



Immunization Strategies for the Control of Histoplasmosis

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Abstract Histoplasmosis is an infection caused by the dimorphic fungus *Histoplasma capsulatum*. Histoplasmosis is typically self-limited and presents asymptotically in most people. Nevertheless, histoplasmosis can cause severe pulmonary disease and death. Histoplasmosis is increasingly found worldwide; however, it is best documented in the endemic region of the Mississippi river valley system in the Eastern part of the United States (US). Epidemiological studies from the US detailing the morbidity, mortality, and cost associated with histoplasmosis underscore the need to develop a vaccine.

Purpose of Review This review will detail some of the major developments in potential vaccines against histoplasmosis, with particular emphasis on those that could be used to immunize immunocompromised hosts. Additionally, this review will highlight some non-traditional vaccine-like ideas for the prevention of diverse mycoses.

Recent Findings Historically, immunization strategies against histoplasmosis have largely focused on identifying immunogenic proteins that confer protection in animal models. More recently, novel active, therapeutic, and immunomodulatory strategies have been explored as potential alternatives for those with various immune deficiencies.

Summary The studies summarized in this review demonstrate that more research is needed to clarify the immunobiology, clinical role, and efficacy of each candidate vaccine in the ever-expanding potential armamentarium against histoplasmosis.

Keywords Histoplasmosis · *Histoplasma capsulatum* · Vaccine · T cells · Antibodies · Immune therapy

Introduction

Histoplasmosis is an infection caused by *Histoplasma capsulatum* (*H. capsulatum*), a thermally dimorphic ascomycete fungus. *H. capsulatum* and, correspondingly, histoplasmosis are increasingly found worldwide [1–3]. Of all the systemic mycoses, histoplasmosis is the most common in North America, with the majority of cases occurring within the endemic region of the Mississippi river valley system in the Eastern part of the United States (US) [4–6]. From 2011 to 2014, a total of 3409 cases of histoplasmosis were documented in health surveillance records across 12 states, including those within and outside the classically defined endemic region of the continental US. State-specific annual incidence

rates of histoplasmosis ranged up to 4.3 cases/100,000 population and were relatively consistent over the 4-year period [6]. Due to a lack of reporting and robust public health surveillance data, however, the true incidence and burden of histoplasmosis in the US may be underestimated.

H. capsulatum infection is a serious public health issue as it can cause severe disease and has a significant risk of mortality. Of all the cases of histoplasmosis reported in the aforementioned study, 57% of patients were hospitalized for a severe infection, of which 7% died [6]. These recent findings are consistent with a previous epidemiological study, conducted in 2002, which found a mortality rate of around 7% for adult patients hospitalized for histoplasmosis within the endemic region of the US [4]. Moreover, significant costs are incurred by patients and the health care system at large for hospitalizations related to histoplasmosis (average hospital charge per adult patient in 2002: \$20,300) [4]. The current morbidity, mortality, and cost associated with histoplasmosis, in combination with the emergence of drug-resistant varieties of *H. capsulatum*, suggest that a robust immunization strategy to prevent cases of histoplasmosis is indicated [6, 7].

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Histoplasmosis

H. capsulatum infection typically occurs after incidental exposure to either contaminated soil or the decaying excreta of bats or certain bird species. *H. capsulatum* enters the host through the inhalation of spores or mycelial fragments into the alveolar spaces of the lung. Spores or active mycelia readily differentiate into budding yeast cells at physiological temperature and are phagocytosed by dendritic cells (DC), polymorphonuclear phagocytes (neutrophils), and alveolar macrophages [8]. Dendritic cells are able to rapidly phagocytose and kill yeast-phase *H. capsulatum* in the lung; neutrophils also play a role in the acute response to *H. capsulatum* infection by inhibiting fungal growth through the release of azurophilic granule contents [9, 10]. A few days after initial infection, phagocytosed *H. capsulatum* yeast cells are principally found within inflammatory macrophages, wherein they are able to survive and proliferate as facultative intracellular pathogens [8]. Control of histoplasmosis is thus classically dependent on the activation of infected mononuclear phagocytes by various type 1 pro-inflammatory cytokines such as IFN- γ , TNF- α , and GM-CSF to kill phagocytosed yeasts or form granulomas encapsulating the infected tissue [11].

The severity of histoplasmosis is largely dependent on the degree of exposure and the patient's immune status. Histoplasmosis is typically asymptomatic and self-limited in immunocompetent hosts. More severe, symptomatic infections characteristically involve some degree of pulmonary disease. *H. capsulatum* infection can also cause a lethal disseminated disease. Disseminated *H. capsulatum* infection is most commonly seen in immunocompromised hosts (e.g., those with comorbid HIV infection and hematologic malignancies, or those receiving some type of immunosuppressive therapy) [12–14]. Nevertheless, disseminated disease can occur in otherwise immunocompetent hosts as well, particularly in the setting of a heavy inoculum exposure. *H. capsulatum* can also establish a latent phase in humans. In this capacity, *H. capsulatum* is able to operate as an opportunistic pathogen causing infection as host conditions permit (e.g., organ transplant, anti-TNF- α therapy) [13, 15, 16]. Thus, the ability to vaccinate even those with various immune deficiencies is paramount in the search for an effective immunization strategy against histoplasmosis.

Currently, there are no vaccines available for the prevention or treatment of any mycosis. However, there are numerous strategies under study. This review will detail developments in both active and passive immunization strategies against histoplasmosis.

Active Immunization Strategies

rHsp60

In an early pioneering study, a detergent extract of the cell wall and membrane of yeast-phase *H. capsulatum* was identified to confer protective immunity against lethal intravenous challenge with *H. capsulatum* in murine models [17]. Subsequent research narrowed in on specific fractions of the extract, testing them systematically for antigenicity and immunogenicity [18, 19]. HIS62, a glycoprotein isolated from the 62-kDa fraction of the extract, was identified as an immunogenic antigen. Additional experiments showed that purified native HIS62 could induce a delayed type hypersensitivity reaction (DTHR) in mice that had been previously immunized with a sublethal inoculum of *H. capsulatum* [18]. Moreover, splenocytes from mice that had been previously immunized with live *H. capsulatum* proliferated in response to exposure to HIS62. Most importantly, however, approximately 80% of mice immunized with purified HIS62 survived the subsequent lethal intravenous challenge of *H. capsulatum* [18].

After documenting the protective capacity of HIS62, the gene encoding the protein was identified and cloned in order to better understand the biology of the native protein and assess the antigenicity and immunogenicity of its recombinant counterpart. The amino acid sequence revealed that HIS62 is about 70% homologous to heat shock protein 60 (Hsp60) found in *S. cerevisiae*; HIS62 was also recognized by a rabbit antiserum raised against *E. coli* Hsp60. Thus, HIS62 was renamed heat shock protein 60 (Hsp60) from *H. capsulatum* and its recombinant counterpart, rHsp60. rHsp60 was documented to have a similar antigenicity and immunogenicity to its native cousin. One hundred percent of mice vaccinated with rHsp60 survived a lethal intranasal challenge with live *H. capsulatum* yeast cells [20].

Subsequent studies were carried out to isolate the specific amino acid sequence of rHsp60 responsible for its immunogenicity. To do this, rHsp60 was broken down into four overlapping polypeptide fragments, all of which could stimulate cell proliferation of pre-sensitized splenocytes. Only fragment three (F3), however, ranging from amino acids 172 to 443, conferred protection, albeit in only 50% of mice, against lethal intranasal challenge with live *H. capsulatum* yeast cells. By comparison, the full-length recombinant protein, rHsp60, provides greater protection against histoplasmosis in murine models than the immunologic fragment, F3, in and of itself [20, 21].

rHsp60 vaccination in murine models works by priming a CD4+ Th1 effector response against histoplasmosis. Induction of the Th1 effector response to *H. capsulatum*

infection is classically dependent on the interleukin 12 (IL-12)/IFN- γ axis. In simplest terms, macrophages and dendritic cells respond to *H. capsulatum* infection by secreting IL-12. IL-12 induces the differentiation of naïve (Th0) CD4+ T cells into Th1 cells, which in turn secrete IFN- γ among other pro-inflammatory cytokines. IFN- γ plays a pivotal role in promoting the cellular immune response to *H. capsulatum* as it is a potent activator of effector macrophages; its absence is correlated directly with decreased survival in murine models of primary histoplasmosis [22–24]. Vaccination with purified rHsp60 requires the presence of CD4+ T cells, a functioning IL-12/IFN- γ axis and IL-10 for an effective inductive phase. Neutralizing any of these mediators with mAb results in a 100% mortality rate upon subsequent lethal challenge with *H. capsulatum* in murine models [25].

The expressive phase of vaccination after lethal challenge with *H. capsulatum* requires the presence of either CD4+ or CD8+ T cells. This finding suggests that even though CD8+ T cells are not necessary for the afferent phase of rHsp60 vaccination, these cells, when present, can also be primed by rHsp60 immunization to carry out an effector response in the expressive phase of the vaccine, even if the CD4+ T cell population has been depleted. MAb-induced depletions of both CD4+ and CD8+ T cell populations after vaccination results in the failure to control a lethal *H. capsulatum* infection in murine models [25].

Vaccination with rHsp60 is critically dependent on a functional host CD4+ T cell population in the afferent phase and thus would have limited efficacy in those with significantly depleted CD4+ T cell counts (e.g., individuals with AIDS). Theoretically, the evidence suggests that vaccination with rHsp60 might still be effective if given before the acquisition of such an immunodeficiency. Though most importantly, this discovery indicates that it may be possible to immunize those with seriously depleted CD4+ T cell counts by directly activating CD8+ T cells to carry out an effector response [25].

Molecular indicators for vaccine efficacy for rHsp60 and F3 of rHsp60 have been studied in some detail. Vaccination of mice with rHsp60 or F3 induces the differentiation and clonal expansion of different subsets of T cell clones. In either case, elimination of the specific clonally expanded Th1 cell population nullifies the protective effect of vaccination. Despite the differences in the T cell clones isolated after vaccination with either rHsp60 or F3, common protective epitope-binding domains were identified in the T cell receptors (TCRs) of both populations. Thus, if the same phenomenon holds true in humans, common TCR domains could potentially serve as primary indicators for effective induction of vaccination, reducing the need for extensive primary clinical efficacy trials [26].

F3 of rHsp60 has additional value in that it is a small protein fragment. It may well be possible to generate immunogenic peptide fragments of F3 as potential subunit vaccines. Peptides are advantageous in that they can be manufactured in mass and be readily modified to improve their stability and enhance their immunogenicity, and most importantly, they are non-infectious [27]. Thus, even in the face of potentially diminished protection from the subunit in and of itself, the pros of using a peptide may outweigh the cons. In the context of a defined biologic marker for efficacy, immunogenic peptide fragments of F3 represent potentially powerful, modifiable, and scalable clinical options for active vaccination against *H. capsulatum*.

H Antigen

An additional protein immunogen, H antigen, has also been shown to confer protective immunity against *H. capsulatum* in murine models. H antigen, a member of the β -glucosidase family, is a surface protein component of the cell wall of *H. capsulatum* [28]. Studies have revealed that H antigen is indeed antigenic [29–31]; however, mice immunized with recombinant H antigen were not protected against sublethal or lethal *intravenous* inoculums of live *H. capsulatum* yeast cells [32]. Nevertheless, it was subsequently shown that immunization with recombinant H antigen does confer protection against sublethal and lethal *intranasal* challenge with *H. capsulatum*. The mechanism by which this differential response occurs is not currently understood [33]. These studies highlight the fact that the efficacy of H antigen vaccination, at least in murine models, is dependent on the route of infection. This is a critical finding in that it identifies a potential limitation of H antigen vaccination against histoplasmosis. More importantly, however, it suggests that future active immunization strategies against histoplasmosis should be assessed in terms of their efficacy against different potential routes of infection.

Cross-priming

Dendritic cells play a critical role in the control of histoplasmosis in murine models. Dendritic cells can phagocytose and kill intracellular *H. capsulatum*, and, as principle multimodal antigen presenting cells, they are able to stimulate effective CD4+ or CD8+ T cell responses to *H. capsulatum* infection. *In vitro* priming of dendritic cells with apoptotic macrophages, which have previously phagocytosed heat-inactivated *H. capsulatum*, followed by auto-transplantation has been shown to produce protective CD4+ or CD8+ T cell responses in murine models of histoplasmosis. This active immunization

strategy, although technically challenging, may be invaluable in the context of actively vaccinating people with HIV/AIDS who might otherwise not be able to mount a sufficient response to *H. capsulatum* infection due to low CD4+ T cells counts [34].

Passive Immunization Strategies

The natural humoral response to histoplasmosis is not well understood. However, that does not preclude the use of antibodies against *H. capsulatum* as a therapeutic tool to control histoplasmosis. *H. capsulatum* infection in murine models induces the production of polyclonal antibodies against a variety of *H. capsulatum* antigens. Nonetheless, passive immunization with polyclonal serum from mice immune to *H. capsulatum* does not protect naïve mice from lethal *H. capsulatum* infection [35]. This may be due to the need of cellular components for an effective response against *H. capsulatum*, but also to the differential composition of polyclonal serum, which has been found to include both protective and non-protective (infection-enhancing) antibodies. A few *H. capsulatum*-specific mAbs, however, have been shown to confer protection against histoplasmosis and are currently being studied as potential passive immunization strategies [36].

IgM Against Histone 2B

Immunization of mice with heat-inactivated *H. capsulatum* yeast cells elicits the production of IgM mAbs against histone 2B (H2B), a protein exposed on the cell surface of *H. capsulatum* [37]. Pre-treatment with these mAbs prior to *H. capsulatum* infection reduces the overall severity of the disease and increases survival in mouse models of histoplasmosis [37, 38]. This protective effect is enhanced with the addition of sub-therapeutic concentrations of amphotericin B; however, even with the addition of amphotericin B, the protection is incomplete with only about 40% survival at 45 days after lethal *H. capsulatum* infection [37]. The treatment of histoplasmosis with IgM is limited by nature due to the large pentameric structure of the immunoglobulin, which impedes its ability to easily move from the bloodstream to the alveolar space. However, when preincubated with *H. capsulatum* prior to intranasal challenge, the IgM mAb to H2B does not only enhance survival, but it also decreases the fungal burden and tissue damage in experimental infections [34••].

IgG Against rHSP60

Protective IgG mAbs against rHsp60 have been raised and are currently under study [39]. Intraperitoneally (IP) administered IgG1 and IgG2a mAbs against rHsp60 have been shown to significantly prolong the survival of mice

challenged with a lethal intranasal inoculum of *H. capsulatum* yeast cells. However, the efficacy of these mAbs is limited. IP administered IgG1 and IgG2a mAbs conferred protection in only about 60% of the recipients. Surprisingly, an IgG2b mAb does not confer protection against lethal *H. capsulatum* challenge in murine models, even though it was demonstrated to bind to the same epitope on rHsp60 as one of the protective IgG1 mAbs. The pre-treatment of mice with the anti-Hsp60 IgG2b has no effect on mice survival after a lethal challenge with *H. capsulatum*, but incubation of the fungus with this mAb prior to infection leads to a 30% survival of infected mice. The pre-incubation of *H. capsulatum* with this IgG2b mAb has no effect on the rate of phagocytosis by macrophages, but the mAb significantly enhances the intracellular growth of the yeast [36]. Currently, research is underway to determine whether the agglutination properties [40], isotype, or other fine specificity issues mediate this differential protective effect.

Pan-Fungal Therapeutic Antibodies

An ever-expanding armamentarium of mAbs is being generated against a diverse set of pan-fungal antigens. Most recently, a lectin (wheat germ agglutinin (WGA)) was fused with the Fc domain of an IgG2a generating the chimera WGA-Fc. WGA has high affinity to chitin oligomers, a product generated by chitin degradation, a main fungal cell wall constituent [41]. Not only does WGA-Fc directly inhibit fungal growth in culture, but it also enhances phagocytosis of diverse fungal species by macrophages *in vitro* [42]. Opsonization of *H. capsulatum* with WGA-Fc prior to co-culture with macrophages decreases the rates of intracellular growth and most importantly, pre-treatment of mice with WGA-Fc completely protects mice against subsequent lethal challenge with an intranasal inoculum of *H. capsulatum* [42••]. Additional examples of pan-anti-fungal mAbs include but are not limited to an IgG2b against 1,3 β -glucan, IgM against the fungal surface antigen glucosylceramide, and an IgM against melanin. These mAbs are not necessarily *H. capsulatum*-specific but they may be useful down the road in combination therapy to treat histoplasmosis [43•–48]. Pan-fungal antibodies represent a novel strategy to control diverse mycoses, including histoplasmosis. Therapeutic antibodies may end up being the most effective way to treat mycoses such as histoplasmosis in immunocompromised patients who might otherwise not benefit from active immunization strategies. Certainly, more research into the safety and efficacy of these antibodies in a variety of experimental models is indicated.

Immune System Modulation

Recently, immune system-based therapeutic approaches to treating cancer are being investigated as new potential tools for treating fungal infections. One new strategy takes advantage of the PD-1 peripheral immunologic tolerance pathway. Binding of PD-1 ligand (PD-L1) to its receptor (PD-1) on T cells activates inhibitory pathways leading to T cell anergy and immunosuppression [49]. *H. capsulatum* infection in mice induces the upregulation of both PD-L1 and PD-L2 on the surface of macrophages and dendritic cells as well as T cells, promoting disease progression. PD-1 knockout mice survive challenge with a lethal inoculum of *H. capsulatum* yeast cells; moreover, 70% of mice treated with mAb against PD-1 survive lethal *H. capsulatum* infection. Although the selective blockade of the PD-L1–PD-1 interaction does not represent a passive immunization strategy, it constitutes a promising use of antibodies to treat histoplasmosis by blocking inhibitory immunologic checkpoints [50].

CAR T cells that target 1,3 β -glucan, using the extracellular domain of Dectin-1, have been made and tested in experimental models of murine aspergillosis. Mice treated with the modified T cells showed a reduction in hyphal expansion of *Aspergillus fumigatus* compared to mice that did not receive the modified T cells [51]. Although this therapy has not been tested in experimental histoplasmosis, the use of CAR T cells to target pan-fungal antigens represents a potentially powerful tool in the armamentarium of the clinician to treat advanced mycoses as access to these biotechnologies becomes more widely available.

Conclusion

There are numerous candidate preventative and therapeutic vaccines against histoplasmosis described in the literature. Additional epidemiologic data will be needed to evaluate who should be vaccinated and when it would be feasible and reasonable to do so. Determining the utility of vaccination in the setting of variable incidences of disease, particularly with an endemic pathogen in and outside of the high incidence regions, and in different settings of immune competency or suppression will be essential for optimal implementation of vaccine strategies against *H. capsulatum*. Additionally, specific work testing efficacy in the absence of different components of the immune system in animal models can aid in predicting which individuals may benefit most from different immunization approaches. Nevertheless, these epidemiological questions should not preclude the much-needed transition from pre-clinical laboratory research to clinical trials. Numerous lives could be saved, and ultimately healthcare costs may be reduced.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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