Coccidioidomycosis

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Abstract 1: Combination therapy I experimental Coccidioidal Meningoencephalitis

D. Pappagianis, R. F Hector

School of Medicine, University of California, Davis, Cutter Labs, Berkeley, CA

In previous studies we had demonstrated that oral administration of nikkomycin Z (NZ) (50mg/Kg b.i.d.) could prolong the life and even cure mice with CM as did amphotericin B (AMB) (3 mg/Kg qd) given intraperitoneally. In the present study, mice were infected with 75 arthroconidia of *C. immitis* Silveira by intracranial route and treatment was begun 48 hours later. Treatment groups were as follows: NZ 50 mg/Kg p.o.-b.i.d.; NZ 300 mg/Kg p.o.-b.i.d.; NZ 50 mg/Kg p.o.-b.i.d. plus AMB 3 mg/Kg i.p.-q.d.; Fluconazole (fluco) 25 mg/Kg p.o.-b.i.d.; fluco 25 mg/Kg p.o.-b.i.d. plus AMB 3 mg/Kg i.p.-q.d.; AMB 3 mg/Kg i.p.-q.d. Treatment was given for 2 weeks or 8 weeks after infection and surviving mice sacrificed 170 days after infection. All control untreated mice were dead by day 12. All mice receiving NZ 50 + AMB survived and none had viable *C. immitis*. However, 90% of mice receiving AMB only survived and were free of *C. immitis*. From these results, it is not clear if there was potentiation of AMB by NZ. AMB + fluco may have been antagonistic as only 30% of mice survived; however, these survivors were free of *C. immitis*. NZ 50 given for 8 weeks or NZ 300 for 2 weeks yielded 20% (all cured) and 30% (20% cured) survivors respectively. NZ 300 for 8 weeks gave 80% survivors for at least 122 days (one mouse died with a lymphoma on day 122), and 70% were free of *C. immitis*. Thus, NZ 300 mg/Kg b.i.d. yielded a high rate of survivors most of whom were free of viable C. immitis, slightly less effective than AMB alone. AMB + NZ may have shown a potentiating (or at least an indifferent effect on each other whereas fluco appeared less effective alone and reduced the efficacy of AMB. However, repetition of studies with fluco plus AMB will be needed to learn whether antagonism occurs regularly between these antifungal agents.

Abstract 2: Interaction of Human Peripheral Blood Cells with *Coccidioides immitis*

N M Ampel, G C Bejarano, J N Galgiani

Tucson VAMC, University of Arizona, Tucson, AZ

The role of human peripheral blood mononuclear cells in controlling infection with *Coccidioides immitis* is not established. We used a unique assay which employs a single fungal particle per well in a 96-well plate to examine fungal killing by polymorphonuclear leukocytes (PMN) and mononuclear cells (MNL) and their fractions in vitro. Blood was obtained from healthy volunteers with known skin-test reactivities to spherulin. MNL were found to kill 25±4% of *C. immitis* arthroconidia. This was significantly greater than the 5±3% seen for PMN from the same donors (N=8, p=0.01). Killing was not dependent on donor spherulin skin-test reactivity. Killing activity of MNL diminished significantly after incubation for 7 days and when MNL were $<5 \times 10^3$/well. Killing activity increased when a population consisting of predominantly monocytes isolated by Percoll density gradient centrifugation was used and diminished when monocytes were removed from MNL using Sephadex G-10. While MNL killed young (24 hr) spherules to an equal or greater extent than arthroconidia, this activity was progressively lost as spherules aged to 96 hr. Incubation of MNL with TNF-a or interferon-gamma failed to result in increased killing of coccidioidal arthroconidia. We conclude the MNL have inherent ability to kill *C. immitis* arthroconidia in vitro and that this activity appears to reside in the monocyte.

Abstract 3: Chitinase - The Coccidioidal CF Antigen

S M Johnson, D. Pappagianis

University of California, Davis, CA

Coccidioides immitis produces an endogenous chitinase which presumably acts to degrade the chitin layer within the spherule wall promoting lysis of the mature cell and release of endospores. This isolated protein has a native molecular weight of >100 kDa which under reducing conditions yields a 48 kDa protein, properties shared with the previously described immunodiffusion-complement fixation antigen (IDCF). We have therefore examined the antigenic identity and serological reactivity associated with the purified chitinase.

The purified chitinase was isolated by adsorption then desorption from chitin. The purified chitinase was subjected to immunoblot (Western blot) analysis following SDS-PAGE. The 48 kDa protein was found to be highly reactive with coccidioidal antibodies and stained most intensely when peroxidase labeled anti-human IgG was used as the secondary antibody. The enzymatically active chitinase reacted with coccidioidal antibodies in the complement fixation and immunodiffusion tests, forming a line of identity with the reference IDCF antigen in the latter method. The antigen-antibody precipitate which formed also retained enzymatic activity. Heating the isolated chitinase to 56°C destroyed the chitinase activity and its antigenicity in the IDCF test.

These results indicate that the endogenous chitinase is the previously described IDCF antigen and that the antigenic epitopes recognized by the coccidioidal antibodies are probably distinct from the enzymatically active sites. The purified chitinase has been reactive as an antigen in an ELISA with human sera.

Abstract 4: Prevalence of Human Serum Antibodies against 33k Da antigen from *Coccidioides immitis* and its expression in different morphologic forms of fungal growth

J N Galgiani, S H Sun, K O Dugger, G G Grace, J Harrison, M A Wieder

Veterans Affairs Medical Center, University of Arizona, Tucson, AZ

A 33 kDa antigen, distinct from antigens used in conventional diagnostic antibody tests, was employed to measure antibodies by ELISA in humans with and without coccidioidal infections (cocci). IgM >1:160 or IgG >1:80 were positive.

Subject Group	n	IgM +	IgG +
Recent cocci	11	7	7
Chronic cocci	26	15	25
No active cocci	107	2	3
Histoplasmosis pts.	16	0	1

Only 2 of 37 cocci pts. were negative for both IgM and IgG; some lgG were > 1:150,000. Anti-33kDa human antibodies were affinity purified with biotinylated antigen bound to an agarose column and used to prepare immunoelectron photomicrographs. Antigen was detected in the inner wall of arthroconidia, in spherules increasingly as they matured, in the glycocalyx on the surface of released endospores, but very little in young mycelia. We conclude that the 33 kDa antigen is displayed prominently to host surveillance, leading to an immune response in most patients with cocci.

Abstract 5: Isolation of chitin synthase gene from *Coccidioides immitis*

J Au Young,1 C Zimmerman,2 R Hector,3

1. University of California, San Francisco, CA
2. University of California, Davis, CA
3. Miles Pharmaceuticals, Berkeley, CA

We have isolated a chitin synthase gene fragment from *C. immitis* by PCR amplification of genomic DNA. The PCR primers used for PCR were deduced from two small regions of complete amino acid sequence identity in the chitin synthase genes of Saccharomyces cerevisiae CHS1 and Candida albicans CHS1. The 600 base pair DNA fragment was sequenced, and the deduced amino acid sequence was compared with chitin synthase fragments from 15 fungal species (Bowen et al., PNAS, 89: 519-23, 1992). The *C. immitis* sequence falls into a distinct group denoted as Class I. Within the class, the chitin synthase gene from *C. immitis* is most closely related to Histoplasma capsulatum (93% identity), Blastomyces dermatiditis and Wangiella dermatiditis (both 92% identity). This similarity between species reflects current phylogenetic relationships. The partial sequence of *C. immitis* chitin synthase gene is homologous to all fungal chitin synthases isolated to date. Isolation of the chitin synthase gene(s) from *C. immitis* will facilitate the study of its function in cell wall synthesis and the analysis of evolutionary relationships of fungal species.

Abstract 6: Phylogeny and identification of pathogenic fungi

J W Taylor,1 B H Bowman,2 M B Berbee,1 T J White,2

1. Department of Plant Biology, University of California, Berkeley, CA
2. Roche Moleculer System, 1145 Atlantic Alameda, CA

Phylogeny. We are using nucleotide sequence of the 18S ribosomal RNA gene to examine the evolutionary relationships among human pathogenic fungi. The hypotheses that we have examined were developed from morphological and physiological studies, but nucleic acid comparison offers a means of testing these hypotheses using cladistic and statistical methods (1). Because all fungi have nucleic acids, sexual and asexual fungi can be included in the same analysis and resulting phylogeny. We have found that the two Ajellomyces species which are better known for their asexual forms, *Histoplasma capsulatum* and *Blastomyces dermatitidis*, and the asexual fungi *Coccidioides immitis* and *Trichophyton rubrum* are all ascomycete fungi in the class Plectomycetes (2, 3). In the case of *C. immitis*, this solves a long standing controversy (4). We also examined *Sporothrix schenckii* and find that it can be accommodated within the genus *Ophiostoma*, home of the agent of Dutch Elm Disease (5). When *Pseudallescheria boydii* is added to the analysis, it is clear that the pathogenic condition has multiple, independent origins (6).

Identification. The same variation in nucleotide sequence that was used for phylogenetics can be used for PCR based identification. We have demonstrated, using DNA isolated from cultures, that species-specific oligonucleotides can correctly identify the plectomycete pathogens (7).

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Abstract 7: Coccidioidal Infection Treated with Amphotericin B Clloidal Dispersion (Amphocil or ABCD)

J S Hostetler, J W Caldwell, R H Johnson, A D Munoz, H E Einstein, R A Larsen, D A Stevens

Santa Clara Valley Med. Ctr, Stanford Univ, Kern Medical Ctr, Univ. of California, Univ. of Southern California

24 courses of ABCD, a new lipid-complexed amphotericin, have been studied in 23 pts with progressive *Coccidioides immitis* infections at 3 centers. Disease sites include chronic pulmonary (8), skin/soft tissue (13), bone/joint (14). HIV(+) pts were excluded. Mean age was 36 yrs, 83% male. initial 2 pts. received 0.5 mg/kg/day x2 wks then 1 mg/kg 3 days/wk xlO wks. 1 Pt. received 1 mg/kg/day x2 wks then 1.5 mg/kg 3 days/wk xl0 wks. All others (88%) received 1 mg/kg/day x2 wks then 2 mg/kg 3 days/wk. Mean duration of therapy was 9 wks (range 3-12 wks). 11/24 (46%) completed 12 wks of therapy thus far (total 5-6 grams ABCD) with a mean of 2 mo. (0-6 mo.) post-therapy follow-up. Outcome was assessed every 2 wks by a standardized scoring system comprising symptoms, serology, culture, and exam. Baseline scores ranged from 9-57 (median 15). The mean score for all pts was reduced by 26% (33% pulmonary, 21% bone/joint, 19% skin/soft tissue). For pts completing 12 wks of therapy the mean score was reduced by 33%. Relapses included 2 pts who received 12 wks of therapy, and 1 Pt who stopped treatment after 4 wks. 3 pts have not shown improvement but continue on therapy. Common adverse experiences (AE) induded chills 79%, fevers 71%, nausea 42%, and decrease in hct. (of >5 vol%) 46%. No pts required transfusion. 4 pts (17%) experienced reversible rises in creat. above 1.5 mg/dl. One Pt. failed due to toxicity after 4 wks with an unacceptable drop in hct. Symptomatic AE's generally decreased over time with 71% and 58% of pts experiencing chills or fever respectively in the first 2 wks, 58% and 50% in the second 2 wks, and 50% and 33% after 4 wks. These preliminary results suggest ABCD is an effective and safe agent for the treatment of progressive coccidioidomycosis. Treatment with higher doses would be of interest.

Abstract 8: Oral Fluconazole for Prevention of Dissemination of Acute Pulmonary Cocci

D W Smilovitz, D Stevig, R Mission, D Van, E Shultz, W Shepard

San Luis Obispo county in central California is an area with upper Sonoran Ecology. As such, it was felt not to represent an endemic cocci area. Early soil studies in 1978 isolated *Coccidioides immitis* from the northern part of the county.

In the fall of 1991, 9 (nine) acute cases were noted at the California Men's Colony in San Luis Obispo who acquired their disease while inmates. Subsequent testing isolated the organism from 4 (four) sites, the first such isolates in San Luis Obispo.

A brief study using fluconazole for cocci pneumonia in dark skinned individuals was begun in an attempt to prevent later dissemination.

Abstract 9: Is it ever safe to stop azole therapy (Rx) of Coccidioides immitis?

D H Dewsnup, J N Galgiani, J R Graybill, M Diaz, A Rendon, D A Stevens, NIAD Mycoses Study Grpoup

Santa Clara Valley Med Ctr, San Jose, CA; Stanford Univ, Stanford, CA; Tucson VA Med Ctr, Univ of Arizona Health Sci Ctr, Tucson, AZ; Audie Murphy VA Med Ctr, Univ of Texas San Antonio Health Sci Ctr, San Antonio, TX; Univ Autonoma de Nuevo Leon, Hospital Universitario, Monterrey, Mexico; and the NIAID Mycoses Study Group, Birmingham, AL

Introduction: Azole Rx of CiM has generally been successful; however relapses following apparently curative Rx have been observed. This has led to concerns that eradication of *C. immitis* from the CNS may not be complete, leading to relapse after stopping Rx. We present a follow-up clinical report of patients who have completed Rx of CiM with azoles.

Methods: Retrospective chart review of 14 patients enrolled in open, prospective, non-comparative trials of ketoconazole (K), fluconazole (F), and itraconazole (I) Rx of CiM. All patients had stopped Rx for the following reasons: a) asymptomatic considered cured by physician (10), b) non-compliance (3), and c) drug side effect (1).

Results: Nine patients relapsed. Patients with both normal and abnormal CSF parameters at completion of azole Rx experienced relapse. Mean azole Rx duration was 41 months (range 8-101 m). All patients treated <24 m relapsed (3/3), 6/11 patients treated >=24 m relapsed but Rx duration did not differ significantly (37 vs. 48 m, respectively, P=0.46). Median time to relapse was 8 m (<1 m - 30 m). Relapse rates were not different among patients treated with different azoles; 4/6, 4/7 and 1/1 relapses were observed with K, F, and I, respectively. Complications of relapse included hydrocephalus (1 patient) and possible sudden death (1 patient). Five patients have not relapsed at 10, 18, 19, 30 and 72 m of follow-up to date. Multivariate analysis did not reveal any clinical parameter, laboratory parameter, or combination of parameters associated with therapeutic success.

Conclusions: The high relapse rate in this patient cohort among patients, irrespective of treatment duration or CSF parameters at time of stopping azoles, is consistent with the hypothesis that azoles are fungistatic in vivo and do not eradicate *C. immitis* from the CNS. Analysis of these data at this time suggest that it is not safe to stop azole Rx of CiM.

Abstract 10: A Fatal Case of Coccidioidal Meningitis initially presenting with Increased Intracranial Pressure without Hydrocephalus

P L Williams, M E River, D A Stevens, J C Aguet

The patient (JL), a 20-year-old Hispanic male, was diagnosed (DX) with CM one month after symptoms of headache, fever, and altered mental state (AMS) began. An initial lumbar puncture (LP) demonstrated 740 white cells lmm3 (100% lymphocytes). The protein was 108 mgldl and the glucose 12 mgldl. There was a positive spinal fluid culture for *C. immitis*. The complement fixation titer was 1:4. JL was begun on fluconazle (F) 400 mg orally daily and initially responded. On day #18, after DX, JL presented with a seizure. An opening pressure (OP) at LP was 500 mm of H20. An MRI scan showed a right (R)-sided CVA consistent with an ischemic event, but no H was observed. A cisternogram was normal. JL was treated with dexamethasone (DXM) and F was increased to 800 mg orally daily with improvement. JL stabilized for one month until admitted day #55 after DX with AMS, aphasia, and R hemiparesis. An LP OP was 350 mm of H20 and, for the first time, H was evident on CT scan of the head. Subsequent treatment with intravenous (IV) and intrathecal (IT) amphotericin-B (AMP-B), DXM, daily LPs with removal of 20 cc of CSF, Diamox, VP shunting, and nimodipine (a calcium channel blocker) failed to successfully resuscitate JL who ultimately expired on day #108 after DX. The total dose of IT AMP-B was 32.75 mg and for IV AMP-B 2.48 grams. Serial MRI scanning throughout JL's final hospitalization demonstrated continuing infarctions bilaterally involving both cortical and subcortical regions of the brain parenchyma. No improvement in JL's clinical course was noted in spite of treatment for ICP and for CM including placement of a VP shunt and aggressive therapy. JL ultimately deteriorated into a clinical status resembling "coma vigil." Vasculitis (V) was presumed to be the basis of JL's CVAs as a post mortem exam was not granted. Possible pathophysiologic mechanisms to explain JLs increased ICP without H were discussed, reflecting a previous experience by one of our authors in AIDS patients with cryptococcal meningitis (OS). Possible therapeutic options were discussed. The best RX for CM with V and increased ICP is problematic; a suitable animal model is urgently needed to study these aspects reflecting complications of CM.

Abstract 11: Markers of Coccidioidomycosis prior to Cardiac or Renal Transplantation and Risks of Recurrence

Kevin A Hall, Jack G Copeland, Charles F Zukoski, Gulshan K Sethi, J N Galgiani

University of Arizona and Veterans Affairs Medical Center, Tucson, AZ

Acquisition of coccidioidomycosis occurs within restricted endemic regions. However, following organ transplantation, recurrence of infection is independent of geography and can be very serious. To assess the risk of reactivation and to formulate a management strategy, we reviewed renal and cardiac recipients from our programs with antecedent lesions, coccidioidal antibodies or skin test reactivity. 9 (8 males; 1 female) of 656 had lesions or serologic findings, and 13 others of 304 cardiac recipients had reactive skin tests. Lesions in 3 were unilateral pulmonary nodules; 4 others had antibodies and no lesions; 2 had a lung cavity or antibodies in the past. Of 5 who received post-operative antifungal drugs, 0 recurred whereas of 4 without treatment 2 had recurrent infection. Both infections which occurred after renal grafting were associated with periods of heightened immunosuppression for acute rejection and were fatal. Patients identified only by skin test reactivity showed no recurrence. We propose i) coccidioidal antibodies be measured in patients with any endemic exposure and ii) antifungal drugs be given after transplantation when pulmonary lesions or antibodies are evident.

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