



Coccidioidomycosis

STUDY GROUP

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**Abstract 1: T-LYMPHOCYTE SUBPOPULATIONS IN
THE CUTANEOUS INFILTRATE OF PATIENTS
WITH DISSEMINATED COCCIDIOIDOMYCOSIS**

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CR Taylor, TH Rea.*

To evaluate cell-mediated immune response *in situ* in patients with disseminated Coccidioidomycosis, T lymphocyte subsets were identified in cryostat sections from three patients using monoclonal antibodies and a two-step immunoperoxidase technique. T lymphocytes constituted 30%-40% of cells in the perivascular infiltrate and approximately 20% of cells in the granulomatous infiltrate. Overall the helper (TH)/suppressor (TS) ratio was 0.5 ± 0.2 . In the granulomas, both TS and TH cells were localized to the mantle surrounding the mononuclear cell aggregates. These findings are in marked contrast to our studies of other epithelioid granulomas (tuberculosis, sarcoidosis and tuberculoid leprosy), in which TS cells were located in the mantle while the more numerous TH cells were distributed throughout the granuloma in intimate contact with macrophages. Our results suggest that disseminated coccidioidomycosis is a granuloma that is relatively ineffective in eliminating the organisms because TH cells are relatively deficient in number and have a disadvantageous microanatomical localization at the periphery of the granuloma.

Abstract 2: Coccidioidin (C) and Spherulin (S) Skin Tests in Pregnant Women (PW)

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We estimated the prevalence of prior coccidioidal infection among PW living in Phoenix by measuring dermal induration 48 hours after injection to 1:100 C and usual test strength S. Ninety-eight PW who consented were skin tested with C & S at parturition, our sample represents," 17% of live births at HMC during the study interval. The racial background of PW is: white 40.8%, black 16.3%, and Spanish surname 39.8%. Compared to non-pregnant skin tested women our sample under represents white women and over represents black women and women with Spanish surnames.

Results:

	S	C
No. tested	98	98
No. react	36 (37%)	37 (38%)
Size reaction (mean \pm SD)	21.3 + 12.4	20.6 \pm 12.2
Reactors \geq 5 mm	94%	97%

A + test is \geq 5mm in duration 48 hours after injection

The prevalence of + tests for S & C in PW and non-pregnant women of similar age and race is:

	P	n	% +	
			S	C
White	P	40	27.5	27.5
	Non P	118	23.7	22.8
Black	P	16	62.5	56.2
	Non P	16	68.7	68.7
Spanish surname	P	39	35.9	41
	Non P	40	45	42.5

We conclude that pregnancy does not influence the prevalence of coccidioidal infection among women of similar racial background. Our results should be interpreted cautiously because our sample is small and racially skewed.

Abstract 3: THIRD ANNUAL PROGRESS REPORT ON COCCIDIOIDOMYCOSIS VACCINE TRIAL

Joanne Walker Nichols. R.N. and the Vaccine Study Group

This double blind, randomized trial of the efficacy of a killed spherule vaccine used to reduce the morbidity of coccidioidomycosis in man is in its third year of enrollment. Funding has been from the state of California through U.C. Davis. The Navy and local support from the community of Bakersfield, Enrollment statistics as of February 25, 1983, are as follows: 4350 persons have attended orientation meetings; of 4059 people who were skin tested, 44% had positive results; 2090 persons have been enrolled in Bakersfield, Tucson, Lemoore Naval Air Station, Visalia, and Lemoore community.

An evaluation has been made of 5016 injections. No discomfort was reported in 48%, mild discomfort in 31%, moderate discomfort in 17%, marked discomfort in 3% and an unacceptable reaction in 0.4%. Five sterile abscesses were seen in participants enrolled during the first year of the study among 474 enrollees. None have been seen since in 1875 additional enrollees. Among the 3.4% who reported a marked or unacceptable local reaction, the following transient systemic signs or symptoms were noted (variably among various individuals): fever, myalgia, arthralgia, fatigue, generalized malaise, rash, headache or nausea. Localized reactions included pain at the injection site, swelling, redness, warmth and limitation of motion of the extremity.

To date we have documented coccidioidal illness in six study participants. An additional three participants had a coccidioidal episode that cannot be evaluated at this time due to the timing of the illness, which occurred during or soon after enrollment.

Continuing to follow participants and document cases will be the major focus for the remainder of the study.

Abstract 4: The Interaction of Human Peripheral Blood Monocytes with *Coccidioides immitis* In Vitro

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Human peripheral blood monocytes were obtained from individuals who did not have a history of exposure to *C. immitis* and did not respond to an injection with coccidioidin. The monocytes readily phagocytized arthroconidia of *C. immitis* without affecting the viability of the-fungus within 5h post infection. Phagolysosome fusion occurred with 11% of the phagocytized arthroconidia as shown by light microscopy, as compared to 20% observed with electron microscopy. Peripheral blood was also obtained from an individual with a known history of infection with *C. immitis* and a definite skin response to coccidioidin. Monocytes were infected with arthroconidia in the presence of lymphocytes. Within 4-6h 35% of the arthroconidia were killed. With light microscopy phagosome-lysosome fusion occurred with 70% of the phagocytized arthroconidia. These results were confirmed by electron microscopy.

Abstract 5: HUMAN NEUTROPHILS BLOCK INCORPORATION OF CELL WALL PRECURSOR IN COCCIDIOIDES IMMITIS

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Polymorphonuclear leukocytes (PMN) surround arthroconidia (A) early in coccidioidal infections, retard mycelial growth, and promote the development of spherules. Because exogenous N-acetylglucosamine (GluNAc) localizes in the developing fungal wall, we examined incorporation of this chitin precursor into TCA-perceivable material by pulse-labelling as a measure of fungal growth. In substrate excess, different strains and pre-labelling conditions resulted in different rates of incorporation (3-550 fm/min/I0(000,000) CFU), yet the substrate concentration for half-maximal incorporation was similar (0.2-0.4 &1). After 2 h incubation with human blood PMN, GluNAc incorporation was inhibited up to 77%+4 (PMN: A=15: I) as compared to leukocyte-free controls. Inhibition was not observed before 10 min and was lost after 6 h of PMN exposure. Homologous serum enhanced this PMN effect. PMN from a patient with chronic granulomatous disease failed to inhibit GluNAc incorporation. Concurrently, PMN readily contacted and engulfed A with or without serum. The oxidative burst of normal PMN elicited by A, as measured by chemiluminescence, was 95% of that caused by S. aureus. However, PMN did not kill A. Microbicidal activity against isolated A was assessed in a micro-well assay of our design (ARRD I25:I75A) and showed only 5.7%+3.8 of fungal units killed as compared to candida, S. aureus, or E. coli (44+9, 40+4, or 63+14).

We conclude that PMN inhibit incorporation of GluNAc, corresponding to PMN inhibition of growth in the mycelial phase. The non-lethal effect appears to require the normally elicited oxidative burst and may be an important early host response to coccidioidal infection.

Abstract 6: A DIRECT COMPARISON OF ORAL TREATMENTS WITH BAY-n-7133, BAY-I-9139 AND KETOCONAZOLE IN EXPERIMENTAL MURINE COCCIDIOIDOMYCOSIS

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Two new experimental antifungal azole drugs were compared with ketoconazole for the management of experimental murine coccidioidomycosis. The first, BAY-n-7133, a triazole, was superior to the second, BAY -I-9139, an imidazole derivative. Neither BAY drug was as effective as ketoconazole in early fulminant coccidioidomycosis of mice, in later disseminated disease and in deep-seated chronic disease. A possible limitation of BAY -n-7133 in the mouse model was its reported capacity to induce enzyme changes that accelerated its clearance from serum. Induction of such an enzyme response in human beings has been reported not to occur.

Abstract 7: Effect of BAY n 7133 (7133), BAY 1 9139 (9139) and Ketoconazole (KET) in Experimental Murine Coccidioidomycosis

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Under pentobarbital anesthesia, 26-30 gram female, Swiss-Webster albino mice were infected by direct intratracheal injection of 150 arthroconidia of Coccidioides immitis (strain Silveira) suspended in 0.05 ml of 0.9% NaCl solution. Treatment of cohorts of 10 mice infected and uninfected (anesthetized, operated, injected with saline)] was begun 72 hours later. Daily IV injections (tail vein) of 0.1 ml of 5% glucose solution delivering either, 0, 2.5, 5.0, or 10.0 mg/kg/ dose were continued for 30 days. Observed daily and weighed weekly, all mice were autopsied with culture of suspensions of the right lung, 0.5 g of liver and entire spleen on Sabouraud's glucose agar.

All of the uninfected mice survived. The mice given KE T had distal necrosis of the tail. The infected mice lost weight initially and progressively if untreated. Treatment allowed gain in weight and decrease in mortality that was significant ($p<0.05$) as compared with controls for 7133 and KET, only with the 2.5 mg/kg dose; however, 7133 and KET were not significantly different from each other. All cultures of organs yielded C. immitis with an apparently decreased density of growth in the treated mice.

In a model of murine coccidioidomycosis that avoided variations in size of inocula and bypassed possible differences in absorption of drugs, the new azole 7133 was as active as KET whereas the new azole 9139 was less effective. All three drugs were coccidiostatic only.

Abstract 8: Present status of NIH multicenter studies of coccidioidomycosis and Pharmacology of high dose ketoconazole in man

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A multicenter, NIAID-sponsored study (protocol 3B) of oral ketoconazole Rx of patients with bone/joint, skin/soft tissue and chronic pulmonary coccidioidomycosis has enrolled 102 patients as of 2/83. Preliminary analysis of 30, 25, 30 points respectively indicates 2, 1, 4 failures thus far. Pts. are randomized initially to either 400 or 800 mg/d; the dose may increase depending On rate of response. Side effects occurred in 37% and 6 had Rx discontinued due to side effects. Data with regard to protocol adherence and relapses will be detailed. Serum was sampled at 2, 4, 6, 8, 12, 24h post-dose and indicates prolonged high levels at these doses. Peak \pm SEM after 400, 800, and 1200 mg doses occurred 4h post-dose, at 8.1 ± 0.8 , 9.7 ± 0.8 and 14.3 ± 3.8 mcg/ml respectively, Drug was detected 12h (1.1, 2.5, 7.9 mcg/ml. respectively) and 24h (0.8, 1.6, 1.3) post-dose. The results to date are very encouraging, suggesting safety and efficacy at these doses. Referral of patients, who may be treated at your center, is urged so that the optimal regimen for ketoconazole can be determined.

Abstract 9: THE EFFECT OF HIGH-DOSE KETOCONAZOLE ON ADRENAL AND TESTICULAR FUNCTION IN HUMANS

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Ketoconazole, an oral antifungal agent, transiently blocks testosterone synthesis and adrenal response to adrenocorticotrophin (ACTH) in man, at conventional doses. We show here that higher therapeutic doses currently under study, even given once daily, cause more prolonged blockade, and in some males, for example, their serum testosterone concentrations are always subnormal. End organ effects were shown, including oligospermia and azospermia after prolonged therapy; impotence, decreased libido and gynecomastia were also noted. Bound and free testosterones are equally diminished. Depressed response to intravenous ACTH was pronounced, as was depression of daily urine cortisol excretion. The blockade appears related to the serum ketoconazole concentration. The few instances of normal hormone levels or responsiveness could be associated with low serum ketoconazole concentrations. The hormonal effects are generally unrelated to duration of therapy, although there may be partial reversibility on continued therapy. These effects and the azospermia appear reversible with discontinuation of therapy. Patients receiving ketoconazole should be considered potentially unable to mount an adrenal stress response, and may require testosterone supplementation. The blockade may be turned to useful effect in other situations, e.g., prostate carcinoma

Abstract 10: THE PERCENT GUANINE plus CYTOSINE IN DNA OF COCCIDIOIDES IMMITIS

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The taxonomic position of C. immitis is uncertain (with some resemblance of spherules to zygomycetous sporangia, and hyphae with some ascomycetous features). We sought to add information pertinent to the classification by determining the molar percent guanine plus cytosine (%GC) in nuclear DNA of C. immitis. This was carried out by extraction of DNA and determination of buoyant density of the extracted DNA in cesium chloride. Four strains of C. immitis, three from the San Joaquin Valley of California (strains Silveira, Jacob and Huckaba) and one from Texas (strain Bates) were cultured in synthetic (modified Converse) medium. The spherule phase of Silveira and mycelial phase of the other strains were compared. Triplicate determinations yielded the following mean %GC values:

Silveira (spherule phase) 49.61 ± 0.12

Bates (mycelial phase) 49.41 ± 0.23

Huckaba (" ") 49.60 ± 0.26

Jacob (" ") 49.51 ± 0.25

Thus, the %GC is very close among these different strains and between morphologic phases. These findings per se do not permit specific placement in a given division of the fungi as they fit in the ranges for a variety of fungi. The %GC lies in the "transition zone" between ascomycetous and basidiomycetous yeasts, and differs from the 50-53% values shown by Arachniotus and Auxarthron whose hyphae-arthrocomidia show some resemblance to those of C. immitis. For the present, it still appears that correct placement of C. immitis may require demonstration of a sexual phase.

Abstract 11: Genetics of Murine Resistance to Intraperitoneal (IP) Infection with *Coccidioides immitis*

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The incidence of disseminated coccidioidomycosis in Blacks and Filipinos is much higher than in Caucasians. This apparent genetic influence on resistance to coccidioidomycosis prompted us to study the genetics of resistance in inbred mice. We found that the LD₅₀ of R.S. strain arthroconidia in BALB/c female mice is 1.7 (log 10) at 28 days and the LD₅₀ in DBA/2 female mice is 5.3. Males of both strains are more susceptible than females. Resistance is the dominant phenotype; the LD₅₀ for (BALB/c x DBA/2) F1 female mice is 4.95. Resistant and susceptible mice can also be differentiated by counting the number of live organisms (CFU) in the lung 14 days after IP infection. To determine the number of genes responsible for the resistant phenotype, (BALB/c x DBA/2) x BALB/c female mice were infected with 5×10^2 arthroconidia IP and compared to BALB/c and F1 mice, with the CFU assay_ 32 of 67 mice were as susceptible to *C. immitis* infection as the BALB/c controls ($>10(00,000)$ CFU/lung on day 14). However, the resistant backcross mice were not as resistant as the F1 parent. The 1:1 distribution of susceptible to resistant mice suggests that a single major gene is required for resistance. The fact that resistant backcross mice were not completely resistant is consistent with several modifying (minor) genes in the BALB/c strain.

Abstract 12: CISTERNAL TREATMENT OF COCCIDIOIDAL MENINGITIS: EIGHT YEAR EXPERIENCE

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Over the last eight years we have treated approximately 28 cases of Coccidioidal meningitis (CM) the age range at onset is 7-76: 20 male, 8 female. The racial categories are as follows: Caucasian 5; Hispanic 14; Black 4; Filipino 2; East Indian 1; Arab 1. The treatment protocol includes a premedication tailored to the patients' reactions. The intrathecal injections consisted of a preinjection of 5mg methylprednisolone, 1cc D5W and an injection of 0.1-0.8mg amphotericin in 4cc D5W. This was given initially, alternating 3X/week lumbar and 3X/week cisternal injections for 2 weeks, and then only 3X/week cisternally. The treatment was continued for at least 3 months and clinical and CSF findings improved. Then the treatment was gradually tapered over no less than 2 years.

Treatment complications encountered include significant subarachnoid hemorrhage, transient neurologic deficits and one instance of culture negative purulent meningitis. Outcomes include 5 alive off therapy; 8 alive on therapy; 13 deaths; 2 lost to follow-up. Of the 13 deaths, 2 were on treatment; 4 elected to discontinue treatment; 4 were never treated (acute deaths); 3 died of other causes. Of those alive and off therapy, 2 work full time; 1 was disabled prior to Coccidioidal and had a recent unrelated cerebral infarct. He has associated hypertension and DM; 1 elderly lady has returned to her premorbid activities; 1 off therapy 3 mo. by his choice and will probably be restarted in the near future.

Hydrocephalus occurred in 8 patients, 1 prior to coccidioidal meningitis. Of these, 6 are dead; 2 are alive and off therapy, 1 with and 1 without a shunt.

Problems with CM include early diagnosis and definition of remission or cure. Additionally, the psychosocial dislocations caused by the disease are severe.

Abstract 13: Methods of Delivering Amphotericin B to the Central Nervous System

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Treatment of coccidioidal meningitis requires intra central nervous system (CNS) Amphotericin B (Amp B). There are three common methods of delivering Amp B into the CNS: (1) lumbar injection with or without 10% glucose, (2) cisternal injection, and (3) Ommaya reservoir injection. Most of the published series are from the Bakersfield area where cisternal therapy predominates. These series report 30-40% mortality from coccidioidal meningitis and infrequent serious side effects from cisternal puncture.

Lumbar injection with 10% glucose is used at some centers but results are published for only a few patients. Side effects may be frequent (up to 50% of cases) and occasionally are serious.

Ommaya reservoirs are used in patients with coccidioidal ventriculitis, a complication of meningitis. Most of these patients had prior treatment with a different method. The side effects of infection, obstruction and migration are frequent. Some physicians are using Ommaya reservoirs as initial therapy but no published results are available.

In my practice I begin treatment of coccidioidal meningitis with lumbar 10% glucose injections and change to cisternal injections if serious side effects occur or if the patient does not improve. Conclusions: The published data are not sufficient for a meaningful comparison among the three methods. I urge physicians using the lumbar 10% glucose method and the Ommaya reservoir method to publish results.

Abstract 14: CHRONIC EFFECTS OF INTRATHECCAL AMPHOTERICIN IN 25 YEAR SURVIVOR OF COCCIDIOIDAL MENINGITIS

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This man had onset of coccy meningitis at age 46, in 1957. He received treatment from 1957 to 1974. Treatment consisted of IV and intraspinal amphotericin from 1957 to 1962, intracisternal 1963 to 1966, via Omaya reservoir 1966 to 1970, and intracisternal 1970 to 1974. He remained free of evidence of disease thereafter. In all he received about 16.4 gm. of amphotericin IV, 50 mgm. intraspinally, 46 mgm. intracisternally, and 10.4 mgm. intraventricularly.

When cisternal therapy was first started, the patient was placed in the prone position after cisternal injection. He had several transient episodes of cranial nerve palsies and then developed permanent deafness in one ear following such treatments. These problems did not occur when he was subsequently placed supine.

All CSF findings were normal for a year before suppressive therapy was discontinued.

He died of unrelated cause in 1982, 25 years after onset. Autopsy revealed calcific lung focus containing calcifying spherules with endospores. The kidneys failed to show any microscopic changes which could be attributed to amphotericin.

The brain was removed with difficulty because of dense adhesion below the tentorium. The ventricles were dilated. There was uniform fibrous thickening of the pia arachnoid of the distal pons, medulla, cervical cord and extending over the inferior surfaces of the cerebellum requiring sharp dissection. No area of calcification or granuloma formation was found within the meninges. The dura of the posterior fossa was thickened 3 to 4 times normal. Microscopic sections of the dura revealed very dense acellular hyalinizing fibrocollagenous thickening.

Abstract 15: An Unusual Case of Coccidioidal Meningitis with Normal CSF

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A 29 year old Mexican man residing in the western San Fernando Valley was diagnosed as having severe disseminated Coccidioides immitis infection, presenting with miliary pulmonary disease, numerous skin lesions and impressive diffuse adenopathy after 3 weeks of malaise, cough, arthralgias, weight loss, and fever. He was anergic with a complement fixation titer of 1:8 initially. Both transbronchial and skin biopsies were culture positive. An admission LP was negative. After an initial response to Amphotericin B (AME) there was a recrudescence with worsening pulmonary status and new skin lesions. This happened after more than one gram of AMB. Approx. 1 month into therapy after more than 1.5 AMB lethargy was noted; an LP & CT scan were negative. 2 weeks later the pt. defervesced & was clinically improving but developed unexplained urinary incontinence & intermittent mental status changes. Another LP was traumatic but initially unremarkable, and CT scan was again negative. A UTI with 2 gram negative rods cleared with appropriate antibiotic therapy. He became febrile again. Three weeks later the CSF was reported positive by complement fixation; a 4th LP at this time was again positive for Coccidioides by compo fix, with otherwise entirely normal CSF. A collodidal gold curve on this CSF was flat and a gallium scan failed to show CNS involvement. Intracisternal therapy with AMB was begun to which the patient responded clinically, with resolution of fever, incontinence and mental status abnormalities. In addition to the local therapy he has continued to receive intravenous AMB - more than 6 grams to date - which he has tolerated well. Questions remain as to the duration of therapy and the possible addition of Ketoconazole.

Abstract 16: THE COCCI REGISTRY - A VALUABLE TOOL

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The idea for a Coccidi Registry came from a need as seen by a medical records statistician. Every medical records department prepares a case abstract on every patient. This abstract is used to produce the disease indices for that facility. A part of this abstract is the ICD-9CM code for Coccidi and other diseases. These case abstracts, as well as procedure codes for Coccidi, could be used as a data base for a Coccidi Registry. In addition to that data base, a new set of data could be formulated by those physicians and health care personnel who would use the registry. This tailored set of data would provide many statistical reports which could be modified for the user to suit his needs. These reports could also be produced at any time frame.

Participation in this Coccidi registry would be voluntary. Proposals for grants in funding will be done. The location of the physical plant for the registry would be determined at a later date. Data requests from interested parties have been submitted and the registry abstracts are being formulated.