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## Immunotherapeutic approaches to treatment of fungal diseases

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Fungal infections cause morbidity worldwide and are associated with an unacceptably high mortality despite the availability of antifungal drugs. The incidence of mycoses is rising because of the HIV pandemic and because immunomodulatory drugs are increasingly used to treat autoimmune diseases and cancer. New classes of antifungal drugs have only been partly successful in improving the prognosis for patients with fungal infection. Adjunctive host-directed therapy is therefore believed to be the only option to further improve patient outcomes. Recent advances in the understanding of complex interactions between fungi and host have led to the design and exploration of novel therapeutic strategies in cytokine therapy, vaccines, and cellular immunotherapy, each of which might become viable adjuncts to existing antifungal regimens. In this report, we discuss immunotherapeutic approaches—the rationale behind their design, the challenges in their use, and the progress that is so urgently needed to overcome the devastating effect of fungal diseases.

## Introduction

Fungal infections occur in more than a quarter of the world's population, yet the enormous burden caused by fungal infections is underestimated. Most fungal infections are superficial and easily treated. However, fungi also cause invasive diseases that are associated with more than 50% mortality, causing an estimated 1.5 million deaths every year.<sup>1,2</sup> This unacceptably high mortality stems primarily from inadequate diagnostics and from clinical shortcomings of available antifungal drugs. Most invasive diseases are caused by fungi from one of four genera (*Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis*), yet no clinical vaccines exist for these fungi.<sup>1</sup>

Most invasive infections occur as a result of altered immune status. The incidence of these diseases has increased primarily as a result of the more widespread use of immunosuppressive drugs and invasive medical interventions and because of the HIV pandemic. The key role of host-immunity defects in fungal disease has sparked interest in adjunctive immunotherapies that could enhance important immune effector functions and improve outcomes. However, comprehensive insight into the host response against fungi is essential before targeted immunotherapies can be developed. Exciting advances in the understanding of protective antifungal immunity mechanisms have provided a fundamental basis for the development of these new approaches. The central role of phagocytic cells in the protective innate host responses and in the development of adaptive antifungal immunity is now widely appreciated.<sup>3,4</sup> For example, fungal infections are life threatening in patients with neutropenia or defects in phagocyte nicotinamide adenine dinucleotide phosphate oxidase (chronic granulomatous disease).<sup>3</sup> The phagocytes are therapeutic targets because their activity can be enhanced or suppressed by soluble immunomodulatory mediators. The importance of pattern recognition receptors, particularly the C-type lectin receptors, and their signalling pathways, in

antifungal defence has also become apparent.<sup>5</sup> For example, polymorphisms in the Toll-like receptors and C-type lectin receptors have been linked to susceptibility to various fungal infections in patients.<sup>6</sup> An understanding of these receptor systems and their cognate fungal ligands will also foster the development of adjuvants and vaccines.

The function of pattern recognition receptors, and the leukocytes that express them, in the modulation of adaptive immunity has been well characterised. For example, interactions of fungi with C-type lectin receptors, such as dectin-1, dectin-2, mannose receptor, or mincle, induce both Th1 and Th17 immunity.<sup>4</sup> Although the effects of natural killer lymphocytes and other innate lymphocytes on fungal infections have been described, their role in clinical disease needs to be assessed. Understanding these systems and the underlying mechanisms will open opportunities for the development of novel immunotherapies, such as adjunctive interferon- $\gamma$  immunotherapy to induce protective CD8<sup>+</sup> responses in patients with AIDS or for the treatment of cryptococcal meningitis.<sup>7,8</sup>

Insight to these systems and mechanisms is limited largely to immune responses to *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis*. Far less is known about immunity to most other fungal pathogens, even those highly prevalent pathogens that cause superficial, non-life-threatening diseases. Immunity to rare fungal pathogens such as the mucorales is also far less understood, despite the extremely high mortality associated with the infection. The importance of fungi to immune homeostasis and the pathology of inflammatory and autoimmune diseases such as colitis is only just becoming apparent.<sup>9</sup>

Novel host-directed therapies have enormous potential for antifungal treatment. In this report, we discuss the recent developments in immunotherapeutic approaches to antifungal disease, including cytokine therapy, vaccines, and cellular immunotherapy.

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	Genetic cause	Mechanism
Candida (systemic or in the CNS)	Chronic granulomatous disease, CARD9-deficiency, MPO deficiency	Defective reactive oxygen species production, defective recruitment of Th17 cells or neutrophils
Chronic mucosal candidiasis	STAT1 gain-of-function mutations, STAT3 deficiency, AIRE defects (APECED), interleukin 17 F or interleukin 17 receptor A deficiency, ACT1 deficiency, interleukin 17 receptor C deficiency, DOCK8 deficiency	Defective recruitment of Th17 cells, autoantibodies against interleukin 17 or interleukin 22
Aspergillosis	Chronic granulomatous disease, STAT3 deficiency, GATA2 deficiency	Defective production of reactive oxygen species, defective regulation of Th17 response or neutrophil function, defective haematopoiesis
Deep dermatophytosis	CARD9-deficiency, STAT1 gain-of-function	Defective recruitment of Th17 cells or neutrophils
Cryptococcosis	Autoantibodies against GM-CSF or interferon $\gamma$ , primary CD4 lymphopenia, GATA2 deficiency	Neutralisation of GM-CSF or interferon $\gamma$ bioactivity, defective Th function, defective haematopoiesis
Dimorphic fungi*	Interleukin-12Rb1 deficiency, interferon $\gamma$ receptor deficiency, STAT1 gain-of-function, GATA2 deficiency	Defective Th1 function, defective Th17 function, defective haematopoiesis
Pneumocystosis	SCID, X-linked CD40 ligand deficiency, CARD11 deficiency, MHC class II deficiency, NEMO deficiency	Defective CD4 lymphocyte function, defective NF $\kappa$ B modulation

\*Infections with dimorphic fungi include histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis. APECED=autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. GM-CSF=granulocyte-macrophage colony-stimulating factor. Th=T-helper lymphocyte. SCID=severe combined immunodeficiency.

**Table: Examples of important genetic causes of severe fungal infections**

### Recombinant cytokines as adjunctive therapy in systemic infections

Host-directed therapy using recombinant cytokines and growth factors is an important strategy against fungal infections. Insight into the genetic causes of fungal infections provides an important source of information: by understanding which immune defects increase susceptibility to fungal infections, appropriate adjunctive therapy can be designed. Many primary immunodeficiencies increase host susceptibility to fungal infections.<sup>10,11</sup> Neutrophils and T-helper lymphocytes have a particularly important function in maintaining antifungal host defences (table). This conclusion is also strongly supported by information from patients with acquired neutrophil immunodeficiencies (eg, from chemotherapy or immunosuppressive therapy) or CD4 T-lymphocyte immunodeficiencies (HIV, immunosuppressive therapy) who are highly susceptible to fungal infections. Strategies to augment the host defence against fungi using colony-stimulating factors and recombinant interferon  $\gamma$  as the main classes of biologicals to enhance antifungal host defences stem from evidence that neutrophils are important in the host defence against candida and aspergillus infection and Th1 and Th17 are essential for the adaptive responses.

Colony-stimulating factors, which have a dual effect on myeloid cell population recovery and neutrophil activation, were among the first antifungal adjunctive therapies to be used in a clinical setting. Granulocyte-macrophage colony-stimulating factor (GM-CSF)

stimulates expression of dectin-1, a C-type lectin, on macrophages and has been shown to ameliorate the course of disseminated murine candidiasis.<sup>12</sup> Adjunctive immunotherapy for refractory mucosal candidiasis in patients with HIV was successful and without adverse events.<sup>13,14</sup> Although GM-CSF has not been tested in patients with invasive candidiasis specifically, either GM-CSF or GM-CSF plus granulocyte colony-stimulating factor (G-CSF) combination therapy is associated with a reduced post-transplant mortality and with reduced incidence of candida infections.<sup>15</sup> A combination treatment with GM-CSF and interferon- $\gamma$  induced immunological and clinical recovery in three patients with invasive aspergillosis.<sup>16</sup> The clinical efficacy of GM-CSF against cryptococcosis has not been systematically assessed, but this is a rational choice given the increase in susceptibility to cryptococcus in patients with neutralising anti-GM-CSF antibodies.<sup>17</sup>

G-CSF also increases neutrophil numbers and enhances their antifungal activity; it is also routinely used to reduce neutropenia during oncological treatments. In a randomised placebo-controlled pilot study with patients who had disseminated candidiasis (but not neutropenia), adjunctive immunotherapy with G-CSF and fluconazole showed a trend for faster resolution of infection than fluconazole alone.<sup>18</sup> Nevertheless, a note of caution has to be given, since rapid neutrophil recovery with G-CSF therapy can lead to severe pulmonary complications,<sup>19</sup> illustrating the delicate balance between restoration of antifungal resistance and induction of pathology.

Interferon  $\gamma$  has been carefully studied as an antifungal adjuvant immunotherapy. Interferon  $\gamma$  can be used as repletion therapy in patients with Th1 synthesis defects (eg, patients with STAT3 or interleukin 12 receptor deficiencies) and in a pharmacological manner, leading to supraphysiological concentrations (eg, in patients with chronic granulomatous disease). Interferon  $\gamma$  also enhances antifungal activity of macrophages and polymorphonuclear neutrophils, and interferon- $\gamma$  immunotherapy reduces fungal burden in murine candidiasis,<sup>20</sup> aspergillosis,<sup>21</sup> and cryptococcosis.<sup>22</sup> The first successful clinical applications of interferon  $\gamma$  as an adjunctive therapy was in patients with chronic granulomatous disease, in whom it gave substantial protection against aspergillosis.<sup>23,24</sup> Interferon- $\gamma$  treatment might also be suitable for patients who have received transplants and have developed systemic fungal infections. Effective clearance of refractory or disseminated fungal infections, especially aspergillosis, has been shown in several patients.<sup>25</sup> These study outcomes are the basis for the use of interferon- $\gamma$  immunotherapy in patients with aspergillosis; however, randomised controlled trials are needed.

The concentration of interferon  $\gamma$  at the site of infection has been found to determine the rate of cryptococcal clearance.<sup>26</sup> Results of a clinical trial<sup>7</sup> of adjuvant

immunotherapy with interferon  $\gamma$  in patients with HIV and cryptococcal meningitis showed faster sterilisation relative to standard therapy alone. Adjunctive, short-course interferon  $\gamma$  has also been found to increase cryptococcal clearance from cerebrospinal fluid without increasing adverse events.<sup>27</sup> The efficacy of interferon- $\gamma$  therapy has also been reported in patients with CD4 lymphopenia<sup>28</sup> suggesting that interferon- $\gamma$  immunotherapy is an important adjuvant in the treatment of cryptococcal meningitis.

The Th1 responses in patients with candida infection suggest a potential benefit of interferon- $\gamma$  immunotherapy for patients with invasive candidiasis. In an open-label, prospective pilot study,<sup>29</sup> six patients with candidaemia received either adjunctive immunotherapy with interferon  $\gamma$  or placebo. Adjunctive interferon- $\gamma$  immunotherapy improved leukocyte innate immune responses (interleukin 1 $\beta$ , tumour necrosis factor  $\alpha$ ), increased production of the T-lymphocyte cytokines interleukin 17 and interleukin 22, and restored monocyte expression of human leukocyte antigen (mHLA-DR). Although this study was not powered to measure mortality, the results show the potential for adjunctive interferon- $\gamma$  immunotherapy to enhance antifungal immunity and restore sepsis-associated immunoparalysis in patients with candidaemia. Interferon  $\gamma$  is known to improve clinical outcomes in patients with chronic granulomatous disease and cryptococcal meningitis.<sup>27</sup> An increasing number of studies suggest that interferon  $\gamma$  improves clinical outcomes in patients with other invasive fungal infections (IFIs), but larger randomised trials are needed.

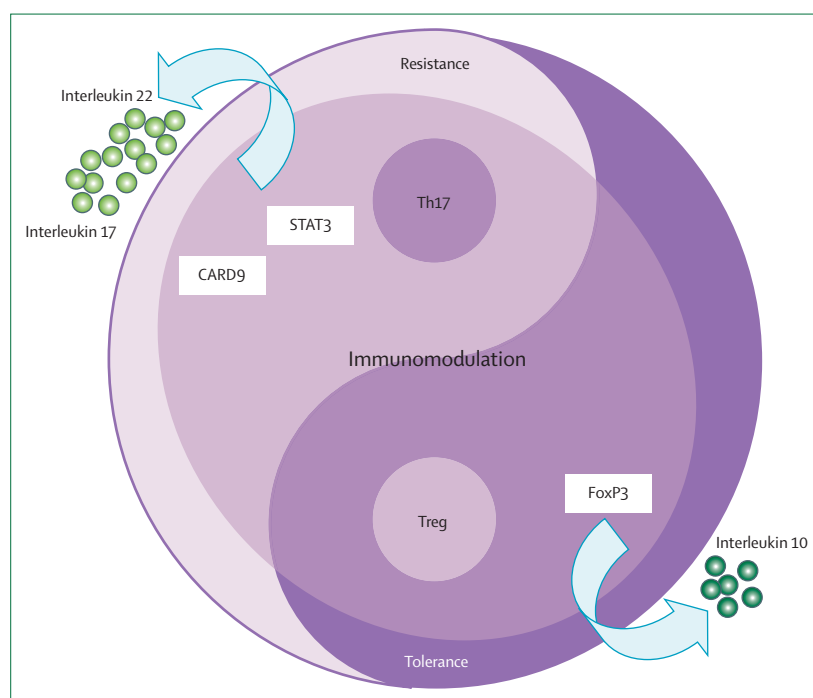
### Immune dysregulation as a risk factor for fungal infections

Although severe induced and acquired immunodeficiencies strongly affect a host's immune defences and increase the risk of IFIs, results of human and mouse studies show that hyper-reactivity of both innate and adaptive branches of the immune response also predispose to IFIs. For example, immune reconstitution inflammatory syndrome is a complication of antiretroviral therapy in patients with HIV who are immunocompromised.<sup>30</sup> Paradoxically, immunosuppression and corticosteroids have been used to treat more severe cases of the syndrome that are associated with concomitant IFIs; by lowering immune reactivity, the fungal infection is once again brought under control. Hence, immune-system dysregulation can be a primary cause of susceptibility to fungal infections, and opportunistic fungal infections can be reconsidered as immune-mediated pathologies.

These intellectual advances have overturned the belief that successful immunity relates only to the blockade of a pathogen's infective capacity. Informed by established theories of evolutionary ecology,<sup>31</sup> the definition of immunity to pathogens can now be revised: a successful immune response is the process by which the host both

resists a microorganism's infective capacity and limits the damage caused either directly or indirectly by the infection. For fungal infections, damage-limiting tolerance and pathogenic resistance have been proposed as two axes that frame the space within which the immune response occurs; the point of their intersection determines the balance of these important factors and therefore the overall benefit of that response to the host.<sup>32,33</sup> Emerging experimental data are refining these theories; however, studies in murine models of candidiasis and aspergillosis have only led to the identification of Th1 cells as key mediators of antifungal resistance and of Treg cells as the cell type that is most involved in damage limitation.<sup>34</sup> Bringing this concept to the clinical arena, immune dysregulation implies alterations of mechanisms that either enhance the immune system's capacity to fight fungal microbes or abrogate the tolerance that controls the self-harm caused by an immune response (immunopathology; figure 1).

Several lines of evidence suggest that maintaining self-tolerance has equal importance to providing pathogen resistance in immunity. One situation with immediate clinical relevance is in patients with primary immunodeficiencies leading to both infection susceptibility and serious autoimmune manifestations.



**Figure 1: The yin and yang of immunomodulation in fungal diseases**

Immune responses to fungal infections can polarise a host defence to either resistance or tolerance modes, promoting or inhibiting antifungal immune reactions, respectively. Resistance to fungal infection is mediated through mucosal Th17 responses leading to interleukin 17 and 22 production, whereas mucosal tolerance to fungi is mediated through Treg responses leading to interleukin 10 production. The concerted and balanced action of the two immune modes confers mammalian host fitness to fungal infection, raising the question whether immunotherapy for human fungal diseases should aim for the same duality.

For example, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a primary immunodeficiency that is characterised by simultaneous dysregulation of immune tolerance and multiple fungal infections.<sup>35</sup> *Candida* infections are very common in patients affected by APECED because autoantibodies are released against cytokines that are involved in both the resistance and tolerance arms of the response.<sup>36</sup> These patients are also often diagnosed with chronic mucocutaneous candidiasis—a clinical syndrome with selectively altered immune responses against *Candida*, consistent with immune dysregulation.<sup>37</sup> APECED is characterised by mutations in the *AIRE* gene, which encodes autoimmune regulator protein that is mainly involved in central tolerance induction. Individuals with cryptococcal meningitis but not HIV have severe disease associated with M2 macrophage polarisation; intrathecal expansion of activated HLA-DR+ CD4+ CD8+ T cells and natural killer cells; high concentrations of interferon  $\gamma$ , interleukin 4, and interleukin 13; and neuronal damage despite good microbial control.<sup>38</sup> These observations give further evidence for immune dysregulation as a major driver of immunopathology in fungal disease.

#### Panel: Best practice and knowledge gaps

##### Best practice

- Judicious use of growth factors (granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor) or interferon  $\gamma$  should be considered in selected immunocompromised groups with invasive fungal disease
- Adjunctive corticosteroids should be used in many cases of chronic disseminated candidiasis
- Granulocyte transfusions should be considered in individuals with prolonged neutropenia and invasive fungal disease
- Interferon- $\gamma$  prophylaxis should be considered in chronic granulomatous disorder patients at risk of aspergillosis

##### Knowledge gaps

- What is the role of B-cell depletion in individuals with cytokine or growth factor autoantibodies and invasive fungal disease?
- Are JAK kinase inhibitors useful in individuals with invasive fungal disease and *STAT1* gain-of-function mutations?
- Is anakinra therapeutically useful in individuals with invasive fungal disease and excessive inflammasome activation?
- What is the role of IgE depletion in allergic bronchopulmonary aspergillosis?
- What is the clinical efficacy of fungal vaccines currently in development and who should receive them?
- Is adoptive fungal-specific T-cell therapy clinically effective and in which groups of patients?

The presence of autoantibodies against endogenous interferon  $\gamma$  has also been linked with fungal infections.<sup>17,39</sup> Accordingly, B-cell depleting therapies (eg, rituximab) have been used successfully in patients with mycobacterial infection;<sup>40</sup> however, this strategy has yet to be tested in patients with fungal infections. Similarly to immune reconstitution inflammatory syndrome, chronic disseminated candidiasis (also known as hepatosplenic candidiasis) is a severe IFI principally observed during neutrophil recovery in patients who were previously treated with immunosuppressant drugs. The addition of corticosteroids to antimicrobial therapy has been found to be more effective than antifungal drugs in reducing inflammation while treating fungal infection.<sup>41</sup> Similarly, pattern recognition receptor polymorphisms are known to predispose towards vulvovaginal candidiasis.<sup>42</sup> Pathogenic inflammasome activity has also been observed in vulvovaginal candidiasis.<sup>43</sup> In murine experimental candidiasis, a pathogenic inflammatory response characterised by hyperactive type-1 interferon signalling promotes inflammatory dendritic cell differentiation and, thereby, fungal infection.<sup>44</sup> Whether a parallel mechanism operates in human beings remains to be seen.

In addition to autoantibodies against interleukin 17, deficiencies in interleukin 17 receptor subunits (interleukin 17 receptor A and interleukin 17 receptor C), interleukin 17F, and the interleukin 17 receptor adapter molecule ACT1, and defects in the *STAT1* adaptor molecule lead to immunodeficiency (panel).<sup>45,46</sup> Whereas some primary immunodeficiencies are characterised by dysregulation of tolerogenic functions, other immunodeficiencies present with altered inflammatory or resistance responses (eg, in Th17 development or function) and concomitant occurrence of fungal infections. Mutations in *STAT3* (in autosomal-dominant hyper IgE syndrome), *CARD9*, *IL17RA*, and *IL17F* dampen the Th17 adaptive inflammatory response and therefore diminish resistance to fungi.<sup>47</sup> *STAT1* gain-of-function mutations cause autosomal dominant chronic mucocutaneous candidiasis and are associated with various features, from autoimmune disease to cancer to cerebral aneurysm formation.<sup>48</sup> JAK inhibitors have been explored in patients with *STAT1* gain-of-function mutation because interferon  $\gamma$  and type 1 interferons signal via JAK1 and activate *STAT1* (panel). Some attempts have been successful.<sup>49,50</sup> Given the broad clinical phenotype and the complexity of the underlying pathophysiology, whether this approach is beneficial in a subset of patients with autosomal dominant, chronic mucocutaneous candidiasis that is due to *STAT1* gain-of-function mutations remains to be investigated. Primary immunodeficiencies can also originate from genetic defects in the primary innate immune response (particularly in phagocytic functions, as in chronic granulomatous disease where phagocytic ability is reduced), from an altered respiratory burst in response



to pathogens, and from a specific defect in LC3-associated phagocytosis (a crucial mechanism for efficient uptake of aspergillus).<sup>51,52</sup> Chronic granulomatous disease also leads to dysregulated Th17 cell expansion, with high concentrations of interleukin 1 and interleukin 17 correlating with susceptibility to aspergillosis.<sup>53,54</sup> Ultimately, immunotherapies might become an important adjunctive strategy in the treatment or prevention of fungal infections in patients with chronic granulomatous disease (eg, interleukin 1 receptor antagonist anakinra to inhibit interleukin 1 signalling) when the main aim is to re-establish the balance between immune resistance and immune tolerance.<sup>52</sup>

### Immunomodulation in fungal allergic diseases

An exaggerated immune response to fungi can cause allergic diseases ranging from atopic dermatitis and asthma with fungal sensitisation to allergic bronchopulmonary aspergillosis (ABPA), a severe allergic pulmonary complications caused by *Aspergillus* spp. Pathological inflammatory responses in ABPA are mainly characterised by excessive Th2 responses, resulting in uncontrollable asthma, mucosal plugging, and bronchiectasis and can in some cases lead to fatal lung damage. ABPA is treated with oral corticosteroids,  $\beta_2$  agonists (to control the underlying asthma), and triazole antifungals.<sup>55,56</sup> Azoles and corticosteroids can have severe side-effects, so new immunotherapies for ABPA are needed urgently and will, in turn, rely on a clear understanding of the underlying immunological mechanisms.

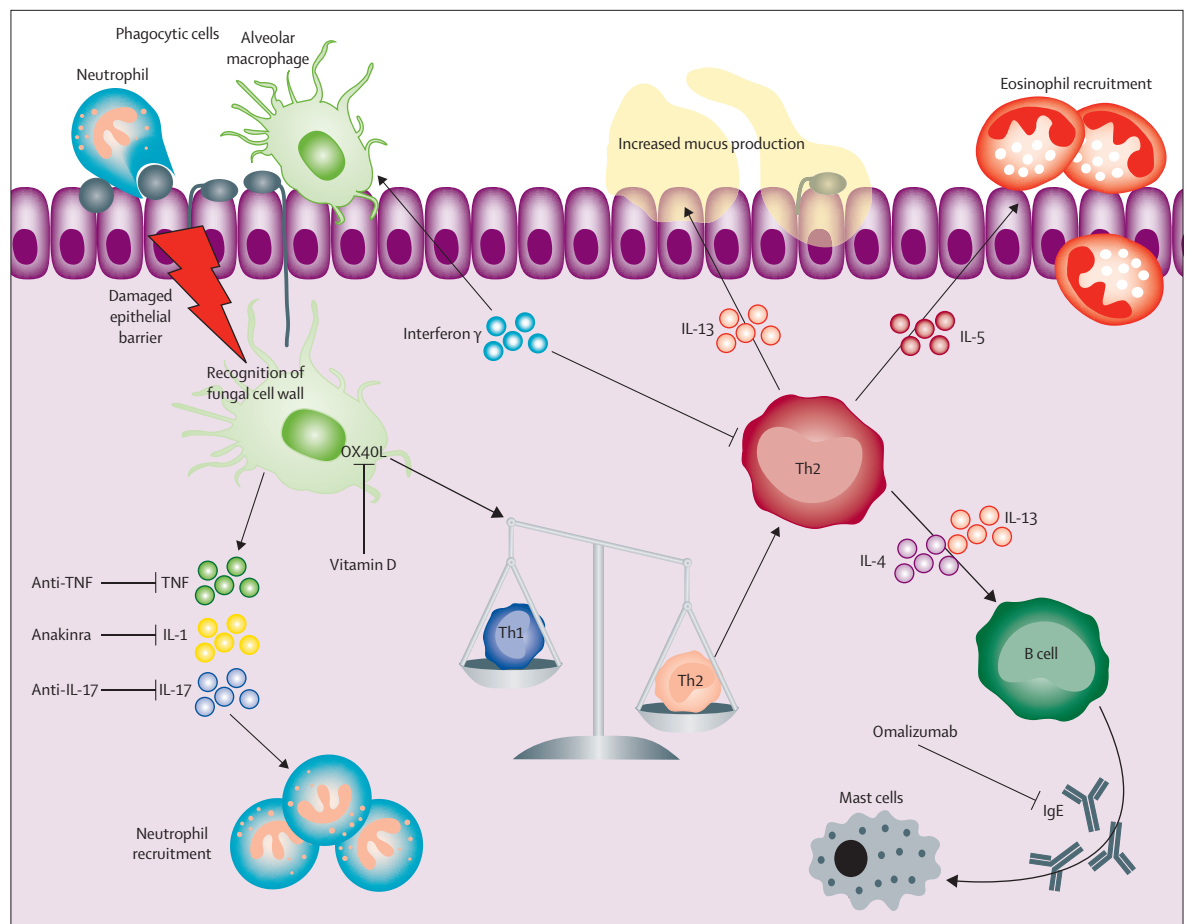
Allergic reactions can be triggered by fungal cell-wall components that stimulate the production of thymic stromal lymphopoietin, which induce chemokines such as TARC (CCL17) that are known to be important in both murine and human asthma with fungal sensitisation.<sup>57–59</sup> TARC binds to its receptor CCR4 and induces a Th2 response that drives mast cell degranulation and increases pulmonary IgE concentration.<sup>60</sup> The hallmark of allergic inflammatory responses is increased production of Th2 cytokines interleukin 4, interleukin 5, and interleukin 13 as well as the production of IgE. OX40L is a co-stimulatory molecule on antigen-presenting cells that can potentiate these Th2 responses.<sup>61</sup> In experimental murine fungal asthma, aspergillus-induced Th2 responses dependent on OX40L, which, in turn, is regulated by vitamin D.<sup>61</sup> Vitamin D decreases OX40L synthesis and reduces Th2 responses and so was tested in a clinical phase 1 trial with patients with cystic fibrosis and ABPA.<sup>62</sup> The outcomes revealed that vitamin D reduced aspergillus-induced interleukin 13 and IgE responses in patients with ABPA and was well tolerated.<sup>62</sup> Another approach to limiting Th2 responses is to target IgE with the monoclonal antibody omalizumab. Although not a clinically licensed indication, omalizumab has been used to treat ABPA in patients with and without cystic fibrosis, allowing a corticosteroid-sparing treatment regimen.<sup>63–66</sup>

Although asthma research has not addressed the fungal allergy component in detail, there is a range of disease endotypes from neutrophilic (Th1/Th17) to eosinophilic asthma (Th2), suggesting that asthma with fungal sensitisation might also have a neutrophilic component. Peripheral blood mononuclear cells isolated from patients with ABPA have decreased production of interferon  $\gamma$  in response to aspergillus and exaggerated Th2 responses.<sup>67</sup> This increased ratio of Th2 to Th1 was also observed in patients with asthma who had aspergillus sensitisation, but not in patients without aspergillus sensitisation.<sup>67</sup> These findings provide a rationale to explore immunomodulatory treatment with interferon  $\gamma$  in aspergillus-driven allergic inflammatory disorders. Findings from other studies suggest that interleukin 33 could be a therapeutic target in patients with severe asthma and fungal sensitisation.<sup>68</sup>

Experimental aspergillus-induced airway inflammation can have various characteristics depending on the mouse strain. BALB/c mice have increased neutrophilia in the lung and more potent tumour necrosis factor  $\alpha$  responses than C57BL/6 mice do.<sup>69</sup> In BALB/c mice, when the pulmonary dendritic cells responsible for the production of tumour necrosis factor  $\alpha$  were depleted, the inflammatory responses switched from a tumour necrosis factor  $\alpha$ -interleukin 17 signature, with predominant neutrophilic infiltrate, to an increased interleukin 5 response with increased eosinophil influx, resembling the inflammatory response in C57BL/6 mice.<sup>69</sup> Fungi can therefore trigger different types of pulmonary responses depending on the host genetic background. Host-directed therapy for fungal allergy diseases can therefore be based on several strategies: dampening neutrophilic inflammation (eg, by blocking tumour necrosis factor  $\alpha$ , interleukin 1, or interleukin 17), inhibiting Th2 responses (blocking IgE or interleukin 13), or augmenting antifungal immune responses (interferon  $\gamma$  or GM-CSF; figure 2).

### Fungal vaccines and antibody therapy

Considerable efforts have gone towards developing preventive vaccines and immunotherapies for fungal disease; however, some obstacles have impeded vaccine development. Fungi are ubiquitous in the environment, and the rarity of direct person-to-person spread implies that fungal vaccines cannot be expected to impart herd immunity on a population. Furthermore, most individuals with serious fungal infections are immunocompromised and are likely to mount suboptimal responses to a vaccine.<sup>70</sup> Nevertheless, vaccination could be considered for individuals with relatively intact immunity but who are anticipated to have severe immunosuppression in the future. Examples include persons awaiting solid organ transplant and those with early HIV infection. People living in or traveling to areas endemic for coccidioides and histoplasma are another important target group for vaccination. Finally, efforts are underway to develop vaccines that downregulate the allergic response in people who have asthma with fungal sensitisation.



**Figure 2: Immunomodulatory options in aspergillus-induced pulmonary allergic diseases**

Aspergillus hyphae can trigger excessive Th2 responses. Host-directed therapy with the anti-IgE monoclonal antibody omalizumab and vitamin D treatment to block OX40L expression on antigen-presenting cells might decrease exaggerated Th2 responses induced by aspergillus. Interferon  $\gamma$  could supplement the deficient interferon- $\gamma$  response in patients with allergic bronchopulmonary aspergillosis, increase aspergillus clearance via stimulation of phagocytosis and killing, and dampen Th2 responses. Furthermore, when a predominant neutrophilic signature is present, blocking cytokines such as tumour necrosis factor  $\alpha$ , interleukin 1, and interleukin 17 with biologicals such as anti-tumour necrosis factor  $\alpha$ , anakinra, and anti-interleukin 17 might be beneficial. TNF=tumour necrosis factor. IL=interleukin.

An attractive fungal vaccine strategy targets shared antigens of the most common genera of medically important fungi. For example, a key component of the fungus cell wall is  $\beta$ -1,3-D-glucan; however, this glycan is poorly immunogenic. Nevertheless, mice immunised with the  $\beta$ -1,3-D-glucan laminarin conjugated to diphtheria toxoid mount strong antibody responses and are protected in models of candidiasis, aspergillosis, and cryptococcosis.<sup>71</sup> Furthermore, immunisation of mice with antigens encapsulated in glucan particles results in long-lasting and protective antigen-specific antibody and T-cell responses.<sup>72</sup>

Preclinical studies of vaccines containing live, attenuated fungi have shown promising results.<sup>73</sup> Vaccination of mice with an attenuated strain of the endemic fungus *Blastomyces dermatitidis* protected against subsequent challenge with a virulent strain.<sup>8</sup> Remarkably, protection was seen even in the setting of CD4<sup>+</sup> T-cell depletion because of the emergence of protective CD8<sup>+</sup> T cells. Another live-vaccine

strategy that successfully protected CD4<sup>+</sup> T-cell deficient mice used a *Cryptococcus neoformans* strain engineered to produce murine interferon  $\gamma$ .<sup>74</sup> Such findings have obvious implications for the development of vaccines that protect in the setting of AIDS and other CD4<sup>+</sup> T-cell deficiencies.

A caveat to the use of live vaccines is that the fungi must be sufficiently attenuated to not cause disease, particularly in immunosuppressed hosts. In a phase 3 clinical trial,<sup>75</sup> one quarter of patients receiving a vaccine containing formaldehyde-killed spherules of *Coccidioides immitis* were protected against coccidioidomycosis, but this level of protection was statistically non-significant by comparison with controls. Although these outcomes showed the feasibility of human fungal vaccine trials, they also highlighted that the proinflammatory properties of fungi can elicit unacceptable local inflammatory reactions. For this reason, much of the focus in modern fungal vaccine development has been on subunit vaccines. Two vaccines containing recombinant *Candida*

*albicans*-derived proteins were found to confer immunogenicity in phase 1/2 clinical trials.<sup>76,77</sup>

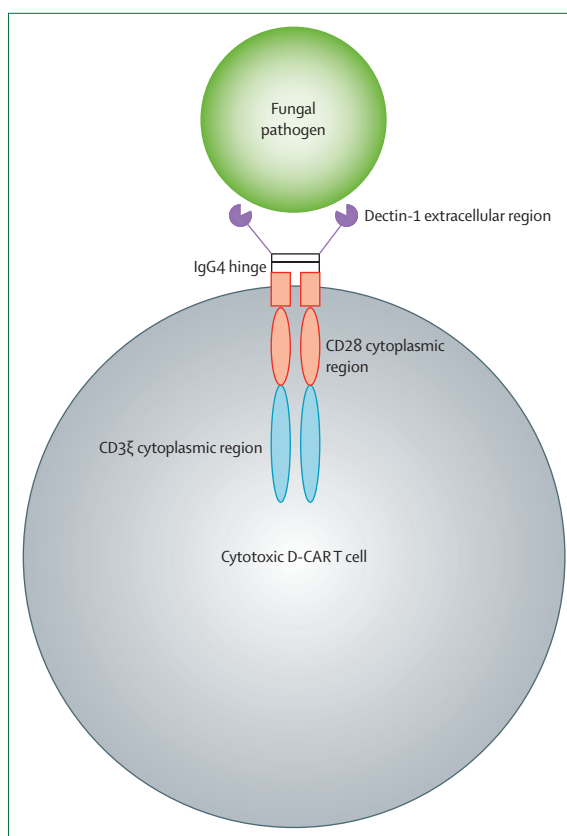
The recognition that antibodies elicited by vaccination could protect against fungal infection has led investigators to develop therapeutic monoclonal antibodies. In mouse models, monoclonal antibodies against cell wall  $\beta$ -1,3-D-glucan and *C neoformans* capsule have protective efficacy; in addition to opsonisation, the antifungal effects of antibodies have been attributed to direct inhibitory effects on fungal growth and metabolism.<sup>71,78</sup> Patients with AIDS and cryptococcal meningitis had a transient reduction in serum cryptococcal antigen titres after receiving monoclonal anticapsular antibody.<sup>79</sup>

### Cellular immunotherapy

Cellular immunotherapy is a promising approach to treating infection that is based on re-engineering immune cells to attack foreign invaders. In the 1960s, adoptive cell transfer (syngeneic or allogeneic transfer of cells into an individual) was developed for the treatment of T-cell malignancies.<sup>80</sup> Major progress was made after the discovery that immunoglobulin T-cell receptor chimaeric molecules could be expressed, heralding the advent of T cells that can be designed to target specific molecular targets.<sup>81</sup> Although T-cell-based therapy holds immense potential, serious risks are associated with the potential for cytokine storms.<sup>82</sup> In parallel, since the initial use of granulocyte transfusions for patients with neutropenic sepsis, innate cell therapy has steadily gathered pace as a promising alternative approach, with the advantage that there is no need to re-engineer antigen specificity.<sup>83</sup>

### Adoptive T-cell therapy and chimaeric antigen receptor T-cell engineering

The rationale behind adoptive T-cell therapy for patients with fungal disease is the observation that anti-aspergillus T cells are slow to engraft after allogeneic haematopoietic stem-cell transplantation.<sup>84</sup> Adoptive transfer of anti-aspergillus T cells has been tested in a clinical study and shown to enhance control of aspergillus antigenaemia and reduce mortality.<sup>85</sup> In further studies of refined selection and expansion protocols for anti-aspergillus T cells, investigators have primarily focused on optimal antigen-based selection protocols.<sup>86</sup> The potential for selecting multi-pathogen T cells against the medically important fungi has also been investigated.<sup>87</sup> The key questions are: whether or not specific, antigen-based selection or broad-repertoire selection using fungal extracts is optimal; what are the relative uses of CD4 and CD8 T cells; and what is the ability of specific antigens to induce specific Th1, Th2, or Th17 cells? One possible drawback of T-cell therapy is the potential for alloreactivity with the recipient; however, study findings so far indicate this alloreactivity is unlikely to be a serious problem.<sup>85</sup>



**Figure 3: Engineered T-cells for fungal therapy and the molecular anatomy of a D-CART cell**

Re-engineering T cells with specificity for fungal pathogens through the exploitation of C-type lectin receptor function is an exciting and novel approach for fungal disease therapy. In proof-of-principle studies,<sup>89</sup> the classic C-type lectin fungal receptor Dectin-1 extracellular region was expressed in combination with CD28 and CD3ζ cytoplasmic regions to enable  $\beta$ -1,3-D-glucan-specific activation of T cells in response to *Aspergillus fumigatus*.

Re-engineering of T cells with chimaeric antigen receptors has huge potential for the treatment of individuals with B-cell malignancies.<sup>88</sup> Fundamentally, chimaeric antigen-receptor technology is based on the manipulation of an extracellular ligand recognition domain, typically a single-chain variable fragment, bound to an intracellular signalling complex including CD3ζ to enable T-cell activation after antigen binding.<sup>88</sup> Enhanced activation can be achieved by adoption of further co-stimulatory molecules such as CD28, 4-1BB, or OX40. Taking this approach, Kumaresan and colleagues<sup>89</sup> substituted the variable fragment for the fungal-specific C-type lectin receptor Dectin-1 (figure 3). The resulting D-CAR T cells had specificity for  $\beta$ -1,3-D-glucan and were effective against *Aspergillus fumigatus* in vitro and in vivo (using murine models). This pivotal finding provides proof of principle that that chimaeric antigen-receptor technology can be extended to encompass C-type lectins, important innate pattern recognition receptors with broad repertoires against fungal pathogens.

### Innate cellular therapy

The association between neutropenia and severe sepsis has long been recognised, particularly in haematology medicine. Granulocyte transfusion was developed as a rational approach to treatment in the 1970s but its continued use was hindered by toxicity issues and was superseded by multiple broad-spectrum antimicrobial agents and growth factors in the 1980s. However, there has been renewed interest in granulocyte transfusion, especially with the availability of recombinant G-CSF and GM-CSF, which increase the yield of granulocytes from donors.<sup>83</sup> Two randomised studies of febrile neutropenia have been undertaken. In one study,<sup>90</sup> patients were randomly assigned to standard of care or standard of care plus granulocyte transfusions; no obvious difference in survival was found among the 55 patients with fungal disease, but the study was beset by logistical difficulties such as delays in granulocyte transfusions and was closed early. In the more recent multicentre, randomised study<sup>91</sup> of granulocyte transfusion for patients with febrile neutropenia, no difference was found in the overall success between granulocyte transfusion and antibiotics alone, although individuals who received the highest doses of granulocytes tended to have the best outcomes. Again, however, the study failed to meet enrolment targets.

Most research of dendritic cells for fungal immunotherapy is pre-clinical, with promising studies in murine transplantation models.<sup>92</sup> Priming dendritic cells with either aspergillus conidia or fungal RNA activates fungal-specific Th1 T cells and enhances protection from invasive aspergillosis. The advantage of using natural killer cells is that they do not cause graft-versus-host disease during adoptive transfer. Natural killer lymphocytes are also able to damage the hyphae of *A. fumigatus* and *Rhizopus arrhizus* by releasing perforin, and they have a crucial role in models of neutropenic aspergillosis.<sup>93,94</sup> As clinical trials are already underway to assess natural killer cell-adoptive therapy in malignancies, future studies to address their use in fungal disease are a new goal.<sup>95</sup> Other approaches include innate stimulation, for example with imiquimod for chromoblastomycosis, a chronic skin infection caused by impaired Toll-like receptor sensing of black fungi.<sup>96,97</sup>

### Immunotherapy and risk-stratification of patients

Outcomes of numerous studies provide evidence of an association between common genetic polymorphisms and increased risk for fungal infections, especially in patients who have received transplants.<sup>98,99</sup> It might be possible to stratify patient risk of infection on the basis of immunogenetics. These patients would benefit from intensive diagnostic screening or prophylactic antifungal therapy, and by knowing the exact defective immune pathway, targeted immunotherapy can be prescribed to circumvent the defect.

The fact that immunotherapies target specific immune defects or dysregulated components of the antifungal

host response highlights the importance of characterising the host immune status. With progression of the infection and the introduction of immune therapy, the antifungal host response can change with time, and the immunotherapy might need to be stopped or adjusted. New functional immunoassays are needed to define host deficits and to provide mechanistic insight to how these immunological deficits respond to immunotherapy.<sup>100,101</sup>

### Conclusions

Fungal diseases cause life-threatening infections in the context of primary and acquired immunodeficiencies all over the world. The close relation between infection susceptibility and immunocompromised status, combined with poor outcomes and increasing resistance to conventional antifungal chemotherapy, has pushed immunotherapy to the fore. The rapid progress in clinical immunotherapy research is creating unprecedented opportunities to exploit existing approaches for treatment of fungal disease—from recombinant cytokines to vaccines, monoclonal antibodies, and engineered T cells. Yet the biggest challenge in the next decade will be to test the use of immunotherapy for fungal diseases in carefully designed clinical trials.

### Contributors

All authors contributed to the writing of this paper. MSG, TZ, and DA-J designed the figures.

### Declaration of interests

We declare no competing interests.

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