Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *Advances Against Aspergillosis*

Inflammation in aspergillosis: the good, the bad, and the therapeutic

Agostinho Carvalho, Cristina Cunha, Rossana G. Iannitti, Antonella De Luca, Gloria Giovannini, Francesco Bistoni, and Luigina Romani

Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy

Address for correspondence: Luigina Romani, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Via del Giochetto, 06122 Perugia, Italy. lromani@unipg.it

Aspergillosis includes a spectrum of diseases caused by different *Aspergillus* spp. New insights into the cellular and molecular mechanisms of resistance and immune tolerance to the fungus in infection and allergy have been obtained in experimental settings. The fact that virulence factors, traditionally viewed as fungal attributes, are contingent upon microbial adaptation to various environmental stresses encountered in the human host implies that the host and fungus are jointly responsible for pathogenicity. Ultimately, despite the occurrence of severe aspergillosis in immunocompromised patients, clinical evidence indicates that aspergillosis also occurs in the setting of a heightened inflammatory response, in which immunity occurs at the expense of host damage and pathogen eradication. Thus, targeting pathogenicity rather than microbial growth, tolerance rather than resistance mechanisms of defense may pave the way to targeted anti-inflammatory strategies in difficult-to-treat patients. The challenge now is to translate promising results from experimental models to the clinic.

Keywords: *Aspergillus*; inflammation; Th cells; PAMPs; DAMPs; tolerance

Introduction

Aspergillus is a large genus containing more than 180 species that are ubiquitous in nature. Among the species, *Aspergillus fumigatus* remains the most common cause of human diseases, followed by other species including *A. flavus, A. niger, A. nidulans,* and *A. terreus*. Diseases caused by *Aspergillus* include saprophytic colonization of preexisting cavities (aspergilloma), allergic asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis (ABPA) occurring as a complication of bronchial asthma or cystic fibrosis, and invasive aspergillosis. Invasive aspergillosis is associated with a high mortality rate in patients with dysregulated immunity, such as patients with hematological malignancies, recipients of solid organs and stem cell transplants, and patients admitted to the intensive care unit.[1] Although the past decade has witnessed significant progress in the management of invasive aspergillosis, the infection continues to be a deadly disease.[1] The main reasons for this include intrinsic or acquired antifungal resistance and the deleterious effect of a dysregulated inflammation.

Current understanding of the pathophysiology underlying *Aspergillus* infection and disease highlights the multiple cell populations and cell-signaling pathways involved in these complex conditions. Applying systems biology approaches to these complex processes has resulted in a better appreciation of the intricate cross talk provided by temporal changes in mediators, metabolites, and cell phenotypes underlining the coordinated processes.[2] In addition to the pathogen-derived ligands (PAMPs) of pattern recognition receptors (PRRs),[3] primary metabolites associated with fungal growth and adaptation[4] and damage-associated molecular patterns (DAMPs)[5] all contribute to fungal pathogenesis and generation of antifungal immune response. These emerging themes in fungal pathogenesis better accommodate the susceptibility to fungal diseases in primary immunodeficiencies,

doi: 10.1111/j.1749-6632.2012.06754.x

Ann. N.Y. Acad. Sci. 1273 (2012) 52–59

such as chronic granulomatous disease (CGD)[6,7] and cystic fibrosis (CF),[8] in which susceptibility to *Aspergillus* diseases occurs in the face of high-level inflammation. In this regard, despite the occurrence of severe aspergillosis in immunocompromised patients, clinical evidence indicates that aspergillosis also occurs in the setting of a heightened inflammatory response, in which immunity occurs at the expense of host damage and pathogen eradication.[9] The main implication of these findings is that, at least in specific clinical settings, it is a heightened inflammatory response that likely compromises a patient's ability to eradicate infection, not an intrinsic susceptibility to infection that determines a state of chronic or intractable disease.[10] The conceptual principle highlighting the truly bipolar nature of the inflammatory process in infection is best exemplified by the occurrence of severe fungal infections in patients with immune reconstitution syndrome, an entity characterized by localized and systemic inflammatory reactions and worsening disease in opportunistic and nonopportunistic infections, which are associated with immunological recovery.[9,11] In addition, a high incidence of fungal infections and sensitization to *Aspergillus* spp. has been described in hyperimmunoglobulinemia E (hyper-IgE) syndrome[12,13] in which increased levels of proinflammatory gene transcripts have recently been described.[13] For *A. fumigatus* the association of persistent inflammation with intractable infection is common in nonneutropenic patients after allogeneic hematopoietic stem cell transplantation,[14] as well as in allergic fungal diseases.[15] Therefore, paradoxically, increased inflammatory innate responses may predispose to either fungal infections or dysregulated immune responses to the fungus. In addition, the status of innate host immunity may also contribute significantly to the histological patterns associated with fungal infections.[1] Thus, although host immunity is crucial in the eradication of infection, immunological recovery can also be detrimental and may contribute to worsening disease.

Immunity to *Aspergillus*

Immunocompetent and nonatopic subjects are relatively resistant to *Aspergillus* infections; disease occurs only in the setting of host damage.[1] Thus, a high degree of coexistence occurs between airborne fungi and their mammalian hosts, which deviates into overt disease only under specific conditions, most prominently deficits in immunity. The inflammatory response, initiated by cells of the innate immune system, is followed by adaptive immunity, which responds to and, at the same time, regulates signals emanating from the innate system. The inflammatory allergic manifestations that follow the contact or inhalation of *Aspergillus* spp. all constitute compelling evidence of the pathogenic role of T cell dysreactivity in fungal diseases.

Innate recognition: PAMPs, DAMPs, and beyond

The physical barrier of the respiratory tract provides a first line of resistance to hundreds of conidia inhaled daily. However, due to the small size of resting *Aspergillus* conidia (2–5 μm in diameter), inhaled conidia may go beyond the ciliated epithelium to reach sites where they stimulate cells of the innate immune system, such as resident alveolar macrophages and dendritic cells (DCs), as well as recruited inflammatory cells, mainly polymorphonuclear neutrophils (PMNs), by means of soluble and cell-bound pattern recognition receptors (PRRs).[3,16,17] Thus, antigen-independent recognition of the fungus by the innate immune system leads to the immediate mobilization of immune effector mechanisms that provide the host with the establishment of a first line of defense and an appropriate adaptive immune response. This implies that immunity to the fungus is a dynamic interplay between every arm of the immune system in which how to respond is primarily determined by interactions between the fungus and cells of the innate immune system; T cells, however, feed back into this dynamic equilibrium to regulate antifungal effector functions and the balance between proinflammatory and anti-inflammatory signals.

PRRs on host cells include Toll-like receptors (TLRs), C-type lectin receptors, nucleotide oligomerization domain (NOD)-like receptors,[18] and NALP3 inflammasome,[19] all of which sense PAMPs and DAMPs (Fig. 1). Both murine and human studies have confirmed the susceptibility to *Aspergillus* infection and diseases associated with genetic deficiency of selected PRRs.[3] By varying the composition with the morphotype, growth stage, environment sensing, and fungal species the cell wall provides the prime sources of PAMPs that, as such, are ideal targets for recognition as nonself by

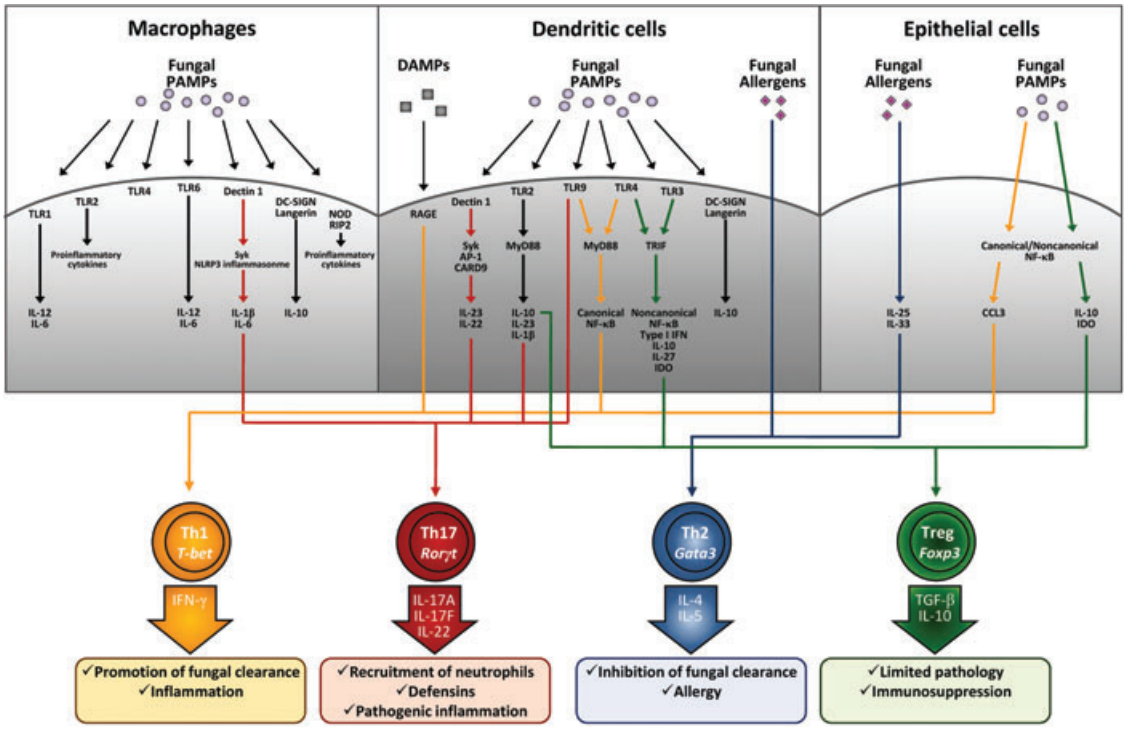

Figure 1. Innate and adaptive cell–mediated immunity to *Aspergillus*. The figure is an update of the innate and adaptive responses mediated by different Th cell subsets, their transcription factors, and possible effector functions in aspergillosis. Through the production of distinct sets of cytokines and other mediators, T cells can act as immune effectors and as master regulators of the inflammatory and effector responses of innate cells. The ability of dendritic cells, macrophages, and epithelial cells to respond to the different fungal PAMPs, fungal allergens, or DAMPs with flexible intracellular signaling pathways confers unexpected plasticity to the system and contributes to shaping T cell responses. CARD9, caspase recruitment domain-containing protein 9; CCL3, chemokine (C–C motif) ligand 3; DAMPs, damage-associated molecular patterns; IDO, indoleamine 2,3-dioxygenase; nucleotide oligomerization domain (NOD)–like receptors; NLRP3, NOD-like receptor family, pyrin domain-containing 3; PAMPs, pathogen-associated molecular patterns; RAGE, receptor for advanced glycation end products.

mammalian cells.[20] To achieve optimal activation of antigen-specific adaptive immunity it is first necessary to activate the pathogen-detection mechanisms of the innate immune response. However, by hyper-inducing proinflammatory cytokines, facilitating tissue damage, or impairing protective immunity, PRR activation itself is a double-edged sword, and this may explain why PMNs, although essential in the initiation and execution of the acute inflammatory response and subsequent resolution, may also act as double-edged swords, as the excessive release of oxidants and proteases may be responsible for injury to organs and fungal sepsis. As a matter of fact, despite extensive fungal growth, pulmonary pathology is reduced in conditions of PMN deficiency.[21] Thus, although the inflammatory response to fungi may serve to limit infection, an overzealous or heightened inflammatory response may contribute to pathogenicity and, paradoxically, favor the infection.[7,22,23] A hyperinflammatory response does in fact enhance virulence of many *Aspergillus* spp.[24] This implies that the fungus may exploit PRRs to divert and subvert host inflammatory immune responses to become a frank pathogen. Accordingly, it has been shown that activation of TLRs by the fungus unmasks an essential role for the protease-activated receptors (PAR) 2 in downstream signaling and inflammation.[25] Conceptually, the model is consistent with a binary signaling pathway of mammalian recognition of fungi, as observed in *Drosophila* and plants.

The unexpected convergence of molecular pathways responsible for the recognition of PAMPs and DAMPs raised the question of whether and how

the host discriminates between PAMPs and DAMPs and the relative contribution of either one to inflammation, immune homeostasis, and mechanisms of repair during infection. A mechanism that discriminates between pathogen- and danger-induced immune responses via the spatiotemporal integration of signals from TLRs and the receptor for advanced glycation end products (RAGE) has recently been described in mice, and a genetically determined hyperfunction of DAMP signaling was associated with invasive aspergillosis in hematopoietic stem cell transplanted (HSCT) patients.[26] The mechanism exploits a previously unrecognized role for the S100B/RAGE axis that, in sensing danger, plays a critical and unanticipated role as a fine modulator of inflammation in *Aspergillus* pneumonia. Thus, the crosstalk between RAGE and TLRs details an evolving braking circuit whereby an endogenous danger protects the host against pathogen-induced inflammation and a pathogen-sensing mechanism terminates danger-induced inflammation.

The ability of fungi to colonize almost every niche within the human body involves specific reprogramming events to adapt to environmental conditions, to fight for nutrient acquisition, and to use and deal with stresses generated by host defense mechanisms.[27] Genomic and transcriptomic approaches have revealed links among fungal metabolism, morphogenesis, and the response to stress in adaptation to the host environment. Thus, understanding *in vivo* fungal metabolism in the different host microenvironments is critical for new therapeutic approaches. Recent evidence has indicated how the ability of *Aspergillus* to adapt to oxygen-limited or hypoxic microenvironments has a great impact on fungal pathogenesis.[4] In these conditions, the fungus activates glycolysis, transcriptional downregulation of the TCA cycle, and oxidative phosphorylation and produces secondary metabolites that promote lung inflammation, exacerbate the infection, and influence subsequent host immune responses.[28,29]

Adaptive T helper cell immunity

Different types of T helper (Th) cell subsets are locally activated upon repeated conidia inhalation in mice[30] and are present in the human T cell repertoire.[31] The ability of DCs to respond with flexible activating programs and activation of distinct intracellular signaling pathways to the different PRR/PAMP combinations confers unexpected plasticity to the DC system and pivotally contributes in shaping the different Th cell responses (Fig. 1). Although epithelial cells (ECs) are not dedicated antigen-presenting cells they may play critical immunological roles by expressing PRRs and providing the machinery required for the induction of T cell tolerance (Fig. 1). Th1 responses driven by IL-12 are essentially required for the expression of protective immunity to the fungus in mice and humans,[32] as well as to fungal vaccines.[6] Through the production of the signature cytokine interferon-gamma (IFN-γ), the activation of Th1 cells is instrumental in the optimal activation of phagocytes and of a tolerogenic environment at sites of infection. The importance of IFN-γ in response to respiratory fungal pathogens has been recently recognized.[33] Allergy is an overzealous Th2 response to environmental airborne allergens. The inflammatory allergic manifestations that follow the contact or inhalation of fungi constitute compelling evidence of the pathogenic role of T cell dysregulated reactivity in fungal diseases. In patients with a defective IL-12/IFN-γ pathway, such as those with hyperimmunoglobulinemia E syndrome, fungal infections and allergy are observed.[34] Although normally induced upon conidia inhalation in mice,[35] Th2 responses are associated with aspergillosis in murine[36] and human[37,38] CF. We have recently shown that Th17 responses are also associated with susceptibility to ABPA and *Aspergillus* infection in experimental CF.[8] Thus, similar to what is observed in experimental CGD,[7] Th17 cell activation correlates with *Aspergillus* diseases in immunodeficiencies. These findings are consistent with the observation that the repeated intranasal exposure to *A. fumigatus* conidia in mice results in a chronic pulmonary inflammatory response characterized by eosinophilia, goblet cell metaplasia, and sustained Th17 cell activation that drives Th2-type inflammation.[39] However, IL-23 production and Th17 responses also negatively regulate the allergic inflammatory response in chronic fungal-induced asthma,[40] a finding indicating that the actual role of the Th17 pathway and cytokines in fungal infection and allergy needs further elucidation. Indeed, repeated exposure to *A. fumigatus* conidia induced the codevelopment of Th1, Th2, and Th17 cell responses in the lungs of immunocompetent mice,[30,41,42] arguing for a

redundancy in protective Th mechanisms of antifungal immunity. In humans, *A. fumigatus* did not stimulate production of IL-17, and human host defense against aspergillosis has been shown to rely on potent Th1 responses.[43]

Protective tolerance to *Aspergillus*: regulatory T cells and the tryptophan metabolic pathway

Continued integration of proinflammatory and antiinflammatory stimuli is mandatory for proper control of infection, tissue, and immune homeostasis. A major role of the innate and adaptive immune systems is to maintain tissue homeostasis by stopping and/or preventing cell damage, caused by invading pathogens, and promoting tissue repair following a lesion. The inherent resistance to infections caused by *A. fumigatus* suggests the occurrence of regulatory mechanisms that provide the host with adequate defense without necessarily eliminating the fungus or causing unacceptable levels of host damage.[10,44] The consequence of regulatory activity is less damage to the host but also fungal persistence due to the impaired efficacy of protective immunity, as evidenced by a number of clinical observations suggesting an inverse relationship between IFN-γ and IL-10 production in patients with aspergillosis.[45,46] In addition, consistent with the inflammatory state, patients with pulmonary aspergillosis are genetically low producers of both TGF-β and IL-10.[47] Regulatory T (T_{reg}) cells with tolerogenic activity have been described in murine aspergillosis;[44] in these mice, fungal growth, inflammatory immunity, and tolerance to *Aspergillus* were controlled by the coordinate activation of natural (n)T_{reg} cells, which limited early inflammation at the sites of infection, and pathogen-induced (i) T_{reg} cells that regulated the expression of adaptive Th immunity in secondary lymphoid organs. Early in infection, inflammation was controlled by the expansion, activation, and local recruitment of nT_{reg} cells that suppressed PMNs through the combined actions of IL-10 and CTLA-4 acting on dendritic cells to produce indoleamine 2,3-dioxygenase (IDO), an enzyme that has revealed an unexpected potential in controlling inflammation, infection, and allergic airway inflammation.[48] Mechanistically, the levels of inflammation and IFN-γ in the early stage of contact with the fungus set the subsequent adaptive stage by conditioning the IDO-dependent tolerogenic program of DCs and the subsequent activation and expansion of tolerogenic T_{reg} cells that prevent allergy to the fungus. Asymptomatic atopy associated with increased IDO activity and IL-10 production in seasonal allergen exposure has been described.[49] Late in infection, and similarly in allergy, inflammatory immunity was modulated by inducible (i) T_{reg} cells, which acted through activation of IDO in DCs and prevented Th17 cell development.[44,50] Thus, protective tolerance to fungi is achieved through the balanced act of T_{reg} cells over effector Th1 and Th17 cells, with the contribution of IDO. Recent evidence have indicated a role for ECs in this balance via the IFN-γ/IDO axis culminating in the activation of T_{reg} cells and inhibition of Th17 cell responses.[51] With pathogens like fungi that have a complex pathogenesis, multiple types of regulatory cells are likely to influence the outcome. We have recently found that antigen-specific $CD4^+$ T_{reg} cells, which are developmentally, phenotypically, and functionally distinct from $Foxp3^+$ T_{reg} cells, are induced in response to *A. fumigatus* in mice and humans (R.G. Iannitti, unpublished observation).

Inflammation in aspergillosis: the therapeutic

Medical treatments that increase host resistance, such as antibiotics, place selective pressures on pathogens. As tolerance mechanisms are not expected to have the same selective pressure on pathogens, new drugs that target tolerance will provide therapies to which pathogens will not develop resistance. Thus, further understanding of the cooperation of various multiple innate and adaptive immune responses that lead to protective tolerance will provide the basis for novel immunomodulatory therapies that are no longer just promising as adjuncts in the management of fungal infections but are strictly required to balance protective immunity and inflammatory pathology in infections. Despite the heterogeneity of patient populations at risk for invasive aspergillosis, the greatest challenge is to pave the way from promising results in experimental models to the clinic. The following targeted anti-inflammatory strategies and vaccines are potential future additions to the arsenal against *Aspergillus* diseases.

Restraining pathogenic inflammation by exogenous kynurenines

In experimental CGD, IL-17A neutralization increased fungal clearance, ameliorated inflammatory pathology, and restored protective Th1 antifungal resistance.[7] 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.[7] Similar results have been obtained in experimental CF.[8]

Targeting IDO in fungal allergy

Consistent with the responsiveness of ABPA to corticosteroid treatment,[52] the Th2 allergic phenotype is attenuated by dexamethasone, which enhanced production of IL-10 and Foxp3 transcripts, both markers of protective T_{reg} cell activity in *Aspergillus* allergy. These data demonstrated that dexamethasone downregulates exacerbating Th2 cell responses in ABPA by inhibiting the expansion and activation of Th2 cells and by upregulating the expression of Foxp3 via mechanisms that require tryptophan catabolism.[50] Thus, induction of IDO could be an important mechanism underlying the anti-inflammatory action of corticosteroids.

Targeting inflammatory pathways via siRNA

It has been demonstrated that the activation of distinct signaling pathways in DCs and other antigen-presenting cells translates recognition of the fungus into distinct inflammatory and adaptive immune responses[42] (Fig. 1). Thus, the screening of signaling pathways in DCs through a systems biology approach could be exploited for the development of therapeutics to attenuate inflammation in respiratory fungal infections and diseases. Indeed, the study showed the utility of the DC systems biology approach for quick screening of therapeutic siRNA to attenuate inflammation in *Aspergillus* infection and allergy. The intranasal administration of siRNA has opened new avenues in drug delivery and respiratory therapy.[53]

Exploiting IDO + DCs as fungal vaccines in transplantation

The finding that *Aspergillus*-pulsed DCs could be used as a vaccine in hematopoietic transplantation[54] raised excitement about vaccination against aspergillosis.[55] Within the instructive model of DC-mediated regulation of the Th repertoire, it is conceivable that an improved understanding of the pathogen/DC interaction will allow the potential use of pathogen- or TLR-conditioned DCs for the induction of patient-tailored T_{reg} cells with indirect antidonor allospecificity. Over recent years, experimental models have shown that it is possible to exploit the mechanisms that normally maintain immune homeostasis and tolerance to self-antigens to induce tolerance to alloantigens.[56] Like natural tolerance, transplantation tolerance is achieved through control of T cell reactivity by central and peripheral mechanisms of tolerance. We have recently found that this goal is achievable by the adoptive cellular therapy of *Aspergillus*-pulsed DCs + IDO that could induce antifungal resistance within a regulatory environment.[57] Tolerogenic DCs + IDO proved to be pivotal in the generation of some form of dominant regulation that ultimately controlled inflammation, pathogen immunity, and tolerance in transplant recipients, eventually leading to prevention of graft-versus-host reaction and reduction of aspergillosis incidence rates.

Conclusions

In summary, because immunity is neither a set of discrete signaling pathways activated by pathogens nor simply a function of the host, the use of systems biology approaches has proved to be useful for the generation of new therapeutics that targets pathogenicity rather than microbial growth, targets the host–pathogen interface rather than the pathogen, and promotes protective immune responses. It is now clear that genetic variants of molecules involved in innate recognition of fungi may account, in part, for the inherited differences in human susceptibility to fungal infections.[58,59] Thus, the integration of systems biology approaches with immunogenetic screenings may promote the development of targeted immunotherapeutic strategies in high-risk patients.

Acknowledgments

We thank Dr. Cristina Massi Benedetti for digital art and editing. This work was supported by the Specific Targeted Research Project ALLFUN (FP7–HEALTH–2009 Contract Number 260338) and the Italian Grant Application 2010 Fondazione per la Ricerca sulla Fibrosi Cistica (Research Project FFC#21/2010), with the contribution of the

Francesca Guadagnin, Coca Cola Light Tribute to Fashion and Delegazione FFC di Belluno.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Latge, J.P. & W.J. Steinbach. 2009. *Aspergillus fumigatus and Aspergillosis*. ASM Press. Washington, DC.
2. Santamaria, R. *et al*. 2011. Systems biology of infectious diseases: a focus on fungal infections. *Immunobiology* **216:** 1212–1227.
3. Romani, L. 2011. Immunity to fungal infections. *Nat. Rev. Immunol.* **11:** 275–288.
4. Grahl, N. *et al*. 2011. *In vivo* hypoxia and a fungal alcohol dehydrogenase influence the pathogenesis of invasive pulmonary aspergillosis. *PLoS Pathog.* **7:** e1002145.
5. Sorci, G. *et al*. 2011. The danger signal S100B integrates pathogen- and danger-sensing pathways to restrain inflammation. *PLoS Pathog.* **7:** e1001315.
6. De Luca, A. *et al*. 2012. CD4 T cell vaccination overcomes defective cross-presentation of fungal antigens in murine chronic granulomatous disease. *J. Clin. Invest.* **122:** 1816–1831.
7. Romani, L. *et al*. 2008. Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease. *Nature* **451:** 211–215.
8. Iannitti, R.G. *et al*. 2012. Aspergillosis in cystic fibrosis: a multifactorial disease? In *Proceedings of the 5th Advances Against Aspergillosis Conference*. Istanbul, Turkey.
9. Perfect, J.R. 2012. The impact of the host on fungal infections. *Am. J. Med.* **125:** S39–S51.
10. Romani, L. & P. Puccetti. 2006. Protective tolerance to fungi: the role of IL-10 and tryptophan catabolism. *Trends Microbiol.* **14:** 183–189.
11. Gupta, A.O. & N. Singh. 2011. Immune reconstitution syndrome and fungal infections. *Curr. Opin. Infect. Dis.* **24:** 527–533.
12. Antachopoulos, C., T.J. Walsh & E. Roilides. 2007. Fungal infections in primary immunodeficiencies. *Eur. J. Pediatr.* **166:** 1099–1117.
13. Holland, S.M. *et al*. 2007. STAT3 mutations in the hyper-IgE syndrome. *N. Engl. J. Med.* **357:** 1608–1619.
14. Ortega, M. *et al*. 2006. Prospective evaluation of procalcitonin in adults with non-neutropenic fever after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* **37:** 499–502.
15. Schubert, M.S. 2006. Allergic fungal sinusitis. *Clin. Rev. Allergy Immunol.* **30:** 205–216.
16. Park, S.J. & B. Mehrad. 2009. Innate immunity to *Aspergillus* species. *Clin. Microbiol. Rev.* **22:** 535–551.
17. Balloy, V. & M. Chignard. 2009. The innate immune response to *Aspergillus fumigatus*. *Microbes Infect.* **11:** 919–927.
18. Li, Z.Z. *et al*. 2012. Role of NOD2 in regulating the immune response to *Aspergillus fumigatus*. *Inflamm. Res.* **61:** 643–648.
19. Said-Sadier, N. *et al*. 2010. *Aspergillus fumigatus* stimulates the NLRP3 inflammasome through a pathway requiring ROS production and the Syk tyrosine kinase. *PloS One* **5:** e10008.
20. Latge, J.P. 2010. Tasting the fungal cell wall. *Cell. Microbiol.* **12:** 863–872.
21. Balloy, V. *et al*. 2005. Differences in patterns of infection and inflammation for corticosteroid treatment and chemotherapy in experimental invasive pulmonary aspergillosis. *Infect. Immun.* **73:** 494–503.
22. Romani, L. & P. Puccetti. 2007. Controlling pathogenic inflammation to fungi. *Expert Rev. Anti Infect. Ther.* **5:** 1007–1017.
23. Singh, N. & J.R. Perfect. 2007. Immune reconstitution syndrome associated with opportunistic mycoses. *Lancet Infect. Dis.* **7:** 395–401.
24. Bignell, E. *et al*. 2005. Virulence comparisons of *Aspergillus nidulans* mutants are confounded by the inflammatory response of p47phox-/- mice. *Infect. Immun.* **73:** 5204–5207.
25. Moretti, S. *et al*. 2008. The contribution of PARs to inflammation and immunity to fungi. *Mucosal Immunol.* **1:** 156–168.
26. Cunha, C. *et al*. 2011. Genetically-determined hyperfunction of the S100B/RAGE axis is a risk factor for aspergillosis in stem cell transplant recipients. *PloS One* **6:** e27962.
27. Cooney, N.M. & B.S. Klein. 2008. Fungal adaptation to the mammalian host: it is a new world, after all. *Curr. Opin. Microbiol*. **11:** 511–516.
28. Barker, B.M. *et al*. 2012. Transcriptomic and proteomic analyses of the *Aspergillus fumigatus* hypoxia response using an oxygen-controlled fermenter. *BMC Genomics.* **13:** 62.
29. Grahl, N. *et al*. 2012. Hypoxia and fungal pathogenesis: to air or not to air? *Eukaryot Cell* **11:** 560–570.
30. Murdock, B.J. *et al*. 2011. Coevolution of TH1, TH2, and TH17 responses during repeated pulmonary exposure to *Aspergillus fumigatus* conidia. *Infect. Immun.* **79:** 125–135.
31. Chaudhary, N., J.F. Staab & K.A. Marr. 2010. Healthy human T-cell responses to *Aspergillus fumigatus* antigens. *PloS One* **5:** e9036.
32. Potenza, L. *et al*. 2008. Assessment of *Aspergillus*-specific T cells for diagnosis of invasive aspergillosis in a leukemic child with liver lesions mimicking hepatosplenic candidiasis. *Clin. Vaccine Immunol.* **15:** 1625–1628.
33. Cohen, N.R. *et al*. 2011. Innate recognition of cell wall beta-glucans drives invariant natural killer T cell responses against fungi. *Cell Host Microbe.* **10:** 437–450.
34. Filipe-Santos, O. *et al*. 2006. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. *Semin. Immunol.* **18:** 347–361.
35. Porter, P.C. *et al*. 2011. Necessary and sufficient role for T helper cells to prevent fungal dissemination in allergic lung disease. *Infect. Immun.* **79:** 4459–4471.
36. Allard, J.B. *et al*. 2006. *Aspergillus fumigatus* generates an enhanced Th2-biased immune response in mice with defective cystic fibrosis transmembrane conductance regulator. *J. Immunol.* **177:** 5186–5194.
37. Mueller, C. *et al*. 2011. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am. J. Respir. Cell Mol. Biol.* **44:** 922–929.
38. Kreindler, J.L. *et al*. 2010. Vitamin D3 attenuates Th2 responses to *Aspergillus fumigatus* mounted by CD4+ T cells

from cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J. Clin. Invest.* **120:** 3242–3254.
39. Murdock, B.J. *et al*. 2012. Interleukin-17 drives pulmonary eosinophilia following repeated exposure to *Aspergillus fumigatus Conidia. Infect. Immun.* **80:** 1424–1436.
40. Moreira, A.P. *et al*. 2011. The protective role of TLR6 in a mouse model of asthma is mediated by IL-23 and IL-17A. *J. Clin. Invest.* **121:** 4420–4432.
41. Zelante, T. *et al*. 2007. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur. J. Immunol*. **37:** 2695–2706.
42. Bonifazi, P. *et al*. 2010. Intranasally delivered siRNA targeting PI3K/Akt/mTOR inflammatory pathways protects from aspergillosis. *Mucosal Immunol.* **3:** 193–205.
43. Chai, L.Y. *et al*. 2010. Anti-*Aspergillus* human host defence relies on type 1 T helper (Th1), rather than type 17 T helper (Th17), cellular immunity. *Immunology* **130:** 46–54.
44. Montagnoli, C. *et al*. 2006. Immunity and tolerance to *Aspergillus* involve functionally distinct regulatory T cells and tryptophan catabolism. *J. Immunol.* **176:** 1712–1723.
45. Roilides, E. *et al*. 1998. Increased serum concentrations of interleukin-10 in patients with hepatosplenic candidiasis. *J. Infect. Dis.* **178:** 589–592.
46. Hebart, H. *et al*. 2002. Analysis of T-cell responses to *Aspergillus fumigatus* antigens in healthy individuals and patients with hematologic malignancies. *Blood* **100:** 4521–4528.
47. Sambatakou, H. *et al*. 2006. Cytokine profiling of pulmonary aspergillosis. *Int. J. Immunogenet.* **33:** 297–302.
48. Puccetti, P. & U. Grohmann. 2007. IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. *Nat. Rev. Immunol.* **7:** 817–823.
49. von Bubnoff, D. *et al.* 2004. Asymptomatic atopy is associated with increased indoleamine 2,3-dioxygenase activity and interleukin-10 production during seasonal allergen exposure. *Clin. Exp. Allergy* **34:** 1056–1063.
50. Grohmann, U. *et al*. 2007. Reverse signaling through GITR ligand enables dexamethasone to activate IDO in allergy. *Nat. Med.* **13:** 579–586.
51. De Luca, A. *et al*. 2010. Non-hematopoietic cells contribute to protective tolerance to *Aspergillus fumigatus* via a TRIF pathway converging on IDO. *Cell. Mol. Immunol.* **7:** 459–470.
52. Judson, M.A. & D.A. Stevens. 2001. Current pharmacotherapy of allergic bronchopulmonary aspergillosis. *Expert Opin. Pharmacother.* **2:** 1065–1071.
53. Bitko, V. & S. Barik. 2008. Nasal delivery of siRNA. *Methods Mol. Biol.* **442:** 75–82.
54. Bozza, S. *et al*. 2003. A dendritic cell vaccine against invasive aspergillosis in allogeneic hematopoietic transplantation. *Blood* **102:** 3807–3814.
55. Stevens, D.A. 2004. Vaccinate against aspergillosis! A call to arms of the immune system. *Clin. Infect. Dis.* **38:** 1131–1136.
56. Waldmann, H. & S. Cobbold. 2004. Exploiting tolerance processes in transplantation. *Science* **305:** 209–212.
57. Montagnoli, C. *et al*. 2008. Provision of antifungal immunity and concomitant alloantigen tolerization by conditioned dendritic cells in experimental hematopoietic transplantation. *Blood Cells Mol. Dis.* **40:** 55–62.
58. Carvalho, A. *et al*. 2009. Polymorphisms in Toll-like receptor genes and susceptibility to infections in allogeneic stem cell transplantation. *Exp. Hematol.* **37:** 1022–1029.
59. Mezger, M., H. Einsele & J. Loeffler. 2010. Genetic susceptibility to infections with *Aspergillus fumigatus*. *Crit. Rev. Microbiol.* **36:** 168–177.