Dust Devil: The Life and Times of the Fungus That Causes Valley Fever

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Coccidioides Biology

Coccidioides immitis and C. posadasii are pathogenic, dimorphic, soil-dwelling Ascomycetes in the Onygenales order. On average, both Coccidioides species have 29 Mb haploid genomes, containing approximately 10,000 open reading frames (ORFs) on five chromosomes [1]. Coccidioides’ most recent common ancestor underwent gene family expansions for proteases and keratinases, membrane biology genes, and toxin production, all likely utilized for survival in animal tissues and morphological changes; and a loss of genes associated with degradation of plant tissue, such as tannases, cellulases, and cutinases [1]. Coccidioides and other fungi in the family Onygenaceae are able to degrade keratin and may cause skin disease in humans and animals. Both species of Coccidioides are distantly related to other dimorphic human pathogens, such as Histoplasma (Ajellomyces) capsulatum, in the new family Ajellomycetaceae [2].

Both Coccidioides species have similar biology, with a well-characterized asexual life cycle with distinct saprobic and parasitic stages, and only molecular evidence of a sexual cycle (Fig 1). In the saprobiic phase, Coccidioides cycles between mycelial and arthroconidial stages. Arthroconidia are abscised and become airborne by soil disturbance. Inhalation of arthroconidia by a potential host can lead to coccidioidomycosis, commonly known as (San Joaquin) Valley fever. In an infected host, Coccidioides cycles between uninucleate endospores and multinucleate spherules (Fig 1). Differential phenotypes between the species, including temperature sensitivity and salt tolerance, have been described [3] (personal communication, Marc Orbach to B. Barker). No differential disease phenotypes have been investigated, although extreme variation in virulence among strains is documented [4]. The most pathogenic strains can cause fatal disease within eight days with as few as 50 arthroconidia administered intranasally in immunocompetent mice, and some cause much later onset of disease symptoms and death [4–6]. For humans, minimum dosage is not known, but it has been stated that the infectious dose is a single arthroconidium [7]. Both species have been shown to infect a wide variety of mammals, with varying levels of disease [8].

Molecular evidence of mating includes identification and characterization of mating type loci, recombination, and distinct gene genealogies [9–11]. In addition to intra-species sexual recombination, population genomics revealed signatures of hybridization and gene introgression between the two species [12]. At this time, no laboratory-controlled genetic recombination or sexual structures have been described.
Coccidioides Ecology and Population Genetics

The recognition that the genus *Coccidioides* contains two species is a relatively recent discovery [3]. *C. immitis* is the name of the first species described, with isolates initially being categorized as California and non-California *C. immitis*. Non-California *C. immitis* was later found to be another species, *C. posadasii* [3]. *C. immitis* is found primarily in the San Joaquin Valley of California. However, recent work has also identified this species in Utah and eastern Washington state [13,14]. Whether the fungus has always been present, or if this reflects a more recent migration, is unknown. *C. posadasii* is found from Arizona to Texas, and throughout Mexico into Central and South America. Similarity of genotypes from Texas and South America indicates a more recent introduction of *C. posadasii* into this region [15]. However, a more complete study of patient and environmental isolates is needed before strong conclusions can be drawn [16]. Indeed, the majority of analyses to date have relied on fungal isolates obtained from human patients, which likely does not represent the overall diversity that occurs in nature.

Multiple analyses of many strains from several geographic locations reveal a high degree of diversity, with little to no clonal structure [3,11,17]. The only nearly identical genome sequences are multiple isolations from the same patient, tracking organ transplant from donor to recipient, and a match of a soil isolate to a patient [13,17,18]. In fact, analysis at a single
10-meter square area in Tucson, Arizona, revealed multiple genotypes present in a single environmental location [11]. Genetic diversity supports the idea that the recent increase in human coccidioidomycosis in the endemic regions is not due to an emerging hyper-virulent strain, but rather an overall increase in exposure of susceptible hosts to environmentally occurring arthroconidia [16,17].

The small number of studies focused on identifying the natural host makes it difficult at this time to make a general statement regarding the ecological niche of Coccidioides. As a pathogen infecting mainly mammals, the suggestion has been made that desert rodents, specifically the heteromyids, are the primary host species for Coccidioides [1,11,19]. Many North American soils that test positive for Coccidioides are associated with rodent burrows or rodent activity [11,20,21]. Studies in South America also found Coccidioides associated with armadillos and bats [22,23]. At this time, the natural host and ecology of the organism is not well understood.

Coccidioidomycosis

Coccidioidomycosis is an endemic disease and Coccidioides fungi are biosafety level 3 organisms, just recently removed from the Federal Select Agent list (1995–2013). Based on early studies in which a skin test measured a delayed type hypersensitivity (DTH) to Coccidioides antigen to indicate previous infection, approximately 60% of natural human infections are asymptomatic [20]. In the 1990s, the United States discontinued the DTH skin test for clinical use, but recently it has received new U.S. Food and Drug Administration (FDA) approval (FDA approval letter 07/29/2011). As of yet, a comprehensive study of infection rates in the general population in all endemic areas has not been conducted. The primary clinical presentation of coccidioidomycosis is pneumonia, which generally resolves without treatment. Some hosts will carry lung nodules or cavities of viable Coccidioides; however, incidence and long-term consequences of asymptomatic carriage are unknown. Others suffer chronic disease, sometimes requiring life-long antifungal treatment. Infections disseminate in fewer than 1% of cases, and create lesions at a single body site or potentially affect multiple organ systems. Central nervous system involvement is often fatal if left untreated [20]. Coccidioidomycosis is diagnosed by serology, microscopy, antigen detection, and/or culture, all of which have limitations [24,25].

Cellular immunity, generated by a Th1 response and manifested by DTH, is essential to defense against coccidioidomycosis and long-lived protection from reinfection [26]. Several studies show that response to Coccidioides is more effective in the presence of immune factors such as interferon gamma (IFNγ) released by Th1 cells [27]. Recent data substantiate an essential role for Th17 pathway induction in mice for long-term immunity as well [28]. Patients with chronic disease appear to mount a non-protective Th2-type humoral immune response [27]. Immunodeficiency, either genetic or acquired, is a major risk factor for disseminated disease [29,30].

Many known and unknown factors shape the immune response to Coccidioides and manifestation of disease. For reasons that are not well understood, African Americans and Filipinos appear at a greater risk for disseminated coccidioidomycosis than other ethnicities [27,31]. Underlying health issues and elder age are also known risk factors for more severe disease [20]. Different strains of Coccidioides affect the magnitude of the immediate immune response [27,32]. Few studies have elucidated roles of other important cell types, such as natural killer cells or dendritic cells, which produce IFNγ to activate macrophages and IL-12 to activate T cells. The current data suggest that a complex combination of host immune factors determines the advancement or clearance of infection [26].
Initial Stages of Infection

The largest gap in our knowledge of host immunity is the first five days of the innate response. The initial encounter of inhaled arthroconidia with the host lung is not well understood. It is believed that arthroconidia reach the alveoli where mucociliary clearance factors are encountered. Lung epithelial cells are equipped with pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and Dectin-1, which are capable of inducing immediate effector responses [33], and these cells influence alveolar macrophage regulation [34]. Yet, the role of epithelial cells in response to Coccidioides inhalation is unknown.

In the lungs, innate immune cells recognize fungal components using multiple receptors, inducing phagocytosis and production of reactive oxygen species (ROS). Resident alveolar macrophages are naturally tolerant to prevent overreaction, with low phagocytic activity and respiratory burst [34]. Upon encounter with arthroconidia, non-fungicidal macrophage engulfment occurs. Within hours, there is a Coccidioides-antigen activated influx of polymorphonuclear neutrophils (PMNs) [35], which may enhance spherule formation [36]. PMNs respond in a similar manner as macrophages, engulfing arthroconidia without killing them [37]. Direct observation implicates failure of phagosome-lysosome fusion, relieving the arthroconidia from contact with lytic enzymes. When activated in vitro with IFN-γ or T cells from an immune host, the phagocytes’ ability to kill arthroconidia increases [37,38]. This may be a contributing factor to the success of IFN-γ as a therapy for disseminated coccidioidomycosis [39].

Based on in vitro and in vivo observations, arthroconidia enlarge and transform into immature spherules (Fig 1, parasitic life cycle). In addition to the induction of phagocytosis and ROS production, TLR-2 and Dectin-1, when bound by spherules, collaborate to trigger cascades mediated by MyD88 and Card-9 intracellular adaptors [40]. Subsequent activation of transcription factor NFκB produces proinflammatory cytokines TNFα, MIP-2, and IL-6, which are essential effectors of Th1 and Th17 cellular responses [40,41]. Dectin-1 also mediates production of critical Th1 cytokines IL-12 and IFN-γ, and Th17 cytokines IL-23, IL-17a, IL-22, and IL-1β [41]. Spherules increase mRNA expression and other factors for evasion of host Dectin-1, as well as resistance to, and suppression of, host oxidative defenses [42].

For the next few days (days two to three), spherules undergo free nuclear division, and mature into large (30 to 80 μm) septate cells containing developing endospores. Some literature suggests that growing intracellular spherules lyse their phagocytes, but direct evidence is lacking. Regardless, spherules are probably too large for phagocytosis. Additionally, spherules produce an alkaline extracellular matrix (ECM) that prevents PMN contact and degranulation-induced damage [32,43].

On day four or five, the mature spherules rupture to release endospores, although this process may take longer in vitro. A renewed host response comprises another influx of PMNs. PMNs and macrophages readily engulf the endospores. However, phagocytosis is still impeded as endospores can remain in large clusters tied by fibrillar structures originating from the spherule outer wall [37] and are protected by the spherule ECM [32,43]. From this point, the Coccidioides parasitic cycle continues (Fig 1). At this time, host and/or pathogen mechanisms terminating this cycle in the majority of hosts are unknown.

Future Directions

Coccidioides species and the mycoses they cause have been studied for over a hundred years. With this review, we attempt to summarize some of the more recent findings that have taken place in this field of research. Nevertheless, there is still a dearth of information about these fungal organisms and how they survive within the environment and inside host organisms.
Multiple research aims can greatly advance this research field. Analyzing environmental *Coccidioides* isolates would increase our understanding of the ecology of these fungi. Further characterizing host and pathogen genetics that influence the course of disease could lead to a better understanding of mycosis and development of immunotherapies for disease. Screening *Coccidioides* for susceptibility to novel therapeutics, particularly those that are being developed for other fungi, will aid in developing new treatments to eliminate the fungus from the patient to prevent reactivation of disease.

Many of the previous immunological studies employed various in vitro methodologies to answer scientific questions while adhering to the biosafety regulations that governed *Coccidioides* research at the time. Though these studies provided the vital foundation of our current knowledge of *Coccidioides* immunology, the answers provided by those studies may not entirely capture in vivo or in situ events. Previous studies that examined potential vaccine candidates in murine models of coccidioidomycosis were also very beneficial to our understanding of coccidioidomycosis and vaccinology [44]; however, our knowledge of a naïve host immune response to infection remains limited. Now that *Coccidioides* is no longer a Select Agent, and-omic technologies are cheaper and more efficient than ever, the time has come to study the in vivo and in situ host immune response to coccidioidomycosis.

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**References**

1. Sharpton TJ, Stajich JE, Rounsley SD, Gardner MJ, Wortman JR, Jordan VS, et al. (2009) Comparative genomic analyses of the human fungal pathogens *Coccidioides* and their relatives. Genome Research 19: 1722–1731. doi: 10.1101/gr.087551.108 PMID: 19717792
2. Untereiner WA, Scott JA, Naveau FA, Sigler L, Bachewich J, Angus A. (2004) The Ajellomycetaceae, a new family of vertebrate-associated Onygenales. Mycologia 96: 812–821. PMID: 21148901
3. Fisher MC, Koenig GL, White TJ, Taylor JW (2002) Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. Mycologia 94: 73–84. PMID: 21156479
4. Friedman L, Smith CE, Gordon LE (1955) The assay of virulence of *Coccidioides* in white mice. The Journal of Infectious Diseases 97: 311–316. PMID: 13286489
5. Muhammed M, Feldmesser M, Shubitz LF, Lionakis MS, Sil A, Wang Y, et al. (2012) Mouse models for the study of fungal pneumonia: a collection of detailed experimental protocols for the study of *Coccidioides*, *Cryptococcus*, *Fusarium*, *Histoplasma* and combined infection due to *Aspergillus-Rhizopus*. Virulence 3: 329–338. doi: 10.4161/viru.20142 PMID: 22546902
6. Shubitz L, Peng T, Perrill R, Simons J, Orsborn K, Galgiani JN. (2002) Protection of mice against *Coccidioides immitis* intranasal infection by vaccination with recombinant antigen 2/PRA. Infection and Immunity 70: 3287–3289. PMID: 12011027
7. Nicas M, Hubbard A (2002) A risk analysis for airborne pathogens with low infectious doses: application to respirator selection against *Coccidioides immitis* spores. Risk Analysis 22: 1153–1163. PMID: 12530785
8. Shubitz LF (2007) Comparative aspects of coccidioidomycosis in animals and humans. Annals of the New York Academy of Sciences 1111: 395–403. PMID: 17332082
9. Burt A, Dechairo BM, Koenig GL, Carter DA, White TJ, Taylor JW. (1997) Molecular markers reveal differentiation among isolates of *Coccidioides immitis* from California, Arizona and Texas. Molecular Ecology 6: 781–786. PMID: 9262014
10. Koufopanou V, Burt A, Taylor JW (1997) Concordance of gene genealogies reveals reproductive isolation in the pathogenic fungus *Coccidioides immitis*. Proceedings of the National Academy of Science 94: 5478–5482. PMID: 9144263
11. Barker BM, Tabor JA, Shubitz LF, Perrill R, Orbach MJ (2012) Detection and phylogenetic analysis of *Coccidioides posadasii* in Arizona soil samples. Fungal Ecology 5: 163–176.
12. Neafsey DE, Barker BM, Sharpton TJ, Stajich JE, Park DJ, Whiston E, et al. (2010) Population genomic sequencing of *Coccidioides* fungi reveals recent hybridization and transposon control. Genome Research 20: 938–946. doi: 10.1101/gr.103911.109 PMID: 2056208

13. Litvintseva AP, Marsden-Haug N, Hurst S, Hill H, Gade L, Driebe EM, et al. (2014) Valley fever: Finding new places for an old disease: *Coccidioides immitis* found in Washington state soil associated with recent human infection. Clinical Infectious Disease 60(1):e1–e3.

14. Johnson SM, Carlson EL, Fisher FS, Pappagianis D (2014) Demonstration of *Coccidioides immitis* and *Coccidioides posadasii* DNA in soil samples collected from Dinosaur National Monument, Utah. Medical Mycology 52: 610–617. doi: 10.1093/mmy/myu004 PMID: 2484703

15. Fisher MC, Koenig GL, White TJ, San-Blas G, Negroni R, Alvarez IG, et al. (2001) Biogeographic range expansion into South America by *Coccidioides immitis* mirrors New World patterns of human migration. Proceedings of the National Academy of Science 98: 4558–4562. PMID: 1128764

16. Barker BM, Jewell KA, Kroken S, Orbach MJ (2007) The population biology of *Coccidioides*: epidemiologic implications for disease outbreaks. Annals of the New York Academy of Sciences 1111: 147–163. PMID: 1734453

17. Jewell K, Cheshier R, Cage GD (2008) Genetic diversity among clinical *Coccidioides* spp. isolates in Arizona. Medical Mycology 46: 449–455. doi: 10.1080/13693780801961337 PMID: 18608919

18. Engelthaler DM, Chiller T, Schupp JA, Colvin J, Beckstrom-Sternberg SM, Driebe EM, et al. (2011) Next-generation sequencing of *Coccidioides immitis* isolated during cluster investigation. Emerging Infectious Disease 17: 227–232. doi: 10.3201/eid1702.100620 PMID: 21219593

19. Catalán-Dibene J, Johnson SM, Eaton R, Romero-Olives AL, Baptista-Rosas RC, Pappagianis D, et al. (2014) Detection of coccidioidal antibodies in serum of a small rodent community in Baja California, Mexico. Fungal Biology 118: 330–339. doi: 10.1016/j.funbio.2014.01.006 PMID: 2460735

20. Nguyen C, Barker BM, Hoover S, Nix DE, Ampel NM, Frelinger JA, et al. (2013) Recent advances in our understanding of the environmental, epidemiological, immunological, and clinical dimensions of coccidioidomycosis. Clinical Microbiology Reviews 26: 505–525. doi: 10.1128/CMR.00005-13 PMID: 2382437

21. Baptista-Rosas RC, Catalán-Dibene J, Romero-Olives AL, Hinajosa A, Cavazos T, Riquelme M (2012) Molecular detection of *Coccidioides* spp. from environmental samples in Baja California: linking Valley Fever to soil and climate conditions. Fungal Ecology 5: 177–190.

22. Brillhante RSN, Moreira RE Filho, Rocha MFG, Castelo-Branco DdSCM, Fechine MA, Lima RA, et al. (2012) Coccidioidomycosis in armadillo hunters from the state of Ceará, Brazil. Memórias do Instituto Oswaldo Cruz 107: 813–815.

23. Cordeiro R A, Silva KRC, Brilhante RSN, Moura FBP, Duarte NFH, Marques FJF, et al. (2012) *Coccidioides posadasii* infection in bats, Brazil. Emerging Infectious Disease Journal 18: 668. doi: 10.3201/ eid1804.111641 PMID: 22469192

24. Blair JE, Mendoza N, Force S, Chang YH, Grye TE (2013) Clinical specificity of the enzyme immunoassay test for coccidioidomycosis varies according to the reason for its performance. Clinical and Vaccine Immunology 20: 95–98. doi: 10.1128 CVI.00531-12 PMID: 2315512

25. Kubserski T, Herrig J, Pappagianis D (2010) False-positive IgM serology in coccidioidomycosis. Journal of Clinical Microbiology 48: 2047–2049. doi: 10.1128/JCM.01843-09 PMID: 20357210

26. Ampel NM (2007) The complex immunology of human coccidioidomycosis. Annals of the New York Academy of Sciences 1111: 245–258. PMID: 17363432

27. Borchers AT, Gershwin ME (2010) The immune response in coccidioidomycosis. Autoimmunity Reviews 10: 94–102. doi: 10.1016/j.autrev.2010.08.010 PMID: 20728582

28. Wang H, LeBert V, Hung CY, Galles K, Saijo S, Lin X, et al. (2014) C-type lectin receptors differentially induce Th17 cells and vaccine immunity to the endemic mycosis of North America. Journal of Immunology 192: 1107–1119. doi: 10.4049/jimmunol.1302314 PMID: 2439121

29. Sampaio EP, Hsu AP, Peachek J, Bax HI, Dias DL, Paulson ML, et al. (2013) Signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations and disseminated coccidioidomycosis and histoplasmosis. Journal of Allergy and Clinical Immunology 131: 1624–1634. doi: 10.1016/j.jaci.2013.01.052 PMID: 2354132

30. Vinh DC, Schwartz B, Hsu AP, Miranda DJ, Valdez PA, Fink D, et al. (2011) Interleukin-12 receptor β-1 deficiency predisposing to disseminated coccidioidomycosis. Clinical Infectious Disease 52: e99–e102. doi: 10.1093/cid/ciq215 PMID: 21258095

31. Wheeler C, Lucas KD, Mohle-Boetani JC (2015) Rates and risk factors for coccidioidomycosis among prison inmates, California, USA, 2011. Emerging Infectious Diseases 21.
32. Frey CL, Drutz DJ (1986) Influence of fungal surface components on the interaction of Coccidioides immitis with polymorphonuclear neutrophils. Journal of Infectious Diseases 153: 933–943. PMID: 3701107

33. Sun WK, Lu X, Li X, Sun QY, Su X, Song Y, et al. (2012) Dectin-1 is inducible and plays a crucial role in Aspergillus-induced innate immune responses in human bronchial epithelial cells. European Journal of Clinical Microbiology and Infectious Diseases 31: 2755–2764. PMID: 22562430

34. Hussell T, Bell TJ (2014) Alveolar macrophages: plasticity in a tissue-specific context. Nature Reviews Immunology 14: 81–93. doi: 10.1038/nri3600 PMID: 2445666

35. Galgiani JN, Isenberg RA, Stevens DA (1978) Chemotaxigenic activity of extracts from the mycelial and spherule phases of Coccidioides immitis for human polymorphonuclear leukocytes. Infection and Immunity 21: 862–865. PMID: 711340

36. Galgiani JN, Hayden R, Payne CM (1982) Leukocyte effects on the dimorphism of Coccidioides immitis. Journal of Infectious Diseases 146: 56–63. PMID: 7086205

37. Drutz DJ, Huppert M (1983) Coccidioidomycosis: factors affecting the host-parasite interaction. Journal of Infectious Diseases 147: 372–390. PMID: 6300253

38. Beaman L (1987) Fungicidal activation of murine macrophages by recombinant gamma interferon. Infection and Immunity 55: 2951–2955. PMID: 3119493

39. Nesbit LA, Knox KS, Nguyen CT, Roesh J, Wheat LJ, Johnson SM, et al. (2013) Immunological characterization of bronchoalveolar lavage fluid in patients with acute pulmonary coccidioidomycosis. Journal of Infectious Diseases 208: 857–863. doi: 10.1093/infdis/jit246 PMID: 23737603

40. Hung CY, Jiménez-Alzate Mdel P, Gonzalez A, Wüthrich M, Klein BS, Cole GT (2014) Interleukin-1 receptor but not Toll-like receptor 2 is essential for MyD88-dependent Th17 immunity to Coccidioides infection. Infection and Immunity 82: 2106–2114. doi: 10.1128/IAI.01579-13 PMID: 24614655

41. Viriyakosol S, Jimenez Mdel P, Gurney MA, Ashbaugh ME, Fierer J (2013) Dectin-1 is required for resistance to coccidioidomycosis in mice. mBio 4: e00597–00512. doi: 10.1128/mBio.00597-12 PMID: 23993437

42. Whiston E, Zhang Wise H, Sharpston TJ, Jui G, Cole GT, Taylor JW, (2012) Comparative transcriptomics of the saprobic and parasitic growth phases in Coccidioides spp. PLoS One 7: e41034. doi: 10.1371/journal.pone.0041034 PMID: 22911737

43. Wise HZ, Hung CY, Whiston E, Taylor JW, Cole GT (2013) Extracellular ammonia at sites of pulmonary infection with Coccidioides posadasii contributes to severity of the respiratory disease. Microbial Pathogens 59–60: 19–28.

44. Cole GT, Hung CY, Sanderson SD, Hurtgen BJ, Wuthrich M, Klein BS, et al. (2013) Novel strategies to enhance vaccine immunity against coccidioidomycosis. PLoS Pathogens 9: e1003768. doi: 10.1371/journal.ppat.1003768 PMID: 24367252

45. Munoz-Hernandez B, Palma-Cortes G, Cabello-Gutierrez C, Martinez-Rivera MA (2014) Parasitic polymorphism of Coccidioides spp. BMC Infectious Diseases 14: 213. doi: 10.1186/1471-2334-14-213 PMID: 24750998