

ANIMAL MODELS OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

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Aspergillus fumigatus is a ubiquitously distributed fungus.^{45, 57} Spores can be found in the soil and in other organic substances such as hay, animal bedding,¹⁰⁰ compost,⁵⁰ wood,^{27, 64} and wool.² Mold growth that is dependent on the moisture and nutrients in the substrate is caused by the growth of *A. fumigatus*, other related fungi, and thermoactinomyces species. Depending on the ventilation and the quality of food and bedding, the concentration of *A. fumigatus* spores in the air can be very high in barns.^{12, 100} This organism (and other closely related species) is an opportunistic fungal pathogen for humans and animals, causing life-threatening systemic infections in immunocompromised patients (HIV-infected patients⁴⁶) and patients undergoing immunoablative treatment.³⁸ Alternatively, the fungus can colonize mucosal sites in predisposed patients (e.g., patients affected with cystic fibrosis⁹⁷ and other structural lung diseases) and in otherwise apparently normal individuals. A variety of proteins from *A. fumigatus* are highly antigenic and cause lung hypersensitivity responses in man and animals with or without colonization of lungs or sinuses.^{45, 56}

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Lung hypersensitivity responses to *A. fumigatus* may present as one of several distinct clinical entities.⁵⁶ Asthma presents as recurrent bouts of dyspnea caused by bronchoconstriction and is often, but not always, accompanied by high serum immunoglobulin E (IgE) levels and peripheral blood and tissue eosinophilia. Hypersensitivity pneumonitis, in contrast, is characterized by bouts of dyspnea caused by alveolar filling and pulmonary restriction, often in the setting of influenza-like symptoms (e.g., fever, fatigue). Serum IgE titers are often low in hypersensitivity pneumonitis, and eosinophilia, if present, is usually less than in asthma. Immunologically, asthma has been associated with an exaggerated response of T helper 2 (Th2) lymphocytes.¹¹ Th2 cells make a specific array of cytokines, most prominently IL-4 and IL-5.⁷³ IL-4 is a critical mediator of IgE synthesis,⁷³ and IL-5 is probably the most essential factor for eosinophil infiltration into the tissues.^{19, 74} IL-4 and IgE are thought to be instrumental for the activation of mast cells.⁹¹ In contrast, hypersensitivity pneumonitis is characterized by neutrophil influx into the lungs at the acute phase and T cell and macrophage influx during the chronic phase of the disease. It is thought to be caused by excessive macrophage activation because of an immune complex-mediated Arthus reaction together with the response of T helper 1 lymphocytes (Th1).^{86, 87} Th1 cells mediate functions that are often antagonistic to functions mediated by Th2 cells.⁷³ Interferon gamma (IFN- γ), the most prominent cytokine made by Th1 cells, activates macrophages and promotes the secretion of IgG2a antibodies.¹ Hypersensitivity to *A. fumigatus* antigens in human patients can lead to allergic bronchopulmonary aspergillosis (ABPA).⁵⁶ Patients with ABPA resemble asthmatics and their airways may be colonized by *A. fumigatus*. The immunopathology of ABPA is characteristic of activated Th2 cells^{14, 48, 95}; however, IgG-mediated Arthus reactions⁵⁶ and autoimmune reactions²⁵ also can contribute to the pathogenesis.

The study of animal models of ABPA allows comparative research of naturally occurring diseases. Both common and distinct pathologic features are of great interest because they serve to identify key elements in the pathogenesis. Animal model studies also afford research of experimentally induced diseases. Only experimentally induced diseases can be modeled in different ways (e.g., changing the genetic background, the route of sensitization) and altered by treatment with specific antibodies. Furthermore, animals with targeted gene-deletions or with insertion of transgenes can be studied. The purpose of this type of research is to identify the roles of specific cells, receptors, or soluble mediators in disease pathogenesis. The conclusions then are used to formulate hypotheses that have to be tested for their application to human disease.

ASPERGILLUS-INDUCED DISEASE IN DOMESTIC ANIMALS

A. fumigatus is a major infectious organism of the respiratory tract in birds (domestic, zoo, or wild).^{6, 28, 29} In domestic mammals, however,

A. fumigatus is a rare opportunistic infectious organism for many species (e.g., dogs, cats, or horses),^{33, 99} occurring mostly on mucosal surfaces of immunocompromised animals. Cattle are the exception because they have a much higher incidence of infectious mucosal aspergillosis than other domestic mammals. It is thought that the port of entry of the organism is the stomach.^{42, 84} Infection and colonization of the stomachs,^{42, 68} the uterus^{43, 47, 51, 88} (leading to placentitis and abortions), and the udder^{7, 44, 85} (causing mastitis) are quite common and associated with the overzealous use of antibiotics. It is thought that the disruption of the natural balance in the microbial flora predisposes the animals to fungal overgrowth. Reports on allergic respiratory diseases mediated by the fungus in cattle are uncommon and are mostly concerned with hypersensitivity pneumonitis.^{24, 80, 82}

In contrast, *A. fumigatus* is a well recognized respiratory allergen for horses.⁷¹ Allergic lung diseases are very common in horses (> 60% of all animals maintained in stables).¹⁰ The most severe form, termed *heaves*, is associated with recurrent bouts of dyspnea. Both healthy controls and affected horses have positive skin tests following intradermal challenge with *A. fumigatus* antigens.⁷⁰ This result reflects probable prior exposure to the organism through contaminated feed or bedding of all animals. In contrast, only in horses affected with heaves but not in controls can airway constriction be elicited by provocative challenge with aerosolized *A. fumigatus* antigens.⁷¹ Similarly, antibodies specific for *A. fumigatus* are more frequently found in bronchoalveolar lavage fluids (BAL) from affected horses than in controls.³⁵ Interestingly, as in humans, sensitization with *A. fumigatus* can be associated with hypersensitivity pneumonitis in horses, although this form of antigen-induced immunopathology is quite rare relative to allergic disease.^{25, 81} It is not known why horses but not cattle suffer from allergic disease despite both species being exposed to the barn environment and to moldy food and bedding. A provocative hypothesis may be that cattle but not horses develop some form of immunologic tolerance to the organism. Increasing the likelihood of infection and colonization, and building tolerance, may prevent cattle from developing allergies.

EXPERIMENTAL ASPERGILLOSIS

To study the pathogenesis of *A. fumigatus* induced diseases in more detail, experimental models have been developed. There are two main lines of investigation: infectious aspergillosis and allergic aspergillosis. Experimental infectious aspergillosis is most commonly studied in immunosuppressed animals using treatments with corticosteroids or irradiation. Subsequently, the animals are infected systemically or locally. Models of infectious aspergillosis have been developed in the monkey,⁶⁹ rabbit,⁸ guinea pig,⁵ rat,¹⁸ and mouse. In the murine model, protection depends on the secretion of IFN- γ and tumor necrosis factor- α (TNF- α)⁷⁸ and on the absence of interleukin-4 (IL-4) secreting CD4+ cells.¹³

In this article we concentrate on allergic aspergillosis. The aim of studying experimental allergic aspergillosis is to dissect the roles of specific cells, antibodies, cytokines, or other mediators in the pathogenesis of selected disease characteristics (e.g., airway inflammation or airway hyperreactivity). In mice, experimental hypersensitivity to *A. fumigatus* is elicited by repeated exposures to soluble extracts, or spores, or a mixture of both. In monkeys, an antibody-mediated form of lung pathology induced by inhalation of *A. fumigatus* antigens has been described. The main features of these models are described in detail. We concentrate on murine models, but data gleaned from the monkey model is added where indicated.

ANTIGEN EXTRACT

Although a variety of recombinant antigens have been characterized and are available in pure form, soluble, crude antigen extract is still most commonly used for the induction of the experimental disease. Spores, or plastic beads coated with crude antigen extract, are good inducers of respiratory pathology as well.^{53, 62} The crude extract is a mixture of culture supernatant and mycelial extract. It is filtered to remove all of the infectious spores and hyphae.^{34, 58} The use of extract containing infectious material has been reported as well.^{15-17, 96} Culture supernatants or mycelial extracts alone are less potent in causing disease.⁵⁶ Most protocols use exposures to *A. fumigatus* antigens over a period of 2 to 5 weeks,⁵⁴ and the longest reported experiments span over 10 to 12 weeks.⁹⁶

Most of the important serologic antigens appear to be constituents of the crude extract.^{60, 61} Analysis by Western blotting using sera from human patients affected with ABPA reveal bands that correspond in size to known antigenic components, including Asp f1, a ribotoxin,⁵⁸ proteases, and carboxidases.⁶¹ Thus the extract is highly antigenic and contains biologically active substances. Interestingly, potent sensitization to the extract occurs in the absence of exogenous adjuvants.⁵⁹ The ribotoxin and proteases in the extract may serve as adjuvants, perhaps by inducing epithelial damage and allowing normally excluded antigens to bypass the mucosal barrier.⁹³ Because proteases have been implicated to preferentially induce Th2 over Th1 responses, they may be involved in skewing the response to *A. fumigatus* to a more allergic phenotype.³¹

Crude extracts can contain toxic molecules including hemolysin, fumitremorgin, fumigallin, helvolic acid, and gliotoxin.^{3, 66} We found that extracts containing Asp f1 may be very toxic for mice as well (Viswanath P. Kurup, unpublished results). Extracts prepared from certain *A. fumigatus* strains under culture conditions that promote the secretion of Asp f1 induce morbidity and mortality in mice (including T- and B-cell deficient mice) after relatively few exposures. Extracts enriched for Asp f1 are also toxic for cells in culture at concentrations that are otherwise well tolerated when Asp f1 concentrations are low.

ANTIBODY RESPONSES

Sensitization with *A. fumigatus* antigens induces a large increase in serum IgE and IgG1.⁵⁸ In contrast, serum IgG2a levels are often only minimally elevated. The increase in the total serum concentration of antibodies is accompanied by an increase in antigen-specific IgG1 titers. Antigen-specific IgE titers, although probably elevated, are more difficult to measure accurately. The magnitude of the antibody response depends on the mouse strain,^{34, 56} the route of sensitization,³⁴ and, in some experiments, on the physical form (soluble versus particulate) of the antigen.⁶²

T-CELL RESPONSES

As predicted from the predominant IgE and IgG1 responses induced by sensitization with *A. fumigatus*, the dominant T-cell response is of the Th2 type.⁶² The T-cell response has been analyzed using different methods. Cell suspensions from the lungs, draining lymph nodes, or spleens have been restimulated in vitro with immobilized anti-CD3 antibodies or with crude antigen extract. Cell suspensions from sensitized but not from control mice produce significant amounts of Th2 cytokines, such as IL-4, IL-5, IL-6, and IL-10.^{34, 62} On the other hand, the Th1 cytokine IFN- γ is made in equivalent amounts by cells from controls and sensitized mice. Similar results have been obtained from the analysis of lung T cells for the presence of intracellular cytokines or from the examination of bronchoalveolar lavage fluids for secreted cytokines.³⁴ Compared with controls, sensitized animals have a larger number of Th2 lymphocytes in the lung tissue, and antigen challenge induces the release of Th2 cytokines into the airway lumen. Immunohistochemical analysis¹⁶ has shown that exposure to *A. fumigatus* antigens induces peribronchial and perivascular infiltration of T cells that make IL-4 or IL-5.

Little is known about T-cell migration and homing in the lungs in experimental ABPA. Intercellular adhesion molecule (ICAM-1), an adhesion molecule of the immunoglobulin superfamily, may be a candidate as the numbers of T cells in the lung tissues of mice exposed to *A. fumigatus* antigens were positively correlated with ICAM-1 expression.¹⁶

LUNG INFLAMMATION

Challenge of sensitized mice with *A. fumigatus* antigens induces a strong eosinophilic inflammation in the lungs that persists over several days (Fig. 1).^{16, 34, 52, 53, 58, 59, 62, 72, 76, 96} Microscopic changes differ in severity depending on the route of sensitization, the frequency of sensitization, the form of the antigen, and the mouse strain. Pathologic lesions tend to be multifocal in less severely affected mice but coalesce and become more diffuse in severely affected animals. Lesions affect the surface of the airways, the interstitial tissue of the lungs, and the alveoli. Airway

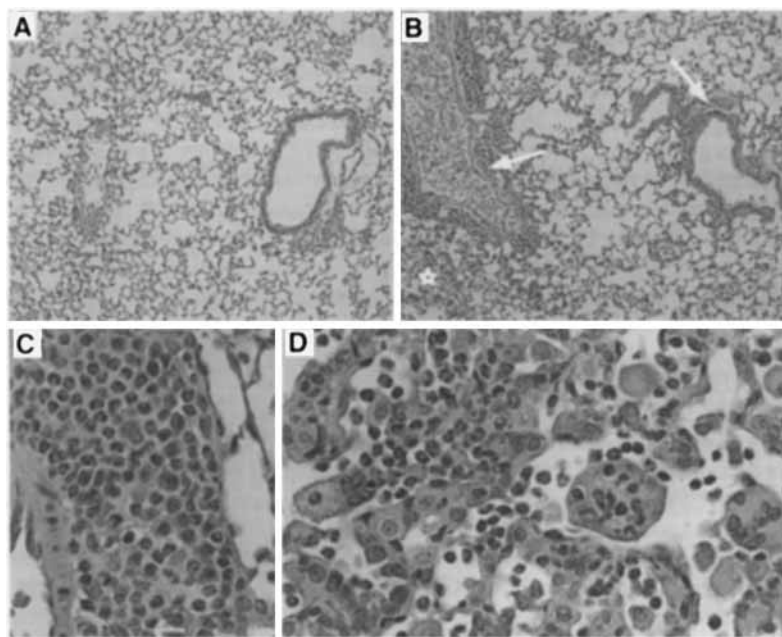


Figure 1. Lung lesions induced by sensitization with *A. fumigatus* antigens (formaldehyde-fixed lung sections stained with hematoxylin and eosin). *A*, Lung section from an unsensitized control mouse (original magnification $\times 80$). *B*, Lung section from a sensitized mouse (original magnification $\times 80$). Perivascular and peribronchial infiltrates (arrows); an area with alveolar inflammation (star). *C*, Higher magnification ($\times 320$) of the cells infiltrating perivascularly; same cell types are found in the peribronchial infiltrates. *D*, Higher magnification ($\times 320$) of severe alveolar lesions.

lesions are characterized by emigration of eosinophils and mononuclear cells into the lumen, goblet cell hyperplasia, and, in severe cases, epithelial metaplasia. Inflammatory infiltrates are found in the interstitial tissues of the airways, blood vessels, and alveoli. They consist primarily of eosinophils, accompanied by smaller numbers of lymphocytes, macrophages, plasma cells, and neutrophils. In cases of severe lung inflammation, alveolar septa in affected areas are thickened by inflammatory cells. Alveolar inflammation in these areas becomes granulomatous with the accumulation of multinucleated giant cells. Lung lesions are reversible (Gabriele Grünig, unpublished results). When mice are examined 3 or 4 weeks after the last antigen exposure, the vast majority of the microscopic lesions have disappeared.

The type of lung inflammation changes over time as mice are repeatedly exposed to *A. fumigatus* antigens intranasally³⁶ (our unpublished results). Analysis of BAL fluids reveals that naive mice have a strong neutrophil and macrophage response after the first intranasal exposure to *A. fumigatus* antigens. After few additional exposures, more than 50% of the cells in the BAL fluid are eosinophils and the remainder

are small mononuclear cells and macrophages. At that point, neutrophils are rare. The infiltrates within the lung tissue are dominated by eosinophils at that time. If intranasal exposures to *A. fumigatus* antigens are continued for a prolonged period of time, numbers of mononuclear cells in the BAL fluid increase, and numbers of eosinophils decrease, although they remain well above saline controls. In the lung tissues, the accumulation of mononuclear cells is more prominent relative to eosinophils. In some of the mouse strains, multiple exposures to *A. fumigatus* antigens over a prolonged time period will lead to an attenuation of the histologic lesions when compared with the acute response induced after a few exposures.⁵⁴

AIRWAY HYPERREACTIVITY

Sensitization with soluble *A. fumigatus* antigens consistently induces very strong airway hyperreactivity.^{22, 34, 72} The relative potency of *A. fumigatus* antigens in inducing airway hyperreactivity appears to be increased relative to other antigens, such as ovalbumin. For example, *A. fumigatus* antigens have been successfully used to induce airway hyperreactivity in all of the inbred strains of mice tested thus far (e.g., C57BL/6, 129 SvEv, BALB/C, crosses of these strains). In contrast, ovalbumin, administered according to comparable protocols, elicits robust airway hyperreactivity in BALB/C^{21, 103} but not in C57BL/6 mice (D.B. Corry, unpublished results). Administered far more frequently and in much greater quantities, ovalbumin has been reported to induce airway hyperreactivity in C57BL/6 mice.^{30, 40}

PARTICULATE VERSUS SOLUBLE ANTIGENS

Immune responses induced by particulate antigens may differ from responses to soluble extracts. Particulate antigens are more likely to stimulate alveolar macrophages and may have a longer "half life" in the lungs. Therefore, preparations of spores of *A. fumigatus* and polystyrene beads coated with the antigens from the soluble extract have been used to elicit lung responses.^{53, 58, 62} The greatest and most consistent differences between particulate and soluble antigen preparations are seen in the severity and type of microscopic lesions.^{53, 62} Compared with the soluble form, particulate antigens induce increased numbers of eosinophils to accumulate peribronchially or perivascularly. Furthermore, non-necrotizing granulomatous inflammation is induced more frequently in response to particulate antigens. The effect of the form of antigen on antibody levels, or cytokine responses, varies between studies.

STRAIN DIFFERENCES

Strain differences in the response to *A. fumigatus* antigens are most prominent in antibody responses and lung inflammation. BALB/C mice and 129 SvEv mice respond with very high serum antibody titers to *A. fumigatus* antigens; in contrast, the magnitude of the antibody response is quite attenuated in C57BL/6 mice (Viswanath P. Kurup and Gabriele Grünig, unpublished results). This genetic difference in the antibody response also has been described using other immunogens (e.g., ovalbumin).¹⁰³ Chromosomal localizations of traits that confer "high antibody responsiveness" have been mapped to murine chromosomes 1, 5, 9, 11, 13, and 19.^{94, 101}

Microscopic lesions induced by repeated intranasal exposures vary between different mouse strains as well. In 129SvEv mice, intranasal sensitization will produce a marked alveolitis in addition to very pronounced inflammatory infiltrates surrounding bronchi and blood vessels. Parallel experiments have shown that all of the inflammatory lesions, but in particular the alveolar lesions, are more attenuated in C57BL/6 mice (Gabriele Grünig, DVM, PhD, unpublished results). When comparing C3H/HeN and C57BL/6 mice, inflammatory lesions in C3H/HeN mice were much more attenuated than in C57BL/6 mice.⁵⁴

ROUTE OF SENSITIZATION

Another factor that affects the antibody and inflammatory responses is the route of sensitization. Intraperitoneally primed and intranasally challenged C57BL/6 mice have approximately a fivefold lower serum antibody response (IgE and IgG1 isotypes) when compared with mice sensitized via the intranasal route exclusively³⁴; the reason for this is not clear yet. However, different lymphoid tissues are targeted depending on the route of sensitization. It is conceivable that the lymphoid tissues associated with the respiratory tract (nose and bronchus associated lymphoid tissue, the submandibular and bronchial lymph nodes) are particularly efficient in eliciting antibody responses compared with the lymphoid tissue associated with the peritoneal cavity. The inflammatory response, however, tends to be stronger after one intranasal exposure in intraperitoneally primed C57BL/6 mice when compared with mice that have been exposed by the intranasal route only.³⁴

ESSENTIAL COMPONENTS OF THE IMMUNE SYSTEM MEDIATING THE RESPONSES INDUCED BY *A. FUMIGATUS* ANTIGENS

Neutralizing anti-cytokine antibodies and gene-targeted mice with specific deletions have been used to determine the cells and mediators critical for prominent features of experimental ABPA (IgE secretion, lung

inflammation, or airway hyperreactivity). The results are summarized in Table 1 and Figure 2, and are presented in upcoming sections.

Antibodies

How are the antibody responses are generated? As has been shown using a number of other immunogens, IL-4 is absolutely critical for the production of IgE in experimental ABPA. IL-4-deficient mice (IL-4^{-/-})⁵⁵ or mice treated with neutralizing anti-IL-4 antibodies throughout the sensitization period⁵⁹ do not have an increased serum IgE response. These mice, however, make similar levels of IgG1 antibodies and increased levels of IgG2a antibodies when compared with sensitized wild-type mice.^{55, 59}

Table 1. SUMMARY OF EXPERIMENTS TESTING THE CONTRIBUTION OF SPECIFIC CELLS AND CYTOKINES*

Mouse	Treatment	Serum IgE	Lung Inflammation	Airway Hyper-reactivity	Cytokine Response	References
Wild type	No	+++	+++	+++	IL-4, IL-5	22, 34, 52, 53, 72, 76
Wild type† or IL-5-	Anti-IL-5	+++	++ (no eos)	+++	NR	22, 53, 76
Wild type or IL-4-	Anti-IL-4	-	++	-	NR	22, 52, 53
IL-10-‡	No	+++	++++	+++	↑ IL-5, IFN-γ IL-4 as WT	34
IgE-	No	-	+++	+++	IL-4, IL-5	72
B cell -	No	-	+++	+++	IL-4, IL-5 (<WT)	22
B cell -	Anti-IL-5	-	+++ (no eos)	+++	NR	22
B cell -	Anti-IL-4	-	++	-	NR	22
RAG-§	No	-	--+	-	-	22
RAG-	Transfer of CD4+ T cells	-	++++	+--+	IL-4, IL-5, IFN-γ	22

*The results summarized in this table are from mice repeatedly sensitized with *A. fumigatus* antigens: saline-exposed mice served as controls. Results are depicted semiquantitatively from negative (-) to very severe (++++), or very high. Wild-type mice are the genetic control for the knock-out animals. Mice homozygous for a disrupted gene are denoted as - (e.g., IgE- is a mouse homozygous for the disruption of the ϵ -immunoglobulin heavy chain).

†As an example this line reads: In sensitized wild-type mice treated with anti-IL-5 antibodies or in IL-5 knock-out mice, IgE serum levels are high, airway inflammation is moderate with much reduced eosinophil numbers, and airway hyperreactivity is fully present. Nothing has been reported on the cytokine response in these mice.

‡IL-10 knock-out mice from different strains and sensitized via different routes differ in their responses; therefore, a trend is summarized.

§Recombinase activating gene (RAG)-deficient animals have no T or B cells because of the absence of the RAG that is absolutely critical for making T-cell receptors and immunoglobulins.

NR = nothing reported; WT = wild type.

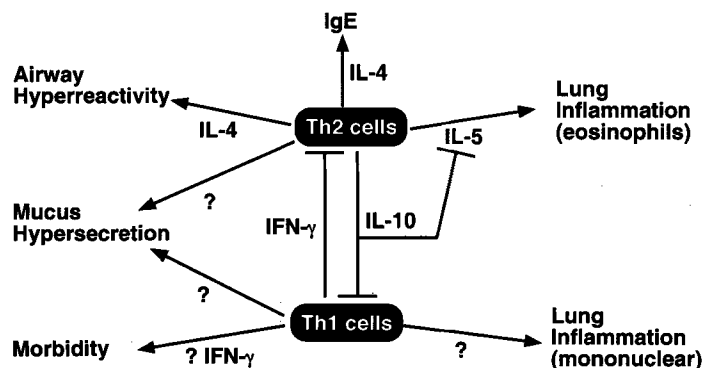


Figure 2. Current hypothesis of how T cells mediate immunopathology in experimental ABPA. Enhancing effects (arrows); inhibitory effects (T-shaped lines). Th2 cells induce airway hyperreactivity through the secretion of IL-4. The effect of IL-4, however, is indirect because neutralization of IL-4 late in the sensitization process does not reverse airway hyperreactivity. IL-4 is crucial for IgE synthesis, and IL-5 is crucial for the accumulation of eosinophils in the lungs. Forming a negative feedback on Th2 responses, IL-10 downregulates IL-5-mediated lung inflammation. IL-10 also downregulates Th1 responses (e.g., IFN- γ secretion). We do not know which cells (Th2 or Th1 or both) affect mucus hypersecretion in experimental ABPA. The role of Th1 cells in experimental ABPA is less well characterized. Th1 cells can cause lung inflammation. IFN- γ is thought to downregulate Th2 responses, but may indirectly induce morbidity when over produced.

The role of antibodies in the pathogenesis of ABPA was addressed using mice with a targeted deletion of the ϵ^{72} or μ^{22} chain. 129SvEv ϵ -chain-deficient mice are not capable of making IgE. The C57BL/6 μ -chain-deficient mice not only lack antibodies of all isotypes but also mature B cells. Sensitization with *A. fumigatus* antigens induces histologic lesions that are similar in quality and extent in both types of gene-targeted mice relative to wild-type mice. These lesions typically consist of airway inflammation and perivascular and peribronchial infiltrates. These results accord with data obtained with B-cell deficient mice exposed to ovalbumin.^{37, 49} Furthermore, prominent alveolar lesions, including granulomas, were induced in response to *A. fumigatus* antigens in the absence of mature B cells and antibodies, as shown in an outbred mouse strain (129/C57BL/6) with targeted deletion of the μ chain (G. Grünig, unpublished results).

However, there is no doubt that *A. fumigatus* antigen-antibody complexes can enhance T-cell responses in the lungs. In the experiments described previously, lower numbers of IL-4-producing T cells consistently accumulated in the lungs of sensitized B-cell deficient mice relative to wild-type mice.²² In a monkey model of ABPA, allergic human serum or control serum was infused into primed animals. Inhalation of *A. fumigatus* antigens led to the development of severe lung lesions typical of ABPA only in the monkey that had received allergic human

serum.⁸⁹ Interestingly, this monkey did not develop airway hyperreactivity.

Both IgE-deficient mice and B-cell deficient mice sensitized with *A. fumigatus* antigens developed airway hyperreactivity to the same extent as did wild-type mice.^{22, 72} The published data using ovalbumin as immunogen are controversial. Ovalbumin sensitization of B-cell deficient mice did not lead to airway hyperreactivity, although the histologic lesions were comparable to wild-type mice.^{36, 37} When mice were hyperimmunized with ovalbumin, however, airway hyperreactivity developed to a similar extent in mice incapable of switching to the IgG or IgE antibody isotypes (CD40 deficient) as in wild-type mice.⁴⁰ It appears that there is a reduction (albeit slight) in the inflammatory response in the lungs of B-cell or antibody-deficient mice. The reduction in the response may affect the development of airway hyperreactivity in animals exposed to a weak immunogen (ovalbumin) but not in mice exposed to a strong immunogen, such as *A. fumigatus* antigens, or in mice hyperimmunized with the weak immunogen (ovalbumin).

Eosinophils

Because eosinophil emigration into the airways and eosinophil infiltration into the interstitium of the lungs is such a prominent feature of human and experimental ABPA, many studies have addressed the requirements for eosinophil accumulation and function. Wild-type mice exposed to *A. fumigatus* antigens develop not only lung eosinophilia but also blood and bone marrow eosinophilia.⁷⁵⁻⁷⁷ If these animals are treated with neutralizing antibodies to IL-5, the bone marrow, blood, and lung eosinophilia is abolished.^{53, 76} The essential requirement for IL-5 has also been demonstrated using many other immunogens (e.g., parasites,¹⁹ ovalbumin³⁰). Granulocyte-macrophage colony-stimulating factor (GM-CSF) is another cytokine that can activate and maintain eosinophils in the tissues. It has been shown that GM-CSF-producing T cells accumulate in the lungs of animals exposed to *A. fumigatus* antigens.¹⁵ GM-CSF, however, must be ordered in hierarchy behind IL-5, because endogenous GM-CSF production cannot support eosinophilia in animals treated with neutralizing anti-IL-5 antibodies^{19, 53, 76} or in IL-5 gene knock-out mice.³⁰

The role of chemokines and adhesion molecules in mediating lung eosinophilia has not been investigated thoroughly in experimental ABPA. A report showed that mRNA for RANTES is up regulated following sensitization of mice with *A. fumigatus* antigens.⁵⁵ It will be of great interest to determine the role of eotaxin because it has been shown to be a major chemoattractant for eosinophils in the lungs.³² MIP-1 α , RANTES,⁶⁷ and MCP-4⁹⁰ are other chemokines that may be instrumental in eosinophil recruitment to the lungs. The ability of eotaxin to induce eosinophil accumulation is still dependent on IL-5 because the infusion of eotaxin at a dose that will elicit eosinophilia in wild-type mice does not cause eosinophilia in IL-5-deficient animals.⁷⁴

Eosinophils *in vitro* have been shown to secrete mediators that are proinflammatory or cytotoxic.²⁵ In experimental ABPA, eosinophils do not appear to be critical to mediate tissue injury because IL-5 deficient animals, or animals treated with anti-IL-5 antibodies, still develop the full spectrum of microscopic lesions.²² The infiltrates consisting of mononuclear cells instead of eosinophils appear to be just as substantial as in wild-type mice. The changes in the airway epithelium (goblet cell hyperplasia) are still present to the full extent. Furthermore, eosinophils are not required to mediate airway hyperreactivity. IL-5-deficient animals or mice treated with neutralizing anti-IL-5 antibodies develop airway hyperreactivity to the full extent relative to wild-type mice when exposed to *A. fumigatus* antigens.²² In models using ovalbumin as immunogen, the requirements for eosinophils and IL-5 in mediating airway hyperreactivity are very controversial^{21, 30, 36, 39} and appear to depend on the mouse strain and the sensitization protocol. It may be that for immunogens that induce relatively poor immune responses in certain mouse strains (e.g., ovalbumin in C57BL/6 mice) the proinflammatory and toxic mediators released by eosinophils are necessary substitutes for the proteases and toxins that are constituents of many natural allergens such as *A. fumigatus* antigens. Thus, in experimental ABPA the consequences of lung eosinophilia are still unclear. In ABPA, eosinophils may be responsible for tissue injury and destruction in chronic disease (e.g., lung injury maintained through repeated exposures over several months).

T Cells

The role of T cells was investigated using a reductionist approach.²² We examined mice with targeted disruption of the recombina-activating genes (RAG deficient) to look at the roles of T or B cells. Because recombina- is critically involved in the gene rearrangement of immunoglobulins and T-cell receptors, and these molecules in turn are critically important for B- and T-cell development, RAG-deficient mice are devoid of mature B and T cells. Even after intense exposure to *A. fumigatus* antigens, RAG-deficient mice do not develop significant lung lesions or airway hyperreactivity. When RAG-deficient mice are reconstituted with highly purified CD4+ T cells, sensitization will induce severe eosinophilic inflammation of the lungs; moreover, these mice will also develop airway hyperreactivity. Thus CD4+ T cells are absolutely critical for the development of peribronchial, perivascular, and alveolar lesions and also of airway hyperreactivity in experimental ABPA.²² A similar conclusion has been reached in the ovalbumin system, as ablation of CD4+ T cells will prevent the development of lung lesions and airway hyperreactivity.³¹ The data obtained with *Aspergillus*-exposed RAG-deficient mice are still surprising because the components of the *A. fumigatus* antigen extract are thought to have the capability to induce tissue injury and to activate cells of the innate immune system. Quite a substantial number

of eosinophils can be found in BAL fluids of RAG-deficient mice exposed to *A. fumigatus* antigens (about 20- to 30-fold more than in unchallenged mice and approximately three- to fivefold less than in sensitized wild-type mice).²² Additionally, exposure to *A. fumigatus* antigens will induce mild goblet cell hyperplasia in RAG-deficient animals (David B. Corry, MD, and Gabriele Grünig, DVM, PhD, unpublished results). The lung tissues (peribronchial, perivascular, or alveolar regions) of exposed RAG-deficient mice, however, are virtually devoid of aggregates of eosinophils or mononuclear cells.²²

In mice sensitized with ovalbumin, it has been shown that Th2 cytokines cause allergic inflammation and hyperreactivity; however, it is controversial whether IL-4 or IL-5 are critical.^{20, 29, 36, 39} In the ovalbumin system, Th1 cells are believed to be much more benign. Cytokines made by Th1 cells, in particular IFN- γ , can prevent airway hyperreactivity.^{41, 63, 65} In experimental murine ABPA, the cytokine requirements are very clear and appear to be the same regardless of mouse strain or sensitization protocol: It is IL-4.²² This cytokine is required to induce the development of Th2 cells that mediate the events leading to allergic lung inflammation and airway hyperreactivity. IL-4 itself becomes redundant after repeated exposures to *A. fumigatus* antigens because delayed depletion of IL-4 using neutralizing antibodies does not reduce airway hyperreactivity or BAL cellularity (Gabriele Grünig and David B. Corry, unpublished results). Similar findings have been reported for the ovalbumin model.²¹ The critical secondary mediators are still unknown because neither IgE, eosinophils, nor IL-5 are required for airway hyperreactivity or lung inflammation in experimental ABPA.²²

Negative Regulators of Immunopathology

Very little is known about mediators that down regulate the immune response to *A. fumigatus* antigens. IL-10 is known as a potent immunosuppressive molecule, and it is constitutively produced by bronchial epithelial cells.⁹ IL-10 has been shown to inhibit Th1 responses in mice¹ and cytokine production of Th1 and Th2 lymphocytes in humans.^{24, 102} IL-10 is thought to exert its suppressive function by inhibiting antigen presenting cells (such as dendritic cells or macrophages).^{4, 79, 92, 98, 104} In experimental ABPA, the major role of endogenous IL-10 is to inhibit lung inflammation.³⁴ In a mouse (mixture of C57BL/6 and 129SvEv) that developed a mixed T-cell response (Th2 and some Th1), endogenous IL-10 prevented mortality and restricted the secretion of IL-5 and IFN- γ into the airway lumen. In this model, production of IFN- γ was detrimental to the IL-10-deficient animals because neutralizing antibodies to IFN- γ reduced mortality (Gabriele Grünig, DVM, PhD, unpublished results). In C57BL/6 mice primed intraperitoneally and challenged intranasally, endogenous IL-10 limited IL-5 production and lung eosinophilia. In the same mouse strain (C57BL/6), however, endogenous IL-10 had no detectable effect on the response to intranasal exposure to *A. fumigatus*.

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References

1. Abbas AK, Murphy KM, Sher A: Functional diversity of helper T lymphocytes. *Nature* 383:787-793, 1996
2. al Musallam AA, Radwan SS: Wool-colonizing microorganisms capable of utilizing wool-lipids and fatty acids as sole sources of carbon and energy. *J Appl Bacteriol* 69:806-813, 1990
3. Amitani R, Taylor G, Elezis EN, et al: Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect Immun* 63:3266-3271, 1995
4. Armstrong L, Jordan N, Millar A: Interleukin 10 (IL-10) regulation of tumour necrosis factor α (TNF- α) from human alveolar macrophages and peripheral blood monocytes. *Thorax* 51:143-149, 1996
5. Arrese JE, Delvenne P, Van Cutsem J, et al: Experimental aspergillosis in guinea pigs: Influence of itraconazole on fungaemia and invasive fungal growth. *Mycoses* 37:117-122, 1994
6. Astorga RJ, Cubero MJ, Leon L, et al: Serological survey of infections in waterfowl in the Guadalquivir marshes (Spain). *Avian Dis* 38:371-375, 1994
7. Bauer J, Gareis M, Bott A, et al: Isolation of a mycotoxin (gliotoxin) from a bovine udder infected with *Aspergillus fumigatus*. *J Med Vet Mycol* 27:45-50, 1989
8. Berenguer J, Allende MC, Lee JW, et al: Pathogenesis of pulmonary aspergillosis: Granulocytopenia versus cyclosporine and methylprednisolone-induced immunosuppression. *Am J Respir Crit Care Med* 152:1079-1086, 1995
9. Bonfield TL, Konstan MW, Burfeind P, et al: Normal bronchial epithelial cells constitutively produce the anti-inflammatory cytokine interleukin-10, which is downregulated in cystic fibrosis. *Am J Respir Cell Mol Biol* 13:257-261, 1995
10. Bracher V, von Fellenberg R, Winder CN, et al: An investigation of the incidence of chronic obstructive pulmonary disease (COPD) in random populations of Swiss horses. *Equine Vet J* 23:136-141, 1991
11. Busse WW, Coffman RL, Gelfand EW, et al: Mechanisms of persistent airway inflammation in asthma. A role for T cells and T-cell products. *Am J Respir Crit Care Med* 152:388-393, 1995
12. Campbell AR, Swanson MC, Fernandez-Caldas E, et al: Aeroallergens in dairy barns near Cooperstown, New York and Rochester, Minnesota. *American Review of Respiratory Diseases* 140:317-320, 1989
13. Cenci E, Perito S, Enssle KH, et al: Th1 and Th2 cytokines in mice with invasive aspergillosis. *Infect Immun* 65:564-570, 1997
14. Chauhan B, Knutsen A, Hutcheson PS, et al: T-cell subsets, epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. *J Clin Invest* 97:2324-2331, 1996
15. Chu HW, Wang JM, Boutet M, et al: Immunohistochemical detection of GM-CSF, IL-4 and IL-5 in a murine model of allergic bronchopulmonary aspergillosis. *Clin Exp Allergy* 26:461-468, 1996
16. Chu HW, Wang JM, Boutet M, et al: Increased expression of intercellular adhesion molecule-1 (ICAM-1) in a murine model of pulmonary eosinophilia and high IgE level. *Clin Exp Immunol* 100:319-324, 1995
17. Chu HW, Wang JM, Boutet M, et al: Tumor necrosis factor-alpha and interleukin-1 alpha expression in a murine model of allergic bronchopulmonary aspergillosis. *Lab Anim Sci* 46:42-47, 1996
18. Cicogna CE, White MH, Bernard EM, et al: Efficacy of prophylactic aerosol amphoteri-

- cin B lipid complex in a rat model of pulmonary aspergillosis. *Antimicrob Agents Chemother* 41:259–261, 1997
19. Coffman RL, Seymour BW, Hudak S, et al: Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 245:308–310, 1989
 20. Comoy EE, Pestel J, Duez C, et al: The house dust mite allergen, *Dermatophagoides pteronyssinus*, promotes type 2 responses by modulating the balance between IL-4 and IFN- α . *J Immunol* 160:2456–2462, 1998
 21. Corry DB, Folkesson HG, Warnock ML, et al: Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 183:109–117, 1996
 22. Corry DB, Grünig G, Hadeiba H, et al: Requirements for allergen-induced airway hyperreactivity in T- and B-cell-deficient mice. *Molecular Medicine* May, 1998
 23. Cramer R, Faith A, Hemmann S, et al: Humoral and cell-mediated autoimmunity in allergy to *Aspergillus fumigatus*. *J Exp Med* 184:265–270, 1996
 24. de Waal Malefyt R, Figdor CG, de Vries JE: Regulation of human monocyte functions by Interleukin 10. In de Vries JE, de Waal Malefyt R (eds): *Interleukin 10*. Heidelberg, Springer-Verlag, 1995, pp 37–52
 25. Dick HM, Dawson CO, Campbell JD: Farmer's lung: A comparison of simple diagnostic techniques and antigen preparation in human and bovine disease. *Clin Allergy* 3:209–216, 1973
 26. Drazen JM, Arm JP, Austen KF: Sorting out the cytokines of asthma. *J Exp Med* 183:1–6, 1996
 27. Eduard W, Sandven P, Levy F: Serum IgG antibodies to mold spores in two Norwegian sawmill populations: Relationship to respiratory and other work-related symptoms. *Am J Ind Med* 24:207–222, 1993
 28. Flach EJ, Stevenson MF, Henderson GM: Aspergillosis in gentoo penguins (*Pygoscelis papua*) at Edinburgh Zoo: 1964 to 1988. *Vet Rec* 126:81–85, 1990
 29. Forbes NA, Simpson GN, Goudswaard MF: Diagnosis of avian aspergillosis and treatment with itraconazole [letter, comment]. *Vet Rec* 130:519–520, 1992
 30. Foster PS, Hogan SP, Ramsay AJ, et al: Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 183:195–201, 1996
 31. Gavett SH, Chen X, Finkelman F, et al: Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 10:587–593, 1994
 32. Gonzalo JA, Lloyd CM, Kremer L, et al: Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J Clin Invest* 98:2332–2345, 1996
 33. Greet TR: Nasal aspergillosis in three horses. *Vet Rec* 109:487–489, 1981
 34. Grünig G, Corry DB, Leach MW, et al: Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J Exp Med* 185:1089–1099, 1997
 35. Halliwell RE, McGorum BC, Irving P, et al: Local and systemic antibody production in horses affected with chronic obstructive pulmonary disease. *Vet Immunol Immunopathol* 38:201–15, 1993
 36. Hamelmann E, Oshiba A, Schwarze J, et al: Allergen-specific IgE and IL-5 are essential for the development of airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 16:674–682, 1997
 37. Hamelmann E, Vella AT, Oshiba A, et al: Allergic airway sensitization induces T-cell activation but not airway hyperresponsiveness in B-cell-deficient mice. *Proc Natl Acad Sci USA* 94:1350–1355, 1997
 38. Hibberd PL, Rubin RH: Clinical aspects of fungal infection in organ transplant recipients. *Clin Infect Dis* 19(suppl):S33–40, 1994
 39. Hogan SP, Koskinen A, Foster PS: Interleukin-5 and eosinophils induce airway damage and bronchial hyperreactivity during allergic airway inflammation in BALB/c mice. *Immunol Cell Biol* 75:284–288, 1997
 40. Hogan SP, Mould A, Kikutani H, et al: Aeroallergen-induced eosinophilic inflamma-

- tion, lung damage, and airways hyperreactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. *J Clin Invest* 99:1329–1339, 1997
41. Iwamoto I, Nakajima H, Endo H, et al: Interferon- γ regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4+ T cells. *J Exp Med* 177:573–576, 1993
 42. Jensen HE, Aalbaek B, Basse A, et al: The occurrence of fungi in bovine tissues in relation to portals of entry and environmental factors. *J Comp Pathol* 107:127–140, 1992
 43. Johnson CT, Lupson GR, Lawrence KE: The bovine placentome in bacterial and mycotic abortions. *Vet Rec* 134:263–266, 1994
 44. Katamoto H, Shimada Y: Intra-arterial and intramammary injection of miconazole for bovine mastitis caused by *Aspergillus fumigatus*. *Br Vet J* 146:354–357, 1990
 45. Kauffman HF, Tomee JFC, Van der Werf TS, et al: Review of fungus-induced asthmatic reactions. *Am J Respir Crit Care Med* 151:2109–2116, 1995
 46. Keating JJ, Rogers T, Petrou M, et al: Management of pulmonary aspergillosis in AIDS: An emerging clinical problem. *J Clin Pathol* 47:805–809, 1994
 47. Knudtson WU, Kirkbride CA: Fungi associated with bovine abortion in the northern plains states (USA). *J Vet Diagn Invest* 4:181–185, 1992
 48. Knutsen AP, Mueller KR, Levine AD, et al: Asp f1 CD4+ TH2-like T-cell lines in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 94:215–221, 1994
 49. Korsgren M, Erjefalt JS, Korsgren O, et al: Allergic eosinophil-rich inflammation develops in lungs and airways of B-cell-deficient mice. *J Exp Med* 185:885–892, 1997
 50. Kramer MN, Kurup VP, Fink JN: Allergic bronchopulmonary aspergillosis from a contaminated dump site. *American Review of Respiratory Diseases* 140:1086–1088, 1989
 51. Krogh HV: Bovine mycotic abortion in Denmark. *Nordic Veterinary Journal* 37:27–33, 1985
 52. Kurup VP, Choi H, Murali PS, et al: IgE and eosinophil regulation in a murine model of allergic aspergillosis. *J Leukoc Biol* 56:593–598, 1994
 53. Kurup VP, Choi H, Murali PS, et al: Role of particulate antigens of *Aspergillus* in murine eosinophilia. *Int Arch Allergy Immunol* 112:270–278, 1997
 54. Kurup VP, Choi H, Resnick A, et al: Immunopathological response of C57BL/6 and C3H/HeN mice to *Aspergillus fumigatus* antigens. *Int Arch Allergy Immunol* 91:145–154, 1990
 55. Kurup V, Guo J, Murali P, et al: Immunopathological responses to *Aspergillus* antigen in interleukin-4 knockout mice. *J Lab Clin Med* 130:567–575, 1997
 56. Kurup VP, Kumar A: Immunodiagnosis of aspergillosis. *Clin Microbiol Rev* 4:439–456, 1991
 57. Kurup VP, Kumar A, Kenealy WR, et al: *Aspergillus* ribotoxins react with IgE and IgG antibodies of patients with allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 123:749–756, 1994
 58. Kurup VP, Mauze S, Choi H, et al: A murine model of allergic bronchopulmonary aspergillosis with elevated eosinophils and IgE. *J Immunol* 148:3783–3788, 1992
 59. Kurup V, Murali P, Guo J, et al: Anti-IL-4 and IL-5 antibodies downregulate IgE and eosinophilia in mice exposed to *Aspergillus* antigens. *Allergy* 52:1215–1221, 1997
 60. Kurup VP, Ramasamy M, Greenberger PA, et al: Isolation and characterization of a relevant *Aspergillus fumigatus* antigen with IgG- and IgE-binding activity. *International Archives of Allergy and Applied Immunology* 86:176–182, 1988
 61. Kurup VP, Resnick A, Scribner GH, et al: Enzyme profile and immunochemical characterization of *Aspergillus fumigatus* antigens. *J Allergy Clin Immunol* 78:1166–1173, 1986
 62. Kurup VP, Seymour BW, Choi H, et al: Particulate *Aspergillus fumigatus* antigens elicit a TH2 response in BALB/c mice. *J Allergy Clin Immunol* 93:1013–1020, 1994
 63. Lack G, Bradley KL, Hamelmann E, et al: Nebulized IFN- γ inhibits the development of secondary allergic responses in mice. *J Immunol* 157:1432–1439, 1996
 64. Land CJ, Lundstrom H, Werner S: Production of tremorgenic mycotoxins by isolates of *Aspergillus fumigatus* from sawmills in Sweden. *Mycopathologia* 124:87–93, 1993

65. Li X-M, Chopra RK, Chou T-Y, et al: Mucosal IFN- γ transfer inhibits pulmonary allergic responses in mice. *J Immunol* 157:3216–3219, 1996
66. Liu J, Yang ZJ, Meng ZH: The isolation, purification, and identification of fumitremorgin B produced by *Aspergillus fumigatus*. *Biomed Environ Sci* 9:1–11, 1996
67. Lukacs NW, Strieter RM, Warmington K, et al: Differential recruitment of leukocyte populations and alteration of airway hyperreactivity by C-C family chemokines in allergic airway inflammation. *J Immunol* 158:4398–4404, 1997
68. Lund A: Yeasts and moulds in the bovine rumen. *Journal of General Microbiology* 81:453–462, 1974
69. Mahajan VM, Dayal Y, Patra CP, et al: Experimental aspergillosis in monkeys. *Sabouraudia* 16:199–201, 1978
70. McGorum BC, Dixon PM, Halliwell RE: Evaluation of intradermal mold antigen testing in the diagnosis of equine chronic obstructive pulmonary disease. *Equine Vet J* 25:273–275, 1993
71. McGorum BC, Dixon PM, Halliwell RE: Responses of horses affected with chronic obstructive pulmonary disease to inhalation challenges with mold antigens. *Equine Vet J* 25:261–267, 1993
72. Mehlhop PD, van de Rijn M, Goldberg AB, et al: Allergen-induced bronchial hyper-reactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci USA* 94:1344–1349, 1997
73. Mosmann TR, Coffman RL: Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv Immunol* 46:111–147, 1989
74. Mould AW, Matthaei KI, Young IG, et al: Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J Clin Invest* 99:1064–1071, 1997
75. Murali PS, Dai G, Kumar A, et al: *Aspergillus* antigen-induced eosinophil differentiation in a murine model. *Infect Immun* 60:1952–1956, 1992
76. Murali PS, Kumar A, Choi H, et al: *Aspergillus fumigatus* antigen-induced eosinophilia in mice is abrogated by anti-IL-5 antibody. *J Leukoc Biol* 53:264–267, 1993
77. Murali PS, Kurup VP, Guo J, et al: Development of bone marrow eosinophilia in mice induced by *Aspergillus fumigatus* antigens. *Clin Immunol Immunopathol* 84:216–220, 1997
78. Nagai H, Guo J, Choi H, et al: Interferon- γ and tumor necrosis factor- α protect mice from invasive aspergillosis. *J Infect Dis* 172:1554–1560, 1995
79. Nicod LP, el Habre F, Dayer JM, et al: Interleukin-10 decreases tumor necrosis factor α and β in alloreactions induced by human lung dendritic cells and macrophages. *Am J Respir Cell Mol Biol* 13:83–90, 1995
80. Nicolet J, Haller RD, Herzog J: Serological investigations of a bovine respiratory disease (urmer pneumonie) resembling farmer's lung. *Infect Immun* 6:38–42, 1972
81. Pauli B, Gerber H, Schatzmann U: Farmer's lung' in the horse. *Pathologia et Microbiologia (Basel)* 38:200–214, 1972
82. Pirie HM, Dawson CO, Breeze RG, et al: A bovine disease similar to farmer's lung: Extrinsic allergic alveolitis. *Vet Rec* 88:346–351, 1971
83. Reiner SL, Locksley RM: The regulation of immunity to *Leishmania major*. *Annu Rev Immunol* 13:151–177, 1995
84. Sarfati J, Jensen HE, Latge JP: Route of infections in bovine aspergillosis. *J Med Vet Mycol* 34:379–383, 1996
85. Schallibaum M, Nicolet J, König H: *Aspergillus nidulans* and *Aspergillus fumigatus* as causal agents of bovine mastitis. *Sabouraudia* 18:33–38, 1980
86. Schuyler M, Gott K, Cherne A, et al: Th1 CD4 + cells adoptively transfer experimental hypersensitivity pneumonitis. *Cell Immunol* 177:169–175, 1997
87. Schuyler M, Gott K, Shopp G, et al: CD3+, CD4+, CD8–, Ia– T cells adoptively transfer murine experimental hypersensitivity pneumonitis. *Chest* 103:143S–145S, 1993
88. Sheridan JJ, White DS, McGarvie QD: The occurrence of and organisms concerned with bovine mycotic abortion in some counties of Ireland. *Vet Res Commun* 9:221–226, 1985
89. Slavin RG, Fischer VW, Levine EA, et al: A primate model of allergic bronchopulmo-

antigens, most probably because of compensatory mechanisms. IL-10 may be beneficial in restricting the inflammation induced by inhalation of *A. fumigatus* antigens.³⁴ More work, however, needs to be done to define additional regulators of the T-cell response to *A. fumigatus* antigens that ultimately suppress lung inflammation and airway hyperreactivity.

CONCLUSION

The studies in murine models clearly reveal that Th2 cells are at the center of the immunopathology (inflammation and airway hyperreactivity) induced by exposure to *A. fumigatus* antigens. In experimental ABPA, however, a strong Th1 response can also cause immunopathology (e.g., inflammation). Therefore, in experimental ABPA, pathology and protection apparently do not follow the strict dichotomy of Th1 versus Th2 responses. In contrast, a Th1 versus Th2 dichotomy has been demonstrated in some parasite infections. For example, Th1 responses are beneficial and Th2 responses detrimental in infections with intracellular parasites like *Leishmania major*.⁸³

In experimental ABPA, antibodies and B cells may augment T-cell responses and consequently increase inflammation. The role for eosinophils in the pathogenesis of the disease remains elusive. It is possible that B cells and antibodies, as well as eosinophils, play a more important role in other aspects of the relationship between the immune system and the fungus, as in infectious aspergillosis or in chronic ABPA.

How do the conclusions drawn from the animal models relate to human disease? Ultimately, only a close examination of the human disease can provide the answer. The survey of the natural diseases in various animal species caused by *A. fumigatus* raises the intriguing possibility that a strong T-cell response to *A. fumigatus* evolved as a double-edged sword. The highly evolved immune system of mammals provides the host with protection from infection or colonization with the fungus, yet predisposes the host to allergies. Cattle appear to be the exception to this rule, in that mucosal colonization and infections with *A. fumigatus* occur frequently, whereas allergies are rare. Murine models of ABPA show that there is a difference in the response depending on the nature of the antigen used (particulate versus soluble) and on the mouse strain examined. In human patients, genetic traits⁹⁷ and differences in the nature of priming and sensitization may be important determinants of pathology and pathophysiology. Data obtained from the murine models point to T cells as major targets for immunologic intervention. The future challenge is to develop immunotherapy for *A. fumigatus* that restricts the T-cell response that causes allergies without impairing the T-cell response that protects from infection.

- nary aspergillosis. *International Archives of Allergy and Applied Immunology* 56:325-333, 1978
90. Stellato C, Collins P, Ponath PD, et al: Production of the novel C-C chemokine MCP-4 by airway cells and comparison of its biological activity to other C-C chemokines. *J Clin Invest* 99:926-936, 1997
 91. Stevens RL, Austen KF: Recent advances in the cellular and molecular biology of mast cells. *Immunol Today* 10:381-386, 1989
 92. Thomassen MJ, Divis LT, Fisher CJ: Regulation of human alveolar macrophage inflammatory cytokine production by Interleukin-10. *Clin Immunol Immunopathol* 80:321-324, 1996
 93. Tomee JF, Wierenga AT, Hiemstra PS, et al: Proteases from *Aspergillus fumigatus* induce release of proinflammatory cytokines and cell detachment in airway epithelial cell lines. *J Infect Dis* 176:300-303, 1997
 94. Vyse TJ, Morel L, Tanner FJ, et al: Backcross analysis of genes linked to autoantibody production in New Zealand White mice. *J Immunol* 157:2719-2727, 1996
 95. Walker C, Bauer W, Braun RK, et al: Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. *Am J Respir Crit Care Med* 150:1038-1048, 1994
 96. Wang JM, Denis M, Fournier M, et al: Experimental allergic bronchopulmonary aspergillosis in the mouse: Immunological and histological features. *Scand J Immunol* 39:19-26, 1994
 97. Weiner Miller P, Hamosh A, Macek M, et al: Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 59:45-51, 1996
 98. Wilkes DS, Neimeier M, Mathur PN, et al: Effect of human lung allograft alveolar macrophages on IgG production: Immunoregulatory role of interleukin-10, transforming growth factor-beta, and interleukin-6. *Am J Respir Cell Mol Biol* 13:621-628, 1995
 99. Wolf AM: Fungal diseases of the nasal cavity of the dog and cat. *Vet Clin North Am Small Anim Pract* 22:1119-1132, 1992
 100. Woods PS, Robinson NE, Swanson MC, et al: Airborne dust and aeroallergen concentration in a horse stable under two different management systems. *Equine Vet J* 25:208-213, 1993
 101. Wu J, Longmate JA, Adamus G, et al: Interval mapping of quantitative trait loci controlling humoral immunity to exogenous antigens: Evidence that non-MHC immune response genes may also influence susceptibility to autoimmunity. *J Immunol* 157:2498-2505, 1996
 102. Yssel H, De Waal Malefyt R: IL-10 and human T cells. *In* de Vries JE, de Waal Malefyt R (eds): *Interleukin 10*. Heidelberg, Springer-Verlag, 1995, pp 19-27
 103. Zhang Y, Lamm WJ, Albert RK, et al: Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am J Respir Crit Care Med* 155:661-669, 1997
 104. Zissel G, Schlaak J, Schlaak M, et al: Regulation of cytokine release by alveolar macrophages treated with interleukin-4, interleukin-10, or transforming growth factor β . *Eur Cytokine Netw* 7:59-66, 1996

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