**Chlamydia pneumoniae** Infection and Atherosclerosis: Methodological Considerations

To the Editor:

In contrast to several previously published studies, 1–3 the one by Caligiuri et al 4 failed to observe an accelerating effect of *Chlamydia pneumoniae* infection on atherosclerotic lesion development in apolipoprotein E–deficient mice. There are several critical differences in experimental design that likely account for these contrasting observations.

In their initial studies, Caligiuri et al 4 administered a single, nonpurified, low titer dose of *C pneumoniae* (1 x 10^7 ifu/mouse). This method is in contrast to the other studies where multiple inoculations of higher titers of purified *C pneumoniae* (5 x 10^7 to 3 x 10^7 ifu/mouse) were administered at intervals of 1 or 2 weeks. We have previously demonstrated that a single inoculation in animals at 8 weeks of age does not produce a sustained infection in the aorta, whereas multiple inoculations lead to a sustained infection for up to 20 weeks. 5 The age of the animals at the time of inoculation, the interval between inoculations, and the length of time post-inoculation are also critical elements for accelerating lesion development. 1–3 For example, we have demonstrated that there is a more significant increase in lesion area in the aortic arch in animals at 6 weeks as compared with 10 weeks after inoculation. 1 In the study by Caligiuri et al, 4 the combination of a single inoculation administered to 6– to 8-week-old mice, coupled with the prolonged duration post-inoculation (22 weeks), may have accounted for their failure to observe an acceleration of lesion development. Additionally, in the second arm of their study, a “booster” inoculation was administered at 18 weeks. This dose was likely administered much too late to have any contributory effect on lesion development.

It is clear from serological evidence that humans are subject to repeat infections throughout life. At present, the time intervals of infection that best simulate the impact of *Chlamydia pneumoniae* infection on experimental atherosclerosis. This fact by itself challenges the notion that *C pneumoniae* is a major etiological or pathogenetic factor in this disease.

A wide range of *C pneumoniae* doses have been used in the studies on apolipoprotein E (apoE) knockout mice, and the one chosen by us 2 (1 x 10^7 ifu/mouse) is intermediate. We accomplished sustained infection, as demonstrated by persistence of *C pneumoniae* DNA in the lungs and high immunoglobulin G-anti-*C pneumoniae* titers in all mice. Kuo et al, who did not perform tissue polymerase chain reaction (PCR) or antibody assays in their study, 1 propose that a sustained infection in the artery wall is needed for *C pneumoniae* to promote atherosclerosis. This possibility is not supported by the work of Burnett et al, 3 who found that mice positive for *C pneumoniae* by aortic tissue PCR did not have larger lesions than negative mice.

Our study is not the only one that reports that *C pneumoniae* does not accelerate atherosclerosis. Aalto-Setälä et al 4 recently found that infection of apoE knockout mice with *C pneumoniae*, either by single or repeated inoculations, did not increase lesions. The observations that normocholesterolemic mice, when infected with *C pneumoniae*, do not develop atherosclerosis 2 and that germ-free apoE knockout mice develop lesions to the same extent as those exposed to microbes 3 show that *C pneumoniae* is does not in itself cause atherosclerosis.

It remains possible that *C pneumoniae* may activate plaques and elicit acute coronary syndromes. Furthermore, experimental conditions in the mouse may not reflect those in humans with respect to *C pneumoniae*. Results from the ongoing clinical trials using antibiotics to prevent coronary heart disease in humans are therefore awaited with great interest.

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Response

In their letter, Kuo et al point out technical differences that may account for the discrepancy between their findings 1 and ours. 2 We agree that very precise experimental conditions may be needed to detect an effect of *Chlamydia pneumoniae* on experimental atherosclerosis. This fact by itself challenges the notion that *C pneumoniae* is a major etiological or pathogenetic factor in this disease.


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