



# Coccidioidomycosis

STUDY GROUP

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# PROCEEDINGS OF THE ANNUAL COCCIDIOIDOMYCOSIS STUDY GROUP MEETING

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## Abstract 1: Human Mononuclear Cells Inhibit N-acetyl Glucosamine (GlcNAc) Uptake by *Coccidioides immitis* (CI) Arthroconidia

*NM AMPEL, JN GALGIANI, G BEJARANO, R HAYDEN*

Tucson VAMC and University of Arizona Tucson AZ

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The role of mononuclear cells (MNL) in human immunity to CI is not defined. In order to explore this we compared peripheral blood MNL to polymorphonuclear leukocytes (PMN) in their ability to inhibit the uptake by CI arthroconidia and spherules of G1cNAc, a chitin precursor. MNL and PMN were obtained from healthy donors of known skin test reactivity and purified using Percoll and Ficoll-Hypaque. MNL and PMN were incubated with  $2 \times 10^6$  CI arthroconidia for 45 minutes. Labeled GlcNAc was added for 20 min, and the cpm of TGA precipitates was determined. MNL inhibited uptake of GlcNAc by CI arthroconidia in a dose-dependent manner; maximal inhibition occurred at  $12 \times 10^6$  MNL with a mean  $\pm$  SEM Inhibition of  $70 \pm 9\%$  (n=5) compared with  $76 \pm 4\%$  (n=5) for  $2 \times 10^6$  PMN. Inhibition was equivalent using MNL from skin-test positive or negative donors. Neither PMN nor MNL inhibited GlcNAc uptake by CI spherules. When monocytes were depleted from MNL using a Sephadex G-10 column, mean inhibition was decreased by  $49 \pm 6\%$  compared to non-depleted MNL ( $P < 0.01$ , n=5). In conclusion, MNL inhibit GlcNAc incorporation by CI arthroconidia. Monocytes appear to account for this inhibition and it is independent of spherulin skin-test reactivity.

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## Abstract 2: Possible role of a proteinase in endosporulation of *Coccidioides immitis*

*L. Yuan and Garry T. Cole, Dept. of Botany*

University of Texas, Austin TX 78713

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We previously reported isolation of a serine proteinase from the soluble conidial wall fraction of *Coccidioides immitis*. The purified proteinase was identified as a polypeptide band of 36,000 M r sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In this study, we raised monospecific antiserum in rabbits against the purified proteinase for use in immunoelectron microscopy. We showed that immunolabel was localized in the cell wall of both the saprobic and parasitic phases but was most concentrated in the wall of the segmentation apparatus of spherules just prior to endospore differentiation. The total wall fractions of the mycelial phase, as well as those of presegmented and endosporulating spherules, were isolated from in vitro grown cells and then treated with a proteinase inhibitor (phenylmethylsulfonyl fluoride [PMSF]) which irreversibly binds to the residual proteolytic enzyme in the wall isolates. Each fraction was dialyzed, lyophilized, and separately incubated with the active, purified 36,000-M r proteinase. The reaction mixtures were examined spectrophotometrically (A<sub>280</sub>) for decomposition of the substrates. Only the PMSF-treated wall isolated from endosporulating spherules was significantly digested. Active, 36,000-M r proteinase was isolated from intact and viable, endosporulating spherules by brief extraction of the cells with 1% octyl-B-D-thioglucoside, a nonionic detergent. The serine proteinase may be partly responsible for autolysis of the segmentation apparatus of mature spherules, a morphogenetic process which is pivotal for release of endospores and subsequent proliferation of the pathogen.

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### Abstract 3: Separation of Spherule Antigens by Ion-Exchange Chromatography

*John N Galgiani, Kris O. Dugger, John H Law, and Neil M Ampel*

VA Medical Center and University of Arizona, Tucson AZ

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We have recently described the release of antigens from spherules by treatment with toluene (*Diagn Microbiol Infect Dis* 1988; 11: in press). To further resolve specific antigens of this crude starting material, ion exchange chromatography was performed utilizing DEAE-Biogel A. Starting conditions were pH 7.0, conductivity = 330  $\mu\Omega$  and elution was carried out with a linear NaCl gradient (0.0 - 1.0 M). Four peaks were identified of which the first three contained virtually all of the antigen activity detectable by immunoblot and immunodiffusion assays for tube precipitin (TP) antigen. The second of these peaks was reappplied to a DEAE-Biogel A column using a shallower gradient (0.0 - 0.6 M NaCl). This resulted in two major peaks. The first peak has relatively concentrated TP activity as compared to the second whereas proliferative responses by peripheral blood mononuclear cells from coccidioidal skin-test positive patients was relatively similar after stimulation by the two pools. By immunoblot analysis, several bands were recovered in very high purity in various fractions. These included a 100 kDa and an 80 kDa antigen. These fractions could serve as the basis for efficient production of monoclonal antibodies.

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## Abstract 4: Immunoaffinity Isolation of the Coccidioidal tube precipitin-ID-TP antigen

*D. Pappagianis and B. Zimmer*

University of California, Davis, CA

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Immunoaffinity isolation of the coccidioidal antigen responsible for the serodiagnostic tube precipitin (TP) and its immunodiffusion correlate (IDTP) was undertaken. We demonstrated that precipitate produced by TP reaction could be digested with the proteolytic pronase leaving the antigen intact. The soluble antigen was separated from the digested antibody and pronase, was found to be larger than 60 kD after passage through polysaccharide P60, and yielded 2 peaks, both with ID-TP activity when separated by ionic charge on DEAE-sepharose. Further separation by size on Sephacryl 400 indicated molecular weights of 140 kD and 225 kD. Both of these could be adsorbed by concanavalin A from which they were liberated by alpha methylmannoside indicating presence of asparagine linked polysaccharides containing mannose and/or glucose previously shown to be major and minor components respectively of coccidioidal polysaccharide. Neither of the ID-TP reactive components was detectable by SDS-PAGE or immunoblots and did not correspond to any SDS-PAGE band previously described.

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## Abstract 5: Cytological Studies of *Coccidioides immitis* Tube Precipitin Antigen by Immunoelectron Monospecific Antibody

*Jeff Hairson Sung Huang Sun and Rebecca A. Cox*

Audie Murphy Memorial Veterans Hospital and San Antonio State Chest Hospital,  
San Antonio, TX

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Primary infection with *Coccidioides immitis* results, in the production of IgM tube precipitin (TP) antibody. The identification of the coccidioidal antigen that reacts with TP antibody and the production of goat antiserum specific for this serodiagnostic antigen have been reported previously. In the present study, goat anti-TP antibody was employed to assess the cellular location of the TP antigen in the saprobic (mycelia/arthroconidia) and parasitic (spherules / endospores) phases of *C. immitis* by immunoelectron Microscopy. Ultrathin sections of *C. immitis* cell types were reacted with goat anti-TP on for a negative control, normal goat serum and then with rabbit anti-goat immunoglobulin, labeled with colloidal gold particles (15nm). The results established the presence of the TP antigen in the cell walls of *C. immitis*, with a quantitative increase in the walls of Spherules as compared to mycelia. In addition, the TP antigen was detected within cytoplasmic organelles having morphologic and staining characteristics consistent with those of the Golgi apparatus. These results, taken together, indicate that the TP antigen, which is predominantly polysaccharide in composition, is synthesized or glycosylated within the Golgi Bodies.

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## Abstract 6: EVALUATION OF LEATHERS-AWASTHI MEDIUM FOR THE ISOLATION OF COCCIDIOIDES IMMITIS FROM CLINICAL SPECIMENS

*Chester R. Leathers, Mary L. Fried, and Jeff R. Griswold*

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Over 2,600 clinical specimens were evaluated on Leathers - Awasthi Medium (LAM) and a routine battery consisting of four media, for the presence of C. immitis. The following results were obtained:

Total specimens processed	2613
Number positive for <u>C. immitis</u> (3%)	82
LAM positive (70%)	57
Routine Battery positive (87%)	71
LAM and/or Battery positive (96%)	79
Positive specimens undetected	
By LAM and/or Battery (4%)	3

LAM's sensitivity was compared to seven agar media for the detection of C. immitis using separate spore suspensions of five isolates, with the following results:

- LAM was as sensitive as PDA, Blood, IMA, BHI-CC-R. and Littman's Ovgall.
- LAM was as sensitive as SDA-M in 80% of the comparisons.
- Mycosel was more sensitive than LAM in 60% of the comparisons.

Twenty five isolates of C. immitis were exoantigen-tested using extracts obtained from LAM and SDA-M. All unconcentrated extracts from LAM and SDA-M tested positive. Eleven genera (165 isolates) of pathogenic fungi were evaluated on LAM. B. dermatitidis, C. albicans, C. neoformans, H. capsulatum, P. brasiliensis, S. schenckii, and T. beigelii did not discolor LAM. Although A. fumigatus, P. boydii, and G. candidum, did discolor LAM within 7 days. Only the latter yielded some false positives.

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## Abstract 7: DISSEMINATED COCCIDIOIDOMYCOSIS

*David Salkin MD and Milton Huppert PhD*

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In 1965, the VA and Armed Forces started a cooperative study of patients in their hospitals during the 4 years 1955 through 1958 to prepare for the chemotherapy era. We had a 12- to 15- year follow-up.

Of the 706 patients in the study, 111 had extrapulmonary dissemination. The study was shown in a series of tables and featured those with Central Nervous System involvement. The tables show the state of the lungs at the time of dissemination, the racial factors present, the major extrapulmonary sites, the pathogenetic factors, and various phases of meningitis such as the incidence, diagnosis, symptoms, treatment, and prognosis. There were also 3 cases of pseudomeningitis.

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For a copy of the study, write to David Salkin MD, 660 South Fair Oaks, Pasadena, CA 91105  
Telephone: (818) 397-8529

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## Abstract 8: Coccidioidomycosis in HIV-Infected Patients: A Prospective study in an Endemic Area

*CS KAPIAN, NM AMPEL & JN GAIGIANI*

Tucson VAMC and University of Arizona, Tucson AZ

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We have begun a prospective evaluation of HIV seropositive patients to assess the relative contribution of primary vs. reactivated coccidioidomycosis (coccy). To date, 19 AITS, ARC or asymptomatic HIV patients have enrolled, with absolute CD4+ T-lymphocyte counts ranging from 21 to 496 cells/ul (median = 187). Coccidioidal antibodies were transiently detected in one asymptomatic patient with 301 CD4+ cells/ul, who had been in the endemic area for 9 years. Another patient, a lifelong Arizona resident with 101 CD4 + cells/ul, developed disseminated coccy concomitant with seroconversion. No patients had cutaneous reactivity or in vitro lymphocyte proliferative response to coccidioidal antigens. However, of those tested, 77% (10/13) were anergic to 3 other common antigens, and blunted lymphocyte proliferation was detected to Con-A in 56% (5/9), and to FHA in 22% (2/9). Our results support further prospective evaluation and follow-up of HIV seropositive populations, which may elucidate the epidemiology of coccy in this setting. Assessment of cellular immunity to coccy in HIV patients may require the enrollment of patients with higher CD4+ cell counts and with less impaired cellular immunity.

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**Abstract 9: A Microtiter method for MIC testing of spherule-endospore phase *Coccidioides immitis***

*R. Hector. B. Zimmer and D. Pappagianis*

Cutter Labs and UC Davis

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A method was developed for susceptibility testing with spherule-endospore phase *Coccidioides immitis* using a microtiter format. Isolated endospores were used to inoculate wells containing modified Converse medium with varying concentrations of azole or nikkomycin antifungal substances, then were sealed with an acetate film. The plate was incubated at 37°C with shaking for 96 h. after which control wells had visible turbidity, and endpoints were discernible. Microscopic examination revealed that both control and treatment wells maintained cells predominantly in the spherule-endospore phase of growth.

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**Abstract 10: THE USE OF R3783 FOR TREATMENT OF  
COCCIDIOIDOMYCOSIS IN A JUVENILE CHIMPANZEE (Pan troglodytes)**

*H. Hogan and J. Fritz*

Primate Foundation of Arizona, P.O. Box 86, Tempe, Arizona 85281

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The experimental triazole, Bay R3783, was used to treat an established, case of pulmonary coccidioidomycosis in a four and a half year old male chimpanzee (Pan-troglodytes). The dose rate was 10 mg/kg orally, once a day for 93 days. The medication was most rapidly and completely consumed in a koolaid and sugar mixture. By the second week of treatment, the animal's cough had subsided, appetite had returned to normal, and he was no longer lethargic. By the seventh week of treatment, pulmonary densities on radiograph had decreased significantly, the animal's condition was excellent and the cocci titer was decreasing. Therefore, the treatment was terminated by the middle of the fourteenth week. The only noted adverse side effect was a splenomegaly that reduced upon completion of the treatment.

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## Abstract 11: Nikkomycins in therapy of Murine Coccidioidomycosis

*R. Hector, B. Zimmer, D. Pappagianis*

Cutter Labs and UC Davis

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Nikkomycins X & Z (NX and NZI, compounds known to inhibit chitin synthesis in fungi, were employed in in vitro and in vivo experiments against Coccidioides immitis to determine the efficacy of these agents. The MIC's of both these agents were determined versus the spherule-endospore phase to be 0.7 ug/ml, while the MLC was 3.5 ug/ml for NZ and 32 ug/ml for NX. In a murine model of pulmonary coccidioidomycosis in which the animals were infected with approximately  $9 \times 10^3$  arthroconidia therapy initiated with a 48h delay with 20 and 50 mg/kg NZ B.I.D. for 10 days completely prevented deaths while 5 mg/kg resulted in 33% survival. NX at 50 mg/kg resulted in only 44% survival, with fewer survivors at the lower doses. 100% of controls died. Short-term organ loads with NZ at 50 mg/kg showed the lungs of animals infected with  $4 \times 10^3$  arthroconidia were essentially sterilized of the infecting organism with five days therapy. Thus, the nikkomycins deserve consideration at the therapeutic agents against C. immitis.

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## Abstract 12: Intraconazole (IZ) Therapy (Rx) of progressive Coccidioidomycosis (C)

*R. M. TUCKER, W. DENNING, MD M. G. RINALDI, L.H. HANSON, D. A. STEVENS*

Santa Clara Valley Medical Center Stanford University Inst. Medicinal Resistant San Jose and Stanford, CA, University of Texas Health Science Center, San Antonio, TX

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IZ Rx 50 mg/d-200 mg BID was given mean 8.2 mo in 62 courses to 60 patients (pts) with C. Evaluation of serologic, culture, clinical parameters of disease at 6 mo Rx showed responses (R) in 23/38 (61) evaluable (E) courses; partial response (PR) in 6 (16%); non-response (N) in 9 (24%) of pts without prior Rx 12/12 were R; 11/26 with prior Rx were R, 6 PR, N. Mean duration disease in R pts was 68 mo; 92 in N. Of 80 total sites, 52 are E. R or PR were seen in 11/17 lung infections, 9/12 skin, 10/11 bone, 7/9 nodal, 1/1 urogenital. Two of 2 E pts with refractory meningitis responded and intrathecal Amphotericin was tapered (discontinued in 1). IZ had equivalent efficacy in pts with single or multiple sites. Nine/23 are off Rx (mean 12 mos); 2 have relapsed. Limited toxicity included mild GI disturbance in 5; rash 2; hyperbilirubinemia 2; gynecomastia 1. Mean serum peak level 6.8 mcg/ml IZ occurs 4h after dose on 299 mg BID with flat kinetics in steady-state. MIC and MFC for 50 isolates varied from 0.018-2.5 mcg/ml. IZ is well tolerated, fungicidal in vitro and clinically efficacious against C, with IZ N only in pts with N to prior Rx.

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## Abstract 13: Treatment of Coccidioidal Meningitis (CM) with Fluconazole (Flu)

*RICHARD M. TUCKER, MD, LINDA H. HANSON, DAVID W. DENNING, MD, PAUL L. WILLIAMS, MD, MARK R. ECKMAN, MD, DAVID A. STEVENS, MD*

Santa Clara Valley Medical Center Stanford University Inst. Medicinal Resistant San Jose and Stanford, CA. Visalia Medical Clinic, Visalia, CA. Duluth Clinic, Duluth, MN.

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Flu is a recently developed oral triazole that has shown impressive activity in murine models of meningeal and pulmonary coccidioidomycosis. We have treated 11 patients with serologically documented chronic CM with fluconazole at doses of 50- 400 mg/d. Patients includes 9 males and 2 females aged 17-71 years (mean 4½ years). Duration of disease upon entry varied from 3-78 mo. with a mean of 35 mos. All had received intravenous Amphotericin B (AMB); 10 of 11, intrathecal (IT) AMB; 8, ketoconazole; 1 itraconazole and 1, flucytosine. Seven of 11 were receiving concomitant therapy with IT AMB when Flu was begun. A total of 96 patient months of therapy have been administered for a mean of 7 mos. Toxicity has been minimal. Six patients remain on fluconazole, two have died, and two have failed and are on alternative therapy and one is without evidence of disease 5 mos. after completing 12 mos. of therapy. Five patients have shown a response to therapy, 3 no response, and 3 are unevaluable. Two patients have responded to therapy with Flu alone; concomitant therapy was discontinued in 2 others. Treatment failures include 2 on Flu alone and one on AMB IT and Flu; Coccidioides immitis was cultured from CSF; of 2 of these while on therapy. Flu peak concentrations range 2.5-3.5, 4.5-8.0, 8.0-16.5 mcg/ml in serum and 2.0-2.3, 3.4-6.2, 8.0-14.5 mcg/ml in cerebrospinal fluid (CSF) on 50, 100 and 200 mg/d respectively. Mean percentage of CSF to serum concentration was 73.8 at 50 mg/d, 88.7 at 100 mg/d and 77.1 at 200 mg/d. A prolonged half life was seen at all doses in both CSF <end serum. Flu is orally absorbed, achieves high CSF penetration, and has low toxicity. Initial clinical results are encouraging in CM although response is not universal.

## Other presentations Abstracts Not Published:

- Coccidioidal skin test responses in patients at the King Khaled Eye Hospital, Riyadh, Saudi Arabi:  
*Halde C.*
- Coccidioidomycosis in students at the University of Arizona, Student Health Center:  
*Gatto KM.*
- Miliary coccidioidomycosi: *Munoz A, Johnson R, Henrich M, Nemechek P.*
- Use of the triazole R 3783 in a dog with coccidioidal meningocerebral infection: *Pedroia V.*
- Comparison of three triazoles in murine coccidioidomycosis  
*Pappagianis D, Zimmer B, Hector R*
- Fluconazole treatment of coccidioidomycosis  
*Catanzaro A.*
- Experience with fluconazole treatment of patients with coccidioidal meningitis:  
*Galgiani JN, Graybill JR, NIAID Mycoses Study*