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 450 north and 300 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 disseminated 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].

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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.

The formation of granulomas is a complex biological process, and the signals that lead to their creation are just beginning to be delineated. Interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) 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-α, 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 β2 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 alkalinizes 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-γ. 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-γ [24]. Coincubation with IFN-γ and TNF-α does not induce anti-*Histoplasma* activity in either splenic or alveolar murine macrophages [12,25]. The antifungal effect of murine macrophages stimulated with IFN-γ 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-γ *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-α and IFN-γ. With regards to histoplasmosis, SCID mice infected with yeast cells exhibit accelerated mortality upon depletion with either IL-12 or IFN-γ [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 αβ 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, β2-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-γ [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-γ results in increased mortality of mice infected intravenously [15]. Similarly, naive mice with a disruption of the IFN-γ gene are unable to control pulmonary infection with *H. capsulatum* [11]. Interestingly, IFN-γ knockout mice infected intranasally do not manifest impaired production of either IL-12 or TNF-α, two other critical cytokines involved in host resistance [11]. During secondary infection, IFN-γ knockout mice infected intravenously maintain the ability to clear infection [15]. In contrast, in the absence of IFN-γ , 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 αβ TCR+ or CD4+ T cells in naive mice infected with *H. capsulatum* results in decreased IFN-γ protein levels in lungs, whereas depletion of CD8+ T cells demonstrates a marked increase in IFN-γ 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-γ 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-γ 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-γ mAb [31,41]. These results demonstrate that the effect of IL-12 is mediated through induction of IFN-γ. Although inhibition of IL-12 decreases production of IFN-γ it has no effect on generation of GM-CSF or TNF-α 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-γ 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 beginnning 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-α is fundamental in the protective immune response to *H. capsulatum* infection. Neutralization of TNF-α with polyclonal or monoclonal anti-TNF-α 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-α depleted mice, spleens and livers do not differ from infected controls at week 1 of primary infection [12]. The influence of TNF-α appears to be compartmentalized and a differential requirement for TNF-α among organs is operative [12]. In secondary infection, neutralization of TNF-α impairs clearance of the fungus in all organs compared to controls. One possible cause for the accelerated mortality in TNF-α-deficient mice would be a decrease in IFN-γ, since TNF-α has been shown to induce this cytokine [47]. However, depletion of TNF-α in pulmonary histoplasmosis is not associated with an impairment of IFN-γ generation during the primary and secondary immune response [12]. To the contrary, IFN-γ levels in lungs are markedly increased in TNF-α-neutralized mice [12]. The compensatory elevation of IFN-γ is not sufficient for resolution of infection as TNF-α-depleted mice exhibit decreased survival. These findings suggest that TNF-α and IFN-γ are regulated independently and both cytokines must be present *in vivo* in adequate amounts for optimal clearance.

TNF-α contributes to the production of nitric oxide, which is a potent killing mechanism against several intracellular pathogens [48-50]. TNF-α enhances either alone or in conjunction with IFN-γ the production of nitric oxide [50]. In primary pulmonary histoplasmosis, neutralization of TNF-α is associated with a marked decrease in production of reactive nitrogen intermediates by alveolar macrophages stimulated with LPS or with IFN-γ plus LPS, suggesting that during primary infection TNF-α must be present to synergize with IFN-γ 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-α [12]. These findings imply that the presence of IFN-γ alone is sufficient to generate reactive nitrogen intermediates in the absence of TNF-α. They also suggest that the accelerated mortality in TNF-α-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-α 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-α 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-α-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-α-deficient mice are similar to those of controls. Conversely, significant increases in both cytokines in immune mice treated with anti-TNF-α 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-α-depleted immune mice [12]. Neutralization of either IL-4

or IL-10 alone does not abrogate mortality of TNF-α-deficient mice [12]. It may seem surprising that treatment with anti-IL-4 and anti-IL10 mAb can restore protective immunity, since IFN-γ levels are high in TNF-α-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-γ 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-α.

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-α and IL-12. Subsequently, induction of IFN-γ 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-α and IFN-γ 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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References

1. Maresca B, Kobayashi GS. Dimorphism in *Histoplasma capsulatum*: a model for the study of cell differentiation in pathogenic fungi. Microbiol Rev 1989; 53: 186-209.
2. Deepe GS Jr, Bullock WE. Histoplasmosis: a granulomatous inflammatory response. In Gallin JI, Goldstein LM, Snyderman R. (Eds.) Inflammation: Basic Principles and Clinical Correlates (2nd ed). New York, Raven Press, 1992: 943-948.
3. Procknow JJ, Page MI, Loosli CG. Early pathogenesis of experimental histoplasmosis. Arch Pathol 1960; 69: 413-426.
4. Baughman RP, Kim CK, Vinegar A, Hendricks DE, Schmidt DJ, Bullock WE. The pathogenesis of experimental pulmonary histoplasmosis. Correlative studies of histopathology, bronchoalveolar lavage and respiratory function. Am Rev Resp Dis 1986; 134: 771-776.
5. Cain JA, Deepe GS Jr. Evolution of the primary immune response to *Histoplasma capsulatum* in murine lung. Infect Immun 1998; 66: 1473-1481.
6. Newman SL, Bucher C, Rhodes, J, Bullock WE. Phagocytosis of *Histoplasma capsulatum* yeasts and microconidia by human cultured macrophages and alveolar macrophages. Cellular cytoskeleton requirement for attachment and ingestion. J Clin Invest 1990; 85: 223-230.
7. Schnur RA, Newman SL. The respiratory burst response to *Histoplasma capsulatum* by human neutrophils. Evidence for intracellular trapping. J Immunol 1990; 144: 4765-4772.
8. Kimberlin CL, Hariri AR, Hempel HO, Goodman NL. Interactions between *Histoplasma capsulatum* and macrophages from normal and treated mice: comparison of the mycelial and yeast phases in alveolar and peritoneal macrophages. Infect Immun 1981; 34: 6-10.
9. Kindler V, Sappino AP, Grau GE, Piguet PF, Vasalli P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell 1989; 56: 731-740.
10. Chensue SW, Warmington KS, Ruth JH, Lincoln P Kunkel SL. Cytokine function during mycobacterial and schistosomal antigen-induced pulmonary granuloma formation: local and regional participation of IFN-γ, IL-10 and TNF. J Immunol 1995; 154: 5969-5976.
11. Allendoerfer R, Deepe GS Jr. Intrapulmonary response to *Histoplasma capsulatum* in gamma interferon knockout mice. Infect Immun 1997; 65: 2564-2569.
12. Allendoerfer R, Deepe GS Jr. Blockade of endogenous TNF-α exacerbates primary and secondary pulmonary histoplasmosis by differential mechanisms. J Immunol 1998; 160: 6072-6082.
13. Couto MA, Liu L, Lehrer RI, Ganz T. Inhibition of intracellular *Histoplasma capsulatum* replication by murine macrophages that produce human defensin. Infect Immun 1994; 62: 2375-2378.
14. Newman SL, Gootee L, Gabay JE. Human neutrophil-mediated fungistasis against *Histoplasma capsulatum*. Localization of fungistatic activity to the azurophil granules. J Clin Invest 1993; 92: 1422-1429
15. Zhou P, Miller G, Seder RA. Factors involved in regulating primary and secondary immunity to infection with *Histoplasma capsulatum*: TNF-α plays a critical role in maintaining secondary immunity in the absence of IFN-γ. J Immunol 1998; 160: 1359-1368.
16. Newman SL, Kidd C. Neutrophils are essential in host defense against *Histoplasma capsulatum*. 97th General Meeting of the American Society for Microbiology, Miami Beach, Fla, 1997. Abstract F-98.
17. Lloyd AR, Oppenheim JJ. Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response. Immunol Today 1992; 13: 169-172.
18. Bullock WE, Wright SD. Role of adherence promoting receptors, CR3, LFA-1, and p150, 95 in binding of *Histoplasma capsulatum* by human macrophages. J Exp Med 1987; 165: 195-210.
19. Gildea L, Newman SL. Human dendritic cells phagocytose *Histoplasma capsulatum* (Hc) yeasts and stimulate lymphocyte proliferation in a secondary immune response. 98th Meeting of the American Society for Microbiology, Atlanta GA. 1998, Abstract F-27a.
20. Eissenberg LG, Schlesinger PH, Goldman WE. Phagosome-lysosome fusion in P388D1 macrophages infected with *Histoplasma capsulatum*. J Leukocyte Biol 1988; 43: 483-91.
21. Eissenberg LG, Goldman WE, and Schlesinger PH. *Histoplasma capsulatum* modulates the acidification of phagolysosomes. J Exp Med 1993; 177: 1605-11.
22. Newman SL, Gootee L, Brunner GD, Deepe GS Jr. Chloroquine induces the human macrophage killing of *Histoplasma capsulatum* by limiting the availability of intracellular iron and is therapeutic in a murine model of histoplasmosis. J Clin Invest 1994; 93: 1422-1429
23. Wu-Hsieh BA, Howard DH. Inhibition of the intracellular growth of *Histoplasma capsulatum* by recombinant murine gamma interferon Infect Immun 1987; 55: 1014-1016.
24. Lane TE, Wu-Hsieh BA, Howard DH. Gamma interferon cooperates with lipopolysaccharide to activate mouse splenic macrophages to an antihistoplasma state. Infect Immun 1994; 61: 1468-1473.
25. Wu-Hsieh BA, Lee GS, Franco M, Hofman FM. Early activation of splenic macrophages by tumor necrosis factor alpha is important in determining the outcome of experimental histoplasmosis in mice. Infect Immun 1992; 60: 4230-4238.
26. Lane TE, Otero GC, Wu-Hsieh BA, Howard DH. Expression of inducible nitric oxide synthase by stimulated macrophages correlates with their antihistoplasma activity. Infect Immun 1994; 62: 1478-1479.
27. Lane TE, Wu-Hsieh BA, Howard DH Antihistoplasma effect of activated mouse splenic macrophages involves production of reactive nitrogen intermediates. Infect Immun 1994; 62: 1940-1945.
28. Newman SL, Gootee L. Colony-stimulating factors activate human macrophages to inhibit intracellular growth of *Histoplasma capsulatum* yeasts. Infect Immun 1992; 60: 4593-4597.
29. Fleischman J, Wu-Hsieh B, Howard DH.

The intracellular fate of *Histoplasma capsulatum* in human macrophages is unaffected by recombinant human interferon-γ. J Infect Dis 1990; 161: 143-145.
30. Newman SL, Gootee L, Kidd C, Ciraolo GM, Morris R. Activation of human macrophage fungistatic activity against *Histoplasma capsulatum* upon adherence to type I collagen matrices. J Immunol 1997; 158: 1779-1786.
31. Zhou P, Sieve MC, Tewari RP, Seder RA. Interleukin-12 modulates the protective immune response to SCID mice infected with *Histoplasma capsulatum*. Infect Immun 1997; 65: 936-942.
32. Patino MM, Williams DM, Ahrens J, Graybill JR. Experimental histoplasmosis in the beige mouse. J Leuco Biol 1987; 41: 228-235
33. Wheat LJ, Connolly-Springfield PA, Baker RL, *et al*. Disseminated histoplasmosis in the acquired immune deficiency syndrome: clinical findings, diagnosis and treatment, and a review of the literature. Medicine 1990; 69: 361-374.
34. Graybill JR. Histoplasmosis and AIDS. J Infect Dis 1988; 158: 623-628.
35. Williams DM, Graybill JR, Drutz DJ. *Histoplasma capsulatum* infection in nude mice. Infect Immun 1978; 21: 973-977.
36. Allendoerfer R. Unpublished observations.
37. Gomez AM, Bullock WE, Taylor CL, Deepe GS Jr. The role of L3T4+ T cells in host defense against *Histoplasma capsulatum*. Infect Immun 1988; 59: 330-336.
38. Allendoerfer R, Magee DM, Deepe GS Jr, Graybill JR. Transfer of protective immunity in murine histoplasmosis by a CD4+ T-cell clone. Infect Immun 1993; 61: 714-718.
39. Deepe GS Jr. Role of CD8+ T cells in host resistance to systemic infection with *Histoplasma capsulatum* in mice. J Immunol 1994; 152: 3491-3500.
40. Wu-Hsieh B. Relative susceptibilities of inbred mouse strains C57BL/6 and A/J to infection with *Histoplasma capsulatum*. Infect Immun 1989; 57: 3788-3792
41. Zhou P, Sieve MC, Bennett J, *et al*. IL-12 prevents mortality in mice infected with *Histoplasma capsulatum* through induction of IFN-γ. J Immunol 1995; 155: 785-795.
42. Lane TE, Wu-Hsieh BA, Howard DH. Iron limitation and the gamma interferon-mediated antihistoplasma state of murine macrophages. Infect Immun 1991; 59: 2274-2278.
43. Allendoerfer R, Brunner GD, Deepe GS Jr. Cytokine profiles in lungs of immunodeficient mice with pulmonary histoplasmosis. 98th General Meeting of the American Society for Microbiology, Atlanta, GA, 1998. Abstract F-5.
44. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 1995; 13: 251-276.
45. Allendoerfer R, Boivin GP, Deepe GS Jr. Modulation of immune responses in murine pulmonary histoplasmosis. J Infect Dis 1997; 175: 905-914.
46. Smith JG, Magee DM, Williams DM, Graybill JR. Tumor necrosis factor-a plays an important role in host defense against *Histoplasma capsulatum*. J Infect Dis 1990; 162: 1349-1353.
47. Wherry JC, Schreiber RD, Unanue ER. Regulation of gamma interferon production by natural killer cells in scid mice: Roles of tumor necrosis factor and bacterial stimuli. Infect Immun 1991; 59: 1709-1715.
48. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, Schreiber R, Mak TW, Bloom BR. Tumor necrosis factor-a is required in the protective immune response against *Mycobacterium tuberculosis* in mice. Immunity 1995; 2: 561-572.
49. Liew FY, Millott S. Tumor necrosis factor-a synergizes with IFN-γ in mediating killing of *Leishmania major* through the induction of nitric oxide. J Immunol 1990; 145:4306-4310.
50. Green SJ, Crawford RM, Hockmeyer JT, Meltzer MS, Nacy CA. *Leishmania major* amastigotes initiate the L-arginine-dependent killing mechanism in IFN-γ-stimulated macrophages by induction of tumor necrosis factor-α. J Immunol 1990; 145: 4290-4297.
51. Saslow S, Schaeffer J. Survival of *Histoplasma capsulatum* in experimental histoplasmosis in mice. Proc Soc Exp Biol 1956; 91: 412-414.

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