The production of chemokines at the site of a fungal infection is critical for effective recruitment of leukocytes to that site. Over 40 chemokines and 20 chemokine receptors have been identified. The most intriguing biological property of chemokines is that they often play non-redundant roles in vivo even though they are highly related, have multiple activities and bind multiple chemokine receptors. Almost all of the chemokine studies to date have concentrated on responses to Cryptococcus, Candida, Aspergillus or Pneumocystis. The role of chemokines in infections caused by fungi such as Histoplasma, Blastomyces, Coccidioides and Paracoccidioides remains to be explored. In this review we have summarized what is currently known about the role of chemokines during fungal infection, including the influence of these signaling proteins on effector cell recruitment and development of cell-mediated immunity.

Keywords  chemokine, chemotaxis, fungi, leukocyte

Chemokines

The production of chemotactic factors at the site of a fungal infection is critical for effective recruitment of leukocytes to that site. A number of molecules are chemotactic for leukocytes and these molecules can be categorized into two groups. The first group includes: (i) peptides derived from activation of the complement pathway (C3a, C5a and C5a-desArg), (ii) leukotrienes and (iii) fungal products. There are also sporadic reports indicating that certain cytokines have secondary chemotactic activity. The second group includes chemokines, a supergene family of small inducible peptides with potent chemotactic activity for leukocyte subpopulations (reviewed in [1-4]).

Over 40 chemokines and nearly 20 chemokine receptors have been identified (Table 1) [1-4]. Chemokines are produced by a variety of cells including leukocytes, epithelial cells, endothelial cells, fibroblasts and smooth muscle cells following stimulation by cytokines or microbial products. Some chemokines such as monocyte chemoattractant protein-1 (MCP-1) are produced by almost all nucleated cells in the body, others such as monocyte inflammatory protein-1α (MIP-1α) are restricted to hematopoietic cells, while some of the more recently identified chemokines are only produced by a limited population of cells. The most intriguing biological property of chemokines is that they often play non-redundant roles in vivo even though they are highly related, have multiple activities, and bind multiple chemokine receptors. The same is true for the chemokine receptors; these receptors bind more than one chemokine but appear to mediate distinct biological activities.

Chemokines have been grouped into four different families based on the position of the first two conserved cysteine residues [2]: (i) XC (previously known as C), (ii) CC, (iii) CXC and (iv) CX3C. Over 90% of the chemokines identified to date are CC or CXC. Since many of the chemokines have been reported under more than one name, a revised nomenclature for chemokines and chemokine receptors has been proposed and submitted to the International Union of Immunological Societies [2,4]. Under the revised nomenclature, the CC chemokines I-309/TCA-3, MCP-1, MIP-1α, MIP-1β,
RANTES and eotaxin will be renamed CC chemokine ligand (CCL)1, CCL2, CCL3, CCL4, CCL5 and CCL11, respectively. The C-X-C chemokines melanoma growth stimulatory activity (MGSA–α/gro–α, PF4, ENA-78/LIX, neutrophil activating protein (NAP)-2, interleukin (IL)-8 and interferon-γ-induced protein (IP)-10 will be renamed CXC chemokine ligand (CXCL)1, CXCL4, CXCL5, CXCL7, CXCL8 and CXCL10, respectively. The use of the historical names for these chemokines longer accurately generalizes the biological activities of these two families of chemokines. There is much to be learned about the roles of chemokines and chemokine receptors during fungal infection. Almost all of the chemokine studies have concentrated on responses to Cryptococcus, Candida, Aspergillus or Pneumocystis. The role of chemokines in infections caused by fungi such as Histoplasma, Blastomyces, Coccidioides and Paracoccidioides remains to be explored. We have attempted to summarize the current understanding of the field in this review.

Cryptococcus neoformans

Cryptococcus neoformans is a widespread fungus found in such environmental niches as soil, eucalyptus trees and avian excreta [5]. C. neoformans is an opportunistic pathogen generally affecting patients that are immunodeficient, although infection occasionally occurs in apparently normal individuals [6]. The primary route of entry for C. neoformans is via the lung where the organism may establish a primary infection [7]. If the initial pulmonary infection is not controlled, C. neoformans can disseminate to other organs and the central nervous system (CNS), resulting in fatal cryptococcal meningoencephalitis [6,8]. Clearance of C. neoformans infection requires the development of a Th1-type cell-mediated immunity (CMI) and the subsequent pulmonary recruitment and activation of leukocytes [9–12] (T1

### Table 1  Leukocyte expression and primary ligands of the chemokine receptors

<table>
<thead>
<tr>
<th>Chemokine receptor</th>
<th>Cell type</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR1</td>
<td>Neutrophil, monocyte, DC</td>
<td>IL-8/MIP-2 (CXCL8); GCP-2/LIX (CXCL6)</td>
</tr>
<tr>
<td>CXCR2</td>
<td>Neutrophil</td>
<td>IL-8/MIP-2 (CXCL8); GCP-2/LIX (CXCL6); GRO-α, β, γ/KC (CXCL1, 2, 3); ENA-78 (CXCL5); NAP-2 (CXCL7)</td>
</tr>
<tr>
<td>CXCR3</td>
<td>Th1, NK</td>
<td>MIG (CXCL9); IP-10 (CXCL10); I-TAC (CXCL11)</td>
</tr>
<tr>
<td>CXCR4</td>
<td>Monocyte, DC, resting T cell</td>
<td>SDF-1 (CXCL12)</td>
</tr>
<tr>
<td>CXCR5</td>
<td>B cell, activated T cell</td>
<td>BLB/BCA-1 (CXCL13)</td>
</tr>
<tr>
<td>CCR1</td>
<td>Monocyte, DC, Th1, eosinophil, basophil</td>
<td>MIP-1α (CCL3); RANTES (CCL5); MCP-3 (CCL7); HCC-1 (CCL14); HCC-2/Lkn-1/MIP-1γ (CCL15); HCC-4/LEC/LCC-1 (CCL16); MPIF-1 (CCL23)</td>
</tr>
<tr>
<td>CCR2</td>
<td>Monocyte, DC, activated T cell, NK, basophil</td>
<td>MCP-1/JE (CCL2); MCP-3 (CCL7); MCP-4 (CCL13); MCP-5 (CCL12); RANTES (CCL5); MCP-3 (CCL7); MCP-4 (CCL13); eotaxin (CCL11); HCC-4/LEC/LCC-1 (CCL16); MPIF-1/eotaxin-2 (CCL24); eotaxin-3 (CCL26)</td>
</tr>
<tr>
<td>CCR3</td>
<td>Th2, eosinophils, basophils</td>
<td>TARC (CCL17); MDC/STCP-1/ABCD-1 (CCL22)</td>
</tr>
<tr>
<td>CCR4</td>
<td>DC, basophil, Th2</td>
<td>TARC (CCL17); MDC/STCP-1/ABCD-1 (CCL22)</td>
</tr>
<tr>
<td>CCR5</td>
<td>Monocyte, DC, Th1</td>
<td>MIP-1α (CCL3); MIP-1β (CCL4); RANTES (CCL5)</td>
</tr>
<tr>
<td>CCR6</td>
<td>DC</td>
<td>MIP-3α/LARC/exodus-1 (CCL20)</td>
</tr>
<tr>
<td>CCR7</td>
<td>DC, naïve T cell, B cell</td>
<td>MIP-3β/ELC/exodus-3 (CCL19); 6ckine/SLC/exodus-2/TCA-4 (CCL21)</td>
</tr>
<tr>
<td>CCR8</td>
<td>Th2, monocyte</td>
<td>I-309/TCA-3/P500 (CCL1)</td>
</tr>
<tr>
<td>CCR9</td>
<td>Immature T cell</td>
<td>TECK (CCL25)</td>
</tr>
<tr>
<td>CCR10</td>
<td>naïve T cell, monocytes</td>
<td>CTACK/ ILC/ALP/Eskine (CCL27); CCL28</td>
</tr>
<tr>
<td>XCR1</td>
<td>T cell, NK</td>
<td>Lymphotactin/SCM-1α/ATAC (XCL1); SCM-1α (XCL2)</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>NK, DC, monocytes, activated T and B cells</td>
<td>Fractalkine/neurotactin (CX3CL1)</td>
</tr>
</tbody>
</table>

Ligands are presented with the historical or common names (human or murine) first and followed by the name based on the new classification system [2] in parentheses. DC, dendritic cell; NK, natural killer cell; Th1, type 1 helper T cell; Th2, type 2 helper T cell.
and T2 refer to the general characteristics of the immune response in terms of cytokines produced or specific cell types recruited; the specific effector cell has not been examined. The leukocytic infiltrate in response to cryptococcal infection includes a mix of myeloid and lymphoid cells, all of which are capable of inhibiting the growth of, or killing, the organism in vitro [10,13]. However, macrophages, which are recruited and activated by immune T cells, appear to be the predominant cellular mediators of cryptococcal killing in vivo [13–15]. Recent investigations have focused on the role of chemokines in the development of CMI in response to C. neoformans and have revealed that not only are these signaling proteins involved in leukocyte recruitment, but they can also be important in T1/T2 immune response polarization.

Several studies have established that C. neoformans and its major capsular component, glucuronoxylomannan (GXM), can induce the production of chemokines in human leukocytes in vitro. C. neoformans has been shown to induce the release of MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4 and RANTES/CCL5 from human peripheral blood monocytes [16,17]. Furthermore, the levels of chemokine release are comparable between monocytes from human immunodeficiency syndrome (HIV)-positive and negative patients, suggesting that the lack of an inflammatory response seen in acquired immune deficiency syndrome (AIDS) patients with cryptococcosis is not secondary to reduced β-chemokine release from these cells [16,17]. Interestingly, C. neoformans did not induce MCP-1 release from alveolar macrophages; this is in contrast to lipopolysaccharide (LPS) which induced MCP-1 from both blood monocytes and alveolar macrophages [17]. GXM was found to induce the release of IL-8/CXCL8 from human polymorphonuclear (PMN) cells in a complement- and concentration-dependent manner [18,19]. Similarly, the level of IL-8 secretion was found to correlate with C. neoformans capsular size, suggesting that the amount of encapsulation/GXM produced by C. neoformans may be important in inducing chemokine secretion in vivo [18]. GXM also increases IL-8 secretion from fetal microglia but inhibits IL-8 induced PMN migration, possibly via a mechanism involving cross-desensitization [20]. This finding may explain the clinical observation that the cerebrospinal fluid of patients with cryptococcal meningoencephalitis contains high levels of IL-8 but few PMN [20,21]. Thus, these in vitro investigations demonstrate that C. neoformans and its capsular components can induce the production of chemokines from human cells.

In vivo investigations using murine infection models have provided insight into the role of chemokines during the immune response to C. neoformans. Intrapulmonary levels of MCP-1, MIP-1α and ENA-78/CXCL5 have been found to increase following intratracheal inoculation of C. neoformans [12,22–24]. MCP-1 levels are increased during the first 1–2 weeks of infection, whereas MIP-1α and ENA-78 are more delayed appearing at 2 and 3 weeks, respectively [12,22,24]. Administration of neutralizing antibodies to either MCP-1 or MIP-1α during the effenter phase of immunity (days 5–11) resulted in a 3-fold increase in fungal burden at 2 weeks post-infection [22,24]. Neutralization of MCP-1 abolished the recruitment of macrophages and reduced CD4+ T cell recruitment by 76% [22]. Similarly, MIP-1α neutralization reduced macrophage and neutrophil recruitment by 66% and 42%, respectively [24]. MCP-1α was also found to be required for the cellular recruitment phase of a recall response to cryptococcal antigen in the lungs of immunized mice [24]. Induction of MIP-1α is largely dependent on MCP-1 production given that MCP-1 levels were not affected by neutralization of MIP-1α, whereas neutralization of MCP-1 significantly decreased MIP-1α within the lungs of infected mice [24]. Thus, MCP-1, MIP-1α and ENA-78 are involved in the effector phase of CMI for maximal phagocyte recruitment into the lungs and subsequent clearance of C. neoformans.

Recent studies from our laboratory have shown that chemokine signaling can also play a role in T1/T2 immune response polarization during C. neoformans infection [12]. In mice lacking CCR2, the primary receptor for MCP-1 [25], intratracheal inoculation with C. neoformans resulted in delayed macrophage and CD8+ T-cell recruitment, as well as a lack of cryptococcal clearance through 6 weeks post-infection when compared to wild-type mice [12]. CCR2-deficient mice also exhibited significant dissemination of C. neoformans to the spleen and brain at 6 weeks post-infection. In contrast to the T1-type response generated by CCR2-expressing mice, CCR2-deficient mice produced a strong T2 immune response to pulmonary C. neoformans infection. The immune response in CCR2−/− mice was characterized by chronic pulmonary eosinophilia, crystal deposition in the lungs, pulmonary leukocyte production of IL-4 and IL-5 but not interferon-γ (IFN-γ), and increased serum immunoglobulin (Ig)E [12]. These results demonstrate that expression of CCR2 is required for the development of a T1-type response to C. neoformans infection and lack of CCR2 results in a switch to a T2-type response.

One possible mechanism that could account for the switch to T2 immunity in CCR2-deficient mice may involve the defective macrophage and CD8+ T-cell trafficking/activation observed in these mice. It has previously been shown that depletion of CD8+ T cells...
during *C. neoformans* infection results in the production of predominantly T2 type cytokines by CD4+ T cells [10]. Therefore, IFN-γ production by CD8+ T cells could be important for the development of Th1-type CD4+ T-cell immunity to *C. neoformans*. It remains to be determined whether the CD8+ T-cell defect observed in the lungs of CCR2−/− mice is also observed in the lung-associated lymph nodes, the likely site of T1/T2 differentiation during pulmonary *C. neoformans* infection [26]. Activated macrophages are an important source of cytokines, such as IL-12, that influence a developing Th1 response in the lungs and lymph nodes. IL-12 has been shown to induce IFN-γ-dependent increases in both mononuclear cell infiltration and MCP-1 production, resulting in a protective response to pulmonary *C. neoformans* infection [27,28]. Since monocytes/macrophages and CD8+ T cells express CCR2 [29] and a lack of CCR2 expression prevents recruitment of these cells into the lungs following *C. neoformans* infection, it is possible that there are similar trafficking defects of these and other antigen-presenting cells in the lymph nodes. Thus, these trafficking defects may account for the development of a T2 type immune response to *C. neoformans* in CCR2-deficient mice.

A second mechanism that could account for the switch to a T2 type response in CCR2-deficient mice might involve the interaction of MCP-1 with a receptor other than CCR2. A recent study has identified a second functional receptor for MCP-1 termed CCR11 [30]. Both uninfected and *C. neoformans*-infected CCR2-deficient mice exhibit lung levels of MCP-1 which are at least 3-fold higher than those seen in similarly treated wild-type mice [12]. Therefore, it is possible that in the absence of CCR2, MCP-1 interactions with CCR11 may predominate to drive a T2-type response during a pulmonary *C. neoformans* infection. Several studies have demonstrated that MCP-1 may drive Th2 differentiation both *in vitro* [31,32] and *in vivo* [33]. We have preliminary data that MCP-1 neutralization produces an effect in CCR2-deficient mice, confirming the presence of at least one other MCP-1 receptor that modulates the immune response.

Chemokines have also been found to be involved in the anti-cryptococcal delayed-type hypersensitivity (DTH) response [34,35]. Using implanted gelatin sponges injected with cryptococcal antigen, Doyle & Murphy [34,35] found that both MIP-1α protein levels and TCA3/I-309/CCL1 messenger RNA (mRNA) increased significantly during the resulting DTH response in immunized mice. The time course for the chemokine increases correlated with the recruitment of neutrophils and lymphocytes into the DTH-reactive sponges. Immunized mice treated with neutralizing antibody to either MIP-1α or TCA3 before sponge injection had reduced numbers of neutrophils and lymphocytes in the DTH-reactive sponges and also showed reduced clearance of *C. neoformans* from the lungs and brains when compared with controls. Injection of recombinant MIP-1α (rMIP-1α) into sponges in naive mice resulted in an increase in the influx of neutrophils and lymphocytes into the sponges while injection of recombinant T cell activation 3 (rTCA3) only produced an increase in neutrophil influx. Because TCA3/I-309/CCL1 neutralization reduced sponge levels of MIP-1α, but not MCP-1, it is likely that TCA3 induces neutrophil directly and influences lymphocyte recruitment indirectly via MIP-1α production. Thus, the chemokines MIP-1α and TCA3 are important components of the anti-cryptococcal DTH response.

The signaling pathways involved in chemokine production during *C. neoformans* infection are beginning to be delineated. Production of the early response cytokine tumor necrosis factor (TNF)−α is required for the development of T1 immunity and the subsequent clearance of *C. neoformans* from the lung [14,15,36,37]. Although TNF-α is not directly chemotactic for macrophages, treatment of *C. neoformans*-infected mice with antibodies against TNF-α significantly reduces macrophage recruitment into the lungs [36]. Neutralization of TNF-α also reduces lung levels of MCP-1 and MIP-1α, which likely explains the reduction in macrophage influx seen following this treatment [38]. These results, along with findings demonstrating that TNF-α can regulate chemokine production *in vitro* [39], support the hypothesis that TNF-α is an important proximal signal for the induction of MCP-1 and MIP-1α during a *C. neoformans* infection.

The Th1 type cytokines IL-12 and IFN-γ have also been found to be important in chemokine induction during *C. neoformans* infection. Highly virulent strains of *C. neoformans* have the ability to evade detection and prevent the development of an adequate immune response [28,40,41]. Pulmonary infection by highly virulent *C. neoformans* is characterized by limited leukocytic influx and very little production of chemokines resulting in dissemination of the organism and fatal meningoencephalitis [14,28,40–42]. Interestingly, administration of IL-12 from the onset of infection was found to induce macrophage and CD4+ T cell infiltration into the lungs, reduce pulmonary cryptococcal burden, and prevent meningoencephalitis [28,42]. Furthermore, IL-12 was also found to increase the lung levels of the chemokines MCP-1, RANTES, MIP-1α, MIP-1β and IP-10 [27,42]. Neutralization of IFN-γ completely abrogated the positive effects of IL-12 administration
including chemokine induction [27]. These results demonstrate that IL-12 can induce chemokine production via an IFN-γ-dependent pathway during a pulmonary *C. neoformans* infection.

**Candida albicans**

*Candida albicans* is part of the normal microbial flora associated with the mucosal surfaces of the oral cavity, gastrointestinal tract and vagina [43,44]. Immune dysfunction can allow *C. albicans* to switch from a commensal to a pathogenic organism capable of infecting a variety of tissues and possibly causing fatal systemic disease [43–45]. Resistance to *C. albicans* infection is associated with Th1 CMI, whereas Th2 immunity is associated with susceptibility to symptomatic infection [43,45,46]. Neutrophils are considered to be the primary effector cells for *C. albicans* killing in vivo [47–49] although macrophages are also involved in CMI to control infection [44,50]. In addition to their role as effector cells, neutrophils may also play an immunoregulatory role in Th response development [43,51]. The fact that neutrophils are (i) abundant at the sites of *C. albicans* infection and (ii) capable of selectively producing the directive cytokines IL-12 and IL-10, suggest that these cells may be important in determining the type of anti-*Candida* Th cell response [43]. Cytokines such as TNF-α, IL-6, granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage (GM)-CSF have been determined to be important for neutrophil recruitment during candidiasis [49]. However, the role of chemokines during *C. albicans* infection, and whether they mediate cytokine driven neutrophil influx, is relatively unknown at this time.

Although specific roles for chemokines in the control of *C. albicans* are just beginning to be studied, it has been shown that this organism can induce the production of chemokines. *In vitro* studies have shown that peripheral blood mononuclear cells produce MCP-1, MIP-1α, MIP-1β, MIP-2, RANTES, KC and IL-8 when treated with *C. albicans* [16,52–54]. *C. albicans* can also induce increases in MIP-1α and MCP-1 in human alveolar macrophages [52] and MIP-1β, MIP-2 and KC in murine peritoneal macrophages [55]. Neutrophils increase IL-8 and MIP-1α production [56,57], and endothelial cells produce MCP-1 and IL-8 [58] in the presence of *C. albicans*. Thus, chemokine production is induced in several cell types by *C. albicans* suggesting a role for these proteins in controlling infection.

Results from a study utilizing an experimental vaginal candidiasis model suggest that MCP-1 may have a role in controlling this type of infection [59]. A developing hypothesis for control of vaginal candidiasis is that local vaginal CMI functions independently to control *C. albicans* [60]. This is supported by the findings that (i) vaginal candidiasis can occur in women with normal levels of Th1 *Candida*-specific immunity and (ii) vaginal tissue contains T cells phenotypically distinct from systemic T cells [60]. Studies focusing on local CMI following intravaginal inoculation with *C. albicans* have demonstrated that MCP-1 mRNA and protein levels, but not MIP-1α, MIP-2 or RANTES levels, are increased in vaginal tissue following infection [59]. Furthermore, intravaginal neutralization of MCP-1 resulted in a significant increase in vaginal fungal burden early during infection. However, there were no differences in vaginal lavage leukocyte profiles between infected and uninfected mice. These findings suggest that MCP-1 may function to control vaginal *C. albicans* infection in a manner independent of its cellular chemotactic activity.

Recent studies demonstrated that truncated CXC chemokines might aid in the clearance of systemic *C. albicans* infection [61]. It was originally reported that the hematoregulatory peptide SK&F 107647 could provide protection to lethal doses of *C. albicans* in both immunosuppressed and immunocompetent mice by indirectly stimulating bone marrow progenitor cells and phagocytic cells [62]. SK&F 107647 was found to stimulate the release of stromal cell-derived hematopoietic synergistic factors (HSF) from both murine and human cell lines [61]. These HSFs were isolated and it was determined that the murine HSF is a truncated form of KC, while the human HSF was identified as a truncated form growth-related oncogene β (GROβ). Both of these truncated CXC chemokines had neutrophil activating properties in vitro as represented by increased superoxide and candidacidal activities. In addition, the truncated forms were at least 10 000-fold more potent than the full-length chemokines in producing enhancement of effector cell activities. *In vivo* administration of truncated KC increased survival of mice infected with a lethal dose of intravenous (i.v.) *Candida*. The increased survival produced by truncated KC is likely attributed to enhanced effector cell function. Thus, this study demonstrated that truncated CXC chemokines can provide protection from *C. albicans* infection [61].

The signaling pathways and regulation of chemokine production during *C. albicans* infection are also just beginning to be investigated. The early response cytokine TNF-α has been shown to enhance IL-8 and MIP-1α production by isolated human neutrophils [57]. Neutrophils also demonstrated increased IL-8 production when incubated with IL-15, a cytokine that augmented the candidacidal activity of these cells [63]. The *C. albicans*-induced enhancement of MIP-1β, MIP-2 and KC production in peritoneal macrophages was found...
to occur independently of mannose receptor activation, whereas this receptor was found to mediate *C. albicans*-induced increases in IL-1β, IL-6 and GM-CSF [55]. This finding demonstrates that signaling pathways involved in chemokine production can be distinct from those involved in cytokine production during *C. albicans* infection. It has also been shown that in human macrophages/monocytes, the *C. albicans*-induced production of MIP-1α and MCP-1, as well as TNF-α and IL-1β, can be down regulated by surfactant protein A [52]. The downregulation of chemokines and cytokines produced by surfactant protein A is apparently occurring at the transcriptional level. This finding suggests that surfactant protein A limits excessive pro-inflammatory cytokine release by macrophages/monocytes exposed to *C. albicans* in the alveolar space. Continued study will lead to further elucidation of the signaling pathways involved in chemokine production during *C. albicans* infection.

**Aspergillus fumigatus**

A ubiquitous and opportunistic fungus, *Aspergillus fumigatus* can cause several types of respiratory disorders including allergic responses and invasive infection [64,65]. *A. fumigatus*-induced allergic responses, such as allergic bronchopulmonary aspergillosis (ABPA), can be triggered following the germination of inhaled spores trapped within the luminal mucus of the bronchial airway [64]. Antigens released from the resulting *A. fumigatus* mycelium have been found to promote Th2 responses in sensitive individuals [64]. It has been suggested that CC chemokines may play a role in the increased number/activity of *A. fumigatus*-specific Th2 cells during ABPA [64], based on the general findings that Th1 and Th2 cells differentially express chemokine receptors [3,66]. Chemokines are also likely to play a role in the recruitment of another primary effector cell of ABPA, the eosinophil [64]. One study has shown that mRNA for RANTES is upregulated following sensitization of mice with *A. fumigatus* antigen [67]. In another study, intratracheal administration of *A. fumigatus* antigen into *A. fumigatus*-sensitized mice resulted in significantly increased lung levels of C10, MCP-1 and eotaxin [68]. The Th2-associated chemokine C10 was found to stimulate *in vitro* chemotaxis of eosinophils. This correlated with the finding that *in vivo* immunoneutralization of C10 prior to challenge produced significant reductions not only in peribronchial eosinophilia, but also in lymphocyte recruitment, bronchial hyper-responsiveness, MCP-1 and eotaxin levels. Furthermore, C10 neutralization inhibited IL-13 production but did not affect the IL-10 or IgE levels after challenge. Thus, C10 modulates many aspects of experimental ABPA including eosinophil recruitment and the production of other chemokines. Further investigation is needed to clarify the roles of chemokines in both Th2 development and effector cell recruitment during *A. fumigatus*-induced allergic responses.

In addition to allergy, *A. fumigatus* can also cause destructive invasive infection, most commonly involving the respiratory system [65,69]. Normally, the innate immune system is effective in controlling *A. fumigatus* once conidia have entered the alveoli [69]. The first line of defense is alveolar macrophages, which are capable of phagocytosing and killing conidia through mostly non-oxidative mechanisms [69]. In circumstances where conidia do happen to germinate, neutrophils and infiltrating mononuclear cells are capable of attacking and destroying the resulting hyphae by secreting both oxidative and non-oxidative microbicidal metabolites, thus preventing invasive infection [69]. Although CMI seems to play a secondary role in resistance to *A. fumigatus* in humans, Th1 type CMI is required for protection in murine models [65]. Defective innate immunity, such as neutropenia or that induced by immunosuppressive therapy, may result in invasive pulmonary aspergillosis (IPA) [65,69]. IPA is characterized by hyphal invasion and destruction of pulmonary tissue following attachment of *A. fumigatus* conidia to alveolar epithelium or submucosa [65,69]. Recent studies have shown that chemokine production is both induced by *A. fumigatus* and required for the prevention of IPA in murine models.

*A. fumigatus* has been found to stimulate chemokine production both *in vitro* and *in vivo*. Proteases from *A. fumigatus* induce the production of IL-8 and MCP-1 from human pulmonary epithelial cells lines [70,71]. The protease-induced production of IL-8 was found to occur via transcriptional mechanisms [70]. Induction of IL-8 is likely to be important for protection given that this chemokine is involved in both the recruitment of neutrophils and the stimulation of neutrophil phagocytosis of *A. fumigatus* conidia [72]. Isolated alveolar macrophages have been shown to produce MIP-1α, MIP-2 and KC, but not MCP-1 or IP-10 when exposed to conidia [73,74]. Two *in vivo* studies have demonstrated that intratracheal inoculation of mice with *A. fumigatus* conidia results in increased production of MCP-1, MIP-1α and MIP-2 by bronchoalveolar lavage (BAL) cells or as measured in whole lung homogenates [74,75]. The *A. fumigatus*-induced chemokine production peaked at day 1 post-infection and returned to near baseline levels by day 4 [75,76]. The increases in chemokine production were found to be, at least partially, mediated by the early response cytokine TNF-α [75]. KC was also induced by
A. fumigatus in BALB/c mice [76] but not in C57BL/6 mice [74,75]. However, when neutrophil-depleted, either strain of mouse was found to produce KC in the face of A. fumigatus challenge [75,76]. Although these studies demonstrate that chemokines are produced by cells of the respiratory tract following exposure to A. fumigatus, the role of chemokines in clearing the fungus are just beginning to be investigated.

Two studies have demonstrated that chemokines play a role in mediating neutrophil recruitment during A. fumigatus infection. The first study by Gao et al. [77] demonstrated that neutrophils from CCR1 knockout mice did not chemotax in vitro or mobilize into peripheral blood in response to MIP-1α. Consistent with this neutrophil defect, i.v. administration of A. fumigatus in CCR1 knockout mice resulted in accelerated mortality compared to wild-type mice [77]. These results suggest that one or more CC chemokines that bind CCR1 (MIP-1α, MCP-2 or RANTES) may be involved in neutrophil recruitment during systemic A. fumigatus infection in the mouse. In the second study, Mehrad et al. [76] found that ELR+ CXC chemokines that bind CXCR2, such as MIP-2 or KC, are important in preventing murine IPA. Neutralization of CXCR2 prior to intratracheal inoculation with A. fumigatus conidia resulted in invasive infection indistinguishable from that observed in neutrophil-depleted animals. CXCR2 neutralization was associated with reduced lung neutrophil influx and produced a significant increase in mortality compared with control animals. In contrast, animals with constitutive lung-specific expression of KC were resistant to A. fumigatus infection following neutrophil depletion. These animals had reduced mortality, a lower lung burden of fungus and increased neutrophil recruitment at sites of hyphal growth suggesting that KC can enhance the recruitment of the limited pool of available neutrophils to the lung. Thus, this study demonstrates that CXCR2 ligands are essential mediators of host defense against pulmonary A. fumigatus infection in the mouse. From these studies it is clear that chemokines play a role in neutrophil recruitment during pulmonary or systemic A. fumigatus infection. However, there is no current data for the role of chemokines in monocyte recruitment/activation following A. fumigatus infection.

It is apparent that most immunocompetent individuals have been exposed to P. carinii, although primary infection does not produce recognized illness [78,79]. Development of CMI and subsequent clearance of P. carinii requires CD4+ T cells and involves the cytokines TNF-α, IL-1 and IFN-γ [79,80]. A major role of CD4+ T cells is likely to be the activation of alveolar macrophages, given that these are the primary effector cells involved in P. carinii clearance. To date, very little is known about the role of chemokines in the development of effective CMI to P. carinii infection. However, there is mounting evidence that suggests the CXC chemokine IL-8 may be involved in PCP-induced lung destruction in immunocompromised patients.

One report provides support for a role for chemokines in the clearance of a pulmonary P. carinii infection [81]. This study utilized the severe combined immunodeficiency (SCID) model of infection. SCID mice lack T and B cells and are susceptible to PCP [80]. Administration of immunocompetent CD4+ T cells results in expression of pro-inflammatory cytokines and the clearance of P. carinii in infected SCID mice [82,83]. In the study by Wright et al. [81], the expression of several chemokines was examined in both lymphocyte-reconstituted and non-reconstituted P. carinii-infected SCID mice. It was determined that lack of detectable pulmonary inflammation in infected, non-reconstituted SCID mice correlated with a lack of pulmonary expression of chemokines. In contrast, lymphocyte-reconstitution of infected SCID mice resulted in signs of focal pulmonary inflammation and increased mRNA levels of RANTES, MCP-1, lymphotactin, MIP-1α, MIP-1β and MIP-2. The time course for chemokine mRNA abundance coincided with that of the inflammatory response and clearance of P. carinii. Chemokine levels were increased by 10 days post-reconstitution, reached a maximum at day 12, and then returned to baseline by day 22. In situ hybridization demonstrated that RANTES gene expression was localized to sites of inflammatory cell infiltration and P. carinii infection during the peak of inflammation. Thus, this study demonstrates that the presence of T lymphocytes is important in initiating an inflammatory cascade that includes the pulmonary expression of chemokines. Furthermore, chemokines are likely to play a role in the recruitment of leukocytes to the sites of infection.

Although the study of the role of chemokines during an effective CMI response to P. carinii infection is just beginning, there is more known about one CXC chemokine, IL-8, and its potential role in the pathogenesis of lung injury during PCP. Several studies have demonstrated a positive correlation between BAL levels of IL-8, BAL neutrophilia and P(A-a)O2 in HIV-positive patients with PCP [84,85]. Furthermore, IL-8 levels in

Pneumocystis carinii

Pneumocystis carinii pneumonia (PCP) is a hallmark opportunistic infection of AIDS but may also occur in other patients with impaired CMI [78]. Although the major environmental source of this atypical fungus is not known, the primary route of transmission is thought to occur animal-to-animal via the respiratory tract [78,79].
BAL fluid correlate to the clinical severity of PCP and are a predictor of mortality and severe respiratory compromise [86]. BAL IL-8 has been confirmed to mediate neutrophil chemotaxis in vitro and is a likely mediator of pulmonary neutrophil recruitment during AIDS-associated PCP [87]. The source of IL-8 during PCP could be alveolar epithelial cells or macrophages-monocytes given that *P. carinii* gpA has been found to stimulate IL-8 production in these cells [88,89]. The mannose receptor was found to mediate the gpA-induced monocyte production of IL-8 [90]. These studies correlate IL-8 with lung pathogenesis during PCP in the immuno-omprised patient. However, it is not known if the increases in IL-8 are causative of, or a result of, lung destruction. The role of the neutrophil in lung pathogenesis is also unclear.

**Conclusions and summary**

The importance of recruitment of specific leukocyte populations in protection from fungal infection has been well established. Macrophages are the primary cells involved in fungal killing during *C. neoformans* and *P. carinii* infection, whereas neutrophils are the primary effector cell in preventing *C. albicans* and *A. fumigatus* infection. In addition to the type(s) of leukocyte(s), it has also been recognized that the type of CMI induced is critical in determining resistance or susceptibility to fungal infection. In general, Th1 type CMI is required for clearance of a fungal infection while Th2 immunity usually results in susceptibility to infection or fungal allergic responses [43,46,51,64,65]. Chemokines regulate a wide array of biological activities in addition to chemotaxis such as hematopoiesis, angiogenesis, cytokine induction, antigen-presentation and Th cell differentiation. All of these activities are important in acute and chronic fungal infections. In this review we have summarized what is currently known about the role of chemokines during fungal infection, including the influence of these signaling proteins on effector cell recruitment and development of CMI. Future research will continue to elucidate the roles of chemokines and chemokine receptors during infection by *Cryptococcus, Aspergillus* and *Pneumocystis* but also determine their role in infections caused by fungi such as *Histoplasma, Blastomyces, Coccioidioides* and *Paracoccidioides* (to name a few).

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**References**


26 Hoag KA, Lipscomb MF, Izzo AA, et al. IL-12 and IFN-gamma are required for initiating the protective Th1 response to pulmonary cryptococcosis in resistant C.B-17 mice. *Am J Respir Cell Mol Biol* 1997; 17: 733–739.


82 Roiths JB, Sidman CL. Both immunity and hyperresponsiveness to Pneumocystis carinii result from transfer of CD4+ but not CD8+ T cells into severe combined immunodeficiency mice. J Clin Invest 1992; 90: 673–678.


