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Is *C. pneumoniae* research in peril?

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Chlamydia pneumoniae is an obligate intracellular parasite which infects mucosal surfaces of the human respiratory tract causing sinusitis, pharyngitis, bronchitis, and pneumonia. Although the bacterium causes acute disease, mildly symptomatic, asymptomatic, or unrecognized infections are most common (Kuo et al., 1995). *C. pneumoniae* infections are widespread among children 5–14 years of age and by age 20 years about 50% of young adults have detectable antibodies to the microorganism. The seroprevalence to *C. pneumoniae* continues to rise in the population and reaches approximately 75% in the elderly. Moreover, the epidemiological data suggests that most people are infected and re-infected throughout life (Kuo et al., 1995). *C. pneumoniae* was established as a human respiratory pathogen in 1986 (Grayston et al., 1986) and initially research on this pathogen was rigorous, especially due to its association with variety of chronic diseases such as Reiter's syndrome, sarcoidosis, asthma, chronic obstructive pulmonary disease (COPD), multiple sclerosis, Alzheimer disease, and atherosclerosis. However in recent years, the interest in basic as well as clinical research on *C. pneumoniae* has undergone a sharp decline. One of the major factors that could have contributed to this decline may include frequent discrepancies in published data. Multiple studies have described the presence of *C. pneumoniae* in patient samples from those who suffered from the chronic diseases listed above by either serology, polymerase chain reaction (PCR), RT-PCR, immunocytochemistry (ICC) or electron microscopy, and occasionally even by direct isolation of the bacterium. Conversely, there are laboratories which could not confirm these findings. Inter-laboratory variations in sample collection, processing and *C. pneumoniae* detection methods are likely to be responsible for these inconsistencies (reviewed in Boman and Hammerschlag, 2002). However, numerous studies published on *C. pneumoniae*, describing the presence or absence of the microorganism, often lacked appropriate positive and/or negative controls. Using only one or two methods for *C. pneumoniae* detection in clinical specimens is insufficient and may have led to inaccurate conclusions (Puolakkainen et al., 1996; Mills et al., 1998; Sriram et al., 1998; Fainardi et al., 2008). Furthermore, positive labeling of cells or tissue for *C. pneumoniae* antigen(s) with an antibody does not necessarily represent an intact bacterium. It has been demonstrated that chlamydiae-infected cells stay positive for several of the chlamydial antigens, for example LPS, weeks after the bacterium has been degraded by lysosomes (Wolf et al., 2005). Direct isolation of *C. pneumoniae* from a patient's sample still represents the most conclusive, yet the most difficult method of detection of this pathogen. In spite of these inconsistencies there may also be another aspect which could have contributed to the current discrepancies existing in the *C. pneumoniae* field. As mentioned above, the bacterium frequently causes mild or asymptomatic infections, which often remain unrecognized and consequently untreated. In cases such as these, it cannot be conclusively determined at what point during their lifetime the studied patients actually suffered from an acute *C. pneumoniae* infection or re-infection. Many of these concerns could hypothetically be addressed by serology. According to the CDC standards for diagnostic detection of *C. pneumoniae* in clinical samples by micro-immuno-fluorescence (MIF), fourfold rise in IgG titer or an IgM titer ≥16 indicates an acute infection and an IgG titer of ≥16 suggests past exposure to the microorganism. Elevated IgA was excluded as a valid indicator of persistent or chronic infection (Dowell et al., 2001). Unfortunately, the MIF, which is considered the "gold standard" for detection of *C. pneumoniae* in humans does not seem to be absolutely reliable either. Cases of acute, culture-positive *C. pneumoniae* illness without seroconversion have been reported (Kutlin et al., 1998; Hammerschlag and Roblin, 2000). Conversely, the presence of an acute infection detected by MIF has been discovered among subjectively healthy individuals (Hyman et al., 1995). Thus, it is likely that the outcome of *C. pneumoniae* infection depends on the infectious dose and more importantly on fitness of the immune system of each individual. Numerous researchers tend to link various chronic diseases to so called chlamydial persistence. However, very little is known about the actual pathology caused to tissues and/or organs by productive *C. pneumoniae* infection in humans. This considerable lack of basic knowledge concerning the pathogen and its effects on the human body has led many studies to questionable conclusions.

One example of this is represented by reports on secondary prevention of coronary heart disease by treatment of patients who suffered from myocardial infarction with azithromycin (O'Connor et al., 2003; Grayston et al., 2005). In view of the fact that cardiovascular disease is one of the leading causes of fatalities in the developed world, it is not surprising that an association of *C. pneumoniae* with atherosclerosis was a priority to investigate. Participants enrolled in these studies, were largely represented by the older population, who had previously suffered from myocardial infarction and had a *C. pneumoniae* titer of IgG 1:16 or more, indicative of past infection. No data concerning the presence of acute sera was provided (Dunne, 2000; O'Connor et al., 2003; Grayston et al., 2005). The fact that an active *C. pneumoniae* infection was not established in this group of patients is a critical issue. Unfortunately, no validated serological marker for persistent or chronic *C. pneumoniae* infection is currently available (Dowell et al., 2001) and it is highly likely that most of these participants were infected or re-infected with the microorganism in the past, long before they received azithromycin. These studies clearly demonstrated that treatment of patients who had suffered from cardiovascular disease with azithromycin did not reduce or alter the risk

of recurrence of cardiac events. However, they completely failed to address the role, if any, of *C. pneumoniae* in the course of cardiovascular disease (O'Connor et al., 2003; Grayston et al., 2005). An atherosclerotic lesion takes many years to develop during which the patient could have encountered asymptomatic *C. pneumoniae* infection(s) at any time. If the bacterium does contribute to various chronic diseases one must not disregard the possibility that an injury to the arteries and other parts of our body may be achieved during the course of an acute respiratory infection with *C. pneumoniae*. If the microorganism is ever shown to cause or worsen any of the proposed chronic diseases, research on this pathogen must first properly investigate primary infection within the human respiratory tract. Similarly to *C. trachomatis*, infections with *C. pneumoniae* are often asymptomatic meaning that the infected person is unlikely to receive proper antibiotic treatment. Therefore clearance of the pathogen must entirely depend on the immune response of the host. It still remains unclear whether any of the diseases, respiratory or non-respiratory, linked to *C. pneumoniae* are caused directly by chlamydial growth or by the immune system's attempt to resolve the infection or both. The recent body of evidence suggests that *C. pneumoniae* employs sophisticated, species-specific strategies, which are comparatively more efficient than those utilized by *C. trachomatis*, in order to avoid recognition by the host innate immune system (Wolf et al., 2009 and unpublished data).

Although all chlamydiae share similarities in biology, they also display extraordinary diversity in tissue tropism and disease manifestation. Based on comparisons among sequenced chlamydial genomes, it seems that *C. pneumoniae* represents an intriguing chlamydial species with unique features, all of which are worthy of further investigation. For example, the *C. pneumoniae* chromosome contains a plasticity zone, a region with a higher rate of DNA reorganization, which is three times larger than the plasticity zone of *C. trachomatis* (~160 versus ~50 kb, respectively; Read et al., 2000). It is postulated that the gene content within the plasticity zone significantly contributes to the virulence of each chlamydial species. Moreover, *C. pneumoniae* contains 21 *pmp* genes encoding chlamydial polymorphic membrane proteins compared to only nine detected in *C. trachomatis*. Overall, the *C. pneumoniae* genome is ~0.15 Mb larger than that of *C. trachomatis* and contains 214 coding sequences which are not found in *C. trachomatis* (Kalman et al., 1999). *C. pneumoniae* is an established pathogen causing a significant number of respiratory infections in humans. However, at this point there is insufficient data with regard to the basic biology of this microorganism that would help to elucidate pathogenic strategies employed by *C. pneumoniae* in order to achieve successful invasion of its human host. In conclusion, more rigorous and thorough research on *C. pneumoniae* is absolutely essential for better understanding of its virulence within the human respiratory tract and its potential association with various chronic diseases.

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