<u>Proposal for an Attenuated Vaccine of *Chlamydia Trachomatis*</u>

<u>Literature Review</u>

Sexually transmitted diseases have caused major health issues in the past and still remain today as a major threat to people, especially sexually active men and women. *Chlamydia trachomatis* is one of the more prevalent sexually transmitted diseases that is being spread around the world today. Roughly four million new cases each year are reported in both sexually active men and women. C*hlamydia* is a disease that is most common of all the sexually transmitted disease, but it can easily be treated. There is a problem with *Chlamydia* though because the majority of the infected patients show no clinical signs of infection causing the disease to spread to the fallopian tubes in females and to the testicles in males causing serious health risks in both. No symptoms of this infection also increases the chances of spreading the pathogen to their partners. *Chlamydia* is not just a sexual transmitted disease; it can spread from person to person by numerous ways such as through vectors or from mother to child. Therefore a vaccine must be created to stop this pathogen from spreading and to protect people at a young age from exposure to this disease. (Currie 2007)

The pathogenic structure of *Chlamydia* has two morphological forms; elementary bodies (EB) and reticulate bodies (RB). The elementary body is the infectious form of the pathogen and the reticulate body form remains latent in the host until the opportunity to presents itself. Elementary bodies are very small in size and usually are shaped as a round figure or a pea shaped structure with an approximate size of 0.3-0.4um. They have an outer membrane that is cross-linked by many disulfide bonds which enables the pathogen to survive in harsh environmental conditions. The elementary bodies are inert until the host takes them up, which means that this pathogen can not carry out its function by itself. Reticulate bodies have a more fragile membrane lacking the disulfide bonds but are roughly the same in size as the elementary bodies. Once this pathogen is inside the endosome of the cell, glycogen is produced from the bacteria that cause the elementary bodies to become the inactive form RB. They have an incubation period of 7-21 days in the host. They replicate and divide by binary fission every 2-3 hours per generation. After the division, the RB switches back to the elementary bodies which are then released into the body through exocytosis. (Gomes, 2006)

Chlamydia trachomatis has 15 different serotypes in the outer membrane protein. Serovars A, B, Ba and C are largely found in Africa and Asia that cause trachoma, an ocular conjunctivitis. This form of the pathogen is transmitted mostly through hand to eye contact and from flies seeking the moisture from the eyes. Another form of the pathogen is serovars D-K which is transmitted by sexual and parental contact. This form cause many health risks such as cervicitis, urethritis, endometriosis, pelvic inflammatory disease, tubal infertility, ectopic pregnancy, neonatal conjunctivitis, infant pneumonia, and infant blindness. The last form of the pathogen is serovars L1, L2, and L3 which are also transmitted by sexual contact. All these forms of this pathogen exist all over the world today. This pathogen has the same mechanism of infection throughout all the serovars but the mode of transmission varies. (Gomes 2006)

Table 1 | ***Chlamydia trachomatis* serovars and their associated human diseases**

Serovars	Human disease	Method of spread	Pathology
A, B, Ba and C	Ocular trachoma	Hand to eye, fomites and eye-seeking flies	Conjunctivitis, and conjunctival and corneal scarring
D, Da and E, F, G, H, I, Ia, J, Ja and K	Oculogenital disease	Sexual and perinatal	Cervicitis, urethritis, endometritis, pelvic inflammatory disease, tubal infertility, ectopic pregnancy, neonatal conjunctivitis and infant pneumonia
L1, L2 and L3	Lymphogranuloma venereum	Sexual	Submucosa and lymph-node invasion, with necrotizing granulomas and fibrosis

Chlamydia trachomatis causes ocular trachoma and several sexually transmitted diseases. It has 18 main serovars, as determined by DNA-sequence analysis and immunotyping of the *C. trachomatis* major outer-membrane protein. Serovars A, B, Ba and C cause trachoma, a leading cause of blindness worldwide. Serovars D to K mainly cause sexually transmitted diseases. Serovars L1 to L3 cause lymphogranuloma venereum.

This particular pathogen has a very fascinating disease mechanism. Since this is an obligate intracellular pathogen, it has to enter the host cell to infect and survive in order to continue to carry out its function. The two forms of this pathogen (EB and RB) are the reason why this pathogen is so successful in evading the immune system. First the pathogen enters through the host epithelial cells by binding to the cells receptors, which is then taken in by phagocytosis. It is still unclear by scientists how the pathogen actually binds to the receptors that causes the phagocytosis and allowing the pathogen to enter the cell. Once *Chlamydia* is actually inside the host cell, the elementary bodies reorganize themselves so they are now in the latent form of RB. (Prebeck ,2003) They can only go through this transition and survive if the IFN gamma is low in the cell (Brunham). To evade intracellular killing by the host cell, it inhibits the fusion of the endosomes with lysosomes that would normally kill a pathogen. Once *Chlamydia* has achieved this task it can now go onto replicate in the cytoplasmic vesicles within the epithelial cells and spread to other cells where they will become infected. The sexually transmitted form of *Chlamydia* can move as far up as the fallopian tubes in females. The actual immune response to this pathogen in the fallopian tubes causes the scarring. This pathogen can also cross the placenta at birth causing problems with infants.

This pathogen should be treated as soon as possible because our immune system could cause more damage in response to this pathogen than itself. Our body initially amounts an immune response once the epithelial cells have been infected by releasing a variety of inflammatory cytokines. These cytokines are CXCL1, CXCL8 (IL-8), CXCL16, GM-CSF, IL-1α, IL-6, and TNF (Brunham). These cytokines help the immune recognize what kind of an infection it is so that it can get to work and destroy the invading pathogen. Some other cytokines that are released are IFNα, IFNβ, and IL-12. The epithelial cells are not the only cells that are releasing the help messages but macrophages and dendritic cells are releasing them as well. Macrophages release TNF and IL-6 while dendritic cells secrete IL-12. Dendritic cells also secrete cytokines for T-cell help which are CXCL10 and CCR7 (Brunham). Some of the inflammation in the female fallopian tubes is the actual cause of the scarring that leads to infertility. This pathogen is the leading cause for PID (Pelvic Inflammatory Disease), which can lead to other serious health issues such as cancer.

Chlamydia is the most frequently reported STD in the US. It is estimated that 2.8 million Americans are infected with this pathogen each year. This includes all the people that don't know they have it since this pathogen doesn't show symptoms in many infections. Asymptomatic infections of this pathogen allow it to be easily spread from person to person. The population at risk for *Chlamydia* is any sexually active male or female. Perhaps the more prevalent at risk are teenage girls and young women since their cervix has not fully matured to mount a defense against a pathogen of this sort. Another population at risk would be the homosexual community since this bacterial STD is transmitted through oral and sexually (vaginally and anal). There is a treatment for this pathogen if it is caught early before serious damage has been done. A single dose of antibiotics such as Azythromycin or Doxycycline BID will be enough to stop the infection and completely eradicates the pathogen. Subsequent testing and treatment of the sexual partner(s) must be taken into consideration to prevent re-infection. If left untreated, the infection can spread in both male and females leading to more serious issues such as infertility in both males and females. The only way to totally avoid this pathogen is though abstinence and knowledge of the potential health risks of a *Chlamydia* infection. .

The ideal potential target for the vaccine would be males and females who are non-sexually active or have had limited sexual partners. The targeted age for the vaccine study would be between the ages of 10 and 15 since this is when sexual activity usually first occurs. This vaccine would be most effective if given at an early age before they have been exposed, limiting the potential risk of an asymptomatic infection. Screening vaccine participants will be administered before inoculation to test for an active and current infection using cytology from the mucosal membranes. This can also be diagnosed through in some case urine but mainly a small specimen is taken from the infected area usually in the penis or the cervix. Any participant with an active infection will be treated with the appropriate antibiotics before being administered with the vaccine. (Millman 2004)

Description of Vaccine

The *C. trachomatis* vaccine will contain an attenuated organism and be delivered by inhalation. An attenuated vaccine suits the needs of the immune system in combating this pathogen because it induces the appropriate memory and humoral immune responses and can be delivered orally. Oral administration of the vaccine is helpful because it induces production of IgA, the antibody against mucosal infections like *C. trachomatis*, more efficaciously than other methods.

Target serotypes include D, E, F and Ia. Of the 11 strains of *C. trachomatis* that give rise to Chlamydia, these four serotypes are the most common and/or virulent. The antigens used to create antibodies will be polymorphic protein D (PmpD), a serotype common antigen (D.D. Crane et al, 2006, p. 1895).

To begin activation, pre-B cells receive an antigen binding signal via ITAMs. The B cell then clones itself producing cells with identical μ chain. Once the cells have ceased to divide, expression of L chain is possible and the pre-B cell becomes an immature B cell, ready for antigen binding. B cells cross-linked to antigen, in this case PmpD, will proliferate into antibody as well as memory PmpD to respond to reinfection. Positive selection of pre-B cells requires that the cell bind the appropriate antigen. B cells also undergo negative selection whereby B cells that bind self are induced to commit apoptosis.

Pre-T cells divide in the thymus. They express CD2 but not CD4 or CD8, markers for Th and Tc cells respectively. Signaling cascades cause the pre-T cell to start and stop β chain, followed by expression of Th and Tc receptors. The T-cells then move into the cortico-medullary junction where selection commits the cells to express either CD4 or CD8 but not both. Th1 cells are associated with an effective immune response to Chlamydia (D.D. Crane et al, 2006 p. 1894).

The target population is preadolescent males and females between 10 and 15 years of age. By targeting this demographic, we can protect the at-risk population before sexual activity becomes a factor. Vaccinating sexually inactive teens and young adults is also suggested.

A barrier to proper vaccination of the target population is that an attenuated vaccine cannot be administered to the immunosuppressed. In this instance, a subunit vaccine could be formulated and used. Even so, attenuated vaccines stimulate mucosal immunity and IgA production; which is of greatest importance against this disease.

It has been suggested by D.D. Crane et al that production of antibodies other than PmpD, as might be obtained with whole virus vaccines, may inhibit the ability of anti-PmpD to control the level of infection but use of the antigen still seems plausible (Crane,p. 1896).

Description of immunity

Innate immunity.

C. trachomatis is an obligate intracellular gram-negative bacterium that infects humans in many ways. Colonization of *Chlamydia* begins with attachment to sialic acid receptors on the genitalia. This infection persists at body sites that are inaccessible to phagocytes. Chlamydia is also able to inhibit phagolysome fusion in phagocytes because of it cell wall.Following colonization, the body responds to this infection through innate immunity and adaptive immunity. Immunity is the process by which the body fights pathogens. Innate immunity is always found right at the site of infection. The first innate immune cells that respond to *Chlamydia* infections are inflamments. In *Chlamydia* infection, inflammation response is noted at the infection site through characteristics such as pain, and heat which are cause by changes in local blood vessels. Blood vessels in this area become permeable to fluid swelling, showing an accumulation of proteins that help in protecting the body from the pathogen. These blood vessels are stimulated to express cell adhesions; inflammation is mediated by signaling molecules such as cytokines and chemokines.

Inflammatory chemokines attract leukocytes to the infectious sites. Chemokines receptors allow the movement of dendritic cells to lympiod organs. The infected cells produce inflammatory cytokines categorized into type, this is based on their function. Interleucine-1 (IL) which activates helper T cell also acts as an attractor of phagocytes to the inflammation system. IL-2 which is important in development of B cells and in stimulation of cytotoxic T cells, along with IL-8 (CXCL1, CXCL8) which engages in chemoattraction stimulation of CXCL16, GM-CSF, IL-6 and TNF regulate secretion of

CCL5, CCL7 and CXCL10 that cause lympocytes attraction. Cytokines also produce IFNβ, IFNα and IL-12 which are involve in the production of CD4 T cell.
Infected macrophage and neutrophiles cells affect the replication of Chlamydia by producing toxic substances that are poisonous. These toxic chemicals include nitric oxide (NO), the superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2). Since *Chlamydia* is an intracellular pathogen, macrophage and neutrophile do not eliminate *Chlamydia,* instead macrophage activate other cells for example T cells.

Complement is another important defense mechanism in innate immunity, it is involved in the pathogen elimination process. Complement is initiated from the liver. Complementation can be activated in some of these ways: the classical pathway activates complement by allowing antibodies to bind to bacterial surfaces forming a complex binding that activates C1. The C1 activates C4 and C2. C4 splits into C4a and C4b whereas C2 splits into C2a C2b. C4b and C2b activate C3 by spitting it into C3a and C3b. C3 is responsible for the start of cytolysis, inflammation and opsonization. The next activation of complement is the alternative pathway which involves antibody production and activates complement by binding proteins and pathogens. This pathway is known as the lectin pathway. Lectin is a protein that links to sugars such as mannose-binding lectin (MBL) which is found on the bacterial cell wall, where it function as an opsonine to phagocytosis . MBL activates C2 and C4 respectively. When C3 is activated, it binds to the surface of the pathogen where it is cleaved to form C3b which is the major effector molecule for complement. C3b binds tightly to the pathogen making it ready for phagocytic cell to kill the pathogen. C3b binds to C3 to form C5 which split into C5a and C5b. C5b bind to C6 and C7 which then bind to the plasma membrane of the bacterium. C3a and C5a bind to mast cells and cause them to release histamine and other chemicals that increase blood vessel permeability during inflammation. C5a also has chemotactic factor that draws phagocytes to the site of infection. Activate dendritic cells present infected cell to the cytotoxic T cell to kill the pathogen.

Adaptive immunity.

Adaptive immunity started with antigen-presenting dendritic cells. Dendritic cells present antigen to lymph nodes which activates lymphocyte. DCs activate T cells and carry them to the infection site by binding MadCAM1, VCAM1 which are expressed in infectious cells. Intracellular pathogens which are inaccessible to phagocytes proceed to the second stage of the immune system. There are two types of adaptive immunities namely cellular T cells and humoral B cells. Both cells originated from stem cell but develop separately. T cell developed in the thymus whereas B cell developed in the bone marrow.

Before they work, T cells are first activated by antigen-present cells. APCs use CD80 and CD86 to deliver co-stimulatory signals via CD28. Signals received through CD28 activate T cells. Once activated, T cells secrete helper T cells which then stimulate cytotoxic T cells. The activated Cytotoxic T cells kill the cells infected by *Chlamydia* and infect the nearby cells. Helper T cell activate other defensive cells e.g. macrophages, and B cells. Helper T cells and cytotoxic T cells are activated to become effector cells. This process takes place at the lymphoid organs, suited on the surface of antigen-presenting cells. Cytotoxic T cells can also eliminate *Chlamydia* by inducing the targeted cells to kill themselves by apoptosis. Cytotoxic T cell releases pore-forming protein known as perforin to plasma membrane to form a transmembrane channel.

When they activate cytotoxic T cell, helper T cells proceed and activate cellular immunity. Helper T cells develop into T_{H^1} and T_{H^2} respectively. T_{H^1} cells activate macrophage and cytotoxic T cell, while T_{H^2} activates B cell. In general, T cell detects foreign peptides that are bound to MHC proteins. Co-receptors play important role in activating T cells. These co-receptors are CD4 and CD8. CD4 is expressed on helper T cells and are always bound to class II MHC protein. CD8 is expressed on cytotoxic T cell is bound to class I MHC proteins. T cells are the key eliminator for *Chlamydia*. Not only because it targets the microbe, but also because it kills the cells that have been infected.

B cells are other types of adaptive immunity. B cells produce antibodies which neutralize or block antigens, preventing the interaction between antigens and cells. To be activated, B cell binds to MHC class II molecules. MHC class II molecules are recognized by helper T cells and activate them to produce protein that result in B cell proliferation. Once helper T cells are activated, they then stimulate B cell to initiate naïve B cell. On B cell, there are markers which serve as signaling molecules. They include CD19 which is a signal receptor, CD21 which is a receptor for complement.

The activation of B cell by helper T cells alone can not eliminate *Chlamydia* completely. At least signals from CD4 T are required. CD40L binds to the B cell surface molecule CD40; this binding leads to the B cell cycle in the body. The interaction between B cell receptor and CD40L together with IL-4 and other cytokines signal molecules from the T cell lead to the production of B cells. B cells migrate to the lymph node where they start secreting Igs. The most abundant Ig produced by B cell in response to *Chlamydia* infection are IgA which guard the body from being infected again organisms like *Chlamydia*. Presence of IgA in the serum can neutralize *Chlamydia*. Patient that lack B cells can not produces antibodies and therefore can not eliminate *Chlamydia*, patient with enough number of B cells can eliminate *Chlamydia* and these patients are at less of a risk for *Chlamydia* to causes a secondary infection.

Flow cytometry test

Flow cytometry is the test for finding out the level of cytotoxic T cells. Flow cytometry is a method which could be used for analyzing a large population of Tc cells in the patient's blood. Flow cytometry is the best technique for quantifying either CD8 or CD4 presence in the culture. This test can also be used to count MHC peptide binding T cells. To do this test, cytokine-producing cells are incubated in drugs to block secretion. These fixed cells are then cross link the cytokine and are kept inside the cells; treatment with detergent opens the cell membrane to allow antibodies to enter and bind the cytokine. Wash the suspension. Blood sample from the patients that have been vaccinated are used for the analysis of T cell and memory B cells. Positives and negatives are recommended for comparision. For a positive control, the known T cell specific for MHC peptide proteins is used. And for negative controls, something different is used. Negative control is used to regulate antibodies. The suspension is run under flow cytometer. As the cells pass through the detector, the quantity of fluorescence on those cells is quantified and diverted to electronic signals which are logically used for recording or plotting. Fluorescence intensity should be monitored carefully because this is what would determine the amount of markers available in the cells. The higher the fluorescence intensity, the more specific MHC peptide protein the cells have. Cells are then separated by fluorescence – activated cell sorter. Based on fluorescence, it is easy to tell if the vaccine has enough CD4 or CD8 to start cellular immunity.

Test

Due to the effective problems that might occur, this vaccine should not be applied directly to human. It is assumed that side effects may result which could end in death as shown by some animal in which DNA resembles that of human being. The animal that should be used to test the effectiveness of this vaccine would be monkeys. Monkey's genes are closely related to humane gene. Humans and monkey could have the same immune response if they have closely related DNA. So if the monkey's immune system responds very bad to this vaccine, human being response should be the same. If no symptoms are seen on the monkey it means that it will be working fine in the human system. Basically, this study will take about three years because it will involve series of tests. *Chlamydia* is one of the top leading infectors of many young people worldwide. For this reason, researchers are looking for possible vaccine for this pathogen

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