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***Histoplasma capsulatum*, lung infection and immunity**

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Abstract

Histoplasma capsulatum, an environmental fungus, is the most common endemic pulmonary mycosis in the USA. Disease is most frequently observed in immunocompromised patients living in endemic areas. We present the mechanisms of fungal recognition, innate immune response and adaptive immune response that lead to protection or exacerbation of disease. Current understanding of these mechanisms is the result of a continuing dialogue between clinical observations and murine studies. Mice are a powerful model to study the immune response to *H. capsulatum* alone or in the presence of immunomodulatory drugs. Vigilance for histoplasmosis should be exercised with novel immunosuppressive agents that target the important immune pathways identified here.

Keywords

chemokines; cytokines; dendritic cells; fungal disease; *Histoplasma capsulatum*; macrophages; T-cell-mediated immunity

***Histoplasma capsulatum*: a global fungal pathogen**

Infection with *Histoplasma capsulatum* causes significant morbidity and mortality worldwide. This fungus is a dimorphic ascomycete that grows in its hyphal form in soil and bird and bat guano. Upon inhalation of spores, *H. capsulatum* transforms into the pathogenic yeast phase. This form replicates within macrophages that carry the yeast from lungs to virtually any organ [1, 2]. Induction of adaptive immunity, particularly the Th1 response, is required for activation of macrophages and efficient clearance of the yeast. Exposure to *H. capsulatum* usually results in symptomless clearance; however, histoplasmosis can manifest as an acute flu-like pulmonary illness, a chronic cavitary lung disease or a progressive

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disseminated form [1]. Immunocompromised individuals are especially at risk for disseminated infection; the HIV pandemic resulted in a dramatic increase in lethal histoplasmosis. Today, highly active antiretroviral therapy (HAART) reduces this risk [2]. Clinical disease develops in immunocompetent individuals with outbreaks occurring when there is a localized exposure to a large infectious dose [3].

● Distribution & variation

Cases of histoplasmosis occur worldwide, but are concentrated in endemic regions. The North American area is centered on the Ohio and Mississippi river valleys. Large surveys of skin testing in the 1960s revealed that 80% of young adult men from this region were positive for *H. capsulatum* exposure [1, 4]. Disease burden is regional; an analysis of US hospital records estimated 3370 inpatient stays and 254 deaths associated with histoplasmosis in 2002, with almost 90% of hospitalizations occurring in midwestern and southern states [5]. Central and South America contain large endemic areas, and reviews have brought attention to the under-diagnosed burden of HIV-associated histoplasmosis in these regions [6]. Additional endemic foci are in China, southeast Asia, the Indian subcontinent, Australia and Africa [7].

H. capsulatum isolates exhibit considerable genotypic and phenotypic variability between and within these regions. Genetic analysis has revealed that *H. capsulatum* is not monophyletic and can be classified into seven or eight distinct clades [8]. Genetic differences between clades lead to differences in phenotype and virulence. Most isolates of *H. capsulatum* have both α - and β -glucan in their cell walls, but isolates from the North American 2 clade lack α -glucan [9]. While α -glucan⁻ strains account for the majority of infections in immunocompetent individuals in North America, α -glucan⁺ infections are associated with HIV [10]. In mouse models α -glucan⁻ yeast causes more severe disease than α -glucan⁺, but only at high infectious inocula [11]. Thus, strains of *H. capsulatum* may depend on factors such as infectious dose or defective adaptive immune response to establish infection.

● *H. capsulatum* virulence

H. capsulatum is not contagious. Despite infection usually being a 'dead end' for fungal replication, *H. capsulatum* appears specifically adapted to mammalian hosts. The transformation from mycelial to yeast phase at 37°C is crucial for infection; strains lacking this ability are avirulent [10]. The yeast is equipped for evading intracellular killing by phagocytes, with mechanisms to degrade reactive oxygen species (ROS), regulate lysosomal pH and capture essential nutrients that might otherwise be deprived [12–15]. Many mammalian species are infected with *H. capsulatum* and are accidental hosts that must cope with the yeast's capability for survival within macrophages. In both human and mouse infection, macrophages provide a niche for *H. capsulatum* proliferation that cannot be halted without adaptive immunity.

Innate immune response

● Recognition & phagocytosis

Immune cells use a variety of surface receptors to recognize and ingest *H. capsulatum*. Pattern recognition receptors, such as C-type lectin receptors and toll like receptors are necessary for innate immune cytokine production and/or phagocytosis of pathogenic fungi [16, 17]. Dectin-1 recognition of β -(1,3)-glucan is required for optimal proinflammatory cytokine production, but not phagocytosis of *H. capsulatum*; α -glucan on *H. capsulatum* masks β -glucan recognition [18, 19]. In humans, a rare mutation that decreases surface expression of dectin-1 is not associated with a higher incidence of histoplasmosis. This suggests that dectin-1 is not needed to combat infection or that these patients have not come in contact with the fungus [20]. Other C-type lectin receptors such as dectin-2 and mincle bind *H. capsulatum*, but their role in immunity has not been elucidated [21].

While macrophages and dendritic cells (DCs) exhibit overlapping expression of many surface receptors, those utilized for phagocytosis of *H. capsulatum* are cell specific. Macrophages bind and ingest yeasts via CD11/CD18 integrins, while DCs utilize VLA-5 to recognize *H. capsulatum* ligands heat shock protein 60 and cyclophilin A, respectively [22–24]. CD11/CD18 blockade reduces, but does not prevent, *H. capsulatum* uptake by both human and murine macrophages; this finding suggests that other receptors are capable of driving phagocytosis – at least in the absence of CD11/CD18. In contrast to macrophages, human DCs rely on VLA-5 for fungal recognition [25]. Differential recognition of *H. capsulatum* by macrophages and DCs may trigger unique signaling cascades. CD11b/CD18 triggers activation of the tyrosine kinase Syk and downstream production of proinflammatory cytokines in macrophages [19]. VLA-5, on the other hand, activates kinases that regulate proliferation and survival including ERK/MAPK and PI3K/Akt. This leads to production of anti-apoptotic Bcl-2, which may prevent fungal-driven apoptosis [26]. Thus, engagement of different receptors on these phagocytes may account for the contrasting intracellular fate.

● Antifungal activity in neutrophils & macrophages

Following *H. capsulatum* recognition, appropriate cell mobilization is required for an effective immune response. Neutrophils and macrophages are recruited early to the site of infection. Human neutrophils are fungistatic, not fungicidal, against *H. capsulatum* [27]. This activity relies on cathepsin G, defensins and bactericidal-permeability-increasing protein within azurophil granules [27]. Neutrophil depletion studies in the murine model have addressed the influence of these cells in host defense. However, the antibody used in these studies is now known to recognize both neutrophils and inflammatory monocytes; therefore, the role of neutrophils is still unsettled [28]. Although neutropenic patients are at risk for some fungi, histoplasmosis is not among them [29].

After cellular activation via Th1 cytokines IFN- γ and GM-CSF, macrophages inhibit intracellular growth of *H. capsulatum* [30]. The mechanisms deployed by murine and human macrophages are distinct. While phagosome acidification is used by murine macrophages, it is dispensable within human macrophages [31]. *H. capsulatum* phagocytosis stimulates a

respiratory burst within human, but not murine macrophages. However, studies utilizing NADPH oxidase-deficient mice suggest that inhibition of fungal replication *in vivo* may require ROS production [32]. Additional mechanisms of inhibition suggested by *in vitro* studies include reactive nitrogen species and metal deprivation [33–35]. Even with activation, macrophages cannot efficiently sterilize tissues and thus harbor *H. capsulatum* [30]. Infected macrophages induce granuloma formation in immunocompetent patients [1, 36]. While granulomas were thought to be a form of host protection, recent evidence suggests that *Mycobacterium tuberculosis*-induced granulomas serve as a repository for pathogenic organisms [37]. Reactivation histoplasmosis is a recognized clinical entity particularly in individuals that have left endemic areas into regions that are not known to contain the fungus [38, 39]. Although studies in the 1950s indicated that healed granulomas only contained dead organisms, the efficacy of organism recovery is unknown [40]. Thus, the source of organisms that cause reactivation remains enigmatic.

Adaptive immune response

● Induction of immunity

As the most potent antigen-presenting cells, DCs provide a link between innate and adaptive immunity. Human DCs are capable of killing *H. capsulatum* [25]. This fungicidal activity was dependent on lysosomal hydrolases, but not the respiratory burst or nitric oxide production. Human DCs drive CD4⁺ or CD8⁺ T-cell proliferation by presenting *H. capsulatum* antigen directly or from apoptotic macrophages, respectively [25, 41]. Adoptively transferred *H. capsulatum*-loaded DCs are able to suppress maladaptive IL-4 production and improve survival following CD4⁺ depletion [42]. Thus, the DC ability to kill yeast and present antigen in the absence of activating cytokines indicates they are drivers of an effective T-cell response.

Early studies demonstrated that CD4⁺ and CD8⁺ T cells are necessary for an effective immune response; nude mice that lack T cells exhibit high mortality following a low dose *H. capsulatum* infection [43]. CD4⁺ T-cell depletion during primary infection led to murine death; loss of CD8⁺ T cells decreased clearance efficiency [44]. Following vaccination, CD8⁺ T cells confer protection while CD4⁺ T cells are dispensable [45]. Increased mortality and fungal burden in the absence of T cells is caused by a lack of protective cytokines such as IFN- γ and TNF- α [44, 46]. An increased incidence of histoplasmosis in AIDS patients supports the protective role of CD4⁺ T cells in humans. CD4⁺ and CD8⁺ T cells influence reactivation; elimination of both 6 weeks after infection elevates fungal burden [47]. These studies mimic the human situation in which AIDS patients living in nonendemic areas exhibit reactivation disease concomitant with low CD4⁺ T-cell counts [38].

The protective effect of antibody that develops after infection is minor at best. B-cell knockout (KO) mice exhibit no change in fungal burden during primary infection but exhibit elevated fungal burden and a prolonged resolution in secondary disease [44]. Others have reported that monoclonal antibodies to *H. capsulatum* surface ligands are protective [48].

● Th1 response: protagonists in clearance of *H. capsulatum*

During infection, CD4⁺ T cells polarize into several helper phenotypes such as Th1, Th2, Th17 and Treg with unique cytokine profiles. IL-12 promotes Th1 differentiation and IFN- γ production. In murine histoplasmosis, IL-12 blockade accelerates murine mortality in an IFN- γ -dependent manner [49]. In histoplasmosis, IFN- γ is produced primarily by Th1 cells, activates intracellular killing and is necessary for control of primary infection [50]. IFN- γ also contributes to survival in secondary infection [50, 51]. Lipid mediators may be part of the Th1 response; leukotriene inhibition in murine histoplasmosis results in decreased IFN- γ , IL-12 and survival [52]. The importance of IFN- γ signaling in humans has been established with reports linking genetic deficiency in the receptor to disseminated disease [53].

TNF- α , another protective Th1 cytokine, exerts multiple effects including activation of phagocytic cells, induction of apoptosis and control of the CD4⁺ phenotype. TNF- α neutralization impairs survival of mice [54]. In primary infection, TNF- α blockade reduces nitric oxide production by macrophages. By contrast, the major defect in secondary infection is overproduction of detrimental IL-4 and IL-10 [54]. In both primary and secondary infection, TNF- α neutralization abolishes the ability of murine T cells to mediate protection from *H. capsulatum* [55]. TNF- α induces caspase activation, additional TNF- α production, and apoptosis in infected macrophages [56]. This result suggests a protective mechanism in which yeast replicating in permissive macrophages are released by apoptosis, along with an additional burst of TNF- α to neighboring cells. Studies in mice regarding the importance of TNF- α are confirmed by clinical use of TNF- α blockers. These medications increase patient susceptibility to *H. capsulatum*, and patients are especially at risk for severe disseminated infection [57].

GM-CSF is another important inflammatory cytokine produced by multiple cell types, including Th1 cells. GM-CSF promotes both differentiation and activation of myeloid cells such as macrophages and neutrophils; neutralization leads to murine death following *H. capsulatum* infection [58]. GM-CSF acts at least in part by promoting fungistatic activity in macrophages through sequestration of zinc, a novel mechanism for pathogen control [34]. Loss of IFN- γ , TNF- α , GM-CSF or IL-12 can each independently increase murine mortality in histoplasmosis.

● Th17 response: supporting characters in *H. capsulatum* control

Th17 cells produce inflammatory cytokines such as IL-17, IL-6 and GM-CSF. The Th17 response is important for controlling many fungal infections and may be beneficial, but not essential, for control of *H. capsulatum*. In wild-type mice, IL-17 neutralization results in a larger fungal burden but does not alter resolution of infection [59]. Conversely, elevated IL-17 improves fungal clearance in the CCR5 KO mouse [60]. In humans, Hyper-IgE syndrome (HIES) is defined by a mutation in STAT3 leading to decreased Th17 cells. Even though HIES is a rare disorder, there are several case reports of HIES patients that developed disseminated or gastrointestinal histoplasmosis [61]. The fact that the gastrointestinal tissue is a target in most of these patients suggests that, as with mucosal

candidiasis, Th17 cells may be more important in the regulation of mucosal rather than systemic immunity for *H. capsulatum*.

● Th2 & Treg: villains in histoplasmosis

In contrast to Th1 and Th17, cytokines representative of Th2 responses exacerbate histoplasmosis. Th1 and Th2 responses are mutually antagonistic. Th2-associated cytokines polarize macrophages to a M2 phenotype, and these cells assist in tissue repair but fail to kill various intracellular pathogens [62]. IL-4 is a critical type 2 cytokine, and transgenic mice overexpressing IL-4 exhibit delayed fungal clearance [63]. CCR2 KO mice also exhibit increased IL-4 production and impaired clearance [64]. In these mice IL-4 triggers production of another Th2-associated cytokine, IL-33, by infected macrophages [65]. Subsequent to these elevated cytokines, a shift to the maladaptive M2 macrophage phenotype occurs. Neutralization of either IL-4 or IL-33 improves *H. capsulatum* clearance in this model.

Tregs mediate immune suppression in several ways, including cell–cell interactions and production of cytokines such as IL-10 and TGF- β . While important for limiting excessive immune activation, IL-10 impedes clearance of *H. capsulatum* [66]. This cytokine negatively affects development of the protective Th1 response; in histoplasmosis, IL-10 KO mice develop more IFN- γ^+ CD4 $^+$ cells and clear the infection more rapidly [66]. Treg balance with other T-cell subsets is important. CCR5 KO mice exhibit a shift toward Th17 which leads to decreased Treg numbers and IL-10 production [60]. These mice have accelerated clearance of *H. capsulatum*; both the elevation in Th17 and depression of Treg response may improve intracellular killing. Since IL-17 appears to be dispensable for *H. capsulatum* clearance, decreased Tregs are likely responsible for this phenotype.

● Metals & immunity in histoplasmosis

Deprivation of trace metals is an effective immune strategy to slow or stop an infection. In murine histoplasmosis, activation of macrophages involves mechanisms that limit at least two essential minerals: iron and zinc. *H. capsulatum* must obtain iron bound to ferritin or transferrin in the phagosome/lysosome compartment. It possesses several means to accomplish this including siderophores, ferric reductases and maintenance of favorable pH [13–15, 67]. Murine macrophages activated with IFN- γ and lipopolysaccharide counteract *H. capsulatum* iron acquisition, possibly via NO production; conversely, increasing iron availability increases yeast survival [33, 35]. This mechanism of *H. capsulatum* control has not been reported in human macrophages, and the importance of iron in clinical histoplasmosis is unresolved.

Zinc must be obtained by an intracellular invader for survival and growth. Both human and murine macrophages activated with GM-CSF upregulate metallothioneins, small metal-binding proteins which reduce intracellular free zinc [34, 68]. Metallothionein-driven reduction of zinc supports production of ROS by increasing activity of the phagosomal H $^+$ channel [34]. Thus, GM-CSF's ability to inhibit yeast growth in macrophages may be due to zinc deprivation and enhanced ROS. In contrast to GM-CSF, IL-4 increases zinc availability to intracellular yeast [68].

Translation from mouse to human

● Importance of CD4⁺ T cells in clinical histoplasmosis

Our understanding of the importance of CD4⁺ cells in histoplasmosis has emerged from observations in both mice and humans. By 1971, physicians noted that patients with histoplasmosis usually exhibited an increase in T or B lymphocytes, while patients with progressive disseminated disease tended to lack this response [43]. Early studies in athymic mice confirmed the importance of T cells for control of histoplasmosis [43]. In the 1980s, the HIV epidemic brought a dramatic increase in histoplasmosis incidence, severity and awareness. An impaired CD4⁺ response is the major risk factor; progressive disseminated histoplasmosis typically presents in those with low CD4⁺ T-cell counts (<100 cells/mm³). Today most instances occur in patients not receiving HAART. Mortality in this group may approach 50% [2].

Patients taking medications that suppress CD4⁺ cell number or function are another major population with increased risk for histoplasmosis, particularly disseminated disease. Glucocorticoids effect all immune cell types, but have an especially dramatic influence on T-cell number and function [69]. High-dose glucocorticoids either alone or in combination with other immunosuppressive agents have been linked to histoplasmosis [70–72]. Immunosuppressants that target T cells including folic acid antimetabolites, calcineurin inhibitors and monoclonal antibodies targeting lymphocytes are associated with cases of histoplasmosis [73–75]. Exact assessment of risk is difficult because many patients take multiple medications, have an underlying condition that may influence immunity, or both.

● TNF- α neutralization & human disease

Our understanding of the mechanism of TNF- α in histoplasmosis has evolved through both murine studies and clinical observations. The requirement of TNF- α for optimal control of histoplasmosis was first observed in mice, and the ability of TNF- α to suppress detrimental cytokines such as IL-4 and IL-10 was elucidated in this model [54]. These findings predicted the relevance for human disease, which was eventually revealed by the introduction of TNF- α blocking therapeutics. Histoplasmosis is the most commonly reported fungal infection associated with TNF-blockers, and affected patients are at risk for severe disseminated histoplasmosis [57, 76]. Clinical studies revealed the ability of TNF- α to antagonize Tregs: reduced Treg number and function in rheumatoid arthritis patients is restored by TNF- α blockers [77]. This observation prompted further studies in murine histoplasmosis; TNF- α blockade in mice was found to induce an expanded pool of *H. capsulatum*-specific suppressive T cells [78]. Unexpectedly, these T cells did not have typical Treg markers; therefore TNF- α blockade may also act by promoting suppressor function in non-Treg CD4⁺ populations [78]. Recent human studies have reflected this finding, showing that TNF- α blockers can induce immunosuppressive features in Th17 cells [79].

Conclusion

Innate immune recognition of *H. capsulatum* by macrophages and dendritic cells is critical for both early cytokine and chemokine production and phagocytosis. These innate cells

express effector cell function and are necessary for promoting differentiation and recruitment of Th1 cells. The failure to generate a robust Th1 response results in a collapse of immunity. Enhanced generation of Th2 or Treg cells antagonizes the development of protective immunity to this fungus in part by counter-acting the action of Th1 cells. Clinical evidence supports the importance of Th1 cells and TNF- α in host defenses to this pathogen. The overlap between murine data and clinical studies suggest that studies in mice are useful for delineating the human immune response to *H. capsulatum* even in the context of novel immunosuppressive drugs.

Future perspective

Since the murine model seems to accurately reflect the clinical picture, murine and clinical studies for *H. capsulatum* should continue to inform each other to advance future research. This dialogue has already led to important insights. Murine models correctly predicted that individuals taking anti-TNF- α drugs would have an increased susceptibility to histoplasmosis; these models also helped explore the role of an expanded Treg population following treatment. Targeted antibody therapies that block specific cytokines or receptors provide powerful new tools that target autoimmune diseases or transplant rejection. However, these medications put the patient at risk for infection. Knowledge from murine studies will help predict risk for histoplasmosis as new therapies are introduced. Mavrilimumab, the first in a new class of GM-CSF blocking therapeutics, has shown promising results in Phase I and II clinical trials for treatment of rheumatoid arthritis [80]. If these therapies are utilized in endemic regions, awareness of histoplasmosis is warranted.

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References

Papers of special note have been highlighted as:

• of interest

1. Kauffman CA. Histoplasmosis: a clinical and laboratory update. Clin. Microbiol. Rev. 2007; 20(1): 115–132. [PubMed: 17223625] • Provides an historical perspective and reviews histoplasmosis presentation and treatment.
2. Adenis AA, Aznar C, Couppié P. Histoplasmosis in HIV-infected patients: a review of new developments and remaining gaps. Curr. Trop. Med. Rep. 2014; 1:119–128. [PubMed: 24860719]
3. Chamany S, Mirza SA, Fleming JW, et al. A large histoplasmosis outbreak among high school students in Indiana, 2001. Pediatr. Infect. Dis. J. 2004; 23(10):909–914. [PubMed: 15602189]
4. Edwards LB, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. Am. Rev. Respir. Dis. 1969; 99(Suppl. 4):1–132. [PubMed: 5767603]
5. Chu JH, Feudtner C, Heydon K, Walsh TJ, Zaoutis TE. Hospitalizations for endemic mycoses: a population-based national study. Clin. Infect. Dis. 2006; 42(6):822–825. [PubMed: 16477560] • Analyzes inpatient histoplasmosis in the USA by region, age and immunocompetency.
6. Nacher M, Adenis A, Aznar C, et al. How many have died from undiagnosed human immunodeficiency virus-associated histoplasmosis, a treatable disease? Time to act. Am. J. Trop. Med. Hyg. 2014; 90(2):193–194. [PubMed: 24277783]

7. Antinori S. *Histoplasma capsulatum*: more widespread than previously thought. Am. J. Trop. Med. Hyg. 2014; 90(6):982–983. [PubMed: 24778192]
8. Kasuga T, White TJ, Koenig G, et al. Phylogeography of the fungal pathogen *Histoplasma capsulatum*. Mol. Ecol. 2003; 12(12):3383–3401. [PubMed: 14629354]
9. Edwards JA, Allore EA, Rappleye CA. The yeast-phase virulence requirement for α -glucan synthase differs among *Histoplasma capsulatum* chemotypes. Eukaryot. Cell. 2010; 10(1):87–97. [PubMed: 21037179]
10. Edwards JA, Rappleye CA. *Histoplasma* mechanisms of pathogenesis – one portfolio doesn't fit all. FEMS Microbiol. Lett. 2011; 324(1):1–9. [PubMed: 22092757]
11. Sepúlveda VE, Williams CL, Goldman WE. Comparison of phylogenetically distinct *Histoplasma* strains reveals evolutionarily divergent virulence strategies. mBio. 2014; 5(4):e01376–e1314. [PubMed: 24987093] • Investigates interdependence of strain, α -(1,3)-glucan expression, and inoculum size in *Histoplasma capsulatum* virulence.
12. Holbrook ED, Smolnycki KA, Youseff BH, Rappleye CA. Redundant catalases detoxify phagocyte reactive oxygen and facilitate *Histoplasma capsulatum* pathogenesis. Infect. Immun. 2013; 81(7):2334–2346. [PubMed: 23589579]
13. Eissenberg LG, Goldman WE, Schlesinger PH, Schlesinger PH. *Histoplasma capsulatum* modulates the acidification of phagolysosomes. J. Exp. Med. 1993; 177(6):1605–1611. [PubMed: 8496679]
14. Hilty J, George Smulian A, Newman SL. *Histoplasma capsulatum* utilizes siderophores for intracellular iron acquisition in macrophages. Med. Mycol. 2011; 49(6):633–642. [PubMed: 21341981]
15. Zarnowski R, Cooper KG, Brunold LS, Calaycay J, Woods JP. *Histoplasma capsulatum* secreted γ -glutamyltransferase reduces iron by generating an efficient ferric reductant. Mol. Microbiol. 2008; 70(2):352–368. [PubMed: 18761625]
16. Sieling PA, Modlin RL. Toll-like receptors: mammalian “taste receptors” for a smorgasbord of microbial invaders. Curr. Opin. Microbiol. 2002; 5(1):70–75. [PubMed: 11834372]
17. Sancho D, Reis e, Sousa C. Signaling by myeloid C-type lectin receptors in immunity and homeostasis. Annu. Rev. Immunol. 2012; 30:491–529. [PubMed: 22224766]
18. Rappleye CA, Eissenberg LG, Goldman WE. *Histoplasma capsulatum* α -(1,3)-glucan blocks innate immune recognition by the β -glucan receptor. Proc. Natl Acad. Sci. USA. 2007; 104(4):1366–1370. [PubMed: 17227865]
19. Lin J-S, Huang J-H, Hung L-Y, Wu S-Y, Wu-Hsieh BA. Distinct roles of complement receptor 3, Dectin-1, and sialic acids in murine macrophage interaction with *Histoplasma* yeast. J. Leukoc. Biol. 2010; 88(1):95–106. [PubMed: 20360401] • Dissects the contribution of various macrophage receptors to phagocytosis and cytokine production.
20. Ferwerda B, Ferwerda G, Plantinga TS, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. N. Engl. J. Med. 2009; 361(18):1760–1767. [PubMed: 19864674]
21. Wang H, LeBert V, Hung CY, et al. C-type lectin receptors differentially induce Th17 cells and vaccine immunity to the endemic mycosis of North America. J. Immunol. 2014; 192(3):1107–1119. [PubMed: 24391211]
22. Long KH, Gomez FJ, Morris RE, Newman SL. Identification of heat shock protein 60 as the ligand on *Histoplasma capsulatum* that mediates binding to CD18 receptors on human macrophages. J. Immunol. 2003; 170(1):487–494. [PubMed: 12496435]
23. Gomez FJ, Pilcher-Roberts R, Alborzi A, Newman SL. *Histoplasma capsulatum* Cyclophilin A mediates attachment to dendritic cell VLA-5. J. Immunol. 2008; 181(10):7106–7114. [PubMed: 18981131]
24. Bullock WE, Wright SD. Role of the adherence-promoting receptors, CR3, LFA-1, and p150,95, in binding of *Histoplasma capsulatum* by human macrophages. J. Exp. Med. 1987; 165(1):195–210. [PubMed: 3025331]
25. Gildea LA, Morris RE, Newman SL. *Histoplasma capsulatum* yeasts are phagocytosed via very late antigen-5, killed, and processed for antigen presentation by human dendritic cells. J. Immunol. 2001; 166(2):1049–1056. [PubMed: 11145684] • Identified very late activation antigen-5 as the dendritic cell receptor for *H. capsulatum* phagocytosis.

26. Lee B-H, Ruoslahti E. $\alpha 5\beta 1$ integrin stimulates Bcl-2 expression and cell survival through Akt, focal adhesion kinase, and Ca^{2+} /calmodulin-dependent protein kinase IV. *J. Cell. Biochem.* 2005; 95(6):1214–1223. [PubMed: 15962308]
27. Newman SL, Gootee L, Gabay JE, Selsted ME. Identification of constituents of human neutrophil azurophil granules that mediate fungistasis against *Histoplasma capsulatum*. *Infect. Immun.* 2000; 68(10):5668–5672. [PubMed: 10992469]
28. Sá-Nunes A, Medeiros AI, Sorgi CA, et al. Gr-1+ cells play an essential role in an experimental model of disseminated histoplasmosis. *Microbes Infect.* 2007; 9(12–13):1393–1401. [PubMed: 17296322]
29. Sipsas NV, Bodey GP, Kontoyiannis DP. Perspectives for the management of febrile neutropenic patients with cancer in the 21st century. *Cancer.* 2005; 103(6):1103–1113. [PubMed: 15666328]
30. Deepe GSJ, Gibbons RS, Smulian AG. *Histoplasma capsulatum* manifests preferential invasion of phagocytic subpopulations in murine lungs. *J. Leukoc. Biol.* 2008; 84(3):669–678. [PubMed: 18577715]
31. Newman S, Gootee L. Human macrophages do not require phagosome acidification to mediate fungistatic/fungicidal activity against *Histoplasma capsulatum*. *J. Immunol.* 2006; 176(3):1806–1813. [PubMed: 16424211]
32. Youseff BH, Holbrook ED, Smolnycki KA, Rappleye CA. Extracellular superoxide dismutase protects *Histoplasma* yeast cells from host-derived oxidative stress. *PLoS Pathog.* 2012; 8(5):e1002713. [PubMed: 22615571]
33. Lane TE, Wu-Hsieh BA, Howard DH. Antihistoplasma effect of activated mouse splenic macrophages involves production of reactive nitrogen intermediates. *Infect. Immun.* 1994; 62(5):1940–1945. [PubMed: 8168960]
34. Subramanian Vignesh K, Landero Figueroa JA, Porollo A, Caruso JA, Deepe GS Jr. Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. *Immunity.* 2013; 39(4):697–710. [PubMed: 24138881] • Identifies zinc sequestration as a macrophage defense mechanism against *H. capsulatum*.
35. Lane TE, Wu-Hsieh BA, Howard DH. Gamma interferon cooperates with lipopolysaccharide to activate mouse splenic macrophages to an antihistoplasma state. *Infect. Immun.* 1993; 61(4):1468–1473. [PubMed: 8454351]
36. Heninger E, Hogan LH, Karman J, et al. Characterization of the *Histoplasma capsulatum*-induced granuloma. *J. Immunol.* 2006; 177(5):3303–3313. [PubMed: 16920971]
37. Ramakrishnan L. Revisiting the role of the granuloma in tuberculosis. *Nat. Rev. Immunol.* 2012; 12(5):352–366. [PubMed: 22517424]
38. Martin-Iguacel R, Kurtzhals J, Jouvion G, Nielsen SD, Llibre JM. Progressive disseminated histoplasmosis in the HIV population in Europe in the HAART era. Case report and literature review. *Infection.* 2014; 42(4):611–620. [PubMed: 24627267]
39. Mandell W, Goldberg DM, Neu HC. Histoplasmosis in patients with the acquired immune deficiency syndrome. *Am. J. Med.* 1986; 81(6):974–978. [PubMed: 3799657]
40. Okudaira M, Straub M, Schwarz J. The etiology of discrete splenic and hepatic calcifications in an endemic area of histoplasmosis. *Am. J. Pathol.* 1961; 39(5):599–611. [PubMed: 19971008]
41. Lin J-S, Yang C-W, Wu-Hsieh BA, Wang D-W. Dendritic cells cross-present exogenous fungal antigens to stimulate a protective CD8 T cell response in infection by *Histoplasma capsulatum*. *J. Immunol.* 2005; 174(10):6282–6291. [PubMed: 15879127]
42. Szymczak WA, Deepe GSJ. Antigenpresenting dendritic cells rescue CD4-depleted CCR2^{-/-} mice from lethal *Histoplasma capsulatum* infection. *Infect. Immun.* 2010; 78(5):2125–2137. [PubMed: 20194586]
43. Williams DM, Graybill JR, Drutz DJ. *Histoplasma capsulatum* infection in nude mice. *Infect. Immun.* 1978; 21(3):973–977. [PubMed: 309438]
44. Allendörfer R, Brunner GD, Deepe GS. Complex requirements for nascent and memory immunity in pulmonary histoplasmosis. *J. Immunol.* 1999; 162(12):7389–7396. [PubMed: 10358191]
45. Wuthrich M, Filutowicz HI, Warner T, Deepe GS, Klein BS. Vaccine immunity to pathogenic fungi overcomes the requirement for CD4 help in exogenous antigen presentation to CD8⁺ T cells:

- implications for vaccine development in immune-deficient hosts. *J. Exp. Med.* 2003; 197(11): 1405–1416. [PubMed: 12782709]
46. Wu-Hsieh BA, Howard DH. Inhibition of the intracellular growth of *Histoplasma capsulatum* by recombinant murine gamma interferon. *Infect. Immun.* 1987; 55(4):1014–1016. [PubMed: 3104206]
 47. Allen HL, Deepe GS. B cells and CD4-CD8-T cells are key regulators of the severity of reactivation histoplasmosis. *J. Immunol.* 2006; 177(3):1763–1771. [PubMed: 16849486]
 48. Nosanchuk JD, Zancopé-Oliveira RM, Hamilton AJ, Guimarães AJ. Antibody therapy for histoplasmosis. *Front. Microbiol.* 2012; 3:21. [PubMed: 22347215]
 49. Zhou P, Sieve MC, Bennett J, et al. IL-12 prevents mortality in mice infected with *Histoplasma capsulatum* through induction of IFN- γ . *J. Immunol.* 1995; 155(2):785–795. [PubMed: 7608555]
 50. Allendoerfer R, Deepe GS. Intrapulmonary response to *Histoplasma capsulatum* in gamma interferon knockout mice. *Infect. Immun.* 1997; 65(7):2564–2569. [PubMed: 9199420]
 51. Zhou P, Miller G, Seder RA. Factors involved in regulating primary and secondary immunity to infection with *Histoplasma capsulatum*: TNF-alpha plays a critical role in maintaining secondary immunity in the absence of IFN-gamma. *J. Immunol.* 1998; 160(3):1359–1368. [PubMed: 9570555]
 52. Medeiros AI, Sá-Nunes A, Soares EG, Peres CM, Silva CL, Faccioli LH. Blockade of endogenous leukotrienes exacerbates pulmonary histoplasmosis. *Infect. Immun.* 2004; 72(3):1637–1644. [PubMed: 14977971]
 53. Zerbe CS, Holland SM. Disseminated histoplasmosis in persons with interferon-gamma receptor 1 deficiency. *Clin. Infect. Dis.* 2005; 41(4):e38–e41. [PubMed: 16028145]
 54. Allendoerfer R, Deepe GS. Blockade of endogenous TNF- α exacerbates primary and secondary pulmonary histoplasmosis by differential mechanisms. *J. Immunol.* 1998; 160(12):6072–6082. [PubMed: 9637524]
 55. Deepe GS, Gibbons RS. T cells require tumor necrosis factor- α to provide protective immunity in mice infected with *Histoplasma capsulatum*. *J. Infect. Dis.* 2006; 193(2):322–330. [PubMed: 16362898]
 56. Deepe GS, Buesing WR. Deciphering the pathways of death of *Histoplasma capsulatum*-infected macrophages: implications for the immunopathogenesis of early infection. *J. Immunol.* 2012; 188(1):334–344. [PubMed: 22102723]
 57. Tsiodras S, Samonis G, Boumpas DT, Kontoyiannis DP. Fungal infections complicating tumor necrosis factor alpha blockade therapy. *Mayo Clin. Proc.* 2008; 83(2):181–194. [PubMed: 18241628]
 58. Deepe GSJ, Gibbons R, Woodward E. Neutralization of endogenous granulocytemacrophage colony-stimulating factor subverts the protective immune response to *Histoplasma capsulatum*. *J. Immunol.* 1999; 163(9):4985–4993. [PubMed: 10528203]
 59. Deepe GS, Gibbons RS. Interleukins 17 and 23 influence the host response to *Histoplasma capsulatum*. *J. Infect. Dis.* 2009; 200(1):142–151. [PubMed: 19469707]
 60. Kroetz DN, Deepe GS. CCR5 dictates the equilibrium of proinflammatory IL-17+ and regulatory Foxp3+ T cells in fungal infection. *J. Immunol.* 2010; 184(9):5224–5231. [PubMed: 20335531]
 61. Robinson WS, Arnold SR, Michael CF, et al. Case report of a young child with disseminated histoplasmosis and review of hyper immunoglobulin E syndrome (HIES). *Clin. Mol. Allergy.* 2011; 9(1):14–21. [PubMed: 22126402]
 62. Muraille E, Leo O, Moser M. Th1/Th2 paradigm extended: macrophage polarization as an unappreciated pathogen-driven escape mechanism? *Front. Immunol.* 2014; 5:603. [PubMed: 25505468]
 63. Gildea LA, Gibbons R, Finkelman FD, Deepe GS. Overexpression of interleukin-4 in lungs of mice impairs elimination of *Histoplasma capsulatum*. *Infect. Immun.* 2003; 71(7):3787–3793. [PubMed: 12819061]
 64. Szymczak WA, Deepe GS. The CCL7-CCL2-CCR2 axis regulates IL-4 production in lungs and fungal immunity. *J. Immunol.* 2009; 183(3):1964–1974. [PubMed: 19587014]

65. Verma A, Kroetz DN, Tweedle JL, Deepe GS. Type II cytokines impair host defense against an intracellular fungal pathogen by amplifying macrophage generation of IL-33. *Mucosal Immunol.* 2014; 8(2):380–389. [PubMed: 25118166]
66. Deepe GS, Gibbons RS. Protective and memory immunity to *Histoplasma capsulatum* in the absence of IL-10. *J. Immunol.* 2003; 171(10):5353–5362. [PubMed: 14607938]
67. Hwang LH, Mayfield JA, Rine J, Sil A. *Histoplasma* requires SID1, a member of an iron-regulated siderophore gene cluster, for host colonization. *PLoS Pathog.* 2008; 4(4):e1000044. [PubMed: 18404210]
68. Winters MS, Chan Q, Caruso JA, Deepe GS. Metallomic analysis of macrophages infected with *Histoplasma capsulatum* reveals a fundamental role for zinc in host defenses. *J. Infect. Dis.* 2010; 202(7):1136–1145. [PubMed: 20731582]
69. Herold MJ, McPherson KG, Reichardt HM. Glucocorticoids in T cell apoptosis and function. *Cell. Mol. Life Sci.* 2006; 63(1):60–72. [PubMed: 16314919]
70. Dismukes WE, Royal SA, Tynes BS. Disseminated histoplasmosis in corticosteroid-treated patients. Report of five cases. *JAMA.* 1978; 240(14):1495–1498. [PubMed: 682353]
71. Kauffman CA, Israel KS, Smith JW, White AC, Schwarz J, Brooks GF. Histoplasmosis in immunosuppressed patients. *Am. J. Med.* 1978; 64(6):923–932. [PubMed: 350045]
72. Hansen KE, St Clair EW. Disseminated histoplasmosis in systemic lupus erythematosus: case report and review of the literature. *Semin. Arthritis Rheum.* 1998; 28(3):193–199. [PubMed: 9872480]
73. Witty LA, Steiner F, Curfman M, Webb D, Wheat LJ. Disseminated histoplasmosis in patients receiving low-dose methotrexate therapy for psoriasis. *Arch. Dermatol.* 1992; 128(1):91–93. [PubMed: 1739293]
74. Freifeld AG, Iwen PC, Lesiak BL, Gilroy RK, Stevens RB, Kalil AC. Histoplasmosis in solid organ transplant recipients at a large Midwestern university transplant center. *Transpl. Infect. Dis.* 2005; 7(3–4):109–115. [PubMed: 16390398]
75. Nath DS, Kandaswamy R, Gruessner R, Sutherland DER, Dunn DL, Humar A. Fungal infections in transplant recipients receiving alemtuzumab. *Transplant. Proc.* 2005; 37(2):934–936. [PubMed: 15848579]
76. Hage CA, Bowyer S, Tarvin SE, Helper D, Kleiman MB, Wheat LJ. Recognition, diagnosis, and treatment of histoplasmosis complicating tumor necrosis factor blocker therapy. *Clin. Infect. Dis.* 2010; 50(1):85–92. [PubMed: 19951231]
77. Ehrenstein MR, Evans JG, Singh A, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNF- α therapy. *J. Exp. Med.* 2004; 200(3):277–285. [PubMed: 15280421]
78. Deepe GS, Gibbons RS. TNF- α antagonism generates a population of antigen-specific CD4⁺CD25⁺ T cells that inhibit protective immunity in murine histoplasmosis. *J. Immunol.* 2008; 180(2):1088–1097. [PubMed: 18178849] • Demonstrates generation of immunosuppressive T cells by TNF- α inhibition, reflecting clinical data.
79. Evans HG, Roostalu U, Walter GJ, et al. TNF- α blockade induces IL-10 expression in human CD4⁺ T cells. *Nat. Commun.* 2014; 5:3199. [PubMed: 24492460]
80. Di Franco M, Gerardi MC, Lucchino B, Conti F. Mavrilimumab: an evidence based review of its potential in the treatment of rheumatoid arthritis. *Core Evid.* 2014; 9:41–48. [PubMed: 24648832]

Executive summary

Histoplasma capsulatum: a global fungal pathogen

- Histoplasmosis typically presents in immunocompromised patients living in endemic regions.
- Strains of *Histoplasma capsulatum* are phenotypically unique, which may alter the infection and immune response.
- *H. capsulatum* yeasts are adapted to infect mammalian macrophages.

Innate immune response

- Macrophages and dendritic cells utilize different surface receptors to phagocytose *H. capsulatum*, which activate different signaling cascades and may lead to differential response.
- Macrophages must be activated to inhibit *H. capsulatum* survival.
- Granulomas represent a potential source of pathogens for reactivation disease.

Adaptive immune response

- Antigen presentation and induction of *H. capsulatum*-specific CD4⁺ T-cell response is essential for clearance of infection.
- Protection requires polarization of the CD4⁺ T-cell response for production of protective Th1-associated cytokines, and suppression of detrimental Th2- and Treg-associated cytokines.
- Th17 response may be important in the human response to *H. capsulatum* although it is dispensable in murine disease.
- Protective cytokines trigger iron and zinc sequestration from *H. capsulatum* to facilitate clearance of infection.

Translation from mouse to human

- Clinical studies support murine data that CD4⁺ T cells are critical for host protection from histoplasmosis.
- In histoplasmosis, TNF- α is an essential cytokine first identified in murine models and confirmed by clinical use of TNF- α inhibitors.