PROCEEDINGS OF THE ANNUAL COCCIDIOIDOMYCOSIS STUDY GROUP MEETING

Meeting Number 45
March 31, 2001
University of Arizona
Tucson, Arizona

John N. Galgiani, M.D.
Secretary, Cocci Study Group
Editor of Proceedings

Robert J. Brauer, Jr., M.A.
Production Editor

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List of Annual Meetings

MEETING	DATE	LOCATION	IN CONJUCTION WITH:
1	07/18/56	San Francisco	
2	21/5 - 6/57	Los Angeles	
3	12/4 - 5/58	Los Angeles	
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9	12/10 -11/64	Los Angeles	CTS
10	12/07/65	Phoenix	2nd Coccidioidomycosis Conference
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22	5/18/77	San Francisco	ALA
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Conferences held in conjunction with:

Abbreviations: CTS = California Thoracic Society
CCTG = Coccidioidomycosis Cooperative Treatment Group
ALA = American Lung Association

PROCEEDINGS OF THE

FORTY FIFTH ANNUAL

COCCIDIOIDOMYCOSIS STUDY GROUP MEETING

ABSTRACTS

Meeting Number 45

March 31, 2001

University of Arizona

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Hillel B. Levine, Ph.D.
Chairholder

John N. Galgiani, M.D.
Secretary

Robert J. Brauer, Jr., M.A., CQA
Production Editor

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Address editorial correspondence to:

Dr. John N. Galgiani, M.D.

Medical Service (MS1-111)

Southern Arizona VA Health Care System

3601 South 6th. Ave.

Tucson, AZ 85723 USA

Program for the 45th Coccidioidomycosis Study Group
Tucson, Arizona, March 31, 2001

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Chairperson: Hans Einstein

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2. Ampel NM, Carroll C. On-going prospective clinical evaluation of coccidioidomycosis in a Veterans Affairs Medical Center within the coccidioidal endemic area.
3. Hoffman KM, Fritz J, Howell S, Murphy J. The effect of coccidioidomycosis on blood chemistry in captive chimpanzees (Pan troglodytes).
4. Kolviras K, Comrie A. Development of a climate-coccidioidomycosis predictive model.
5. Fisher FS, Bultman MW. Definitive parameters and modeling of the saprophytic habitat of *Coccidioides immitis* in Arizona with a spatial fuzzy system.
6. Cano MV, Lyon III GM, M'ikanatha N, Lindsley M, Pappagianis D, Hajjeh RA. Long-term storage of *Coccidioides immitis*.

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9. Williams PL, Zuker K, Leib SL, Leppert D, Kamberi P, Sobel RA, Clemons KV, Stevens DA. Production of cytokines within the CSF and within the brain ventral artery of rabbits with coccidioidal meningitis/vasculitis.
10. Peng T, Shubitz LF, Perrill R, Simons J, Orsborn KO, Galgiani JN. Recent studies of Ag2/PRA as a vaccine candidate.

11. Kerekes K, Johnson S, Ampel NM, Oamek M, Zimmermann CR, Pappagianis D. Further (ion-exchange) fractionation of the 27K coccidioidal vaccine: immuneprotection and *in vitro* lymphocyte stimulation.

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15. Shubitz LF, Matz ME. Use of Abelcet to treat dogs with severe coccidioidomycosis: Open-label uncontrolled clinical trial.
16. Kamberi P, Sobel RA, Clemons KV, Stevens DA, Williams PL. A murine model of coccidioidal meningitis.
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1. Coccidioidomycosis in the Liver Transplant Clinic at Mayo Clinic Hospital: A Preliminary Review

Janis E. Blair, MD
Division of Infectious Diseases
Mayo Clinic Hospital, Phoenix, AZ

Background: Coccidioidomycosis (cocci) in patients with solid organ transplantation is most often seen in the first post-transplant year, is often disseminated, and has significant morbidity and mortality. Risk factors include prior history of cocci, positive serology, high dose steroids, and treatment of acute rejection.

Aim: To describe the incidence and clinical characteristics of cocci in the liver transplant candidates and recipients attending the Liver Transplant Clinic at Mayo Clinic Hospital.

Results: From June 1999 through March 2001, 127 patients were placed on the waiting list for liver transplantation. Three of these patients (2.3%) presented initially with asymptomatic, but active coccidioidal infection, and during the wait for transplantation, 2 new cocci infections occurred (overall incidence 5/127, or 3.9%). As of March 2001, 55 patients had undergone liver transplantation; 53 patients did not require cocci prophylaxis, 2 received prophylaxis (1 for recently-active cocci, 1 for prior history). One new case of cocci infection occurred in this period (1/55, or 1.8% overall incidence), and no asymptomatic cocci infections were identified by routine monitoring. A total of 112 patients have been followed post transplantation (55 from MCH, 58 from other programs). Of these, 3 patients have had new cocci infections (4/112, 4%) following transplantation. All 3 patients had disseminated infection, and none died. No infections occurred after the first year post transplantation. Twenty-seven patients who had liver transplants performed in other (non-endemic areas) states relocated to Arizona more than one year after transplantation. None of these 27 developed cocci.

Conclusions: The incidence of cocci in patients awaiting liver transplantation is higher than anticipated. Targeted prophylaxis for cocci is a reasonable approach for patients undergoing liver transplantation in an endemic area. Relocation of transplant patients to Arizona is safe and does not require prophylaxis. Further follow up on of each of these patient groups is needed to solidify findings.

2. Coccidioidomycosis in a Veterans Affairs clinic: an on-going project.

Neil M. Ampel, Deborah S. Carroll.

Southern Arizona Veterans Affairs Health Care System (SAVAHCS) and the University of Arizona, Tucson, AZ.

We continue to follow patients with various forms of coccidioidomycosis in a referral clinic at a Veterans Affairs Medical Center within the coccidioidal endemic region. Study methodology included active recruitment of all patients with a diagnosis of coccidioidomycosis at SAVAHCS based on either direct referral by the patient's primary care provider or by review of pathology, serology or culture reports followed by direct patient recruitment. On entry, all subjects provided informed consent and the study was approved by the IRB of the University of Arizona. Subjects were followed as clinically indicated and all laboratory tests were obtained at clinical discretion. By March 14, 2001, a total of 113 subjects have been recruited into the clinic, a gain of 44 from the previous year. The mean age was 60 years (range: 25 - 84) and 84 were white, 25 African-Americans and 5 were Amerindian. Of these subjects, 106 were non-hispanic and 107 were male. Seventy-three of the subjects had some form of pulmonary coccidioidomycosis, including 22 with nodules, 22 with primary focal pulmonary disease, 11 with chronic pulmonary disease, 11 with cavities, and 4 with diffuse pulmonary disease. Of the 40 with extrathoracic disseminated coccidioidomycosis, 14 had bone or joint involvement, 15 had soft tissue disease, and 11 had meningitis. The median time of coccidioidal diagnosis was 2.2 years (range: 2.3 - 25) and the median IDCF titer was 2 (range: 0 - 256). Forty-eight were on no antifungal therapy, while 55 were receiving fluconazole; 7 itraconazole; 2 ketoconazole; and 1 intravenous amphotericin B therapy. There was a significant difference in the type of clinical disease and the antifungal therapy, with all subjects with meningitis receiving fluconazole ($p < .001$). There was a significant difference between the race of the subject and the type of coccidioidomycosis, with African-Americans being more likely to have disseminated coccidioidomycosis than other races ($p = 0.026$). These data represent a continued analysis of an on-going study. Follow-up up to 122 months has been acquired.

3. The Effect of Coccidioidomycosis on Blood Chemistry in Captive Chimpanzees (Pan troglodytes)

K.M. Hoffman, J. Fritz, S. Howell, J. Murphy
Primate Foundation of Arizona

This study considers the effect of coccidioidomycosis on blood chemistry values for chimpanzees at the Primate Foundation of Arizona (PFA). PFA is located in a desert region near Phoenix, Arizona -- an area endemic for coccidioidomycosis. In this study, we compared blood chemistry values for 30 chimpanzees treated for coccidioidomycosis to those derived from 91 healthy chimpanzees gathered during the same period (1974 to 2000) to better understand the effects of this systemic infection on bodily function. Blood chemistry values comprised more than 50 variables including those related to hematology, leukocyte values, serum electrolyte values, and serum chemistry related to pancreatic, renal and liver function. Treatment modalities entailed antifungal agents currently used (imazoles: ketoconozole, fluconozole, and amphotericin B or a combination) and those tested for possible future use for humans (the triazole Bay R 3780, nikkomycin Z). Bay R 3783 and ketoconozole were the most common treatments. Values were compared using a *t*-test ($p \leq 0.01$). Equality of variances were tested using the folded F method. When variances were equal, a pooled test was conducted; when variances were unequal, the Satterthwaite test was used. While most results were within established normal ranges based on human blood chemistry, we found hemoglobin, MCV, MCH, bilirubin/total, bilirubin/indirect and iron were significantly lower in coccidioidomycosis subjects than their normal counterparts. In contrast, potassium (T/pooled = -3.38, p = 0.0011) was significantly higher in coccidioidomycosis subjects than their normal counterparts. Results support a diagnosis of anemia of chronic disease and thrombocytosis secondary to a cocci-induced inflammatory response. Further exploration of the cause of the hyperkalemia is being investigated.

4. Development of a climate-coccidioidomycosis predictive model

Kolviras, K., Comrie, A.

Previous studies indicate a relationship between temperature and precipitation, particularly alternating periods of wet and dry conditions, and valley fever incidence. Given the large number of valley fever infections within the United States, as well as high treatment cost, researchers have attempted to anecdotally estimate the number of cases based on climate conditions. In this research, exploratory data analysis, including bivariate analysis and compositing of antecedent climate conditions, aided in the understanding of the relationship between climate and valley fever incidence. Monthly predictive models

were then created using multivariate regression to estimate incidence based on current or forecast climate conditions, using temperature and precipitation variables. The models were tested on independent data, and the best model results were found for the months with the highest incidence. The models can provide government health officials and health care providers with a warning of possible increased incidence in the coming months particularly when used in conjunction with climate forecasts.

5. Definitive parameters and modeling of the saprophytic habitat of *Coccidioides immitis* in Arizona with a spatial fuzzy system

Frederick S. Fisher[1] and Mark W. Bultman[2]
[1]USGS Contractor and [2] U.S. Geological Survey, Tucson, AZ

Coccidioidomycosis is a public health issue of increasing importance to humans in the southwestern U.S. and in parts of Central and South America. It also affects domestic animals and wildlife. It is caused by *Coccidioides immitis,* a dimorphic soil-inhabiting fungus. The saprophytic phase of the fungus is characterized by branching segmented hyphae that form a network of mycelium in the upper (5 - 20 cm) horizons of soils. As the fungus matures arthroconidia (spores), 2 to 5 microns in size, are formed as barrel shaped, rectangular segments of the hyphae that can be easily separated by soil disturbance (natural or anthropogenic) and consequently dispersed by the wind. If airborne arthroconidia are inhaled by an appropriate host primary infection may occur and the parasitic phase of the *C. immitis* lifecycle is initiated.

Soil characteristics that provide the necessary conditions for the growth of *C. immitis* are; 1) soil temperature (controlled by the amount of sunlight, vegetation cover, soil color, rainfall, cloud cover, elevation, slope, and sun aspect); 2) soil textures that provide adequate pore space for moisture, oxygen, and growth; 3) soils containing some organic material content for the supply of carbon and nitrogen; and 4) soils with a capacity to hold some moisture in the upper (30 cm) parts of the profile. In Arizona, the combination of these essential factors is mostly present in soil families mapped as hyperthermic arid, thermic arid, and thermic semiarid soils. All of the known *C. immitis* growth sites in Arizona are located in these three families. These soils cover approximately 16,443,000 hectares in Arizona and are generally distributed throughout the southern third, along the western border, and along the bottoms of the deeper canyons of the state. The mean annual soil temperatures of these soils ranges from 15 degrees C to over 22 degrees C and they receive annual precipitation from less than 250 mm to 410 mm.

Habitat modeling of the saprophytic phase of the *C. immitis* life cycle is difficult due to the limited number of known growth sites. This confounds the establishment of statistical relationships of the physical, chemical, and biological habitat parameters. Therefore, habitat modeling is being accomplished using analysis of the physical properties of known *C. immitis* sites and a spatial fuzzy system. The fuzzy system is capable of translating structured knowledge into a flexible numerical framework and processing it with a series of if-then rules called fuzzy associative memory rules. In effect, the fuzzy system reduces the dimensionality of the system to a manageable set of fuzzy variables.

The fuzzy system was applied to each 30-meter spatial cell over the study area, Organ Pipe Cactus National Monument, Arizona. The resulting product is a map depicting each cell's favorableness for hosting *C. immitis* based on a scale of 0 to 1, which we define as its fuzzy habitat suitability index. The fuzzy system allows modelers to change and update relationships between the variables as more is learned about *C. immitis* habitat. It also allows dynamic representation of climate related variables and can be used to predict changes in habitat with changing climate.

6. Coccidioidomycosis in travelers returning from Mexico--Pennsylvania, 2000.

M. V. Cano[1], G. M. Lyon III[1], N. M'ikanatha[2], M. Lindsley[1], D. Pappagianis[3], R. A. Hajjeh[1]

[1]CDC, Atlanta, GA; [2]Pennsylvania Dept. of Health, Harrisburg, PA; [3]Univ. of California, Davis, CA

Background: On January 24, 2000, 35 church members from Pennsylvania traveled to Hermosillo, Mexico to construct a church. Within 2 weeks of returning home, most of the travelers experienced influenza-like symptoms. Prompted by clinical suspicion, initial testing of serum specimens at CDC suggested a diagnosis of coccidioidomycosis. To determine the extent of the outbreak and to identify potential risk factors for developing coccidioidomycosis, the Pennsylvania Department of Health and CDC conducted a cohort study among church members.

Methods: Acute- and convalescent-phase serum samples from consenting church members were tested for antibodies to *Coccidioides immitis* by immunodiffusion and complement fixation at both CDC and the University of California-Davis. A case was defined as a positive serologic test for coccidioidal antibodies by 1) detection of coccidioidal immunoglobulin M by immunodiffusion (IDTP) or 2) detection of rising titer of coccidioidal immunoglobulin G by complement fixation or a positive immunodifusion (IDCF) in a church

member from Pennsylvania who had traveled to Hermosillo during January 24-February 2, 2000. All participants completed a standardized questionnaire about medical and travel history, environmental exposures and activities while in Mexico.

Results: A questionnaire and at least one serum sample were obtained for 30 (86%) of the 35 church members. Twenty-nine (97%) were men; mean age was 45 years (range: 18-62). Based on serologic testing, eight (27%) persons met the case definition, seven of whom were symptomatic. The most common symptoms were fatigue, fever, arthalgias and myalgias (71% in each). The median duration of symptoms was 7 days (range: 2-35). Eighteen (78%) of 23 ill persons sought care from at least one health care provider. Six (50%) of 12 persons who had chest radiographs had abnormal findings. One person required hospitalization in an intensive care unit for 1 day. No activities or other conditions were significantly associated with infection or symptomatic disease. However, 22 (73%) church members reported working in extremely dusty conditions. Although 11 (37%) persons reported history of previous travel to coccidioidomycosis-endemic areas, none of the travelers had prior knowledge of the disease and its risk factors.

Conclusion: This is the second outbreak of coccidioidomycosis among church group members traveling to Mexico reported in the last 3 years. As travel by similar groups to coccidioidomycosis-endemic areas increases, it underscores the need for increased awareness of coccidioidomycosis and its risk factors among susceptible persons, especially among persons who engage in construction work or other activities in dusty environments. Health care providers should consider coccidioidomycosis in travelers returning from areas where the disease is endemic and who present with influenza-like illness.

7. Flow cytometric analysis of cellular immunity in human coccidioidomycosis.

Neil M. Ampel, Lara A. Kramer, Lijin Li, Deborah S. Carroll, Kathleen M. Kerekes, Suzanne M. Johnson, Demosthenes Pappagianis.

Southern Arizona Veterans Affairs Health Care System (SAVAHCS), the University of Arizona, Tucson, AZ; and the Department of Microbiology/Immunology at the University of California at Davis, Davis CA.

Assessment of cell-mediated immunity (CMI) in coccidioidomycosis is important for both determining prevalence of coccidioidal infection in a geographic area as well as examining immunologic function among persons with symptomatic coccidioidomycosis. Skin-testing has been the standard method by which coccidioidal CMI has been determined. However, this test is not currently available in the United States. We examined the use of flow cytometric analysis of expression of the activation marker CD69 on CD3 lymphocytes after stimulation with the coccidioidal antigen preparation T27K as a technique to measure coccidioidal CMI. A total of 46 subjects with various forms of coccidioidomycosis were studied. There was no significant difference in the percent of cells expressing CD69 between 21 subjects with disseminated coccidioidomycosis compared to 25 with pulmonary coccidioidomycosis ($p = 0.358$), but there was a trend toward higher percentage of cells expressing CD69 among those currently not receiving antifungal therapy compared to those who were receiving therapy ($p = 0.053$). The log IDCF titer was inversely proportional to the the percentage of cells expressing CD69 and, among those who responded, CD69 expression increased with increasing concentrations of the coccidioidal antigen T27K. There was a highly significant association between CD69 expression and simultaneous production of the Th1 cytokines interferon-g and IL-2 but not with the production of the Th2 cytokines IL-4, IL-5, and IL-10. In summary, measurement of CD69 expression on CD3 lymphocytes using flow cytometry is a promising technique for assessing cell-mediated immunity among donors with various forms of coccidioidomycosis.

8. Dendritic cells pulsed with coccidioidal antigens induce lymphocyte activation from individuals not exposed to *Coccidioides immitis*.

John O. Richards, Neil M. Ampel, John N. Galgiani and Douglas F. Lake

Dendritic cells (DC) are potent antigen presenting cell and are responsible for initiating an immune response. DC are very efficient at antigen uptake and undergo maturation to make them potent activators of T lymphocytes. The mechanism of T cell priming has not been characterized in *C. immitis* infection. T lymphocytes from non-immune individuals have failed to induce lymphocyte proliferation. Monocyte derived DC were generated with GM-CSF and IL-4 from non-immune individuals. DC were then pulsed with either TSL, T27K or left untreated. DC pulsed with TSL induced DC maturation whereas DC pulsed with T27K remained immature. TSL matured DC induced T cell proliferation from non-immune individuals. T27K pulsed DC required maturation with tumor necrosis factor-a and prostaglandin E2 in order to stimulate a T lymphocyte response. DC matured during TSL pulsing or pulsed with T27K and TNF-a/PgE2 were both capable of stimulating IFN-g secretion from non-immune lymphocytes. These data demonstrate that DC are capable of stimulating T lymphocyte expansion and cell-mediated immunity from individuals previously not exposed to *C. immitis* infection.

9. Production of metalloproteinase 9 (MMP-9) within cerebrospinal fluid and assessment of specific cytokines/chemokines within the brain ventral artery (VA) of *C. immitis* (Ci) infected rabbits demonstrating coccidioidal meningitis/vasculitis (CM/V).

Authors: Williams PL*, Zucker K, Leib SL***, Leppert D***, Bifrare YD***, Kamberi P†‡, Sobel RA †‡, Clemons KV †‡„ Calderon L†, Stevens DA †‡**

***Kaweal Delta Health Care District, Visalia, CA; **Valley Chidren's Hospital, Fresno, CA; ***Univer of Bern, Switzerland; †Calif Instit Med Res and Santa Clara Val Med Ctr, San Jose, CA; ††Dept Vet Aff Health Care Sys, Palo Alto, CA; ‡Stanford Univer, Stanford, CA**

MMP-9 is released from inflammatory cells and resident brain and vascular cells and is implicated in inducing meningeal inflammation, vasculitis, and break-down of the blood-brain barrier in a variety of non-*C. immitis* CNS infections. Using zymography, we were able to demonstrate significant production of MMP-9 within CSF over a 2-week period in 5 New Zealand white male rabbits following an intracisternal injection of 5 x10^4 Ci arthroconidia when compared to 5 controls. All infected rabbits manifested impaired mobility, abnormal posturing, weight loss, fever, CSF leukocytosis, and positive CSF, brain, and spinal cord

cultures. Four infected rabbits survived 15 days; all showed CM histologically and 3 rabbits had vasculitis. CSF was obtained on days 0, 3, 7, 10, and 15. The ventral artery was harvested in all rabbits at time of euthanasia, day 15 post infection. PCR demonstrated up-regulation of IFN gamma, IL-2, and iNOS with a trend for up-regulation of MMP-9 whereas CCRI, IL-1, IL-6, IL-10, MCP-1, TGF-beta, TNF-alpha, and PDGF-F were not up-regulated when compared to controls. Future studies will address MMP-9 blockers in addition to further studies of the temporal production of the above gene products at earlier and later times.

10. Recent Studies of Antigen 2/PRA.

Peng T, Shubitz LF, Perrill R, Simons J, Orsborn KO, Galgiani JN.

Valley Fever Center for Excellence, Southern Arizona VA Health Care System, and University of Arizona, Tucson, AZ.

The proline-rich protein component of Antigen 2 (Ag2/PRA) has been studied as a purified recombinant antigen (rAg2/PRA) for vaccine in both BALB/c and C57B/6 mice to protect against intranasal infection with arthroconidia of *Coccidioides immitis*. Subcutaneous vaccination produced significant prolongation of survival in BALB/c mice. However, protection was not evident if infecting inoculum sizes of greater than 10 arthroconidia per mouse were used. Administering vaccine intranasally extended protection in BALB/c mice to inoculum sizes of up to 84 spores per animal. Furthermore, subcutaneous immunization of C57B/6 mice afforded protection with inoculum sizes of up to 145 arthroconidia. The inoculum effect may reconcile previously noted differences in results between laboratories with respect to Ag2/PRA eliciting protection against intranasal infection.

In other studies, DNA vaccination was carried using portions of Ag2/PRA in Vical vector 1020 to immunize BALB/c against intraperitoneal infection. Significant reduction in pulmonary fungal burden was evident in mice immunized with cDNA encoding amino acid 1-106 and 27-106 but not with sequences encoding 90-194 or 90-151. These studies, which suggest that the protective effect resides in the N-terminal half of the protein, will be extended in future studies by using recombinant antigens in place of DNA as vaccines.

Studies to date with Ag2/PRA continue to support this protein as a vaccine candidate antigen for use in future clinical trials.

11. Further (ion-exchange) fractionation of the 27K coccidioidal vaccine: immunoprotection and *in vitro* lymphocyte stimulation

Kerekes K, Johnson S, Ampel N, Oamek M, Zimmermann C, Pappagianis D

The 27K vaccine derived from whole killed spherule of *C. immitis* (*ci*) was fractionated by fast protein liquid chromatography. This yielded a gel filtration fraction I (GFI) that when administered with alum was protective against intranasal (i.n.) challenge of mice with *ci*. Further fractionating GFI by anion exchange (AE) chromatography yielded six fractions (AE1-AE6). An additional fraction, CW, was recovered by washing the AE column with various electrolyte solutions. Of these fractions, AE6 and CW were the most protective against a lethal i.n. challenge (1500 arthroconidia).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of AE6 yielded discrete protein bands when stained with silver. When the same gel was stained with a glycoprotein stain, only one band at approximately 5 kDa stained, indicating limited presence of carbohydrates.

Using flow cytometry, AE6 was tested for its stimulation in vitro of human lymphocytes from immune and non-immune donors. AE6 specifically stimulated only the immune cells and the stimulation was proportional to the concentration of AE6 antigen.

Further fractionation of AE6 is underway to identify individual antigens that protect against lethal in. challenge with arthroconidia.

12. Status of the Valley Fever Vaccine Project.

Rutherford G, Hector R.F.

The Valley Fever Vaccine Project is an academic-based, cooperative effort to discover and develop a safe and effective acellular vaccine for the prevention of coccidioidomycosis in humans. Research is funded at five institutions, with two additional institutions, CSUB and UCSF, serving administrative and scientific leadership roles. A broad range of approaches, ranging from classical biochemical fractionation to genomics, have been applied to these efforts. Once isolated, antigens are evaluated using a variety of murine models, with those showing promise being circulated amongst the other laboratories for confirmation of activity. To date, four patent applications for antigens have been filed, based on these efforts. As a prelude to testing of candidate antigens in a higher species, a study has been initiated at the California Regional Primate Center, UC Davis, to establish a survival model of coccidioidomycosis in cynomolgous monkeys. Additionally, the project has commissioned a prevalence and incidence study of naturally-acquired coccidioidomycosis in dogs in the Tucson/Phoenix area. Based on the results, it may

prove possible to evaluate the vaccine in dogs prior to human trials. Lastly, in order to make coccidioidin available in support of the vaccine trials, an IND was file with the FDA last year, and Phase 1 trials are currently in progress at the Naval Medical Center, San Diego, to establish the safety and suitability of this skin-test antigen. Initial results indicate a high rate of response to the thimerosal preservative, necessitating changes in the formulation before studies resume.

13. Successful Treatment of a Critically Ill Patient with Disseminated Coccidioidomycosis Using Adjunctive Gamma Interferon

Timothy Kuberski, M.D. and Phillip Rubin, M.D.
Good Samaritan Hospital and Medical Center, Phoenix, Arizona

A critically ill patient with disseminated Coccidioides immitis and respiratory failure was hospitalized one hundred thirty-seven days in the intensive care unit (ICU). The patient could not be weaned from the ventilator despite treatment with conventional anti-fungals. The addition of gamma interferon to the therapeutic regimen after seventy days in the ICU resulted in gradual clinical improvement. Improvement was felt to be related to the use of gamma interferon because the patient had not been improving despite two months of anti-fungals, the treatment overcame the poor prognosis associated with an extended time in the ICU and improvement was associated with a decrease in complement fixation antibody titers to C. immitis. Gamma interferon is an immune modulating drug and enhancement of the immune response in this patient may have been enough to resolve a previously refractory C. immitis infection.

14. Chitobiase present in the 27K coccidioidal vaccine: molecular sequencing

M. Oamek, S. Johnson, K. Kerekes, C.R. Zimmermann, D. Pappagianis

Current studies in our laboratory have involved a two part approach to vaccine development for *Coccidioides immitis*. The first is to separate the 27K vaccine into fractions and the second is to isolate and clone individual protein components from the 27K vaccine. Recently, chitobiase activity was detected in the 27K vaccine using a chitobiase assay on an isoelectric focusing gel. Upon further examination, chitobiase activity was also detected in the anion exchange fractions 1 and 3. To characterize further the chitobiase protein found in the 27K vaccine, degenerate primers were designed to obtain a PCR product from which a full length chitobiase cDNA was eventually obtained. The full length chitobiase cDNA was cloned and sequenced and represents a 2,113 base pair product. A BLAST search on the full length cDNA sequence showed a 65% homology to the gene of *Aspergillus nidulans* and a 59% homology to

Penicillium chrysogenum. The amino acid sequence was deduced from the full length nucleotide sequence and was calculated to be a 595 amino acid protein with a molecular weight of 68 kDa and a pI of 9.3. Future studies include the expression of the recombinant protein as a vaccine candidate.

15. Use of Abelcet to treat dogs with severe coccidioidomycosis: an open-label, uncontrolled clinical trial.

L.F. Shubitz[1] and M.E. Matz[2]

[1]Department of Veterinary Science and Microbiology, University of Arizona, and [2]Southwest Veterinary Specialty Center, Tucson, AZ

Coccidioidomycosis in dogs can be a severe, life-threatening disease. It is routinely treated with oral ketoconazole, fluconazole, or itraconazole, but some dogs fail to improve or have progressive disease in spite of medication. While amphotericin B deoxycholate might benefit these cases, the drug is used with great reluctance in veterinary medicine because of the sensitivity of the canine kidney to the drug. Abelcet, a lipid-complexed form of amphotericin B, offers the option of treating severely ill dogs with amphotericin B with a greatly reduced risk of renal impairment. The Liposome Company agreed to donate Abelcet. Eighteen dogs with demonstrated coccidioidal disease and normal kidney function that had failed treatment with one or more azole drugs were enrolled in the study. Median age was 5 years (range 8 mos-8 yrs) and median cocci titer at entrance into the study was 1:8 (range 1:2-1:128). There were 8 females and 7 males. Dogs were treated with 1 mg/kg or 2 mg/kg of Abelcet for 15 treatments. At necropsy, 2 dogs had disseminated aspergillosis and 1 had primary lung neoplasia; 7/15 dogs with coccidioidomycosis were alive more than 12 months after Abelcet treatment. Three dogs died or were euthanized during treatment for progression of coccidioidomycosis. Two dogs developed renal toxicity and one of these remained in chronic renal failure; this dog received only 4 doses of Abelcet. In summary, Abelcet appears to be relatively safe at doses of 1-2 mg/kg/treatment in the dog, and it offers a treatment option for dogs with severe coccidioidomycosis that are failing oral antifungal medications.

16. A Murine Model of Coccidioidal Meningitis

P. Kamberi,[1, 2] R. A. Sobel,[2] K. V. Clemons,[1, 2, 3] D. A. Stevens,[1, 2, 3] and P. L. Williams[6]

[1]California Institute for Medical Research, San Jose; [2] Stanford University, Stanford; [3] Santa Clara Valley Medical Center, San Jose; [4]Kaweah Delta District Hospital, Visalia

Coccidioidal meningitis (CM) is a lethal human disease. Recent development of rabbit model enabled study of pathogenesis and Rx, but expense and handling of animals are limiting. The mouse is best-defined animal species, and immunological reagents and genetically manipulated animals are available, however, access to the tiny CSF is formidable. A reproducible CM model was established by intrathecal injection of arthroconidia in 9 wk. CD-1 mice. CSF was sampled by cisternal puncture, producing $\geq$ 5 ml. Lethal infection developed in all 40 mice given 10, 15, 30 or 60 arthroconidia, with dose-responsive survival times from 7 to 15 days. Ruffled fur, lethargy, ataxia or paralysis preceded euthanasia or death in all animals. Quantitative organ cultures revealed a mean of 3.3-5.3 $\log_{10}$ CFU/g brain, in a dose-responsive manner, and dissemination to the lungs, spleen and kidney. Histopathology showed acute CM in brain and cord, with some parenchymal invasion. Subsequent temporal studies after 27 arthroconidia challenge revealed brain (4/5) and cord (2/5) culture positive on day 3, with mean $\log_{10}$ CFU/organ <1.09; CSF wbc/mm^3 and 33 CFU/ml; histopathology was unremarkable. By day 8, all mice examined were culture positive, with mean $\log_{10}$ CFU/organ of 5.0 and 4.1 in brain and cord, respectively. CSF showed 4833 wbc/mm^3 and 3425 CFU/ml, extrameningeal dissemination had occurred. Histopathology revealed non-thrombotic meningeal arteritis and intraparenchymal abscesses in addition to meningitis. In other studies, groups given 27 arthroconidia and ketoconazole 50 mg/kg b.i.d. postinfection for 14 days had prolonged survival ($p<0.05$) vs. controls, largely by suppression of lung disease, and granulomatous CM plus abscesses, enabling development of a chronic CM model. With development of these models, studies of pathogenesis, host response and therapy are now possible.

17. COCCIDIOIDAL MENINGITIS: EVALUATION OF INTRATHECAL AMPHOTERICIN B AFTER NON-RESPONSE TO FLUCONAZOLE

V. Shirvani, D. J. Gaucher, R. H. Johnson, J. W. Caldwell,
P. L. Williams, H. Einstein
Department of Medicine, Kern Medical Center,
Bakersfield, California

Objectives:
To determine the response rate to fluconazole within 12 months of therapy, and to evaluate the response rate to intrathecal amphotericin B in fluconazole non-responders.

Methods:
Retrospective chart review of 122 cases of coccidioidal meningitis diagnosed by compatible clinical findings, CSF pattern, serum complement fixation titer, from July 1991 to December 2000, at Kern Medical Center, Bakersfield, California. The standard scoring system of Mycoses Study Group (MSG) was used. Patients were grouped at 12 months into responders to fluconazole, non-responders to fluconazole, and unevaluable. Response was defined as MSG score reduction of $\geq$40% from baseline. Non-responders were divided into 2 sub-groups, one continued on fluconazole alone, and the other on intrathecal amphotericin B. Patients were scored at 3, 6, 9 and 12 months after start of treatment. Data

was entered in Epi Info version 6.0, analyzed by chi square, and p values < 0.05 were considered significant.

Results:
43% of evaluable cases of coccidioidal meningitis did not respond to fluconazole within 12 months of therapy. Among the non-responders who were started on intrathecal amphotericin B, 65% had a ≥40% reduction in MSG score within 3 months and 91% after 9 months of follow up. The response was largely related to the decrease in CSF CF titer and protein, and the increase in CSF glucose level, rather than the change in CSF
WBC count.

Conclusions:
Fluconazole non-response rate in this study is somewhat higher than previous reports. A very high percentage of these non-responders can be salvaged by intrathecal amphotericin B.

18. Cost-benefit analysis of treating acute coccidioidal pneumonia with azole drugs

Rafael Laniado-Laborín MD, MPH,
Universidad Autónoma de Baja California

In the US, there are an estimated 100,000 *Coccidioides immitis* infections annually; 5 % of those will develop a clinical problem that will need treatment at an estimated total cost of 120 million dollars. Unfortunately, the rates of failure and relapse after treatment of chronic pulmonary or disseminated Coccidioidomycosis are disappointingly high.

Currently, patients without evidence of extensive coccidioidal infection, or risk factors for dissemination do not receive antifungal therapy. Should we treat acute coccidioidal pneumonia? This is a controversial topic, and currently, it is impossible to answer this question. Our current understanding of optimal management of acute coccidioidal pneumonia is severely limited by the absence of randomized comparative trials that evaluate the different forms of therapy.

Despite the time-honored empiric recommendation that mild and moderate cases of Coccidioidomycosis do not benefit from antifungal therapy, there is no valid evidence that proves that antifungal therapy for such patients will not prevent the development of complications. Recent data from a nonrandomized trial suggest that primary illness may be averted by early treatment of acute disease with an abbreviated course of a triazole for one to six months.

Conclusions
1. Current rates of failure and relapse after treatment of chronic pulmonary or disseminated Coccidioidomycosis are unacceptably high.
2. A randomized clinical trial is needed to determine if azole treatment of acute pulmonary forms could prevent the development of secondary forms.

Annual Presentations of Interesting Coccidioidomycosis Cases:

Cocci pneumonia in a neonate

Peter M. Cole
Clinical Assoc Prof of Pediatrics
College of Medicine, University of Arizona
Attending Pediatrician, St. Joseph's Hospital, Phoenix, AZ

Patient JD (DOB=8/1/00) was an apparently normal infant until fever was noted at 19 days of age at which time he was hospitalized and treated for a developing right upper lobe pneumonia. Interestingly, he had few clinical respiratory symptoms, documented on several examinations. Despite intravenous treatment with multiple antibiotics, he remained febrile in the 102F-103F range, and was transferred on 8/28/00 to St. Joseph's Hospital in Phoenix.

His chest x-ray worsened, with a prominent focal right upper lobe process. Diflucan, in addition to other antimicrobials, was started empirically prior to bronchoscopy. Washings obtained at bronchoscopy subsequently grew out Coccidioides immitis and the infant's serum complement fixation coccidioidal titer became positive in a 1:2 dilution. Treatment was continued with IV Diflucan. CSF culture was negative for Coccidioides.

The infant remained febrile for 15 consecutive days. Subsequently he has remained clinically well and is maintained on oral Diflucan. The chest x-ray improved considerably, but residual changes are evident in the upper lobes. Growth and development have been normal.

The infant had a history of direct exposure to a local dust storm during the first week of life. Mother's cocci AB by CF done 10/25/00=<1:2, making it unlikely that this was congenital cocci.

A Case of Coccidioides Fungemia

Gary Skankey, MD
University of Nevada

Hx: 64 yo Indonesian Male saw orthopedist for non-healing Fx of thumb. X-ray revealed lytic lesion. Bx revealed coccidioides spherules. Pt. Referred to ID specialist 2/13/97. Symptoms included thumb pain, mild dry cough, no F/C/S.
PMHx: Diabetes, pneumonia 11/96, cocci IgM+
SHx: Lives in Pahrump, carpet cleaner for MGM Grand
PE: ecchymotic thumb, no open wounds, no gross deformity
CXR: 11/96 - LUL infiltrate, 2/97-clear
Lab: Cocci CF 1:32

Treatment and course: Started Ampho B 2/13/97, switched to Abelcet for increased Cr. Cocci CF decreased to 1:2 by 6/97. Switched to fluconazole. Follow up Cocci CF <1:2. Then, pt. lost to follow up.

2/20/99- Saw private MD in Pahrump for pain in thumb. Was treated with prednisone 60mg PO QD.
3/2/99- Admitted to hospital in septic shock, fever, coma, fluctuent mass right hand.
X-ray: destruction of 4th metacarpel
To OR: hand I&D revealed frank pus, gross osteomyelitis
Lumbar Puncture: 72 WBC (55% segs), 1545 RBC, Protein=148, Glucose=153 (serum=269), fungus smear negative, coccidioides titers negative.
Cultures: Blood 3/1 and 3/4/99 = coccidioides immitis
Pus from hand = coccidioides immitis
Cocci CF: 1:64
Treatment and course: Fluconazole 800 mg QD and Amphotericin B 40 mg QD. Shock resolved after five days. CSF pleocytosis decreased from 72 WBC on 3/3/99 to 19 WBC on 3/19/99.

Unfortunately, pt. Never regained consciousness and a had prolonged hospitalization complicated by multiple episodes of bacterial sepsis. Pt. Expired in 6/99.

46Th. Annual Meeting - April 6, 2002
Davis, California

The participants of this year's meeting voted to hold the next meeting **(46th annual) in Davis, California** on **Saturday, April 6, 2002.** Autumn Davidson and Richard Hector have graciously offered to co-host our meeting which will be held on the University of California, Davis campus.

Meeting Location: **170 Schalm Hall University of California, Davis**
(Visitor Parking Lot 50)

Lunch: **Lunch will be provided** (picnic style).

There will also be a lunch time tour of the Veterinary Medical Teaching Hospital & The Center for Companion Animal Health, School of Veterinary Medicine, University of California, Davis (adjacent to Schalm Hall).

Saturday Dinner location: **University Club Conference Center, University of California, Davis**
(Visitor Parking Lot 5)

Questions may be directed to:

Richard Hector or Autumn Davidson
Rhector@psg.ucsf.edu Adavidson@guidedogs.com

The "Most Unusual Cases of Cocci" session, which has been part of the program for the past twelve years, will be continued. **Paul Williams** will again moderate, and will select participants from cases submitted to him directly. In the past, emphasis has been on the most unexpected cases of cocci and on very difficult cases -- either to solicit suggestions from the group or to show the value (or lack of value) of a specific therapeutic approaches. **Please call or write to Paul if you are interested in presenting a case at this session. He can be reached at the Visalia Medical Clinic, 5400 W. Hillsdale, Visalia, CA 93291; tel (209)738-7568.**

To plan the rest of the program, I need to know what you might like to present and/or what you might like others to present. As usual, the program is open to papers on everything from purely basic science to purely clinical, and all points in between. Data of interest presented elsewhere, or updates of work previously presented are welcome. Our meeting, as always will be open. **Please send full titles and authorship** as you would like them to appear in the program to me at the following address:

John Galgiani, M.D.
Valley Fever Center for Excellence (1-111)
Southern Arizona VA Health Care System
3601 South Sixth Street
Tucson, AZ 85723

vfever@arl.arizona.edu