



Coccidioidomycosis

STUDY GROUP

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Abstract 1: ENZYME LINKED IMMUNOSORBENT ASSAY OF COCCIDIOIDAL ANTIBODY IN CSF SPECIMENS

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Coccidioidal antibodies in CSF can be detected by the ELISA procedure. Coccidioidin antigen was absorbed onto the surface of polystyrene wells and a 1:10 dilution of cerebrospinal fluid added. Attachment of coccidioidal antibody was then detected by alkaline phosphatase labeled anti-human globulin. The substrate, P-nitrophenyl phosphate, was acted upon by the alkaline phosphatase producing a yellow color the intensity of which was measured spectrophotometrically.

When specimens were tested in parallel with the complement fixation procedure, the ELISA was shown to have greater sensitivity with 94% specificity. Twenty three pulmonary coccidioidomycosis cases and 17 patients with coccidioidal meningitis were tested periodically for one year by the ELISA to ascertain clinical correlation.

The greater sensitivity of the ELISA did not prove to be a benefit in the early diagnosis of suspected cases of coccidioidal meningitis. For 78% of the patients with serum complement fixation antibody titers of $\geq 1:16$ also had detectable CSF antibody by ELISA and no detectable CSF antibody by the complement fixation procedure. None of these patients ever developed coccidioidal meningitis. The coccidioidal antibody detected in the CSF by the ELISA was not of CSF origin and did not represent CNS infection. Coccidioidal meningitis cannot be detected with reliability any earlier with the ELISA procedure than with the standard complement fixation procedure.

Abstract 2: Cocci Skin Testing as an Epidemiologic Tool - 48 years experience in Kern County

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Continued skin testing in this area over 48 years shows that the annual per capita "attack rate of 10% in 1938-9 dropped to 4% in 1959, to 3% in '64 and was showing less than 1% in 1979 among students (age 6-16)." However, this was not confirmed in adults** and studies in 1980-84 indicate an attack rate of 2-3% now.

Second lesson learned: Students just don't turn out for such things like they used to. The poor turnout for a well planned program (313 students out of 1700 eligible) gave figures too small to be useful, though not inconsistent with prior results.

If your area does not have an ongoing or periodic Coccy skin testing program, it's easy to do in your own private practice, in a Health Dept. or school, at Health Fairs (a good service as well as good P.R. for the Lung Ass'n) or such. Please write to me for the forms we've found effective, and/or share yours with me. Some consistency in how we do this will make comparison of results much easier.

Abstract 3: Evaluation of the protective efficacy of the killed *Coccidioides immitis* spherule-vaccine in man

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The formalin-killed spherule vaccine previously shown by Levine et al. to provide protection against development of lethal coccidioidomycosis (coccy) in laboratory animals was evaluated for its protective effects against coccidioidomycosis in humans. Based on previous safety trials, 3 doses of 1.7 mg (dry weight) of lot KM vaccine were selected for intramuscular injection. The placebo was to consist of 3 doses of 0.85% NaCl solution. Healthy volunteers 18 to 60 years Of age were drawn from the various study sites (Kern County, Lemoore, Lemoore, Lemoore Naval Air Station and Visalia, Calif., and Tucson, Arizona). Participants were selected based on negative skin tests with coccidioidal antigens, negative chest roentgenograms, and no history of prior coccidioidal infection. The participants were divided at random into vaccine recipients (n=1,436) and placebo recipients (n=1,431), in the period of the study (1980-1985), we concluded that there were 21 cases of coccy, 9 in those given vaccine, 12 in the placebo recipients. Additionally, 22 suspected cases were detected with a similar distribution i.e. 9 in the vaccinated group, 13 in the placebo group. The infections occurred from less than 2 months to 43 months after the third injection. The Cases were generally mild.

Under the conditions of this study there was no significant difference in the rate of occurrence of clinically apparent coccidioidomycosis among those receiving vaccine and those receiving placebo; and because of the mild clinical aspects it is not possible to tell whether the vaccine could have an ameliorating effect on the course of coccy.

Abstract 4: Pertussis-Derived Adenylate Cyclase Blocks Human Neutrophil Oxidative Burst and Inhibition of arthroconidia without Alteration of Phagocytosis: Differential Sensitivities of Chemiluminescence and Superoxide Release

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Bordetella pertussis adenylate cyclase toxin (BACT), as extracted by 4 M urea, inhibits polymorphonuclear leukocyte (PMN) superoxide release (O_2^-) and Staphylococcus aureus killing. BACT was evaluated over a final concentration range of 0.1- 260 ug/ml for its effect on PMN functions: inhibition of N-acetylglucosamine incorporation into arthroconidia of Coccidioides immitis. O_2^- release as measured by cytochrome c reduction, luminol-enhanced chemiluminescence (CL), and phagocytosis of Candida glabrata as measured by two methods. O_2^- release after staphylococcal or arthroconidial stimulation required 13 - 26% BACT for half-maximal inhibition. PMN inhibition of arthroconidia paralleled that of PMN O_2^- release. In contrast, 50% inhibition of CL was achieved with ten-fold lower BACT concentrations. At all concentrations, BACT had no effect on phagocytosis of yeast. We conclude that in this system, both O_2^- and CL were inhibited by BACT concentrations that do not affect phagocytosis. That CL was more sensitive than O_2^- generation may be related to the multiple sequential steps involved in the generation of products that CL measures. PMN inhibition of arthroconidial GlcNAc incorporation correlates closely with changes in the oxidative burst

Abstract 5: Coccidioidomycosis (C) in AIDS

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21 cases of AIDS were seen at MMC. Among these 18 had pulmonary (p) infections (i) and 4 had C as proven by isolation of fungus (Ci) from p tissue or respiratory tract secretions (rs).

2 of these 4 had concurrent pi with other pathogens including Pneumocystis and CMV. Both patients (pts) were lymphopenic (\downarrow 1). Neither developed precipitin or complement fixing (cf) antibody (ab) to Ci. Extrapulmonary (ep) C was not detected. Both pts died within 2 mos. In these pts C was but 1 of several pi associated with AIDS.

The 2 remaining pts had C as the predominant i manifestation of AIDS. Both were in high risk groups, were \downarrow 1, had cutaneous anergy, and were HTLV III ab +. 1 pt had a miliary pattern on chest x-ray (CXR), and rs revealed only Ci. The cf ab titer to Ci was + at 1:8. No ep C was found. The other pt had focal pC in 9/84 with mild \downarrow 1 and an inverted T: T ratio (iTr). In 1/85 he developed meningeal 4/c 8/with a serum Cf ab of 1:128. He remained \downarrow 1 with iTr. In 7/85 he had miliary CXR pattern, rs grew Ci and CMV. He died in 1 mo.

Adequate host defense against C requires T cells. AIDS pts living in the endemic zone of Ci may contract C as a manifestation of their immune suppression.

Abstract 6: Coccidioidomycosis in Patients with the Acquired Immuno Deficiency Syndrome

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Of 27 patients with the acquired immunodeficiency syndrome (AIDS) in Tucson, 7 had concurrent coccidioidomycosis* as of 12/85. In each patient, AIDS and cocci were diagnosed \leq 16 months apart. Six manifested their infection with diffuse nodular pulmonary infiltrates and *Coccidioides immitis* in many extra-thoracic sites (blood, liver, spleen, kidney, urine, bone marrow, thyroid, brainstem). By comparison, a retrospective review of 300 patients hospitalized with coccidioidal infection identified only 13 non-AIDS patients with the same extent of infection, and only 3 of these were without immunosuppressing conditions. Antibodies for coccidioidal antigens at serum dilutions as high as 1:2048 were detected in five of the seven AIDS patients; the other two had AIDS related tumors and were receiving chemotherapy. Six had temporary responses to amphotericin B with or without ketoconazole, but all died within 14 months of their diagnosis of coccidioidomycosis. Since annual rates of coccidioidal infection in Tucson area patient groups are \leq 6%, an apparent rate of 27% (7 cases during 26 AIDS-years of risk) suggests frequent reactivation or enhanced susceptibility to endemic exposure in this group.

Abstract 7: Acute Coccidioidal Meningitis

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Two young adult immunocompetent males with symptomatic acute coccidioidal meningitis (CM) are presented. Both patients (P) presented with severe headache, fever, consistent CSF findings, and complement fixing antibody for *C. immitis* in the CSF. Abnormal sensorial manifestations with normal neurological examinations developed in both. Disorientation and erratic behavior developed in the first P. He later developed seizures and coma unresponsive to decadron (D) and amphotericin B (A) therapy. Auditory hallucinations developed in the second P responsive to D and A begun empirically when other differential considerations were eliminated or judged unlikely prior to serologic confirmation. Previous literature describing acute and subacute CM was discussed. Histopathologic findings in autopsy studies are reviewed and point to common vasculitis association involving small and medium sized arteries within the brain parenchyma. Similarities between CM and the steroid responsive idiopathic disease granulomatous arteritis are described. We conclude: 1. Arteritis is a common accompaniment of fatal acute, subacute, and chronic CM and contributes to focal and global manifestations, seizures, and death. 2. High dose D and empiric A should be strongly considered in patients presenting with acute confusional states, focal neurological findings, or seizures proven or suspected to be secondary to CM when other correctable considerations have been excluded.

**Abstract 8: PULMONARY CAVITARY COCCIDIOIDOMYCOSIS AND
KETACONAZOLE**

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Disseminated coccidioidomycosis is usually treated with Amphotericin B. Ketaconazole appears to be effective against *C immitis*, and our experience in cavitary disease is presented. Five cases are outlined, three black and two Caucasian. Two were started on Ketaconazole. The uses of Ketaconazole are discussed. The economic impact of therapy is described. The exact place for Ketaconazole in therapy of valley fever is still to be determined. Further study and evaluation is necessary.

Abstract 9: Symptoms and Nonspecific Laboratory Results Significantly Associated with Coccidioidomycosis

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Currently, coccidioidal pneumonia (Cocci) is difficult to diagnose on initial symptoms or routine lab findings alone. We devised a 40-Question form which was completed by 530 college students seeking medical care for respiratory complaints. Of 530 such patients 286 had serology and skin testing and of these 29 (10.1%) were found to have coccy. Variables were analyzed both individually and by logistic regression to assess dependence. By the latter procedure, skin rashes (not *E. nodosum*), increased WBC, acuteness of symptoms, chest pain with breathing, and absence of hoarse voice were factors positively associated with coccy ($p < 0.05$). Other factors which individually (but not independently) were significantly associated included: abnormal chest X-ray, decreased percentage of lymphocytes on CBC, elevated erythrocyte sedimentation rate, colleges of engineering or mines, fever, absence of stuffy nose, "red lumps on shins" (patient's perception), absence of watery eyes, and recent arrival to endemic area. We conclude that specific initial symptoms and findings in readily available laboratory tests, if incorporated into a probability model. May improve the early diagnosis of coccy.

Abstract 10: Cocci Peritonitis associated with Chronic Ambulatory Peritoneal Dialysis. (CAPO)

U. Varanasi, M.D. and T. Larwood, M.D

31 year old Caucasian woman with severe primary coccy with E.N. in 1975, seemingly subsided. Mesangial proliferative G.N. dxed 5/80; renal failure 8/82 and hemodialysis begun. Changed to CAPO 6/84. Peritonitis (the most expected complication) appeared 12/84 - dxed coccy 1/85. Ketoconazole (p.o.) tolerated to 400 mg. daily for 6 weeks - no apparent effect. Intraperitoneal Amphotericin-B (0.25 - 0.5 mg per Liter, 2L exchange 4-5 times daily) given for a few days in 3 courses over 10 weeks but an intense peritoneal reaction occurred and a white, curdy exudate finally plugged the catheter and required its replacement so 1500 mg. Amphotericin was given I.V. over the next 2 months. Peritoneal catheter removed 1 month later: neg. culture but myceliae seen. Peritoneal C. culture + 1/85 to 6/5/85, neg. 3 times later. On hemodialysis since; works; does not desire peritoneal tap but abdomen remains soft. Blood serology 4+ @1:8 in 1/85 higher later but stabilized and no other evidence of dissemination.

This case seems to follow the pattern of relative benignity that the rare cases of C. peritonitis more often than not show." In the absence of useful kidneys, Ampho, use would be safer than usual but its peritoneal use was not tolerated well here.

- Coccidioidomycosis, Ajello - 2nd Coccy Symposium, 1965. 21 cases gathered and there have been 11 or more reported since, with some others also known.

Abstract 11: Ketoconazole (K) and corticosteroids are additive in their suppression of mitogen-induced lymphocyte proliferation in vitro

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In addition to antifungal activity, K affects mammalian cells. This includes inhibition of steroidogenesis, immunomodulation of lymphocyte blastogenesis, and competition for glucocorticoid receptor, where it is an antagonist, e.g., glucocorticoid-induced tyrosine amino transferase expression. Because glucocorticoids are potent immunosuppressants and K antagonizes glucocorticoid functions, we examined the effects of K and corticosterone (C), and their interactions, on mitogenstimulated lymphocyte blastogenesis. 2×10^5 (200,000) human blood mononuclear cells were stimulated with 5 mcg/ml concanavalin A (Con A) alone or with K, C, or K and C. After 48 h each well was pulsed with ^3H -thymidine, incubated for 24 h, and thymidine incorporation assessed. K alone inhibited blastogenesis in a dose-responsive manner. Significant inhibition of thymidine incorporation occurred at concentrations of $9.4 \times 10^{-6}\text{M}$ and $18.8 \times 10^{-6}\text{M}$ ($p < 0.05$) but not $4.7 \times 10^{-6}\text{M}$ ($p > 0.05$). Similar results were observed when K was added 1 h prior to or simultaneously with Con A. To see if K antagonized the immunosuppressive effect of C on blastogenesis, various suppressive concentrations of K were tested in conjunction with a concentration of C (6.5×10^{-6} (0.000,000)M) which inhibits mitogen responsiveness 50%. Although each compound suppressed the Con A response, in combination an additive suppressive effect was seen. The results agree with reports that K suppresses mitogenstimulated lymphocyte blastogenesis. However, in this suppression K and glucocorticoids are not antagonistic.

Abstract 12: Initial experience with itraconazole in coccidioidomycosis

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Seven patients, not included in multicenter studies, were evaluated. Five had failed prior chemotherapy. Two had chronic and 1 acute pulmonary, 1 epididymitis, 1 meningitis and 2 lymphadenitis disease. Two (lymphadenitis, epididymitis) responded, 4 had partial responses (in the meningitis patient decreased pleocytosis has permitted tapering of intrathecal therapy.), 1 (chronic pulmonary) has not responded. Six are still receiving treatment (400 mg/day). Possible side effects include exacerbation of pre-existing decreased libido and impotence, urinary frequency (2), anorexia and nausea, transient acneiform rash. Mild transient elevations of liver function tests were also noted. Two C. immitis isolates had MIC's of 0.23 and 1.5 mcg/ml. Peak serum levels were at 4-6 hrs., and $t_{1/2}$ is prolonged. We plan to continue therapy a minimum of 1 year, or 6 mos. after disease inactivity (whichever is longer). The potency of this oral drug in vitro and in vivo and the promising results in this study thus far and the lack of toxicity, warrant expanded study of this drug.

Abstract 13: A lumbar reservoir makes it possible to give prolonged intrathecal therapy safely and conveniently when lumbar punctures are impossible

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A 51 year old man developed coccidioidal meningitis in January 1985. Lumbar intrathecal amphotericin therapy began in February. In March, A ventriculoperitoneal shunt was required because of hydrocephalus, and an Ommaya reservoir was placed. Intrathecal therapy continued by lumbar and ventricular routes until June, when lumbar therapy became technically impossible, an indwelling lumbar reservoir (using Spetzler lumbar-peritoneal shunt and flushing reservoir) was placed, surgically entering the L4-5 interspace. Amphotericin injections were subsequently made into the reservoir with a #27 needle. The entire injection procedure requires only 10 min., is painless, and the patient position comfortable. Traumatic bloody taps do not occur, and this and the absence of repetitive needle trauma may lessen arachnoiditis. The patient has received 43 treatments through the lumbar reservoir over 8 mos., without complications and with continuous clinical improvement. Use of a similar device has been reported in carcinomatous meningitis.

Abstract 14: Inhibition of *Coccidioides immitis* by plumbagin, a chitin synthetase inhibitor

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Plumbagin, a naphthoquinone from the root of Plumbago europea, is recognized as a chitin synthetase inhibitor. It was shown to inhibit microorganisms including mycelial phase C. immitis by de Saint-Rat et al. in 1946, and inhibited chitin synthetase of insects (Kubo et al.) Previous studies (Hector and Pappagianis) had shown an adverse effect of another chitin synthetase inhibitor, polyoxin D, on C. immitis. From a solution containing 25 mg/ml plumbagin in 95% ethanol dilutions were made in the spherule medium of Levine et al. The concentrations ranged from 0.25 ug to 5.0 ug/ml In a part of the study 2-deoxy-D-glucose was also tested at concentrations of 0.25 to 12.5 ug/ml because of its inhibition of cell wall synthesis in Histoplasma capsulatum (Berliner et al.).

With an inoculum of 10⁴ to 10⁵ power endospores per ml of medium, plumbagin alone was lethal to C. immitis at concentrations of 0.25 to 5.0 ug/ml. Microscopically the effect of plumbagin was to produce swollen endospores, and/or swollen immature spherules that had a retracted, compact cytoplasm and apparently thinned wall. The influence of 2-deoxy-D-glucose is unclear. By itself it did not affect viability of C. immitis, but it may have had a potentiating effect on plumbagin at low concentrations of the latter.

Some toxicologic data are available for plumbagin in laboratory animals and will prompt its evaluation in murine coccidioidomycosis

Abstract 15: Ketoconazole Therapy of Coccidioidomycosis Long-term follow-up after successful treatment with 400 - 1600 mg per day

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From 1980 until March, 1984, 122 patients with soft tissue (n=33), skeletal (n=44), or chronic pulmonary (n=35) coccidioidomycosis randomly received ketoconazole (keto) 400 or 800 mg/day; failures could continue to be evaluated with larger doses. 10 other patients were assigned 800 mg/day without randomization. Overall, 45 patients achieved a successful response (400 mg not significantly different from 800 mg/day) and data is available on 39 after stopping treatment. Successful treatment courses ranged from 174 to 1149 days using 105 - 620 grams of keto. Median follow-up was over 1 year (range: 49 - 1110 days). Only 1 of 13 patients who responded to 400 mg/day has relapsed as compared to 7 of 21 relapses in patients who responded to initial 800 mg/day therapy. Conversely, of 7 patients failing 400 mg/day and subsequently successful with higher doses, 4 have relapsed. 1 of 4 has relapsed after successful treatment above initial 800 mg/day. Relapses have been detected between 31 and 660 days after therapy was discontinued with 11 of the 13 occurring within nine months. Relapses occurred with each major site of infection: 4 with skeletal, 3 with pulmonary and 6 with soft tissue lesions. In most patients, relapses occurred at previous sites of infection.

We conclude that relapses are frequent after discontinuing effective ketoconazole therapy at all doses. Patients requiring higher doses of ketoconazole to achieve a response, appear more likely to relapse.

Abstract 16: Pharmacokinetics and toxicity of high dose ketoconazole

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160 patients receiving 400-2000 mg ketoconazole once daily in 2 multicenter protocols for nonmeningeal and meningeal coccidioidomycosis were studied. Drug concentrations were determined by bioassay. For 24 h, at all doses, mean serum levels exceeded *C. immitis* MICs (>1 mcg/ml). Mean peaks occurred 4-6 h post dose, ranging from 7 to 17 mcg/ml for 400 to 2000 mg. Incremental increases in serum peak levels were greatest \leq 1200 mg. As long term Rx may alter pharmacokinetics, serial data were studied by several methods. The results suggested a trend to increased serum levels with prolonged therapy, but were not statistically significant. All 168 meningitis CSF samples were \leq 2.9 mcg/ml, and only 6 were >1 mcg/ml. There was no clear relation of dose, time after dose, site of sampling, or concurrent inflammation to CSF level. Penetration was best into lumbar CSF (8% of serum level). Toxicity was reversible, and principal side effects were nausea and vomiting (50%), gynecomastia (21%), decreased libido (11%), alopecia (8%) elevated liver function tests (6%), pruritus (6%), and rash (5%). Gastrointestinal and endocrinologic toxicity was dose related, and markedly increased >800 mg. Cumulative percent toxicity requiring discontinuing drug was 6, 17, 23 and 56% at 400, 800, 1200, 1600 mg. Response/nonresponse to Rx did not correlate with peak serum level or dose in mg/kg. As response to initial doses of 400 or 800 mg is not different in chronic pulmonary, bone/joint, skin/soft tissue disease, the increased toxicity at high doses suggest no enhanced benefit of doses >400 mg. Whether high doses improve meningitis results awaits further studies.

Other presentations Abstracts not Published:

- Natural killer cells in coccidioidomycosis
A. Catanzaro
- Soluble extracts of *C. immitis* Cell Walls are antigenic for T lymphocytes.
T. Kirkland, G. Cole
- Immunoblot analysis of immuno- diffusion antigens of *C. immitis*
B. Zimmer
- Late dissemination of coccidioidomycosis associated with pregnancy
H. Einstein
- Initiation of a randomized trial of ketoconazole in primary Coccidioidomycosis
P.L. Williams, et al
- Initiation of a randomized trial in coccidioidal meningitis with intrathecal therapy \pm ketoconazole
J.R. Graybill, et al