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The role of cytokines and chemokines in *Histoplasma capsulatum* infection

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Abstract

Histoplasma capsulatum is a prevalent fungal pathogen in the United States, infecting approximately 500,000 individuals each year. Host protection requires an intact cell-mediated immune response. In this review, we will discuss how cytokines and chemokines influence protective immunity in *H. capsulatum* infection.

Keywords

Fungus; infection; cytokine; chemokine

1. Introduction

The dimorphic fungus *Histoplasma capsulatum* is found worldwide, but is endemic in the Midwest and Southeast regions of the United States. Epidemiological studies have shown that as many as 90% of individuals in endemic areas have been exposed to *H. capsulatum* (1, 2). The majority of people infected with *H. capsulatum* develop an acute pulmonary infection that is asymptomatic or associated with mild flu-like symptoms, including fever, malaise, headache, and weakness. This self-limiting infection normally occurs in children or individuals exposed to the organism for the first time. However, in immunocompromised individuals, infection with *H. capsulatum* often results in a disseminated infection that is lethal (3). Infection typically occurs upon inhalation of microconidia and mycelial fragments upon disruption of soil containing the organism. Once deposited in the lungs, *H. capsulatum* is internalized by resident and recruited phagocytes, including macrophages (M ϕ), dendritic cells (DCs), and neutrophils. The intracellular fate of the organism is divergent in these cellular populations. While neutrophils and DCs have fungistatic and fungicidal activity (4–7), respectively, *H. capsulatum* survives in M ϕ prior to cellular activation by regulating the pH of the intraphagosomal environment (8). Phagocytes facilitate dissemination of *H. capsulatum* to several organs, including the spleen, liver, bone marrow, and lymph nodes. Thus, to control intracellular growth of the organism, the host must mount a robust proinflammatory response. In this review, we will discuss how cytokines and chemokines

interact with host immune cells, trigger or inhibit anti-fungal activity, and ultimately dictate the outcome of infection.

2. Protective cytokines in *H. capsulatum* infection

2.1. Th1 response

The development of a Th1 immune response is required to successfully control *H. capsulatum* infection (Figure 1). Early production of IL-12 by innate cells is required for the initiation and amplification of a Th1 response. DCs and neutrophils are likely the predominant source of IL-12 since *H. capsulatum* downregulates IL-12 production by M ϕ by binding to the complement receptor 3 (CR3, CD11b/CD18) to gain entry (9). IL-12 stimulates the synthesis of IFN- γ by CD4⁺ T, CD8⁺ T cells, as well as NK cells (10, 11). CD4⁺ T cells are the predominant producers of IFN- γ in *H. capsulatum* infection. Depletion of this population accelerates mortality as a result of defective IFN- γ production. Although CD8⁺ T cells contribute to IFN- γ in infected lungs, this population appears to simultaneously regulate IFN- γ production, preventing immunopathology (12). A major function of IFN- γ is the activation of phagocytes at the site of infection. IFN- γ inhibits intracellular growth of *H. capsulatum* by stimulating murine M ϕ to produce nitric oxide (NO) (13, 14). Another mechanism by which IFN- γ controls intracellular replication is by limiting the amount of iron and zinc accessible to *H. capsulatum* (15, 16). Regulation of iron and zinc transporters is likely how IFN- γ dictates intracellular metal concentrations. In other models, it has been demonstrated that IFN- γ downregulates the transferrin receptor, which limits the amount of iron available to the organism, impairing intracellular replication (17, 18).

The proinflammatory cytokine TNF- α is also required for the development of a protective immune response during *H. capsulatum* infection (19). T cells are the predominant source of TNF- α upon infection. Other populations including monocytes, M ϕ , and DC also contribute to TNF- α production in the lungs (20, 21). The use of TNF- α antagonist, including infliximab and etanercept, for the treatment of inflammatory diseases such as rheumatoid arthritis and Crohn's disease can have detrimental consequences if an individual has previously been infected or is subsequently exposed to *H. capsulatum*. Blockade of TNF- α signaling facilitates reactivation *H. capsulatum* or enhances susceptibility to the organism, potentially causing a severe disseminated infection that can ultimately be lethal (22).

During primary infection, TNF- α stimulates NO production to regulate intracellular growth. Additionally, signaling through TNFR1 promotes the influx of immune cells to lungs during infection, as the cellular infiltrate is altered in TNFR1^{-/-} mice. The expression of several chemokines and chemokine receptors is influenced by TNF- α . In *H. capsulatum* infection, chemokine receptors such as CCR2 and CCR5 have been identified as potent mediators of inflammatory cell recruitment to the lungs (23, 24). Thus, it seems likely TNF- α indirectly controls cellular migration by controlling chemokine production. TNF- α also controls the secretion of both pro- and anti-inflammatory cytokines in *H. capsulatum* infection. In primary infection, signaling through TNFR2 regulates IFN- γ expression, as the concentration of IFN- γ is reduced in TNFR2^{-/-} lungs. Administration of recombinant IFN- γ significantly lowers fungal burden and enhances survival in TNFR2^{-/-} mice. In contrast, IFN- γ is elevated in TNFR1^{-/-} mice. However, enhanced IFN- γ is not sufficient to rescue these animals suggesting TNF- α and IFN- γ work in synergy to promote protective immunity. During secondary infection, the anti-inflammatory cytokines IL-4 and IL-10 are upregulated in TNFR1^{-/-} mice, dampening the Th1 immune response, resulting in unrestricted fungal growth.

TNF- α also dictates the immune response to *H. capsulatum* by regulating the emergence of regulatory T cells (Tregs) and the magnitude of apoptosis (25, 26). Administration of a monoclonal antibody to TNF- α results in elevated the proportion of Tregs in the lungs during primary and secondary histoplasmosis. Tregs in TNF- α -neutralized mice dampen the protective immune response in an IL-10-dependent manner (26). How TNF- α regulates the emergence of this population remains unresolved. One possibility is that TNF- α limits Treg expansion. IL-2, which is involved in the maintenance and expansion of peripheral Tregs (27), is augmented in the lungs after neutralization of TNF- α . Thus, TNF- α may dictate Treg expansion by regulating cytokine expression. Alternatively, since TNF- α influences the expression of CCR5 and its ligands, which have been identified as potent mediators of Treg chemotaxis, it is possible trafficking is improved in the absence of TNF- α . Additionally, TNF- α signals through TNFR1 to trigger apoptosis in infected lungs. The severity of infection is influenced by the proportion of apoptotic lung leukocytes. Administration of a caspase inhibitor to block apoptosis exacerbates infection in mice. In mice lacking TNF- α or TNFR1^{-/-}, diminished apoptosis correlates with elevated fungal burden.

Other Th1-associated cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-1 β influence host protection in *H. capsulatum* infection. Neutralization of either GM-CSF or IL-1 β is associated with an altered cytokine profile and fewer effector T cells in infected lungs. Mice lacking either cytokine have depressed expression of proinflammatory cytokines IFN- γ and TNF- α , which correlates with diminished NO production. In contrast, production of cytokines with anti-inflammatory roles, including IL-4 and IL-10, are elevated in GM-CSF-neutralized and IL-1R^{-/-} mice infected with *H. capsulatum*. These alterations in cytokine expression increase the severity of infection and cause a significant proportion of animals to succumb to infection (28, 29). Furthermore, it is possible that the Th17 response is dampened since signaling through IL-1R has been implicated in the induction of Th17 cells (30). However, it seems unlikely that depressed IL-17 production in the absence of IL-1 signaling would contribute to the lethal outcome since IL-17 plays only a minor role in controlling *H. capsulatum* infection.

GM-CSF, along with M-CSF and IL-3, stimulates M ϕ fungistatic activity against *H. capsulatum* yeast (31). One mechanism by which GM-CSF controls intracellular growth is by regulating M ϕ metal levels (16). Metallomic analysis of infected M ϕ revealed a fundamental role for zinc in response to *H. capsulatum*. Treatment with a zinc chelator inhibits both extracellular and intracellular yeast replication. Upon activation of M ϕ with GM-CSF, the amount of zinc, as well as iron available to the organism is diminished. Additionally, a reduced amount of intracellular zinc after activation with GM-CSF is accompanied by alterations in the zinc binding species profile. IL-4, which promotes the growth of *H. capsulatum* in M ϕ , elevates intracellular zinc and reverses the anti-fungal activity of GM-CSF.

2.2. Th17 response

The significance of a Th17 response in controlling *H. capsulatum* infection was recently elucidated (24, 32). Th17 cells are a distinct subset of T cells that arise from naïve T cells in the presence of IL-6 and TGF- β and require the transcription factor retinoic acid receptor-related orphan receptor γ t (33). Although their name originates from their ability to secrete IL-17, other cytokines, including IL-21, IL-22, TNF- α , and IL-6, are also released from these cells (34). IL-17 promotes the production of proinflammatory cytokines, including TNF- α and IL-1 β , as well CXC chemokines involved in recruitment and activation of M ϕ and neutrophils in several models of infection (35, 36). Others, however, have identified a negative role for IL-17 during fungal infection. In *Aspergillus fumigatus* infection, IL-23-deficient mice are less susceptible to infection as a result of increased IL-12 and IFN- γ

production (37). In these models, the presence of IL-23 and IL-17 suppress the Th1-mediated immune response and the fungicidal activity of neutrophils.

In *H. capsulatum* infection, initial studies identified IL-17 in hepatic granulomas (38). Subsequent studies demonstrated that the concentration of cytokines associated with a Th17 response including IL-6, IL-23, and IL-17 gradually increase the first week of infection and declines thereafter in pulmonary histoplasmosis. CD4⁺ and CD8⁺ T cells expressing CD25 are the predominant source of IL-17 during infection. Although IL-6 is considered to be critical in the generation of Th17 cells, the proportion and number of IL-17⁺ cells is unaltered in IL-6^{-/-} mice during infection (32). IL-21 in synergy with TGF- β can induce an alternative pathway of Th17 generation (39). Thus, it is possible that IL-21 rather than IL-6 promotes Th17 in *H. capsulatum* infection. IL-17 mediates the influx of inflammatory cells to the lungs during infection; however, plays a minimal role in host protection to primary and secondary infection. Mice lacking endogenous IL-17 resolve infection despite manifesting a modest elevation in fungal burden at day 7 post-infection. Interestingly, in the absence of IL-12, IL-23 promotes the development of a protective Th17 response. IL-12p35^{-/-} mice, which lack IL-12, have prolonged survival compared to IL-12p40^{-/-} mice, which lack both IL-12 and IL-23. Enhanced survival of IL-12p35^{-/-} mice is not observed after treatment with anti-IL-17, highlighting the significance of the Th17 response in protective immunity if the Th1 response is compromised (32). In contrast, a vital role for Th17 cells in vaccine-mediated resistance to several fungi including *H. capsulatum* has been described. Signaling through IL-17RA controls fungal resolution by promoting the homing and effector function of neutrophils and M ϕ after challenge in vaccinated mice (40).

3. Exacerbating cytokines in *H. capsulatum* infection

Cytokines with anti-inflammatory functions such as IL-4 and IL-10 are important for controlling the proinflammatory response associated with *H. capsulatum* infection (41, 42). IL-10 is a pleiotropic cytokine that is produced by phagocytes and T cells in response to *H. capsulatum*. In both primary and secondary infection, clearance of *H. capsulatum* from the lungs and spleen is accelerated in the absence of endogenous IL-10. Enhanced production of protective cytokines, including IFN- γ and GM-CSF, likely facilitate clearance in IL-10^{-/-} mice. Despite elevation of Th1 cytokines, animals do not display immunopathology in the absence of endogenous IL-10. These data suggest other cytokines or regulatory populations function to limit the Th1 response and ultimately, prevent immune mediated-tissue damage.

In addition to dampening the protective Th1 response, IL-4 impairs fungal resolution by promoting a Th2 response. A dominant Th2 response delays fungal clearance from the lungs and spleen; however, does not prevent animals from resolving infection (41, 43). Interestingly, an amplified IL-4 response in *H. capsulatum* infection is not associated with characteristic features such as goblet cell hyperplasia or increased mucus production that is observed in allergy models. IL-4 activates M ϕ to have an alternative phenotype which is permissive to fungal growth. IL-4 can promote survival of intracellular pathogens by reducing NO production (44). Additionally, IL-4 is known to dictate the intracellular concentration of metals including calcium, iron, and zinc in M ϕ (16, 45, 46). In response to *H. capsulatum*, zinc and calcium are elevated in IL-4-activated M ϕ . IL-4-driven regulation of intracellular zinc controls yeast replication. A higher concentration of zinc after IL-4 treatment partially restores fungal growth (16). Additionally, IFN- β , which is produced by M ϕ in response to *H. capsulatum* conidia, has been shown to dampen immunity during infection. Mice lacking the type I IFN receptor IFNAR1 have a reduced fungal burden in the lungs and spleen (47).

Although anti-inflammatory cytokines may depress the protective immune response, their function may be important in controlling particular pathological conditions associated with infection. Syndromes such as mediastinal fibrosis, pericarditis, or presumed ocular histoplasmosis result from an overly exuberant host response rather than uncontrolled infection (48). The driving forces that propel these hyperinflammatory responses have not been identified largely because a suitable animal model has not been developed. But, it is quite likely that a Th1 and/or Th17 response provokes the immune pathology. In these clinical conditions, anti-inflammatory cytokines do not appear to be functioning optimally to suppress the mobilization of inflammation. IL-10 and other anti-inflammatory mediators would subserve a highly useful function in these uncommon but devastating forms of histoplasmosis.

4. The role of chemokines in host protection against *H. capsulatum*

Although the roles of several proinflammatory cytokines have been extensively characterized in *H. capsulatum* infection, the role of chemokines remains unresolved. Chemokines, a family of chemotactic cytokines, are small proteins (8 to 12-kDa) that are most notably known for their ability to mediate leukocyte migration and activation (49). The chemokine superfamily is divided into subfamilies based on the spacing between conserved cysteine residues in their N-terminal domain and are classified as CXC (α), CC (β), C (γ), and CX₃C (δ) chemokines (50). Chemokines induce a complex signaling cascade upon binding to pertussis toxin-sensitive G protein-coupled receptors expressed on the surface of a variety of cell types. Chemokines can also interact with glycosaminoglycans on endothelial surfaces and extracellular matrix to facilitate chemotaxis by establishing a chemokine gradient (51). In addition to promoting chemotaxis, specific chemokines have been shown to activate leukocytes, induce cytokine production, promote T cell differentiation and polarization, and regulate apoptosis (52, 53).

4.1. CCR5

The influx of inflammatory cells to the site of *H. capsulatum* infection is associated with the upregulation of multiple chemokines and chemokine receptors. CCR5, which serves as a receptor for CCL3, CCL4, and CCL5, is expressed on both hemopoietic and non-hemopoietic cells and is differentially regulated by pro- and anti-inflammatory cytokines, including IL-12, IFN- γ , and TNF- α and IL-4 and IL-10, respectively (54). In a peritoneal cavity model of infection, *H. capsulatum* rapidly induces the expression of CCL3. Injection of live *H. capsulatum*, but not β -glucan isolated from the cell wall, promotes CCL3 production as early as 4 hours after infection (55). Consistently, high concentrations of CCL3 and CCL4 are detected in the lungs by day 3 post-infection in a model of pulmonary histoplasmosis. In contrast, CCL5, which also signals through CCR5, is not upregulated until one week after infection, suggesting it is not required for the influx of innate cells to the lungs.

Signaling through CCR5 promotes the migration of several inflammatory cell populations, including neutrophils, M ϕ , DCs, NK cells, and CD8⁺ T cells, to the lungs after *H. capsulatum* infection. A reduced proportion and absolute number of each of these populations in mice deficient for CCR5 or given a neutralizing antibody to CCL4 enhances the severity of infection during the innate response. However, CCR5 is not required for fungal resolution. The absence of this CCR5 signaling in the adaptive response promotes accelerated clearance due to defective emergence of Tregs. The involvement of CCR5 in Treg recruitment has been characterized during infection with several pathogens (24, 56, 57). CD4⁺ CD25⁺ T cells express high levels of CCR5 and are chemotactic towards CCL3, CCL4, and CCL5. CCL4, which signals exclusively via CCR5, is the most potent chemoattractant for Tregs currently known (58). In *H. capsulatum* infection, fewer Tregs in

CCR5^{-/-} lungs stems from a defect in trafficking, as well as local expansion in the lungs (24, 59). Although a reduction in Tregs is associated with an amplified Th1 response in other models, CCR5^{-/-} mice infected with *H. capsulatum* manifest an enhanced Th17 response which accelerates fungal clearance (Figure 2).

CCR5 likely controls the Treg-Th17 equilibrium by regulating the local cytokine environment. An amplified Th17 response in CCR5^{-/-} mice is also associated with enhanced production of IL-6 and IL-23. IL-6, in synergy with TGF-β, promotes CD4⁺ T cells to commit to the Th17 lineage and inhibits Foxp3 expression/Treg development (60), while IL-23 is involved with Th17 maintenance (61). The paucity of Tregs in CCR5^{-/-} mice correlates with a higher concentration of IL-17 in the lungs. Remarkably, neutralization of IL-17 restores the number of Tregs in CCR5^{-/-} lungs during *H. capsulatum* infection and Treg proliferation is augmented by nearly 3-fold. These data identifies a potential role for IL-17 in regulating the generation and/or homing of Tregs and consequently, the Treg-Th17 balance. The ability of IL-17 to directly hinder Treg emergence has not been described. Although the aforementioned is a possibility, it seems more likely that this observation is an indirect effect of IL-17. In CCR5^{-/-} mice given anti-IL-17, the concentration of cytokines involved in Treg development and/or function, including IL-2, IL-10, and TGF-β, are elevated in the lungs. Thus, we postulate these cytokines regulate the emergence of Tregs rather than IL-17 itself. Furthermore, since IL-17 regulates the expression of several chemokines, the possibility of IL-17 regulating Treg chemotaxis should be explored.

H. capsulatum infection results in mild to moderate multifocal peribronchiolar and perivascular mixed inflammatory cuffing in wild-type and CCR5^{-/-} lungs. Coincident with fungal resolution, IL-6, IL-17, IL-22, and IL-23 are restored to wild-type levels by day 21 or earlier, suggesting that elevated Th17 cytokine production is transient, reducing the potential of detrimental inflammation in CCR5^{-/-} mice. However, the mechanism by which inflammation is regulated is unresolved. One possibility is that anti-inflammatory cytokines prevent hyper-inflammation in CCR5^{-/-} mice. IL-27 and IL-35 are members of the IL-12 family and are heterodimeric proteins composed of Ebi3 (Epstein-Barr virus-induced gene 3) and p28 or p35, respectively (62, 63). IL-27 is predominantly produced by antigen-presenting cells, whereas IL-35 is produced by Tregs. Since both cytokines have been shown to regulate the Th17 response (64, 65), it is plausible that IL-27 and/or IL-35 dampen the Th17 response and restore the Treg-Th17 equilibrium in the absence of CCR5.

4.2. CCR2

In addition to CCR5, CCR2 and its ligands influence inflammatory cell recruitment and cytokine production during infection. In the absence of CCR2, mice succumb to infection due to the inability to control *H. capsulatum* infection. A dampened protective immune response in CCR2^{-/-} mice is associated with fewer inflammatory cells and elevated IL-4 in the lungs (23). In contrast to other models, amplified IL-4 production does not dampen the expression of Th1 cytokines such IFN-γ and TNF-α. Although CD4⁺ T cells are the predominant source of IL-4 in *H. capsulatum* infection, other cellular populations produce this Th2 cytokine. In the absence of CCR2, an enhanced proportion of Mφ and DC secrete IL-4 during infection. The negative consequences associated with excess production of IL-4 can be reversed by neutralizing IL-4. Administration of an antibody to IL-4 rescues CCR2^{-/-} mice; however, does not restore inflammatory cell infiltration.

CCL2 and CCL7 are the CCR2 ligands that signal through CCR2 to regulate IL-4 expression. The absence of CCR2 is associated with elevated IL-4 transcript and protein during the first week of infection. A comparable increase in IL-4 is observed when CCL2^{-/-} mice are given a neutralizing antibody to CCL7. Together, CCL2 and CCL7 promote the migration of CD8⁺ conventional DCs to the lungs, as this population is diminished in

CCR2^{-/-} and CCL7-neutralized CCL2^{-/-} mice. Adoptive transfer of antigen-specific DC suppresses IL-4 production in CCR2^{-/-} mice. These results indicate CCR2-dependent recruitment of DCs suppresses local IL-4 production in the lungs. However, although IL-4 levels are restored, CCR2^{-/-} animals still succumb to infection suggesting other factors regulate fungal clearance in the absence of CCR2.

Enhanced IL-4 skews the immunity to a Th2 response. Since cytokines such as IL-25, IL-33, and TSLP are known to initiate and amplify the Th2 response, it is possible CCR2 signaling regulates their expression. The presence of IL-4 is detrimental in *H. capsulatum* infection because it activates Mφ to have an alternative phenotype. This Mφ population is characterized by augmented expression of Arg1, which is disadvantageous for fungal clearance. Administration of an Arg1 inhibitor modestly reduces fungal burden, but does not enhance survival of CCR2^{-/-} mice. These data suggest IL-4 dictates fungal resolution in an Arg1-independent manner as well.

5. Conclusions

Cytokines and chemokines exert a pivotal role in dictating the outcome of *H. capsulatum* infection. The outcome of infection is largely determined by the early response to invasion. Dissecting the influence and interactions among the various soluble mediators will provide information on why and how the host fails to contain infection. This knowledge can be utilized to create therapeutic agents that transform the response from one that is ineffective to one that can constrain the progressive nature of invasion. The paradigms that arise from work with this intracellular fungal pathogen may extend to a numerous other intracellular microbes.

Highlights

- The involvement of Th1 and Th17 cytokines in mediating a protective immune response to *H. capsulatum*.
- How cytokines such as IL-4 and IL-10 dampen protective immunity in *H. capsulatum* infection.
- The role of chemokines in the recruitment of immune cells and regulation of cytokine production.

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***H. capsulatum* is internalized by resident & recruited phagocytes**

Figure 1. *H. capsulatum* induces a proinflammatory response

Upon inhalation, the *H. capsulatum* is internalized by host phagocytes. Whereas polymorphonuclear (PMN) and DCs inhibit fungal growth, the organism replicates within Mφ prior to cellular activation. Cytokines such as IL-12, IL-6, and IL-23 are produced during the innate response and are involved in the polarization of naïve T cells. Th1 cells, characterized by IFN-γ, are the predominant T cell population during infection. A smaller population of Th17 population emerges, but only plays a minor role in resolving infection. In addition to IFN-γ, other Th1 cytokines including TNF-α, IL-1β, and GM-CSF activate Mφ to prevent intracellular growth.

Figure 2. CCR5 mediates Treg trafficking and expansion during *H. capsulatum* infection
 The absence of CCR5 results in a reduced proportion and number of Tregs in the lungs during infection. Fewer Tregs in CCR5^{-/-} lungs is a result of impaired trafficking and diminished proliferation. A reduction in Tregs is associated with a cytokine profile that favors a Th17 response. Thus, CCR5 functions to maintain the Treg-Th17 equilibrium to promote pathogen persistence.