Murine models of Aspergillosis: Role of collectins in host defense

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Aspergillus fumigatus, a ubiquitous fungus, causes a wide spectrum of clinical conditions ranging from allergic to invasive aspergillosis depending upon the hosts’ immune status. Several animal models have been generated to mimic the human clinical conditions in allergic and invasive aspergillosis. The onset, duration and severity of the disease developed in models varied depending on the animal strain/fungal isolate, quantity and mode of administration of fungal antigens/spores, duration of the treatment, and type of immunosuppressive agent used. These models provide insight into host and pathogen factors and prove to be useful for evaluation of diagnostic markers and effective therapies. A series of studies established the protective role of collectins in murine models of Allergic Bronchopulmonary Aspergillosis and Invasive Pulmonary Aspergillosis. Collectins, namely surfactant protein A (SP-A), surfactant protein D (SP-D) and mannan binding lectin (MBL), are pattern recognition molecules regulating both innate and adaptive immune response against pathogens. In the present review, we discussed various murine models of allergic and invasive aspergillosis and the role of collectins in host defense against aspergillosis.

Keywords: ABPA, Aspergillus fumigatus, Concanavalin A, Cystic fibrosis, Fungus, IFN-γ, IgE, IgG, IPA, MBL, Surfactant proteins, TLRs, TNF-α.

Aspergillus fumigatus, a fungal pathogen

Aspergillus has been the organism of interest and has been extremely exploited for industrial importance. However, several species of Aspergilli, such as Aspergillus flavus, A. fumigatus, A. niger and A. terreus can act as human pathogens. A. fumigatus spores, 2-3 μm in diameter, are universal in distribution, and with their counts from 0.1-22% of total spores in the air, can easily deposit in the lungs up to the alveolar level. However, A. fumigatus shows higher pathogenic potential due to production of large numbers of small conidia, presence of number of conidial adhesins, resistance to oxidative stress and thermostolerance. Also, it produces gliotoxin that affects the circulating neutrophils, inhibits phagocytosis and enhances dissemination of the organism. Another virulence factor produced by A. fumigatus is pigment melanin that inactivates the C3 component of the complement system.

A. fumigatus, a major opportunistic fungal pathogen, causes a spectrum of respiratory diseases depending on the immune status of the host. In healthy host, the inhaled conidia of Aspergillus seldom cause any adverse effects. The innate and adaptive immune responses in a healthy individual result in the mucociliary clearance and phagocytosis of spores. In the hypersensitive individuals, the fungal sensitization mostly results in allergic manifestations with allergic bronchopulmonary aspergillosis (ABPA). About 2% of patients with...
asthma and 1-15% of patients with cystic fibrosis develop ABPA. In the immunocompromised host, the fungus invades various organs leading to systemic form of infection, termed as invasive pulmonary aspergillosis (IPA). The hyphal invasion causes destruction of pulmonary tissue, followed by dissemination of fungus to other organs in approximately 20% cases. The number of IPA cases has risen with the increasing incidence of AIDS, organ transplantations and aplastic anemia, etc. The immunity of host is compromised due to pathogens such as HIV or prolonged treatment with corticosteroids or aggressive antineoplastic chemotherapeutic regimens. IPA results in > 80% mortality, and up to 95% among patients of bone marrow transplantation.

Murine models of diseases have been predominantly the choice among researchers because of their striking similarity to humans in anatomy, physiology, and genetics, where over 95% of the mouse genome is similar to that of humans. In addition, possibility of manipulating the mouse genome provides a powerful tool to model specific diseases and help study the host’s inherent factors associated with disease. Further, the cost, ease of handling, short generation time and availability of genetically defined strains of mice makes this model system ideal for studies. However, the murine models too fail in mimicking human disease owing to known discrepancies in both innate and adaptive immunity. Some of the discrepancies lie in: balance of leukocyte subsets, defensins, toll receptors, the NK inhibitory receptor families, Ig subsets, the B cell and T cell signaling pathway components, cytokines and cytokine receptors, Th1/Th2 differentiation, co-stimulatory molecule expression and function, etc. The spectrum of murine models available for ABPA and IPA, and their successful applications in depicting the molecular mechanisms of the diseases, and in evaluation of therapeutic agents are discussed in the current review.

**Collectins as pattern recognition proteins of host defense**

To understand the role of collectins, pattern recognition proteins integral to innate immune defense, several researchers have successfully used the murine models of ABPA and IPA in wild type and collectin deficient mice. Collectins such as SP-A, SP-D and MBL have been well studied. The genes for all the three proteins are located on chromosome 14 in mouse and on chromosome 10 in humans. Collectins recognize pathogen through carbohydrate patterns and interact with various immune cells through receptors and thus, regulate the host immune response to pathogens. Contrary to the earlier theory that SP-A and SP-D are secreted by pulmonary Type II epithelial cells in lungs, and MBL is secreted by hepatocytes (present as serum activating lectin complement pathway), it is now established that all the three proteins are secreted by the epithelial cell linings of various organs and tissues almost ubiquitously.

Host defense against *Aspergillus* infections is mediated by phagocytic cells including macrophages and neutrophils. Macrophages can bind and phagocytose *A. fumigatus* conidia, and kill conidia as well as hyphae. Innate immune molecules, such as collectins, toll-like receptors and pentraxin-3 are known to be involved in lung resistance to *A. fumigatus* infection. SP-A and SP-D bind and agglutinate *A. fumigatus* conidia in vitro in a sugar and calcium dependent manner and thus, enhance killing of conidia by circulating neutrophils and alveolar macrophages via phagocytosis and superoxidative burst. In vitro interactions of SP-A and SP-D with *A. fumigatus* allergens/antigens suggest their hierarchical role at various levels that involves allergen scavenging, inhibition of allergen-IgE cross-linking and histamine release, suppression of the activation of sensitized basophils or mast cells. SP-A and SP-D can also modulate host immune response by suppression of B- and T-cell proliferation, modulation of dendritic cells and macrophages, and Th cell polarization.

MBL interacts directly with the glycosylated cell wall components of the *A. fumigatus* conidia via its lectin domain. MBL-bound *A. fumigatus* conidia show increased uptake by the PMNs similar to SP-A and SP-D in the absence of serum. MBL alone does not increase oxidative burst in PMNs or PMN-mediated conidia killing in serum free conditions. However, increasing amounts of MBL added to MBL deficient serum result in increased deposition of C4b on conidia and hyphae. This dose dependent complement deposition suggests that MBL-bound *A. fumigatus* may participate in the activation of lectin complement pathway. The associated increased conidial and hyphal damage further indicates the role of MBL-mediated complement activity. These in vitro studies highlight the relevance of collectins in host defense against *A. fumigatus*. 

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1. INDkin J Exp Biol, November 2015
2. Reference numbers are cited in the text for further reading.
Allergic bronchopulmonary aspergillosis (ABPA) and immune hallmarks

Allergic aspergillosis comprises a spectrum of allergic diseases induced by Aspergillus species in non-immunocompromised patients and are defined as hypersensitivity disorders. ABPA is the most severe and fatal form of allergic aspergillosis and occurs predominantly in patients predisposed with lung diseases such as cystic fibrosis or bronchial asthma. In these lung diseases, the A. fumigatus spores are not effectively cleared off. They germinate and grow to hyphae in the mucus plugs. The host suffers from an allergic disorder characterized by Type I and Type III hypersensitivity response to the secreted A. fumigatus allergens, antigens toxins and hyphae. The common features of Type I hypersensitivity in ABPA are upregulation of TH2 cytokine pathway that includes IL-4, IL-5, IL-6, IL-10 and IL-13 cytokines. Presence of IgE antibodies specific to A. fumigatus sensitizes and causes mast cell degranulation resulting in bronchoconstriction and increased capillary permeability. Further, immune complexes are formed because of type III reaction and the inflammatory cells get deposited in the mucous membranes leading to necrosis and eosinophilic infiltration of the airways.

Briefly, clinical ABPA is characterized by Rosenberg et al. in terms of episodic bronchial hyper-reactivity, reversible airway obstruction, positive immediate skin reactivity, peripheral and pulmonary eosinophilia, central bronchiectasis and history of expectorating brown plugs or flecks, etc.

Murine models of allergic aspergillosis

Experimental murine models of ABPA have been generated in various strains of mice. It is now well illustrated that the genetic factors contribute variably to the ability of individuals to respond to potential pathogens, including fungi. The development of ABPA has been linked to a number of genetic risks, which include polymorphisms in the genes of HLA-DR and HLA-DQ, surfactant protein A2 (SP-A2), IL-4 receptor alpha chain (IL-4RA), IL-10-1082GA promoter, etc. Cystic fibrosis transmembrane conductance regulator gene (CFTR) mutations and TLR polymorphisms are also linked to ABPA. The effect of genetic variation is also observable in murine fungal exposure studies. Of the three congenic mouse strains (BALB/c, CBA/J, C57BL/6), BALB/c mice exhibit the strongest inflammatory response. The BALB/c species is more sensitive to hypersensitivity disorders, while C57BL/6 mice are comparatively more resistant. An enhanced lung SP-D production has been predicted to attenuate airway hyperresponsiveness to allergic airway sensitization in C57BL/6 mice, suggesting an important role of SP-D in regulating pathogenesis in ABPA.

It is a peculiar task to develop a true ABPA model in a predisposed asthma and CFTR—conditions. Important features of ABPA model can be developed in mice genetically predisposed to atopy (e.g. BALB/c) by the continual exposure and sensitization of the airways to A. fumigatus allergens. Type I and Type III immune responses are present in these models. In case of natural disease, this happens by the growth of A. fumigatus spores into the hyphal structures in the lungs. Spores present in central and up to distal parts of the lungs release allergens continuously which in turn lead to clinical symptoms of ABPA. A variety of experimental animal models of ABPA have been studied depending on the pathogen form, inoculum size (conidia/hyphae/allergens) and the frequency and route of exposure in addition to variations on the basis of strain of animals. The available information has been summarized in Table 1.

We used BALB/c mice and a relevant strain of A. fumigatus (a clinical isolate, strain 285, from an ABPA patient) to study the effect of collectins in vivo in the murine model of allergic aspergillosis.

The BALB/c murine models of ABPA have demonstrated nine-fold increase in SP-D levels on A. fumigatus-induced allergic airway inflammation suggestive of its role in pathophysiology. Increase in serum SP-D levels is also associated with allergic bronchial inflammation in allergic patients. Serum SP-D levels are helpful to follow pulmonary function and lung injury patients of cystic fibrosis. However, these levels do not change significantly in the patients who develop ABPA.

Role of SP-D and SP-A in ABPA murine models

Treatment with SP-A and SP-D drastically decreases the serum A. fumigatus IgG and A. fumigatus IgE levels in murine models of ABPA. The decrease in specific IgG and IgE levels persist till 16 days in the SP-D treated ABPA mice, but only 4 days in SP-A treated mice. SP-D treated murine ABPA models show significant decline in peripheral blood eosinophilia and lung eosinophil peroxidase activity, hallmarks of Type I hypersensitivity. The chronic inflammatory infiltrates disappear and the
Table 1—Murine models of allergic bronchopulmonary aspergillosis.

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Antigen preparation</th>
<th>Mice sensitization/Immunization</th>
<th>Booster/Challenge</th>
<th>Features of ABPA murine models</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>CBA/J</td>
<td>Commercially obtained A. fumigatus crude antigen (Ag) from Greer Laboratories, Lenoir, NC</td>
<td>Soluble 10 μg crude Ag per 0.2 ml of incomplete Freund’s adjuvant. One-half of this preparation deposited in the peritoneal cavity (i.p.), and the remainder delivered subcutaneously (s.c).</td>
<td>After 2 wk, 20 μg A. fumigatus antigens administered via the intranasal route (i.n.). After 4 days, 20 μg A. fumigatus Ag in normal saline administered via the intratracheal route (i.t.).</td>
<td>Increased systemic IgE, blood eosinophilia and leukocyte lung infiltration.</td>
<td>53</td>
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<tr>
<td>BALB/c, Mixture of culture filtrate + mycelia antigen</td>
<td>50 μg Ag/50 μl preparation given i.n.</td>
<td>The sensitization carried out for 4 wk with twice weekly exposures. Additional i.n. antigen given 5 days before killing the animal.</td>
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<tr>
<td>BALB/c/ByJ Mixture of culture filtrate + mycelia antigen</td>
<td>Two groups of animals (i) Given i.p. with 200 μg of A. fumigatus antigen mixture adsorbed to 2 mg of alum. or (ii) in. administration of 200 μg of soluble A. fumigatus antigen.</td>
<td>Earlier procedure repeated twice a week for 4 weeks. This was followed by a challenge with 50 μl of 10 x 10⁸ antigen-coupled beads, where 4 mg of protein was coupled to 1.68 x 10⁹ beads/ml. This was administered either: i.n.-Only one i.n. instillation or i.v. (intravenously) as above.</td>
<td>Mice immunized i.n. with soluble Ag produced low levels of serum IgE than animals given alum precipitated Ag i.p. i.p. administration also produced higher levels of serum A. fumigatus-specific IgG1 than soluble A. fumigatus given i.p. or i.n. Blood and lung eosinophilia was detected in i.n but not on i.p. administration. Particulate A. fumigatus Ag challenge also induced striking blood and lung eosinophilia and elevated levels of serum IgE.</td>
<td></td>
<td>54</td>
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<tr>
<td>BALB/c Mixture of culture filtrate + mycelia antigen</td>
<td>50 μl of the Ag mixture applied to the nostrils. 100 μl of Ag mixture i.p.</td>
<td>Intrasinal instillations and i.p. injections given twice a week to each mouse for 4 wk.</td>
<td>Produced significant levels of A. fumigatus specific IgE and IgG1 antibodies. Extensive eosinophilia in blood and bone marrow.</td>
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<td>55</td>
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<tr>
<td>BALB/c A. fumigatus crude protein extract</td>
<td>10 μg of antigen per 0.2 ml incomplete Freund’s adjuvant. Half of this was given i.p., and the remainder was delivered s.c.</td>
<td>Two weeks later, 20 μg of Ag in normal saline was administered via i.n. route. To initiate the acute fungal asthma model, mice received 20 μg of A. fumigatus Ag in normal saline via the i.t. route 4 days after one i.n. challenge. To initiate chronic fungal asthma model, A. fumigatus-sensitized mice received 5.0 x 10⁹ A. fumigatus conidia in 30 μl of 0.1% Tween 80 via the i.t. route 7 days after the third intranasal challenge.</td>
<td>Initiation to both acute and chronic model lead to eosinophil and T-lymphocyte recruitment to the lungs. Model helpful in study of chemokines, cytokines and the leukocytes involved in the acute and chronic fungal lung allergy disease. Helpful in study of early allergic and chronic allergic responses to A. fumigatus. i.t. conidia challenge in A. fumigatus-sensitized mice did not precipitate to Invasive Pulmonary Aspergillosis. Conidia challenge in mice previously sensitized showed persistent airway hyperresponsiveness.</td>
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<td>C57BL/6:A. fumigatus primary CFTR knockout crude protein extract</td>
<td>i.p. injections of 200 μg of A. fumigatus Ag on days 0 and 14.</td>
<td>Aerosol challenge was performed with 0.25% A. fumigatus for 20 min in a 30×30×20 cm acrylic chamber using a Pari model LC jet nebulizer with an air flow of 6 l/min on days 28, 29 and 30.</td>
<td>Hyper-IgE response and high serum IgG1 levels. Partial correction of CFTR-dependent ABPA model via targeted gene delivery. Splenocytes from CFTR-corrected mice also secreted less IL-13, INFγ, TNFα, RANTES and GM-CSF after ConA stimulation.</td>
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<td>BALB/c 3 wk culture filtrate (27 mg/ml) of A. fumigatus (strain 285, from sputum of an ABPA patient).</td>
<td>100 μg/50μl of the Ag applied i.n. Mice also received 200 μg/100 μl of the same Ag by i.p. administration.</td>
<td>i.n. and i.p. administration of Ag given twice a week for 4 wk.</td>
<td>ABPA mice model showed higher levels of A. fumigatus specific IgE and IgG levels. Higher levels of peripheral blood eosinophilia. Increased ratios of IL-2, IL-4 and IL-5 levels, while a decrease in IFN-γ in splenic supernatants of the untreated ABPA mice compared to their respective controls.</td>
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<td>58</td>
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bronchi and parenchyma appear normal in the lungs. Treatment with collectins decrease the levels of IL-2, IL-4, and IL-5 cytokines, while the level of IFN-γ increase in the splenic supernatants indicating a marked shift from Th2 to Th1 response. Thus, collectins have a major role in modulation of allergic reactions in ABPA.

Similar study in C57BL/6 mice sensitized with A. fumigatus allergens from 1-wk culture filtrate indicate a significant reduction in levels of IgE, IgG1, peripheral blood eosinophilia as well as peribronchial lymphocytic infiltration and airway hyper-responsiveness on treatment with truncated recombinant human SP-D (rSP-D) (Kaur S et al., unpublished data). Also, cellular eosinophil infiltration around perivascular areas in the lung sections on allergen treatment. Increased levels of IL-13, IL-5, and IL-2. However, IL-4, IL-10, IL-12, and TNF-α levels in both AKO and DKO mice do not show significant changes compared to WT mice challenged with allergens. The intranasal treatment with SP-D is effective in rescuing the A. fumigatus sensitized DKO mice. A. fumigatus IgE levels, peripheral eosinophilic count, pulmonary eosinophilia and EPO activity also decrease. SP-D treatment to DKO mice also reduces IL-13 and IL-5 cytokines and increases IFN-γ to IL-4 ratio. SP-A-treated A. fumigatus-sensitized AKO mice develop several fold elevated levels of IL-13 and IL-5, resulting in increased pulmonary eosinophilia and lung tissue damage. Hence, SP-A and SP-D may have an important role in treating ABPA, as evident from studies of murine models.

**Susceptibility of SP-A and SP-D gene knockout mice to allergic challenge**

Murine models of ABPA in SP-A gene deficient (AKO), SP-D gene deficient (DKO), and WT mice in C57BL/6 background were used for such studies. The studies show that both AKO and DKO mice exhibit intrinsic hypereosinophilia and several fold increase in levels of IL-5 and IL-13, and decrease in IFN-γ to IL-4 ratio in the lungs. DKO mice develop chronic inflammation, foamy alveolar macrophages secreting 10-fold higher levels of hydrogen peroxide, increased activity of metalloproteinases, emphysema, and fibrosis in the lungs. Administration of SP-A or SP-D to the respective knockout mice decrease peripheral eosinophil count, EPO activity, IL-13, IL-5, and increases TNF-α, IFN-γ levels. The Th2 bias in AKO or DKO mice was reversible on treatment with SP-A or SP-D, respectively.

The A. fumigatus allergen sensitization of AKO and DKO mice further increases the Th2 response, as opposed to the predominantly Th1 profile of the WT C57BL/6 mice. DKO mice are more susceptible than WT mice to pulmonary hypersensitivity induced by A. fumigatus allergens. DKO mice also develop 2-fold elevated peripheral eosinophil count with increased eosinophil infiltration around perivascular areas in the lung sections on allergen treatment. A more pronounced Th2 bias of DKO mice is also evident by decrease in IFN-γ levels and increase in IL-13, IL-5, and IL-2. However, IL-4, IL-10, IL-12, and TNF-α levels in both AKO and DKO mice do not show significant changes compared to WT mice challenged with allergens. The intranasal treatment with SP-D is effective in rescuing the A. fumigatus sensitized DKO mice. A. fumigatus IgE levels, peripheral eosinophilic count, pulmonary eosinophilia and EPO activity also decrease. SP-D treatment to DKO mice also reduces IL-13 and IL-5 cytokines and increases IFN-γ to IL-4 ratio. SP-A-treated A. fumigatus-sensitized AKO mice develop several fold elevated levels of IL-13 and IL-5, resulting in increased pulmonary eosinophilia and lung tissue damage. Hence, SP-A and SP-D may have an important role in treating ABPA, as evident from studies of murine models.

**MBL and allergic bronchopulmonary aspergillosis**

High levels of MBL lead to greater complement activation and subsequent allergic responses while lower levels of MBL are less effective. The cystic fibrosis (CF) patients with optimum serum MBL levels show maximum lung function, while patients with high and low extremes of MBL levels have reduced lung function. Patients of bronchial asthma with allergic rhinitis and ABPA have significantly increased MBL levels and MBL pathway activity (measured as C4b deposition activity on a mannan surface). The increased MBL levels and activity has a significant positive correlation with the increased peripheral blood eosinophilia of the allergic patients. Plasma mouse MBL-A (mMBL-A) levels are increased in A. fumigatus sensitized mice compared to non-sensitized mice, suggesting it to be a mediator of inflammation in vivo. mMBL-A deficient mice (mMBL-A−/−) experience a significant decline in the airway hyper-responsiveness to A. fumigatus conidia as compared to the control mice (mMBL-A+/+). MBL has also been reported to have a deleterious effect on double MBL knockout (MBL-A and MBL-C) mice model of systemic allergic aspergillosis. Mortality is enhanced in both wild type and MBL double knockout mice, when infected intravenously with A. fumigatus conidia.

However, intranasal administration of rhMBL in ABPA mice leads to a marked decrease in A. fumigatus-IgE and A. fumigatus-IgG levels and peripheral blood eosinophil (PBE) counts, indicating a downregulation of the disease process (Fig. 1 A-D) (Kaur S et al., unpublished data). Also, cellular
infiltration consisting of lymphocytes and eosinophils in the lung sections is markedly reduced in the ABPA mice treated with rhMBL, suggestive of a possible role of MBL in combating the airway remodeling in ABPA.

Invasive pulmonary aspergillosis- immune response

Deficiency of innate and adaptive immune response, induced by infectious agents such as HIV or the organ transplant immunosuppressive regimens, makes the host susceptible to germination and invasion of inhaled fungal conidia. In the patients of IPA, there is low or almost no humoral immunity while the adaptive immune response is of Th2 type. Recovery of these patients on empirical treatment with antifungal drugs is accompanied by revival of innate immunity and a protective Th1 type of response.

Murine models of Invasive pulmonary aspergillosis

To mimic the human IPA, murine model of IPA can be generated by administration of immunosuppressive agents, such as cyclophosphamide, hydrocortisone acetate and FK506, etc., followed by fatal spore challenge(s) (Table-2)\textsuperscript{71-75}. The survival rate and enumeration of pulmonary colony forming unit (CFU) serve as the markers of therapeutic evaluation.

**Role of SP-D in strengthening the host defense in IPA murine model**

Intranasal administration of SP-D or a recombinant fragment of SP-D composed of trimeric neck and CRD regions (rSP-D) have protective effect in a murine model of IPA, where mice are immunosuppressed with hydrocortisone and challenged intranasally with *A. fumigatus* conidia. Untreated IPA mice have 100% mortality in 7 days, whereas SP-D or rSP-D treatment rescued 80% of the IPA mice. Interestingly, SP-A does not have significant effect on survival, while SP-D plays an important role in the ability of host to resist *A. fumigatus* challenge and subsequent infection\textsuperscript{71,72}. The rSP-D treatment lowers IL-4, IL-5 and increases TNF-α, IFN-γ levels in the lung cell suspension, as compared to untreated IPA mice.
Table 2—Murine models of invasive pulmonary aspergillosis for evaluation of therapeutic molecules.

<table>
<thead>
<tr>
<th>Mice Breed</th>
<th>Methodology</th>
<th>Strain of the fungus</th>
<th>Objective</th>
<th>Ref</th>
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<tr>
<td>Male BALB/c mice, 20 to 22 g</td>
<td>Mice were immunosuppressed with cyclophosphamide (250 mg/kg, IP (8 mg/mice), on day 2 prior to infection, and 200 mg/kg on day 3 post infection and cortisone acetate (200 mg/kg, SC (10mg/mice), on day 2 prior and day 3 post infection). To prevent bacterial infection, all immunosuppressed mice received ceftarizime (5 mg/day subcutaneously) from days 1-6 after infection.</td>
<td>Aspergillus fumigatus AF293, the inoculum, was grown on SDA plates for 2 wk at 37°C. Conidia were collected in sterile PBS containing 0.2% (v/v) Tween 80.</td>
<td>To evaluate the reproducibility of this model, and to compare the time course of mortality and fungal burden and the efficacy of liposomal amphotericin B in two different laboratories.</td>
<td>73</td>
</tr>
<tr>
<td>BALB/c, C57BL/6, DBA/1, DBA/2, CBA, A/Sn</td>
<td>Cyclophosphamide was dissolved in sterile saline (20 mg/mL) and 100-150 micro liter (2-2.5mg/mice) (100 mg/kg)) was administered i.p. before inoculation of A. fumigatus conidia on days 4 and 1, and after inoculation of A. fumigatus conidia on days 2, 5, and 11 to keep mice neutropenic. Cortisone acetate was suspended in sterile PBS (20 mg mL, 100 mg/kg (2-2.5mg/mice) subcutaneous injection on day 1 before infection</td>
<td>A. fumigatus CBS 144.89 (Institut Pasteur, Paris, France) was maintained on Sabouraud maltose agar plates supplemented with chloramphenicol at 37°C.(1x10^8/mice)</td>
<td>To compare six genetically different mouse strains in their susceptibility to IPA and to determine possible mechanisms involved in the pathogenesis of this infection. Immunosuppressed BALB/c and C57BL/6 mice infected with A. fumigatus conidia were more resistant to IPA than DBA/1, DBA/2, CBA, and A/Sn strains.</td>
<td>74</td>
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<tr>
<td>BALB/c or C57BL/6 male mice (18-22 g)</td>
<td>Mice received hydrocortisone by s.c. injection (2.5 mg/mice/day) for 3 days and FK506 i.p injection (20 ng/mice/day). All mice received 1 mg/L tetracycline hydrochloride and 64 mg/L ciprofloxacil in drinking water as prophylaxis against bacterial infections.</td>
<td>A. fumigatus CEA10 was the primary isolate used. (5x10^2 conidia/mice/day) for 3 consecutive days</td>
<td>To show that the combination of steroid and calcineurine inhibitor (FK506) impairs innate immune responses and leads to an incremental increase in susceptibility to IFIs in murine model of IPA during solid organ transplant as compared to the group treated with steroid only.</td>
<td>75</td>
</tr>
<tr>
<td>Male BALB/c mice mice (20-22 g)</td>
<td>Mice were immunosuppressed by 3 consecutive intradermal injections of 2.5 mg/mouse/day (125 mg/kg body wt.) of hydrocortisone acetate 1 day before, on the day and the day after conidia challenge</td>
<td>A. fumigatus 285, isolated and cultured from ABPA patients (1×10^5/mice)</td>
<td>To evaluate the potential of lung surfactant protein-D (SP-D) in host defense against A. fumigatus induced IPA.</td>
<td>71</td>
</tr>
<tr>
<td>Male and female C57BL/6 mice (6-8 wk old SP-A and SP-D gene deficient mice)</td>
<td>Mice were immunosuppressed by 3 consecutive intradermal injections of 2.5 mg/mouse/day (125 mg/kg body wt.) of hydrocortisone acetate 1 day before, on the day and the day after conidia challenge</td>
<td>A. fumigatus 285, isolated and cultured from ABPA patients (1×10^5/mice)</td>
<td>To evaluate the key role of SP-A and SP-D in pulmonary host defense against A. fumigatus induced IPA.</td>
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Susceptibility of SP-A and SP-D gene knockout mice to Invasive pulmonary aspergillosis

The immune status of AKO as well as DKO mice alters distinctly following steroid treatment prior to A. fumigatus conidia challenge. DKO mice have increased susceptibility to the IPA pathogenesis than the WT mice while AKO mice are more resistant than WT mice. Intranasal treatment with SP-D or rSP-D was effective in ameliorating the pathology in the case of DKO mice, whereas the SP-A treated A. fumigatus challenged AKO mice have increased mortality. The survival data also reflected on the CFU counts and hyphal burden in the lung, consistent with the lung cytokine profiles. Thus under immuno-suppressed or immunocompromised conditions, the SP-A or SP-D deficiency may have profound but contrasting effects on host immune response.
Protective role of MBL in IPA murine model

Similar to SP-D, MBL has shown protective effect in murine model of IPA\textsuperscript{76}. The survival rate significantly increases along with a concomitant reduction in the pulmonary fungal hyphae density and pulmonary fungal load after the administration of recombinant human MBL (rhMBL). Levels of IL-1β, TNF-α, IL-10 and IFN-γ also increase significantly in rhMBL-treated IPA mice. MBL, thus acts as an immunoregulatory molecule against \textit{A. fumigatus} restoring the fine balance between pro-inflammatory and anti-inflammatory cytokines\textsuperscript{73}. Further, MBL deficiency does not affect the survival of immunocompetent mice following the intratracheal challenge with \textit{A. fumigatus} conidia\textsuperscript{69}. However, in conditions of immunodeficiency, the administration of MBL strengthens host defense and proves to be protective against \textit{A. fumigatus}\textsuperscript{76,77}.

Conclusion

Murine models of IPA and ABPA, although may not replicate all the disease characteristics observed in patients, may provide useful information on mechanisms of pathogenesis and efficacy of novel therapeutic agents. Collectins are integral to the host defense in both allergic and invasive aspergillosis. ABPA and IPA models would be further useful in evaluating safety and efficacy of formulations comprising recombinant human SP-D and MBL.

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