Coccidioidomycosis

STUDY GROUP

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Abstract 1: Exploring links between climate and Valley Fever

Comrie AC, Kolivras KN.

Previous research, mainly from the 1950s and 1960s, indicates that Valley Fever varies with climatic conditions. However the precise relationship has not been thoroughly examined. Our research aims to understand how the timing and magnitude of seasonal climate variations affect the growth, release, and wind dispersal of the fungal spores that cause the disease. Precipitation and temperature appear to be the most important climatic controls on the fungus, and particular sequences of wet and dry conditions impact its variability. By comparing the influence of individual climate variables (including temperature, precipitation, and a drought index) on incidence, we determine which variables are important to the growth and dispersal of the fungus during different times of the year. We also examine the climatic conditions leading up to a month with particularly high or particularly low incidence.

Preliminary results indicate that summer temperatures positively influence incidence in the months that follow. In other words, particularly high summer temperatures may lead to increased incidence in the following months. Also, precipitation is important in both long and short time periods. Moisture is required for the fungus to grow and reproduce, while a short-term dry spell is necessary for the fungus to become airborne.

Given an understanding of the information from these analyses, we will develop a model that will aid in the forecast of Valley Fever incidence in future months based on current climate conditions.

Abstract 2: Epidemiologic and Geographic Assessment of Human Coccidioidomycosis in Pima County, AZ 1998

K.Smith, S.Rogan, K.Komatsu, S.Yool, N.Ampel

Table 1: Number of coccidioidomycosis cases in Arizona, by year.*

1990	1991	1992	1993	1994	1995	1996	1997	1998
256	287	444	580	578	623	655	958	1436

Table 2: Incidence of selected infectious diseases in Arizona, 1998.*

Disease	Incidence of Disease (per 100,000)
Malaria	**0.32**
Tuberculosis	**5.38**
Chickenpox	**35.43**
Coccidioidomycosis	**30.41**

*Information in tables obtained from Arizona Department of Health Services website: www.hs.state.az.us

Objective: To build a predictive model of coccidioidomycosis risk by identifying significant risk factors for the disease

Method used to achieve objective: Combining environmental data and epidemiologic data (obtained from a case-control study) in a geographic information system (GIS)

Risk	Environment	Epidemiology
Low	Climate	Human Activity
Medium	Soil	Land Use
High	Geology	Health History
	Vegetation	Race/Ethinicity
	Topography	SES
	Soil disturbance	

*Assumption in development of cocci risk model is that exposure to the disease occurred at or around the location of an individual's residence

See also: G.E. Glass, et al. (1995) Environmental Risk Factors for Lyme Disease Identified with Geographic Information Systems. Am J Public Health 85: 944-948

I. Epidemiologic Assessment:

Case-control study of cocci in Pima County, AZ, 1998

Case Subjects:

*278 cases of cocci reported for Pima County in 1998

Breakdown of attempts at contact made for inclusion in the case-control study as a case subject:

1. No telephone number available: 133/278 (47.8%)

2. Telephone number available: 145/278 (52.2%)

a. Phone disconnected/not at # anymore: 52/278 (18.7%)

b. No contact made after 5 attempts: 14/278 (5.0%)

c. Refused to answer: 10/278 (3.6%)

d. Unable to answer: 5/278 (1.8%)

e. Died: 13/278 (4.7%)

10 males

3 females

f. Administered questionnaire: 51/278 (18.4%)

Control subjects:

Obtained 1:1 ratio of cases to controls (51 cases, 51 controls)

1. Same household controls: 11/51 (21.6%)

2. Random neighbor controls: 40/51 (78.4%)

***Skin tests were not performed on control subjects. Since approximately 60% of individuals that develop cocci are asymptomatic, some of these controls may actually have had the disease. Therefore, this study is more accurately examining the risk of developing moderate to severe cocci.**

Questionnaire:

***7 pages, 26 questions**

***Average time of 10-15 minutes to administer**

***5 major sections: Baseline Data, Residency, Activities,**

Medical History, and Demographic Data

***2 interviewers**

Statistical Analyses of Case-Control Study Results

(Performed using SPSS; SPSS for Windows, Rel. 9.0.0. 1998. Chicago: SPSS Inc.)

UNIVARIATE ANALYSIS

***162 variables examined**

1. Independent t test for continuous variables

2. Chi-square test for categorical variables

Variables found significant in univariate analysis:

Variable	**p-value**
Total years in Arizona	**0.023**
Visited Arizona before living in the state	**0.001**
Performed yard-work	**0.044**
Asian/Pacific Islander	**0.050**
Some post-college education	**<0.001**
Other Education (Masters/PhD)	**0.022**
Income <$9,999 per year	**0.001**
Income $10,000 - 19,999 per year	0.002
Income $20,000 - 29,999 per year	<0.001
Income $30,000 - 139,999 per year	0.001
Income $50,000 - 74,999 per year	0.002
Income $75,000 - 99,999 per year	0.001
income >$100,000 per year	0.002

MULTIVARIATE ANALYSIS

Logistic Regression:

*Included the variables found significant in univariate analysis, along with age and gender, in logistic regression model as independent variables

*Development of cocci (moderate to severe) was the dependent variable (case vs. control status)

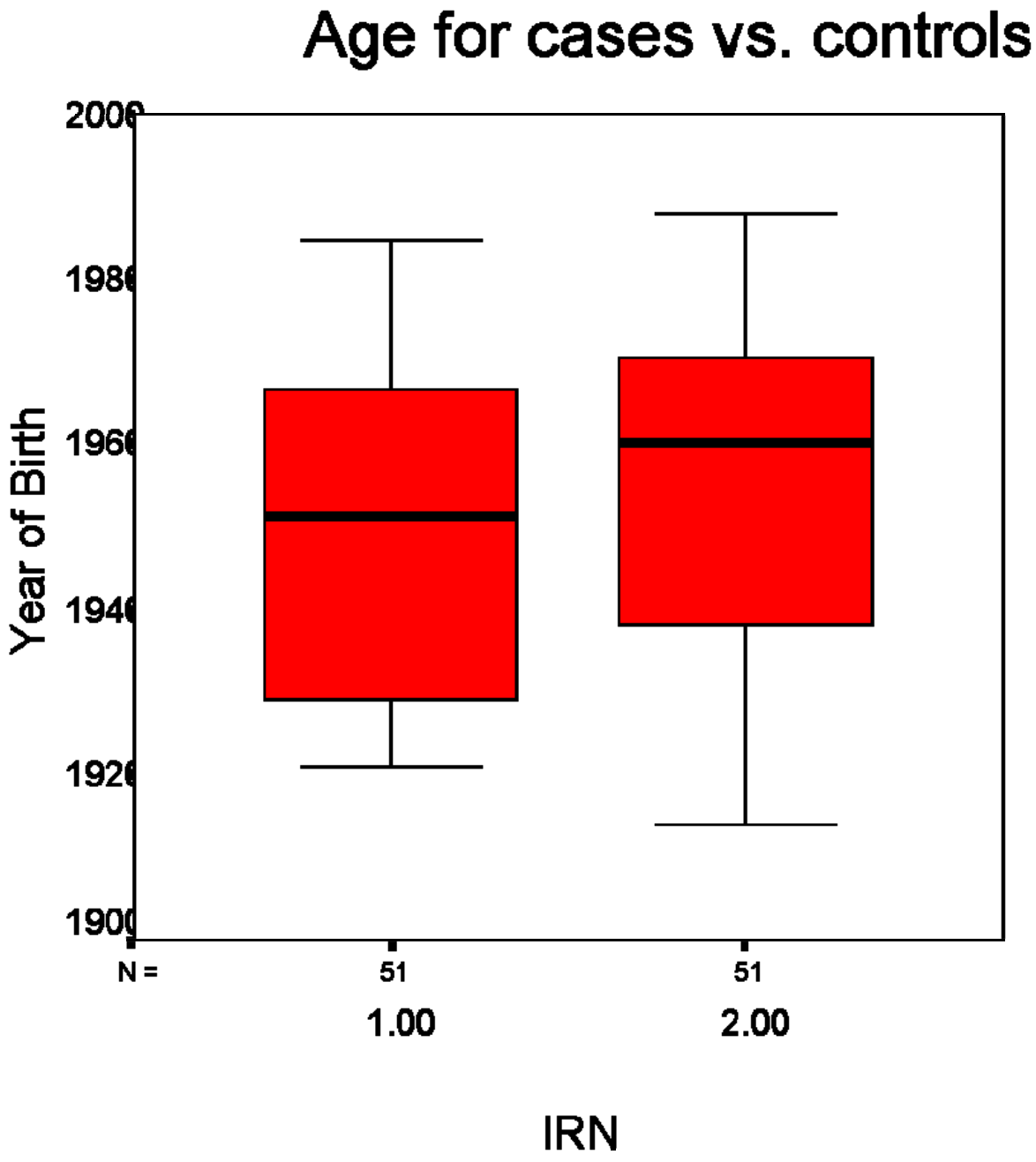
**Final model included total number of years living in Arizona and age as significant explanatory variables (test for interaction between age and total years living in AZ was not significant)

Total years living in Arizona

p=0.0114
Odds Ratio=1.0442, 95% CI (1.0098-1.0797) **Birthyear (age)**

p=0.0318
Odds Ratio=1.0235, 95% CI (1.0020-1.0454)

****Study by Leake, et al, Risk Factors for Acute Coccidioidomycosis Among Older Adults in Arizona, 1996-1997, also found duration of residence in Arizona to be a significant factor for increased risk of developing cocci**



II. Geographic Assessment using GIS

*Geocoding addresses (address matching) for 278 Pima County cocci cases produced the following results:

1. No address available: 73/278 (26.3%)

2. Geocodable addresses: 191/278 (68.7%)

3. Address not geocodable: 14/278 (5.0%)

(P.O. Box/Rural route numbers)

*Addresses for all of the 51 cases and 51 controls in the case-control study were geocodable

*Addresses were matched using PCLIS database, not U.S. Census TIGER files. PCLIS is more comprehensive than the TIGER files, and is produced and updated every 4 months by the Pima County Land Information office.

*Used latitude and longitude of parcel centroids to match addresses, instead of matching along a line feature which interpolates address ranges. Geocoding by parcel gives more precise information about the actual location of a residence.

Evaluation of Spatial Clusters:

1) Overlay Rectangular Grid

2) Measure Distance from Each Point on Grid to:

    a) Numerator - Health Event

    b) Denominator - 1990 Census Block Population

3) Compute Disease Rate for Each Grid Point at Varying Distances from the Grid Point

4) Interpolate Grid Output

5) Map Observed Disease Rates

*Test for Significance of Mapped Rates:

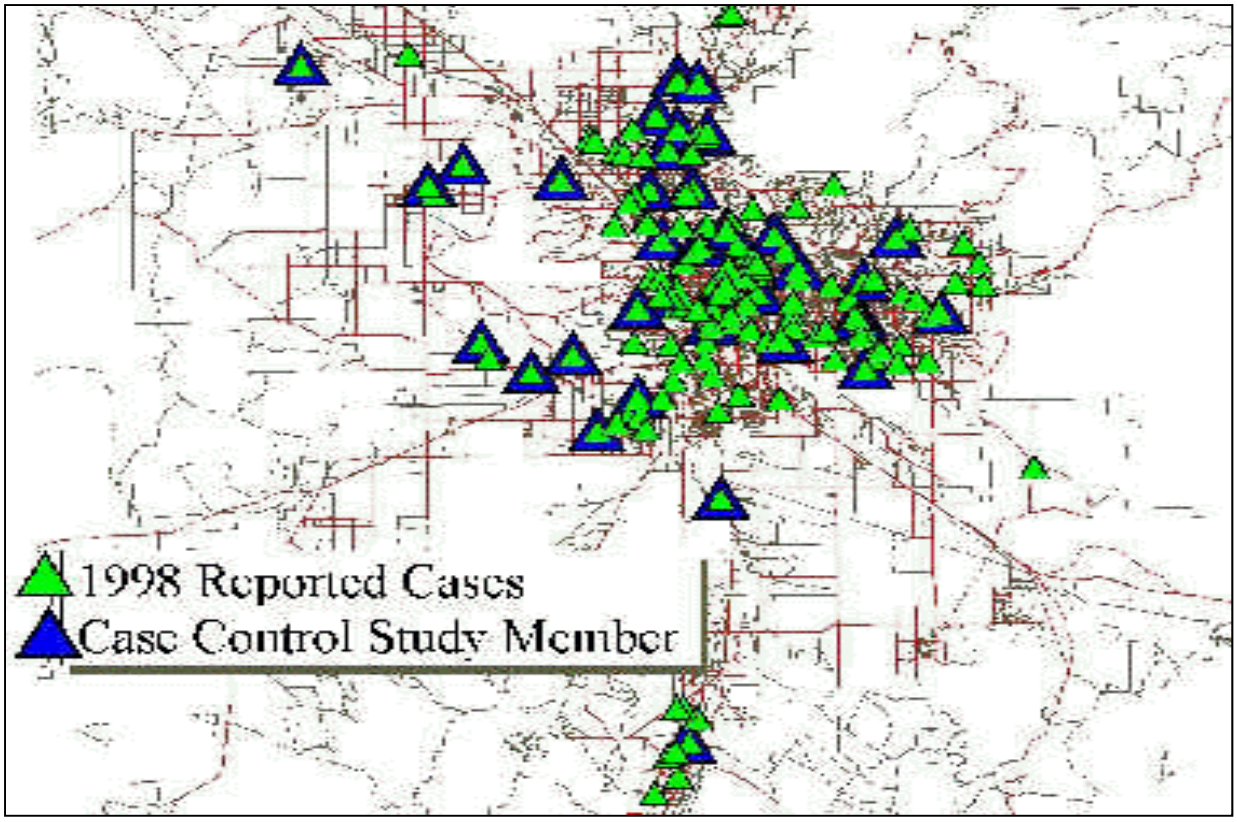
Monte Carlo Simulation

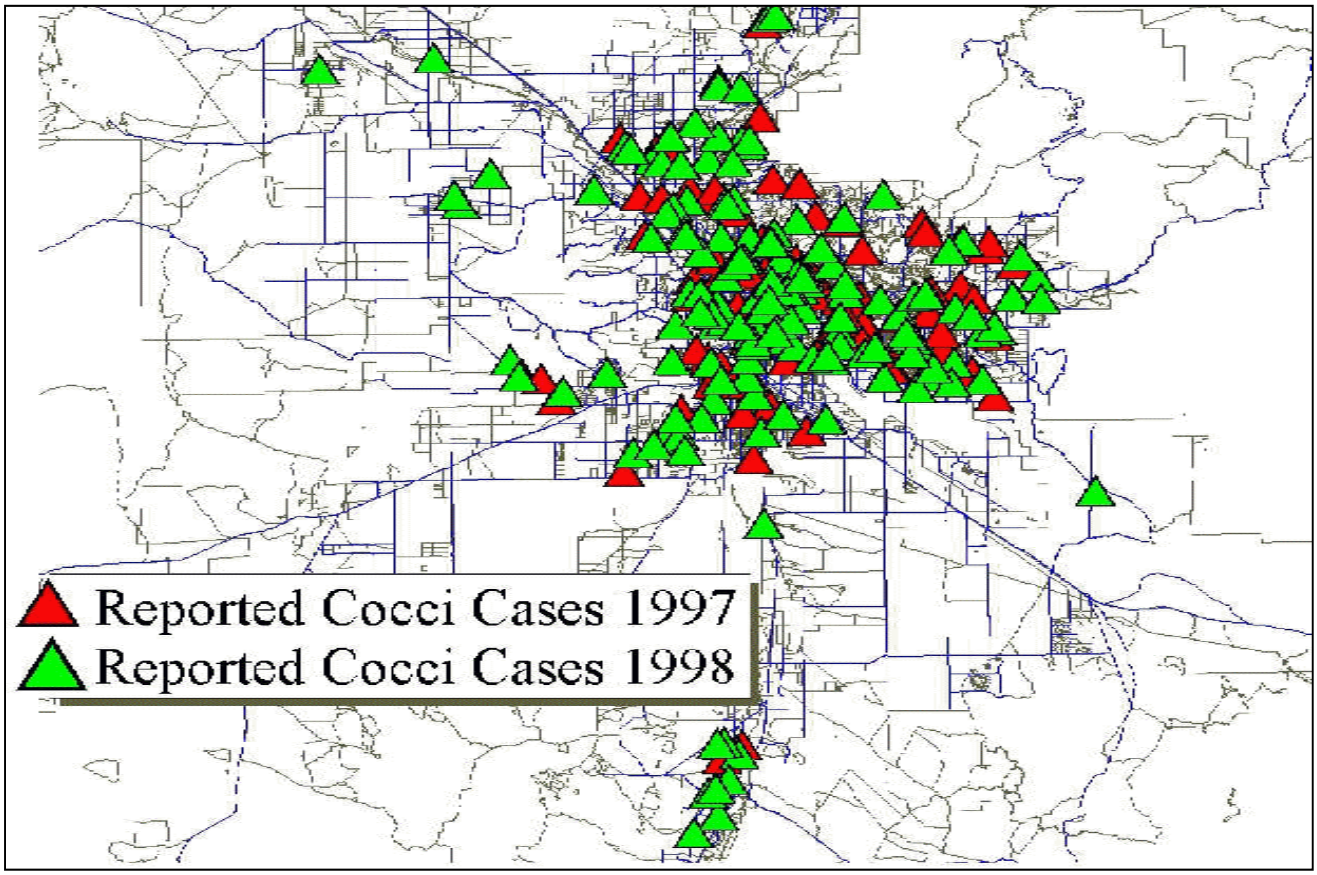
Significance= Proportion (Simulated Rates<Observed)

See also:

Rushton, G., and Lolonis, P. 1996. Exploratory Spatial Analysis of Birth Defect Rates in an Urban Population. Statistics in Medicine 15: 717-726

Rushton, G., and Krishnamurthy, R., Krishnamurti, D., Lolonis, P., and Song, Hu. 1996. The Spatial Relationship Between Infant Mortality and Birth Defect Rates in a U.S. City. Statistics in Medicine 15:1907-1919


1998 Reported Cases
Case Control Study Member


Reported Cocci Cases 1997
Reported Cocci Cases 1998

Abstract 3: MICROSATELLITES IN *COCCIDIOIDES IMMITIS* THROUGHOUT ITS NEW WORLD RANGE

M. C. Fisher, J. L. Koenig†, T. J. White† and J. W. Taylor**

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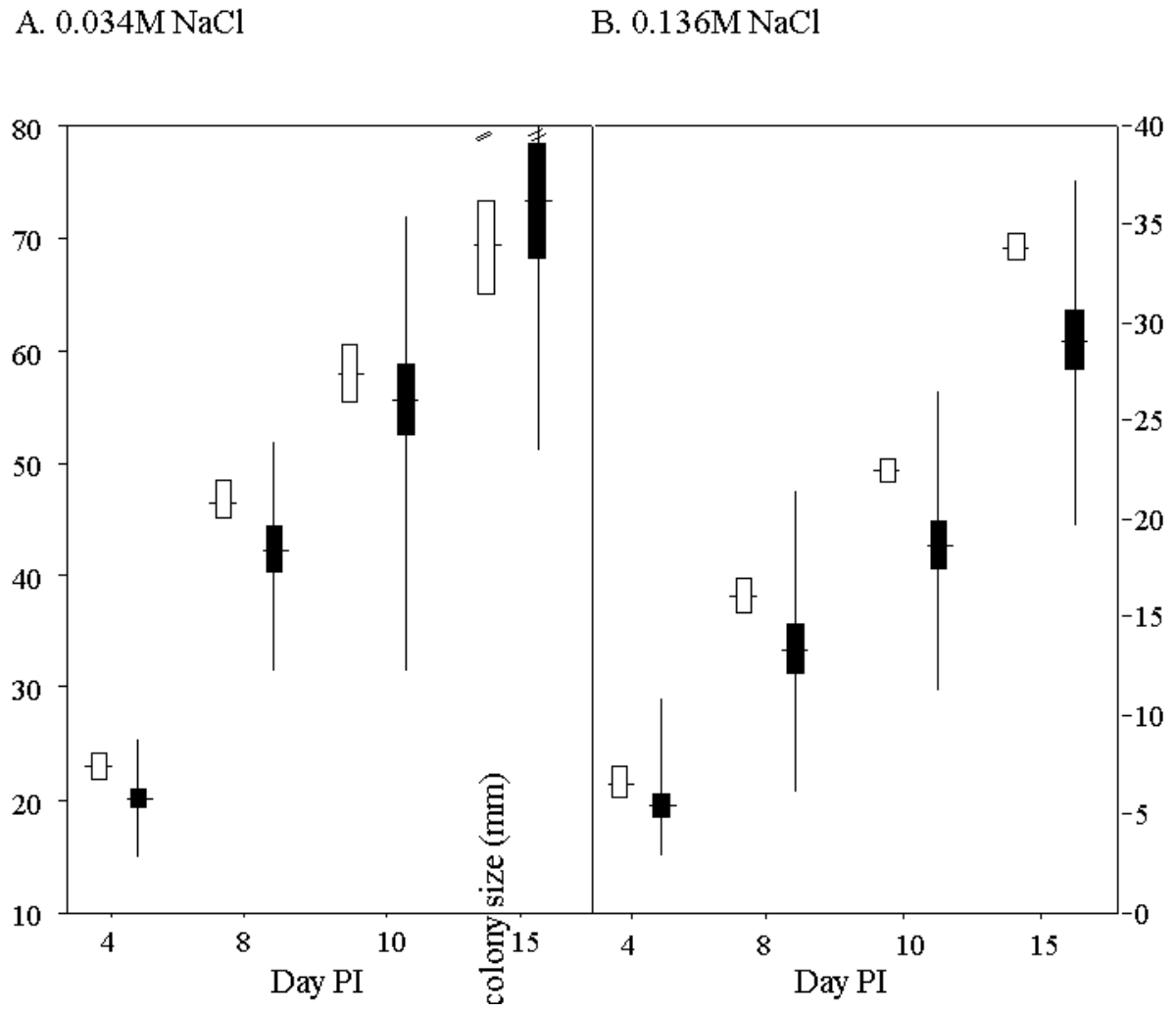
† Roche Molecular systems, 1145 Atlantic Avenue, Alameda, California 94501 USA

We have isolated nine microsatellite-containing loci from *Coccidioides immitis* and determined allele-frequencies at each locus from a panel of 167 isolates collected over the entire New World range. This has shown that *C. immitis* consists of two phylogenetic species, reproductively isolated from one another for the past 12 million years. The first species, *California*, appears to be centered in the Southern San Joaquin valley although isolates also appear in the San Diego and Northern Mexico area. The second species, *non-California* has a much wider range and occurs in Southern California, Arizona, Texas, North, Central and Southern Mexico and South America. Growth experiments on high salt-containing media have shown that *California C. immitis* grows significantly faster (Figure 1), suggesting that other, perhaps clinically important, differences may exist between the two species.

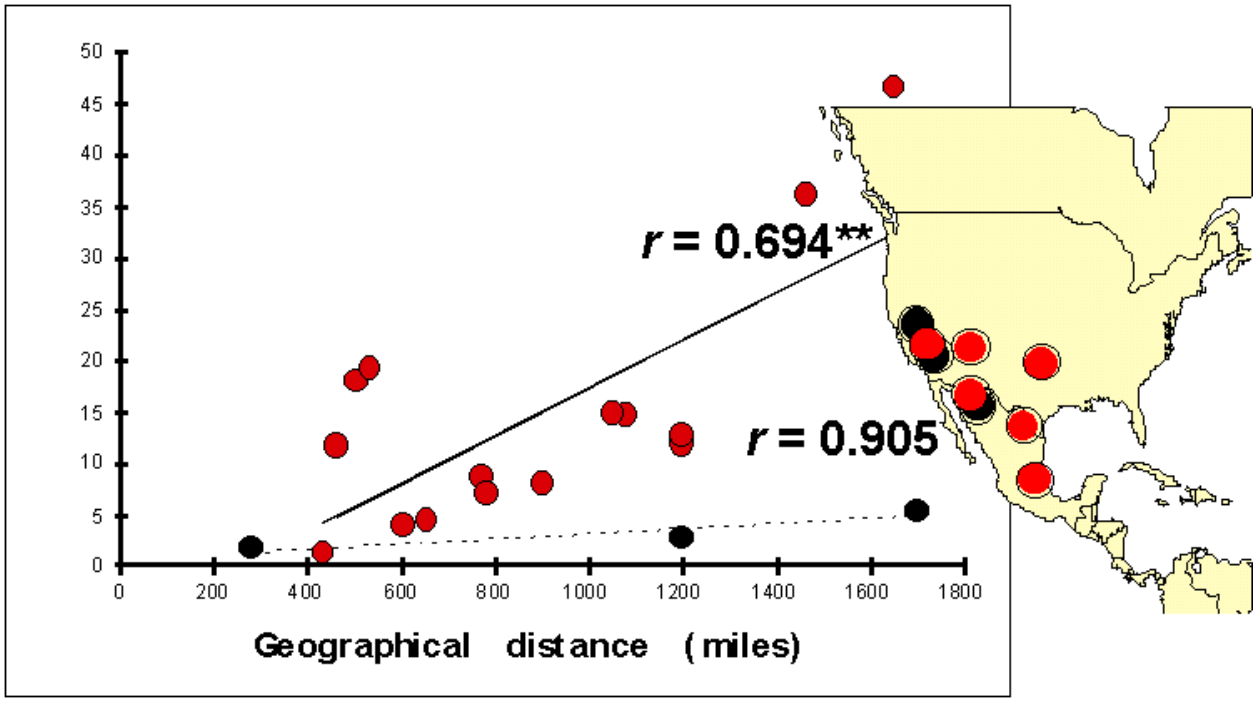
Genetic analyses show that within each species there occur a number of isolated populations, showing that gene-flow is not great. There are strong positive correlations between geographic and genetic distance for both *California* and *non-California* (Figure 2). This shows that geography is important in determining how far isolates disperse. Interestingly, this pattern of isolation-by-distance breaks down when isolates from South America are included (all *non-California*). These isolates are all closely related to those within North America despite the large geographical distance between these populations. We speculate here that South American *C. immitis* may recently (within the past 9 000 to 134 000 years) have originated from populations in North America, an event that is possibly related to the end of the last glaciation and the subsequent radiation of mammalian hosts.

Figure 1. Growth on different media of *California* (white bars) and *non-California* (black bars) *C. immitis*.



notes: Summary of means ± 95% confidence intervals (rectangles) and total ranges (lines) on A. low (0.034M) and B. high (0.136M) salt-containing YEG media. White = *California C. immitis* (*CA*; *n* = 10) and black circles = *non-California C. immitis* (*non-CA*; *n* = 10).

Figure 2. North American and Mexico *C. immitis* show isolation-by-distance (*California* in black, *non-California* in red).



Abstract 4: Genetic transformation of *Coccidioides immitis* by *Agrobacterium tumefaciens*

Abuodeh RO[1,2,4,a], Orsborn KI[2], Orbach MJ[1,3], Mandel MA[1,3],

Das A[5], Galgiani JN[1,2,4]

[1]Valley Fever Center for Excellence; [2]Medical and Research Services, Southern Arizona Veterans Affairs Health Care System; and [3]Dept. of Plant Pathology; College of Agriculture, and [4]Dept. of Internal Medicine, College of Medicine, University of Arizona; [5]Dept. of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, St. Paul; [a]College of Health Sciences, University of Sharjah, Sharjah, United Arab Emirates

Molecular genetic approaches have revolutionized the study of virulence and pathogenesis of a wide variety of organisms. As a prelude to targeted gene disruption, we have utilized the DNA-transfer machinery of the plant pathogenic bacterium *A. tumefaciens* to develop an efficient system to transform *C. immitis. A. tumefaciens*, a gram-negative soil bacterium, can transfer a portion of its own Ti plasmid DNA (T-DNA) to plant cells. This property has been exploited to deliver foreign genes into the genome of plants and more recently into fungi as well. The *Escherichia coli* hygromycin phosphotransferase (*hph*) gene, under the control of the *Neurospora crassa* cpc-1 promoter, was cloned between two T-DNA border sequences of a Ti-plasmid vector and introduced into *A. tumefaciens*. Co-cultivation of plasmid-bearing *A. tumefaciens* with 10^7 previously germinated arthroconidia of *C. immitis* under conditions favoring T-DNA mobilization yielded numerous hygromycin-resistant (hyg-R) fungal colonies. Enzymatic removal of the fungal cell wall prior to cocultivation was not essential for transformation. A transformation efficiency of up to 10 transformants per 10^6 arthroconida was obtained by varying the germination time of *C. immitis* and the ratio of *A. tumefaciens* to *C. immitis* germlings. The hyg-R phenotype appeared to be mitotically stable, consistent with Southern analysis indicating that in most cases examined, a single copy of the T-DNA containing the *hph* gene was integrated into the *C. immitis* genome. *A. tumefaciens* mediated gene transfer is relatively simple and requires no special equipment. This approach should prove useful for the development of tagged mutagenesis and targeted gene disruption technologies for *C. immitis* and other fungi of medical importance.

Abstract 5: Long-term storage of *Coccidioides immitis*

Koenig, Gina

Roche Molecular Systems

Goal

- Preservation of unique isolates with a minimum of ongoing maintenance
- Currently seven years of stability data

Cryopreservation Process

- Isolate pure culture
- Grow until conidiation occurs
- Suspend in fungal freeze medium
- Aliquot
- Freeze slowly
- Check viability one week later
- Thaw frozen vial in 37C water bath

Fungal Freeze Medium

- Potato dextrose broth
- 0.01% Tween 20
- 15% glycerol

Contacts

- Freezing container VWR 55710-200
- Cryovials VWR 66021-986
- Laser Tough-Tags USA/Scientific Plastics 9187-2016
- Color cap inserts VWR 24270-120
- Cryoboxed VWR 55710-252

Abstract 6: Cost-effectiveness of a potential vaccine against *Coccidioides immitis*

Barnato AE, Sanders GD, Owens DK

Background: Coccidioidomycosis is a systemic fungal infection that affects Americans living in the Southwest for which vaccines are under development. We evaluated the cost-effectiveness of a potential vaccine against *Coccidioides immitis*.

Methods: We developed a decision model to evaluate the health and economic consequences of withholding the vaccine, screening and vaccinating only those susceptible to infection (screen/vaccinate), or vaccinating all eligible persons.

Results: Among infants, 15 year-old residents, 15 year-old immigrants, and 35 year-old immigrants, vaccination increased quality-adjusted life expectancy by 1.2, 1, 1.1, and 0.75 days and saved $260, $186, $202, and $90 per person over no vaccination, respectively. For resident 35 year-olds, a screen/vaccinate strategy increased quality-adjusted life expectancy by 0.5 days and saved $29 per person over no vaccination. Vaccination for immigrant 65 year-olds and a screen/vaccinate program for 65 year-old residents cost $158,000 and $310,000 per quality-adjusted life year (QALY), respectively. If a one-year birth cohort in highly endemic counties received the vaccine, 9 deaths would be averted and $23 million saved.

Conclusions: Vaccination against *C. immitis* reduced morbidity, mortality, and health care costs for infants, teens, and immigrants 35 and younger in endemic regions. Although the magnitude of reductions is low for individual persons, the aggregate health benefits and economic savings at the county and state level would be substantial.

Abstract 7: Flow Cytometric Assessment of the Human Immune Response to Coccidioidal Antigens

Ampel, Neil M.

Southern Arizona Veterans Affairs Health Care System (SAVAHCS)

and the University of Arizona, Tucson, AZ.

Protective immunity against human coccidioidomycosis appears to reside in the cellular immune response. Past studies have indicated that peripheral blood mononuclear cells (PBMC) from healthy, immune individuals produce interferon-gamma (IFN-) in response to coccidioidal antigen. Flow cytometry offers the ability to examine the immune response on a cell-to-cell level. In initial studies, we used PBMC isolated by Ficoll-Hypaque¨ and CD3+ lymphocytes (CD3) after incubation with the coccidioidal antigen preparation TSL (a gift from Dr. J. Galgiani), comparing healthy immune and non-immune donors. In data already published (Ampel & Christian, Med Mycol 2000; 38:127-32), we found that CD3 incubated with TSL from coccidioidal immune donors showed increased surface expression of the activation markers CD69, CD25, and HLA-DR as well as increased intracellular production of IFN- compared to non-immune donors. To explore this further, we began incubating whole blood samples with the coccidioidal antigen preparation T27K (a gift from Dr. D. Pappagianis) and examined various groups of donors. Whole blood assay has the advantage of rapid preparation and maintenance of the cells in a physiologic milieu. T27K antigen preparation has been shown to act as a protective vaccine in mice (Zimmermann, et al; Infect Immun 1998; 66:2342) but has not been previously examined in humans. Using this system, we also found IFN- production by CD3 was increased among immune donors. Moreover, there was a hierarchy of response, with CD3 from donors with either chronic pulmonary or disseminated coccidioidomycosis producing significantly less IFN- than immune donors but increased amounts when compared to healthy, non-immune donors. Examination of surface expression of CD69 yielded similar results. These data indicate that flow cytometric analysis of whole blood is a rapid method of assessing cellular immune response to coccidioidal antigens.

Abstract 8: Fractionation and characterization of T27K vaccine:

Selecting immunoprotective components against *Coccidoides immitis*.

Kerekes KM, Johnson SM, Williams RH, Zimmermann CR, Ampel NM, Christian L and Pappagianis D.

Department of Medical Microbiology and Immunology University of California at Davis, Davis CA; and Southern Arizona VA Health Care System and University of Arizona, Tucson, AZ

A subcellular vaccine, T27K, derived from thimerosal-killed whole spherules of *C. immitis* when administered with alum was protective in mice against lethal intranasal (i.n.) respiratory challenge with arthroconidia. When subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the T27K yielded discrete protein bands. Further studies have been completed to separate and identify immunoprotective components present in the T27K vaccine. Fractionation was carried-out (1) by ammonium sulfate (AS) precipitation using 30% saturated AS followed by 50% saturated AS, and (2) by fast protein liquid chromatography (FPLC).

From the AS precipitation four fractions were obtained: 30% AS precipitate and 30% AS supernatant; and 50% AS precipitate and 50% AS supernatant. By SDS-PAGE, the two supernatants were virtually identical, while the redissolved 30% and 50% precipitates differed markedly. *In vitro*, all four fractions stimulated lymphocytes from skin test positive (immune) humans. The 30% AS and 50% AS precipitates were comparable to whole 27K in stimulating the lymphocytes; the supernatants were substantially less stimulatory.

FPLC gel filtration, which separates proteins according to molecular size, yielded seven pooled fractions from the T27K vaccine. Enzymatic analysis demonstrated that chitobiase activity localized in fraction I. Furthermore, a serine protease that localized in fraction VI has been identified through amino acid sequencing as the CS-antigen (Resnick, et al, 1988 and Cole, et al, 1995).

The *in vitro* studies were performed to determine if there was a correlation between lymphocyte stimulation and *in vivo* protection of mice. Lymphocyte proliferation assays showed that fractions I and IV were the most stimulatory while fractions II and III were only slightly stimulatory and fractions V-VII were cytotoxic. Gel filtration fraction I was equally as stimulatory as the whole T27K vaccine *in vitro*; and 100 mg doses of fraction I protected 100% of mice from death when challenged i.n. with either 500 or 1500 arthroconidia, markedly more protective than any other fraction. However, 100 mg doses of fraction IV did not protect mice against intranasal arthroconidia challenge. Fractions II-III and V-VII were not protective in mice.

Further fractionation of FPLC gel filtration fraction I was accomplished using FPLC anion exchange. Elution of negatively charged proteins using a salt gradient resulted in several more fractions comprising discrete bands on SDS-PAGE, thus permitting further characterization of the immunoprotective fraction I.

Abstract 9: Fractionation and characterization of T27K vaccine: II.

Immunoprotective fraction with determination of effective adjuvant dose in mice

Johnson SM, Kerekes KM, Williams RH, Zimmermann CR, Pappagianis D.

University of California, Davis.

A soluble vaccine derived by mechanical disruption of thimerosal-killed *C. immitis* strain Silveira spherules followed by centrifugation at 27,000 x *g* (27K) has previously been shown to provide protection against a lethal respiratory *C. immitis* challenge when given with alum adjuvant. In an effort to determine the immunoprotective components of the 27K vaccine, fractionation methods have been applied and the resulting fractions tested using the murine model. Also, the effect of vaccine/adjuvant dosage was studied.

Fractions of the 27K vaccine were prepared by ammonium sulfate precipitation or gel filtration chromatography. Fractions obtained by precipitation with 30% and 50% ammonium sulfate (3 x 100 mg doses plus adjuvant) protected as well as the whole 27K vaccine (3 x 250 mg doses plus adjuvant) when challenged with either 500 or 1,500 arthroconidia. In contrast, both resulting supernatants of the ammonium sulfate precipitation showed much lower protective ability. Gel filtration chromatography yielded seven fraction pools. One hundred percent of mice immunized with fraction I (3 x 100 mg doses plus adjuvant), which contains the largest protein species, survived following challenge with 500 or 1,500 arthroconidia. Although fraction II did show protection against 500 arthroconidia challenge (100% survival), it did not protect as well against 1,500 arthroconidia (29% survival). All other fractions showed little or no protection at either challenge levels. A dose titration study of whole 27K vaccine showed that 250 mg x 3 doses gave a higher level of protection, superior to the standard 1 mg x 3 doses that was previously used. The adjuvant alum was also titrated with accompanying doses of 27K, and the optimal dose of the adjumant was 1.2 mg, the dose we have previously used.

Studies are now underway to define the immunoprotective components that comprise gel filtration fraction I.

Abstract 10: Further studies with the recombinant antigen, rAg2/PRA, as a vaccine candidate to prevent coccidioidomycosis

Shubitz L, Peng T, Perrill R, Orsborn KI, Galgiani JN.

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Ag2/PRA is a proline-rich 194 amino acid sequence contained in a spherule glycoprotein historically identified as Antigen 2. Recombinant antigen (rAg2/PRA) used in these studies was purified from its fusion partner after expression in pET-32. Female BALB/c mice were immunized at 6-7 weeks of age and boosted 4 weeks later. Subcutaneous immunizations were prepared with monophosphoryl lipid A (MPL) in an oil emulsion (MPL-SE, Corixa, Hamilton, MT); intranasal immunizations were prepared with MPL in an aqueous formulation (MPL-AF, Corixa). Four weeks after boosting, mice were challenged with arthroconidia of *C. immitis*, either intraperitoneally (IP) or intranasally (IN). Mice for quantitative studies were sacrificed at 14 days, while animals in survival studies were kept for 56 days. *In vitro* markers of interferon-, lymphocyte proliferation, and immunoglobulin G (IgG) subtypes were evaluated for some studies.

A quantitative organ culture study with rAg2/PRA doses of 50g, 5.0g, and 0.5g demonstrated improved protection against IP infection (50 spores per mouse) with lower doses; 0.5g was statistically better than 50g. A second study comparing doses of 5g, 0.5g, and 0.05g showed all to be effective against 12 spores IP, and vaccination appeared to prevent dissemination to the lungs.

After subcutaneous vaccination with doses of 5g or 0.5g of rAg2/PRA and IN infection with 7 spores per mouse, survival 56 days later was 70% and 90%, respectively, significantly greater than than for control mice (30%, $p<0.01$). IN immunization was also evaluated using 5g, 0.5g, or 0.05g of rAg2/PRA in MPL-AF adjuvant. When infected IN with low numbers of challenging arthroconidia, fungal burden of vaccinated mice was significantly reduced (P=0.012, Kruskal-Wallis). Of *in vitro* markers evaluated in our lab, IgG_{2a}:IgG_1 ratios 1 correlate most consistently with protective immunity. In two studies with variable doses of rAg2/PRA IN or SC, levels of IFN- increase from stimulated splenocytes with increasing dose of antigen. Stimulation indices of splenocytes are also elevated, two- to three-fold above background after vaccination with rAg2/PRA.

These studies support rAg2/PRA as a vaccine candidate to protect against coccidioidal infection.

Abstract 11: HLA class I association with coccidioidomycosis disease severity in Kern County

Jackman R[1], Hajjeh R[2], Johnson R[3], Vugia D[4], Werner B[4], Louie L[1].

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While most patients with symptomatic coccidioidomycosis experience a mild, flu-like illness, progression to severe pulmonary or disseminated disease occurs in 1 to 10%, depending on ethnicity. Previous work shows risk of symptomatic disease is associated with ABO blood group and class II HLA loci variation. We now examine the role of class I HLA genes. Participants included Caucasians, Hispanics, and African-Americans with mild or severe disseminated coccidioidomycosis from Kern County, California. Ethnically matched population controls were selected from the literature and from the National Marrow Donor Program database.

Heterogeneity between cases and controls was observed within all three ethnic groups to varying degrees, and several alleles were associated with either increased or decreased risk of mild or severe forms of the disease. C*07 was associated with decreased risk of both severe and mild disease vs. controls among Caucasians (OR = 0.5, $p < 0.05$, and OR = 0.3, $p < 0.001$, respectively), and with decreased risk of mild disease vs. controls among Hispanics (OR = 0.2, $p < 0.01$). A*34/66 was associated with increased risk of severe disease vs. controls among African Americans (OR = 9.5, $p < 0.05$) and increased risk of both severe and mild disease vs. controls among Caucasians (OR = 8.6, $p < 0.01$, and OR = 14.6, $p < 0.05$, respectively). In addition, C*08 was associated with increased risk of both severe and mild disease vs. controls among Caucasians (OR = 3.1, $p < 0.05$, and OR = 4.1, $p < 0.001$, respectively), and B*38 was associated with increased risk of both severe and mild disease vs. controls among Hispanics (OR = 11.0, $p < 0.01$, and OR = 22.3, $p < 0.001$). Additional alleles were found to be associated with risk of disease between only one ethnic/disease severity group compared to controls.

These results are consistent with data for class II HLA, in that the allelic frequencies of mild and severe cases do not differ from each other, but both differ from those of controls. These data suggest the involvement of class I HLA genes in the pathogenesis of coccidioidomycosis, indicating that CTL responses play a role in elimination or control of *C. immitis*, and further supporting the hypothesis that host genes influence susceptibility to coccidioidomycosis.

Abstract 12: Coccidioidomycosis in a Veterans Affairs clinic: An on-going project

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The natural history of symptomatic coccidioidomycosis is not completely defined. To further understand this, we have established a referral clinic in a single Veterans Affairs Medical Center within the coccidioidal endemic region. Study methodology included active recruitment of all patients with a diagnosis of coccidioidomycosis at SAVAHCS based on either direct referral by the patientÕs primary care provider or by review of pathology and serology reports and direct patient recruitment. On entry, all subjects provided informed consent and the study was approved by the Human Subjects Committee of the University of Arizona. Subjects were followed as clinically indicated and all laboratory tests were obtained at clinical discretion. By March 14, 2000, a total of 69 patients have been referred to the clinic. The mean age was 59 years (range: 28 - 84) and 48 were white, 17 African-Americans and 4 were Amerindian. Of the 69 subjects, 63 were non-hispanic and 63 were male. Forty-four of the subjects had some form of pulmonary coccidioidomycosis, including 17 with nodules, 14 with primary focal pulmonary disease, six with chronic pulmonary disease, four with diffuse pulmonary disease, and three with cavities. Of the 25 with extrathoracic disseminated coccidioidomycosis, 10 had bone or joint involvement, nine had soft tissue disease, and six had meningitis. The median time of coccidioidal diagnosis was 1.4 years (range: 0 - 24) and the median IDCF titer was 2 (range: 0 - 32). Twenty-nine were on no antifungal therapy, while 34 were receiving fluconazole; three itraconazole; two ketoconazole; and one intravenous amphotericin B therapy. There was a significant difference in the type of clinical disease and the antifungal therapy, with all subjects with meningitis receiving fluconazole ($p = .01$). There was a significant difference between the race of the subject and the type of coccidioidomycosis, with African-Americans being more likely to have disseminated coccidioidomycosis than other races ($p < .001$). These data represent an early analysis of an on-going study. In the future, more information will be collected and multiple visit follow-up data will accrue, allowing for more detailed analysis and assessment.

Abstract 13: Recent Epidemiologic Trends of Coccidiodimycosis in Arizona

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Coccidioidomycosis has been a reportable disease in Arizona since 1954. During those years a case was define as a physician's diagnosis of coccidioidomycosis. In September 1996, the Centers for Disease Control and Prevention (CDC) and the Council of State and Territorial Epidemiologists (CSTE) developed a case definition for public health surveillance. The definition is composed of clinical and laboratory criteria. The clinical criteria are: influenza-like signs and symptoms, including fever, chest pain, cough, myalgia, headache; pneumonia or other pulmonary lesion, by chest x-ray; rashes, including erythema nodosum or erythema multiforme; meningitis; or involvement of viscera and lymph nodes. The laboratory criteria requires having one of the following: cultural, histopathologic, or molecular evidence of *C. immitis*; or immunologic evidence of infection (serologic testing of serum, cerebrospinal fluid, etc., detection of cocci IgM by immunodiffusion, enzyme immunosorbant assay, latex agglutination or tube precipitin; or detection of cocci IgG by immunodiffusion, enzyme immunosorbant assay, or complement fixation; or skin test conversion after onset of symptoms).

The rate of reported cases of cocci has increased dramatically since 1996 (see figure 1). A portion of that increase is attributable to the implementation of a regulation requiring clinical laboratories to report positive cocci cultures and serologies. The geographic distribution shows the majority of cases reported from the lower half of the state with Pinal (53.8 per 100,000), Pima (46.8 per 100,000) and Maricopa (44.9 per 100,000) counties having the highest rates. As expected rate of cocci increases with age, with those over 60 years of age having twice the rate of persons under 40.

Abstract 14: Coccidioidimycosis in New York:

Unique challenges posed by an endemic mycosis in non-endemic areas

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Coccidioidomycosis, a systemic fungal disease caused by *Coccidioides immitis,* is endemic in the southwestern United States and in parts of Mexico and Central and South America. Only sporadic cases have been reported in areas (including New York) where the disease is not endemic. We used hospital discharge records and state mycology laboratory data to investigate the characteristics of *C. immitis* infections among New York State residents. From 1992 to 1997, 161 persons had hospital discharge diagnoses of coccidioidomycosis (ICD9 Code 114.0 - 114.5, 114.9). From 1989 to 1997, 49 cultures from patients were confirmed as *C. immitis*; 26 of these patients had traveled to disease-endemic areas. Fourteen of 16 isolates had multilocus genotypes similar to those of Arizona isolates, which corroborates the travel-related acquisition of the disease. Our results indicate that coccidioidomycosis may be more common in New York residents than previously recognized. Increased awareness among health-care providers should improve timely diagnosis of coccidioidomycosis and prevention of associated illnesses and deaths among patients in nondisease-endemic areas.

Abstract 15: Host Genetic Influences on Severity of Coccidioidomycosis.

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Coccidioidomycosis is usually expressed as a mild flu-like illness in ~40% of infected individuals, progressing to severe pulmonary or disseminated disease in 1-10% of symptomatic cases, depending on race. This report examines host genetic influences on disease severity among class II HLA loci and the ABO blood group. Subjects include African American, Caucasian, and Hispanic individuals with mild or severe disseminated coccidioidomycosis from Kern County, California. Among Hispanics, predisposition to symptomatic disease and severe disseminated disease is associated with blood types A and B, respectively. HLA class II allele DRB1*1301 allele marks predisposition to severe disseminated disease within each of the three ethnic groups. Reduced risk of severe disease is associated with DRB1*0301-DQB1*0201 among Caucasians and Hispanics, and with DRB1*1501-DQB1*0602 among African Americans. These data support the hypothesis that host genes, in particular HLA class II and the ABO blood group, play a role in susceptibility to severe coccidioidomycosis.

Abstract 16: Comparison of intravenous AmBisome or conventional Amphotericin B with oral fluconazole for the treatment of experimental coccidioidal meningitis in the rabbit.

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The efficacy of AmBisome (AmBi) against coccidioidal meningitis was assessed in comparison with oral fluconazole (FCZ) and conventional amphotericin B (AmB). Male NZW rabbits were immunosuppressed with 2 mg/kg of Solu-Cortef (days -1 to 4) and infected by intracisternal inoculation of 5 x 10^4 arthroconidia of *Coccidioides immitis* Silveira. Treatments began 5 days later. In the first study, groups of 8 to 10 received 15 mg/kg of AmBi i.v. (3x/wk for 3 wks), 80 mg/kg of fluconazole (FCZ) PO for 19 days or D5W i.v. In the second study, 8 were given either 1 mg/kg of AmB i.v (3x/wk for 3wks), which approaches the maximally tolerated dose, or D5W. One week after the cessation of therapy all survivors were euthanatized and the number of CFU remaining in the spinal cord and brain determined, and histological analyses performed. All AmBi, FCZ or AmB-treated survived and had prolonged survival, whereas 75% of controls died (P < .0005). Clinical parameters showed that animals in each regimen had significantly fewer WBC and lower protein concentrations in the CSF than controls (P < .01-.0005), and fewer clinical signs of infection (e.g., weight loss, elevated temperature, neurological abnormalities, or mobility difficulties); AmBi treatment was better than FCZ. AmBi-treated animals had minimal disease pathology and lower histologic scores than animals given FCZ or D5W (P < .016 or .0005, respectively); AmB-treated were better than control (P < .0005) and had pathology similar to FCZ-treated. All regimens reduced CFU in the brain and spinal cord versus controls (P < .0003 or .0005). AmBi-treated had 3- to 11-fold lower CFU than FCZ and 6- to 35-fold lower than AmB. Three of 8 AmBi-treated had no detectable infection in either tissue (5 cured in spinal cord and 4 in brain), whereas no FCZ-treated were free of infection in both organs (1 cured in spinal cord, 2 in brain); AmB cleared no animals of infection in either tissue. Overall, these data indicate that against experimental coccidioidal meningitis i.v. AmBi was superior to oral FCZ and i.v. AmB; FCZ was better than AmB. Since i.v. AmB alone is insufficient therapy of human coccidioidal meningitis, these data are an encouraging basis for a novel, possibly curative regimen using intravenously administered AmBi.

Abstract 17: Fibro-cavitary Valley Fever

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The Valley Fever epidemic in Kern County, California during 1991 and 1994 has called into question the incidence of various presentations and complications of Valley Fever. One such complication has been the development of cavitary and fibro-cavitary disease. Dr. J.W. Birsner presented the x-ray manifestations of 500 Valley Fever cases in 1954. Cavities represented 12%, fibrotic lesions 3.2% and Tuberculous/Valley Fever combined lesions were 3.4%. The patients ranged in age from young children to adults over 60 years old. The predominate age group was between 14 and 60 years of age. The ethnic groups included Caucasian, African-American and Hispanic (Mexican). The Caucasian and Hispanic groups made up the majority of cases. This report concerns 1,803 patients treated Kern Medical Center from 1992 to March 2000. There were 68 cavitary lesion, of which 15 had fibro-cavitary lesions and 5 tuberculosis and Valley Fever combined. The cavitary lesions were 3.7% and the other two categories were less than 1% of the cases. The majority of patients were men. The presentation was highest in the sixth and seventh decade for the fibro-cavitary lesions as compared to the fourth decade for cavitary lesions in total. The majority of the patients were Hispanic and Caucasian in ethnicity. Co-existing conditions included: HIV, diabetes and substance abuse (alcohol, cigarettes and intravenous drugs). There were two disseminated cases (cns, skin). Symptoms were cough, hemoptysis, sob and chest pain. The CF titers varied from 1:2 to 1:512. The lesions were single or multiple and either lung was involved evenly. The predominant treat was azoles and three patients required the addition of Amphotericin B. Seven responded well to treatment, 2 died, 5 were lost to follow up and one is doing poorly. The deaths occurred in the patients with HIV.

Abstract 18: Cavitary Coccidioidimycosis

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Between 1991 and 1994 over 10,000 documented cases of coccidioidomycosis were seen in Kern County California. One of the striking features of the epidemic was the small number of cases that presented with pulmonary cavitation compared to experienced over the past 60 years. This was marked contrast to other coccidioidal complications which failed to show major variations in previously recognized complication rates.

In studying this phenomenon, and going back to the work of Auerbach, Chamberlain, Salkin, and others, in the changing pulmonary reactions to the tubercle bacillus following the introduction of chemotherapy in the 1940's, the following hypothesis is proposed.

The above investigators clearly showed that the action of streptomycin and the later drugs was most active at the broncho-cavitary junction, thus permitting drainage of cavitary contents rather than producing obstruction and enlargement of cavities. We have previously reported the extensive use of anti-fungal agents, especially azoles, early in the disease in many of the local cases. It is proposed, therefore, that a fungistatic action at the broncho-cavitary junction similarly prevents blockage, bleeding, rupture, and secondary infection in these cavities with resulting fibrosis.

Since these cases, as stated above currently come to surgery in only a minority of cases pathological confirmation of this thesis is not feasible and epidemiological evidence will have to suffice.

Abstract 19: Coccidiodimycosis and Prosthetic Knee Infections

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The knee is one of the most common joints involved by disseminated Coccidioides immitis. Involvement of a prosthetic knee by C. immitis has not been reported except in an abstract which gave no details. We report three cases of C. immitis involving prosthetic knee replacements. The circumstances for each were different. One case appeared to be infection of a prosthetic knee by disseminated disease; the second case, a prosthetic knee was inadvertently placed in a knee infected with C. immitis and in the third case, a prosthetic knee was placed in a joint known to be infected with C. immitis. All patients received Amphotericin B followed by indefinite oral fluconazole. At four year follow up all patients were doing well. These cases illustrate various presentations for an infection likely to become more common in older individuals in endemic areas who have reconstructive arthroplasty.

Abstract 20: SCH 56592 as treatment for coccidioidomycosis: Preliminary analysis

Catanzaro, A and the Fungal Trials Study Group.

Background:

- Open label study
- Entry criteria
    - Chronic pulmonary
    - Soft tissue
    - Skeletal cocci
    - Culture or histology positive rr serology with compatible clinical picture
- SCH56592 400mg/day for 12 months
- optional: could start at 80 mg/day for 2weeks
- 20 patients enrolled
    - 9 chronic pulmonary 6 soft tissue 7 skeletal
    - 2 had multiple organs involved
- New enrollment halted 4/24/98 due to adverse effects in animal studies
- Patients on study given option of completing 6 months of therapy

15 subject completed 24 weeks of therapy

Results: Post treatment:

- 15 subject completed 24 weeks of therapy
- 5 did not complete 24 weeks
    - 1 died on treatment of rheumatoid lung disease
    - 4 elected to switch despite a satisfactory response
    - 3 were switched to fluconazole

- 1was switched to itraconazole

- 15 patients completed 24 weeks Rx
  - 16 weeks overall scores
    - mean 33%
    - range 0-89%
    - 10 patients below 50%
  - 24 weeks overall scores
    - mean 24% range 0-50%
    - all below 50%

- Of the 15 who completed 24 months of treatment with SCH 56592
  - 10 were followed up follow off all anti fungal treatment
  - 10 remained well
  - follow up exceeds 12 month in all.
  - Some experienced continued resolution of lesions off treatment

- Reactivations were seen in 3/10 followed up off treatment
  - 2 developed new nodules
  - 1 had drainage of an old lesion

- Reactivations developed early (2-4 months)

Adverse events:

- Monitored for
  - standard blood testing
  - Bone density
  - ACTH stimulation
  - Pelvic ultrasound

- 10/20 reported no AEs
  - AEs relationship to study drug judged by PI to be-
    - Probable in 5 patients

- Possibly in 5 patients

***Adverse Events*:**

- Probable in 5 patients
    - 3 had dryness
    - 1 each
        - lips
        - mouth
        - nose
    - 1 each
        - diarrhea
        - heartburn
        - insomnia
- Possibly in 5 patients
    - 6 CNS Sx
    - 3 headache
        - 1 @ light headed, mood change,fatigue
    - 5 Dermatologic Sx
        - rash,itchy scalp,worsening acne,dry palms, chapped lips
    - 2 Rheumatologic Sx
        - joint pain, myalgia
    - 4 Miscellaneous
        - 1 @ increase bilirubin, thirst, sore throat, rhinorrhea & conjunctivitis

Conclusions:

- SCH 56592 warrants further study for the treatment of coccidiooidomycosis