognized that the organism may reside in tissues in a latent, metabolically inactive, persistent state. Utilization of corticosteroids several days or weeks before sacrifice or inhibition of γ-interferon (by monoclonal antibodies) are measures that should be considered as they may induce reactivation or proliferation of the organism.

2. The animal models so far have been relatively short-term and have demonstrated mild, early, atherosclerotic lesions, although more diffuse macroscopic lesions can be seen when a cholesterol-enriched diet is added. Longer-term studies are needed to define the natural history (in terms of years) of the arteriopathy and to determine whether it is feasible to produce stenotic lesions or vulnerable plaques, which may have more clinical application.

3. Multiple risk factors have been associated with atherosclerosis and cardiovascular disease. It is likely these cofactors have additive or synergistic effects on the development or progression of atherosclerosis. So far, *C. pneumoniae* have been demonstrated to accelerate atherosclerosis in the rabbit and murine model in the presence of hypercholesterolemia. Further animal studies are needed to explore the interaction of *C. pneumoniae* infection with other established risk factors (e.g., smoking, hypertension, diabetes mellitus) and newly recognized cofactors, such as hyperhomocystinemia.

4. Other infectious diseases (cytomegalovirus, herpes simplex virus, periodontitis and *Helicobacter pylori*) have been associated with cardiovascular disease in humans, and the effect of coinfections with *C. pneumoniae* and one or more agents should be explored and compared with the effect of *C. pneumoniae* alone in animal models. Other microbial agents not associated with cardiovascular disease should be used as controls in these models to assess nonspecific effect. To date, organisms used as controls in animal studies include *C. trachomatis*, *Mycoplasma pneumoniae*, and *Pasturella multocida*. Neither *C. trachomatis* nor *M. pneumoniae* have been found to induce or enhance atherosclerosis in animal models, but *P. multocida* can accelerate the atherosclerotic changes induced by a cholesterol-enriched diet in the rabbit.

5. The implications of an infectious disease playing a major role in cardiovascular disease are enormous. Large-scale clinical trials have already been started with the newer macrolides to determine their efficacy in reducing secondary cardiovascular events. Animal models are important for providing guidelines concerning the most effective antimicrobial agent or combination of agents (especially with rifampin) as well as the optimal dose and the duration of therapy to be used in clinical trials. Studies in animals should also examine the benefit(s) of using antibiotics combined with a lipid-lowering agent (“statins”), antioxidants (e.g., vitamin E), or anti-inflammatory agents.

6. The ultimate strategy to prevent cardiovascular disease, besides alteration of lifestyle, would be to develop a vaccine. Animal models are crucial for testing the efficacy and safety of a vaccine before clinical trials. The primary objective of a vaccine program would likely be to prevent respiratory disease, with a secondary aim of also reducing atherosclerosis and cardiovascular disease. Primary prevention and secondary prevention studies could be conducted in different animal models. The potential of a therapeutic vaccine (after establishing infection) with and without antibiotics should be explored. The possibility of a vaccine inducing myocarditis or worsening vascular damage needs to be rigorously examined in animal models.

**Conclusion**

In many aspects, animal models are crucial to our understanding the role of *C. pneumoniae* in the development or progression of atherosclerosis and cardiovascular disease. The members of the workshop committee strongly urge governmental and other funding agencies and pharmaceutical companies to support these important and essential animal studies. In addition, animal studies regarding causality between *C. pneumoniae* and atherosclerosis and the mechanisms would be facilitated and earlier answers would be enabled if a central resource was established to provide early information to investigators on the availability of new transgenic animals and investigative and diagnostic tools.