

**Formalin-killed spherule (FKS) Vaccine Implemented With an ISCOM Adjuvant
against *Coccidioidomycosis immitis***

Literature Review

Valley Fever is a disease caused by the fungus *Coccidioides immitis*. *Coccidioidomycosis* is a systemic mycosis initially affecting the lungs. Hematogenous spread of cocci leads to infection of skin, bones, joints, lymph nodes, adrenal glands, and central nervous system (Stevens, 1995). Some early symptoms dogs show after inhalation can be coughing, fever, weight loss, lack of appetite, and lack of energy.

Cocci are prominent in areas with arid climates, alkaline soils, hot summers, and few freezings. Outbreaks of coccidioidomycosis usually occur after earthquakes, dust storms, and any disruption of the earth where the spores reside (Drutz, 1978). Anyone who is outside a lot is more prone to infection. Some examples of at risk people are construction workers and outside animals. Infection is caused by inhalation of the airborne arthroconidia, the spore form of cocci. Once inside the lungs, the arthroconidia transform into spherules. The tissue around the spherules will have a granulomatous inflammation reaction. Rupture of spherules causes acute suppurative reaction (Pappagianis, 1998).

Like most diseases the body can have a protective immune response to valley fever in the form of memory cells. Memory cells form when the body is first introduced to the infection and produces antibodies to it. These memory cells also last a long time in the body just in case the disease comes back then they recognize the infection and get rid of it fast. There are test that are done to see if an animal has been exposed to valley fever. This testing is done to see if there are any antibodies made to the fungus that

causes valley fever. If the test is positive it shows the animal has been exposed to the disease and further test are done. Then next test that is done is a titer test, which measures how much antibody is being produced in the animal. X-rays can also be done to detect the cocci. An affected animal will have light patches in the lung area indicating and infection from Valley fever (Drutz, 1998).

Many animals can acquire Valley fever but dogs are most affected by it. Valley fever is usually asymptomatic in about 70% of dogs infected. The rest of the infected dogs can show acute symptomatic symptoms. When the disease travels outside the lungs it is a more serious form of the disease and can cause systemic or dissemination. Some signs of this can be lameness or swelling of the limbs, seizures, and eye inflammation. Treatment for this disease has so far been to use ketoconazole, itraconazole and fluconazole. Amphotericin B is another treatment for this disease and can be used for the management of meningitis. Some animal experiments suggest that caspofungin, sordarins, and nikkomycins are also treatments of this disease. Therapy for this disease should be at least 1 year (Galgiani, 1993).

When this pathogen is grown at 25 degrees Celsius hyphae and arthroconidia are produced. The hyphae are septate and thin. The arthroconidia are thick-walled, barrel-shaped and about 3-6 Mm in size. At 37 degrees Celsius the spherules can be observed. They are large, round, thick-walled spherules about 10-80 um in diameter. They are filled with endospores, which are 2-5 um in diameter (Pappagianis, 1998).

This pathogen was chosen due to the increasing number of cases. The amount of cases is rapidly rising due to the growing population in the southwest. This population growth causes new developments and this disruption of the soil causes more spores to be

released into the air. Also, because this disease mainly occurs in the southwest and this is where we live it affects our own personal lives. Our population we are targeting is animals.

Description of Vaccine.

Coccidioidomycosis is a cause of serious illness and even death in dogs. And although dogs account for the majority of coccidioidomycosis in animals, little attention has been devoted to understanding this disease in the canine species.

Canine coccidioidomycosis is caused by two genetic subgroups, *C. immitis* and *C. posadasii*. *C. immitis* is geographically limited to California's San Joaquin Valley, whereas *C. posadasii* formally known as "non-California *C. immitis*" is found in the desert southwest and Mexico. No difference has been detected in antigenicity, virulence, or morphology between these two coccidioides species. Data from experimental immunization of laboratory animals with genetically different isolates indicate that immunization can be accomplished with exposure to a single isolate of *C. immitis* (Pappagianis 2001). Yet since the incidence of *C. immitis* is higher in the desert southwest region, the vaccine will be tailored specifically to the *C. immitis* subgroup.

The importance of a vaccine against *C. immitis* relies on the fact that this pathogen contains a variety of components that make it antiphagocytic and consequently increase its pathogenicity in the canine host. The resistance towards phagocytosis is due to several components of the pathogen's cell wall in the conidial phase, which inhibit phagosome-lysosome fusion (Cox and Magee 2004). The cell wall also provides a substrate that reacts with the destructive enzymes of neutrophils and other phagocytes, counteracting the lethal effects of these enzymes. It is also known that in the spherule

form, the pathogen possesses certain proteases that are able to digest antibodies and opsonins produced by the host immune system, further contributing to the organism's pathogenicity (Immunol, 1987).

A second concern about the pathogenesis of *C. immitis* is the lack of a recognizable toxin. Unlike other bacterial or fungal pathogens, *C. immitis* does not possess a known toxin. This can be a problem because without a recognizable toxin, it is very unlikely that the humoral immunity be activated at all. In previous research, it has been shown that antibodies can be detected during a *C. immitis* infection, but the contributions of such antibodies are seen as minimal. *C. immitis*-specific antibodies are used more often as a diagnostic tool, where high antibody titers are associated with worsening of the infection and dissemination of disease (Immunol, 1987).

As with the development of other fungal vaccines, the threat of pathogen survival within the host must be addressed. It has been noted that *C. immitis* may remain viable for years in the host postinfection, and upon reactivation cause a recurrence of disease. A vaccine to combat coccidioidomycosis must prevent the establishment of a dormant state within the naïve canine host and protect against reactivation in those who have been infected (Deepe 1997). *In vivo* *C. immitis* is seen as a spherule, which represents the pathogen's parasitic phase (Kirkland et al 2006); thus whether a new infection or a reactivated infection, the spherule will be present. If used to initiate protective immunity the spherule would promote the elimination of new and reactivated infection.

Therefore for the development of the new vaccine against *C. immitis* infection, a formalin-killed spherules (FKS) was chosen as the antigen. Non-viable preparation of the spherule with formalin treatment will ensure added safety in administering the

vaccine by inhibiting its replication within the host. Previous research has shown that experimental animals have been successfully vaccinated with formalin-killed spherules (Kirkland et al 2006); therefore the use of FKS in a canine vaccine is plausible.

In order to produce a more efficient and specific immune response against *C. immitis*, a recombinant ISCOM adjuvant will be used in conjunction with FKS. The recombinant ISCOM adjuvant will contain a deglycosylated protein isolated from the spherule's cell wall of *C. immitis* (Galgiani., 1993). The recombinant ISCOM, like the regular protein virus-containing ISCOMs, is going to load peptides and protein into the cell cytoplasm with the minimal toxicity, but with the difference that the recombinant ISCOM will stimulate both class I and class II responses in T lymphocyte. As a result, the immune response obtained by the vaccine will be much broader and efficient, stimulating the activation of the innate, cellular and humoral immunity (Parham, 2005).

Intranasal delivery of the FKS-ISCOM vaccine will likely produced the desired immune response by mimicking the natural route of infection with coccidioidomycosis, by way of the respiratory mucosal surfaces. Despite involving local immune response at the site of entry, intranasal vaccines usually tend to be less traumatic form of vaccination due to the absence of needles.

Innate Immunity

Upon intranasal delivery of vaccine, the first response of the body is directed by the innate immune system, specifically by the mucosa-associated lymphoid tissues (MALT) in the respiratory system. The presence of the FKS antigen will activate the alternative complement cascade through zymosan and other surface molecules on the fungal cell wall. Complement will opsonize the FKS to increase macrophage

phagocytosis and stimulate the inflammatory response. Ultimately, the alternative complement cascade will result in the formation of a membrane attack complex (MAC), which will insert itself into the membrane of the FKS and initiate osmotic lysis.

Macrophages that have bound and engulfed FKS will process and present FKS peptides on Class I MHC and produce and release cytokines IL-1, IL-6, IL-8, IL-12, and TNF α . The release of IL-1, IL-6 and TNF α initiate the inflammatory response, which recruits other leukocytes to the site of infection, stimulates blood vessel dilation, and signals the endothelial cells to express more cell adhesion molecules (CAMs). CAMs along with the dilation of blood capillaries allow leukocytes that arrive at the inoculation site to stick and move between the cells into the tissues. IL-1, IL-6 and TNF α signal the liver to release acute phase proteins that opsonize the FKS for enhanced phagocytosis and further, signal the liver to produce mannose-binding lectin (MBL) which will initiate the MBL complement cascade. Dendritic cells that have taken up FKS are signaled by IL-1, IL-6 and TNF α to migrate to lymphoid tissue for the presentation of antigen to T lymphocytes.

IL-12 release will activate natural killer (NK) cells. NK cells will bind to altered Class I MHC with FKS peptide present on macrophages. These macrophages become target cells, and cell binding will signal the NK cells to kill; releasing perforins that form pores in the target cells membrane and granzymes that induce apoptosis.

Cellular Immunity

After inoculation with the formalin-killed spherule vaccine containing an ISCOM adjuvant (FKS-ISCOM), cellular immunity on dogs starts to develop, activating both cytotoxic T cells and Helper T cells. During the activation of cytotoxic T cells (CD8 T

cells), the formalin-killed spherule from *Coccidioidomycosis immitis* is phagocited by macrophages, dendritic cells and other antigen presenting cells by the immune system. After uptake of the FKS-ISCOM, the antigen is broken down into smaller peptides by a proteasome. Then the peptide is transported into the endoplasmic reticulum by TAP. The peptide then binds to MHC class I molecule, and it is presented on the surface of the antigen presenting cells, more likely on the surface of dendritic cells. After expression of antigen on the cell surface, dendritic cells migrate to the secondary lymphoid tissue where it presents the FKS-ISCOM antigen to naïve T cells. Naïve CD8 T cells recognize the FKS-ISCOM antigen presented on MHC class I molecule and it is activated by a strong co-stimulatory signal provided the binding of the CD28 ligand to the B7. Such co-stimulatory signal induces the release of cytokine IL-2, which stimulates the proliferation and differentiation of CD8 T cells. Once activated, cytotoxic T cells go to site of infection, identify infected cells and eliminate infected cells by *C. immitis* through the use of perforins and granzymes (Parham, 2005).

In a similar pathway as CD8 T cells are activated, Helper T cells (CD4 T cells) activation starts with the uptake of FKS-ISCOM antigen by macrophage and dendritic cells. Then the FKS-ISCOM complex is broken down into peptides in the phagolysosome and binds to MHC class II form the cell's vesicle. Then the FKS-SCOM antigen is presented on dendritic cells surface and migrates to the secondary lymphoid tissue. Naïve CD4 cells recognize the antigen presented on MHC class II molecule and it is activated by a strong co-stimulatory signal provided by the binding of the CD28 and B7 ligand. Activation and proliferation of helper T cells results in to types: Th1 which leads to the enhancement and aid of the innate immune response. Activation and proliferation of Th1

cells by the FKS-ISCOM vaccine induce the secretion IFN- γ and CD40 ligand which enhance the phagocytosis of *C. immitis* by macrophages, TNF- α which increase the amount of adhesion molecules produced by macrophages and therefore produce a better inflammatory response. Also IL-2 is secreted by Th1 T cells are secreted to induce further proliferation of T cells, and CXCL2 which induce macrophage agglomeration at site of infection caused by *C. immitis*. The second type of helper T cells is the Th2, which is involved in the development of humoral immunity (Parham, 2005).

Humoral immunity

B cell activation requires two signals to initiate its response. The first signal comes from the direct interaction of the FSK-ISCOM antigen provided by the vaccine with the specific BCR. Naïve mature B cells travels to the secondary lymphoid tissue and encounter the FKS-ISCOM antigen. Once the FKS-ISCOM antigen is bound to the specific BCR, it is internalized, degraded and combined with MHC class II molecule. Th2 cells provide the second signal towards B cell activation. B cells are assisted by Th2 cells, through the communication of the MHC class II molecule and the co-signal provided by the binding of the CD40 and B7 ligand. Once such communication between B cells and Th2 cells have been completed, B cells undergoes somatic hypermutation, in which several immune complexes are displayed on follicular dendritic cells in order for B cells to recognize the correct FKS-ISCOM antigen. After such display, B cells undergo a selection process in which B cell with high affinity for the FKS-ISCOM antigen are accepted for its proliferation. Effector molecules secreted by Th2 cells include IL-4 and CD40 ligand, which initiates proliferation and clonal expansion of B cells. IL-5 and IL-6 which induce differentiation of plasma cells (Parham, 2005). Once B cells are mature, the

production of IgM and IgD antibodies initiates. But because *C. immitis* produce a pulmonary infection on dogs, B cells switch its isotpye to IgA. IgA antibodies produced in the mucous membrane of the respiratory tract will serve two main purposes: to opsonize the *C. immitis* pathogen and neutralize the pathogenic spherules produced by *C. immitis*. After elimination of the pathogen, B cells undergo a process of memory cells generation in the secondary lymphoid tissue (Galgiani, 1993).

Description of Immunity Assessment

The intranasal vaccine should be administered once at approx. 16 weeks of age. Ideally, the dog should be fully vaccinated by this time and likely to be going outside more and more. As long as the dog is staying in an endemic area, the vaccine should only need to be given one time, as the animal should continue to be exposed to the antigen in the environment. However, dogs that leave the endemic area for an extended period of time, a year or more, should be re-vaccinated before returning to boost immunity.

An initial trial of ten fully vaccinated (rabies, Da2PP, and Bordatella) puppies at sixteen to twenty weeks of age will have the intranasal vaccination administered. In order for puppies to be eligible to participate they must meet the age and vaccination requirements and test negative for coccidioidomycosis. Serum samples will be taken at seven, ten, fourteen, twenty-eight, and sixty days after the administration of the vaccine. Each sample will be tested for IgM and IgG anti-coccidioidomycosis antibodies using a paired titer after the initial sampling. The IgM will test for the initial response to the antigen and IgG will test for long term antibodies. Antibodies will be tested for using an

ELISA test performed by IDEXX laboratory. An increase in T-cells will be tested for using cytometry to detect antigen specific CD4 and CD8 T-cells.

After the sixty day sampling, a high dose of live coccidioidomycosis will be administered intranasally and serum samples will again be taken at seven, ten, fourteen, twenty-eight, and sixty days using paired titer tests. The serum samples will be tested for anti-coccidioidomycosis IgM and IgG antibodies using ELISA testing performed at IDEXX laboratory. An increase in T-cells will be tested for using cytometry. X-rays will be taken of any dogs that become symptomatic for coccidioidomycosis; the dogs will be treated with a regimen of anti-fungal medication, such as Fluconazole.

A possible problem with this kind of vaccination will be that testing for coccidioidomycosis will be made more difficult with low titers becoming a gray area for diagnosis of active infection. High antibody titers along with positive X-ray studies and ongoing symptoms will be needed for clinical diagnosis. In addition, there will probably need to be a lag period after vaccination in which high coccidioidomycosis titers should be ignored as effects of the vaccine. This time period will be defined from when the titer count levels out in the serum samplings.

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