Coccidioidomycosis

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Abstract 1: Human *in Vitro* Cell-Mediated Immune Responses in Coccidioidomycosis

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Clinical experience as well as animal studies indicates that cellular immunity is critical to successful host response to infection with *"Coccidioides immitis.* We performed a variety of studies in an attempt to study human cellular immune response *in vitro* using cells from donors with known skin-test responses to spherulin. We have previously found that mononuclear cells (MNL) from spherulin-positive donors (SK+) incubated with the coccidioidal antigen toluene spherule lysate (TSL) demonstrate lymphocyte transformation (LT) in a dose dependent manner, with an optimal response between 50 and 100 ug/mLof TSL [Galgiani *et al.,* Diagn Microbiol Infect Dis 1988; 11 :65]. We have subsequently demonstrated that MNL from SK + donors stimulated with TSL are capable of producing y-interferon (y-IFN) in a dose-dependent manner similar to the response seen using LT. Further, MNL from these same donors were able produce interleukin-2 (IL-2) after incubation with TSL MNL from skin-test negative (SK-) donors did not produce either y-IFN or IL-2. In further work, we found that monocytes from either SK + or SK- donors were able to phagocytize coccidioidal arthroconidia, inhibit the uptake of the chitin precursor N-acetyl glucosamine, and to decrease coccidioidal growth. These results indicate that human peripheral blood mononuclear cells have several demonstrable *in vitro* effects on coccidioidal arthroconidia and probably play a role in early immune response to coccidioidal infection.

Abstract 2: Characteristics of Concanavalin A-bound Spherule Antigen (CAB)

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The concanavalin A-bound (CAB) fraction of Converse medium following 96 hours of spherule growth produced soluble antigens with protein and carbohydrate concentrations of .105 and 7.6 mg/mL. By double-immunodiffusion, tube precipitating-type (TP) antigen was detected at a 1:50 dilution of CAB. Two-dimensional tandem immunoelectrophoresis of CAB demonstrated no cross-reactivity with any of 15 antigens in reference coccidioidin, whereas four other TP-containing extracts of either coccidioidal mycelia or spherules showed multiple shared antigens. Immunoblots of CAB with TP-reactive human sera showed diffuse antigenic activity between 55 and > 200 kDa with more focused activity at 67 kDa; rabbit anti-spherule antiserum without TP antibodies detected three additional bands (23, 54, and 59 kDa). Analogous immunoblots of other coccidioidal extracts were much more complex. Isoelectric focusing of CAB demonstrated at least 2 silver-staining bands with pIs between pH 4 and pH 6. We have also measured anti-CAB antibody concentrations in sera from uninfected patients and from patients with anti-TP antibodies or other diagnostic evidence (TP-) of coccidioidal infection. 0.1 µg of CAB was used to coat wells and, 2-fold dilutions of sera were added. Results are shown as % tested with concentrations> 1:80 (median titer):

Patient Group	# tested	IgM	IgG
Infected, TP+	34	96%(1:1280)	76%(1:1280)
Infected, TP -	26	58%(1: 160)	96%(1: 640)
Not infected	43	2 %	0%

Serial measurements of some patients indicated that ELISA titers decreased with therapy. We conclude that CAB material elaborated from growing spherules is rich in TP antigen, contains relatively few contaminating antigens. It may be a useful starting preparation for purification and analysis of TP antigen structure or as a diagnostic reagent.

Abstract 3: A REVIEW AND EVALUATION OF THE BAY R 3783 TREATMENT PROTOCOL FOR THE TREATMENT OF COCCIDIOIDOMYCOSIS IN THE CHIMPANZEE (Pan troglodytes)

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Twelve chimpanzees (Pan troglodytes) were treated for coccidioidomycosis with the experimental triazole, Bay R 3783. The dosage rate was 10 mg/kg orally once a day. Although the white blood cell counts and Cocci titers continued to rise for 2 to 4 weeks after initiating treatment, all animals showed marked gross improvements in the first few weeks of treatment. Liver enzymes remained normal throughout treatment. After 12 weeks of treatment, treatment interruptions were initiated if the serum level of the active metabolite, Bay U 3625, was less than 4 ug/ml Upon reinitiation of treatment, serum levels of the active metabolite tended to be higher (in some cases 2-3 times higher) than serum levels achieved during the first course of treatment. After treatment had been, terminated, some animals, which were presumed "cured," began to relapse three to nine months later. Treatment termination criterion were then modified: 1) a Cocci titer of no more than (1 :16) be maintained or show steady reduction for a period of eight (8) weeks, and 2) sedimentation rates remaining in the normal range for the same eight (8) week period. To obtain a more definitive view of the pharmacokinetics of Bay R 3783, four treatment regimes have been instituted: 1) alternating 3 weeks on Bay R 3783, 1 week off; 2) alternating 2 weeks on, 2 weeks off; 3) Monday, Wednesday, and Friday treatments; and 4) the original daily regime. Preliminary results indicate Bay R 3783 may be a fungistatic drug.

Abstract 4: EFFICACY OF SCH39304 IN A SYSTEMIC MODEL OF MURINE COCCIDIOIDOMYCOSIS

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The efficacy of a new oral azole, SCH39304 (SCH) 1 was tested in comparison with fluconazole (FLU) in a murine model of systemic coccidioidomycosis. Female CD-I mice (6 wks. old) were infected i.v. with 252 (LD90) arthroconidia of C. immitis (Silveira). Therapy with SCH at 2, 10, 25 or 50 mg/kg/day (I1KD) or FLU 10 or 100 MKD began 2 days post-infection by gavage for 19 days (Rx QD). Controls received no therapy. Organ burdens of C. immitis were determined in survivors 52 days post-infection. Survival was prolonged ($p<.001$) by both drugs at all doses over that of controls. However, 5/10 treated with SCH 2 MKD, 3/10 FLU 10 MKD and 9/10 control mice died. All mice receiving SCH 10, 25, and 50 MKD and FLU 100 MKD survived. Residual CFU of C. immitis in the lungs, liver and spleen of SCH 25 and 50 MKD treated mice were significantly lower than FLU 100 MKD ($p<.001$). No C. immitis was recovered from the organs of 8/10 and 1/10 mice treated with SCH 50 and 25 MKD, respectively; all others were infected. Serum levels of SCH peaked 2 h post-dose at 45 mcg/ml after a single dose and 64 mcg/ml after 19 days of therapy. These results indicate that SCH 39304 is an effective therapy for coccidioidomycosis and superior to FLU in this model.

Abstract 5: Nikkomycin Z-induced resolution of meningocerebral coccidioidomycosis in the mouse

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Coccidioidal meningitis remains the most serious challenge to successful treatment of coccidioidomycosis. Nikkomycin Z (NZ), a chitin synthase inhibitor, was evaluated for antifungal activity in meningocerebral coccidioidomycosis in the mouse, a model approximating coccidioidal meningitis. Mice were infected by intracranial injection of approximately 100 arthroconidia. Therapy was begun 2 days after infection. Untreated controls were all dead within 11 days. In all experiments, some mice treated with NZ died early after commencement of therapy. However, when treatment was carried out for 2 weeks, NZ at 50 mg/Kg BID was more effective than keto-, itra- and fluconazole at 25 mg/Kg BID in prolonging life. When carried out for 3 weeks, NZ was more effective than fluconazole. Prolonged therapy (56 days) was then carried out. Fluconazole 25 mg/Kg BID gave initial protection, but all mice died by 54 days after infection. By contrast 70-80% of mice treated with NZ 50 mg/Kg BID survived at least 72 days at which time the mice were sacrificed and the brain, lungs, liver and spleen were cultured. The brains of 8/18 (44%) surviving mice were free of viable C. *immitis,* the lungs in 17/18 (94%) livers in all 18 mice, and spleen in 16/18 (89%). NZ, therefore, has been shown to effect "biocure" of the brain in a substantial proportion of mice, a response not previously established with other antifungal agents.

Abstract 6: The Pulmonary Cavity in the Coccidioidomycosis Cooperative Study Group

David Salkin, MD and Milton Huppert, PhD

This study was of 5 years duration from 1954 through 1958, involving 16 hospitals (12 VA, 2 Army, 1 Air Force, 1 Navy), located in the endemic area to learn the history of the disease prior to the chemotherapy era. A total of 706 patients were hospitalized, of which 267 had cavities.

Time of cavity development: 86 within 6 mos of the primary disease (32%); 26 later (10%); 154 unknown (58%)

Mode of formation: No primary history in 122 cases (46%)

Course of the cavity: stationary 73 (27%); enlarged 57 (21%); variable size 51 (19%); disappeared 16 (6%); became nodular 41 (15%); ruptured 9 (3%); decreased 6 (2%); unknown 14(15%). Total 98%

Clinical classification: symptomatic 60%; 40% are asymptomatic are asymptomatic

No. cavities present: 1 (217) 81%; 2 or more 19%

Thickness of cavity wall: thin 42%; thick 31%; variable 20%

Fibrocavernous disease: 29 patients (4%)

Coccy plus TB: 62 patients (9%)

Abstract 7: Coccidioidomycosis during HIV infection. A continuing prospective study

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We have initiated a prospective study which follows HIV seropositive individuals living in Tucson or Phoenix, AZ, an area endemic for coccidioidomycosis, in an attempt to define the epidemiology and immunology of C. *immitis* infection among infected patients. As of March, 1990, 137 subjects have been entered into the study. Subjects were placed into 5 groups based on their CD4 count and immunologic evidence of prior coccidioidomycosis (positive skin test or lymphocyte transformation): *Group* 1: CD4≥2001J.1L & evidence of prior coccidioidomycosis [N=22]; *Group* 2: CD4≥200/u.L & no evidence of prior coccidioidomycosis [N=44]; *Group* 3: *CD4<200/uL* [N=37]; *Group* 4: data incomplete [N=28]. These first four groups had no evidence of active coccidioidomycosis on entry into the study. Subjects in *Group* 5 entered the study with active coccidioidomycosis [N=6]. Group 2 subjects differed significantly from Group 1 subjects in time spent in the endemic area but did not differ in Karnofsky score, weight or serum albumin. Group 3 subjects had significantly lower Karnofsky scores, less non-coccidioidal skin-test positivity and were less reactive to concanavalin A and *Candida* antigen in lymphocyte stimulation assay. Follow-up for all subjects has gone on for a median of 7.2 months (range 0 - 20.9). Over this time, 4 of 109 evaluable subjects (3.7%) have developed active coccidioidomycosis. None had a history or any evidence of prior coccidioidomycosis and were in either Group 2[N=2] or Group 3 [N=2] on entry into the study. Two had focal pulmonary disease while two had diffuse pulmonary involvement. All four had CD4 counts <200/IIL at the time of development of coccidioidomycosis. There has no relation between development of infection and the time in the endemic area. These data suggest that a large number of HIV + subjects in the endemic area have evidence of prior coccidioidomycosis but that active disease is associated with CD4 count <200/IIL and lack of evidence of prior infection with C. *immitis.*

Abstract 8: ITRACONAZOLE THERAPY FOR NONMENINGEAL COCCIDIOIDOMYCOSIS: CLINICAL AND LABORATORY OBSERVATIONS

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Itraconazole, a new oral triazole antifungal agent, was administered in 75 courses to patients with chronic coccidioidomycosis at doses of 50 to 400 mg/day for a median duration of 10 months. Assessment of efficacy was made with a standardized scoring system. Responses were seen in 42 of 58 assessable courses (72%). Non-response occurred exclusively in patients who had failed previous therapy and was most common in pulmonary disease. Toxicity was minimal at the doses studied. Pharmacokinetic analysis of itraconazole in serum at steady state showed negligible circadian variation; differences in serum concentrations among patients were large. Clinical isolates of Coccidioides immitis showed uniform in vitro susceptibility to itraconazole. Itraconazole shows impressive activity in this series of patients with refractory coccidioidomycosis. Further evaluation of itraconazole in this and in other systemic mycoses is in order.

Abstract 9: DRUG INTERACTION BETWEEN CYCLOSPORINE ANDITRACONAZOLE IN HEART-LUNG, HEART, AND LUNG TRANSPLANT RECIPIENTS WITH FUNGAL DISEASE

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Itraconazole is a new broad-spectrum antifungal agent. Its low frequency of toxicity compared to other antifungals, in particular lack of nephrotoxicity, and oral formulation make it potentially useful in organ transplant recipients receiving cyclosposine therapy. We report a clinically significant interaction of itraconazole with cyclosporine in 7 patients with fungal infections following heart lung, heart, or lung transplants. During concomitant therapy, an immediate interaction was noted in all. Serum cyclosporine concentrations increased up to threefold within 48 hours of commencing itraconazole, with resulting deterioration in renal function in 6. The mechanism of this interaction is not clear but may be related to interference in hepatic cyclosporine metabolism. We recommend reduction of cyclosporine dosage to 50% of baseline when initiating therapy with itraconazole with careful monitoring of cyclosporine concentrations and renal function.

Abstract 10: Fluconazole Therapy for Coccidioidomycosis

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We have used oral fluconazole to treat 71 patients with chronic pulmonary (41), soft tissue (18) or skeletal (12) lesions of Coccidioides immitis and 38 patients with coccidioidal meningitis (19 as initial therapy, 10 with AIDS). Nonmeningeal infections were first treated with 200 mg/day and efficacy evaluated by standardized composite assessment of symptoms, appearance of lesions, fungal culture results, and antibody concentrations. Abnormalities improved 40% by 4 months and 48 % by 8 months. Successful (any improvement within 4 months and 2:: 50 % improvement by 8 months) occurred in 61 % of patients. Of 9 failing patients raised to 400 mg, 4 subsequently responded. For CNS infections treated with 400 mg/day, CSF WBC mean ± sem(# of patients) at 0, 2, 4, 8, and 12 months were 247±52(34), 115±36(23), 55±13(20), 1l±4(13), and 9±4(9) cells/mm3; CSF protein and glucose abnormalities showed similar trends. To date only 1 patient with meningitis has failed to respond, 4 have died (3 AIDS, 1 stroke), and 1 lost to follow-up. All other patients are clinically stable on therapy. Toxicity from therapy was negligible. From this interim analysis of these ongoing studies, fluconazole appears effective in many complicated coccidioidal infections and should be compared to other antifungals.

Abstract 11: COMPARATIVE EFFICACY OF M1PHOTERICIN B COLLOIDAL DISPERSION (ABCD) AND AHPHOTERICIN B TREATMENT OF MURINE COCCIDIOIDOMYCOSIS

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The efficacy of ABCD (Liposome Technology Inc.) was compared to amphotericin B (Fungizone, F) therapy of systemic coccidioidomycosis. Groups of 10 CD-l female mice (6 wk old) were inoculated IV with 180 arthroconidia of Coccidioides immitis. Three days post infection, therapy (3x/wk, 2 wks) was initiated IV with ABCD at 10, 0.66 or 0.22, or F at 2.0, 0.66 or 0.22 mg/kg/dose (MPK). Controls received either no therapy or only diluent. Deaths were scored for 49 days and the burdens of C. immitis in the spleen, liver and lungs of survivors determined. All doses of .ABCD or F prolonged survival over that of controls ($p<.01$). While all 0.66 or 0.22 MPK ABCD treated mice were infected in one or more organs, C. immitis was cleared from the organs of 100% of mice treated with ABCD at 10 MPK or all F at 2.0 MPK survivors as well as 50 and 0% of 0.66 or 0.22 MPK F-treated mice, respectively. Burdens (log 10CFU/organ) in livers and lungs of 0.66 MPK F-treated (1.1 and 0.6, respectively) were lower than 0.66 MPK ABC-treated (3.1 and 3.2) ($p<.01$). Mice treated with ABCD or F at 0.22 MPK had equivalent burdens in liver ($p>.05$), but burdens were lower in spleen or lung of F-treated animals ($p<.05$). All organs in controls were infected. ABCD at 10 MPK showed no toxicity, whereas F at 2.0 MPK killed 50% of the mice. Though not equivalent to F on an MPK basis, ABCD was >5X less toxic, allowing the total of Amphotericin B as ABCD to be increased to an efficacious level. ABCD shows promise for the therapy of disseminated mycoses and should be tested in other models and clinically.