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Mononuclear Phagocyte-Mediated Antifungal Immunity: The Role of Chemotactic Receptors and Ligands

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Abstract

Over the past two decades, fungal infections have emerged as significant causes of morbidity and mortality in patients with hematological malignancies, hematopoietic stem cell or solid organ transplantation and acquired immunodeficiency syndrome. Besides neutrophils and CD4⁺ T lymphocytes, which have long been known to play an indispensable role in promoting protective antifungal immunity, mononuclear phagocytes are now being increasingly recognized as critical mediators of host defense against fungi. Thus, a recent surge of research studies has focused on understanding the mechanisms by which resident and recruited monocytes, macrophages and dendritic cells accumulate and become activated at the sites of fungal infection. Herein, we critically review how a variety of G-protein coupled chemoattractant receptors and their ligands mediate mononuclear phagocyte recruitment and effector function during infection by the most common human fungal pathogens.

Keywords

fungal infections; monocytes; macrophages; dendritic cells; chemotactic factors; candidiasis; aspergillosis; cryptococcosis

Introduction

Fungi can cause a wide variety of infections in humans. On one hand, self-limited pulmonary disease by dimorphic fungi or superficial candidal and dermatophytic infections affecting the skin, nails, and mucosal surfaces may occur in immunocompetent individuals. On the other hand, life-threatening systemic infections caused by *Candida*, *Aspergillus* and other molds, *Pneumocystis*, *Cryptococcus*, and the endemic dimorphic fungi *Coccidioides*, *Histoplasma* and *Blastomyces* may develop in immunocompromised patients involving deep-seated tissues such as the brain, lungs, kidneys, spleen and/or liver [1–6]

Modern medical advances have resulted in appreciable increases in lifespan and thereby in an expansion of the populations of immunosuppressed and debilitated individuals at risk for fungal infections, contributing to a considerable rise in their incidence. Hence, patients with the acquired immunodeficiency syndrome (AIDS), those receiving immunosuppressive

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therapies for cancer or autoimmune disorders, recipients of hematopoietic stem cell and solid organ transplantation, as well as individuals undergoing major abdominal surgical procedures and premature low-birth weight infants are all at heightened risk for developing fungal infections [6–8]. Nonetheless, despite recent advances in diagnostic modalities and the availability of several classes of antifungal agents with potent *in vitro* and preclinical antifungal activity, disseminated fungal infections remain an unmet medical condition and represent a significant cause of morbidity and mortality in affected patients [5,6].

Therefore, the increase in opportunistic systemic fungal infections, the complexity and heterogeneity of the patient populations affected, the diversity of fungal pathogens involved and the lack of effective treatment in the clinic, collectively underscore the importance of better understanding of the molecular and cellular basis of antifungal immunity with a goal to improve patient outcomes by devising immune- and vaccine-based strategies for prevention, risk assessment, prognostication, diagnosis and treatment of human fungal disease.

The Emerging Role of Mononuclear Phagocytes in Antifungal Host Defense

The crucial role of neutrophils in host defense against systemic fungal infections became apparent in the 1950–1960s soon after the introduction of neutropenia-inducing cytotoxic chemotherapy for the treatment of hematological malignancies [9,10]. Indeed, patients with inherited or acquired neutropenia or qualitative neutrophil defects such as those with chronic granulomatous disease are at increased risk for development of invasive mold infections and systemic candidiasis and suffer poor outcomes from these infections [11–13]. Neutrophils protect against mold fungi and *Candida* via direct potent fungicidal activity mediated by both oxidative and non-oxidative mechanisms [14].

In the 1980–1990s, the AIDS epidemic uncovered the critical contribution of the CD4⁺ T lymphocyte in host defense against mucosal and systemic fungal disease. Indeed, patients with AIDS as well as those with idiopathic CD4 lymphocytopenia are susceptible to mucosal candidiasis and systemic infections caused by *Cryptococcus*, *Pneumocystis* and the endemic dimorphic fungi [15–17]. The production of IL-17 and IL-22 by T cells of the Th17 differentiation program is now known to account for T-cell-mediated mucosal anti-*Candida* host defense [18]. On the other hand, T-lymphocyte-dependent protection against *Cryptococcus*, *Pneumocystis* and endemic dimorphic fungi is thought to be indirect via production of cytokines that prime mononuclear phagocytes to exert effector function against these fungi. In fact, the T lymphocyte-mononuclear phagocyte cross-talk in this context was a first inference in humans that mononuclear phagocytes such as monocytes, macrophages and dendritic cells (DCs) are key players in antifungal host defense.

Further demonstration that mononuclear phagocytes mediate protective immune responses against fungi emerged recently via the characterization of certain inherited and acquired conditions that lead to enhanced susceptibility to fungal disease. Indeed, patients with inherited mutations in the IL-12/IFN- γ pathways are susceptible to infections by the intracellular dimorphic fungi and by *Cryptococcus* as a result of impaired macrophage activation and effector function [18–20]. In addition, patients with *GATA2* mutations who

feature severe monocytopenia and lack of circulating and tissue-resident DCs have been recently reported to develop systemic fungal disease by dimorphic fungi, *Cryptococcus* and *Aspergillus* [21]. Last, putatively immunocompetent patients with neutralizing autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF) were recently reported to develop disseminated cryptococcal infections highlighting the role of mononuclear phagocytes in anti-cryptococcal host defense [22], as granulocytes are dispensable for protection against these yeast fungi in humans.

How do mononuclear phagocytes exert their antifungal effector functions? First, they express an array of pattern recognition receptors such as C-type lectin receptors and Toll-like receptors that recognize conserved pathogen-associated molecular patterns of fungi and activate the innate immune system via the production of a variety of pro-inflammatory cytokines and chemokines [1,14]. As part of the innate immune response, mononuclear phagocytes can directly internalize and kill fungi by both production of reactive oxygen species and generation of non-oxidative antimicrobial products [14]. In addition, uptake of fungal pathogens by mononuclear phagocytes promotes the development of an adaptive immune response against the invading fungus [14,23]. Thus, identification of the molecular factors that modulate the effector function of mononuclear phagocytes during fungal infection is a research direction of major importance in order to gain insight into the mechanisms by which these cells mediate fungus-specific protective immunity.

The Study of Chemotactic Factors as a Platform to Probe Mononuclear Phagocyte-Fungal Interactions

A critical step for mononuclear phagocytes to execute their antifungal activity is their effective recruitment and activation at the site of infection. These processes are mediated by the production of a variety of chemokines, the largest subgroup of structurally related cytokines, which are 8–10kDa proteins that collectively coordinate leukocyte trafficking and activation in health and disease conditions such as infection, autoimmunity and cancer by binding to G-protein coupled chemokine receptors [24]. Currently there are 50 endogenous chemokine ligands identified in mouse and humans that signal through 20 chemokine receptors. In addition there are 5 non-signaling chemokine receptors including Duffy and D6 receptor [25], as well as several chemoattractant receptors such as C5a receptor (C5aR), leukotriene B4 receptor 1 (LTB4R1), formyl-peptide receptor (FPR) 1 and 2, and platelet-activating factor receptor (PAFR). Chemokines are produced by a variety of hematopoietic and non-hematopoietic cells [24] and are divided into four subgroups based on the position of the first two conserved cysteine residues; XC, CC, CXC and CX3C. While CXC chemokines are predominantly chemotactic for granulocytes, XC, CC and CX3C chemokines are principally involved in mononuclear phagocyte and lymphocyte recruitment [24]. In addition to chemoattraction, chemotactic factors have recently been shown to regulate a wide array of other cellular biological functions such as survival, proliferation, differentiation, phagocytosis and killing, cytokine production, hematopoiesis and angiogenesis [24,25]. Since chemotactic factors are integral to the generation of any immune response, their study offers an opportunity to probe the host-fungal interface in the context of fungal disease.

A major hurdle in mononuclear phagocyte research has historically been the lack of adequate tools to specifically evaluate their function *in vivo*. Administration of clodronate-loaded liposomes has been the predominant method used to deplete mononuclear phagocytes and examine their *in vivo* role in the context of infection or inflammation in mice [26]. However, systemic administration of clodronate-loaded liposomes depletes monocytes, macrophages and DCs non-selectively, thus not allowing for the investigation of the role of specific mononuclear phagocytes in host defense. Moreover, clodronate-induced mononuclear phagocyte depletion does not provide information on specific molecular factors that mediate mononuclear phagocyte immunity nor does it address the relative contribution of recruited and resident mononuclear phagocytes in host defense.

Therefore, the investigation of mononuclear phagocyte-targeted chemotactic factors (Table 1) is appealing for dissecting the mechanisms of mononuclear phagocyte-mediated antifungal immunity at the mucosal level and systemically. Some of these factors such as CCR2, CCR4, CCR5, CCR6, CCR7 and CX3CR1 are not expressed by neutrophils [24]. In addition, CCR2 and CX3CR1 are “signature” chemokine receptors on inflammatory and resident monocytes/macrophages, respectively [27], thus offering the advantage of interrogating the relative contribution of recruited inflammatory versus resident mononuclear phagocytes in host defense against fungi. CCR7 is particularly useful for the study of innate and adaptive immune functions of DCs as it is expressed predominantly by these cells and not by monocytes/macrophages among mononuclear phagocytes [24]. The study of other mononuclear phagocyte-targeted chemokine receptors such as CCR1, CXCR1, CXCR2, C5aR, LTB4R1, FPR1 and FPR2 is also informative but it is confounded by their parallel expression on the surface of granulocytes [24].

In recent years there has been a surge of studies on the role of some of these mononuclear phagocyte-targeted chemotactic factors in antifungal immunity that have helped to shed light on the mechanisms by which monocytes, macrophages and DCs respond to fungal insult. In this review, we synthesize and critically review our current understanding on the role of mononuclear phagocyte-targeted chemotactic factors on the host immune response during infection by the most common human fungal pathogens: *Aspergillus*, *Candida*, *Cryptococcus*, the endemic dimorphic fungi and *Pneumocystis*.

Invasive Aspergillosis

Aspergillus is a filamentous fungus that is ubiquitous in the environment and its conidia are continuously inhaled and reach the human terminal airways due to their small diameter. However, immunocompetent individuals are not affected by invasive aspergillosis, as the innate immune system is able to effectively control the fungus once it enters the lungs. The first line of defense is thought to be alveolar macrophages and other resident mononuclear phagocytes, which internalize and destroy conidia via oxidative and non-oxidative cytotoxic mechanisms [3,28]. In situations when conidia escape resident mononuclear phagocyte control and germinate into hyphae, recruited neutrophils and mononuclear cells represent an additional critical line of innate host defense [29], which prevents invasive pulmonary infection and dissemination to distal organs [28]. Not surprisingly, patients with neutropenia or those receiving corticosteroid treatment that impairs granulocyte and mononuclear

phagocyte effector function are at high risk to develop invasive pulmonary infection and disseminated aspergillosis [2,3,12]. Indeed, invasive aspergillosis is the leading cause of infection-related mortality in recipients of allogeneic hematopoietic stem cell transplantation, with case-fatality rates that exceed 50% despite the administration of antifungal therapy [30].

Although quantitative and qualitative defects of neutrophils are known major risk factors for the development of invasive aspergillosis [12,18,31] several mouse and human mononuclear phagocytes have also been shown to exert anti-*Aspergillus* activity *ex vivo*. For example, mouse monocytes, resident alveolar macrophages and DCs are able to internalize and kill *Aspergillus* conidia and damage *Aspergillus* hyphae extracellularly, and to secrete a wide variety of pro-inflammatory cytokines and chemokines following *Aspergillus* exposure [32–35]. In humans, both CD14⁺⁺CD16⁻ classical and CD14⁺CD16⁺ non-classical monocytes exhibit anti-*Aspergillus* activity and are able to effectively phagocytose conidia; however, CD14⁺CD16⁻ monocytes are more efficient in controlling *Aspergillus* conidial germination, while CD14⁺CD16⁺ monocytes lack conidial germination inhibitory capacity, but are crucial in inducing an inflammatory response via secretion of mediators, such as TNF- α [36]. Although chemokines and their receptors have been shown to be important during pulmonary allergic *Aspergillus* disease [37], this review will focus only on the specific role of mononuclear phagocyte-targeted chemotactic factors during invasive aspergillosis.

Elegant *in vivo* studies from the Hohl lab have recently demonstrated that not all pulmonary mononuclear phagocyte populations contribute equally to effective anti-*Aspergillus* host defense in the lung. It was traditionally thought that because corticosteroid-treated patients are at risk for invasive aspergillosis and corticosteroids adversely affect alveolar macrophage function [3,38] that these cells play a preponderant role in host defense against *Aspergillus*. However, corticosteroids do not only affect alveolar macrophage function but also impair neutrophil and recruited mononuclear phagocyte trafficking and effector function [3]. Therefore, a series of experiments using intratracheal clodronate administration, which specifically depletes alveolar macrophages but not monocytes, DCs or neutrophils in the mouse lung [39], were set to determine the direct contribution of alveolar macrophages in anti-*Aspergillus* host defense. Remarkably, alveolar macrophage depletion did not impair the production of neutrophil-recruiting chemokines and pro-inflammatory cytokines, did not increase pulmonary fungal growth, and did not enhance mortality after *Aspergillus* infection [39]. Moreover, depletion of alveolar macrophages in neutropenic mice did not further enhance host susceptibility compared to that seen in neutropenic mice alone. Collectively, these data established for the first time that mouse alveolar macrophages exert redundant functions in anti-*Aspergillus* host defense *in vivo*, which can be compensated by other resident and recruited phagocyte cell populations.

In a follow-up study by the same research group, it was shown that depletion of Ccr2-expressing monocytes and monocyte-derived DCs using Ccr2-deleter mice results in a dramatic increase in fungal proliferation in the lung and mouse mortality, comparable to that seen in *Aspergillus*-infected neutropenic mice [40]. Monocytes and monocyte-derived DCs exert anti-*Aspergillus* host defense *in vivo* via two independent mechanisms; a direct one by mediating *Aspergillus* killing and production of pro-inflammatory cytokines including tumor

necrotic factor (TNF)- α , and an indirect one via priming the conidicidal activity of neutrophils, as shown by imaging the *in vivo* interaction of *Aspergillus* and neutrophils in the infected lung [40]. The mechanism by which Ccr2-expressing mononuclear phagocytes activate neutrophils in the *Aspergillus*-infected lung is not yet elucidated. However, the mechanism appears distinct from that shown during systemic candidiasis, in which IL-23p19 produced by mononuclear phagocytes induces the production of GM-CSF by natural killer (NK) cells, which in turn enhances the candidacidal activity of neutrophils in the kidney [41]. Instead, in *Aspergillus*-infected mice, depletion of NK cells does not phenocopy the marked susceptibility seen in mice depleted of Ccr2-expressing monocytes and monocyte-derived cells [40]. This indicates that either the source of GM-CSF in the lung during aspergillosis is different from that seen in *Candida*-infected kidneys, and/or that factors other than GM-CSF mediate the mononuclear phagocyte-neutrophil cross-talk in the lung.

Besides the critical role of Ccr2-expressing mononuclear phagocytes in innate immune control of *Aspergillus* infection, these cells were also reported to be critical for the induction of protective adaptive immune responses following *Aspergillus* challenge [42], a finding which may have clinical implications for vaccine development in patients at risk for invasive mold disease [43]. Specifically, Ccr2⁺ inflammatory monocytes rapidly differentiate into CD11b⁺ DCs in the infected lung upon exposure to *Aspergillus*, transport the conidia to the draining mediastinal lymph nodes and prime pathogen-specific CD4⁺ T cell adaptive immune responses [42]. Interestingly, Ccr2 plays a compartment-specific role in modulating pathogen-specific adaptive immune responses following *Aspergillus* infection, as CD4⁺ T cell priming was severely impaired in lung draining lymph nodes of Ccr2-depleter mice infected with *Aspergillus* conidia via the intratracheal route but not in the spleen of Ccr2-depleter mice infected with *Aspergillus* conidia via the intravenous route [42]. Therefore, Ccr2-expressing mononuclear phagocytes collectively affect the innate and adaptive immune arms of pulmonary *Aspergillus* infection. Besides Ccr2, because several mononuclear phagocyte populations in the lung express Cx3cr1 at steady state and during inflammation [44], the role of this receptor in priming innate and adaptive immune responses during invasive aspergillosis merits future investigation.

Besides Ccr2 and its ligands Ccl2, Ccl7 and Ccl12, whose individual roles in Ccr2-mediated mononuclear phagocyte immunity during aspergillosis remain unknown, the chemokine Ccl3 has been reported to mediate accumulation of mononuclear phagocytes in the lung of *Aspergillus*-infected mice, but only in the setting of neutropenic aspergillosis and not in mice with intact neutrophil counts [45]. Several studies have revealed that Ccl3 is significantly induced in *Aspergillus*-infected lungs [45–47] and neutralization of Ccl3 in neutropenic mice led to decreased pulmonary accumulation of mononuclear phagocytes and increased mortality relative to that seen in neutropenic mice alone, attesting to the importance of Ccl3-mediated mononuclear phagocyte trafficking and effector function in controlling the infection during neutropenia [45]. Future studies should elucidate the specific mononuclear phagocyte subsets whose recruitment is preferentially impaired in the lungs of mice during Ccl3 neutralization.

Ccl3 is a ligand to both Ccr1 and Ccr5. However, *Aspergillus*-infected Ccr1-deficient neutropenic mice did not develop heightened susceptibility as opposed to *Aspergillus*-

infected Ccl3-neutralized neutropenic mice [45]. Therefore, it is likely that Ccr5 may mediate Ccl3-dependent mononuclear phagocyte immunity during neutropenic aspergillosis, and future studies should examine the role of Ccr5 in pulmonary host defense against aspergillosis. Of interest, despite the dispensable role of Ccr1 in pulmonary anti-*Aspergillus* immunity, Ccr1 is crucial for host defense during systemic challenge with *Aspergillus* [48], as *Ccr1*^{-/-} mice develop uncontrolled fungal proliferation in the kidney and brain and lethal infection after intravenous *Aspergillus* challenge. These data underscore the compartmentalized role of Ccr1 in tissue-specific immunity against the same fungal pathogen and merit further study to determine whether the protective effects of Ccr1 in anti-*Aspergillus* host defense in the brain and kidneys are mediated by neutrophils, mononuclear phagocytes, or both.

Ccr6 and Ccr7 are two other chemotactic factors that have further highlighted the important role of DC accumulation in anti-*Aspergillus* host defense in the lung during neutropenia. Work at the Mehrad lab [49] found that Ccr6 deficiency resulted in greater pulmonary fungal burden and increased mortality after invasive aspergillosis under neutropenic conditions but not in animals with intact neutrophil counts [49]. Ccr6-deficient neutropenic animals exhibited a marked decrease in the recruitment of myeloid DCs and inflammatory monocytes/macrophages but no difference in the accumulation of resident alveolar macrophages. Using a series of adoptive transfer and monocyte/macrophage depletion strategies *in vivo*, it was shown that Ccr6 deficiency results in enhanced susceptibility to pulmonary aspergillosis due to impaired recruitment of DCs, but not monocytes/macrophages [49]. While DC recruitment in the lung was Ccr6-dependent, *Ccr6*^{-/-} DCs had unaffected effector function as they exhibited intact phagocytosis, maturation and production of pro-inflammatory cytokines upon conidial exposure. Consistent with the protective Ccr6-mediated influx of DCs into the *Aspergillus*-infected lung, neutralization of Ccl20, the sole ligand for Ccr6, led to impaired DC recruitment and increased fungal lung load in neutropenic mice [49].

Of interest, the observation that Ccr6 deficiency resulted in increased susceptibility to aspergillosis only in the presence of neutropenia led to further studies on the immunomodulatory cross-talk between neutrophils and DCs in the lung during *Aspergillus* infection. It was shown that in the absence of neutrophils, a striking accumulation of DCs occurs in *Aspergillus*-infected lungs of both mice and humans [50]. Neutropenia-related enhanced DC accumulation in the lung was the result of both enhanced DC recruitment via TNF- α -mediated induction of Ccl2 and Ccl20 [50], and impaired DC maturation and egress to mediastinal lymph nodes [51].

Moreover, uptake of *Aspergillus* conidia by DCs results in up-regulation of Ccr7, which in turn facilitates Ccl19/Ccl21-mediated egress of DCs into the draining lymph nodes [24,52]. Therefore, Ccr7-deficient mice had increased pulmonary accumulation of DCs due to impaired DC efflux out of the lungs, which was associated with control of pulmonary fungal proliferation and improved host survival during neutropenic aspergillosis [53]. Besides increased DC accumulation in the setting of Ccr7 deficiency, *Ccr7*^{-/-} DCs also exhibited a more activated and mature phenotype, which may also contribute to improved outcome following *Aspergillus* infection [53]. The detrimental role of Ccr7 during aspergillosis was

further demonstrated in a mouse model of the infection after transplantation of hematopoietic stem cells (HSC) and myeloid progenitor cells (MPC) [54]. Specifically, mice transplanted with *Ccr7*^{-/-} HSC and MPC were less susceptible to invasive aspergillosis compared to mice transplanted with wild-type cells. *Ccr7*^{-/-} HSC were able to reconstitute the myeloid cell compartment more effectively thereby able to better control the infection [54]. Thus, future studies will be required to determine whether CCR7 inhibition may be a viable adjunct treatment option for invasive aspergillosis in neutropenic patients and/or hematopoietic stem cell transplant recipients.

Besides *Ccr2*, *Ccl3*, *Ccr6* and *Ccr7*, which are mononuclear phagocyte- but not neutrophil-targeted chemotactic factors, neutralization of *Cxcr2*, which is expressed on both neutrophils and mononuclear phagocytes, resulted in substantially increased susceptibility following pulmonary aspergillosis [55]. Mice with neutralized *Cxcr2* had markedly impaired neutrophil recruitment in the *Aspergillus*-infected lung accounting for the heightened susceptibility to infection. However, overexpression of *Cxcl1*, a major ligand for *Cxcr2*, led to marked improvement in host survival and control of fungal load in the lung of *Aspergillus*-infected neutropenic mice. Therefore, the survival and microbiological benefit seen with *Cxcl1* overexpression in the setting of neutropenic aspergillosis raises the possibility that the *Cxcl1/Cxcr2* axis may, at least in part, mediate its anti-*Aspergillus* immune effects in a mononuclear phagocyte-dependent manner. The recent development of *Cxcr2*-floxed mice [56] will be helpful in delineating whether the *Cxcr2*-dependent protection during invasive aspergillosis is exclusively mediated by neutrophils or whether mononuclear phagocytes may also play a role.

All studies on *Aspergillus* immunity have thus far been performed using *A. fumigatus*, the most common species of human invasive aspergillosis. However, the emergence of infections by non-*fumigatus Aspergillus* species and other molds such as *Mucorales*, *Fusarium* and *Scedosporium*, [3,8,57–59] which are often recalcitrant to antifungal therapy with mortality rates that exceed 70–80%, highlights the importance for future research on the molecular mechanisms of host defense against these molds, which are ill-defined, including the study of mononuclear phagocyte-targeted chemotactic factors.

Candidiasis

Candida is a commensal organism residing in the mucosal surfaces of the gastrointestinal tract, skin and/or female genital tract in the majority of humans. When perturbations in immunity and/or microbial flora occur, *Candida* can convert to an opportunistic pathogen and cause mucosal or systemic infections. On one hand, although not life-threatening, mucosal candidiasis has a substantial global disease burden as the majority of healthy women will develop at least one episode of vaginal candidiasis during their lifetime, and >90% of AIDS patients, if left untreated, will develop oral candidiasis [60]. On the other hand, invasion of *Candida* from the mucosa into the systemic circulation results in systemic infection, the most common deep-seated invasive mycosis in the developed world and the 3rd leading cause of nosocomial bloodstream infection in modern intensive care units [61]. Despite administration of antifungal treatment, mortality due to systemic candidiasis remains above 30–40% [61].

In recent years, it has become apparent via mouse immunological studies [62,63] and the genetic characterization of human monogenic disorders with susceptibility to chronic mucocutaneous candidiasis [18] that IL-17 signaling is crucial for effective host defense against *Candida* at the mucosal level via the local induction of potent antifungal antimicrobial peptides. In stark contrast, neutrophils play a principal role in the control of systemic candidiasis and neutropenia is a major risk factor for the development of the infection and adverse outcome after systemic candidiasis [61]. In agreement with this segregation in host factor dependence for mucosal versus systemic immune protection against *Candida*, patients with AIDS are at risk for mucosal but not systemic candidiasis, whereas patients with neutropenia or chronic granulomatous disease develop systemic but not mucosal candidiasis [64].

Although the role of mononuclear phagocytes in mucosal anti-*Candida* host defense has not been thoroughly investigated thus far, several lines of emerging evidence have highlighted the indispensable contribution of these cells in the systemic control of *Candida* infection. Firstly, depletion of mononuclear phagocytes by systemic administration of clodronate-loaded liposomes was shown to result in significantly increased renal and splenic fungal load and accelerated mortality [65]. Secondly, brain microglial cells and blood monocytes have been shown to exert direct neutrophil-independent anti-*Candida* protection *in vivo* [66,67]. Thirdly, genetic abrogation of Syk signaling in CD11c-expressing renal mononuclear phagocytes was recently shown to dramatically enhance host susceptibility after systemic *Candida* challenge [41]. These CD11c-expressing cells represent both macrophages and DCs as recent studies have highlighted the plasticity and overlapping macrophage and DC features within the resident mononuclear phagocytic system in the kidney, in which CD11c is expressed by both macrophage and DC cell subsets [68,69].

How do mononuclear phagocytes mediate anti-*Candida* host defense? Several mouse mononuclear phagocyte populations including bone marrow monocytes and renal resident macrophages, which both come in direct contact with the pathogen *in vivo*, have been shown to effectively internalize and kill *Candida* [69,70]. In addition to direct fungal killing, mononuclear phagocytes also promote *Candida* killing indirectly via IL-23p19-induced production of GM-CSF by NK cells, which primes neutrophil candidacidal activity at the site of infection [41]. Furthermore, monocytes, macrophages and DCs are also able to produce a variety of pro-inflammatory cytokines and chemokines via Toll-like receptor- and C-type lectin receptor-mediated fungal recognition [14,71]. These innate antifungal effects are also seen in human CD14⁺⁺CD16⁻ classical and CD14⁺CD16⁺ non-classical blood monocytes [72]; although these cell subsets have comparable capacity for *Candida* internalization and killing, CD14⁺⁺CD16⁻ monocytes appear more effective in production of pro-inflammatory cytokines and induction of Th17 responses in response *Candida* stimulation [72].

By employing intravital imaging in *Candida*-infected kidney, the target organ of systemic candidiasis [73], we recently characterized the *in vivo* interaction of renal mononuclear phagocytes with dTomato-expressing *Candida albicans* using *Cx3cr1^{sfp/+}* mice in which almost all renal mononuclear phagocytes express Cx3cr1 [69,74]. We found that *Candida* rapidly invaded the renal interstitium and formed long filamentous elements as early as 2

hours after intravenous injection [69]. At this early time point, >80% of *Candida* yeast and pseudohyphal elements were engulfed or encircled by mononuclear phagocytes, respectively (Figure 1). Over 80–85% of Cx3cr1-expressing cells at this early time-point post-infection consist of resident renal macrophages with the remainder of the cells being resident renal DCs [69]. Strikingly, direct mononuclear phagocyte-*Candida* contact was no longer seen by day 1 post infection, because by then >90% of fungal elements had already invaded within the renal collecting system, and mononuclear phagocytes never entered the renal tubules but instead formed a contiguous network surrounding them [69]. This study demonstrated for the first time that mononuclear phagocytes interact with *Candida* only very early after infection *in vivo* and that the fungus is able to evade the mononuclear phagocyte arm of the innate immune response by entering into the renal collecting system.

Consistent with the early requirement of mononuclear phagocytes for innate control of *Candida* invasion in the kidney, Cx3cr1 deficiency, which results in a 50% reduction in the accumulation of resident macrophages in the kidney but no impairment in accumulation of inflammatory monocytes, led to a significant decrease in early direct macrophage-*Candida* contact *in vivo* and markedly increased fungal proliferation as early as 12 hours after infection, at a time point when inflammatory monocytes and neutrophils are not yet recruited in large numbers in the kidney [69]. Decreased macrophage accumulation in Cx3cr1-deficient kidneys was not due to impaired monocyte recruitment or differentiation or macrophage proliferation in the kidney; instead, Cx3cr1 promotes the survival of renal macrophages by inhibiting caspase-3-dependent apoptosis associated with Akt activation [69]. In addition to reduced accumulation and direct contact with the fungus in *Cx3cr1*^{-/-} kidneys, resident renal macrophages sorted from Cx3cr1-deficient mice also had moderately reduced capability of killing *Candida ex vivo* compared to wild-type macrophages. Collectively, the decreased number of resident macrophages and their compromised killing capacity led to inexorable *Candida* kidney growth, severe renal failure and universal death in *Cx3cr1*^{-/-} mice [69]. Although to a less pronounced extent compared to the kidney, Cx3cr1 deficiency also delayed the ability of brain and liver to control fungal growth. Conversely, consistent with the segregation of immune factors that control mucosal versus systemic *Candida* disease, Cx3cr1 was dispensable for control of mucosal candidiasis in mice [75].

In agreement with the Cx3cr1-dependent host protection during systemic but not mucosal candidiasis in mice, the dysfunctional human *CX3CR1-M280* allele was found to be an independent risk factor for both development of candidemia and disseminated infection in two different patient cohorts of over 650 patients with candidemia and matched control patients without candidemia from the US and Europe, indicating that genetic variation at *CX3CR1* may be a novel factor for risk assessment and prognostication in patients at risk for systemic candidiasis [69]. In addition, similar to mice, the *CX3CR1-M280* allele was not associated with increased risk of mucosal candidiasis by testing a cohort of 280 patients with recurrent vaginal candidiasis and control women without mucosal candidiasis [75]. Research will be required to define the mechanisms by which monocytes/macrophages from individuals carrying the mutant *CX3CR1-M280* allele exhibit altered antifungal innate effector function, which accounts for their heightened susceptibility to systemic candidiasis.

Consistent with the presence of direct mononuclear phagocyte-*Candida* contact in the kidney only within the first hours of infection [69], *Ccr2*^{-/-} mice, which do not mobilize >90% of inflammatory monocytes in the kidney post-infection but have intact accumulation of resident renal macrophage and DC populations had a modest increase in susceptibility after systemic *Candida* challenge relative to that seen in *Cx3cr1*^{-/-} mice [69,70]. The greater dependence on Cx3cr1-expressing resident macrophages over Ccr2-expressing inflammatory monocytes for innate host defense in the kidney is not due to a relative inability of inflammatory monocytes over resident macrophages to kill *Candida* but instead is likely explained by the delay in influx of inflammatory monocytes into the infected organ, which allows critical time for *Candida* to leave the renal interstitium, resulting in a relatively short window of opportunity for inflammatory monocyte-*Candida* direct interaction before the fungus enters the renal collecting system.

When Ccr2-depleter mice were infected with *Candida* they developed an overwhelming infection with excessive fungal invasion in the kidney, far more severe compared to that seen in *Ccr2*^{-/-} mice [70]. Besides the complete lack of inflammatory monocytes, Ccr2-depleter mice also lack subsets of resident renal macrophages and DCs but also NK cells, which have recently emerged as critical mediators of host defense in systemic candidiasis [76]. Elegant adoptive transfer experiments of bone marrow-derived Ly6C^{hi} inflammatory monocytes into *Candida*-infected Ccr2-depleter mice revealed that these mice could only be rescued when monocytes were delivered at the onset of infection but not at day 2 after infection [70], further attesting to the time-dependent early interactions of mononuclear phagocytes with *Candida* before tubular fungal invasion ensues, as shown by our intravital imaging experiments [69]. Intriguingly, depletion of all Ccr2-expressing cells in Ccr2-depleter mice adversely affected the ability of kidney and brain, but not of liver and spleen, to control fungal proliferation, highlighting the differential roles of Ccr2 and Cx3cr1 in modulating hepatic anti-*Candida* innate immunity [69,70].

The role of other mononuclear phagocyte-targeted chemotactic factors in systemic candidiasis has not been thoroughly explored. We have previously reported that Ccr1 does not mediate the accumulation of monocytes, macrophages or DCs in *Candida*-infected tissues [77]. Other studies have shown that, although *Candida* exposure of the human monocyte cell line THP-1 and of human primary peripheral blood mononuclear cells resulted in induction of Ccr5 and its ligands Ccl3 and Ccl4, Ccr5 was dispensable for control of fungal growth and host survival in *Candida*-infected mice; however, the direct role of Ccr5 in accumulation and/or effector of mononuclear phagocytes was not examined in that study [78]. Additional work will be required to study the role of other chemotactic factors expressed by monocytes, macrophages and/or DCs in host defense against systemic and mucosal candidiasis. Furthermore, more studies are needed to shed light on the differential mechanisms by which mononuclear phagocytes respond to non-*albicans* *Candida* species such as *C. glabrata*, *C. tropicalis* and others, which have emerged as significant causes of morbidity and mortality in patients in the era of widespread azole prophylaxis [79].

Cryptococcosis

Cryptococcus is a yeast fungus ubiquitously found in soil and most commonly causes meningoencephalitis in human immunodeficiency Virus (HIV)-infected individuals. Cryptococcal meningitis is an AIDS-defining illness, with mortality rates that exceed 50% in parts of the world without availability of antiretroviral therapy (ART). In fact, cryptococcal meningitis is a leading cause of death in individuals infected with HIV globally, with over 600,000 deaths each year, greater than those caused by diseases such as tuberculosis [80–82]. Less often, *C. neoformans* affects non-AIDS individuals with iatrogenic depression of cell-mediated immunity, whereas *C. gattii* typically infect apparently immunocompetent individuals, some of which have neutralizing autoantibodies against GM-CSF [22,81,83].

The initial infection typically follows inhalation of *Cryptococcus* from the environment, which results in a robust innate and adaptive immune response that requires T cells and mononuclear phagocytes, including macrophages and DCs. When these immune responses are not generated within the lung, *C. neoformans* can disseminate to the systemic circulation, cross the blood-brain barrier, and replicate in the central nervous system (CNS) [81]. Both CD4⁺ and CD8⁺ T cells are essential for clearance of *C. neoformans*; Th1 (via production of IFN- γ and TNF- α) and Th17-associated responses are protective, while Th2-associated responses (via production of IL-4 and IL-13) are detrimental for clearance [82]. T cells appear to play their most prominent role in control of cryptococcosis via orchestration of the recruitment and activation of mononuclear phagocytes in the infected tissues [84].

With regard to mononuclear phagocytes, DCs are thought to be most important in the initiation of adaptive immune responses against *C. neoformans*. On the other hand, alveolar macrophages are the first cells that come in contact with *C. neoformans*, can rapidly internalize yeast cells [85,86], can produce TNF- α upon yeast uptake [85], and are able to directly kill *C. neoformans* [86]. However, there is some evidence to suggest that macrophages may also serve as a reservoir for fungal proliferation [82]. This dichotomy of macrophage function has been suggested to be due to the intrinsic activation status of the macrophage. Hence, classically-activated macrophages, promoted by a Th1 cytokine environment, are necessary for resolution of cryptococcal infection, as compared with alternatively-activated macrophages, promoted by a Th2 cytokine environment, which are unable to mediate fungal clearance [87].

Not surprisingly, several studies have examined the chemotactic factors that mediate trafficking and activation of monocytes and monocyte-derived inflammatory macrophages and DCs during *C. neoformans* infection to gain insight into the mechanisms of their function *in vivo*. One of these studies found that Ccl3 is induced in the lungs upon infection, and its neutralization via antibody during only the second week of the infection reduced the number of macrophages in the lungs by almost 70%, without impairment of T lymphocyte accumulation [88]. This decreased recruitment of macrophages was accompanied by a significant increase in lung fungal burden [88]. Another report confirmed the role of Ccl3 in pulmonary anti-cryptococcal host defense, as mice deficient in Ccl3 developed uncontrolled fungal proliferation in the lung and lethal infection [89]; however, this enhanced

susceptibility was not associated with impaired monocyte/macrophage recruitment in the infected lung as seen with Ccl3 antibody neutralization. Instead, *Ccl3*^{-/-} mice developed detrimental Th2-driven immunity with associated eosinophil-driven immunopathology [89]. Whether the discrepancy in macrophage accumulation between the two studies relates to the time-specific neutralization of Ccl3 via antibody in the former study or is due to compensatory mechanisms operational in *Ccl3*^{-/-} mice is currently unknown.

One of the chemokine receptors that binds Ccl3 is Ccr5. Interestingly, *Ccr5*^{-/-} mice were not a phenocopy of *Ccl3*^{-/-} mice during cryptococcosis [90]. Specifically, while Ccr5 deficiency led to uncontrolled CNS cryptococcal infection, it did not increase pulmonary fungal proliferation nor did it affect the recruitment of leukocytes in the lungs. Instead, Ccr5 deficiency was associated with diminished recruitment of mononuclear cells to the brain during infection, and Ccr5 was found to be necessary for elimination of extracellular polysaccharide from the brain [90]. Interestingly, *Ccr5*^{-/-} macrophages were severely impaired in their ability to be recruited to cryptococcal products, which indicates that Ccr5 may be critical for the ability of macrophages to respond to shed fungal elements in the brain [90]. The dependence on different chemotactic factors for organ-specific immune cell recruitment during cryptococcosis is another example of the highly idiosyncratic tissue-specific antifungal immune responses that have been observed during other fungal infections, such as aspergillosis [45,48] and systemic candidiasis [73,77].

Of interest, the differential organ-specific cellular and microbiological phenotypes observed in *C. neoformans*-infected Ccl3- and Ccr5-deficient animals suggest that Ccr1, the other receptor for Ccl3 [24], might be responsible for lung-dependent cell recruitment and fungal control during cryptococcosis. Thus, future studies should evaluate the role of Ccr1 in host defense against *C. neoformans* in mice. In addition, the heightened susceptibility of *Ccr5*^{-/-} mice to CNS cryptococcosis suggests that caution should be taken when considering the use of CCR5 inhibitors for the treatment of HIV-infected patients with prior or active cryptococcosis or those without a history of cryptococcosis who have low CD4⁺ T cell counts and are at risk for developing the infection [91].

Another chemokine axis important for the recruitment and activation of monocytes/macrophages during inflammation is Ccl2-Ccr2. Ccl2 is induced in the lung during *C. neoformans* infection, and its antibody neutralization during the second week of infection resulted in increased pulmonary fungal burden associated with >95% reduction in the accumulation of macrophages in the lung [92]. Of note, there was also decreased recruitment of CD4⁺ T cells to the *Cryptococcus*-infected lung with Ccl2 neutralization, which may have independently contributed to the increased mouse susceptibility [92].

Consistent with the findings in mice with neutralized Ccl2, *Ccr2*^{-/-} mice were unable to control *C. neoformans* proliferation in the lung and developed disseminated *Cryptococcus* infection in the spleen and brain [93] as opposed to wild-type mice that contained the infection within their lungs. The enhanced susceptibility of *Ccr2*^{-/-} mice to cryptococcosis appears to be due to two major mechanisms. Firstly, *Ccr2*^{-/-} mice had severely reduced numbers of inflammatory macrophages in the lung following infection [93]. This defective macrophage accumulation was not due to decreased proliferation of alveolar macrophages.

Instead, Ly6C^{high} monocytes were unable to egress from the bone marrow to traffic into the lung, where they would differentiate into inflammatory macrophages during infection. In the presence of functional Ccr2, monocytes were recruited into the lung and differentiated into classically-activated inflammatory macrophages, which were directly fungicidal against *C. neoformans* and expressed high levels of protective iNOS and TNF- α [94]. Therefore, the severely reduced accumulation of inflammatory monocyte-derived macrophages in the lungs of *Ccr2*^{-/-} mice impairs pulmonary clearance of *C. neoformans* by depriving the lungs of highly active effector cells during infection.

Secondly, Ccr2 deficiency skews the Th1/Th2 balance in the lung towards a detrimental Th2-type response. In line with this, lungs from infected *Ccr2*^{-/-} mice contained significantly reduced IFN- γ , increased IL-4, IL-5, IL-13, enhanced eosinophil accumulation, and highly elevated serum IgE levels [93]. The mechanism by which this Th2 bias occurs during Ccr2 deficiency appears to be due to defective DC accumulation in the infected lung, as well as decreased DC-CD4⁺ T cell co-localization within bronchovascular infiltrates [95]. As with inflammatory macrophages, impaired DC accumulation in the absence of functional Ccr2 is the result of reduced recruitment of Ly6C^{high} monocytes that differentiate into DCs in the infected lung, not impaired proliferation of lung-resident DCs [96].

Because Ccr2-deficient mice had a more profound impairment in host defense during *C. neoformans* infection compared to mice that underwent Ccl2 neutralization, it is likely that other Ccr2 ligands, including Ccl7 and Ccl12, may also mediate mononuclear phagocyte recruitment and activation and confer protection against *Cryptococcus* infection. Indeed, Ccl2, Ccl7, and Ccl12 are all up-regulated during *C. neoformans* infection, though the time-course and magnitude of induction differ for each of these chemokines [95]. In support of a protective role for Ccr2 ligands other than Ccl2, Tlr9-deficient mice develop increased lung cryptococcal fungal burden, associated with decreased IFN- γ , impaired accumulation and activation of CD11b⁺ DCs and exudate macrophages, and decreased levels of Ccl7 and Ccl12. Interestingly, reconstitution of Ccl7 in Tlr9^{-/-} mice was sufficient to significantly increase the accumulation and activation of CD11b⁺ DCs, increase IFN- γ production, and, at least partially, ameliorate the lung fungal proliferation [97]. As opposed to Ccl2 and Ccl7, the role of Ccl12 in anti-cryptococcal host defense has not been studied and, thus, deserves future investigation.

Interestingly, it has been found that TNF- α can induce the production of both Ccl2 [98] and Ccl3 [99], the major mononuclear phagocyte-targeted chemokines in *Cryptococcus*-infected lung, is highly up-regulated during infection with *C. neoformans*, and is integral for its clearance [82]. In agreement with the dependence of Ccl2 and Ccl3 production on TNF- α , neutralization of TNF- α leads to markedly decreased recruitment of leukocytes, including an almost complete lack of macrophage accumulation in infected mouse tissue [100]. Not surprisingly, patients treated with TNF- α inhibitors such as infliximab and etanercept are at risk for development of cryptococcosis [101] and future studies should determine whether such patients have impaired accumulation of mononuclear phagocytes at the sites of infection. Conversely, depletion of Ccl2 or Ccl3 impairs TNF- α induction in *C. neoformans*-infected lungs [88,92] suggesting that there is reciprocal cross-regulation between TNF- α and mononuclear phagocyte-targeted chemotactic factors during cryptococcosis. Therefore,

more research will be required to define the precise mechanisms by which TNF- α enhances the recruitment of mononuclear phagocytes in the lung and brain of infected mice.

While several studies have examined the molecular basis of mononuclear phagocyte-mediated host defense against *C. neoformans*, much less is known with regard to the molecular factors, including chemoattractants, which orchestrate host defense against *C. gattii* infection. Of note, recent research in mice has shown that, as opposed to what is seen during infection by *C. neoformans*, infection with *C. gattii* primarily targets the lung, but not the brain [102]. Specifically, *C. gattii*-infected mice succumb to inexorable pulmonary infection without uncontrolled proliferation within the CNS, which is the hallmark of *C. neoformans* infection. Mononuclear phagocytes are thought to be critical for host defense against both *C. gattii* and *C. neoformans*, but species-dependent differences in immune responses have not been thoroughly studied to date. Therefore, future studies should aim to characterize the differences in the role of mononuclear phagocyte-targeted chemotactic and other factors between the two agents of cryptococcosis, which target apparently different tissues and cause infection in apparently different hosts.

Infections caused by Endemic Dimorphic Fungi

Histoplasma capsulatum, *Coccidioides immitis* and *Blastomyces dermatitidis* are dimorphic fungi that are endemic in certain parts of the United States [103]. These fungi are facultative intracellular pathogens, which are present in the conidial developmental state in the soil at room temperature and convert to the yeast form when present in the human body. While immunocompetent individuals exposed to these fungi may develop asymptomatic or self-limited pulmonary infection, life-threatening disseminated infections may occur in immunocompromised hosts [104,105]. Among these fungi, *Histoplasma* is the most prevalent infecting ~500,000 individuals per year in the US [104,105].

Alveolar macrophages represent the first line of defense against *Histoplasma* and the other endemic fungi. Both resident macrophages and recruited phagocytes including neutrophils, monocytes/macrophages and dendritic cells are able to internalize *Histoplasma* conidia [106–109]. The outcome of histoplasmosis is determined at large by the activation state of macrophages; hence, classical activation of macrophages by the Th1 cytokines IL-12, IFN- γ , TNF- α and GM-CSF is protective for the host, whereas alternative activation of macrophages by Th2 cytokines such as IL-4 is known to be detrimental for fungal clearance [110]. In fact, in the absence of activation, resting macrophages may serve as a reservoir for the dissemination of the fungus to distant organs [111].

The most studied mononuclear phagocyte-targeted chemotactic factor in the context of histoplasmosis is Ccr2. Szymczak and colleagues [112] showed that intranasal *Histoplasma* inoculation in *Ccr2*^{-/-} mice results in greater mortality compared to wild-type mice, associated with increased pulmonary fungal burden and dissemination of the fungus to the spleen. Ccr2 is critical for the recruitment of mononuclear phagocytes in the *Histoplasma*-infected lung, as the percentage and absolute numbers of inflammatory monocytes, monocyte-derived iNOS-producing DCs and conventional DCs were all markedly decreased in *Ccr2*^{-/-} mice.

In addition to the impaired accumulation of these key effector cells during Ccr2 deficiency, there was a marked increase in the amount of IL-4 produced in the lungs of Ccr2-deficient *Histoplasma*-infected mice, which was pathogenic as neutralization of IL-4 resulted in a reduction in the fungal burden in Ccr2^{-/-} mice [112]. Instead, the production of pro-inflammatory cytokines was not affected by Ccr2 deficiency. Increased IL-4 in Ccr2^{-/-} mice led to alternative activation of alveolar and tissue macrophages resulting in impaired fungal clearance. In fact, a recent study by the Deepe group showed that IL-4 acts in synergy with *Histoplasma* to amplify IL-33 production by macrophages via STAT-6/IRF-4 and dectin-1 signaling [113]. In turn, IL-33-primed macrophages are permissive for increased intracellular fungal growth and survival, and neutralization of IL-33 in Ccr2^{-/-} mice restores host resistance during histoplasmosis [113]. The source of IL-4 in the lungs of Ccr2^{-/-} mice is both mononuclear phagocytes (macrophages and DCs) [112] and CD4⁺ T cells [114]. Of interest, not only Ccr2^{-/-} DCs directly contribute to IL-4 secretion, but they also exhibit impaired expression of MHCII and CD40, which promotes a Th2-biased activation of CD4⁺ T cells that is partly responsible for the observed IL-4 phenotype in *Histoplasma*-infected lungs [114].

Investigation of the role of the Ccr2 ligands Ccl2, Ccl7 and Ccl12 during *Histoplasma* infection using Ccl2^{-/-} mice and antibody neutralization strategies revealed a combined role of Ccl2 and Ccl7 in promoting the detrimental IL-4 response seen in Ccr2^{-/-} mice [112]. Instead, neutralization of Ccl7 alone or Ccl7 along with Ccl12 or of Ccl2 along with Ccl12 did not phenocopy the molecular and microbiological phenotypes seen in Ccr2^{-/-} mice [112].

Besides Ccr2, another mononuclear phagocyte-targeted chemokine receptor that has been investigated in histoplasmosis is Ccr5 and its ligands Ccl3 and Ccl4. *Histoplasma* infection of the peritoneum or the lungs results in induction of Ccl3 and Ccl4 associated with recruitment of mononuclear phagocytes in the infected tissues [115–117]. Although the role of Ccl3 has not been directly investigated during histoplasmosis, *in vivo* studies have examined the role of Ccr5 and its other ligand Ccl4 and have revealed differential Ccr5-dependent responses depending on the phase of the infection. Specifically, in the early innate phase after *Histoplasma* pulmonary infection, mice deficient in Ccr5 or wild-type mice administered a neutralizing antibody against Ccl4 have increased tissue fungal burden and reduced proportion and absolute numbers of macrophages and DCs compared to control mice [116,117]. Despite this early increase in fungal proliferation, Ccr5^{-/-} mice exhibited accelerated clearance of *Histoplasma* compared to wild-type mice during the adaptive phase of the immune response. This late enhanced fungal clearance in the absence of Ccr5 is due to a protective Th17-Treg response that results in increased expression of IL-17 and decreased numbers of Foxp3⁺ regulatory T cells [116,117]. Thus, Ccr5 acts both on the early innate phase of the infection where it mediates a protective role via the recruitment of mononuclear phagocytes and on the late adaptive phase of the infection where it is detrimental via affecting the homing and proliferation of regulatory T cells. In fact, Ccr5 inhibition was recently shown to mitigate the deleterious effects of TNF- α antagonism during histoplasmosis by similarly modulating regulatory T cell responses [118], suggesting that targeted Ccr5 inhibition may be a potential therapeutic intervention in patients receiving

TNF- α antagonists, which are known to predispose to the development of histoplasmosis [119].

In addition to these “conventional” mononuclear phagocyte-targeted chemotactic factors, the immune response against *Histoplasma* infection is also modulated by leukotriene production. Specifically, blockade of leukotriene function either by administration of the leukotriene synthesis inhibitor MK-886 or by using mice deficient in 5-lipoxygenase (5-LO), the enzyme required for downstream leukotriene synthesis, has been shown to increase pulmonary fungal burden and enhance the dissemination of the fungus to the spleen, which collectively lead to decreased host survival [120–122]. Leukotriene inhibition did not affect the recruitment of mononuclear phagocytes in the lungs of *Histoplasma*-infected animals, although there was a reduction in proliferation and blunted T cell recruitment in 5-LO-deficient mice [122]. Instead, leukotrienes were critical for the induction of effective mononuclear phagocyte-mediated immunity. Specifically, macrophages isolated from 5-LO-deficient mice exhibited markedly reduced phagocytosis of both opsonized and unopsonized *Histoplasma* yeast compared to wild-type macrophages [120–122]. In addition, the production of nitric oxide, a critical mediator of intracellular fungal killing, was significantly reduced in both 5-LO-deficient macrophages and macrophages obtained from mice treated with MK-866 relative to wild-type macrophages [120,122]. Last, consistent with the known role of leukotriene signaling in NF- κ B-dependent cytokine secretion, the production of the pro-inflammatory Th1 cytokines IL-2, IL-12 and IFN- γ by T cells was decreased in mice with impaired leukotriene production resulting in a local immunological milieu that was not permissive for protective classical macrophage activation [120,121].

Much less is known about the mechanistic role of chemotactic factors in host defense against the other endemic dimorphic fungi. A study by the Klein lab [123] examined the vaccine response to live attenuated *Blastomyces* when injected subcutaneously and found that Ccl7- and Ccr2-dependent recruitment of Ly6C^{hi} monocyte-derived DCs is critical for the internalization and transfer of fungal antigen to the draining lymph nodes for the development of protective vaccine-mediated immune responses. In contrast, when the same vaccine was administered via the intratracheal route it failed to induce a protective immune response associated with significantly reduced numbers of Ly6C^{hi} monocyte recruitment in the lungs and draining lymph nodes, decreased maturation of monocytes into DCs, and reduced CD4⁺ T cell priming [124]. This blunted response in the lung was due to inactivation of Ccl7 by fungus-induced matrix metalloproteinase (MMP)-2 production and was restored by administration of either recombinant Ccl7 or MMP-2 inhibitors [124]. This study highlights both the importance of mononuclear phagocyte-targeted chemotactic factors in mounting a protective vaccine response and the development of fungal evasion strategies *in vivo* via inactivation of such chemotactic factors. Future studies should expand the investigation on the role of mononuclear phagocyte-targeted chemotactic factors in blastomycosis caused by virulent *Blastomyces* strains, in coccidioidomycosis, as well as in the other endemic dimorphic fungi *Sporothrix schenckii* and *Penicillium marneffei* that cause lymphocutaneous infections in healthy individuals and disseminated infections in AIDS patients, respectively.

***Pneumocystis* Pneumonia (PCP)**

PCP is a major cause of pulmonary infection in patients with AIDS [15], and it is caused by *Pneumocystis jirovecii*, a ubiquitous fungus that shares some biologic characteristics with protozoa. Prior to the widespread use of *Pneumocystis* prophylaxis and ART, PCP occurred in almost 80% of patients with AIDS, typically those with CD4 counts of less than 200 cells/mm³, and was associated with mortality rates of 20–40% [15].

Several *ex vivo* and *in vivo* studies have demonstrated that alveolar macrophages are critical for phagocytosis and killing of *Pneumocystis* and secretion of pro-inflammatory cytokines such as TNF- α and IL-8 upon *Pneumocystis* exposure via dectin-1-, TLR2- and mannose receptor-dependent sensing [125–130]. Interestingly, as opposed to the deleterious effects of alternative macrophage activation during cryptococcosis and histoplasmosis [94,113], it was recently demonstrated that alternative polarization of mouse alveolar macrophages resulted in enhanced killing against *Pneumocystis*, which was augmented by exogenous IL-33 administration [131]. In agreement, IL-33 treatment of wild-type mice led to alternative activation of macrophages in the lung associated with induction of the M2-macrophage chemokine Ccl17 and improved *Pneumocystis* clearance without affecting mononuclear phagocyte recruitment [131]. Therefore, IL-33 has strikingly opposing role in macrophage-dependent host defense against *Histoplasma* and *Pneumocystis* [113,131]. In humans, decreased binding and phagocytosis of *Pneumocystis* by macrophages of HIV-infected patients associated with impaired expression of macrophage mannose receptor, and depressed macrophage oxidative burst in response to *Pneumocystis* have been suggested as potential mechanisms to account for the susceptibility to the infection in AIDS patients [132,133].

Nonetheless, the molecular mechanisms that mediate trafficking and activation of mononuclear phagocytes *in vivo* during PCP are still poorly understood. A recent study utilized *Pneumocystis*-infected wild-type and highly susceptible CD40 ligand-deficient mice to examine the induction of chemotactic factors *in vivo*. It was found that the mononuclear phagocyte-targeted Ccr2 and its ligands Ccl2, Ccl7 and Ccl12, as well as Ccr5 and its ligands Ccl4 and Ccl5 were highly induced after pulmonary *Pneumocystis* challenge [134]. However, no study has thus far examined the role of these and other chemotactic factors *in vivo* and future work will be needed to determine the chemoattractants that mediate protective accumulation and effector function of resident and recruited mononuclear phagocytes during *Pneumocystis* infection.

Conclusions

The first report of the human chemokine receptors CXCR1 and CXCR2 in 1991 spearheaded an explosion in the discovery of several G-protein coupled chemotactic factors and led to a deeper understanding of the mechanisms by which immune cells traffic to sites of infection and inflammation. The recent appreciation that these molecules mediate several cellular biological functions beyond their initially described role in directional cell movement has opened new avenues for defining their pleiotropic roles in immunity. In the context of infection by a variety of human fungal pathogens, the work reviewed here has

emphasized the indispensable role of several chemotactic factors that target mononuclear phagocyte in orchestrating effective antifungal immune responses. Through these studies, not only the central role of mononuclear phagocytes in antifungal immunity was further highlighted but also valuable insight into the mechanisms by which effective antifungal host defense develops has been gained. Collectively, by enhancing our knowledge of how immune cells including mononuclear phagocytes are recruited and activated during fungal invasion should aid in the development of immune-based strategies to augment conventional antifungal therapy in humans suffering from fungal disease.

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Non standard abbreviations

AIDS	acquired immunodeficiency syndrome
DCs	Dendritic cells
TNF	Tumor necrosis factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
C5aR	C5a receptor
LTB4R1	Leukotriene B4 receptor 1
FPR	Formyl-peptide receptor
PAFR	Platelet-activating factor receptor
NK	Natural killer
HSC	Hematopoietic stem cells
ART	Antiretroviral therapy
HIV	Human Immunodeficiency virus
CNS	Central nervous system
5-LO	5-lipoxygenase
MMP	Matrix metalloproteinase
PCP	<i>Pneumocystis pneumonia</i>

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Figure 1. Renal resident mononuclear phagocytes mediate early innate control of *Candida* in the infected kidney

Yeast (left panel) and filamentous (right panel) elements of dTomato-expressing *C. albicans* are engulfed and encircled by $Cx3cr1^{+}$ renal resident mononuclear phagocytes, respectively, 2 hours after intravenous yeast challenge in *Cx3cr1^{gfp/+}* mice. Bars, 5 μ m (left panel); 7 μ m (right panel).

Table 1

Summary of the known mononuclear phagocyte-targeted chemokine receptors, their ligands, their mechanisms of action and their contribution to specific fungal immune responses.

Chemoattractant receptor	Agonists	Functions on mononuclear phagocytes	Fungal infection with mononuclear phagocyte-dependent chemoattractant contribution to host defense
CC Chemokine Receptors			
CCR1	CCL3, CCL5, CCL7, Ccl6 (m), Ccl9 (m), CCL13-16 (h), CCL23 (h)	Monocyte/ macrophage migration, Cytokine production	Systemic (but not mucosal) candidiasis, Disseminated (but not pulmonary) aspergillosis
CCR2	CCL2, CCL7, CCL12, CCL13, CCL16 (h)	Monocyte egress from the bone marrow into the blood	Systemic candidiasis, Pulmonary aspergillosis, Cryptococcosis Histoplasmosis Blastomycosis
CCR4	CCL17, CCL22	Monocyte migration	ND
CCR5	CCL3, CCL4, CCL5, CCL7, CCL14 (h), CCL16 (h)	Monocyte migration	CNS (but not pulmonary) cryptococcosis, Histoplasmosis
CCR6	CCL20	Dendritic cell migration	Pulmonary aspergillosis
CCR7	CCL19, CCL21	Dendritic cell migration and maturation	Pulmonary aspergillosis, blastomycosis
CX3C Chemokine Receptors			
CX3CR1	CX3CL1	Monocyte adhesion, migration and differentiation, Monocyte and macrophage survival, Macrophage killing	Systemic (but not mucosal) candidiasis
CXC Chemokine Receptors			
CXCR1	CXCL5, CXCL6 (h), CXCL8 (h)	Monocyte migration	ND
CXCR2	CXCL1–3, CXCL5, CXCL6 (h) CXCL7, CXCL8 (h)	Monocyte migration	Pulmonary aspergillosis
CXCR4	CXCL12	Monocyte migration, Macrophage phagocytosis	ND
Other Chemoattractant receptors			
C5aR	C5a	Monocyte/macrophage migration	ND

Chemoattractant receptor	Agonists	Functions on mononuclear phagocytes	Fungal infection with mononuclear phagocyte-dependent chemoattractant contribution to host defense
LTB4R1	LTB4	Monocyte, macrophage and DC migration, Monocyte adhesion, Proinflammatory cytokine production by monocytes, Macrophage proliferation and phagocytosis DC activation and cytokine production	Histoplasmosis
FPR1	<i>N</i> -formylated peptides	Monocyte migration and activation	ND
FPR2	<i>N</i> -formylated peptides	Monocyte migration	ND

ND, not determined; CNS, central nervous system; m denotes that the ligand is only present in mice and h denotes that the ligand is only present in humans.

Table 2

Summary of the mechanisms by which chemoattractant receptors and their ligands mediate fungus- and tissue-specific mononuclear phagocyte-mediated antifungal immunity.

Infection	Organ affected	Chemokine /chemokine receptor involved	Mechanism (mononuclear phagocyte involved)
Aspergillosis	Lung	Ccr2 (protective)	1. <i>Aspergillus</i> killing (Monocytes, DC's) 2. Production of pro-inflammatory cytokines, including tumor necrosis factor (TNF)- α (Monocytes, DCs) 3. Priming the conidiacidal activity of neutrophils (Monocytes) 4. <i>Aspergillus</i> -specific CD4 T cell priming (Monocyte derived DCs)
		Ccr6 (protective)	Cell accumulation via recruitment (DCs)
		Ccr7 (detrimental)	Cell efflux out of the lungs to draining lymph nodes (DCs)
Candidiasis	Kidney	Cx3cr1 (protective)	1. Cell accumulation via survival (Macrophages) 2. <i>Candida</i> killing (Macrophages)
		Ccr2 (protective)	Cell accumulation via recruitment (Monocytes)
Cryptococcosis	Lungs	Ccl3 (protective)	1. Cell accumulation* (Macrophages) 2. Suppression of Th2-driven immunity and eosinophil-driven immunopathology
		Ccr2 via Ccl2 and likely Ccl7 (protective)	1. Cell accumulation via recruitment (Monocytes, macrophages, DCs) 2. Development of protective Th1 immunity via DC accumulation and DC-CD4 co-localization
	Brain	Ccr5 (protective)	1. Cell accumulation via recruitment (Macrophages) 2. Elimination of extracellular cryptococcal polysaccharide (Macrophages)
Histoplasmosis	Lungs	Ccr2 via Ccl2 and Ccl7 (protective)	1. Cell accumulation via recruitment (Monocytes, DCs) 2. Classical cell activation (Macrophages) 3. Inhibition of Th2-driven immunity
		Ccr5 via Ccl4 (protective in innate phase and detrimental in adaptive phase)	1. Cell accumulation via recruitment (Macrophages, DCs) 2. Enhanced Th17 and decreased Foxp3 ⁺ regulatory T cell responses during the adaptive phase of the infection
		Leukotrienes (protective)	1. Phagocytosis of opsonized and unopsonised yeast cells (Macrophages) 2. Nitric oxide production (Macrophages)
Blastomycosis	Lungs	Ccr2 (protective vaccine-mediated immune response)	1. Cell accumulation via recruitment (Monocytes, DCs) 2. Fungal antigen internalization and transfer to the draining lymph nodes for the development of protective adaptive immune responses (Monocytes, DCs)

DCs, dendritic cells

* Discrepant results in Ccl3-dependent macrophage accumulation were seen in *Cryptococcus*-infected mice with genetic versus pharmacological inhibition of Ccl3.