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BIO5 Institute
Powering Twenty-First Century Biology

COLLEGE OF MEDICINE
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VALLEY FEVER CENTER FOR EXCELLENCE

Early Diagnosis of Valley Fever By Detecting Fungal Proteins

February 2010

Each year, Valley Fever (coccidioidomycosis) causes 50,000 new illnesses. Yet only a small fraction of these patients are correctly diagnosed. Health professionals partly are responsible because many patients are not tested for this possibility. But even when testing is done, early in the illness standard blood tests often are not yet positive and this can delay diagnosis for many weeks.

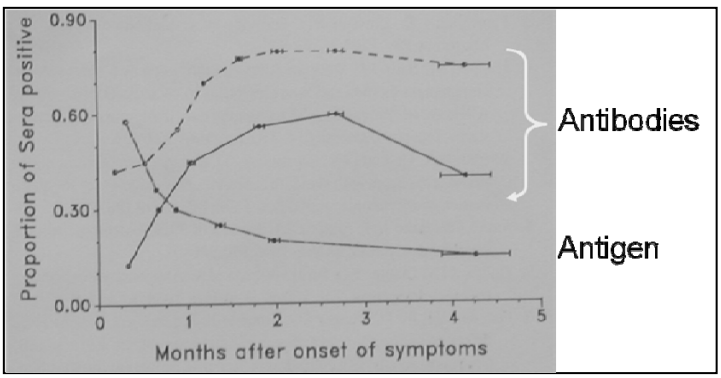
Why are conventional blood tests so often non-diagnostic?

Blood tests now in use originally were designed in the 1940s and are based on finding antibodies in the patient's serum that react to complex extracts prepared directly from the Valley Fever fungus. There are two inherent limitations of this approach. First, because the fungal extracts are made up of many components, some people have antibodies to some of them, unrelated to whether they have developed Valley Fever. To avoid these antibodies causing a false-positive test, the amount of fungal antigen is diluted to the point that only strong antibody responses due to the infection are detected. However, by eliminating false-positive results the sensitivity for true positive test results is reduced.

A second reason that antibody tests can be negative early in a Valley Fever infection is that it takes time for antibodies to develop. This may be the case for antibody tests for any infection but appears to be especially problematic for Valley Fever.

Our approach to improving early diagnosis of Valley Fever

For most infections, the invading microorganism multiplies in large numbers. In Valley Fever it is thought that most infections are caused by a single spore and every four days or so the number of fungal cells increases by a hundred-fold. By the time symptoms first develop (one to three weeks after infection), there likely would be several trillion organisms. With this proliferation, molecules or pieces of the fungus are released into the infected tissue, the blood stream and possibly find their way into the patient's urine. In other diseases detecting these pieces has been a very good way of detecting the infection, often before the body has been able to mount an antibody response. A good example of this is viral hepatitis. In Valley Fever, research has shown a similar pattern of a fungal antigen showing up in the sera of patients weeks before antibodies were detected. However, this observation has not been pursued until recently, primarily because these tests, like those for antibodies, were based on very complex and poorly understood fungal extracts. At BIO5 we thought if we could identify specific fungal products in the tissue of experimentally infected animals, these could serve as the exact targets for a future diagnostic test.



Where are we now?

Different strains of mice have different susceptibility to Valley Fever infections – some get very sick while others have mild infections. We have infected three strains of mice with fungal spores. At different times after infection, the lungs were recovered and analyzed for what fungal proteins could be found. The analysis was carried out by determining protein sequences by a powerful technique called "mass spectrometry." Among the hundreds of mouse proteins, we identified more than 20 fungal proteins, and of these, three had no similar relatives in mammals and very little similarity to proteins of other fungi. These three proteins are therefore very good candidates as biosignatures of a Valley Fever infection.

We have created a prototype assay to test for one of the target proteins. We did this by isolating the gene from the Valley Fever fungus that encodes the protein, integrated it into the DNA of brewer's yeast (*Saccharomyces cerevisiae*), let the yeast produce the protein as it grows and then purified the protein by standard biochemical methods. This "recombinant" purified protein then was used to immunize a goat and the goat's antibodies were used to capture and detect the antigen. When we used this assay to test mouse lungs a quantitative relationship was seen between how much antigen was detected and the severity of the infection in the different mouse strains and after different amounts of time the infection had been present. These results strongly indicate that a similar assay could be developed for all three target proteins.

What are we planning to do next?

We have begun to raise antibodies for all three target proteins that will be the basis for three detection assays. These assays will be used first to determine what results are obtained in sputum, serum and urine specimens for people without Valley Fever. These results will establish how much background activity the tests display and indicate that future results that show more activity above background likely are specific for a Valley Fever infection.

We then will test specimens from individuals who have an illness suggestive of Valley Fever. We also will test these patients by fungal culture and standard antibody tests. These conventional results will be compared to what the antigen detection tests find. From these comparisons the value of the antigen detection tests will be established.

Who is paying for developing these tests?

Original studies were supported by a grant from the Arizona Board of Regents to BIO5 (TRIF grant). Work has been continued with funds from the National Institutes of Health (NIH) through a project within the Pacific Southwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases and from philanthropic support from the JT Tai & Company Foundation. Arizona-based Valley Fever Solutions, Inc., is licensing this technology from the UA and is seeking approximately $1.5 million from investors to continue the commercialization of these tests. If this investment is made, the antigen detection assays could be ready for patient care in less than three years.

Summary

Three Valley Fever-specific proteins have been discovered at BIO5 and prototype evidence indicates that assays to detect these proteins are feasible. Development of these tests could lead to a clinically useful test to improve early diagnosis of Valley Fever pneumonia.

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