Immunotherapy of aspergillosis

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Abstract

Management of invasive aspergillosis in high-risk patients remains challenging. There is an increasing demand for novel therapeutic strategies aimed at enhancing or restoring antifungal immunity in immunocompromised patients. In this regard, modulation of specific innate immune functions and vaccination are promising immunotherapeutic strategies. Recent findings have also provided a compelling rationale for assessment of the contribution of the individual genetic profile to the immunotherapy outcome. Altogether, integration of immunological and genetic data may contribute to the optimization of therapeutic strategies exerting control over immune pathways, ultimately improving the management of fungal infections in high-risk settings.

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Aspergillus fumigatus is a ubiquitous mould that can adapt to a broad range of environmental conditions [1]. *A. fumigatus* can cause a number of diseases, ranging from allergic to invasive, life-threatening forms of infection, which are strictly defined by the immune status of the host. The more severe forms of aspergillosis occur in specific clinical settings, such as patients with haematological malignancies and prolonged and severe neutropenia, or patients on corticosteroid therapy and receiving solid organ or stem cell transplantation. The management of invasive aspergillosis in high-risk settings remains challenging, owing to intrinsic or acquired antifungal resistance, organ dysfunction preventing the use of particular agents, and the deleterious effects of deregulated inflammation. Thus, there is an ever-increasing demand for novel therapeutic strategies to enhance or restore critical immune functions that may be applied to patients at high risk for fungal infections [1].

This review will highlight the conceptual advances made regarding the cellular and immunological mechanisms underlying the host–fungus interaction, and how this knowledge may be exploited for the successful design of immunotherapeutic interventions. The pathophysiological mechanisms underlying susceptibility to invasive aspergillosis are highlighted, along with the awareness that the genetic make-up of the host may also critically contribute to the risk of acquiring this disease [2,3].

Immunology of Aspergillosis: From Innate to Adaptive Immune Responses

In the absence of major immune deficiencies, humans are relatively resistant to infection by *A. fumigatus*, owing to the immunological inertia of resting conidia [4]. This also implies that, for most healthy humans, innate immune mechanisms are sufficient to clear the organism. These include the physical barriers of the respiratory tract and the activation of cellular (alveolar macrophages, dendritic cells (DCs), and recruited neutrophils) and humoral (pentraxin (PTX)3 (see below), complement, and cytokines) effector mechanisms of innate immunity (reviewed in [1]). Germination, however, exposes fungal-specific carbohydrates that serve as targets for invariant, germline-encoded pattern recognition receptors—such as Toll-like receptors (TLRs) and C-type lectin

receptors—that are crucial for the initiation of innate and adaptive T-helper (Th) immune responses [5]. Generation of a dominant Th1 response is essentially required for the expression of protective immunity to the fungus. Through the production of the signature cytokine interferon (IFN)-γ, Th1 cells are instrumental in optimal microbicidal activity of phagocytes. In contrast, allergic manifestations following inhalation of conidia, resulting from overzealous Th2 responses, are classic examples of the pathogenic role of T-cell dysreactivity in fungal diseases. However, a further degree of complexity of cross-regulatory innate and adaptive Th pathways lies beyond the dichotomous Th1/Th2 model. Recent insights have suggested a role for Th17 cells, as a link between T-cell inflammation and the granulocytic influx observed in allergic airway inflammation [5]. The Th17 pathway, playing an inflammatory role previously attributed to uncontrolled Th1 cell reactivity, and regulatory T-cells (T_{reg}s), which are capable of fine-tuning protective antimicrobial immunity in order to minimize harmful immune pathology, are now regarded as integral components of the immune response to the fungus (Fig. 1). The enzyme indoleamine-2,3-dioxygenase (IDO) and kynurenines pivotally contribute to this delicate balance by providing the host with immune mechanisms that are adequate for protection without necessarily eliminating fungal pathogens or causing an unacceptable level of tissue damage (see below). Specific antibodies against *A. fumigatus* have also been detected in patients suffering from invasive aspergillosis, and their role in infection could be of interest. Indeed, a glycoconjugate vaccine was found to efficiently immunize and protect mice against the infection [6], suggesting a role for antibody-mediated protection in aspergillosis.

Aspergillosis as an Example of an Inflammatory Disease

Although the inflammatory response to fungi may serve to limit infection, an overzealous or heightened inflammatory response may contribute to pathogenicity. This conceptual principle is best exemplified by the occurrence of severe fungal infections in patients with immunoreconstitution syndrome, an entity characterized by localized and systemic
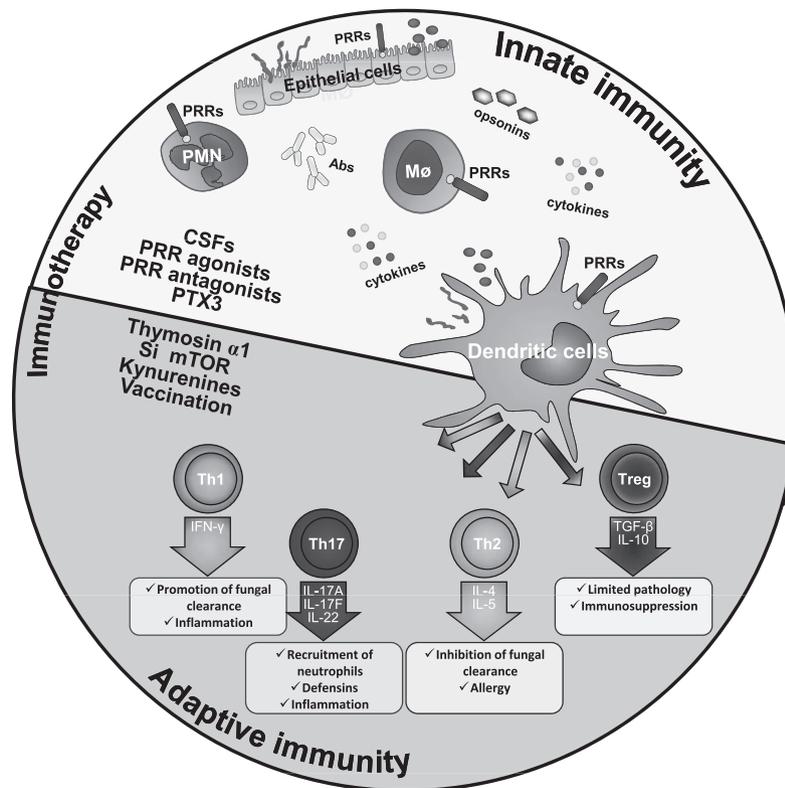

FIG. 1. Immunotherapy in aspergillosis. The figure shows recent findings on the innate and adaptive immune responses to *Aspergillus fumigatus* and the possible strategies for immune intervention. Most of these immunotherapies have only been evaluated in animal models of aspergillosis. Abs, antibodies; CSF, colony-stimulating factor; EC, epithelial cell; IFN, interferon; IL, interleukin; Mø, macrophage; PMN, polymorphonuclear neutrophil; PRR, pattern recognition receptor; PTX3, pentraxin 3; TGF, transforming growth factor; Th, T-helper; T_{reg}, regulatory T-cell. See the text for details.

inflammatory reactions of varying degrees that have both beneficial and deleterious effects on the host [7]. Intriguingly, immunoreconstitution syndrome responses are found after rapid resolution of immunosuppression, indicating that inflammatory responses can result in quiescent or latent infections manifesting as opportunistic mycoses. Thus, although host immunity is crucial in eradicating infection, immunological recovery can also be detrimental and may contribute to worsening disease in opportunistic and non-opportunistic infections [7]. The association of persistent inflammation with intractable *Aspergillus* infection is common in non-neutropenic patients after allogeneic hematopoietic stem cell transplantation and in the hyper-IgE syndrome, in which increased levels of proinflammatory gene transcripts have been described [8]. Therefore, the status of innate host immunity may contribute significantly to the pattern of diseases associated with fungal exposure. Corroborating these findings, recent studies have highlight the role of sterile inflammation—i.e. (auto)inflammation caused by endogenous ligands—as a pathogenic determinant responsible for inflammation-driven pathology in aspergillosis [9]. Most prominently, in patients with chronic granulomatous disease (CGD), a hyperinflammatory phenotype and defective fungal—typically *A. fumigatus*—clearance have long been known to occur. This condition is crucially exemplified by recent findings in CGD mice, in which an intrinsic, genetically determined failure to control inflammation caused by sterile fungal components determines the animals' inability to resolve an actual infection with *A. fumigatus* [10]. These observations highlight the truly bipolar nature of the inflammatory process in infection. Early inflammation prevents or limits infection, but an uncontrolled response may eventually oppose disease eradication. A main implication of these findings is that, at least in specific clinical settings, it is an exaggerated inflammatory response that probably compromises a patient's ability to eradicate infection, and not an 'intrinsic' susceptibility to infection that determines a state of chronic or intractable disease.

Translating Immunity into Clinical Perspectives

Colony-stimulating factors (CSFs), granulocyte transfusion, and cytokines

CSFs and cytokines, mainly IFN-γ, have been utilized in the clinical management of fungal diseases [11]. CSFs and granulocyte transfusions are used to augment the number and the function of circulating neutrophils in neutropenic patients. Studies *in vitro*, studies in animal models and limited patient data provide a rationale for adjunctive IFN-γ for invasive aspergillosis. The limited clinical experience to date shows a possible benefit of these cytokines, and further controlled clinical trials are needed to validate their routine use in cancer patients and stem cell transplant recipients with invasive fungal infections, who are likely to have a poor response to antifungal drug therapy [12]. Pairing IFN-γ with CSFs could be an option in the setting of refractory fungal disease, although clinical experience is anecdotal, and the efficacy of this approach is not established (reviewed in [13]). Moreover, the diverse effects of CSFs on the immune system and types of immune response generated suggest an activity for CSFs beyond their effects on haematopoiesis [14]. For instance, granulocyte–macrophage CSF has recently been shown to play a key role in human DCs for activation of inflammatory Th1 and Th17 cells, and to serve a non-redundant function in the initiation of autoimmune inflammation [15]. Emerging evidence demonstrates the benefits of granulocyte transfusions for treating infections in patients after treatment with high-dose chemotherapy, particularly the chemotherapy associated with conditioning for haematopoietic stem cell transplantion. The increased interest in the use of therapeutic granulocyte transfusion in recent years has stemmed largely from improvements in donor mobilization methods. However, the evidence for clinical efficacy is limited to that of case reports and small series, and the results are not uniform. Randomized controlled clinical trials are needed to determine whether this therapy is useful in either clearing infections or prolonging survival [16,17].

T-cell therapy

T-cell-mediated heterologous immunity to different pathogens is promising for the development of immunotherapeutic strategies. Fungus-specific CD4^{+} Th1 immunity is an appealing strategy for antifungal therapy [18]. Recently, an immunogenic epitope of the *A. fumigatus* cell wall glucanase Crf1 was reported to be capable of inducing major histocompatibility complex class II restricted memory CD4^{+} Th1 cells that are cross-reactive with *Candida albicans* [19]. These data illustrate the existence of T-cell-based cross-protection for the distantly related clinically relevant fungal pathogens that may foster the development of immunotherapeutic strategies. More recently, the usefulness of T_{reg}-based immunotherapeutic approaches in allogeneic transplanted patients has also been investigated. Early adoptive therapy with T_{reg}s followed by conventional T-cells was shown to favour immunological reconstitution, besides preventing graft-versus-host disease without weakening the graft-versus-leukaemia effect [20]. However, whether and how adoptive immunotherapy with T_{reg}s may compromise general immunity and blunt responses to other infectious agents remain to be fully assessed.

Anti-inflammatory therapies

Breakthroughs in our understanding of how immune homeostasis is established, maintained or disrupted in the presence of fungal exposure and/or colonization could be sources of new therapeutics and drugs targeting specific inflammatory or metabolic endpoints. In essence, limiting inflammation to stimulate a protective immune response to fungi should pave the way for the rational design of novel immunomodulatory therapies. Below are examples of new therapeutics that are potentially capable of simultaneously activating antifungal resistance and taming overzealous inflammatory host responses.

PTX3

PTX3 is receiving great attention as a potential therapeutic agent with anti-inflammatory properties in aspergillosis. PTX3 is an opsonin that forms complexes on the conidial surface of *A. fumigatus*, thereby amplifying the innate immune response. Remarkably, PTX3 deficiency rendered immunocompetent mice highly susceptible to *A. fumigatus* infection [21], whereas PTX3 administration protected against invasive aspergillosis in mice with T-cell-depleted allogeneic transplants and potentiated the efficacy of amphotericin B. More recently, early administration of exogenous PTX3 was shown to enhance the conidiocidal activity of neutrophils and limit the inflammatory pathology in NADPH oxidase-deficient mice [22]. Finally, a number of predominantly non-coding polymorphisms have been identified in the *PTX3* gene that could be relevant in clinical fungal infections [23].

IDO and tryptophan metabolites

It has been shown that resistance and tolerance are two types of host defence mechanism to increase fitness in response to fungi [5]. In experimental fungal infections, both defence mechanisms are activated through the delicate equilibrium between Th1 cells (which provide antifungal resistance mechanisms) and T_{reg}s, which limit the consequences of the associated inflammatory pathology [24]. IDO and kynurenines pivotally contribute to this delicate balance by providing the host with immune mechanisms that are adequate for protection without necessarily eliminating fungal pathogens or causing an unacceptable level of tissue damage [24]. Through their capacity to induce T_{reg}s and inhibit Th17, IDO and kynurenines pivotally contribute to cell lineage decisions in experimental fungal infections, and have shown an unexpected potential to control inflammation, allergy and Th17-driven inflammation in these infections [24]. In this context, the Th17 pathway, which downregulates tryptophan catabolism, may instead favour pathology [25] and serve to explain the seemingly paradoxical association of chronic inflammation with fungal disease. In experimental CGD, interleukin-17 neutralization increased

fungal clearance, ameliorated inflammatory pathology, and restored protective Th1 antifungal resistance [10]. Perhaps more importantly, complete cure and reversal of the hyperinflammatory CGD phenotype were achieved by administration of supplemental L-kynurenine, an early amino acid catabolite of L-tryptophan in the IDO-dependent pathway.

Thymosin α1 (Tα1)

Innate signalling may either activate or limit, via induction of T_{reg}s, adaptive immunity. This implies that selected PRR ligands might be useful candidate adjuvants with which to dampen inflammatory responses associated with infections. Tα1, a naturally occurring thymic peptide, which is approved in 30 countries for the treatment of a number of viral infections [26] and as an immune adjuvant, has been found to promote maturation of and cytokine production by DCs via TLR signalling [27]. By controlling the balance between the activity of immunogenic and tolerogenic DCs, Tα1 acted as an endogenous immune regulator that was capable of inducing protective immunity to *A. fumigatus*. In effect, Tα1 was found to induce IDO expression and production of kynurenines, thereby imprinting a tolerogenic programme into inflammatory DCs [27]. Thus, instructive immunotherapy (therapy based on the instruction/education of specific cell types) with Tα1 targeting IDO-competent DCs could allow for balanced control of inflammation and tolerance in aspergillosis.

Targeting inflammatory pathways with small interfering RNAs (siRNAs)

The intranasal administration of siRNAs has opened new avenues in drug delivery and respiratory therapy [28]. Although the *in vivo* use of siRNAs has been limited, they have huge potential as therapeutic agents, given the absence of adverse immune reactions and systemic access. Adding to the potential of siRNAs as therapeutic agents in the areas of cancer and viral infections, a recent study showed the potential of siRNAs to attenuate inflammation in respiratory fungal infections [29]. *In vivo* targeting of inflammatory (phosphoinositide 3-kinase/Akt/mTOR) or anti-inflammatory (STAT3/IDO) pathways by intranasally delivered siRNAs modified inflammation and immunity in aspergillosis. Thus, the screening of signalling pathways through a systems biology approach may be exploited for the development of siRNA therapeutics to attenuate inflammation in respiratory fungal infections and diseases. Ultimately, because immunity is neither simply a set of discrete signalling pathways activated by pathogens nor a function of the host, the utilization of systems biology approaches is useful for the generation of new therapeutics that target pathogenicity rather than microbial growth, the

host–pathogen interface rather than the pathogen, and promote protective immune responses.

Vaccine development

With the exception of a killed spherule vaccine for coccidioidomycosis, no fungal vaccine trials have ever been conducted. However, the level of our understanding of fungal–host interactions has progressed to the point where vaccines against both primary fungal pathogens and the prevalent opportunistic fungi are becoming a reality [30]. In this regard, given the plasticity of the DC system in initiating disparate immune responses to fungi, vaccine-induced education of T-cells could be regarded as a promising strategy to reduce the risk of fungal infection.

Genetic Variability and Immunotherapy Outcome: Towards Personalized Treatments?

Although the dissection of the complex genetic traits modulating susceptibility to fungal infections is a challenge, the contribution of host genetics may hold the key to elucidating genetic markers for fungal infections and diseases occurring in high-risk patients. The lack of understanding regarding which patients are at highest risk of developing a life-threatening infection is at present a major deficiency, and genetic markers will probably assist in risk assessment. A number of studies have investigated the role of genetic variability in defining individual susceptibility to fungal infections [2,3]. A single-nucleotide polymorphism in TLR4 was shown to be a risk factor for chronic aspergillosis in immunocompetent individuals [31], and later for invasive aspergillosis among unrelated patients with allogeneic transplants [32]. More recently, the stop codon polymorphism Y238X in dectin-1 was also found to contribute to increased susceptibility to aspergillosis in the stem cell transplantation setting; this study also revealed the distinct contributions of the haematopoietic/non-haematopoietic cell compartments to the induction of immune protection against the fungus [33]. Although considerable efforts have been made in this direction, there is still the problem of the limited power of the existing studies. Larger, well-designed trials, preferably performed in consecutive patients, are ultimately required to clarify the usefulness of genetic stratification of patients at risk for infection.

Perspectives in Antifungal Immunotherapy

The new discoveries in the field of fungal immunology have offered new grounds for a better comprehension of cells and immune pathways that are amenable to manipulation in patients with or at risk of fungal infections (Fig. 1). By influencing immune responses and inflammation, single-nucleotide polymorphisms in genes concerned with immunity may also affect the outcome of immunotherapeutic interventions. Therefore, when immunotherapeutic strategies are being devised, the new conceptual advances in the knowledge of antifungal immunity should not only be considered, but also accommodated from an immunogenetic standpoint. Identifying the cellular and molecular bases affected by genetic polymorphisms may prove to be very powerful in maximizing the potential of therapeutic targets and prophylactic strategies that exert control over the outcome of immune pathways. Altogether, the genetic screening of at-risk patients may ultimately be used to individualize treatments through the formulation of new targeted and patient-tailored antifungal immunotherapeutics, probably improving the management and outcome of fungal infections.

Acknowledgements

Transparency Declaration

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