2007

Proceedings of the Fifty First Annual Coccidioidomycosis Study Group Meeting

March 31, 2007 • Biodesign Institute • Arizona State University • Tempe, Arizona

Proceedings of the Fifty First Annual Coccidioidomycosis Study Group Meeting

Meeting Number 51
March 31, 2007
Biodesign Institute
Arizona State University
Tempe, Arizona

Antonino Catanzaro, M.D.
Chairperson

Janis E. Blair, M.D.
Secretary

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Phoenix, Arizona 85054

Meeting Program

7:30 a.m. **Registration & Coffee**

8:30 a.m. **Session I: Epidemiology, Environment, Fungal Biology**
Chairperson: Demosthenes Pappagianis

1. Coccidioidomycosis in California: Surveillance, Recent Outbreaks, and the Debate over Making Coccidoidomycosis Laboratory-Reportable
   Wheeler C, Bissell S, Yuan J, Vugia D, Mohle-Boetani J
2. Epidemiology of Coccidioidomycosis in Arizona
   Sunenshine RH, Erhart L, Bronson-Lowe D, Saubolle M, Anderson S, Komatsu K
3. Soil Temperature Profiles of Coccidioides Sites in Arizona, California, and Utah
   Fisher FS, Johnson SM, Bultman MW
4. An Update on Connections Between Coccidioidomycosis Incidence and Climatic Fluctuations
   Talamantes J, Zender C
5. Climate and Satellite Remote Sensing Analyses of the "Grow and Blow" Hypothesis
   Comrie A, Skirvin S, Glueck M, Pianalto S, Johns R, Stacy P, Yool S
6. Coccidioidin and Spherulin Skin Testing in Maricopa County Revisited
   Kelly PC, Rowland VS, Doto I
7. Epitope Prediction from Genome Sequence Data Using a Combined Evidence Approach
   Neafsey DE, Galagan JE

10:15 a.m. **Break**

10:45 a.m. **Session II: Laboratory Studies**
Chairperson: Karl Clemons

8. Immunoproteomic Analysis of the Coccidioidal T27K Vaccine
   Lunetta JM, Johnson SM, Pappagianis D
9. Human Immunogenicity of Specific Fractions of T27K, a Complex, Glycosylated Antigen Preparation Derived from Coccidioides Posadasii
   Johnson SM, Dionne SO, Ampel NM, Pappagianis P
10. Detection of Circulation Coccidioidal Antigen Using an Inhibition ELISA
    Miller CN, Johnson SM, Pappagianis D
11. Assessment of T27K and Recombinant Coccidioidal Antigens Using Human Monocyte-Derived Dendritic Cells
    Dionne SO, Podany A, Ampel NM
12. Immunohistochemical Characterization of Immune Cells in Coccidioidal Lesions of Immunized and Nonimmunized Mice
    Shubitz L, Dial S, Galgiani J, B. Perrill, Casement R

13. Nikkomycin Z Biosynthesis: Molecular Modifications to Improve Manufacturing
Bandarian V, McCarty R, Nix D, Galgiani JN

12:15 p.m. Lunch

1:15 p.m. Business Meeting
Chairperson: Antonino Catanzaro

- Selection of Date, Location, and Hosts for the 52nd Annual Meeting
Catanzaro, A
  – Next year's meeting: April 5, 2008, San Diego, CA
- Status of the Valley Fever Vaccine Project
Hector, R
- The Future of Valley Fever Expertise: Passing the Torch
Larwood, T
- Presentation of proposed CSG Bylaws
- Other business

2:00 p.m. Session III: Interesting Cases
Chairperson: Janis Blair

2:30 p.m. Break

3:00 p.m. Session IV: Clinical Management
Chairperson: Royce Johnson

14. Serum Mannose Binding Lectin (MBL) Levels in Various Types of Coccidioidomycosis
Ampel NM, Dionne SO, Giblin A, Podany A, Chavez S, Klimecki W
15. Nikkomycin Z Clinical Trials: Phase I Single Dose Results and Planned Multidose Protocol
Nix D, Hoover S, Galgiani JN
16. A Survey of the Clinical Practices of Canine Valley Fever
Butkiewicz CD, Dufficy D, Dial SM
17. A Clinical Review of Coccidioidomycosis in the Elderly Population
Blair JE, Dunne R
18. Coccidioidomycosis in Human Immunodeficiency Virus-Infected Individuals in Arizona
Sunenshine RH, Bailey SR, Porter B, Kelly PC
19. Hyphae Forms of Coccidioides SPP Associated to Chronic Pulmonary Coccidioidomycosis and Diabetes Mellitus
Muñoz-Hernández B, Martínez-Rivera MA, Palma Cortés G, Tapia-Díaz A, Manjarrez ME
20. Follow Up on Six Patients with Coccidioides Meningitis Treated with Liposomal Amphotericin B
Kuberski T

5:00 p.m. Scientific Session Adjourned

ABSTRACT 1: Coccidioidomycosis in California: Surveillance, Recent Outbreaks, and the Debate over Making Coccidoidomycosis Laboratory-Reportable

Wheeler C, Bissell S, Yuan J, Vugia D, Mohle-Boetani J

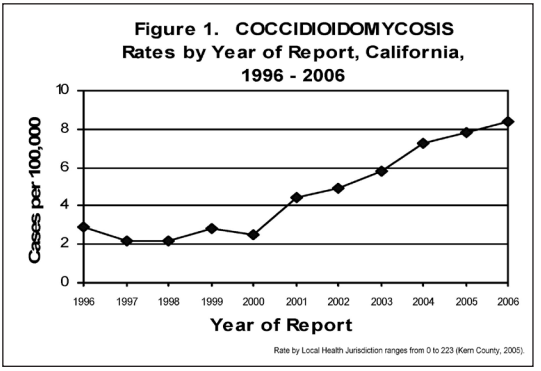
Coccidioidomycosis Epidemiology and Surveillance in California

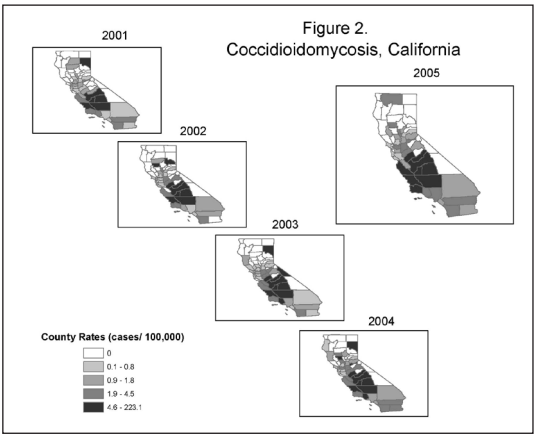
Mark Starr, DVM, MPVM; Charlotte Wheeler, MD, MPH; Stan Bissell, MS; Jean Yuan, MD, MPH; Duc Vugia, MD, MPH; Janet Mohle-Boetani, MD, MPH
Infectious Diseases Branch, California Department of Health Services

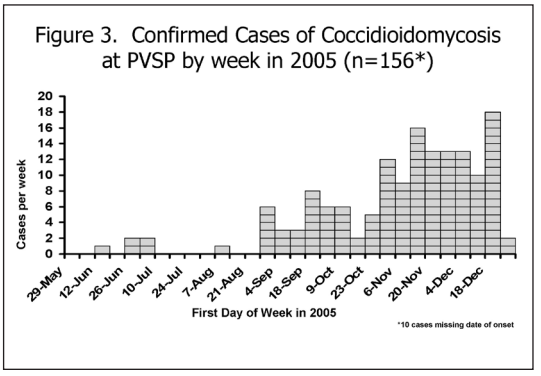
Surveillance based on provider reporting: California has experienced a dramatic rise in reported cases of coccidioidomycosis since 2000, rising from an average of about 800 cases per year during the 1996-2000 period (~2.5 cases per 100,000 population) to over 3000 cases in 2006 (8.4/100,000) (Figure 1). Only during the 1991-1994 outbreak were the state rates higher (peaked at 14.4/100,000). Over 70% of state cases occur in the hyperendemic region of the Southern San Joaquin Valley, where rates are typically over 50/100,000 and exceed 200/100,000 in some jurisdictions (Figure 2). Cases peak in fall and early winter, and age, gender and race distributions of cases are similar to those described elsewhere. There is a reporting bias towards more severe (including disseminated) cases, and the actual number of infections is likely over 10 times the reported number. Weather conditions, population growth with the resultant increase in the proportion susceptible, outdoor activities, construction and other soil disturbance are the likely explanations for the observed increase. **Recent outbreaks:** While not conclusive, some recent outbreak investigations have also pointed to these typical risk factors. Most notable was the investigation of an outbreak at Pleasant Valley State Prison in 2005. The prison, located near the town of Coalinga on the western side of the valley, experienced a dramatic rise in cases in late 2005 (Figure 3). Weather, outdoor activities, increases of susceptible population and, for serious/disseminated disease, black race and chronic conditions, were identified as risk factors. **Other surveillance approaches:** We are currently evaluating enhanced surveillance strategies in California, including those used in Arizona such as considering a single IgG positive result to be laboratory evidence of current or recent infection, we are promoting improvements to the national surveillance case definition with other endemic States and CDC, and we will be considering mandating laboratory reporting. **Valley Fever Vaccine Project:** California continues to support the effort to develop a vaccine, which could be the key to prevention, having contributed over $7 million (>40% of total) to the effort since 1998.

ABSTRACT 1: Coccidioidomycosis in California: Surveillance, Recent Outbreaks, and the Debate over Making Coccidoidomycosis Laboratory-Reportable

Wheeler C, Bissell S, Yuan J, Vugia D, Mohle-Boetani J







ABSTRACT 2: Epidemiology of Coccidioidomycosis in Arizona

Sunenshine RH, Erhart L, Bronson-Lowe D, Saubolle M, Anderson S, Komatsu K

Abstract presented but not submitted.

ABSTRACT 3: Soil Temperature Profiles of *Coccidioides* Sites in Arizona, California, and Utah

Fisher FS, Johnson SM, Bultman MW

Soil Temperature Profiles of *Coccidioides* Sites in Arizona, California, & Utah

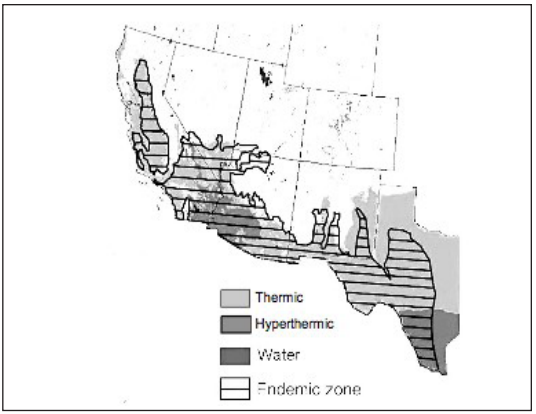
Fisher, F.S.[1], Johnson, S.M.[2], Bultman, M.W.[3], Pappagianis, D[2], Zaborsky, E[4]
[1]Univ. of AZ; [2]Univ. of CA, Davis; [3]U.S.G.S.; [4]B.L.M.

Soil temperatures at known *Coccidioides* sites in Arizona, California, and Utah were measured hourly at depths of 2, 10, and 20 cm for approximately one year (8,760) individual readings). Self-recording thermometers were used to establish the soil temperature profile at each site. The most remarkable finding was the large amount of time that soils at all sites at the 20 cm depth were within temperature regimes (10^{o} - 40^{0} C) favorable for the growth of *Coccidioides*. Also, while surface temperatures at many sites within the endemic zone commonly reached values likely lethal for *Coccidioides* (> 55^{o} C), they moderated quickly below lethal values with depth, often within 2 cm of the surface. Also noteworthy are areas (e.g., Dinosaur National Monument, Utah) (DNM) with ambient winter air temperatures as low as minus 40^{o} C that can still support *Coccidioides* growth at soil depths of 20 cm. Some regional differences were observed. For example at the 20 cm depth, soils at the Swelter Shelter site (DNM), were within the 20^{o} to 40° C temperature range an average of 32 % of the year (2,800 hrs), as compared to the California and Arizona sites that have soil temperatures in this range an average of 56% of the year (~ 4,900 hours), and 66% of the year (~ 5,700 hours) respectively.

Most, perhaps all, of the known *Coccidioides* growth sites throughout the southwestern United States are within soils classified as hyperthermic arid, or thermic arid or thermic semi-arid. Hyperthermic soils have an average annual temperature that is greater than 22° C at 50 cm depth. Thermic soils have an average annual temperature between 15° C and 22°C at 50 cm depth. We believe the temperature regimes of these soils are most favorable for the growth of *Coccidioides* and with rare exceptions, (possibly micro-climate locations), growth sites for *Coccidioides* are restricted to thermic or hyperthermic soils. We further suggest that the endemic zone for *Coccidioides* is better characterized by soil temperature regimes rather than the less rigorous classification into the Lower Sonoran Life Zone.

ABSTRACT 3: Soil Temperature Profiles of *Coccidioides* Sites in Arizona, California, and Utah

Fisher FS, Johnson SM, Bultman MW



ABSTRACT 4: An Update on Connections Between Coccidioidomycosis Incidence and Climatic Fluctuations

Talamantes J, Zender C

An Update on Connections Between Coccidioidomycosis Incidence and Climatic Fluctuations

Jorge Talamantes,[a] Sam Behseta,[b] Francisco Beltran,[b] and Charles S. Zender[c]
[a]Department of Physics and Geology, California State University, Bakersfield
[b]Department of Mathematics, California State University, Bakersfield
[c]Department of Earth System Science, University of California, Irvine

Coccidioidomycosis (valley fever) is a fungal infection found in the southwestern US, northern Mexico, and some places in Central and South America. The fungi which cause it (*Coccidioides immitis* and *Coccidioides posadasii*) are normally soil-dwelling but, if disturbed, become air-borne and infect the host when their spores are inhaled. It is thus natural to surmise that weather conditions which foster the growth and dispersal of *Coccidioides* must have an effect on the rate of infection of humans in the endemic areas. Our work so far has attempted to quantify this relationship in Kern County, California (where *C. immitis* is endemic). We have examined the effect on incidence fluctuations (about a seasonally-varying background) resulting from the following weather parameters: precipitation, surface temperature, and wind speed. We have performed several studies by means of a simple linear correlation analysis,[1] and by a generalized auto regressive moving average model. [2] Our first analysis suggests that linear correlations between climatic parameters and incidence are weak; our second analysis indicates that incidence can be predicted largely by considering only the previous history of incidence in the county – the inclusion of climate- or weather-related time sequences improves the model only to a relatively minor extent. Our last analysis, which includes concentrations of particular matter of size 10 micrometers or less (PM_{10}), in addition to weather parameters, yields somewhat inconsistent results. We suggest that, in accordance with our prior surmise,[3] a coherent, self-consistent picture of the dynamics of the disease can only be achieved by including in the model effects which are not yet well-characterized and are not related to weather. Specifically, the effects we have in mind are i) anthropogenic activities such as new construction resulting in the disturbance of previously-pristine environments where the fungus may be found, and ii) fluctuations in the immunity levels of the population exposed to *Coccidioides* spores. These immunity variations may results for a number of reasons, such as migration of populations with different susceptibilities, changes in the exposure levels of the population as urban areas grow, and possible effects of atmospheric pollution on the immune response.

[1] Zender CS and J Talamantes (2006) Climate controls on valley fever incidence in Kern County, California, International Journal of Biometeorology 50: 174-182, doi: 10.1007/s00484-005-007-6.

[2] Talamantes J, S Behseta and CS Zender (2007) Statistical modeling of valley fever data in Kern County, California, International Journal of Biometeorology 51: 307-315, doi:10.1007/s00484-006-0065-4.

[3] Talamantes J, S Behseta and CS Zender (2007) Fluctuations in Climate and Incidence of Coccidioidomycosis in Kern County, California: a review, in print in Annals of the New York Academy of Sciences, doi: 10.1196/annals.1406.028

ABSTRACT 5: Climate and Satellite Remote Sensing Analyses of the "Grow and Blow" Hypothesis

Comrie A, Skirvin S, Glueck M, Pianalto S, Johns R, Stacy P, Yool S

Climate and Satellite Remote Sensing Analyses of the "Grow and Blow" Hypothesis

Comrie A, Skirvin S, Glueck M, Pianalto S, Johns R, Stacy P, Yool S
University of Arizona

Valley Fever (coccidioidomycosis) is a disease endemic to arid regions in the Western Hemisphere, and is caused by the soil-dwelling fungi, *Coccidioides immitis* and *Coccidioides posadasii*. Arizona is currently experiencing an epidemic with well over 5000 cases in 2006. We are collaborating with the Arizona Department of Health Services to further understanding of the relationships between surface disturbances, climate variability and other environmental factors that may interact to produce coccidioidomycosis outbreaks. Specifically, we are developing seasonal climate-based models and associated databases to anticipate coccidioidomycosis outbreaks and improve public health actions to mitigate them. These decision-support tools are intended to describe the climate contribution to incidence and to epidemics, specify the covariability of atmospheric dust levels and coccidioidomycosis incidence, make seasonal forecasts of disease incidence by geographic area, provide satellite-derived spatial data on surface moisture and derived relationships to disease incidence, and satellite-derived maps of land cover change and disturbance. Preliminary regression-based climate modeling results have shown that about 80% of the variance in seasonal coccidioidomycosis incidence for southern Arizona can be explained by precipitation and dust-related climate scenarios prior to and concurrent with outbreaks. More recent results indicate that while climate, alone, cannot account for the recent broad increasing trend in incidence, precipitation and dust remain useful predictors. Furthermore, results confirm that climate accounts for much of the coccidioidomycosis incidence variability about the trend from 1992-2005. These findings continue to support the "Grow and Blow" hypothesis of coccidioidomycosis outbreaks in which associated spores grow in moist soil (rainy season) and then, become airborne or "blow", during subsequent episodes of dry soil conditions (dry, windy season). Refinement of our models is underway including incorporation of updated case-level coccidioidomycosis data, and intensive exploration of parameterization of environmental data. Here we present a progress report on our efforts to link precipitation and other environmental conditions (e.g., atmospheric dust), remotely-sensed environmental characteristics (e.g. soil moisture from AVHRR and MODIS NDVI, land use change/disturbance from LANDSAT TM, habitat stratification (via rodent records), dust permits) to seasonal coccidioidomycosis incidence.

ABSTRACT 6: Coccidioidin and Spherulin Skin Testing in Maricopa County Revisited

Kelly PC, Rowland VS, Doto I

Coccidioidin and Spherulin Skin Testing in Maricopa County Revisited

Peter C. Kelly, M.D., Virginia S. Rowland, M.D., Irene Doto

In 1978-79 we conducted a skin test survey of 761 volunteer residents of Maricopa County to estimate the prevalence of coccidioidomycosis as measured by dermal reactions to Coccidioidin (C) and Spherulin (S). The mantoux technique was used. Data were recorded in millimeters of induration at 48 hours after intradermal injection of C and S. A reactive test (R) was defined as ≥5mm induration.

The demographics of our subjects were: age range 15 to >65 years with 62% < 40 years; gender 59% females; ethnicity 69% white (W), 21% Hispanic (H), 8% black (B) and 2% other.

Among 526 self selected volunteers 32% reacted to either or both C or S. 28% reacted only to S and 26% only to C.

Males of each ethnic group were more frequently reactive than females; W males 35.7%R, W female 23.4%R; H males 70.5%R, H females 41.2%R; B males 85%R, B females 51.2%R.

Length of residence in Maricopa County increased reaction rates. Subjects with ≥ 10 years residence had 47.5%R to C and 48.5%R to S. Subjects with < 10 years residence had 19.5%R to C and 17%R to S. ($p<0.01$ both C and S)

Subjects who had outdoor occupations (N=164 and includes 137 highway workers) reacted more frequently than those who did not work outdoors. 67%R to C and 64%R to S among outdoor workers compared to 26%R to C and 28%R to S for subjects who did not work outdoors. ($p<0.001$ both C and S)

98 pregnant women were tested at parturition. 35%R to C and 37%R to S.

These data were collected 28 years ago. Since then the population of Maricopa County has more than doubled and land use patterns have changed significantly to accommodate population growth. We believe a new survey is needed to measure the current prevalence coccidioidomycosis in Maricopa County.

ABSTRACT 7: Epitope Prediction from Genome Sequence Data Using a Combined Evidence Approach

Neafsey DE, Galagan JE

Epitope Prediction Using a Combined Evidence Approach

Daniel E. Neafsey
Microbial Analysis Group, Broad Institute of MIT and Harvard, Cambridge, MA
neafsey@broad.mit.edu

Genome sequencing of multiple isolates of *C. immitis* and *C. posadasii* is continuing at the Broad Insitute. In addition to the C735 strain of *C. posadasii* that has been sequenced by TIGR, the Broad Institute has now made available to the community genome assemblies for *C. immitis* strains RS, H538.4, RMSCC 2394, and RMSCC 3703, as well as *C. posadasii* strains RMSCC 3488 and Silveira.

This growing wealth of comparative genomic data may be useful in identifying antigenic genes or epitopes using 'immunoinformatic' approaches. In theory, portions of pathogen proteomes that are epitopes, binding to one or more alleles of MHC I or MHC II, are subject to natural selection favoring amino acid substitutions that decrease MHC binding affinity. These antigenic regions of the proteome may therefore be more highly polymorphic in a sampling of strains than non-antigenic genomic regions. We tested whether this evolutionary signal might be informative for epitope prediction using HIV, as many more sequenced genomes already exist for this virus relative to *Coccidioides* spp. Using a 'naïve Bayes' statistical framework to incorporate multiple data sources, I show that comparative genome sequence data may indeed offer additional power for the computational identification of epitopes in HIV. When sequencing is completed for the remaining *Coccidioides* strains, it is hoped that implementation of this and other immunoinformatic approaches will acceleration epitope identification and vaccine development for this pathogen.

ABSTRACT 8: Immunoproteomic Analysis of the Coccidioidal T27K Vaccine

Lunetta JM, Johnson SM, Pappagianis D

Immunoproteomic Analysis of the Coccidioidal T27K Vaccine

Jennine M. Lunetta, Suzanne M. Johnson and Demosthenes Pappagianis
Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, CA 95616, USA

The coccidioidal T27K vaccine is a soluble, subcellular vaccine prepared from mechanically-disrupted, thimerosal-inactivated endosporulating spherules that has been shown to protect mice against intranasal challenge with *Coccidioides posadasii* arthroconidia. Despite its effectiveness in mice, it is improbable that it will be used as a human vaccine, given that the vaccine is a complex mixture of protein and carbohydrate that has not been fully characterized. However, the vaccine in its crude form does provide a good source of potential protective antigens that can be isolated and evaluated as recombinant vaccines. We have conducted a limited immunoproteomic analysis of the T27K vaccine in order to identify seroreactive proteins present in the vaccine which might also contribute to the protective capacity of the vaccine. The T27K vaccine was separated by two-dimensional gel electrophoresis and then analyzed for seroreactive proteins using immunoblot analysis with sera pooled from patients with coccidioidomycosis. Over 50 spots were recognized by pooled sera obtained from patients positive for the CF-IgG antibody. From these spots, 31 were selected for protein identification using mass spectrometry and a total of 17 proteins were identified by a BLAST search of the GenBank *Coccidioides* nonredundant protein sequences database. The following proteins were identified: 1,3-ß-glucanosyltransferase (Gel1), chitinase (CF-Ag), ELI-antigen 1 (ELI-Ag1), peroxisomal matrix protein (Pmp1), 1,2-α-mannosidase (ManI/Amn1), calnexin, a protein of unknown function and 10 hypothetical proteins. Five of the proteins (Gel1, CF-Ag, ELI-Ag1, Pmp1 and ManI/Amn1) have been previously characterized in *Coccidioides* spp. and the results have been published. Two proteins (calnexin and a protein of unknown function) have been previously cloned and sequenced by our laboratory but the results have not been published. Previous studies have tested the protective ability of recombinant versions of four of the previously characterized proteins (Gel1, ELI-Ag1, Pmp1 and Amn1) and all four were determined to be protective antigens. In other words, of the proteins tested so far, 100% of the proteins identified in this study by immunoproteomic analysis of the T27K vaccine were determined to be protective antigens. These findings demonstrate that immunoproteomics is a useful tool for targeting protective components present within the crude T27K vaccine. Our laboratory has initiated experiments to clone and sequence the cDNA encoding the 10 hypothetical proteins and to prepare recombinant versions of all 12 of the previously uncharacterized proteins in order to evaluate their protective ability in animal experiments.

ABSTRACT 9: Human Immunogenicity of Specific Fractions of T27K, a Complex, Glycosylated Antigen Preparation Derived from *Coccidioides posadasii*

Johnson SM, Dionne SO, Ampel NM, Pappagianis P

Human Immunogenicity of Specific Fractions of T27K, a Complex, Glycosylated Antigen Preparation Derived from *Coccidioides posadasii*

Johnson SM[1], Dionne SO[2], Ampel NM[2], and Pappagianis D[1]
[1]University of California, Davis, [2]University of Arizona, [2]Southern Arizona Veterans Health Care System, Tucson, Arizona

Prior studies have shown that coccidioidal antigen T27K, a complex, highly glycosylated, subcellular mixture prepared from thimerosal-inactivated *C. posadasii* endosporulating spherules, specifically stimulates peripheral blood mononuclear cells (PBMC) from *Coccidioides* immune human donors to secrete IL-2. We hypothesize that glycosylation is responsible at least in part for the stimulation and that deglycosylation will diminish or abrogate reactivity.

T27K was subjected to enzymatic deglycosylation using the E-DEGLY Kit (Sigma) for 0-24 hours at 37°C. Treated (deglycosylated) and untreated T27K was compared for ability to stimulate immune cells. PBMC, derived from venous blood of healthy donors, were incubated in AIM-V medium for 48 hours at a concentration of 2 x 10^6 cells/ml in the presence of the test antigen or appropriate control (Con A). The supernatant was then harvested and the IL-2 concentration measured by ELISA. T27K lost immunogenicity when deglycosylated as demonstrated by a reduction of the IL-2 released from immune cells.

Reduced, denatured T27K was fractionated with respect to size into 5 pools (PC1 – PC5) using continuous elution electrophoresis. One pool, PC2, was further segregated into subpools PC2a and PC2b. When these pools were subjected to SDS-PAGE, discrete bands were discernable and subpools PC2a and PC2b contained single bands. These pools were also used to stimulate PBMC and they retained immunogenic activity.

These results establish the importance of glycosylation with regard to immune stimulation. In addition they demonstrate that the continuous-elution electrophoresis can be used to isolate fractions in a manner that preserves antigenic specificity. Mass spectrometry is being used to identify the peptides present in the fractions to permit preparation of recombinant proteins.

ABSTRACT 10: Detection of Circulation Coccidioidal Antigen Using an Inhibition ELISA

Miller CN, Johnson SM, Pappagianis D

Detection of Circulating Coccidioidal Antigen Using an Inhibition ELISA

Miller CN, Johnson SM, and Pappagianis, D.
University of California, Davis

Currently, detection of antibody is used for the diagnosis of coccidioidomycosis, though some individuals, especially early in the course of their illness, fail to produce sufficient amounts of antibody; therefore, a test that can detect antigen could be useful for an early diagnosis. An inhibition ELISA, using polyclonal equine antibody, was developed that can detect between 5 nanograms and 20 nanograms of circulating coccidioidal antigen in human serum before antibody is observed. In a preliminary study, antigen was detectable prior to antibody in 9 of 27 cases, showing 33.3% sensitivity. Antigen was detected between 1 and 101 days before diagnosis using conventional serology. Additionally, one of 60 negative specimens (later samples were negative for antibody, or no follow-up specimens submitted) was antigen positive, demonstration 98.3% specificity. This test may assist in an earlier diagnosis of coccidioidomycosis, and may directly benefit immunocompromised individuals show antibody response may be weak or delayed.

ABSTRACT 11: Assessment of T27K and Recombinant Coccidioidal Antigens Using Human Monocyte-Derived Dendritic Cells

Dionne SO, Podany A, Ampel NM

Cytokine Assessment of Activation of Peripheral Blood Mononuclear Cells (PBMC) and Human Monocyte-Derived Dendritic Cells (DC) by T27K and Recombinant Coccidioidal Antigens

Sara O. Dionne, Abigail Podany, and Neil M. Ampel
University of Arizona and the Southern Arizona Veterans Affairs Health Care System (SAVAHCS), Tucson, AZ

Cellular immunity plays a critical role in human coccidioidomycosis and can be measured in vitro by incubating coccidioidal antigens with PBMC or lymphocytes incubated with DC. DC are professional antigen presenting cells that can stimulate lymphocytes to specifically respond to antigen. T27K, a complex coccidioidal antigen preparation, has been shown to cause activation of lymphocytes from naïve and anergic donors. However, neither the cytokine pattern of this response nor the effect of recombinant antigens has been studied in this system. To address this, we compared the cytokine expression pattern of lymphocytes obtained from coccidioidal-immune and non-immune donors. The median IL-2 levels released by PBMC from 8 immune donors after incubation with T27K was >1000 pg/ml and significantly higher than after incubation with the Saccharomyces-induced coccidioidal recombinant antigens Ag2PRA and CSA. No IL-2 was released by after incubation of PBMC from 6 nonimmune donors with any antigen. However, TNF-α, IFN-γ and IL-10 were released by immune and non-immune donors after incubation with Ag2PRA and CSA. When antigen was presented by DC, minimal IL-2 release was seen for Ag2PRA or CSA compared to T27K but these recombinant antigens did induce release of IFN-γ. These data suggest that examining multiple cytokines and their pattern of release could be important in determining immunogenic antigens in human coccidioidomycosis.

ABSTRACT 12: Immunohistochemical Characterization of Immune Cells in Coccidioidal Lesions of Immunized and Nonimmunized Mice

Shubitz L, Dial S, Galgiani J, B. Perrill, Casement R

Immunohistochemical Characterization of Immune Cells in Coccidioidal Lesions of Immune and Nonimmune Mice

Lisa Shubitz, Sharon Dial, John Galgiani, Robert Perrill, Rachael Casement. Valley Fever Center for Excellence and Department of Veterinary Science and Microbiology, The University of Arizona, and Southern Arizona Veterans Health Care Administration, Tucson, AZ

Introduction: We hypothesized that immunohistochemistry can aid in assessing a vaccine at the cellular level in the murine model of coccidioidomycosis. To test this, we determined whether vaccination makes a susceptible mouse look like a resistant mouse by comparing vaccinated and unvaccinated susceptible (C57BL/6), intermediate (DBA/2n), and resistant (Swiss-Webster) mice.

Materials and methods: Female C57BL/6, DBA/2n, and Swiss-Webster mice were either vaccinated with recombinant Ag2/PRA_{1-106}-CSA in an adjuvant or were given nothing. Groups were as follows: 16 mice each strain (C57, DBA, SW), each vaccination status (Vx, noVx) and 3 time points (days 12, 18, and 56 post infection - no 56-day unvaccinated C57). Mice were challenged with approximately 50 spores of C. posadasii, strain Silveira. At sacrifice, half the mice had lungs cultured quantitatively and the other half had entire lungs and spleens fixed in zinc acetate for histological studies. Sections were stained with H&E, anti-CD3 (T-cells), Mac2 (macrophages), CD22 (B-cells), CD4 and CD8 T-cell subsets, and anti-Ag2/PRA antibody to identify spherules/endospores. Slides were scored and analyzed by ANOVA (P significant ≤0.05)

Results: Histological scoring of the spherule/endospore slides showed that day 18 noVx C57 had significantly greater spherule scores than DBA and SW; DBA were also significantly higher than SW. Vaccination did not appreciably affect spherule scores in DBA and SW, but reduced them in C57 near to the level of SW mice ($P<0.05$) and all vaccinated C57 mice survived, as did SW, while all noVx C57 died before day 18.

By immunohistochemistry, we scored the quantities of Mac2, CD22, CD3, CD4, and CD8 positive cells. Vaccination significantly increased CD3 and CD22 ($p<0.05$) in C57 mice but not in the other two strains. By day 18, noVx C57 were nearly devoid of both types of lymphocytes, but SW had rising CD3 ($P<0.05$) and CD22 scores with intense peribronchiolar/perivascular infiltrates. By comparison, Vx C57 had significantly higher CD3 and Mac2 scores ($P\leq0.02$) and higher CD22 than SW at day 18. CD4s were significantly higher in Vx vs noVx C57 mice.

Summary: Vaccine-induced immunity to *Coccidioides* spp. in C57 mice is effective at abating infection, but the histologic picture is not the same as in the innately resistant Swiss-Webster mice. B-cells, T-cells, and macrophages are increased compared to SW. Vaccine-induced immunity in C57 mice appears to be related to upregulation of T-cells, $CD4^+$ cells in particular.

ABSTRACT 13: Nikkomycin Z Biosynthesis: Molecular Modifications to Improve Manufacturing

Bandarian V, McCarty R, Nix D, Galgiani JN

Abstract presented but not submitted.

ABSTRACT 14: Serum Mannose Binding Lectin (MBL) Levels in Various Types of Coccidioidomycosis

Ampel NM, Dionne SO, Giblin A, Podany A, Chavez S, Klimecki W

Mannose Binding Lectin Serum Levels are Low in Clinically Active Coccidioidomycosis

Neil M. Ampel, Sara O. Dionne, Andrea Giblin, and Abigail Podany

Mannose binding lectin (MBL) is a plasma protein involved in complement activation and induction of the innate immune response. It is encoded by a single gene associated with 3 major polymorphisms as well as promoter gene polymorphisms that are relatively frequent among various populations. Because of this, serum levels of MBL can vary greatly between individuals. Low levels of MBL have been associated with increased susceptibility to infection, including those due to fungi. However, the serum levels of MBL have not been examined in human coccidioidomycosis. To explore this, we recruited 2 major subject groups: 1) healthy individuals with known coccidioidal immunity who had never had symptomatic coccidioidomycosis; and 2) individuals with symptomatic disease. The latter were divided into a) primary pulmonary disease; b) non-meningeal disseminated illness and c) coccidioidal meningitis. Serum was obtained at a time when all subjects with symptomatic disease were clinically stable and under treatment. MBL was measured by ELISA (Cell Sciences, Canton, NJ). Serum levels of MBL were significantly lower in the 69 subjects with symptomatic disease than in the 29 immune donors ($p = 0.0003$) and each subcategory of symptomatic disease wassignificantly lower than immune donors. When an absolute level of MBL of 70 ng/ml was used, each group of those with active disease were again significantly below the immune donors ($p = 0.009$), as shown below:

Serum MBL level	Healthy, immune	Primary pulmonary	Non-meningeal, disseminated	Meningitis
≤70 ng/ml	7	13	17	7
>70 ng/ml	22	8	10	4

These data indicate that serum MBL levels are markedly depressed in persons with symptomatic coccidioidomycosis. Whether MBL plays a role in the pathogenesis of coccidioidomycosis and whether genetic differences account for these differences in levels remains to be determined.

ABSTRACT 15: Nikkomycin Z Clinical Trials: Phase I Single Dose Results and Planned Multidose Protocol

Nix D, Hoover S, Galgiani JN

Abstract presented but not submitted.

ABSTRACT 16: A Survey of the Clinical Practices of Canine Valley Fever

Butkiewicz CD, Dufficy D, Dial SM

Abstract presented but not submitted.

ABSTRACT 17: A Clinical Review of Coccidioidomycosis in the Elderly Population

Blair JE, Dunne R

Coccidioidomycosis in the Elderly Population

JE Blair, R Dunne
Division of Infectious Diseases, Mayo Clinic Arizona

Background: Persons older than the age of 60 years constitute a growing population within Arizona. Amongst the general population of Arizona, the incidence of coccidioidomycosis (CM) has been rising from 2000-2006, but among older individuals, the incidence is incrementally greater with each 5 years of age. Previous publications indicate that severe pulmonary infection, dissemination and mortality are greater in the elderly population, but little risk stratification with respect to comorbidities has been performed.

Aim: We sought to further define whether poorer outcomes were the result of age or comorbidities among elderly persons with CM.

Methods: A retrospective chart review of all patients identified with coccidioidomycosis from January1999-October 2003. Clinical, microbiological, serological, radiographic, treatment and follow up information were all abstracted. The "healthy elderly" group was without the following comorbidities: diabetes mellitus, HIV infected, solid or blood/marrow transplantation, iatrogenic immunosuppression, hematological malignancy or other cancer in active receipt of chemotherapy.

Results: 284 patients without the previously mentioned comorbidities were identified. The age range was 12-94 years, mean and median ages were 57 and 59.5 years respectively. 142 patients were each present in <60 years and ≥ 60 years age groups. The older group had more Caucasians than the younger (71-74% vs. 85-93%). The rate of pulmonary infiltrates and nodules were similar between the 2 groups, but elderly group was less likely to manifest cavities (16% vs. 8%, $p<0.05$). Serology, cultures and histology were all performed equally among the 2 groups, although the elderly group was less likely to manifest a positive serology (96% vs. 85%, $p<0.01$). Overall dissemination and hospitalization was similar among the 2 groups, however, when incremental age groups were evaluated, hospitalization among patients >80 years was significantly increased (53% $p<0.001$). Both groups received similar rates of medical and surgical therapy. No differences in clinical outcomes were seen with respect to improvement, resolution, progression, relapse or death.

Conclusions: Minor age group related differences were seen among healthy persons with CM older than 60 years when compared to those younger than 60 years. No differences in clinical outcomes were observed.

ABSTRACT 18: Coccidioidomycosis in Human Immunodeficiency Virus-Infected Individuals in Arizona

Sunenshine RH, Bailey SR, Porter B, Kelly PC

Coccidioidomycosis in Human Immunodeficiency Virus-Infected Individuals in Arizona

Sunenshine RH,[1,2] Bailey SR[1], Porter B[1], Kelly PC[1]
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Background: Coccidioidomycosis, a fungal disease endemic to Arizona, can cause debilitating illness and/or death in human immunodeficiency virus (HIV) - infected individuals. Arizona reported coccidioidomycosis rates have increased by more than 50% since 2000, however, data are not available to determine how many of these patients also have HIV infection. The aim of this investigation is to utilize information from HIV surveillance data to determine the proportion of patients with coccidioidomycosis who are co-infected with HIV.

Methods: All laboratory-confirmed cases of HIV and coccidioidomycosis are required by law to be reported to public health in Arizona. While the Arizona coccidioidomycosis surveillance database does not include information about co-morbidities such as HIV, the HIV surveillance database does include comorbidity data regarding coccidioidomycosis. Surveillance data from these two sources from 1992 to 2005 were merged and analyzed to determine the proportion of coccidioidomycosis cases co-infected with HIV, stratified by age and sex. Additionally, the proportion of coccidioidomycosis-related deaths in HIV-infected patients was estimated using two sources; HIV surveillance data and death certificate data from Arizona. We used number of HIV-infected patients diagnosed with coccidioidomycosis within 60 days of death to determine the numerator and number of coccidioidomycosis deaths for the denominator.

Results: Of 23,704 coccidioidomycosis cases in Arizona, 986 (4.2%) patients were co-infected with HIV (OR 20.9). Of 6148 male coccidioidomycosis cases < 50 years old, 773 (12.6%) had a concurrent diagnosis of HIV (OR 27.5). A median of 23.5% (range 14-48%) of deaths related to coccidioidomycosis are estimated to have occurred in HIV-infected patients.

Conclusions: Males under age 50 in Arizona who have coccidioidomycosis are much more likely to be infected with HIV than those who do not have coccidioidomycosis. This emphasizes the importance of testing for HIV in males under age 50 who are diagnosed with coccidioidomycosis. Approximately one quarter of coccidioidomycosis-related deaths occur in HIV co-infected individuals, further emphasizing the need for HIV screening in this population.

ABSTRACT 19: Hyphae Forms of *Coccidioides* Species Associated to Chronic Pulmonary Coccidioidomycosis and Diabetes Mellitus

Muñoz-Hernández B, Martínez-Rivera MA, Palma Cortés G, Tapia-Díaz A, Manjarrez ME

Hyphae Forms of *Coccidioides* Species Associated to Chronic Pulmonary Coccidioidomycosis and Diabetes Mellitus

Muñoz-Hernández B, Martínez-Rivera MA, Palma Cortés G ,Tapia-Díaz A, Manjarrez ME.

Background: *Coccidioides immitis* and *C. posadasii* are etiologic agents of coccidioidomycosis. Major endemic zones are arid and semi-arid climates in North America, such as the northern Mexican states and the southwestern United States. *Coccidioides* spp. is a dimorphic fungus that forms arthroconidia during its mycelial phase while growing in soil. Inhalation of arthroconidia by a susceptible host initiates the parasitic phase. The arthroconidia transform into endospore-containing spherules, which are classically found in *Coccidioides* spp. infected tissue, although parasitic mycelial structures have been identified in some cases.

Methods: The study population was comprised of patients with chronic pneumopathy referred to the National Institute of Respiratory Diseases of Mexico and analyzed association between cases, controls, and risk factors, including co-morbidity. The ambidirectional study included elements of cohort, case, and control studies. A case was defined as any patient who presented with mycelial forms hyphae or hyphae /spherules. A control was defined as any patient who presented only with spherules or in whom no parasitic forms were observed. All patients (n=44) with pulmonary coccidioidomycosis were diagnosed by culture, histopathology, cytology and immunologic methods.

Results: There was a positive association between diabetes mellitus and Coccidioides spp. infection with development of hyphae, and those patients were four times more likely than non-diabetics to develop mycelial forms 95% CI, 0.85-20.10; $P < 0.05$.

Conclusions: We found that any patient presenting pulmonary coccidioidomycosis with diabetes mellitus II and chronic evolution with radiological evidence of cavitary lesions is likely to develop parasitic mycelial forms of *Coccidioides* spp.

ABSTRACT 20: Follow Up on Six Patients with *Coccidioides* Meningitis Treated with Liposomal Amphotericin B

Kuberski T

Follow Up on Six Patiens with *Coccidioides* Meningitis Treated with Liposomal Amphotericin B

Tim Kuberski, M.D., Phoenix, Arizona

Six patients with *Coccidioides* meningitis (CM) treated with liposomal amphotericin B (LAB) were reported at the 2004 meeting of the Coccidioidomycosis Study Group. The objective was to treat CM early and aggressively with LAB to effect a cure. A general treatment dose of 10 mg/kg was used with a target total dose of ≥ 5,000 mg. The total dose of LAB in these patients ranged between 6,300 mg and 17,549 mg. LAB was well tolerated with less of the usual toxicities seen with deoxycholate amphotericin B. One of the six patients is felt to be cured of CM after a five year follow up. This is based on negative immunodiffusion and complement fixation serologies. In addition, the patient is functioning normally off antifungal therapy. Since CM is felt to be a fatal disease untreated and generally incurable with previous modalities, further studies need to be done on the treatment of CM with LAB.

Annual Meetings of the Coccidioidomycosis Study Group

Number	Date(s)	Location	Held In Conjunction With
1	July 18, 1956	San Francisco, CA	
2	December 5-6, 1957	Los Angeles, CA	
3	December 4-5, 1958	Los Angeles, CA	
4	December 3-4, 1959	Los Angeles, CA	
5	December 8-9, 1960	Los Angeles, CA	
6	November 30 – December 1, 1961	Los Angeles, CA	
7	November 29-30, 1962	Los Angeles, CA	
8	December 5-6, 1963	Los Angeles, CA	
9	December 10-11, 1964	Los Angeles, CA	California Thoracic Society
10	December 7, 1965	Phoenix, AZ	2nd Coccidioidomycosis Conference
11	April 19, 1967	Palm Springs, CA	California Thoracic Society
12	May 1, 1968	Fresno, CA	California Thoracic Society
13	April 15, 1969	San Diego, CA	California Thoracic Society
14	April 1, 1970	San Francisco, CA	California Thoracic Society
15	April 6, 1973	Newport Beach, CA	California Thoracic Society
16	April 5, 1974	Sacramento, CA	California Thoracic Society
17	September 30, 1974	San Francisco, CA	Coccidioidomycosis Cooperative Treatment Group
18	April 2, 1975	San Diego, CA	California Thoracic Society
19	July 31, 1975	San Diego, CA	Coccidioidomycosis Cooperative Treatment Group
20	January 14-15, 1976	San Diego, CA	Coccidioidomycosis Cooperative Treatment Group
21	April 7, 1976	Palo Alto, CA	California Thoracic Society
22	May 18, 1977	San Francisco, CA	American Lung Association
23	April 5, 1978	Beverly Hills, CA	California Thoracic Society
24	May 15, 1979	Las Vegas, NV	American Lung Association
25	April 11, 1980	Sacramento, CA	California Thoracic Society
26	March 28, 1981	San Francisco, CA	California Thoracic Society
27	May 15, 1982	Los Angeles, CA	American Lung Association
28	March 20, 1983	La Jolla, CA	California Thoracic Society
29	March 14-17, 1984	San Diego, CA	4th Coccidioidomycosis Conference
30	March 8, 1986	Santa Barbara, CA	
31	April 4, 1987	Los Angeles, CA	
32	April 9, 1988	Los Angeles, CA	
33	April 8, 1989	San Jose, CA	
34	April 7, 1990	Berkeley, CA	
35	April 6, 1991	Tucson, AZ	
36	April 4, 1992	Fresno, CA	
37	April 3, 1993	Tucson, AZ	
38	August 24-27, 1994	Stanford, CA	5th Coccidioidomycosis “Centennial” Conference
39	April 1, 1995	Bakersfield, CA	
40	March 30, 1996	Scottsdale, AZ	
41	March 5, 1997	San Diego, CA	
42	April 4, 1998	Visalia, CA	
43	March 20, 1999	Tijuana, BC, Mexico	
44	April 1, 2000	Berkeley, CA	
45	March 31, 2001	Tucson, AZ	
46	April 6, 2002	Davis, CA	
47	April 3, 2003	Scottsdale, AZ	
48	April 31, 2004	Rosarito Beach, Mexico	
49	April 2, 2005	Bass Lake, CA	
50	August 23-26, 2006	Stanford, CA	6th International Symposium on Coccidioidomycosis
51	March 29, 2007	Tempe, AZ	

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