



# Infection with *Histoplasma capsulatum*: Host-fungus interface

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## Summary

*Histoplasma capsulatum* is a pathogenic fungus that has caused infections in all continents except Antarctica although most disease is found within the Americas. It produces a broad spectrum of illness that can on occasion be fatal. Understanding the interaction between the host and the fungus provides insights into the pathogenic mechanisms as well as the host response to replicating fungi. This knowledge can be used to improve treatment as well as diagnosis. Hence, this review summarizes some of the most recent findings regarding host-fungus interaction.

## Key words

*Histoplasma capsulatum*, Immune Response, Phagocytes, Cytokines, T cells, B cells

## Infección por *Histoplasma capsulatum*: interacción huésped-hongo

## Resumen

*Histoplasma capsulatum* es un hongo patógeno que ha producido infecciones en todos los continentes excepto en la Antártida, aunque la mayoría de las histoplasmosis se dan en América. *Histoplasma* produce un amplio espectro de enfermedades que en ocasiones pueden ser fatales. El conocimiento de la interacción entre el huésped y el hongo nos acerca a los mecanismos patógenos así como a la respuesta del huésped frente al hongo. Este conocimiento puede ser útil para mejorar el tratamiento así como el diagnóstico. Esta revisión resume algunos de los avances más recientes sobre la interacción huésped-hongo.

## Palabras clave

*Histoplasma capsulatum*, Respuesta inmune, Fagocitos, Citokinas, Linfocitos T, Linfocitos B

Infection with the dimorphic pathogenic fungus *Histoplasma capsulatum* is acquired via inhalation. The mycelial form exists in soil whereas the yeast form is essential for parasitic growth in mammals. Endemic areas encompass the midwestern and southeastern regions of the United States and other river valleys of the world between latitudes 45° north and 30° south of the Equator. After inhalation and deposition of the conidia within the alveolar spaces they must convert to the yeast form to become pathogenic. This morphological transition is prompted by a dramatic change in environmental signals with a cascade of biochemical, genetic and physical alterations one of which is clearly the increase in temperature [1].

As many as 500,000 individuals are exposed to the fungus annually in the United States. Clinically, infection with *H. capsulatum* produces a spectrum of illnesses ranging from acute pulmonary disease to a progressive disse-

minated form. In immunocompetent hosts most primary infections are asymptomatic or present as a mild influenza-like illness. In contrast, disseminated histoplasmosis is frequently observed in individuals with an impaired immune system such as persons infected with HIV or treated with immunosuppressive agents, but it can develop in immunocompetent individuals [2].

## INFLAMMATORY RESPONSE TO *H. capsulatum*

The hallmark of a successful tissue response to *H. capsulatum* is the formation of caseating or non-caseating granulomas. Prior to their generation, there is a distinct evolution in the inflammatory response that results in granuloma formation. The early response of mice to intrapulmonary infection with *H. capsulatum* is the influx of macrophages and neutrophils [3-5]. Neutrophils and macrophages bind and phagocytose microconidia via CD11/CD18 receptor rapidly and conversion to the yeast phase most likely transpires within these cells [6-8]. By the second week, the cellular response has shifted and is now characterized by infiltration of lymphocytes, mainly T cells [5]. During this time granuloma formation takes place. The composition of cells creating the granuloma is not precisely defined, but is dominated by the presence of mononuclear cells unlike *Blastomyces dermatitidis* and *Coccidioides immitis* which provoke a pyogranulomatous reaction in tissues.

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The formation of granulomas is a complex biological process, and the signals that lead to their creation are just beginning to be delineated. Interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are two mediators that contribute to granuloma generation in other model systems [9,10]. However, in histoplasmosis, granuloma formation appears to be less dependent on these cytokines, since neutralization of the cytokine or disruption of the corresponding gene encoding the cytokine does not alter substantially the character of the inflammatory response [11,12].

### INTERACTION OF *H. capsulatum* WITH PHAGOCYtic CELLS

Neutrophils are a major component of the inflammatory response to acute infection with *H. capsulatum* and may play a significant role in limiting the spread of yeasts from the lung. Human neutrophils possess fungistatic activity *in vitro*, which is mediated by defensins and proteins localized in the azurophilic granules [13,14]. The importance of neutrophils *in vivo* is spotlighted by studies conducted in mouse models during acute primary infection. Depletion of neutrophils with anti-neutrophil monoclonal antibody (mAb) increases susceptibility of naive mice infected with a sublethal inoculum of *H. capsulatum* [15,16]. However, their eradication during secondary infection does not alter mortality [15]. Neutrophils synthesize and release various cytokines including interleukin 12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNF- $\alpha$ , and thus, the impairment of host response following depletion of neutrophils during primary infection may be associated with alteration of cytokine production [17].

Macrophages and dendritic cells are the principal regulators of the immune response to *H. capsulatum* [6,18,19]. These cells recognize, bind and phagocytose yeasts, thereby providing a permissive intracellular environment within which the organism can multiply. The yeasts bind to the CD11/18 receptors of the  $\beta$ 2 integrin family on the surface of phagocytes and *in vitro* about 80% of the yeasts are ingested in approximately one hour [6,18]. Yeasts multiply within the phagolysosome of murine macrophages despite phagolysosomal fusion [20]. One mechanism by which the organisms survive this utterly hostile environment is by increasing the pH of the phagolysosome [21]. However, a pH exceeding 6.5 deprives iron from the organisms which is required for growth, and consequently yeasts die. That pH regulates iron acquisition is supported by studies in which yeast laden macrophages exposed to chloroquine, a weak base that alkalizes the lysosomes, subsequently causes death of fungal elements [22]. Since the effects of chloroquine can be reversed by exposing macrophages to a source of iron that is independent of pH, these results strongly suggest that maintaining a very tight pH between 6.0 to 6.5 is essential for the organism to multiply, but also for iron acquisition [22].

Murine macrophages are activated by cytokines to exert antifungal activity. One cytokine that directly stimulates murine macrophages to inhibit the growth of *H. capsulatum* is IFN- $\gamma$ . After exposure to this cytokine resident peritoneal macrophages exert fungistatic activity [23]. This macrophage population may be the only one that is directly activated by this cytokine to express anti-*H. capsulatum* activity. An additional signal, lipopolysaccharide (LPS), is required for splenic macrophages to become fully activated following exposure to IFN- $\gamma$  [24].

Coincubation with IFN- $\gamma$  and TNF- $\alpha$  does not induce anti-*Histoplasma* activity in either splenic or alveolar murine macrophages [12,25]. The antifungal effect of murine macrophages stimulated with IFN- $\gamma$  alone or plus lipopolysaccharide is caused by the release of nitric oxide [26,27]. Since nitric oxide can chelate iron, it is likely that it inhibits growth via this mechanism [27]. In contrast to murine macrophages, human macrophages are not stimulated by IFN- $\gamma$  *in vitro* to limit intracellular growth of *H. capsulatum* whereas GM-CSF, M-CSF and IL-3 activate human cells to become fungistatic [28,29]. How these cytokines enhance the antifungal effect of human macrophages is not known.

In addition, human macrophages plated on collagen rather than on plastic inhibit the growth of intracellular yeasts, and this effect is caused by massive phagolysosomal fusion [30]. Thus, the binding of macrophages to collagen transduces signals that result in a merging of phagosomes and lysosomes.

### THE INFLUENCE OF NATURAL KILLER CELLS

Like macrophages and dendritic cells, natural killer (NK) cells are a potential bridge to the acquired immune response. In addition to their cytolytic activity, they produce several cytokines including TNF- $\alpha$  and IFN- $\gamma$ . With regards to histoplasmosis, SCID mice infected with yeast cells exhibit accelerated mortality upon depletion with either IL-12 or IFN- $\gamma$  [31]. In contrast, mortality can be reversed by exogenous treatment of these mice with IL-12 [31]. NK cells seem to play a critical role in host defense to primary infection in an exceptionally immunodeficient mouse strain. In contrast, beige/beige mice which are defective in NK cell activity but otherwise immunocompetent are not more susceptible to infection as their control littermates [32]. Thus, T cells alone may be sufficient for clearance of infection, and the definitive role of NK cells during histoplasmosis remains to be determined. It is quite likely that in an immunocompetent host they serve to amplify the immune response.

### T CELLS AND CYTOKINES

The generation of a T cell-mediated immune response of the host is prerequisite for resistance against *H. capsulatum*. The importance of T cells in humans is indicated by a marked increase in susceptibility to disseminated histoplasmosis in individuals infected by HIV or those whose cellular immunity is depressed by immunosuppressive agents [33,34]. Experimental studies complement the findings in humans. Hence, mice congenitally deficient in T cells are more susceptible to histoplasmosis [35]. In addition, mice depleted of  $\alpha\beta$  T cell receptor (TCR)+ T cells succumb to primary and secondary infection with *H. capsulatum* [36]. The two major phenotypes of T cells, CD4+ and CD8+, manifest distinct functional relevance during infection. Upon elimination of CD4+ T cells host defenses are abrogated; mice exhibit increased mortality during primary and secondary histoplasmosis after exposure to sublethal inocula of yeasts [15,36,37]. Conversely, transfer of CD4+ T cell clones confers protection to naive animals challenged with yeasts [38]. In contrast,  $\beta$ 2-microglobulin knockout mice or mice depleted of CD8+ T cells demonstrate impaired clearance of the fungus, but do not succumb to either primary or secondary infection [39]. Thus, the importance of T-cells can be ranked in histoplasmosis; whereas CD4+ cells are crucial for survival of infection, CD8+ T cells are required for optimal clearance of the fungus.

A principal mechanism by which T cells contribute to the protective immune response is via the release of cytokines that activate macrophages, the principal effector cells, to restrict intracellular growth of the organism. A critical cytokine involved in the protective immune response against *H. capsulatum* is IFN- $\gamma$  [11,15,40,41]. It acts to inhibit intracellular growth by limiting iron availability presumably through induction of nitric oxide synthesis [42]. In primary infection, neutralization of IFN- $\gamma$  results in increased mortality of mice infected intravenously [15]. Similarly, naive mice with a disruption of the IFN- $\gamma$  gene are unable to control pulmonary infection with *H. capsulatum* [11]. Interestingly, IFN- $\gamma$  knockout mice infected intranasally do not manifest impaired production of either IL-12 or TNF- $\alpha$ , two other critical cytokines involved in host resistance [11]. During secondary infection, IFN- $\gamma$  knockout mice infected intravenously maintain the ability to clear infection [15]. In contrast, in the absence of IFN- $\gamma$ , mice reinfected via the pulmonary route demonstrate accelerated mortality [11]. Thus, there exist qualitative differences in the immune response between the pulmonary and systemic models of infection. One possible explanation is that mononuclear cells in the lung are functionally different from those in the circulation or in lymphoid organs.

Preliminary studies indicate that depletion of either  $\alpha\beta$  TCR+ or CD4+ T cells in naive mice infected with *H. capsulatum* results in decreased IFN- $\gamma$  protein levels in lungs, whereas depletion of CD8+ T cells demonstrates a marked increase in IFN- $\gamma$  compared to controls [43]. Generation of other cytokines, including those associated with a Th2 type response, is not impaired between T cell depleted mice and controls [43]. These results underscore the crucial role of IFN- $\gamma$  in mediating intracellular killing of *H. capsulatum*.

Another cytokine implicated in host resistance to *H. capsulatum* is IL-12. It is produced principally by macrophages and is a principal regulator of T cell differentiation and function *in vivo* [44]. It induces the production of IFN- $\gamma$  from T-cells and NK cells [44]. Neutralization of endogenous IL-12 is associated with increased CFU and accelerated mortality during primary infection [41,45]. As a corollary, treatment with recombinant IL-12 rescues mice from a lethal systemic challenge, and this effect can be abrogated by administration with IFN- $\gamma$  mAb [31,41]. These results demonstrate that the effect of IL-12 is mediated through induction of IFN- $\gamma$ . Although inhibition of IL-12 decreases production of IFN- $\gamma$  it has no effect on generation of GM-CSF or TNF- $\alpha$  or Th2 type cytokines involved in host resistance to *H. capsulatum* [45]. However, administration of anti-IL-4 mAb to naive mice infected intranasally ameliorates the inimical effects of depletion of IL-12 [45]. Interestingly, the improved survival is not accompanied by significant elevations in IFN- $\gamma$  levels in the lungs. The beneficial effects of anti-IL-4 mAb in mice lacking IL-12 may be caused by a restoration of the balance between Th1 and Th2 cytokines. Blockade of IL-12 abolishes host defenses when given before or within 3 days of infection. When anti-IL-12 mAb is administered beginning on day 5 of infection, the inimical effects of IL-12 neutralization are not observed [45]. Thus, IL-12-dependent maturation of the protective immune response develops by day 5 postinfection. Neutralization of IL-12 in mice with preexisting immunity to *H. capsulatum* does not result in accelerated mortality, although impaired clearance of the fungus is observed [45]. These results indicate that IL-12 is much less necessary for acquisition of secondary immunity.

TNF- $\alpha$  is fundamental in the protective immune response to *H. capsulatum* infection. Neutralization of TNF- $\alpha$  with polyclonal or monoclonal anti-TNF- $\alpha$  antibody increases susceptibility to primary and secondary infection with *H. capsulatum*, leading to accelerated mortality [12,15,25,46]. Unexpectedly, the fungus burden in lungs is increased in TNF- $\alpha$  depleted mice, spleens and livers do not differ from infected controls at week 1 of primary infection [12]. The influence of TNF- $\alpha$  appears to be compartmentalized and a differential requirement for TNF- $\alpha$  among organs is operative [12]. In secondary infection, neutralization of TNF- $\alpha$  impairs clearance of the fungus in all organs compared to controls. One possible cause for the accelerated mortality in TNF- $\alpha$ -deficient mice would be a decrease in IFN- $\gamma$ , since TNF- $\alpha$  has been shown to induce this cytokine [47]. However, depletion of TNF- $\alpha$  in pulmonary histoplasmosis is not associated with an impairment of IFN- $\gamma$  generation during the primary and secondary immune response [12]. To the contrary, IFN- $\gamma$  levels in lungs are markedly increased in TNF- $\alpha$ -neutralized mice [12]. The compensatory elevation of IFN- $\gamma$  is not sufficient for resolution of infection as TNF- $\alpha$ -depleted mice exhibit decreased survival. These findings suggest that TNF- $\alpha$  and IFN- $\gamma$  are regulated independently and both cytokines must be present *in vivo* in adequate amounts for optimal clearance.

TNF- $\alpha$  contributes to the production of nitric oxide, which is a potent killing mechanism against several intracellular pathogens [48-50]. TNF- $\alpha$  enhances either alone or in conjunction with IFN- $\gamma$  the production of nitric oxide [50]. In primary pulmonary histoplasmosis, neutralization of TNF- $\alpha$  is associated with a marked decrease in production of reactive nitrogen intermediates by alveolar macrophages stimulated with LPS or with IFN- $\gamma$  plus LPS, suggesting that during primary infection TNF- $\alpha$  must be present to synergize with IFN- $\gamma$  to restrict intracellular growth [12]. In secondary pulmonary histoplasmosis, generation of reactive nitrogen intermediates by alveolar macrophages is not impaired in mice depleted of TNF- $\alpha$  [12]. These findings imply that the presence of IFN- $\gamma$  alone is sufficient to generate reactive nitrogen intermediates in the absence of TNF- $\alpha$ . They also suggest that the accelerated mortality in TNF- $\alpha$ -deficient mice is independent of the production of nitric oxide. Furthermore, intracellular killing of the fungus remains impaired in the presence of nitric oxide, and this argument is supported by the inability of inhibitors of nitric oxide to alter protective immunity in secondary infection [15]. The results suggest that another mediator must be responsible for the growth restriction of the fungus in secondary infection. It is unlikely that TNF- $\alpha$  may be directly exerting antifungal activity since exposure of alveolar or splenic macrophages to this cytokine does not alter intracellular growth [12,25]. We postulate that TNF- $\alpha$  initiates production of a mediator or mediators whose biological activity leads to the killing of the fungus by professional phagocytes. At present, the mediator(s) and their pathway remain to be delineated.

Analysis of Th2 type cytokines in TNF- $\alpha$ -deficient mice connotes pronounced differences between primary and secondary infection with *H. capsulatum*. Protein levels of IL-4 and IL-10 in lungs of naive TNF- $\alpha$ -deficient mice are similar to those of controls. Conversely, significant increases in both cytokines in immune mice treated with anti-TNF- $\alpha$  mAb compared to controls are observed [12]. The *in vivo* influence of these two cytokines is highlighted by the fact that neutralization of both IL-4 and IL-10 restores protective immunity in TNF- $\alpha$ -depleted immune mice [12]. Neutralization of either IL-4

or IL-10 alone does not abrogate mortality of TNF- $\alpha$ -deficient mice [12]. It may seem surprising that treatment with anti-IL-4 and anti-IL10 mAb can restore protective immunity, since IFN- $\gamma$  levels are high in TNF- $\alpha$ -depleted mice. Nevertheless, if one analyzes the balance of Th1 and Th2 cytokines rather than absolute values it is evident that the ratio of IFN- $\gamma$  to IL-4 and IL-10 is increased during primary infection. These results indicate that Th2 type cytokines cooperatively contribute to the down-regulation of host resistance in immune mice deficient of endogenous TNF- $\alpha$ .

## B CELLS AND *H. capsulatum*

Very little information exists concerning the influence of B cells. Older studies had indicated that transfer of hyperimmune serum did not modify infection [51]. In a similar vein, opsonization of yeasts with antibody and/or complement has not been shown to greatly augment uptake of yeast cells by macrophages although immune antibody promotes neutrophil uptake [6,7]. More recent exploratory studies have indicated that B cell deficient mice handle primary and secondary infection as well

as B cell sufficient mice [36]. Hence, B cells do not appear to contribute to the overall host defenses that are activated in response to this fungus.

## CONCLUSIONS

Protective immunity to infection with *H. capsulatum* is dependent on the concerted interaction of cytokines and cellular components at different stages during infection. There is early recruitment of neutrophils and macrophages. In the primary response to infection, macrophages and possibly dendritic cells provide the connection to the adaptive immune response via production of several cytokines, most notably TNF- $\alpha$  and IL-12. Subsequently, induction of IFN- $\gamma$  from NK and T cells ensues, and this cytokine contributes to the activation of macrophages to an anti-*H. capsulatum* state. In secondary immunity, there is a reliance upon both TNF- $\alpha$  and IFN- $\gamma$  for optimal protection, but the mechanisms that lead to elimination of the fungus are undoubtedly different from those in primary infection.

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