**Chlamydia pneumoniae**–Induced Atherosclerosis in a Rabbit Model

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In order to establish a causative relationship between *Chlamydia pneumoniae* and atherosclerosis, animal models have been proposed. In a rabbit model, arterial intimal thickening has been induced by direct intravascular and intranasal inoculation with *C. pneumoniae*. *C. pneumoniae* infection can induce significant acceleration of atherosclerosis in a mildly hyperlipidemic rabbit model but is prevented by treatment with azithromycin. Together these preliminary rabbit experiments suggest that *C. pneumoniae* may play a causative role in atherosclerosis. More animal studies are underway that are designed to address further mechanistic and therapeutic questions regarding the association between *C. pneumoniae* and atherosclerosis.

In order to establish a causative relationship between an infectious agent and a disease process, three conditions (termed Koch’s postulates [1]) must be met individually. First, the infectious agent must be found in the majority or all of the patients in which the disease process is manifested. Second, this infectious agent must be introduced into another subject and then result in the development of the proposed disease process. Third, the infectious agent must again be recovered from this new subject now manifesting the disease. With any process as chronic and potentially deadly as atherosclerosis, it is not possible to test Koch’s postulates directly on humans. Therefore, animal models have been proposed as an alternative method of study.

**Nonhyperlipidemic Rabbit Models**

To test the hypothesis that a vascular infection of *C. pneumoniae* can be induced in an animal model, my colleagues and I devised a method of direct intravascular inoculation into the aorta of a rabbit. With a local drug delivery catheter (Infusasleeve; LocalMed, Palo Alto, CA) (see figure 1), we infused 1–6 × 10^8 inclusion-forming units of *C. pneumoniae* at 4 atmospheric pressures directly into the abdominal aortic wall of a rabbit. The rabbit was then maintained on a normal diet and followed for 2 months before the rabbit aorta was removed and evaluated histologically and with direct immunofluorescence for *C. pneumoniae*. Atherosclerotic lesions were detected both within the site of the original local inoculation and also proximally in the thoracic aorta (figure 2). Species-specific *C. pneumoniae* antigen was also detected by direct immunofluorescence—but only at sites of intimal thickening. The lesions detected within the aorta, however, were fairly minor. Other investigators have done similar experiments with similar results but they used a more physiologic intranasal respiratory route of *C. pneumoniae* infection [2, 3]. Those results are reported elsewhere in this issue. While it is unclear how typical of atherosclerosis the early aortic lesions are, these preliminary experiments offer evidence that *C. pneumoniae* infection is capable of causing aortic lesions where they do not otherwise occur.

**Hyperlipidemic Rabbit Models**

We next approached the question slightly differently [4]. Rabbits, if given a high-cholesterol diet (1%–2% cholesterol), will independently develop significant amounts of atherosclerotic plaque [5]. The importance of lipid metabolism in the development of atherosclerosis is known in humans. Thus, we hypothesized that the combination of *C. pneumoniae* infection and small amounts of cholesterol supplementation in the diet of the rabbits might significantly accelerate the development of atherosclerosis.

We evaluated 30 rabbits receiving a small supplement of cholesterol (0.25%) in their diet. Ten rabbits received sham intranasal saline inoculations, 10 received three separate inoculations of *C. pneumoniae*, and 10 were intranasally inoculated with *C. pneumoniae* and then treated with azithromycin, an antibiotic effective against *C. pneumoniae*. The animals were followed for 3 months, then euthanized, and their aortas were evaluated pathologically. Figure 3 shows the study results. Maximal intimal thickness of atherosclerotic plaque was assessed by quantitative microscopic measurement of the radial distance through the intima at the site of most intimal thickening. *C. pneumoniae* infection alone caused significantly accelerated development of aortic atherosclerosis compared with uninfected rabbits or infected rabbits treated with antibiotic. Of interest, even though the antibiotic treatment blunted or eliminated the accelerated atherosclerotic response, direct immunofluorescence testing revealed persistence of Chlamydial antigens within the aortas of infected rabbits, including those receiving antibiotic treatment.
Figure 1. Schematic diagram of percutaneous technique used for local inoculation of C. pneumoniae into abdominal aortic vessel wall of rabbit.

Discussion

Taken together these preliminary rabbit experiments and similar studies in mice ([6] and elsewhere this supplement) suggest that C. pneumoniae may be capable of initiating atherosclerosis and causing an acceleration of the atherosclerotic process. More animal studies are underway, which hopefully will reveal further information regarding the etiologic relationship between C. pneumoniae infection and atherosclerosis and the pathogenic mechanisms that play a role. Potential directions of inquiry for these models may include the influence of C. pneumoniae infection on the development or progression of atherosclerosis (whether it occurs by direct local effects or by an indirect systemic inflammatory response), potential interactions between C. pneumoniae infection and other known risk factors (e.g.,

Figure 2. Photomicrographs of representative aortic sections from rabbit undergoing aortic local delivery infection with C. pneumoniae. A. Site of initial direct local delivery in abdominal aorta. B. Site in thoracic aorta distant to site of initial inoculation. Moderate amounts of intimal thickening in both sites. Sections stained with hematoxylin-cosin. Original magnification, ×1000. Striped arrowheads, luminal surface; arrows, arterial media; solid arrowheads, intimal thickening.
hyperlipidemia, hypertension, and homocysteine), differential effects between a primary *C. pneumoniae* infection of initially normal vessels versus a secondary infection of previously existing atherosclerotic plaque, and a more detailed look at the short- and long-term effect of antibiotics on the entire process. Through these types of animal studies, a greater understanding of the underlying mechanisms of the pathogenic actions of *C. pneumoniae* and of potentially successful therapeutic strategies may be obtained.

References