Development of Valley Fever vaccines and diagnostic tests

These projects are being done in collaboration with Dr. John Galgiani and the Valley Fever Center of Excellence http://www.vfce.arizona.edu/

Valley Fever

Valley Fever, or Coccidioidomycosis, is a human pulmonary infection that normally manifests itself as a flu-like disease caused by infection by the dimorphic fungal pathogens *Coccidioides immitis* and *Coccidioides posadasii*. Both pathogens grow in the soil of semi-arid regions of the US desert southwest as well as parts of Central and South America. It is estimated that between 25,000 and 100,000 new cases of human infection occur each year, with 95% of these cases resolving with no or minimal medical intervention.[1] Approximately 5% of cases result in disseminated disease which can be fatal. Recovery from Valley Fever is associated with lifelong immunity to the disease[2-3] indicating the possibility of producing a human vaccine.

Vaccine Development

A project in collaboration with the Valley Fever Center for Excellence (VFCE) at the Southern Arizona Veteran's Affairs Health Care Center is aimed at finding potential protein vaccine targets utilizing a comprehensive proteomic analysis of the *Coccidioides posadasii* spherule cell wall. The use of both one and two-dimensional liquid chromatography tandem mass spectrometry (LC MS/MS) performed on a linear ion-trap mass spectrometer is being used to identify proteins associated with the exterior cell surface that may come in contact with host immune defenses during infection.

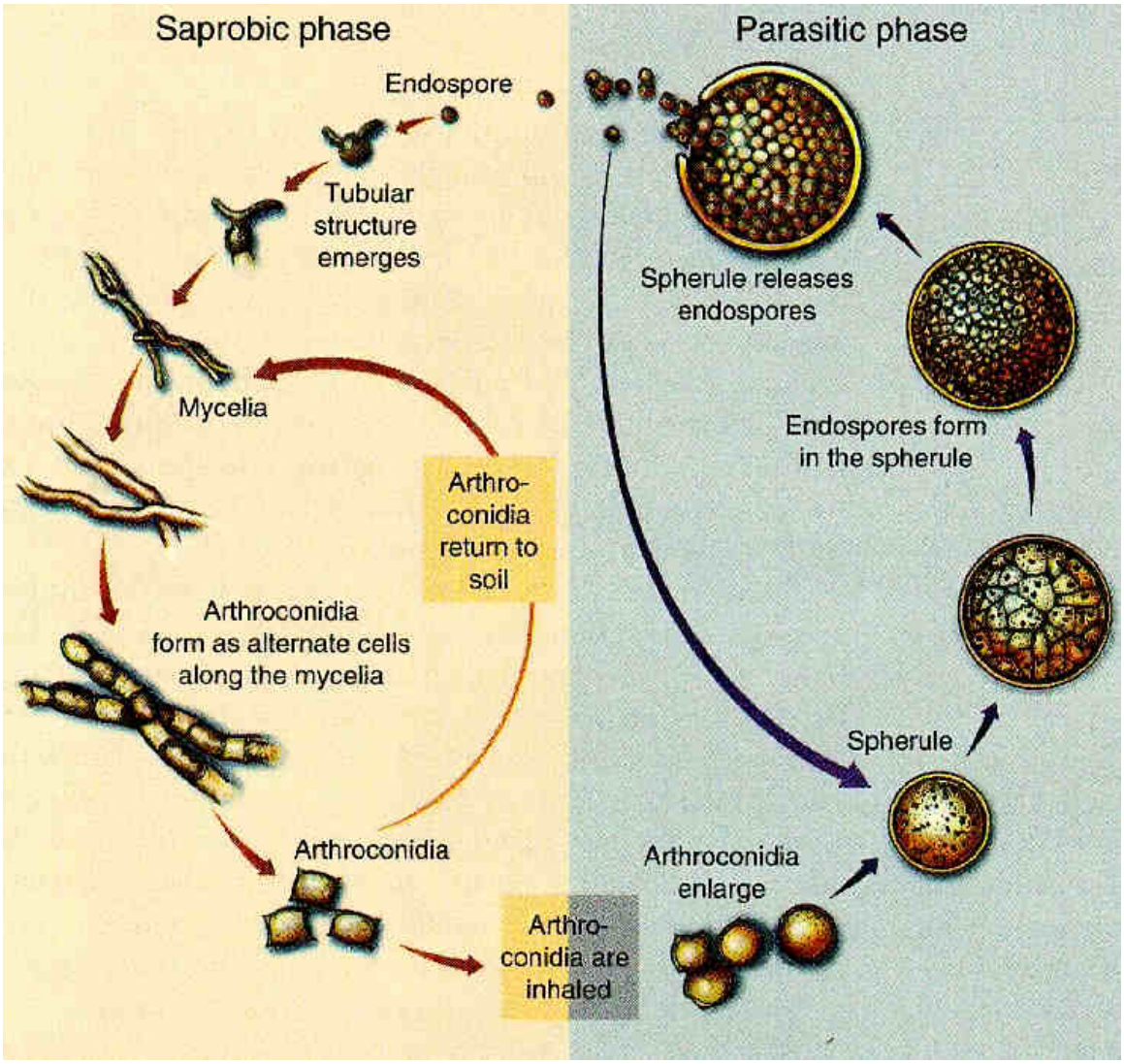
Another project (also in collaboration with VFCE) is aimed at finding proteins that are more highly expressed in the pathogenic (spherule) phase of the fungus compared to the saprobic or soil-dwelling (mycelia) phase. The used of stable-isotope labeling is applied by culturing spherules in ^{15}N-containing media to allow incorporation of the heavy nitrogen atoms into the proteins produced by the spherules. Concurrently, mycelia cells are cultured in normal ^{14}N-containing media. Cytoplasmic proteins from both cell types are collected and analyzed by the Multi-Dimensional Protein Identification Technique (known as MudPIT). MudPIT analysis is a two-dimensional LC MS/MS method used to separate peptides in complex mixtures prior to mass spectrometry analysis. Due to the mass difference of peptides containing ^{15}N compared to those containing only ^{14}N atoms, the relative expression levels of the proteins can be determined.

Disease Diagnosis

A third project in collaboration with VFCE is the identification of coccidioidal proteins in fluid samples from the lungs of infected mice as targets for disease diagnosis. Finding new and robust methods for the early identification of

coccidioidomycosis is important in the medical field due to the difficulty and lack of accuracy of current diagnostic methods. Gel Electrophoresis, LC MS/MS, and MudPIT are utilized to perform these analyses.

Coccidioides Life Cycle [4]



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[4] Stevens, D. A. 1995. Coccidioidomycosis. N Engl J Med **332:** 1077-1082.