

Flying under the radar. Targeting the Achilles heel of *Treponema pallidum*

Literature Review

Overview and Epidemiology:

Syphilis is caused by the bacterium *Treponema pallidum* and is transmitted through sexual contact, and less commonly, blood contact and trans-placental infection (Zeltser and Kurban, 2004). While it is unclear how long syphilis has been around, it is guessed that *Treponema pallidum*, the pathogen responsible for this disease has been infecting since Egyptian and Roman times (Zeltser and Kurban, 2004).

Humans are the only known reservoir for *T. pallidum* with its major risk groups being unborn children and sexually active adults, but faster rises have been seen in particular types of adults. Syphilis, from 2001 to 2002 has been on the rise after a decade of declining infection, increasing 12.4% from 6,103 to 6,862 cases in the US. Interestingly this rise is seen mainly in the “men who have sex with men (MSM)” population, and further, this also came with a rise in HIV infection. This can be explained by how syphilis can increase the chances of getting HIV by 6-7 folds. In congenital infection, the rate in the US has been decreasing since the 1990's. This is most likely a result of the decrease in secondary infection in women. However, in case of infection, early syphilis can infect a fetus and result in death for the fetus in 40% of the cases. Infection without death can occur if the infection is acquired within 4 years of the pregnancy (Zeltser and Kurban, 2004). Due to these high statistics, creating a vaccine can help decrease the rates prenatal death and HIV infection.

Pathogenesis and the Immune System:

Syphilis goes through 3 stages, primary, secondary, and tertiary. The infection is acquired when *T. pallidum* is allowed to penetrate into intact mucosal membranes through a micro abrasion, and it will cause a small blister called a chancre at the site (LaFond and Lukehart, 2006). This begins the primary stage and quickly enters the secondary phase. While typically the site of inoculation is on the penis in the case of men and the labia or cervix in the case of women, but in the MSM population, they can appear in the anus or oral cavity (LaFond and Lukehart, 2006). Also during the early stage, *T. pallidum* can invade the central nervous system (CNS). While the majority of cases of CNS infiltration are resolved by the immune system, some can still develop symptoms. Early symptoms include fever, headache, vomiting, and stiff neck. The disease can then advance to causing cranial nerve damage, blurred vision and photophobia (LaFond and Lukehart, 2006). Since penicillin is inefficient in clearing a CNS syphilis infection, neurosyphilis therapy is used as soon as any cerebral spinal fluid or any other signs of CNS infection are seen (LaFond and Lukehart, 2006).

In the secondary phase, shortly after inoculation, the organism will travel to a variety of tissue but the primary chancre develops after about three weeks after exposure (LaFond and Lukehart, 2006). When this chancre develops, it induces a delayed hypersensitivity. This symptom is mediated by effector T-cells when the antigen to syphilis is seen. This is what causes the tale-tell signs of syphilis infection in the genitals,

swelling and dermatitis for example. The reason it is delayed is because of how few proteins are located on the surface of the organism, about 100 fold less than most gram negative bacteria, so the hypersensitivity is more important than the humoral immune response which is insufficient in clearing the infectious organisms (Zeltser and Kurban, 2004). At this point, antibodies will begin to circulate. It is during this phase that the symptoms such as sore throat, muscle aches, malaise, and weight loss occur because the organism has now become system (LaFond and Lukehart, 2006). The latent stage of infection occurs after about three months of secondary phase activity and the host will become asymptomatic. In 25% of cases, infection can also reenter secondary status. While a serological test will yield positive results in this phase, sexual transmission is unlikely (LaFond and Lukehart, 2006).

Tertiary syphilis is rarely common with the use of antibiotic therapy. However, if left untreated, the infection will reach this stage in about 20 to 40 years after the initial inoculation. There are several ailments associates with tertiary syphilis; these are Gumma, Cardiovascular syphilis and late neurological complications (LaFond and Lukehart, 2006). In Gumma, skin and bone legions will become necrotic. These destructive lesions do not often contain the organism and can also occur in the heart, liver, brain, upper respiratory tract and stomach. While the lesions do not heal spontaneously, antibiotic treatment will often eliminate the legions and no serious complications develop. In cardiovascular syphilis, damage to the ascending aorta causes aortic regurgitation. While this complication only occurs in ten percents of cases, if left untreated, this will result in death, and it is thought to be responsible for the majority of syphilis-related deaths before the discovery of penicillin (LaFond and Lukehart, 2006). Finally, if left untreated CNS infection can advance to mining vascular syphilis. Symptoms include personality change, loss of memory, and hallucinations.

T. Pallidum and Its General Biological Characteristics:

T. Pallidum belongs to the family of microorganisms called *Spirochaetaceae* so named for their spiral shape. This organism is easily seen under dark field microscopy. The organism has a cytoplasmic membrane, which is then surrounded by a loosely associated outer membrane with a thin layer of peptidoglycan to provide structure. On the surface, the organism has endoflagella that allows it to move in a corkscrew motion (LaFond and Lukehart, 2006). To survive, the organism lives by taking up macro molecules using transporter homologs (proteins that mimic the actual transport proteins that host cells use to take up molecules). However, the organism has no homolog proteins, so it is still a mystery of how the molecules taken up get through both layers of the membrane (LaFond and Lukehart, 2006).

Unlike most bacteria, *T. pallidum* has very limited means for metabolism. It can carry out glycolysis, but it lacks other methods, such as an electron transport chain (LaFond and Lukehart, 2006). Another thing that *T. pallidum* does not produce is cytotoxins. Therefore, the virulence associated with syphilis is thought to be a result of the adaptive immune and inflammation responses to infection (LaFond and Lukehart, 2006).

Treatments and Vaccines for Syphilis:

At this time, penicillin is the drug of choice when dealing with a syphilis infection. By binding to the proteins that aid in cell wall synthesis, penicillin can disrupt cell wall formation, killing the organism (42).

In the case of a penicillin allergy, oral doxycycline or tetracycline can be administered. Also, another antibiotic, azithromycin showed promise in the 1990's when it was invented. This was an even more attractive than penicillin because it could be administered orally and in a much smaller dose than penicillin which must be administered intramuscularly. However it has been shown that a single point mutation in the genome of *T. pallidum* in the r3S rRNA gene will allow the organism to become resistant. While azithromycin can still be used, in areas that are affected by resistant strains of *T. pallidum* the patients must be monitored very closely in order to pursue better treatment against resistant strains (42).

While syphilis is normally not life threatening illness, the lesions that it produces are easy gateways for HIV to enter. Also, because macrophages and lymphocytes are localized in the lesion and will fall easy prey to a sudden attack of HIV during inoculation. Further, there is also evidence to support that *T. pallidum* actually aids in the infection and progression of HIV. This is done by the activation of TpN47 lipoprotein, this activation up regulates the replication of the HIV genome in HIV infected monocytes. Therefore, by preventing the spread of syphilis the number of new HIV cases and deaths related to HIV would drop (42).

Sadly though, there is currently no reasonable vaccine available for syphilis infection. A vaccine regiment that had shown any progress in animal models was a sixty injection series of γ -irradiated *T. pallidum* organisms. Also, when subjects were infected with *T. pallidum* molecules did show signs of protection against a challenge of pathogen but the protection, even with high antibodies, were only partial. Therefore, it is clear that an effective vaccine would have to entice a higher cellular immune response in order to properly clear the infection (43).

Innate Immune Response:

Once inoculated by the vaccine, the body would detect the foreign antigen (Tp92) and innate immunity would start to take place. The macrophages are immediately activated and release cytokines to induce the local dilation of blood capillaries, which increases blood flow and causes the skin to warm and redden. They also change the adhesive properties of the vascular endothelium, inviting white blood cells to attach to it and move from the blood to the inflamed tissue. Complement is activated when plasma protein from the liver, activated by IL-6, attach to the Tp92 antigen. Once activated complement causes opsonization of the pathogen. Macrophages and neutrophils have receptors that bind complement; this recognition facilitates the phagocytosis of the pathogen. Macrophages and dendritic cells then process and present the pathogens antigens by MHC class I and II molecules. Once the dendritic cells move to the secondary lymphoid tissue and activate the T-cells, the adaptive immune response begins.

In our approach, innate immunity would be boosted by the virosome which would help deliver the antigen to the antigen presenting cells.

Adaptive Immunity:

When an infection occurs, there is no such thing as a timeline between innate and adaptive immunity. The immune system is dynamic, and everything interacts in the blood, as well as at the infection site. In a typical bacterial encounter, innate immunity starts fighting the pathogen at first, but if it does not get eliminated by macrophages, then infection can occur, which causes stimulation of adaptive immunity. Dendritic cells can actually act as a bridge between innate and adaptive immunity by presenting specific antigens to T cells in the lymph nodes to stimulate them, so they can perform their specialized functions (Reis, Sher, and Kaye, 1999).

In the case of syphilis, the scenario is not as typical. *Treponema pallidum* is a pathogen that has very low levels of surface proteins, so this helps evade the immune system. With our vaccine, we could be providing a strong enough stimulus with the addition of the adjuvant and the selected *T. pallidum* antigen Tp92 to enhance the response of the adaptive immunity to the antigen in the vaccine.

The mechanism by which our vaccine would succeed would begin by having this protein have a specific region which will act as an epitope that will bind to the BCR. B cells will essentially engulf it and end up presenting it on their MHC II, so that maturation of the T cell will occur. At the same time the cytokines released by the T cell will induce the B cell to multiply and differentiate into plasma cells to produce large amounts of antibody. The maturation of B cells will cause changes in gene expression to go from IgM to IgG anti-Tp92 antibodies. Since we expect to have a T cell- dependent activation of B cells, memory cells will be produced, and this will be one more step to create immunity against *Treponema pallidum*.

Although humoral immunity will be acquired, literature suggests it will not be enough to fully protect against this pathogen. (Bishop and Miller 1976) For this reason we are giving the adjuvant in conjunction. This will promote a strong enough signal on the immune system so that macrophages can efficiently engulf the opsonized *T. pallidum* pathogen if ever present on an immunized individual.

Vaccine Description

As mentioned in the literature review, the bacterium *T. Pallidum* has very few antigens on its surface. However, one of them is the antigen Tp92. This antigen stimulates the generation of antibodies that aid in the opsonization of the pathogen. It has been postulated that the main pathway to clear a syphilis infection is through the opsonization and engulfment of the pathogen using Tp92 antigen (Cameron et. al).

The vaccine would consist of the protein Tp92, a virosome, and alum which would be administered intramuscularly. We choose this composition because of the properties of each. The virosome is virtually the influenza virus-envelop, but without the influenza genome. A capability of the virosome is that since it contains Hemagglutinin and neuraminidase (influenza envelope surface proteins), they help amplify fusogenic activity and therefore facilitate the uptake of antigen into the antigen presenting cells. (Previon Biothec Ltd. / www.previon.com/index.php?page=6510n). Alum was also chosen for this vaccine since it has been shown to boost the immune's system capacity to fight pathogens. How alum works isn't fully understood, but it is believed that it plays a

role in the priming of B cells. Priming is necessary for B cells to become plasma cells and secrete antibodies. This addition of the adjuvant and virosome will cause a very strong antibody titer that will prevent *T. pallidum* infection. This titer will eventually decrease if there is no exposure to the pathogen, which is why this vaccine will have to be reinforced constantly.

The protein Tp92 would be obtained by isolating RNA from *T. pallidum*, which would then be converted into cDNA. This cDNA can be amplified using Tp92 specific primers. The amplified product can then be inserted into a plasmid vector (Invitrogen pCR T7/CT) which will then be isolated and inserted into *Rosetta II E. coli* (Novagen Inc.), a protein expression cell line. *E. coli* will be induced to express the protein since the plasmid in it contains the Tp92 gene and also has a lac promoter. This promoter can be turned on and transcription of the gene will occur by using IPTG, a lactose analog, which will bind to the lac repressor and never come off. This allows constant transcription of the gene translating into the Tp92 protein. The protein will be purified and be combined with the virosome and alum, which will be put together for the vaccine.

The type of immunity this vaccine will induce is humoral. The antibodies that would be the most important would be IgA and IgG. The IgA would be located in the mucus membranes of the genital tract where initial infection occurs. Immunoglobulins would be important in opsonizing pathogens before they spread. Also, it has been seen that the Tp92 protein can aid in the hydrogen bonding that the pathogen uses for attachment, suggesting that the opsonizing antibodies will prevent attachment of *T. pallidum* to cells.(Cameron et. al).

Immunity Assessment

ELISA:

The ELISA test would be used to measure the amount of antibody that a subject vaccinated would have. The main targets for the assay will be IgG and IgA antibodies since they will be the ones located where the injection will be. Since we are looking for an analysis of how much antibody is being produced, we will use a competitive inhibition assay. This test will be performed just before vaccination and then about two weeks after to allow time for B cells to produce antibody. The assays should show a marginal increase after vaccination if the antigen is truly stimulating the formation of antibodies.

ELISPOT:

An ELISPOT will be used to assess whether there is any T cell activity or not. First, an anti-IL-5 antibody will be bound to a well and then plasma will be added. The purpose of this is to see any activity of T cells producing IL-5, which can be assumed is being produced to cause isotype switching on B cells to start producing IgG. After the serum has been incubated, T cells will secrete IL-5 which will bind to the antibody, then another antibody specific for IL-5 containing a fluorescent tag will be added and will bind to the cytokine present. The end result of this will be visible dots that can be easily interpreted. If there is color in the well, that means that there is T cell activity.

If this test was performed before vaccination, and then 15 days after vaccination, it should give a significantly different result. It cannot be assumed that the T cell activity is Tp92 specific from doing this test, but it can be assumed that it is specific due to the cytokine production after vaccination.

Opsonization Assay:

Finally, in order to test to see how well the vaccine works by promoting antibody production for *T. pallidum*, primates would be infected to see if illness follows. Primates would be the best candidates for this test because of how similar they are to humans. Not only could it be possible to see how a human-like immune response would behave after immunization, it would also demonstrate hidden side effects that a vaccine may have on primate tissue.

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