T-cell Subsets and Antifungal Host Defenses

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Abstract It has been long appreciated that protective immunity against fungal pathogens is dependent on activation of cellular adaptive immune responses represented by T lymphocytes. The T-helper (Th)1/Th2 paradigm has proven to be essential for the understanding of protective adaptive host responses. Studies that have examined the significance of regulatory T cells in fungal infection, and the recent discovery of a new T-helper subset called Th17 have provided crucial information for understanding the complementary roles played by the various T-helper lymphocytes in systemic versus mucosal antifungal host defense. This review provides an overview of the role of the various T-cell subsets during fungal infections and the reciprocal regulation between the T-cell subsets contributing to the tailored host response against fungal pathogens.

Keywords T helper cells · Gamma delta T cells · Cytotoxic T cells · Regulatory T cells · Th1 · Th17 · Th2 · Fungal infection · Candida · Aspergillus · Histoplasma · Pneumocystis · Cryptococcus · IL-17 · Interferon gamma · IL-4 · IL-10 · Plasticity · Interaction · Adaptive immunity · Cytokine responses

Introduction

The spectrum of fungal infections ranges from superficial skin infection to lethal invasive disease. Pathogenic fungi cause different clinical syndromes dependent on the location of infection, virulence, and host status. Dermatophytes such as Trichophyton rubrum are acquired by direct contact and can cause superficial infection of the skin and nails in immunocompetent patients. Candida albicans is a commensal that can be efficiently controlled by the immune system in normal conditions, but when the immune system becomes compromised, it can cause invasive disease. Filamentous fungi such as Aspergillus spp. are inhaled and in certain conditions will induce an allergic reaction in the lungs known as allergic bronchopulmonary aspergillosis (ABPA), whereas in a neutropenic host they can cause lethal disease. These differences illustrate the large spectrum of infections caused by pathogenic fungi and justify the need to induce tailored innate and/or adaptive immune responses to overcome infection.

T lymphocytes represent an important component of host defense against fungal pathogens, as reflected by the fact that patients with AIDS who lack CD4+ T cells are highly susceptible to C. albicans, Aspergillus fumigatus, Cryptococcus neoformans, Histoplasma capsulatum, and Pneumocystis jiroveci. This review focuses on our current knowledge about the role of T-cell subsets in antifungal host defenses.

T-cell Subsets

Mosmann and Coffman [1] introduced the concept of different sets of T-helper (Th) cells defined on the basis of their function, especially the types of cytokines secreted by each of them. The so-called Th1 cells are characterized by the production of interferon-γ (IFNγ), which is essential for defense against intracellular pathogens, whereas Th2 cells are characterized by the production of interleukin (IL)-4 and are mainly important in allergic disease and host defenses against parasitic infections. Recently, a new subset of T-helper cells has been described, called Th17 cells, and...
these cells are characterized by the production of a distinct cytokine profile, namely IL-17A, IL-17F, IL-21, and IL-22. IL-17A is important for neutrophil recruitment and host defense against extracellular bacteria and fungi. The development of specific T-helper cells from naïve CD4+ T cells is dependent on antigen presentation by professional antigen-presenting cells, engagement of costimulatory molecules, and a specific cytokine milieu. The cytokines IL-23, IL-1β, IL-6, and transforming growth factor (TGF)-β induce the development of Th17 cells; IL-12 and IL-18 are important for Th1 differentiation; and IL-4 drives naïve T cells toward a Th2 phenotype.

In addition to the T-helper activating lymphocytes, other important subsets of T cells are the regulatory T cells (Tregs) and the CD8+ lymphocytes. Tregs serve an important function in host responses by controlling pathogen-specific immune responses and keeping autoreactivity in check. CD8+ T cells play an important role in infectious diseases by producing cytokines, They are also able to directly kill cells—hence the name cytotoxic T-cells. CD8+ T cells recognize peptides derived from pathogens that are presented by MHC class I molecules, and in this way are able to target cells that are infected. In contrast to conventional αβ T cells, γδ T cells are preferentially localized in nonlymphoid tissues such as the lungs and intestine, and their localization and function suggests an important role in the first line of innate host defense.

**Th1 Cells**

IFNγ produced by Th1 lymphocytes is important for stimulation of antifungal activity of neutrophils. The central role of endogenous IFNγ in the resistance against systemic fungal infection is underscored by the observation that knockout mice deficient in IFNγ are highly susceptible to disseminated *C. albicans* infection [2]. In addition, mice deficient in IL-18, which plays a crucial role in the induction of IFNγ, are also more susceptible to disseminated candidiasis [3]. IFNγ can also contribute to anti-*Candida* host defense by inducing nitric oxide (NO) production by macrophages, as well as *Candida*-specific immunoglobulin production [4]. Furthermore, it is well known that the incidence of oropharyngeal candidiasis is very high in patients with HIV who have low CD4+ counts and thus low Th1 responses. These data reflect the importance of Th1 cells in anti-*Candida* host defenses.

IFNγ also appears to be protective in the host defense against *Aspergillus*. Cells producing IFNγ are induced by *Aspergillus* in immunocompetent mice. Live conidia that undergo swelling and germination are able to prime Th1 responses [5]. It has been elegantly demonstrated that CD4+ T cells differentiate during respiratory fungal infection, with Toll-like receptor–mediated signals in the lymph node enhancing the potential for IFNγ production, whereas other signals promote Th1 differentiation in the lung [6]. In a well-designed study, adoptive transfer of dendritic cells pulsed with *Aspergillus* conidia increased resistance to invasive aspergillosis in murine recipients of allogeneic hematopoietic stem cell transplants by activating IFNγ-producing T lymphocytes [7]. Similarly, the adoptive transfer of *Aspergillus*-specific Th1 cells conferred protection against invasive aspergillosis in neutropenic mice that did not receive transplants [8]. There is also accumulating evidence in humans that Th1 responses provide protection against *Aspergillus*. In patients with invasive aspergillosis, a predominant release of IFNγ in culture supernatants on stimulation with *A. fumigatus* antigens, which indicates a Th1 response, was associated with a favorable outcome [9].

Mice deficient in the IFNγ receptor are also highly susceptible to pulmonary cryptococcosis and display increased lung fungal burdens and dissemination to the brain [10]. IL-4/IL-13 double-knockout mice develop robust Th1 and Th17 responses in the absence of Th2 polarization [11]. When these mice are infected with *Cryptococcus*, they display significantly less fungal growth in the lungs than wild-type mice. However, although systemic dissemination was delayed in the IL-4/IL-13 double-knockout mice, the infection progressed with a rate similar to the rate in the wild-type mice. Thus, Th1 responses and Th17 responses play an important role in clearing *Cryptococcus* from the lungs, but they cannot prevent disseminated infection [11]. A critical role for Th1 responses in the host defense against *Histoplasma capsulatum* has been demonstrated by the fact that CD4+ T cells from wild-type mice protect T cell–deficient mice from *Histoplasma* infection, whereas CD4+ T cells from IFNγ-deficient mice were unable to provide protection [12]. Overall, these data demonstrate that Th1 responses play an important protective role during invasive fungal infection.

**Th17 Cells**

The observation that mice deficient in IL-17RA (IL-17R-deficient) show an increased susceptibility to disseminated *C. albicans* infection first demonstrated the critical involvement of Th17 responses in protective anti-*Candida* host defenses [13]. In addition, mice deficient in Th17 responses were highly susceptible to oropharyngeal candidiasis [14•]. Another recent study demonstrated that IL-17A induced by dectin-2, a C-type lectin receptor (CLR) that recognizes *Candida* mannans, is crucial for antifungal host defense in disseminated candidiasis [15•]. Although these studies suggest a protective role for IL-17A and Th17 responses in *Candida* infection, negative effects of
Th17-mediated inflammatory responses to intragastric *C. albicans* infection in mice have also been reported [16]. Furthermore, mice that are deficient in Toll IL1R8 (TIR8), which is a negative regulator of Th17 responses, show higher susceptibility to *Candida* and *Aspergillus* infection and have significantly more immunopathology [17]. On the other hand, patients with impaired *Candida*-specific Th17 responses, such as patients with chronic mucocutaneous candidiasis or hyper-IgE syndrome, are especially susceptible to mucosal *C. albicans* infections [18••, 19]. These observations strongly indicate that Th17 responses are important for human anti-*Candida* mucosal host defense. Th17 induction in response to *Candida* infection is dependent on CLRs such as dectin-1, dectin-2, and mannose receptor. Furthermore, dectin-1 and dectin-2 signal through the Syk-CARD9 pathway, and CARD9 was shown to be involved in *Candida*-induced Th17 responses. Recently, it was reported that patients with genetic defects in dectin-1 or CARD9 suffer from chronic mucosal fungal infections [20••, 21•]. Both dectin-1 and CARD9 deficiency was shown to be associated with a deficient fungal-induced IL-17 response. These data further strengthen the crucial role of Th17 cells in human antifungal mucosal defense.

Patients with chronic granulomatous disease (CGD) lack NADPH oxidase activity and do not generate reactive oxygen species. The result is recurrent bacterial and fungal infections, especially fungal infections with *Aspergillus*. It has been reported that in the setting of deficient reactive oxygen species generation in a mouse model of CGD, the tryptophan metabolism in mice is deficient, which eventually results in increased IL-17 responses [22]. These increased IL-17 responses were suggested to be detrimental to the host when CGD mice were infected with *Aspergillus*. In contrast, *A. fumigatus* does not stimulate strong production of IL-17 in human cells, and human host defenses against aspergillosis may rely on potent Th1 rather than Th17 responses [23].

It has also been suggested that the IL-17 pathway plays an important role in *Pneumocystis carinii* infections. Stimulation of alveolar macrophages with *Pneumocystis carinii* induces IL-23 mRNA, and neutralization of IL-23 or IL-17 increased disease severity during *P. carinii* infection in wild-type mice [24]. Mice deficient in both Th1 and Th17 responses show a much higher mortality when infected with *Cryptococcus* than mice deficient in Th1 responses [25], indicating a protective role of Th17 responses in *C. neoformans* infection. In addition, mice deficient in Toll-like receptor 2 (TLR2) that were infected with *Paracoccidioides brasiliensis* had an increased Th17 response, which was associated with protection [26]. In a murine model of *Histoplasma capsulatum* infection, mice deficient in the chemokine receptor CCR5 had a predominant Th17 response and resolved the fungal infection more efficiently then wild-type mice [27]. Overall, these data indicate that fungi can induce Th17 responses and that the IL-17 pathway plays an important role in protective antifungal host defenses.

**Th2 Cells**

It is generally assumed that Th2 responses in fungal infection are maladaptive or deleterious [28]. It has been long recognized that an optimal balance between Th1 and Th2 is important for antifungal protection. Oropharyngeal candidiasis has been associated with a Th2 cytokine profile in saliva [29]. A recent report highlights the importance of the balance between protective Th1 responses and deleterious Th2 responses in disseminated candidiasis. Overexpression of GATA-3, which is the master transcription factor of Th2 cells, enhances the susceptibility to systemic *Candida* infection, presumably by reducing the production of IFNγ in response to *Candida* infection [30].

Frequent exposure to inhaled fungal spores presents a challenge to the host, and aberrant immune responses to *A. fumigatus* spores can result in allergic bronchopulmonary aspergillosis (ABPA). ABPA is a disease in which Th2 CD4+ T-cell responses play a critical role in the development of an asthma-like disease with pulmonary hypersensitivity and compromised lung function. In patients with allergic fungal rhinosinusitis, fungal antigens that stimulated T-cell activation induced a predominantly Th2 immune response [31]. Furthermore, administration of recombinant IL-4 to mice leads to a significantly increased susceptibility to invasive pulmonary aspergillosis, underscoring the deleterious effects of Th2 responses in systemic infections with filamentous fungi [32]. Thus, *Aspergillus*-induced Th2 responses seem to contribute to ABPA and unfavorable outcome in aspergillosis.

Interestingly, certain fungi have the capacity to modulate T-helper responses. Melanin-producing *Cryptococcus*, for instance, can induce IL-4 and may in this way skew the host defense towards a Th2 type of response [33]. This alteration could be relevant during infection, because Th2 responses are reported to be deleterious in experimental pulmonary cryptococcal infection [34]. In addition, a recent study reported that the host response elicited by macrophages that are activated by prototypical Th2 cytokines is associated with impaired defense against cerebral cryptococcosis [35]. Finally, in a model of experimental *Histoplasma* infection, an excess of IL-4 production and increased Th2 responses were associated with delayed clearance of *H. capsulatum* [36]. These studies show that a predominant Th2 response during fungal infection is generally associated with deleterious effects.
T Regulatory Cells

Tregs suppress inflammatory responses in disseminated candidiasis, and their presence during protective proinflammatory responses results in higher susceptibility to disease in mice that are infected with *C. albicans* [37, 38]. At mucosal sites, however, the tolerance promoted by Tregs seems to be beneficial, and it has been suggested that Tregs have a role in allowing fungal persistence at the mucosal site, which is beneficial for a continuous “state of alertness” of the immune system and durable, protective antifungal host response [39]. Notably, Tregs expand in disseminated and gastrointestinal *Candida* infection. During *Candida* infections, Tregs produce IL-4, IL-10, and TGF-β, which inhibit inflammatory Th1 and Th17 responses [40]. On one hand, Tregs restrict the capacity of the immune system to control *Candida* infection, but on the other hand, they augment host resistance to reinfection.

In experimental *Aspergillus* infection, Tregs that are activated by *Aspergillus* conidia can control inflammation by suppressing potentially deleterious effects of neutrophils through the actions of IL-10 on indoleamine 2,3-dioxygenase [41]. Furthermore, Tregs can inhibit Th2 responses and in this way prevent allergic disease, which may be induced by *Aspergillus* [41]. Tregs also control pulmonary inflammation and protect against lung injury associated with *Pneumocystis* infection in the setting of immune reconstitution as well as during primary infection [42]. The critical balance between proinflammatory responses and Treg responses in fungal infection is further underscored by the observation that a decrease in the number of Tregs in mice infected with *H. capsulatum* is associated with an increase in the Th17 response and more efficient fungal clearance [27]. These data reflect that Treg cells provide suppression of inflammatory responses, which can be deleterious or beneficial, depending on the type or stage of infection.

### CD8+ Cytotoxic T Cells

A protective role of CD8+ T cells in the host defense against mucosal candidiasis has been suggested. CD8+ T cells can inhibit the growth of *C. albicans* hyphae in vitro [43]. Furthermore, CD8+ T cells were important for limiting chronic mucosal carriage of *C. albicans* in one animal model of infection [44]. Notably, in the absence of CD4+ T cells, CD8+ T cells also appear to play an important role in anti-*Candida* host defense [44].

Studies that have investigated host defenses against other fungi also suggest that CD8+ T cells may be protective. IFNγ produced by CD8+ T cells was found to play an important role in controlling cryptococcal infection by limiting growth and survival of *C. neoformans* in macrophages, and these protective effects were independent of CD4+ T cells [45]. The observation that CD8+ T cells can provide protection in the absence of CD4+ T cells has also been reported in *H. capsulatum* infection. Furthermore, CD8+ T cells produce IFNγ in response to *P. carinii* and mediate clearance of the fungal burden [46]. In conclusion, although the role of CD8+ T cells has not been defined as clearly as the role of T-helper responses, they can contribute to protection against fungal pathogens in certain conditions.

### γδ T Cells

In addition to the described T-cell subsets, γδ T cells may play an important role in antifungal host defense by acting as a first line of defense at the mucosal level. Recently, it was described that γδ T cells isolated from HIV patients and healthy controls can proliferate and induce IFNγ and IL-17 in response to *C. albicans* [47]. Because both IFNγ and IL-17 are important for host defense against fungi, γδ T cells could represent an important arm of the mucosal immunity against fungal infections.
In mice, γδ T cells can expand rapidly following intraperitoneal inoculation of *C. albicans*, and γδ T cells can enhance NO production by macrophages in vitro. On the one hand, in vivo depletion of γδ T cells abrogates inducible NO synthase in the mucosa and enhances murine susceptibility to candidiasis [48]. On the other hand, mice that are deficient in γδ T cells are less susceptible to experimental *Candida* vaginitis [49]. These data suggest that γδ T cells can contribute to either protection against candidiasis or susceptibility to it, depending on the site of the infection.

Notably, γδ T cells are responsible for the high IL-17 concentrations observed in CGD mice, as mentioned earlier [22]. Interestingly, another subset of γδ T cells, which produced IL-10 and TGF-β, was associated with protection in the same model. These data suggest that γδ T cells can exert important immunomodulatory functions during pulmonary aspergillosis. Clearance of *C. neoformans* in the lungs of mice was enhanced when mice were depleted of γδ T cells [50]. The increased host defense was associated with an increase in Th1 response in the lungs. These observations suggest that γδ T cells can dampen proinflammatory responses during host defense against cryptococcal infection. Furthermore, human γδ T cells can respond to *Paracoccidioides brasiliensis* and produce factors that support the growth and differentiation of B cells [51]. As observed above, relatively little is known about the role of γδ T cells in antifungal host defense, and the information is scattered. This aspect of antifungal host defense certainly merits further investigation.

Conclusions

Summarizing these data, it has become evident that T cells play a critical role in the host defense against fungi. Although various functions have been described for the T-cell subsets, in general Th1 and Th17 responses contribute to protective antifungal defense, whereas Th2 responses can be potentially deleterious in the setting of a fungal infection (Fig. 1). Furthermore, it has become apparent that Tregs are important in orchestrating the balance between T-cell subsets, and thus Tregs can be good or bad for the host, depending on how they modulate the immune response. Finally, although the importance of cytotoxic T cells and γδ T cells in the host defense against fungi is not yet fully understood, several studies suggest that they also contribute to antifungal host defense. Specifically, γδ T cells may be very important for the first line of defense against fungi at the mucosal and epithelial level; more research is needed to elucidate their importance in antifungal host defense.

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- Of importance
- Of major importance


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