

A Multi-Component Plasmid Vaccine for *Coccidioides immitis*

Part 1: Literature Review:

Coccidioidomycosis, also known as Valley Fever, is a common fungal infection endemic to southern Arizona, central California, southern New Mexico, west Texas, and areas of north eastern Mexico and South America (Kirkland et al 1996). Valley Fever is caused by the fungal agents *Coccidioides immitis* and *Coccidioides posadasii* (Delgado 2003). In endemic areas, *Coccidioides* thrives in alkaline soil high in calcium. This fungus is able to reproduce in areas with ample moisture and temperatures greater than 30 degrees Celsius (Delgado 2003). Even though the spores are continually present in the soil, the risk of infection increases during dust storms and activities that disrupt the earth such as earthquakes, floods, tornadoes, construction and farming.

Coccidioides is a dimorphic fungus that exists as a mycelium in the external environment. However, it is distributed unevenly throughout the soil and is more prevalent in some areas than others. The backbone of the fungus is its hyphae which contain the infective arthroconidia. When the earth is disrupted, the arthroconidia are freed from the hyphae into the air and pose a threat to humans as the risk for fungal inhalation increases. Once inhaled, these arthroconidia enter the lungs and undergo a significant morphological change within 48 to 72 hours. The arthroconidia form spherules that colonize the lung tissue and then rapidly divide to form endospores. Hundreds of thousands of endospores are produced with the ability to develop into mature spherules filled with new endospores. These spherules can then burst and rapidly proceed through this continuous cycle. The virulence of the disease is thus increased because each spore is able to colonize the lung. If enough colonization occurs, however, the disease can disseminate and invade other portions of the body such as the central nervous system, bone marrow, internal organs and the skin. Signs and symptoms of dissemination are meningitis, a low grade chronic cough, calcifications and lesions on lymph nodes, myalgia, miliary disease, bone and joint infection, skin disease and soft tissue abscesses (Kirkland and Freer 1996).

Even though the fungus can infect any person or animal, certain populations are more at risk than others. At the greatest risk are the immunosuppressed, such as those persons with HIV, AIDS, those that have received organ transplants and pregnant women in their third trimester. Also in danger of increased susceptibility are people of African American and Filipino descent. For supposed genetic reasons, these individuals have a chance ten fold greater to contract a serious Coccidioidomycoses infection than other sectors of the population (Kirkland and Freer 1996). These individuals, along with people working in construction, farming or other professions with risk of dust exposure, are at an increased risk of infection.

There are many treatments available for Valley Fever. Until recently, Amphotericin B was one the most common drugs prescribed for Coccidioidomycosis. This drug binds to the ergosterol in the fungal cell membrane resulting in leakage of potassium, magnesium, sugar, and other metabolites from the intracellular space through the damaged cell membrane. The loss of these essential cell components results in cell death. However, current research and use of the drug has proven it to be highly toxic. Common side effects include myalgia, fever, and chills and can culminate into nephrotoxicity, causing an increase in erythropoietin and normachromic anemia.

Amphotericin B is also administered intravenously and can cause thrombophlebitis, or venous blood clots, at the site of infusion. In an attempt to avoid the adverse side effects of Amphotericin B, a group of azole drugs has become a more favorable treatment. As a group these medications work to inhibit cytochrome P450 14 α -demethylase which is involved in the biosynthesis of sterols. Inhibition of this pathway keeps the cell from developing a solid cell wall. Ketoconazole, fluconazole and itraconazole are the three that are most prevalently used. These medications can be given orally or intravenously and treatment usually lasts about a year. However, if the infection is chronic and disseminated, treatment may have to be life long. This is especially true of patients with compromised immune systems. Side effects do exist with these drugs, but are less common making them a safer and more preferable medication. (Dr. Fungus, 2007)

In recent years, research has focused on developing a vaccine for this problematic illness. A killed spherule vaccine has been tested in mice and some other animal species. However, in human trials the amount of killed spherule that could be injected without an unfavorable immune response was very low. As a result, this makes the vaccine unsafe for human use. A 7-3-5-5 cDNA vaccine was created, but it has not been investigated at this point in time (Ivey 2003). It has also been discovered that the whole organism is not an ideal vaccine candidate as the cell wall is almost completely non-antigenic for T lymphocytes.

However, many of the proteins in the cell wall of *C. immitis* are able to stimulate a protective T cell-mediated immune response. Research evidence demonstrates that cytokines such as IFN- γ and IL-12 secreted by Th1 cells are needed to achieve active acquired immunity. Upon entrance of an immunocompetent host, the arthroconidia and endospores of *C. immitis* are phagocytosed by the alveolar macrophages; however, fewer than 1% of these cells are killed. It is thought that the fungi are able to survive intracellularly because of inhibition of the phagosome-lysosome fusion, a technique used by many intracellular parasites to avoid the antimicrobial effects of phagocytes (Cox and Magee 2004). As a result, a humoral immune response has not been found to be efficacious in the eradication of this disease and, in turn, the focus of vaccine research is primarily concerned with enhancing the adaptive immune response.

Part 2: Description of Vaccine

The vaccine will be a recombinant PVR 1012 plasmid that will be taken up by dendritic cells, antigen presenting cells, B-cells and other muscle cells when entering the body (Kimball 2007) & (Jiang 1999). The PVR 1012 plasmid is purchased commercially from Vical Inc. A cDNA 7-3-5-5 clone, IL-12 gene, Ag-2/PRA, spherule outer wall glycoprotein fraction (SOWgp) and a cDNA urease enzyme will also be incorporated into this plasmid. These components are explained below.

cDNA 7-3-5-5 Clone

The cDNA 7-3-5-5 clone is a genetic sequence fractionated from a cDNA library specific to *C. immitis*. In order to obtain this 7-3-5-5 clone, researchers utilized cDNA expression library immunization or ELI. Then, 800-1000 genes from a cDNA library of spherule-phase *C. immitis* DNA was fractionated first into 10 pools. Each pool was tested for immunogenicity for the arthroconidia of the fungus and Pool 7 was found to be the most effective. This pool was further fractionated into 5 smaller sub-libraries of approximately 60 genes each. These pools were again tested for immunogenicity and the process was continued until the 7-3-5-5 pool was determined to be the most protective

cell wall protein gene against the arthroconidia of *C. immitis*. (Ivey 2003). Specifically, the cDNA of *C. immitis* is being used instead of genomic DNA due to the smaller size of cDNA clones and the ability for higher specificity to its arthroconidia.

IL-12 Gene

The IL-12 gene is inserted into the plasmid for its ability to stimulate the production of IFN γ , TNF α and IL-2. The most important of these three cytokines is IFN γ ; a primary cytokine responsible for transforming naïve T-cells into Th1 cells. IL-12 also stimulates the activity of natural killer (NK) cells and antigen presenting cells such as monocytes, macrophages and B cells (Jiang 1999). NK cells and Tc cells are also initiated by IL-12 to enhance their proliferation and cytolytic activity. These cells can also stimulate B cells with antigen presentation from the plasmid.

Antigen 2 Proline-rich Antigen

The antigen 2 proline-rich (Ag2/PRA) antigen has been previously isolated from the *C. immitis* spherule. This particular antigen assists in IFN γ production and stimulates the production of anti-Ag2 IgG antibodies by B cells, thus inducing a humoral response of preventative memory B cells towards *C. immitis*. Ag2 also promotes the creation of *C. immitis* CD4⁺ and CD8⁺ T cells. Studies support that the use of Ag2 cDNA in conjunction with the IL-12 gene, as mentioned above, has “induced a significantly higher level of protection in mice than either Ag2 or IL-12 alone” (Jiang 1999). Therefore, the combination of these two genes is being used in the plasmid.

Urease

Recombinant urease, created from urease DNA isolated from the fungal cell wall of *C. immitis*, is used to elevate the levels of Th1-type cytokine gene expression such as IFN γ (Li 2001). The increased production of IFN γ promotes a cellular immune response eventually leading to the production of memory cells against *C. immitis* antigen.

Spherule Outer Wall Glycoprotein Fraction (SOWgp)

SOWgp is a specific surface antigen on spherules of *C. immitis*. It stimulates peripheral monocytic cells (PBMC) to secrete IFN γ . This cytokine is indicative of a Th1 response that is capable of eliciting both humoral and cellular immunity (Hung 1999).

Adjuvant CpG-ODN

The adjuvant CpG-ODN is used in this multi-component vaccine because it causes the maturation of dendritic cells into professional APC or antigen presenting cells. These APCs trigger a Th1 immune response by stimulating interferon gamma (IFN γ) and interleukin 12 (IL-12) (Chaung 2006). These cytokines, as described above, activate Th1 cells.

A multi-Component Vaccine

This specific type of DNA vaccine was chosen because studies have demonstrated that a T-cell mediated immune response, as opposed to a B-cell mediated response, is most effective against a *Coccidioides spp.* infection (Cox 2001). However, there are a few possible problems concerning this type of vaccine. First, the recombinant plasmid may be comprised of too many genetic components that, when combined, could potentially kill the host. For example, over-expression of IFN γ may cause anaphylactic shock in the host as inflammatory responses of innate immunity overreact upon inoculation. In addition to shock, the vaccine may not cause an effective immune response at all because IFN γ may stimulate an immediate T cell response but induce insufficient memory T cell formation. This problem would be addressed by monitoring

the potency of the T cell response and any negative side-effects noted in the vaccine recipients.

Part 3: Description of Immunity Assessment

This vaccine will be given intramuscularly in order to prevent a premature immune response in the mucosal defenses such as IgA activation. If IgA is activated the components of the vaccine will be opsonized to prevent them from entering the lymph system and systemic circulation needed to stimulate the T cell response. IgA opsonization is a common problem associated with intranasal vaccines. A more effective immune response would occur if the plasmid is inoculated into muscle tissue and then taken up by the muscle cells.

Testing Procedures

The procedures for testing this vaccine will consist of one group of individuals with probable exposure and another group of individuals naive to *Coccidioides*. Both pools are comprised of one hundred participants each. All volunteers will be deemed healthy by a physician. These individuals will be tested for antigen-specific T-cells using flow cytometry prior to the commencement and throughout the study to test for exposure to *C. immitis*.

Flow Cytometry

Flow cytometry is used with an MHC class II tetramer specific to SOWgp. SOWgp as previously mentioned is an antigen found in the cell wall of *C. immitis*. This cell wall protein is attached to a fluorochrome, one such as Fluorophore labeled Streptavidin, and then exposed to MHC class II tetramer. The participant's serum sample is then introduced into a solution with the MHC II and fluorochrome-labeled SOWgp antigen complex. Upon the addition of a patient's serum sample, if present, the CD4 T cells specific to *C. immitis* will bind this MHC tetramer complex. The sample will then be passed through a flow cytometer which will quantify the number of fluorochrome-labeled T cell MHC class II complexes.

Description of Subject Population

The "naïve" group will consist of one hundred individuals with no expected prior exposure to Valley Fever who have committed to relocate to an endemic area. The "exposed" group of participants will consist of one hundred inhabitants of areas endemic for Valley Fever. "Exposed" participants will be selected from areas such as southern Arizona and California for this specific study and will include construction workers, geologists, and others who have frequent exposure to endemic soil. These subjects will also be tested for SOWgp antigen-specific T-cells before beginning the study, in anticipation that these individuals have had natural exposure and pre-existing immunity towards *C. immitis*. This "exposed" population will serve as a juxtaposition of the other group of "naïve" individuals.

Immunity Assay

The immunity assay will occur exactly ten days after the initial vaccination to determine the efficacy of plasmid integration into somatic immune cells. The second injection will be given exactly fourteen days following the original and will also be followed by flow cytometry. This study will continue over the period of one year and will include flow cytometry assays at the three, six and nine month intervals in order to help determine the efficacy for long term immunity of this specific vaccine type.

Establishing both of these groups will identify the presence/increase in SOWgp antigen-specific T cells within hosts who were originally positive or negative for these T-cells specific to *C. immitis*. After vaccination, this test will also help assess the duration of immunity for positive individuals living in both endemic and non-endemic areas by quantifying the amount of SOWgp antigen-specific T cells present.

Part 4: Citations

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