Efficacy of DNA minigene vaccine to protect against respiratory and extra-respiratory spread of *Chlamydia pneumoniae*

Taylor Eddens¹ and Dr. Kerin Fresa-Dillon²

Department of Biology, Washington & Jefferson College¹
50 South Lincoln Street, Washington, PA 15301

Department of Immunology, Philadelphia College of Osteopathic Medicine²,
4170 City Ave., Philadelphia, PA 19131

**INTRODUCTION**

*Chlamydia pneumoniae* is an obligate intracellular gram-negative pathogen which produces an atypical pneumonia in humans (Brooks et al., 2009). *C. pneumoniae* spreads from individual to individual via airborne droplets, often resulting in an infection of nasal or oral mucosa (Brooks et al., 2009). Although severe disease is rarely reported, 70% of the total population in Western countries is seropositive by the age of 65 (Grayston, 2000).

Respiratory infection caused by *C. pneumoniae* is well established in both young and old mice (Little et al., 2005). However, respiratory infection in young mice typically resolves within 28 days (Little et al., 2005). The immune systems of older mice, however, have a decreased ability to eliminate infection. Little et al. found a statistically significant difference between the mean titers in the lungs of 6 and 20 month old mice at 14 days post-infection with *C. pneumoniae*. *C. pneumoniae* has also been linked to extra-respiratory infections, including diseases of the nervous and circulatory systems (Grayston, 2000). In a comprehensive review of 43 studies, *C. pneumoniae* or chlamydial DNA was found in 46% of coronary arteries in individuals with atherosclerosis, but only in 1% of arteries from individuals without coronary artery disease (Campbell & Kuo, 2004). This is especially noteworthy because atherosclerosis is a disease of aging and is more common in elderly individuals. Thus, understanding the implications of age in extra-respiratory spread of *C. pneumoniae* is quite important, especially if a link to atherosclerosis is confirmed.

This study tests the effects of a recently developed vaccine on the ability of aged and young mice to clear both respiratory and extra-respiratory infections. In particular, we were testing a heptavalent CTL minigene vaccine developed by Dr. Benjamin Wizel at the University of Texas at Tyler. After a challenge with *C. pneumoniae*, vaccinated mice exhibited a 3.6 log-reduction in the mean bacterial load found in the lungs (Pinchuk et al., 2005). However, no research had been conducted on the effects of age and vaccination.

**METHODS**

After plating HEp-2 cells with 5 x 10⁵ IFU of *C. pneumoniae*, the cells were inoculated into four well chamber slides at 1.4 x 10⁴ cells/well and infected with dilutions of 10% wt-volume homogenate of heart and ascending aorta tissue. After plating HEp-2 cells into 4-well chamber slides at 1.4 x 10⁴ cells/well and infecting with dilutions of a 10% wt-volume homogenate of heart and ascending aorta tissue, the slides were stained using fluorescently labeled FIT-C antibody specific for *C. pneumoniae* and counterstained with bisbenzamide as described in the Methods. The nuclei (blue) appear to be in a confluent monolayer. The specific punctate binding of the FIT-C antibody (green) indicates the presence of *C. pneumoniae* in the cellular cytoplasm. All other FIT-C staining in this image appears to be non-specific/justified by negative control not shown), as the staining sites are too large or placed over top of a nucleus. Both of these criteria for rejection as inclusions with *C. pneumoniae* should appear small, punctate and extra-nuclear.

**RESULTS**

- **Vaccine showed protection of the lungs at 28 days post-infection in both young and aged mice when compared to unvaccinated mice of the same age (p_young = 0.014, p_aged = 0.00012).**
- **There was no statistically significant difference between the titers found in the lungs of young and aged vaccinated mice at 28 days post infection (p<0.05).** This demonstrates that the vaccine is capable of overcoming immunosenescence in aged animals.
- **Vaccine showed protection of the hearts/ascending aortas at 28 days post infection in both young and aged mice when compared to unvaccinated mice of the same age (p_young = 0.014, p_aged = 0.002).**
- **Aged vaccinated mice had a statistically significant difference in mean titer in the heart when compared to young mice at 28 days post-infection (p = 0.0072).** The hearts of aged mice had a 131-fold higher mean than the young mice, indicating the effects of immunosenescence were not overcome.

**LITERATURE CITED**


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