Review Article
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How *Histoplasma* Evades the Human Immune System

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Abstract
This review summarises the current knowledge of the interactions between *Histoplasma capsulatum* (Hc) and the human immune system, with particular emphasis on host immune responses and fungal immune evasion mechanisms that modulate disease pathogenesis and clinical outcomes. Histoplasmosis is a disease caused by Hc, a fungus found worldwide. Upon inhalation, complex interactions occur between the pathogen and the human immune system, primarily involving the recognition of fungal cell wall components. Both innate and adaptive immune responses are orchestrated to eliminate the fungus through a tightly regulated balance. However, Hc has evolved multiple strategies to evade host defences and establish infection. The clinical spectrum of histoplasmosis varies, ranging from isolated pulmonary involvement to disseminated disease, depending on host factors and pathogen characteristics. **Conclusion.** Overall, host-pathogen interactions between Hc and the human immune system play a central role in determining disease outcomes and represent key targets for improving preventive, diagnostic, and treatment strategies.

Key Words: Histoplasmosis ▪ Immune Evasion ▪ Pathogenesis ▪ Host-Pathogen Interaction.

Introduction

Histoplasmosis is a disease caused by fungi of the genus *Histoplasma*. Histoplasmosis in humans is classically caused by the fungi *Histoplasma capsulatum* (Hc) var. *capsulatum* and Hc var. *duboisii* (1). Histoplasmosis has been found worldwide, including in the Americas, Africa, Asia, Europe, and Australia, with the main endemic areas located in the Ohio and Mississippi River Valleys of the United States (2). In Latin America, positivity rates range from 37% to 90%, especially in Guatemala, Belize, Venezuela, and Brazil (3). The disease is increasingly recognised in Asia and Africa, with histoplasmin positivity rates of up to 86% in Asia and 0–35% in Africa (4, 5). In contrast, incidents in Europe and Australia are less frequent and primarily associated with travel-related cases, although limited endemic foci have been identified in Australia (3). Histoplasmosis can be fatal, especially in immunocompromised individuals, such as those with HIV/AIDS, and is characterised by systemic spread to various organs (6).

Misdiagnosis and co-infection occur due to similarities with other pulmonary diseases. Acute pulmonary histoplasmosis is frequently misdiagnosed as pneumonia, resulting in inappropriate antibiotic treatment that worsens outcomes (7, 8). Chronic pulmonary histoplasmosis may be misdiagnosed or co-infected with pulmonary tuberculosis because of the similarity in clinical and radiographic symptoms, such as cough, fever, weight loss, and chest X-ray findings of patchy pneumonic infiltrates, calcifications, cavities, and pulmonary nodules (7, 8). In a study of 213 patients with suspected pulmonary tuberculosis, 27 (12.7%) tested positive for Hc infection via antigen testing and/or PCR, indicating that histoplasmosis is relatively prevalent in this population. Of the 94 confirmed patients with TB, 7 (7.4%) had

histoplasmosis co-infection. However, 20 of the 119 patients who were not confirmed to have TB had histoplasmosis, suggesting that some cases may have been misdiagnosed as TB (9).

The high positive rate, frequent misdiagnosis, and co-infection in histoplasmosis cases in endemic areas indicate its significance, particularly for physicians and researchers in the prevention and control of infectious diseases. Histoplasmosis requires a comprehensive understanding of its pathogenesis for effective diagnosis, prevention, and treatment. This article outlines the fundamental aspects of the pathogenesis of Hc, including its cell wall, infection mechanisms, and host immune response. Hc develops mechanisms to evade or suppress the human immune response, allowing the infection to become chronic or spread to other organs. Hc has the potential to spread through the host's immune system. In addition, this study presents recent developments in Hc strain identification from diverse geographical regions, particularly Asia. These novel strains exhibit distinct genetic signatures and many virulence and host response variants, with potential implications for the severity of histoplasmosis and future treatments.

This review aims to examine the interactions between Hc and the human immune system, with a particular focus on fungal cell wall components, host immune responses, and immune evasion strategies, and to highlight their implications for prevention, diagnosis, and treatment.

Cell Wall Structure of *H. capsulatum*

The fungal cell wall is an essential structure for survival, morphology, and cell protection. Hc is a dimorphic fungal pathogen with a unique structure in its cell wall that plays a crucial role in its pathogenicity. Upon entering the host's body, Hc undergoes a phase transition to yeast, during which its cell wall contains essential components for communication with the environment and interaction with host immune cells, such as in the processes of phagocytosis and self-defence. The cell wall components of the yeast form of Hc are composed of various key elements, including carbohydrates, proteins, vesicles, lipids, and melanin (Figure 1) (10).

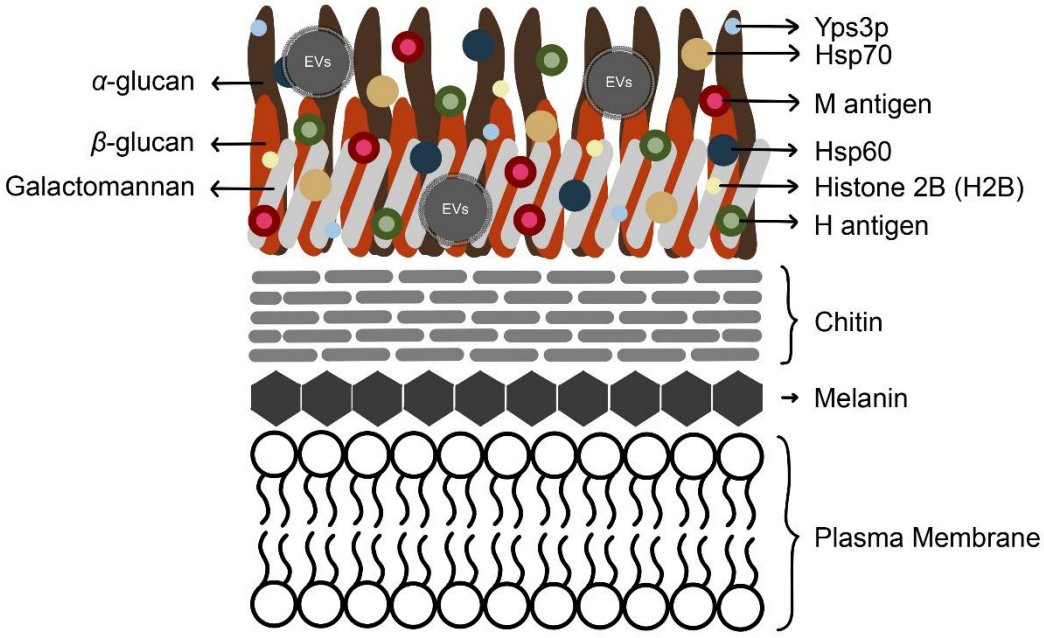

Figure 1. Cell wall schematic structure of *H. capsulatum* yeast (adapted and modified from Guimarães et al. (10)).

The carbohydrates in the yeast cell wall include chitin, glucan, galactomannan, lectin-like components, and mannoproteins. Chitin provides integrity and rigidity to the cell wall, providing structural protection against environmental pressures (10). Glucan is the primary carbohydrate component of the cell wall. They are D-glucose polymers that are connected by α- or β-glycosidic bonds. β-glucan, a major fungal polysaccharide also found in *Pneumocystis carinii* and *Saccharomyces cerevisiae,* has high antigenic properties that can bind to the Dectin-1 receptor on host macrophages and initiate an immune response (11). α-glucan has an important role in the virulence of several pathogenic fungi, such as *Aspergillus fumigatus*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis,* which masks yeast from immune recognition during morphogenesis (12, 13). Under normal conditions, only small amounts of α-glucan are found. However, after infecting the host, the amount of α-glucan increases significantly. This increase is important for helping the pathogen evade the host's immune response (immune evasion). The detailed mechanism and its contribution to successful infection are further discussed in the next section (10, 14).

In macrophage-based experimental models, galactomannan from the Hc cell wall triggers a direct response involving phagocytosis, synthesis of antimicrobial compounds, and release of cytokines, such as the pro-inflammatory cytokine IFN-γ and the regulatory cytokine IL-10. (15, 16). During phagocytosis, components such as lectin activate macrophages and agglutinate the host's erythrocytes (17). Mannoproteins are highly antigenic and induce the maturation and activation of dendritic cells, accompanied by the production of pro-inflammatory cytokines for host tissue adhesion (10).

The Hc cell wall contains protein molecules, such as heat shock protein (Hsp), M antigen, H antigen, histone 2B (H2B), and Yps3p. Heat shock proteins respond to extreme conditions, especially in the human body (10, 18). Several types of Hsp have been identified in the cell wall of Hc, such as Hsp of 60 kDa (Hsp60) and Hsp of 70 kDa (Hsp70). Hsp60 is a major ligand attached to the CR3 receptor on macrophages that triggers phagocytosis. Its expression depends on the response to temperature stress, peaking at 34-37°C. The role of Hsp60 is to support cell wall changes and increase energy gain. Hsp70 expression increases during the mycelial-to-yeast phase transition and peaks at 37°C (10).

M and H antigens are glycoproteins found on the wall of Hc that are homologous to catalase and β-glucosidase (19, 20). Antigen M is the catalase possessed by Hc, both within the cell wall and secreted outside the cell. This catalase is classified as an antigen based on its amino acid sequence and reactivity with monoclonal antibodies (21). There are three catalases in Hc: catalase B (CatB) and catalase A (CatA), secreted outside the fungal cell, and catalase P (CatP), which is secreted inside the cell. CatA is primarily produced during the mycelial phase, whereas CatB and CatP are produced during the yeast and mycelial phases. These three catalases protect Hc from oxidative stress and promote survival in host cells. The H antigen is a β-glucosidase homologue that helps in the breakdown of carbohydrate substrates from the environment to produce glucose as an energy source and cell wall modulation. Both antigens are secreted and react with the patient's serum (20).

The Hc cell wall also contains H2B, which is speculated to be a protein used in cell signalling that modulates the immune response of the fungus (10). Yps3p can bind to chitin and is a virulence factor that can increase the spread of phagocytic cells in tissues. These proteins can be used to characterise Hc with distinctive molecules (10).

The cell wall of Hc yeast produces extracellular vesicles (EVs) that contain various lipids, carbohydrates, proteins, pigments, and nucleic acids. These vesicles can function as "virulence bags" because they concentrate virulence factors that trigger stress responses and pathogenesis, such as urease, phosphatase, catalase, and laccase (22, 23). Additionally, proteins extracted from Hc vesicles react with the immune serum of patients with histoplasmosis, indicating that these vesicles can modulate the immune response (23).

Hc can synthesise melanin in its cell wall, a negatively charged hydrophobic pigment with a high molecular weight formed through the oxidative polymerisation of phenolic and/or indolic compounds. Melanin in Hc reduces susceptibility to host defence mechanisms and antifungal drugs, such as amphotericin B and caspofungin (10, 24, 25). Melanin binds to antifungal molecules and prevents the drug from interacting with ergosterol on the cell membrane, thus localising the antifungal compound in the extracellular space. This pigment is also commonly found in various human pathogenic fungi, including *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Candida albicans*, and *Sporothrix schenckii*, underscoring its critical role in fungal survival and virulence (26). Given its role in virulence and antifungal resistance, melanin could be a potential therapeutic target in histoplasmosis, as inhibition of its biosynthetic pathways may enhance the efficacy of histoplasmosis therapy.

Host Immune Response to *H. capsulatum* Infection

Hc microconidia and hyphal fragments enter the host via inhalation and convert into the yeast form in response to body temperature as they reach the lung tissue (Figure 2). The first line of defence is mucociliary clearance, where mucus traps inhaled particles and cilia expel them; however, Hc can evade this due to its small size. In the alveoli, it faces surfactant proteins, particularly SP-A and SP-D, which opsonise pathogens and enhance phagocytosis by macrophages and neutrophils, as well as exert fungicidal effects by disrupting fungal cell walls. To survive, the fungus hides within macrophages and escapes surfactant-mediated defences (2). Hc also interacts with various cell responses of the innate immune system and later adaptive immune response, as summarised in Table 1.

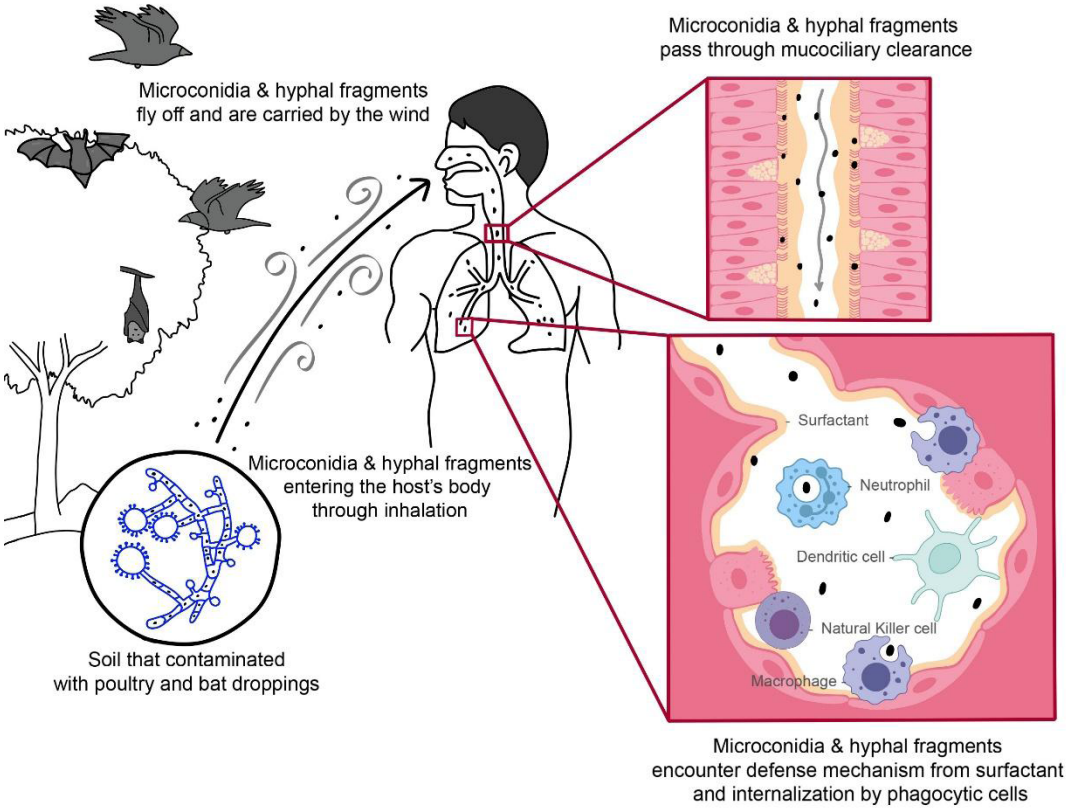

Figure 2. *H. capsulatum* infection in the human body (adapted and modified from Mittal et al. (2)).

Table 1. Immune Response to *H. capsulatum*

Host property	Mechanism
Mucociliary Clearance (2)	The respiratory epithelium produces mucus, which traps, and cilia push microorganisms towards the pharynx to be swallowed or expelled
Surfactant protein (2)	SP-A & SP-D opsonise microbes, thereby increasing phagocytosis by macrophages and neutrophils
	Fungicidal properties by disrupting the fungal cell wall, therefore increasing the permeability
Macrophage (27, 31–33)	CR3 & Hsp60 interaction causing internalisation of Hc
	Dectin-1 & β-glucan interaction causes the release of pro-inflammatory cytokines: IL-6 and TNF-α
	TLR2 & Yps3p interaction causes activation of the NF-κB cascade, which activates the adaptive immune response via T cells
	Dectin-1 & galactomannan interaction causes the release of IFN-γ and IL-10
	Reducing copper availability in the phagosome
	Iron limitation occurs as activated macrophages increase iron binding to transferrin, reducing free plasma iron levels
	GM-CSF induces metallothionein expression, leading to zinc sequestration
Dendritic Cell (34–36)	VLA-5 & CypA interaction causing: Phagocytosis of the fungi by DCs. Inhibit the ability of Hc to control phagolysosome formation Intracellular oxidation through the release of hydrolases
	Dectin-1 & β-glucans also Dectin-2 & α-mannans interaction causing activation of NLRP3 inflammasome protein and further inducing dendritic cells to secrete IL-1β and IL-18, which will mobilise DCs to lymph nodes for antigen presentation & priming of naïve T cells
	TLR7 and TLR9 in dendritic cells detect fungal nucleic acids, promote IFN-1 production
Neutrophil (37–40)	CR1, CR3 & C3B opsonin interaction causes an increase in phagocytosis
	Release of NET to trap & kill Hc
NK Cell (41)	Activating T cells for further adaptive immune response
CD4+ T Cells (41)	IL-12 drives the differentiation of naïve T-cells into Th1 cells and stimulates IFN-γ and TNF-α production by Th1 cells, which activates more macrophages, resulting in oxidative burst
	Th2 cells produce cytokines such as IL-4 & IL-21, which drives immunoglobulin class switching
	Th17 cells enhance fungal clearance by influx of inflammatory cells
Humoral Immune Response (45, 46)	Antibodies towards Histone 2B on fungal walls lower fungal burdens, reduce pulmonary inflammation, and impair Hc's ability to regulate intraphagosomal pH within macrophages
	Antibodies directed against Hsp60 promote agglutination, which in turn hinders the spread of the fungus
	Antibodies targeting the M antigen enhance macrophage-mediated phagocytosis of the yeast and boost the host cell's ability to kill the pathogen

SP-A=Surfactant protein A; SP-D=Surfactant protein D; CR1=Complement receptor 31; CR3=Complement receptor 3; Hsp60=Heat shock protein 60: TNF-α=Tumour necrosis factor alpha; TLR2=Toll-like receptor 2; Yps3p=Yeast phase-specific protein, NF-κB=Nuclear factor-kappa B; IFN-γ=Interferon-gamma; VLA-5=Very late antigen 5; CypA=Cyclophilin A; NLRP3=Nucleotide-binding domain; Leucine-rich–containing family; Pyrin domain–containing-3; NET=Neutrophil extracellular traps.

Macrophages

Macrophages are key in host defence against Hc, internalising the fungus via surface receptors such as CR3 without requiring opsonisation, a crucial mechanism in the low-opsonin environment of the lungs (27). The ligand that interacts with macrophage receptors is Hsp60, which is expressed on the surface of yeast Hc. Heat shock proteins generally play a role in protein folding but also have immunogenic properties in several pathogens, such as *Borrelia burgdorferi, L. pneumophila, Chlamydia trachomatis,* and many others, indicating that this protein is a potent antigen that triggers a host immune response (28). In vitro studies using cultured immune cells have demonstrated that macrophages recognise Hc through several receptors: CR3 binding to Hsp60 mediates fungal internalisation; Dectin-1 binding to *β*-glucan induces IL-6 and TNF-*α* release; and

TLR2 interaction with Yps3p activates the NF-κB cascade, triggering T-cell responses (29). However, Hc is known for its ability to survive and even multiply inside the macrophage through several mechanisms. These mechanisms are further discussed in the next section (30).

Macrophages also function as innate immune cells that recognise the structural components of the Hc cell wall, including galactomannan. A study using peritoneal macrophages extracted from rodents demonstrated that galactomannan is recognised by the C-type lectin receptor Dectin-1, leading to fungal phagocytosis and the induction of IL-10 and IFN-γ, but not TNF-α. IFN-γ subsequently enhances macrophage activation, resulting in reduced intracellular Hc survival. In contrast, IL-10 exerts anti-inflammatory effects that may limit tissue damage by dampening excessive pro-inflammatory responses (31).

Another role of macrophages is to reduce the availability of trace metals within macrophages. A study has shown that activation of macrophages by IFN-γ changes the phagosomal environment to a copper-, iron-, and zinc-deficient environment. Iron, zinc, and copper are essential nutrients for Hc. The limitation of iron is due to a host mechanism that increases the affinity of iron-transferrin binding, thereby reducing free plasma iron concentration (32). An in vitro macrophage experiment reported that zinc sequestration is triggered by granulocyte-macrophage colony-stimulating factor (GM-CSF), which causes the expression of cytoplasmic zinc-binding metallothioneins and redistributes zinc from intraphagosomal Hc yeast to the Golgi compartments. When their availability is restricted, intracellular fungal growth is inhibited. However, Hc can overcome copper deficiency via the Ctr3 transporter mechanism, which is discussed in the following section (33).

Dendritic Cell

The interaction between Hc and DC is mediated by very late antigen 5 (VLA-5), Dectin-1, and Dectin-2 receptors. Using a human dendritic cell culture system, a study demonstrated that the interaction of VLA-5 with its ligand CypA causes phagocytosis and triggers further intracellular signalling to inhibit the ability of Hc to control phagolysosome formation (34). The eradication of Hc in DCs is mainly due to oxidative burst reactions mediated by the release of hydrolases instead of NO. Other DCs receptors, Dectin-1 and Dectin-2, could recognise β-glucans and α-mannans of the Hc cell wall, respectively. This interaction activates the NLRP3 inflammasome protein and further induces DCs to secrete IL-1β and IL-18, which play a role in the mobilisation of DCs to the lymph nodes for antigen presentation and priming of naïve T cells (35). Additionally, dendritic cells have intracellular receptors that, when activated, result in the production of type 1 interferon (IFN-1) (36).

Neutrophil

Neutrophils can phagocytose and release neutrophil extracellular traps (NETs) to eradicate Hc. Neutrophils can phagocytose pathogens through opsonisation mechanisms, such as via CR1, CR3, and FcγRIII (CD16) recognition of C3b opsonins. Neutrophils can also act via opsonin-independent mechanisms, although the specific receptors and pathways involved are not yet fully understood (37). However, this mechanism is unlikely to be the primary cause of the fungistatic effect of neutrophils. This is evidenced by the fact that, in a study with isolated human neutrophils, inhibition of phagocytosis using cytochalasin D still results in the inhibition of Hc (38).

The primary mechanism by which neutrophils exert fungistatic and even fungicidal effects is through the release of neutrophil extracellular traps (NETs), which are a network of extracellular strings consisting of DNA, histones, and antimicrobial proteins that trap pathogenic microorganisms (39). The trapped pathogens are then destroyed by the antimicrobial proteins contained in the neutrophil azurophilic granules. An *in vitro* study using purified neutrophil granules on Hc reported that bactericidal permeability-increasing proteins (BPI) contained in neutrophil azurophilic granules were able to inhibit the growth of

this fungus. In addition to BPI, NETs also contain cathepsin G and defensin proteins, which have been shown to effectively inhibit the growth of Hc yeasts, although the exact mechanisms involved remain unclear (40).

Natural Killer Cell

Macrophages that phagocytose Hc release the cytokine IL-12, which activates NK cells to produce IFN-γ, helping to control the Hc infection. In an in vivo study with mice infected with Hc and treated with IL-12, the investigators found that IL-12 treatment significantly reduced mortality, increased IFN-γ production, decreased fungal burden in spleen cells, and that the protective effect of IL-12 was dependent on IFN-γ. However, its significant role in the elimination of the fungus from the host Hc has yet to be clarified (1).

T Cell

Dendritic cells (DCs) and macrophages function as antigen-presenting cells (APCs) that activate naïve T-cells through antigen presentation in the context of MHC class II molecules. This interaction is enhanced by the production of the key cytokine IL-12 by DCs and macrophages. IL-12 drives the differentiation of naïve T-cells into Th1 cells and stimulates IFN-γ and TNF-α production by Th1 cells, which subsequently strengthens the cellular immune response against intracellular pathogens such as Hc by activating more macrophages, resulting in an oxidative burst (42). A study in mice injected with anti-IFN-γ antibodies and IFN-γ gene knockout mice reported that they were more susceptible to lethal infection than the control group, showing the crucial role of IFN-γ in the host's innate defence against systemic Hc infection (43).

Differentiation into Th2 cells is driven by IL-4. Cytokines with immunosuppressive effects, such as IL-4 and IL-10, can hinder the immune response against Hc. An in vivo study in mice has shown that their combined activity suppresses the development of IFN-γ–producing cells, which are critical for fungal clearance. A longitudinal study found that as IL-4 and IL-10 levels declined, the number of IFN-γ-producing cells induced by Hc increased significantly, leading to improved fungal elimination. Additionally, IL-4 can support the survival of intracellular pathogens by reducing nitric oxide (NO) production and increasing the levels of intracellular metal concentrations in macrophages. This IL-4–mediated zinc, calcium, and iron regulation has been shown to promote fungal replication, with increased metal levels partially restoring yeast growth (44).

Granulomas, formed by macrophages and lymphocytes during Hc infection, restrict fungal replication and prevent systemic spread. In murine models, infiltration begins by day 5, granulomas form by day 7, peak at day 10, and eventually eliminate most fungi, although some latent yeast may persist and reactivate under immunosuppression (45). Studies in mice have shown that reactivation occurs in animals with depleted CD4 and CD8 T cells (44).

In addition to the Th1 immune response, an experimental study conducted in mice has shown that Th17 also plays a minor role in host defence against Hc. Naïve T cells differentiated to Th17 in response to cytokines such as IL-6 and IL-23. Th17-cytokine, IL-17, facilitates further recruitment of inflammatory cells, such as macrophages and neutrophils, to the lungs during infection (44). Wüthrich et al. implied that the fungal load in vaccinated mice without IL-17 receptor was higher than that in vaccinated wild-type controls, revealing its significance in fungal infection (46).

Humoral Immune Response

In general, B cell activation occurs through two pathways: T-independent and T-dependent activation. T-independent activation involves non-protein antigens that directly stimulate B cells through BCR cross-linking and TLR/complement interactions, producing short-lived IgM without memory cells. T-dependent activation requires protein antigens presented by B cells via MHC II to helper T cells, with Th2 cytokines, such as IL-4 and IL-21,

driving class switching to IgG, IgA, and IgE. While T-independent responses provide rapid but temporary protection, T-dependent responses create durable and adaptable immunity. Although the immune response to Hc is primarily driven by cellular immunity, the humoral response also plays a role. An experiment in mice showed that depletion of $CD4^+$ and $CD8^+$ T cells in B-lymphocyte knockout mice resulted in significantly higher fungal burdens in organs than T cell depletion in wild-type mice in a model of secondary histoplasmosis (47).

The cell wall proteins of Hc (melanin, H2B, Hsp60, and M antigen) stimulate antibody production, with IgM, IgA, and IgG peaking by day 21 in murine models. Monoclonal antibodies against H2B and Hsp60 reduce fungal growth, impair survival mechanisms, promote agglutination, and enhance macrophage-mediated phagocytosis, thereby improving host defence (48).

Immune Evasion and Yeast Survivability Factors

Dimorphism is a crucial strategy of Hc to evade the immune system (Figure 3). The transition to its yeast form is primarily triggered by an increase in temperature to 37°C. Stepwise genetic analyses that used random insertional mutagenesis was conducted to identify mutants unable to undergo the mycelial to-yeast transition at 37°C. Following mapping and characterisation of the disrupted loci revealed Drk1, which codes for a hybrid histidine kinase, a protein that integrates environmental sensing and signal transduction. Mutants with Drk1-deficient strains were still in the mycelial phase after being exposed to host temperature. These results established that Drk1 is a crucial signal transducer that mediates temperature-induced pathogenic yeast form (49). These findings were primarily derived from in vitro cell culture-based experiments. Additionally, yeast-phase conversion in synthetic media requires exogenous cysteine, as demonstrated by early chemical complementation experiments showing that cysteine supplementation restores respiration even when the respiratory pathway is inhibited and that the role of cysteine cannot be entirely replaced (50-52). Cysteine, with its sulfhydryl (-SH) group, functions as a reducing agent that reactivates mitochondrial respiration, which is crucial for meeting metabolic demands during the yeast phase. This reactivation is necessary to complete the morphological transition and the pathogenicity of Hc (53, 54).

Furthermore, four transcription factors, Ryp1, Ryp2, Ryp3, and Ryp4, play a role in this differentiation switch by forming an interdependent loop. The binding of multiple Ryp factors to their respective promoter regions, such as Sod3, CatB, CatP, and Yps3, affects the virulence of Hc. These regulatory interactions have primarily been characterised using in vitro cell culture–based models. In addition, Vea1 is also important for yeast-phase morphogenesis in Hc. Mycelial phase factors, such as Wet1, are suppressed at 37°C to prevent hyphal growth (55).

Once Hc has established its yeast phase, it must overcome the unfavourable environments posed by the host's immune system (Table 2). Two of the most well-established mechanisms by which Hc avoids immune system detection are the production of α-glucan and Eng1. Synthesis of α-glucan occurs only during the yeast phase of Hc. The production of this polysaccharide covers the outer layer of the yeast cell wall and covers β-glucan (56). This mechanism is essential for preventing the interaction between Dectin-1 and β-glucan, avoiding the detection of Hc by macrophages, and preventing the production of pro-inflammatory cytokines. In contrast, Eng1 is a protein of Hc yeast that plays a role in hydrolysing β-glucan, thereby shearing off the exposed β-glucan. These immune evasion strategies have been predominantly characterised using in vitro cell culture–based systems, including fungal cultures and host immune cell interaction assays. Similar to α-glucan, this process is proposed to minimise detection by the host immune system (57).

Hc yeast counteracts host-derived oxidative stress by producing multiple antioxidant enzymes, as demonstrated in both in vitro phagocyte infection models and in vivo murine studies. In macrophage and polymorphonuclear neutrophil (PMN)

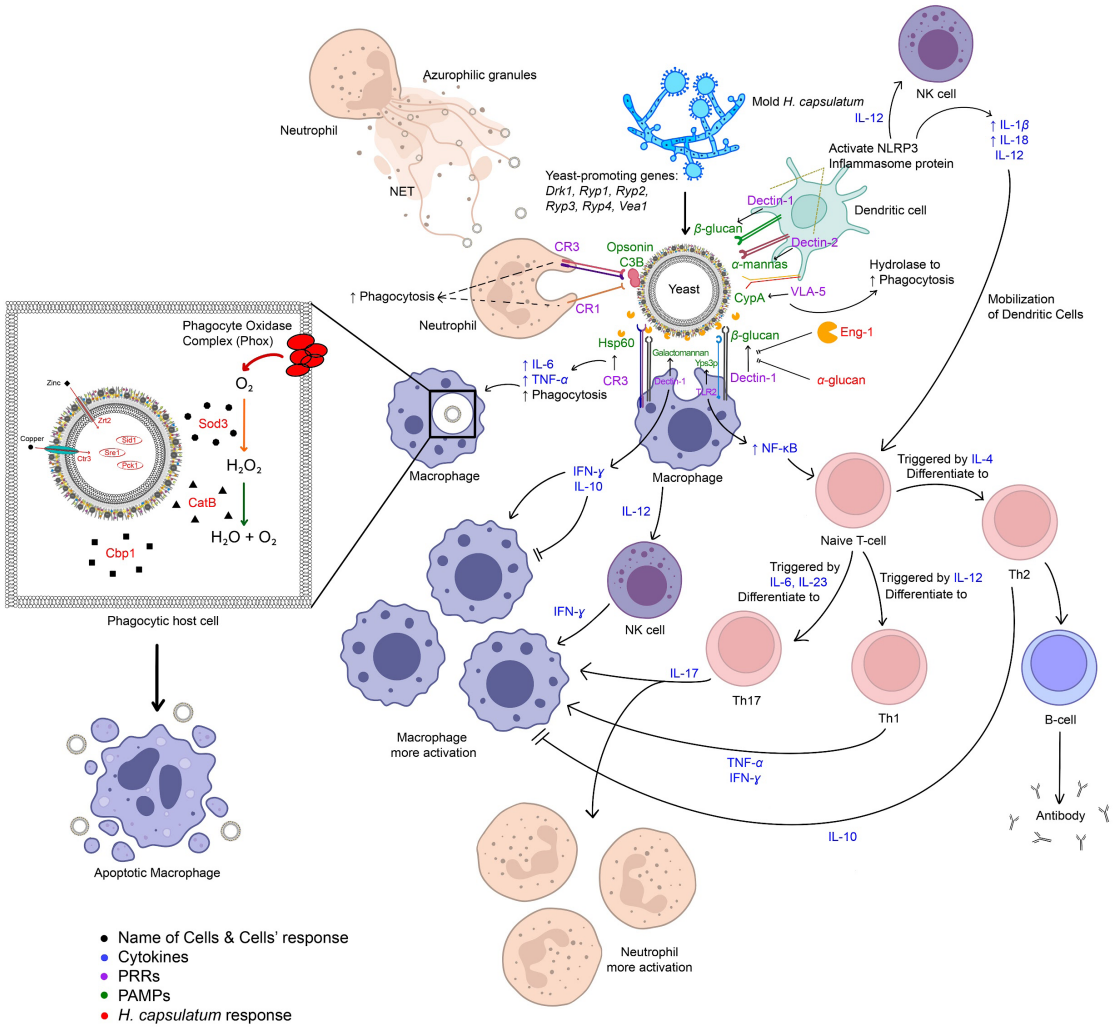

Figure 3. Immune response to *H. capsulatum*. Conceptual framework based on published studies summarised in Tables 1 and 2.

infection assays, Hc yeast secretes an extracellular Cu/Zn-type superoxide dismutase, Sod3, which detoxifies extracellular superoxide generated by host phagocytes, thereby enhancing fungal survival under oxidative stress conditions. (30). Hc yeast also produces CatB and CatP to counteract host cell defence, which function similarly to Sod3. CatB primarily protects against extracellular reactive oxygen species, whereas CatP acts intracellularly. Loss of CatB significantly reduces yeast survival in PMNs and attenuates virulence in mice, whereas deletion of CatP alone has minimal impact. These findings indicate that Hc pathogenesis largely depends on the neutralisation of exogenous oxidative stress (58).

The glutathione system has been shown to participate in stress adaptation and virulence in pathogenic fungi, as demonstrated primarily through in vitro stress-response assays and targeted gene disruption studies, and supported by in vivo infection models. In eukaryotes, glutathione serves as an important metabolite and an essential participant in the protection against oxidative damage. Hc possesses GSH1 and GSH2 genes, which produce γ-glutamylcysteine synthetase and glutathione synthetase enzymes, respectively, which contribute to this system. An intact

Table 2. *H. capsulatum* Immune Evasion and Yeast Survivability Factors

Detection evasion	
Eng1 (54)	Trims excess β-glucan to further minimise host immune system detection
α-glucan (55)	Covers β-glucan layer to avoid detection of Dectin-1
The host immune system is hampering	
Sod3 (53)	Dismutes extracellular superoxide to increase yeast survivability
CatB (57)	Responsible for extracellular catalase activity to increase yeast survivability
CatP (57)	Responsible for intracellular catalase activity to further increase yeast survivability
Gsh1 & Gsh2 (58)	Plays a role in the glutathione system, protecting yeast from oxidative damage
NIT50 and other (59)	Hypoxia-responsive genes increase survivability in hypoxic conditions
Macrophage lysis	
Cbp1 (68)	Lyses macrophage
Yeast survival and proliferation factors	
Ctr3 (32)	Forms a copper transporter in a low-copper environment
Srb1 (59)	Vital for recovering from hypoxic conditions
Sre1 (61)	Forms iron-scavenging siderophores in an iron-limited environment
Zrt2 (62)	Forms a zinc transporter in a zinc-restricted environment
Pck1 and other (65)	Gluconeogenesis for carbon sources
Trp5 (66)	Tryptophan biosynthesis, important for yeast proliferation
Rib2 and other (67)	Vitamin synthesis, important for yeast proliferation

Eng1=Endo-1,3 (4)-beta-glucanase 1, Sod1=Superoxide dismutase 1; CatB=Catalase B; CatP=Catalase P; Gsh1=γ-glutamylcysteine synthetase enzyme; Gsh2=glutathione synthetase enzyme; NIT50=nitrosative stress induced transcription 50; Cbp1=Calcium binding protein 1; Srb1=Sterol regulatory element binding protein 1; Ctr3=Copper uptake protein 1; Sre1=Sterol regulatory element 1; Sid1=Enzyme for siderophore production; Zrt2=Zinc uptake protein 2; Pck1=Phosphoenolpyruvate carboxykinase 1; Trp5=Tryptophan synthase 5; Rib2=Riboflavin synthase.

glutathione system is necessary for successful host adaptation and pathogenicity, according to in vitro experimental analyses that revealed disruption of this pathway reduces virulence in animal models and fungal survival under oxidative stress (59).

Hc could also increase its survival by counteracting the host's adaptive immune response. In murine models, the fungus responds to hypoxia due to inflammation and granuloma formation by producing the Srb1 transcription factor. Srb1 influences the expression of several hypoxia-responsive genes (HRG), such as nitrosative stress-induced transcription 50 (NIT50), an ABC transporter, NADP/FAD oxidoreductase, and an RSP/GEF, which help in survival under hypoxic conditions. Additionally, silencing Srb1 causes hypersensitivity to itraconazole (60).

Hc has several mechanisms to overcome sequestration and the reduction of essential metals. The fungus secretes iron-scavenging siderophores, which are regulated by the GATA transcription factor (Sre1) (61) and require L-ornithine-N^5-monooxygenase (Sid1) in response to low iron levels inside the macrophage, as proven by Hwang et al. and Hilty et al., respectively, using high-iron and low-iron media (61, 62). These siderophores are pivotal in binding iron for further yeast proliferation, while phagosome iron levels decline (62). In the case of zinc sequestration, Hc responds by increasing the transcription of Zrt2, forming a zinc transporter with both high and low affinity (63). Dade found that Zrt2 was crucial on the fifth day post-infection in mice because zinc supply becomes more restricted due to the previously mentioned GM-CSF activation (64). As pathogenesis progresses, copper is restricted by the activation of the host macrophage by IFN-γ. Hc possesses Ctr3, which functions as a copper importer to support yeast growth in low-copper environments. This importer is formed by CTR3 gene expression. It is known that there

are two other genes, CTR1 and CTR2, expressed by Hc yeast. According to Shen et al., all of these genes are expressed equally during the mycelial phase of Hc; however, the expression of CTR3 can reach 10-fold higher in a low-copper environment during the yeast phase, which was proven using a 3M medium containing low or high $CuSO_4$ (33). CTR3 expression is regulated by Mac1, a copper-dependent transcription factor activated in a low-copper environment. A study by Ray et al., utilising both *in vitro* and *in vivo* (murine models) methods, showed that Mac1 is also responsible for Hc virulence, facing the host's adaptive immune response, metal homeostasis, and ROS detoxification (65).

The intracellular environment is characterised by limited carbon sources, requiring Hc yeast to rely on alternative carbon sources for energy. Gluconeogenesis is the most critical pathway for carbon acquisition. Moreover, the primary source of carbon utilised by Hc yeast is derived from amino acids. Shen et al. used an *in vitro* method, which demonstrated that Hc can metabolise single amino acids or short peptides digested by proteinase K or cathepsin D, but not intact proteins. Notably, disruption of phosphoenolpyruvate carboxykinase 1 (Pck1) and fructose-1,6-bisphosphatase (Fbp1) impairs the ability of yeast to utilise gluconeogenic substrates, thereby compromising its virulence. Moreover, this study also found that intracellular Hc did not utilise hexose or fatty acids as carbon sources (66).

Among the various types of amino acids, tryptophan is indispensable for Hc growth and full virulence. Shen et al. evaluated the importance of aromatic amino acid biosynthesis for Hc proliferation. By silencing PHA2, TYR1, and TRP5, Hc lost the ability to encode prephenate dehydratase, prephenate dehydrogenase, and tryptophan synthase, respectively. This depletion also leads to auxotrophy for phenylalanine in Pha2-deficient samples, tyrosine in Tyr1-deficient samples, and tryptophan in Trp5-deficient samples. The study experimented on these auxotrophs by infecting a macrophage population in which Pha2-deficient and Tyr1-deficient Hc were able to kill 80% of the macrophage population, whereas Trp5-deficient Hc were only able to wipe out 20% (67).

Vitamins also contribute to the proliferation of Hc yeast. A study showed that Hc yeast can independently synthesise most vitamins. However, not all synthesised vitamins are essential for proliferation. Garfoot et al. proved that vitamins, such as riboflavin (B2), pantothenate (B5), and biotin (B7), are crucial for Hc yeast proliferation. Silencing their respective genes (RIB2, PAN6, and BIO2) resulted in a severe decrease in the replication rate, fungal burden, and macrophage lysis ability of Hc in mice (68).

Hc can also induce macrophage lysis. The fungus produces Cbp1 protein within macrophages and is fully active in the yeast form (69). Isaac et al. showed that Cbp1 is essential for macrophage lysis and could also prolong yeast cell proliferation in macrophages (69). English et al. found that without Cbp1, Hc yeast cannot trigger host cell death, even with an increasingly high intracellular fungal burden. The same study also proved that Cbp1 affects the virulence of Hc yeast in murine models of histoplasmosis. Subjects infected with lethal doses of wild-type Hc died in 9 days, while subjects with Hc mutants without Cbp1 survived (70).

English et al. also proved that Hc yeast expressing Cbp1 caused macrophages to activate the integrated stress response (ISR) by measuring the level of activating transcription factor 4 (ATF4) in bone marrow-derived macrophages (BMDMs). With an increase in ATF4 levels, the transcription factor C/EBP homologous protein (CHOP) and tribbles pseudokinase 3 (TRIB3) are upregulated (71). These two transcription factors are pro-apoptotic, which explains how Cbp1 can cause macrophage apoptosis (70).

Hc growth also increases in a relatively high amount of CO_2. As mentioned previously, host macrophages produce ROS in an attempt to eradicate endocytosed pathogens. To secrete ROS, the pentose phosphate pathway is activated to produce NADPH, a key component of ROS production, which serves as a reductant in the NADPH oxidase reaction. Subsequently, this pathway excretes CO_2

as a byproduct, thereby increasing the CO2 content within macrophages. Shen et al. discovered that Hc could utilise this increase in CO_2, contributing to its virulence. Hc growth and its antifungal resistance, especially towards itraconazole and caspofungin, were enhanced on solid *Histoplasma*-macrophage medium under 5% CO_2 compared to ambient air (72).

Pathogenesis and Clinical Relevance

The host immune status is a critical determinant of the severity of histoplasmosis. In experimental murine models, depletion of CD4+ T cells, alone or in combination with CD8+ T cell depletion, transforms a self-limited pulmonary infection into a progressive and fatal course characterised by higher fungal burdens and impaired inflammatory responses, recapitulating the features of histoplasmosis in immunocompromised patients (73). These findings parallel clinical observations in individuals with advanced HIV infection and low CD4+ counts, in whom loss of cellular immunity permits the dissemination of Hc beyond the lungs and failure of granulomas to contain the fungus (74). Likewise, pharmacologic immunosuppression disrupts host defence, such as TNF-α inhibitors, which compromise granuloma formation and maintenance and are associated with disseminated histoplasmosis. Long-term corticosteroid therapy has also been associated with more severe and progressive disease due to the suppression of multiple cell-mediated pathways (75).

Disseminated disease generally reflects a high fungal burden; therefore, diagnostic strategies often focus on tests that detect fungal antigens or fungal load rather than host antibody responses. The culture and histopathology of clinical specimens, including blood, bone marrow, respiratory specimens, and tissue biopsies, provide definitive evidence of histoplasmosis. In disseminated disease, these methods have a higher diagnostic yield due to the increased fungal burden, although the results may be delayed, and the sensitivity can vary by specimen type. The detection of Hc galactomannan antigen in urine or serum is highly sensitive for disseminated disease and useful for early diagnosis and monitoring of response to therapy (76, 77). The 100 kDa protein (Hcp100), which has shown promise as a diagnostic antigen with reduced cross-reactivity against other pathogenic fungi, is one of several alternative antigen candidates with improved specificity that have begun to be investigated (74). In contrast, antibody testing against H and M antigens (immunodiffusion test) and the crude yeast/mycelial phase antigen (complement-fixation test) play supportive roles but may be limited in immunosuppressed individuals with impaired humoral responses. Molecular methods, such as PCR, have been used and have shown high sensitivity and specificity, particularly in disseminated disease. However, implementation remains limited due to the lack of a standardised protocol and validated targets (78).

In addition to existing diagnostic tests, there remains a need for methods capable of detecting latent histoplasmosis while retaining sensitivity to active infection. Recent advances have explored diagnostic approaches that leverage knowledge of Hc-specific cellular immunity, including the interferon-gamma release assay (IGRA). This assay detects Hc infection by measuring IFN-γ released from sensitised T cells following stimulation with Hc antigens. Datta et al. evaluated this method in individuals with suspected or confirmed histoplasmosis as well as in healthy controls, reporting a specificity of 100% and a sensitivity of 77.2%, with the ability to identify healthy individuals with evidence of latent Hc infection. Overall, these findings suggest that IGRA represents a promising adjunctive diagnostic tool for Hc infection (79). However, further development and validation are required before routine clinical implementation.

Therapeutic decisions are guided by disease severity and host immune competence. In immunocompetent hosts with mild acute pulmonary histoplasmosis, effective cellular immunity often leads to spontaneous resolution, and antifungal therapy may not be required. In moderate or chronic pulmonary disease, treatment with oral azoles, such as itraconazole, is recommended to inhibit fungal growth (fungistatic) while the host

immune system clears the infection. For severe or disseminated histoplasmosis, especially in immunosuppressed patients, initial therapy with a fungicidal agent, such as liposomal amphotericin B, is recommended, followed by step-down therapy with itraconazole. Immune reconstitution, for example, with antiretroviral therapy in HIV-infected patients, is an essential component of treatment to restore effective cellular immunity (80, 81).

Current research on vaccines and immunotherapies for Hc remains in the preclinical stage, with no licensed human vaccines available. Several cell wall components, such as H antigen, Hsp60, and Hsp70, have been studied for their potential to induce protective immunity. These components have been found to elicit cell-mediated immune response; however, only Hsp60 induce protection against intranasal inoculation of Hc yeast cells in a murine model. Deepe et al. demonstrated protective immunity conferred by a crude alkaline extract of Hc packaged in glucan particles in mice. This study highlights opportunities for further research because only CypA, previously identified as a ligand for DCs, has been investigated among its 20 most abundant components (82). In addition to active vaccination, passive immunotherapy using monoclonal antibodies directed against surface antigens, such as Hsp60 and H2B, has shown potential in experimental models by reducing fungal burden and modulating host immune responses, supporting further exploration as an adjunctive strategy for histoplasmosis (83).

To advance these preventive and therapeutic strategies, a deeper understanding of Hc immune evasion mechanisms and survival pathways is required. The identification of Hc yeast immune evasion and survivability factors has improved our understanding of how Hc could thrive within the human host. Therefore, the development of novel antifungal drugs and vaccines is feasible by utilising this knowledge. A study by Almeida et al. identified several proteins associated with metabolic pathways and enzymes involved in β-glucan elongation as candidates for antifungal drugs and vaccines, respectively (84). Although this study is purely in silico and requires further validation through in vitro and in vivo testing, the potential of utilising crucial proteins and enzymes against Hc survivability opens new possibilities for antifungal drug and vaccine development.

H. capsulatum Strain, Relevance Toward Pathogenesis

Sepúlveda et al. conducted a genomic study on 30 Hc isolates from North America, South America, and Africa, which revealed the presence of several cryptic species, as follows: *H. capsulatum sensu stricto* (Panama), *H. mississippiense* (North America), *H. ohiense* (North America), *H. suramericanum* (South America), and *H. capsulatum* var. duboisii (Africa) (85). A subsequent phenotypic study involving 27 strains representing these species showed distinct species-specific differences, including variation in the α-(1,3)-glucan component in *H. ohiense*, proteolytic activity in *H. mississippiense*, as well as differences in yeast cell size and growth characteristics (86). Another study on *H. suramericanum* revealed a complex, genetically distinct population structure across South America, indicating strong geographic isolation and the possibility of new speciation in this region (87). Together, these genomic and phenotypic findings suggest that genetic divergence among Hc is accompanied by biologically meaningful traits that may contribute to differences in pathogenesis. However, these studies were limited by relatively small sample sizes, a predominance of isolates from the Americas, and a lack of direct correlation with clinical outcomes (85-87).

Based on analyses of a large global dataset of 879 isolates from 47 countries, including 400 sequences analysed using ARF–OLE multilocus typing and 274 sequences included in four-gene analyses (ITS, ARF, OLE, and H-anti), Quan et al. reported a genetically distinct Hc lineage involving isolates from India and Indonesia. These findings suggest a regional population structure among Asian strains. However, the available evidence remains insufficient to support formal taxonomic revision (87, 88). Additionally, current genomic data have not yet systematically demonstrated

consistent differences in disease severity or clinical manifestations across major clades.

Unfortunately, representation from other Asian countries, such as Thailand, Taiwan, Japan, and Malaysia, as well as from Africa, remains very limited. Based on clinical reports and histoplasmin skin test results, exposure to Hc is far more widespread than previously recorded (89). Taiwan, historically not considered endemic, has reported 17 cases of histoplasmosis from 1977 to 2023, including four local cases without a travel history, suggesting local transmission (90). Similar situations occur in other countries, such as Bangladesh, Nepal, and parts of Africa. However, limited testing, low diagnostic capacity, and symptom overlap with tuberculosis lead to the disease being undetected or misdiagnosed (5, 91-93).

Although the genetic diversity of Hc is increasingly recognised, no comprehensive study has directly compared the pathogenesis and clinical relevance of each strain. Consequently, the current strain classification is more taxonomic than clinical. Therefore, further extensive and integrative research combining genomic analysis, clinical data, and pathogenicity studies is needed to better understand the medical impact of Hc strain diversity across different regions worldwide.

Conclusion

A complex interaction occurs between Hc and the host immune response. While the innate and adaptive immune systems can eliminate the fungus in most cases, immune evasion mechanisms allow Hc to survive in hostile host environments and cause disease under certain conditions. Recent reports of histoplasmosis outside the classic endemic regions, such as in parts of Asia and Africa, suggest the existence of distinct strains and alternative pathogenic mechanisms. Advances in proteomic and genomic profiling may facilitate the discovery of novel proteins involved in pathogenesis. Further understanding of these interactions could support the development of improved strategies for prevention, diagnosis, and therapy.

What Is Already Known on This Topic:

Hc is a dimorphic fungus that causes histoplasmosis, a disease ranging from asymptomatic infection to severe disseminated forms. Hc has developed several strategies to evade host immune defences, including modulation of phagolysosomal function, resistance to oxidative stress, and interference with antigen presentation. The host immune response, particularly through innate and adaptive mechanisms, plays a critical role in limiting fungal proliferation. Most data on Hc pathogenesis originate from strains circulating in the Americas, where histoplasmosis is classically endemic. However, recent reports have identified genetically distinct lineages.

What This Study Adds:

This review highlights the complex interactions between the host immune system and H. capsulatum, with particular attention to fungal survival and adaptation under diverse environmental and immunological conditions. The recognition of distinct phylogenetic clades in Asia and Africa suggests potential differences in evolutionary adaptation and pathogenic mechanisms. This review also integrates current findings on cell wall components, host immune responses, and immune evasion strategies of H. capsulatum with their clinical relevance and discusses emerging areas of research with implications for diagnosis and treatment.

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