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Helper T-cell responses and pulmonary fungal infections

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Summary

The mucosal surface of the respiratory tract encounters microbes, such as fungal particles, with every inhaled breath. When pathogenic fungi breach the physical barrier and innate immune system within the lung to establish an infection, adaptive immunity is engaged, often in the form of helper CD4 T-cell responses. Type 1 responses, characterized by interferon-γ production from CD4 cells, promote clearance of *Histoplasma capsulatum* and *Cryptococcus neoformans* infection. Likewise, interleukin-17A (IL-17A) production from Th17 cells promotes immunity to *Blastomyces dermatitidis* and *Coccidioides* species infection by recruiting neutrophils. In contrast the development of T helper type 2 responses, characterized by IL-5 production from T cells and eosinophil influx into the lungs, drives allergic bronchopulmonary aspergillosis and poor outcomes during *C. neoformans* infection. Experimental vaccines against several endemic mycoses, including *Histoplasma capsulatum*, *Coccidioides*, *Cryptococcus* and *Blastomyces dermatitidis*, induce protective T-cell responses and foreshadow the development of vaccines against pulmonary fungal infections for use in humans. Additionally, recent work using antifungal T cells as immunotherapy to protect immune-compromised patients from opportunist fungal infections also shows great promise. This review covers the role of T-cell responses in driving protection and pathology in response to pulmonary fungal infections, and highlights promising therapeutic applications of antifungal T cells.

Keywords: adaptive immunity; fungal infection; lung; mucosal immunity; T cell.

Fungal recognition and T-cell priming the lung

The lungs represent a massive environment-exposed surface in the body, which is challenged with microbes and microbial products with every breath.[1] Fungi represent a medically important class of pathogenic microbes, and 4–11% of the fine-particle mass inhaled into the lungs contains fungal spores.[2] To preserve epithelial integrity and prevent colonization by infectious organisms including fungi, the host has developed several immunological mechanisms in the lungs.[1] Epithelial cells provide barrier immunity, preventing inhaled particles access to deeper tissues and vasculature, while mucus and antimicrobial peptides further deter colonization.[1] Resident alveolar macrophages remove debris from the lungs,[3] maintaining clear airways and preventing the establishment of infection.

The mammalian host employs a suite of pattern recognition receptors (PRRs) that are capable of recognizing fungal ligands and initiating innate inflammatory responses.[4,5] One of the best-characterized PRR–fungal ligand relationships is the recognition of fungal β-glucan by the prototypical C-type lectin receptor (CLR) dectin-1. Likewise, heterodimers of Toll-like receptors (TLR) TLR2 and TLR1 recognize triacylated lipoprotein, whereas TLR2/TLR6 heterodimers recognize diacylated lipoprotein.[5] Mannose receptor recognizes mannose and other sugar moieties on the surface of microbial cells.[6] The CLR dectin-2 has previously been shown to respond to mannan stimulation,[5] and recent work has also identified the glycoprotein Bl-Eng2 of *Blasotmyces dermatitidis* as a bona-fide ligand for this receptor.[7] A newly defined CLR, MelLec enables the host to sense melanin on pathogens including *Aspergillus* spores.[8] These PRR–fungal ligand interactions represent a major means by which the immune system recognizes and initiates immune responses against inhaled pulmonary fungal pathogens.

When these early mechanisms fail and fungal infection is established in the airway, the host responds with a coordinated immune response. Monocyte-derived macrophages and dendritic cells are often recruited into the airway.[9,10] Likewise, other myeloid cell populations, including neutrophils,[11,12] monocytes[13] and eosinophils,[14,15] infiltrate the airways in an effort to combat the infection. In almost all cases of pulmonary fungal infection, the development of adaptive immunity and the engagement of CD4 T-cell help is a key determinant of the outcome on infection.

CD4 T-cell responses can only occur after a carefully orchestrated process involving precise interactions between stromal, myeloid and lymphoid cells. The exact mechanisms underpinning the priming of T-cell responses have been extensively reviewed elsewhere,[16–19] and are only briefly summarized here. Upon uptake of foreign antigen by professional antigen-presenting cells at mucosal sites, the antigen-presenting cells traffic to draining lymph nodes in order to locate and prime naive T cells.[18] CD4 T-cell priming occurs not only by activation of the T-cell receptor by binding its cognate antigen in the context of MHCII, but also through co-stimulatory signals[19] and the cytokine milieu[20] present during T-cell priming. Once T-cell priming is complete, the effector T cells depart the lymph node to enter into the mucosal tissue and carry out their effector function.[17]

There are three major CD4 helper T-cell subsets that we will discuss in the context of fungal immunity.[21–24] Type 1 helper T cells (Th1 cells) are characterized by interferon-γ (IFN-γ) production, and are broadly effective at clearing intracellular pathogens.[25,26] In contrast, Th17 cells produce interleukin-17 (IL-17) and protect against extracellular pathogens in part through the recruitment of neutrophils.[25,27] Production of IL-5 and IL-13 is characteristic of Th2 cells, which are generally believed to promote clearance of helminth worms and other large parasitic organisms, but may also help to clear select fungi or alternatively mediate allergic inflammation in response to inhaled mould and related products (summarized in Fig. 1).[25,26,28–31] This review article will be largely organized along these lines: discussing pulmonary fungal infections grouped by the predominant/protective response of either Th1, Th17 or Th2.

Type 1 responses to pulmonary mycoses

Pneumocystis jiroveci (previously *carinii*) is an opportunistic pathogen and causes *Pneumocystis* pneumonia almost exclusively in immunocompromised hosts. Upon entry into the lung, *Pneumocystis* binds to lung epithelial cells (LECs).[32,33] The LECs play a significant role in the host response to *Pneumocystis*.[30,34–36] *In vitro* studies have demonstrated that LECs can directly respond to *Pneumocystis* by activating the nuclear factor-κB (NF-κB) pathway,[36] and that chemokine production by LECs in response to the fungus is myeloid differentiation primary response 88 (MyD88) -dependent and IL-1R-dependent.[34] Furthermore, the chemokine CCL2 is expressed by LECs in response to infection *in vivo*,[35] and the ablation of NF-κB selectively within LECs significantly impairs clearance of *Pneumocystis* from the lungs,[30] emphasizing the essential role of LECs in mediating a protective response to this fungus.

Though there is robust evidence from both mouse[37] and human[38] studies that strongly suggest that CD4 T-cell responses are required for clearance of *Pneumocystis* infection, the exact CD4 T-cell phenotype(s) required for protection remains a subject of open debate. Numerous groups have suggested a role for IL-17A and Th17 cells in host protection against *Pneumocystis*.[30,39,40] Interleukin-17A-producing CD4 T cells are recruited to the lungs of infected animals,[39] and neutralization of IL-17A or the Th17-promoting cytokine IL-23 both significantly increase lung fungal burden at later time-points.[40] Furthermore, impaired fungal clearance was associated with significantly reduced IL-17$^+$ CD4 T-cell numbers in the lungs of IKK$^{\Delta LEC}$ mice[30] (which lack NF-κB signalling specifically within the lung epithelium), suggesting a potential mechanism by which the lung epithelium marshals protective immunity to *Pneumocystis*.

Other studies have implicated Type 1 and Type 2 immunity in protecting against *Pneumocystis* infection. Some studies have correlated robust Type 2 responses and M2-polarized macrophage responses with protection against *Pneumocystis* and fungal killing.[23,41] Conversely, artificial induction of IFN-γ responses during *Pneumocystis* infection in the absence of CD4 T-cell help restores control of infection,[42] suggesting that Type 1 responses at least have the potential to mediate protection against *Pneumocystis*. Though the precise mechanisms of anti-*Pneumocystis* immunity remain to be fully elucidated, collectively these studies emphasize the critical role of CD4 T-cell-mediated responses in immunity to this fungus.

Histoplasma capsulatum is a dimorphic, primary fungal pathogen capable of causing pulmonary histoplasmosis in immunocompetent hosts.[11,43] As is common among the dimorphic fungi, the spore is the infectious particle, and upon entry into the lung undergoes a phase-transition to the yeast phase to mediate disease.[11,21] Elegant studies using green fluorescent protein-expressing *H. capsulatum* strains reported that dendritic cells were the most likely phagocyte population to be associated with *H. capsulatum* at 1 day post infection,[44] suggesting that dendritic cell–*H. capsulatum* interactions are a key early event in the host response to infection. An important subset includes CD103$^+$ conventional dendritic cells, which are critical for TLR7/9-dependent host defence against *H. capsulatum*.[45] Innate cytokine production is also important in early control of infection, as the neutralization of

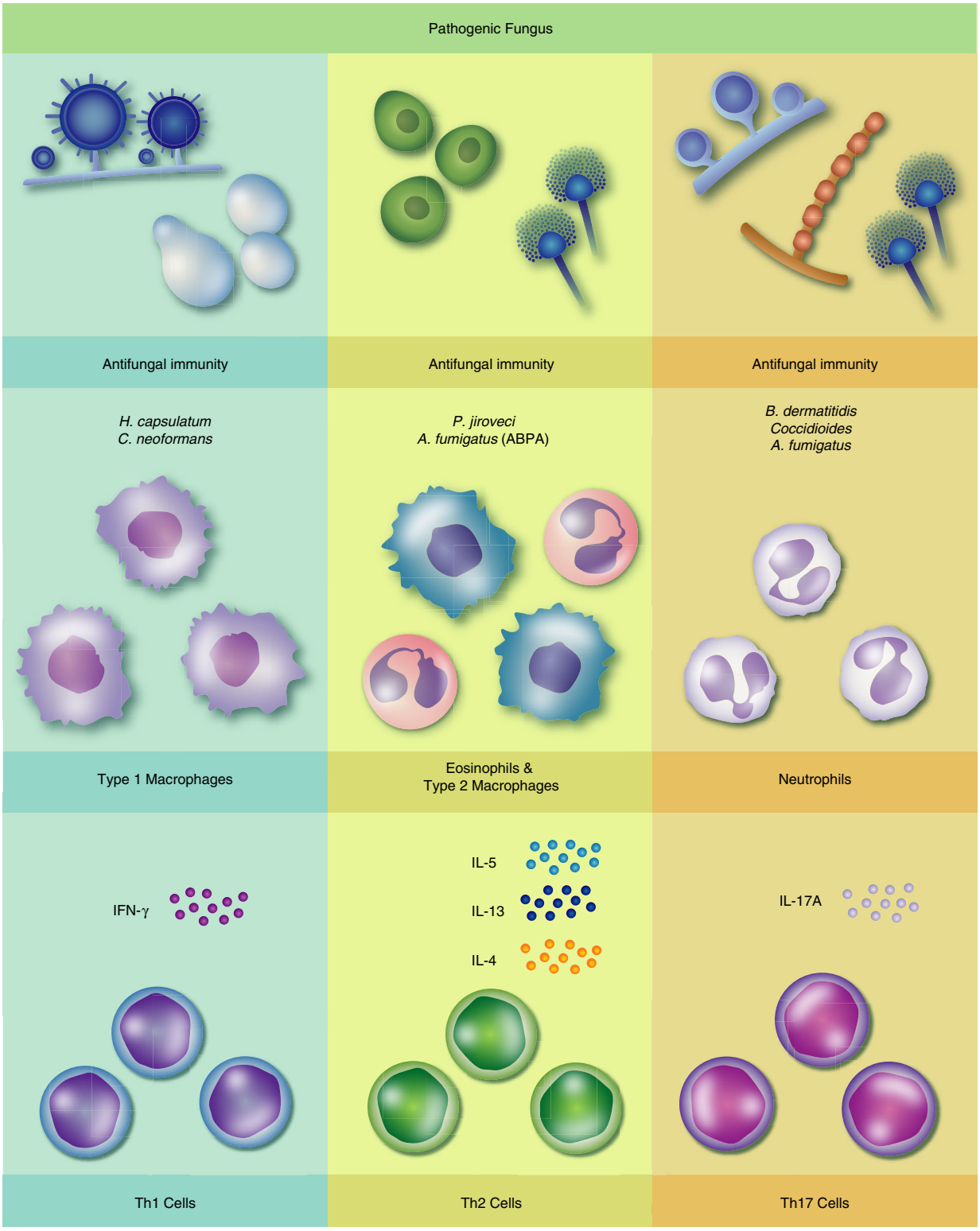

Figure 1. T-cell responses to pulmonary fungal infections. Left column: Type 1 responses, characterized by interferon-γ (IFN-γ) production from CD4 cells and Type 1/classically activated macrophages, mediate antifungal immunity to *Histoplasma capsulatum* and *Cryptococcus neoformans* infection. Centreal column: Eosinophil and alternatively activated macrophages supported by interleukin-4 (IL-4), IL-5 and IL-13 production from T cells protect against *Pneumocystis jiroveci* infection and drive pathology during allergic bronchopulmonary aspergillosis (ABPA). Right column: Host immunity to *Blastomyces dermatitidis*, *Aspergillus fumigatus* and *Coccidioides* species is mediated in part by T helper type 17 cells by IL-17A and neutrophil-dependent mechanisms.

granulocyte–macrophage colony-stimulating factor (GM-CSF) results in an order of magnitude higher fungal burden in the lungs by 1 week post infection.[46] Ablation of GM-CSF also impairs the early production of tumour necrosis factor-α (TNF-α) and IFN-γ,[46] two cytokines that are likewise critical for the control of *H. capsulatum* infection.[47,48] CCR2-dependent inflammatory cell recruitment is also crucial for early control of infection, as $CCR2^{-/-}$ mice show a significant increase in lung fungal burden by 7 days post infection,[49] which involves tilting T helper cell immunity away from Th1 responses.

Numerous studies have demonstrated that Th1 responses are protective against *H. capsulatum* infection.[50,51] Mice deficient in IFN-γ signalling are exquisitely susceptible to experimental *H. capsulatum* infection, dying in little more than a week after experimental infection.[47] In immunocompetent mice, the kinetics of IFN-γ production from CD4 T cells correlates well with the clearance of the fungus from the lungs.[52] Furthermore, recall of these anti-*H. capsulatum* T cells is dependent upon TNF-α, as the neutralization of TNF-α early after re-challenge of immune mice ablates protective immunity and leads to significant mortality.[53] Hence, Th1 cells and their products are paramount in mediating effective immunity against *H. capsulatum* infection.

Although Type 1 immunity is critical for control and clearance of *H. capsulatum* infection, Type 2 responses are uniformly detrimental in this context. Overexpression of IL-4 in transgenic mice was associated with increased lung fungal burden at day 7 post infection, but minimal alteration in the induction of IFN-γ of TNF-α responses,[54] suggesting that enhanced Type 2 responses directly benefit pathogen growth in the absence of impaired Type 1 immunity. Loss of CCR2-dependent signalling is a major determinant driving Type 2 immunity in response to *H. capsulatum.*[49] Though $CCR2^{-/-}$ mice exhibit no defects in lung IFN-γ production, IL-4 levels are markedly increased and associated with significant mortality in a normally non-lethal model of infection.[49] Collectively, these studies indicate that Type 2 immunity is not only inefficient at clearing *H. capsulatum*, but that it actively promotes progression of the infection.

Cryptococcus neoformans is an encapsulated, ubiquitous fungus capable of causing lung disease and meningitis in immunocompromised patients.[55] Initial interactions with resident phagocytes in the lung are crucial for control of *C. neoformans*, as depletion of $CD11c^{+}$ myeloid cells results in significant and rapid mortality in otherwise non-fatal *C. neoformans* lung infection.[56] Likewise, ablation of TNF-α signalling early in infection leads to increased skewing towards a Th2 response with associated impaired clearance of the fungus, probably due to defective maturation of dendritic cells.[57] Collectively, these studies highlight how interactions early in the innate immune response can be critical for host survival and influence the later development and polarization of adaptive immune responses.

While *C. neoformans* infection rarely occurs in the immunocompetent host, infection is common among HIV/AIDS patients,[55] underscoring a role for CD4 T cells in mediating immunity against this fungus. Numerous studies have shown a protective benefit of IFN-γ production and Type 1 immunity during *C. neoformans* infection.[58,59] Chen and colleagues demonstrated that mice deficient in the IFN-γ receptor showed impaired clearance of lung *C. neoformans*, increased dissemination, and increased mortality compared with wild-type animals.[58] This phenotype was largely attributed to decreased fungicidal activity in macrophages in the absence of IFN-γ-mediated activation.[58] Additionally, monocyte recruitment is essential for the development of Th1 immunity, as $CCR2^{-/-}$ mice mount significantly weaker IFN-γ responses.[60] Furthermore, pulmonary infection with a transgenic strain of *C. neoformans* that produces mammalian IFN-γ results in enhanced fungal clearance and survival of an otherwise fatal infection,[59] highlighting the benefit of Type-1 immunity in this infection.

In contrast to Type 1 immunity, Type 2 immune responses are associated with poor outcome during *C. neoformans* infection.[22,28,61] A comparison of several mouse strains infected with the same strain of *C. neoformans* showed that while strong IFN-γ responses were associated with fungal clearance, enhanced IL-4 production and lung eosinophil recruitment was associated with compromised fungal clearance and increased dissemination to the spleen and brain.[61] Additional studies have added further mechanistic insight into the role of Th2 responses in this infection. Mice deficient in the receptor for IL-33, an important signal for Th2 cell function and differentiation, mount a weaker Type 2 response to *C. neoformans* and are consequently better able to control lung fungal colonization and survive infection.[22] Ablation of IL-4 signalling is also associated with reduced eosinophil recruitment and decreased lung fungal burden at later time-points during *C. neoformans* pulmonary infection,[28] further demonstrating the largely detrimental role of Type 2 responses in this context.

Type 17 responses to pulmonary mycoses

Blastomyces dermatitidis is a dimorphic fungus and the causative agent of blastomycosis, a potentially fatal pulmonary infection seen in immunocompetent individuals.[62–65] Innate immune cells play a critical role in regulating the pathogenesis of this infection. Neutrophil recruitment helps to limit the initial growth of the pathogen, as depletion of neutrophils yields an increase in the lung fungal burden by 2 days post infection.[66] Recent studies by Hernandez-Santos *et al.*[67] have also demonstrated a key role for lung epithelial cells in orchestrating

early responses to *B. dermatitidis* infection. Specifically, NF-κB signalling within lung epithelial cells restrains fungal growth in part through the recruitment of IL-17A- and GM-CSF-producing innate lymphoid cells, such as "natural" Th17 (nTh17) cells and $\gamma\delta$ T cells, in the first 2 days of infection. This innate IL-17A and GM-CSF production in turn is required to activate recruited neutrophils and other myeloid cells and enhance their ability to kill fungal cells, highlighting the complex and multifaceted interactions of stromal, myeloid and lymphoid cells in antifungal immunity.

Several studies have interrogated host immunity in response to fungal spores,[66,68,69] but most animal studies of immunity to dimorphic fungal infection to date have been performed with the yeast-like form of the fungi. This is in large part due to technical difficulties in generating pure populations of spores and biosafety concerns of handling infectious spores when performing animal infections. Fungal spores probably represent the infectious particle in most naturally occurring infections. The different biochemical composition and metabolic activity of the spore compared to the yeast probably influences initial interactions with the immune system, including but not limited to ligation of PRRs and interactions with resident phagocytes. Although this discrepancy does not diminish the findings of studies using yeast, future studies delineating the impact of fungal particles on early pathogenesis and immunity will provide further illumination.

While experimental pulmonary infection with *B. dermatitidis* fails to elicit protective adaptive immune responses,[70,71] vaccine models using inoculation with live recombinant, attenuated *B. dermatitidis* have yielded key insights into the protective contribution of CD4 T cells.[70,72–74] Vaccine-elicited CD4 T cells can produce both IFN-γ and IL-17A. However, the protective effects of these T cells are mediated more so by IL-17A production.[73,74] Indeed, the ability of vaccination to protect against lethal challenge with wild-type *B. dermatitidis* is significantly impaired in the absence of IL-17A receptor signalling or the ablation of IL-17A signal directly.[73] Vaccine immunity is dependent upon robust antifungal response from the myeloid compartment, as both phox-deficient[72] and neutrophil-depleted[73] mice show impaired ability to clear *B. dermatitidis* from the lungs of vaccinated animals following an infectious challenge.

Interestingly, CD4 T cells are not the only T-cell subset capable of mediating vaccine immunity against *B. dermatitidis*. In the absence of CD4 T-cell help, CD8 T cells compensate and are sufficient to protect CD4-deficient hosts from otherwise lethal infection.[75] Strikingly, these CD8 T cells produce IL-17A, and mediate immunity against lethal fungal infection by this IL-17A production.[76–78] Impairment of either IL-17A signalling or neutrophil recruitment significantly ablates vaccine immunity in CD4-deficient animals,[77] underscoring the protective role of IL-17A production by CD8 T cells. Furthermore, these IL-17A-producing CD8 T cells (Tc17 cells) display many of the phenotypic characteristics of Th17 cells, including increased expression of Retinoid-related orphan receptor γ T (RORγT) and increased surface CCR6 expression.[77] Hence, both CD4 and CD8 T cells are capable of promoting vaccine-induced clearance of *B. dermatitidis* by the production of IL-17A and the recruitment and activation of neutrophils.

Aspergillus fumigatus is a saprophytic mould found throughout the environment and the causative agent of pulmonary aspergillosis.[79] Humans most commonly encounter *A. fumigatus* by inhaling conidia from the conidiating mould. Immunocompetent hosts rapidly clear conidia through the combined action of the mucociliary escalator, alveolar macrophages and neutrophil recruitment.[79,80] Neutrophil recruitment in particular is essential for the control of *A. fumigatus*, as the germination of *A. fumigatus* conidia is increased in lungs of mice deficient in neutrophils or neutrophil recruitment; for example, in MyD88-deficient or caspase recruitment domain-containing protein 9 (CARD-9) -deficient animals[81] or in CXCR2 deficiency, each of which contribute to neutrophil recruitment.[80] The recruitment of neutrophils also is an inflammasome-dependent process, with IL-1α in particular playing a dominant role in driving initial neutrophil responses.[82]

Although immunocompetent individuals exposed to *A. fumigatus* conidia are usually able to clear these fungal particles without the engagement of adaptive immunity,[79] patients with severe asthma or cystic fibrosis can become consistently colonized with *A. fumigatus* and develop allergic bronchopulmonary aspergillosis (ABPA).[83] ABPA is characterized by eosinophilia, IgE antibody, and the development of *Aspergillus*-specific Th2 cells.[83] High levels of serum IgE, whose production from B cells is driven by Th2 cell-derived IL-4,[84] are often found in patients suffering from ABPA.[83] Experimental models using repeated exposures to *A. fumigatus* conidia have yielded insight into the mechanisms underpinning the development of anti-*A. fumigatus* immune responses.[9,29,31] In these models, mice repeatedly instilled with *A. fumigatus* conidia develop many of the hallmarks of allergic airway inflammation and ABPA, including robust eosinophil recruitment, arterial remodelling, and collagen deposition around airways.[29,31] Interestingly, this inflammatory response is associated with the development of Th1 and Th17 responses in addition to Th2 responses.[29] Interleukin-17A is required for full eosinophil recruitment at the peak of inflammation,[9] underscoring the potential for non-Type 2 cytokines in driving 'allergic' responses following fungal exposure. Recent work has shown that signalling via IL-17RA and IL-17RC may drive divergent allergic outcomes,[85] with the IL-17F–IL-17RC axis favouring respiratory allergy in the proximal

airways. Collectively, these studies demonstrate the potentially varied functions of T-cell responses to fungal challenge, and how repeated exposure to fungi and their products can drive development of allergic airway disease.

Coccidioides posadasii and *Coccidioides immitis* are two closely related species of *Coccidioides*, a dimorphic primary fungal pathogen endemic to the American southwest and California, respectively, and the causative agents of Valley Fever.[86] Infection is initiated when *Coccidioides* arthroconidia are inhaled into the lung, where these infectious particles undergo development and eventually grow into large spherules containing numerous endospores. When the spherule bursts, the newly freed endospores form new spherules, and the infection continues.[86–88] The contribution of innate immunity to protection against *Coccidioides* remains incompletely understood. In experimental models of *Coccidioides* infection, the depletion of neutrophils does not result in increased lung or spleen fungal burden,[89] suggesting that neutrophils may be dispensable in that model. Additionally, although the absence of functional TLR4 is not associated with any increase in lung burden, increased fungal dissemination to the spleen was reported.[90]

Experimental vaccination models have begun to elucidate the protective role of T-cell responses against *Coccidioides* infection.[89,91] Subcutaneous vaccination with spores from an attenuated strain of *Coccidioides* engenders resistance against otherwise lethal pulmonary challenge with the wild-type fungus, in association with robust Type 1, Type 2 and Type 17 responses.[91] Th17 cells appear to be required for protective immunity following vaccination however, as IL-17ra knockout mice exhibit a significant defect in survival during rechallenge after vaccination.[91] Furthermore, MyD88 and CARD-9 are required both for the development of vaccine-induced resistance to infection and the development of robust Th17 responses in the lungs.[89] Additionally, the depletion of neutrophils significantly impairs fungal clearance in vaccinated animals,[89] offering further evidence that antifungal activity following vaccination is driven by Th17 cell activity.

Type 2 responses and pulmonary mycoses

Though Type 2 immunity is largely considered dispensable at best and detrimental at worst in response to pulmonary fungal challenge, numerous groups have reported beneficial facets of Type 2 immunity to a variety of fungal pathogens. Notably, although the absence of IL-4 signalling is associated with improved control of *C. neoformans* lung burden at later time-points post infection, IL-4RaKO mice show increased pathogen burden early in infection,[28] suggesting that IL-4-mediated responses are protective at this early time-point. Additionally, whereas eosinophilia is associated with allergic airway inflammation following repeated exposure to *Aspergillus* conidia,[9,29,31] defects in eosinophil activity are associated with impaired ability to clear *Aspergillus* conidia following installation into the lungs.[92] Furthermore, eosinophils exhibit contact-independent killing of *Aspergillus* conidia *in vitro*,[92] suggesting that recruited eosinophils are capable of protecting the host by killing *Aspergillus* conidia after exposure.

Applications for antifungal T cells

One clinical application for antifungal T cells is the development of T-cell-based vaccines, especially for use in populations living in areas where fungal infections are endemic. Experimental models have identified candidate vaccination strategies against the endemic mycoses *B. dermatitidis*,[70] *H. capsulatum*,[93] and *Coccidioides posadasii*.[94] Wuthrich *et al.*[24] recently demonstrated that T cells specific to an epitope found in fungal calnexin can respond to and expand following stimulation with the fungal pathogens mentioned above as well as *A. fumigatus* conidia. Vaccination with glucan particles loaded with calnexin peptide was capable of eliciting protective immunity against both *B. dermatitidis* and *Coccidioides posadasii* experimental pulmonary infection,[24] demonstrating the potential for vaccination strategies promoting calnexin-specific T-cell responses to protect against multiple endemic fungal infections. Various strategies, including recombinant proteins in glucan particles, engineered attenuated strains and alkaline extracts have shown promise in vaccination against experimental murine *Cryptococcus* infections.[95–97]

An additional clinical application where one might leverage antifungal T-cell responses is the development of immunotherapy treatments, especially in populations at high risk for fungal infections. Invasive aspergillosis is a severe fungal infection in immunocompromised individuals, especially those undergoing corticosteroid treatment, and mortality can be as high as 90%.[79,98] A therapeutic approach with promise is the transplantation of *in vitro* differentiated antifungal T cells to at-risk patient populations.[99] Preclinical models have demonstrated the efficacy of antifungal T-cell responses in protecting mice from otherwise lethal doses of *A. fumigatus*.[99–101] Recent studies by Kumaresan and colleagues have demonstrated the potential of CD8 T cells bioengineered to respond to β-glucan via Dectin-1 to impair *A. fumigatus* growth.[102] Furthermore, human trials using the transplantation of *in vitro* stimulated donor *Aspergillus*-specific T cells as a therapeutic intervention in response to evidence of invasive aspergillosis demonstrated a significant increase in survival compared with control patients,[99,103] so demonstrating the potential for this therapy in improving patient outcomes in an otherwise often intractable disease.

Similar immunotherapeutic strategies, where autologous antigen-specific T cells are expanded *in vitro* and transferred to patients, have proven effective at protecting immunocompromised patients against cytomegalovirus

(CMV) infection.[104,105] One difference between the two infections, and a technological challenge that must be met to develop a viable therapeutic, is the mechanisms by which the immunotherapy would kill the infectious agent. Anti-CMV immunotherapeutic approaches use CMV-specific cytotoxic T lymphocytes,[104,105] which can directly kill infected cells. In contrast, CD4 T helper cells will have to engage arms of innate immunity, such as neutrophils, monocytes, or macrophages,[24] which may be absent or functionally impaired in immunosuppressed individuals, to protect against fungal infection. Hence, antifungal immunotherapeutic strategies may also involve the augmentation of effector myeloid cell responses in conjunction with antifungal T-cell transfers.

Concluding remarks

The development of adaptive immunity and the engagement of CD4 T-cell help is a key determinant of the outcome of numerous pulmonary fungal infections. In the case of *H. capsulatum* or *C. neoformans* infection, naturally developing Th1 responses drive clearance of fungal infections. In other cases, such as the Th2 responses that develop following repeated exposure to *A. fumigatus* conidia, CD4 T-cell responses are dispensable to fungal killing and ultimately contribute to immune pathology. Furthermore in other contexts, including *B. dermatitidis* or *Coccidioides* infection, a failure of the development of robust CD4 T-cell responses is associated with poor outcomes following infection. Hence, the phenotype and strength of antifungal T-cell responses is a major factor in immunity and pathology during pulmonary fungal infections.

These insights are improving our understanding of the basic biology of fungal infections, and also informing the development of next-generation antifungal therapies. As mentioned above, the transfer of antifungal CD4 T cells shows great promise as a therapy for difficult to treat fungal infections in immunocompromised hosts. Experimental vaccines against *B. dermatitidis* or *Coccidioides* are capable of eliciting protective immunity in preclinical models of fatal fungal infection. Future investigations of antifungal CD4 T-cell responses should yield novel insights into the determinants of protective versus pathological host responses.

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