

TOWARD A COMPREHENSIVE UNDERSTANDING OF ALLERGIC LUNG DISEASE

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ABSTRACT

Allergic asthma is a respiratory disease induced by exposure to environmental agents that elicit allergic inflammation and transient airway obstruction and which produce the characteristic symptoms of cough and dyspnea. Prior to the advent of experimental models, asthma was believed to be caused primarily by the degranulation of mast cells and eosinophils primed by antigen-specific immunoglobulin E (IgE). More recent studies in mice have shown that T cells primarily mediate antigen-dependent airway obstruction and allergic inflammation through secretion of the cytokines interleukin 4 (IL- 4) and IL-13. Our additional studies indicate that a major environmental link to asthma may be through exposure to environmental proteinases and especially airway infection by proteinase-producing organisms such as fungi. Pending verification in humans, these findings suggest entirely new therapeutic interventions in asthma that include the restricted use of anti-inflammatory therapy and universal application of anti-fungal agents.

Allergic asthma is a respiratory disease that is induced by exposure to environmental antigens that induce allergic inflammation and intermittent airway obstruction, the latter of which is believed to underlie the characteristic symptoms of cough and dyspnea. Although not a highly fatal disease, asthma is nonetheless one of the most common respiratory diseases and, indeed, is one of the most common of all diseases of adults and children in highly developed societies. Consequently, asthma is a major cause of decreased quality of life in societies where its prevalence is high. The often chronic nature of asthma further contributes to the economic burden it places on the health care delivery system (^{1, 2}).

The pathophysiology of asthma may be best understood in terms of an intrinsic immune-based etiology that ultimately has an environmental

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origin. The majority of asthma patients suffer from atopy, which may be defined as a predilection to react against diverse environmental antigens in assays that detect the presence of antigen-specific antibodies (3). Indeed, atopy is one of the strongest risk factors for acquiring asthma and is often assessed by means of the skin prick test (3). With this commonly used assay, immediate wheal and flare reactions to antigen preparations injected ("pricked") into the skin are interpreted as positive reactions. Such reactions are known to be immunologically mediated through the mechanism of type 1 (immediate) hypersensitivity.

As originally envisaged, the type I hypersensitivity response was believed to underlie most asthmatic reactions. According to this well-understood immunological mechanism, immunoglobulin E (IgE) is captured on the surface of cells expressing a high affinity receptor ($F_{C\epsilon}RI$), where it may persist for weeks or even months. Such IgE-primed cells, which include mast cells, dendritic cells, eosinophils (in humans) and other cells, are triggered to degranulate following exposure to cognate antigen that crosslinks $F_{C\epsilon}RI$ (4). The extruded products of these IgE/antigen-activated cells, including histamine, proteases, cytokines, ribonucleases, leukotrienes and other pro-inflammatory substances, were believed to account for not just the wheal and flare reaction of the skin prick test but also the airway obstruction that underlies the clinical manifestations of asthma (5).

The advent of experimental models of asthma combined with advances in molecular biology that permitted the selective inactivation of genes through homologous recombination have allowed scientists to formally test various hypotheses regarding asthma pathogenesis. Although studies are not unanimous, mice with disruption of type I hypersensitivity mechanisms, including mice lacking IgE and other antibodies (6, 7), B cells (6), eosinophils (8), and mast cells (9), all manifest asthma-like disease that is similar to the disease phenotype of wild type animals, demonstrating that type I hypersensitivity mechanisms are not required for either allergic airway inflammation or the disease feature of airway obstruction. Type I hypersensitivity mechanisms remain relevant in asthma as they most likely do underlie other allergic phenomena, such as upper respiratory tract allergic reactions (allergic rhinitis), hay fever and more serious processes such as systemic anaphylaxis that may contribute in distinct ways to the expression of asthma. Nonetheless, the persistence of lower respiratory tract allergic inflammation and airway obstruction (airway hyperresponsiveness, mucus hyper-secretion) in mice with abrogation of type I hypersensitivity provided the first evidence for the existence of alternate immune mechanisms contributing to airway obstruction in asthma.

Studies from our laboratory eventually established that the major immune mechanism underlying asthma-like disease in mice was a type 4 immune hypersensitivity response in which terminally differentiated T helper cells termed T helper type 2 (Th2) cells ⁽¹⁰⁾ secrete the cytokine interleukin 13 (IL-13), which directly causes airway obstruction by acting directly on constitutive cells of the lung such as airway epithelium ^(11, 12). Efforts to target human IL-13 and the IL-13 signaling pathway are now underway in the pharmaceutical industry and hold promise of providing targeted relief from airway obstruction while not interfering with other vital immune functions.

More recently, increasing attention has been given to the extrinsic causes of asthma, particularly in identifying the environmental agents that provoke airway Th2 responses and IL-13 secretion. In the following sections, we review the major advances in this area, focusing primarily on the work from our laboratories.

METHODS AND RESULTS

Traditional Experimental Allergens. Experimental models of allergic lung disease have been developed using virtually all species of domesticated animals, and diverse antigens have been used to elicit the allergic inflammation that is highly characteristic of this disease. However, the majority of experimental work has been conducted in mice and guinea pigs using the antigen ovalbumin ⁽¹³⁾. Somewhat paradoxically, concerns about the ovalbumin-dependent mouse model began to arise even as it proved to be highly successful in elucidating the fundamental immune and physiological mechanisms of allergic lung disease. First, although ovalbumin is clearly capable of eliciting allergic lung disease, it is inexplicably most effective in only certain mouse genetic backgrounds, including the Balb/c and A/J strains ^(14, 15). Furthermore, ovalbumin will only elicit allergic lung disease if animals are first immunized against ovalbumin precipitated in the adjuvant aluminum potassium sulfate (alum), a highly artificial protocol with uncertain physiological relevance. Once ovalbumin-dependent allergic lung disease has been established, however, the disease phenotype cannot be maintained for periods beyond approximately six weeks, presumably because of contravening tolerogenic mechanisms that may be unique to the lung ⁽¹⁶⁾. A final concern with ovalbumin is that this antigen is not a significant cause of human allergic lung diseases and may, therefore, be less than optimal for exploring the relevant extrinsic causes of asthma.

Proteinases as Allergic Adjuvants. To overcome the limitations of ovalbumin, we analyzed a series of antigens prepared from organ-

isms previously known to be highly allergenic for humans (fungi and pollens) to determine if a common biochemistry might provide the essential link to their allergenic properties. We focused initially on the presence of proteinases, which had previously been noted to be a common biochemical feature of human allergens (Table 1) (17) and which had already been shown to exhibit intriguing immunological properties (18–26). Complex, crude allergens prepared from the culture filtrate of the fungus, *Aspergillus fumigatus*, and the pollen of *Ambrosia artemisiifolia* (common ragweed) proved to be highly allergenic in diverse mouse strains, including C57BL/6 mice that are highly resistant to ovalbumin (Figure 1) (14). Moreover, both allergens contained abundant proteinase activity in addition to other enzymatic activities and potentially allergenic moieties (27).

To determine if proteinase was, in fact, contributing to the allergenic nature of these preparations, we obtained highly purified proteinases from two species of *Aspergillus* in amounts sufficient to administer to mice. As little as seven micrograms of the aspergillopepsin derived from *A. oryzae* was sufficient to induce all features of allergic lung disease in C57BL/6 mice (Figure 1) (27). Moreover, we showed that the allergenic activity of this proteinase required intact proteinase activity (Figure 2) (27). Equally striking, we showed that whereas ovalbumin given intranasally to mice in the absence of prior immunization induces no specific immune reaction, lung inflammation or airway hyperresponsiveness, when given together with *A. oryzae* proteinase, ovalbumin elicits specific IgE and IgG1 responses (Figure 2) (27). Thus, the fungal proteinase acts as an adjuvant to promote Th2-dependent allergic reactions to innocuous antigens that by themselves would elicit no active inflammation. These studies are important because they are the first to implicate an organ-

TABLE 1
Proteolytically Active Allergens (17)

Allergen	Source	Allergen	Source
Api m 7	<i>Apis mellifera</i> (honey bee) venom	Der m 1	<i>D. microceras</i> (dust mite)
Asp f 10	<i>Aspergillus fumigatus</i> (fungus)	Fel d 1	<i>Felis domesticus</i> (domestic cat)
Asp f 13	<i>A. fumigatus</i> (fungus)	Pen b	<i>Penicillium citrinum</i> (fungus)
		13	
Asp f 18	<i>A. fumigatus</i> (fungus)	Pen c 13	<i>P. brevicompactum</i> (fungus)
Asp fl	<i>A. flavus</i> (fungus)	Pen c 2	<i>P. citrinum</i> (fungus)
13			
Asp n	<i>A. niger</i> (fungus)	Pen ch	<i>P. chrysogenum</i> (fungus)
18		13	
Asp o 13	<i>A. oryzae</i> (fungus)	Pen ch	<i>P. chrysogenum</i> (fungus)
		18	
Der f 2	<i>D. farinae</i> (dust mite)	Pen o 18	<i>P. oxalicum</i> (fungus)
Der p 1	<i>D. pteronyssinus</i> (dust mite)	Tri t 4	<i>T. tonsurans</i> (fungus)

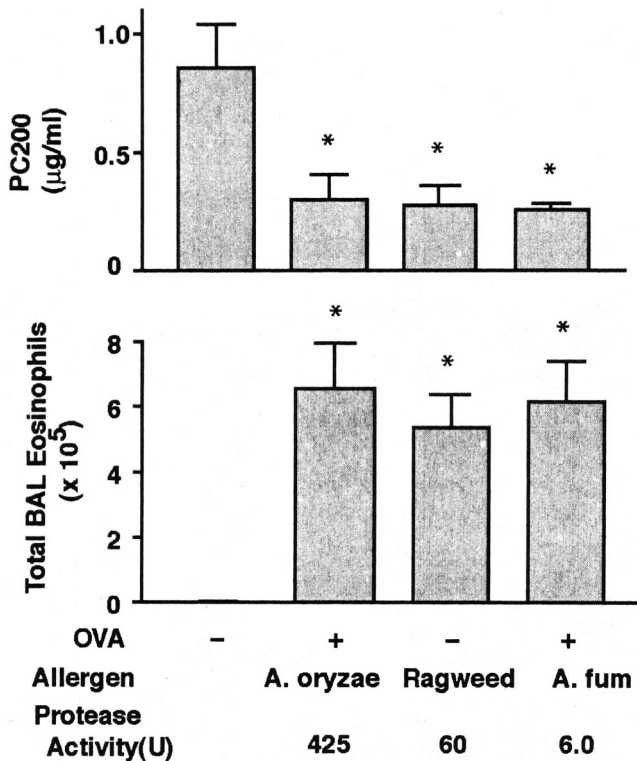


FIG. 1. Comparison of 3 proteinase-containing allergens. Mice were intranasally sensitized with allergens derived from *A. oryzae*, ragweed pollen and *A. fumigatus* (*A. fum*) without or with ovalbumin (OVA, indicated by - or +, respectively) given on a schedule of every 4 days for 5 total challenges and compared to saline- challenged control mice (far left bars). Allergen doses are listed in units (U) of proteinase activity. **Top**, airway responsiveness as assessed by the provocative concentration of acetylcholine that induced a 200% increase in respiratory system resistance (PC₂₀₀). **Bottom**, Total eosinophils influxing into bronchoalveolar lavage (BAL). Data are representative of 3 independent experiments simultaneously comparing the 3 allergens. *:P < 0.05 relative to saline challenged mice (Copyright 2002. The American Association of Immunologists, Inc.).

ism-specific biochemical activity (proteinase activity) as playing a central etiological role in asthma-like disease.

The Search for Environmental Proteinases: Fungi as Infectious Agents. The unexpectedly powerful allergenic effect of fungal and pollen proteinases in mice prompted us to consider the potential relevance of these and related enzymes as human allergens. Analysis of house dust obtained from the homes of asthmatic children in the Houston area revealed that most if not all of these households

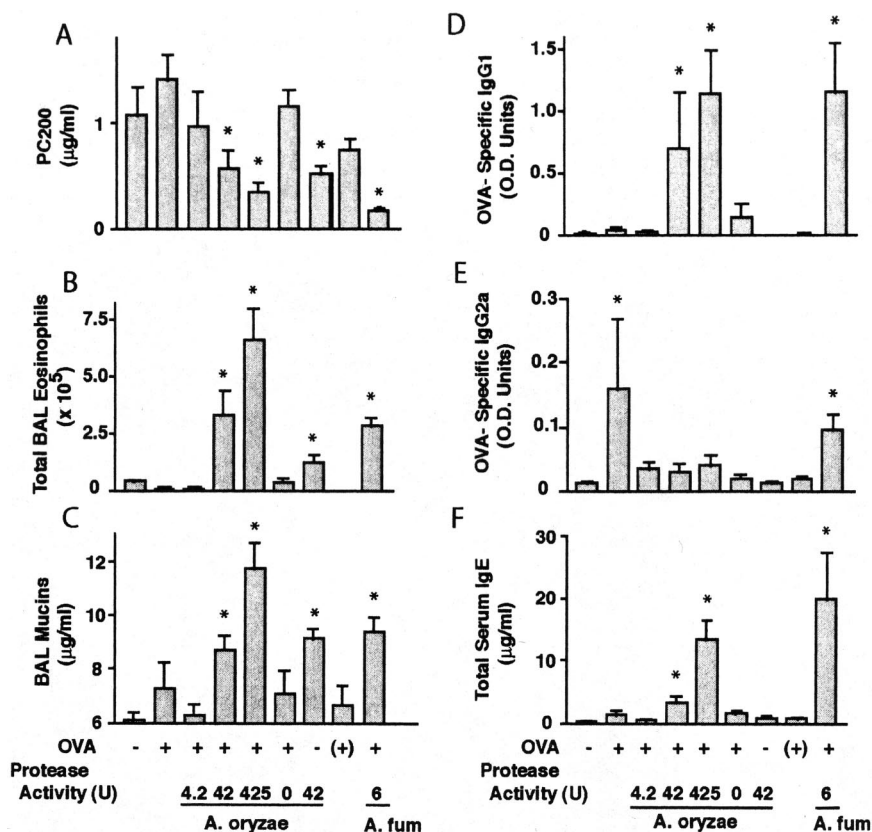


FIG. 2. Requirement of proteinase activity for allergic lung responses. C57BL/6 mice were intranasally challenged with saline (-), or the indicated combinations of ovalbumin (OVA, indicated by +), *A. oryzae* or *A. fumigatus* (*A. fum*) allergens. *A. oryzae* allergen was given in increasing amounts from 4.2U to 425U proteinase activity/dose and following phosphoramidon inactivation of an intermediate dose originally containing 42U proteinase activity (proteinase activity: 0). *A. oryzae* - hydrolyzed fragments of ovalbumin (indicated by (+)) were also administered as an additional control. **A**, airway responsiveness as assessed by PC₂₀₀ values. **B**, Total BAL eosinophils. **C**, BAL mucin concentration. **D-F**, Relative concentrations of ovalbumin-specific serum IgG1 and IgG2a (**D** and **E**, respectively) in optical density (O.D.) units and total serum IgE in mg/ml (**F**) Data are representative of 3 independent experiments; *: P ≤ 0.05 relative to saline-challenged mice. (Copyright 2002. The American Association of Immunologists, Inc.).

contained active dust proteinases (28). Further analysis revealed that a major source of many house dust proteinases was fungi, especially one fungus, *A. niger*, which we have found in every house examined thus far (20/20). We further confirmed that proteinases from dust mites

(e.g., Der p 1), which have long been linked to human asthma (²⁹), were present in many of our house dust samples. However, a surprise from these studies was that the dust mite proteinases were not enzymatically active, as the fungal proteinases were. This finding is important because enzymatic activity is required for allergenic activity in mice, at least in the case of fungal proteinases (²⁷). Our findings, therefore, suggested that fungal proteinases, especially from *A. niger*, were potentially more relevant to human disease than the dust mite proteinases.

In experimental systems, active fungal proteinases possess potent allergic disease adjuvant activity (Figures 1 and 2) (²⁷). However, further analyses indicated that similar enzymes present in house dust likely did not exist in quantities sufficient to elicit allergic disease directly by inhalation (²⁸). We, therefore, considered alternative means by which fungi could be linked to allergic lung disease through their secreted proteinases. Many species of *Aspergillus* have previously been shown to infect the human respiratory tract and cause invasive disease, but invasive disease caused by *A. niger* is an exceedingly rare event, almost always observed in the setting of severe immune compromise (^{30, 31}). As many as 32% of immunologically normal asthma subjects demonstrate serum or skin prick test-based reactivity to fungal conidia antigens (³²). Strikingly, however, fungal-specific IgE may be detected in up to 66% of children with asthma or allergies (³³). These studies indicate that sensitization to fungi is both common and widespread in human societies. However, such sensitization is currently perceived to reflect hypersensitivity to immunogenic, but not necessarily infectious, fungal antigens that are ubiquitous in human environments (³⁴).

To distinguish between the twin possibilities of fungal infection or mere hypersensitivity to fungal antigens and the role that secreted proteinases may play in either process, we next developed an infectious model of allergic lung disease using mice. For this model, mice were challenged intranasally with the conidia of an *A. niger* isolate obtained from a house dust sample. These studies showed that a minimum dose of 50×10^3 conidia given daily was necessary to induce the complete spectrum of allergic lung disease (allergic airway inflammation and airway obstruction), although even much smaller doses were sufficient to induce significant allergic inflammation (Figure 1) (²⁸). Moreover, although conidia sterilized by irradiation induced substantial eosinophilia and airway mucus secretion, they were unable to elicit highly polarized lung IL-4 responses or airway hyperresponsiveness, even if given in very high doses (²⁸), producing instead a lung phenotype more

closely resembling the distinct lung syndrome of hypersensitivity pneumonitis and not asthma ⁽³⁵⁾.

These studies, therefore, showed that the conidia of *A. niger* produced a true infection that was required to elicit the full spectrum of allergic lung disease. To determine if proteinases secreted by *A. niger* were required for disease expression, we next challenged mice with the conidia of genetic mutants of *A. niger* lacking secreted proteinases ⁽³⁶⁾. Lack of one or more proteinases did not affect the ability of *A. niger* to grow in the mouse airway, as equal or greater numbers of organisms were recovered from lung tissue after two weeks of challenge with conidia, irrespective of the fungus' ability to secrete proteinases ⁽²⁸⁾. However, proteinase-deficient *A. niger* mutants were severely impaired in their ability to elicit allergic lung disease, as they were unable to elicit airway hyperreactivity, pronounced airway eosinophilia or highly polarized lung IL-4 responses (Figure 3) ⁽²⁸⁾. Thus, these studies confirm that secreted fungal proteinases are essential for eliciting allergic lung disease in the setting of active airway infection due to a common household fungus.

Finally, we considered the possibility that the asthma-like response to active fungal infection was protective and not maladaptive, as asthma is typically perceived ⁽³⁴⁾. We, therefore, tested the hypothesis that eosinophils are fungicidal and protective against airway fungal infection. We incubated eosinophils isolated from bronchoalveolar lavage fluid of proteinase challenged mice together with the conidia of *A. niger* in various ratios and determined the degree to which fungal growth was suppressed after 48 hours of culture. These studies demonstrated that eosinophils were able to completely suppress fungal growth at an eosinophil:conidia ratio of 100:1, but exhibited noticeable anti-fungal activity at a ratio as low as 13:1 ⁽²⁸⁾. Eosinophils, therefore, exhibit potent anti-fungal activity in vitro and may protect against airway infection with *A. niger* and other fungi.

DISCUSSION

Asthma is defined uniquely by the highly Th2-polarized nature of the immune response that underlies the airway obstruction and dyspnea that are its major clinical features. A major challenge in unraveling the pathophysiology of asthma has been to identify the molecular characteristics of antigens that lead to robust Th2 responses following airway exposure. Our investigations have led to the discovery of secreted fungal proteinases as major adjuvant factors uniquely capable of inducing the signature Th2 responses that are required for disease

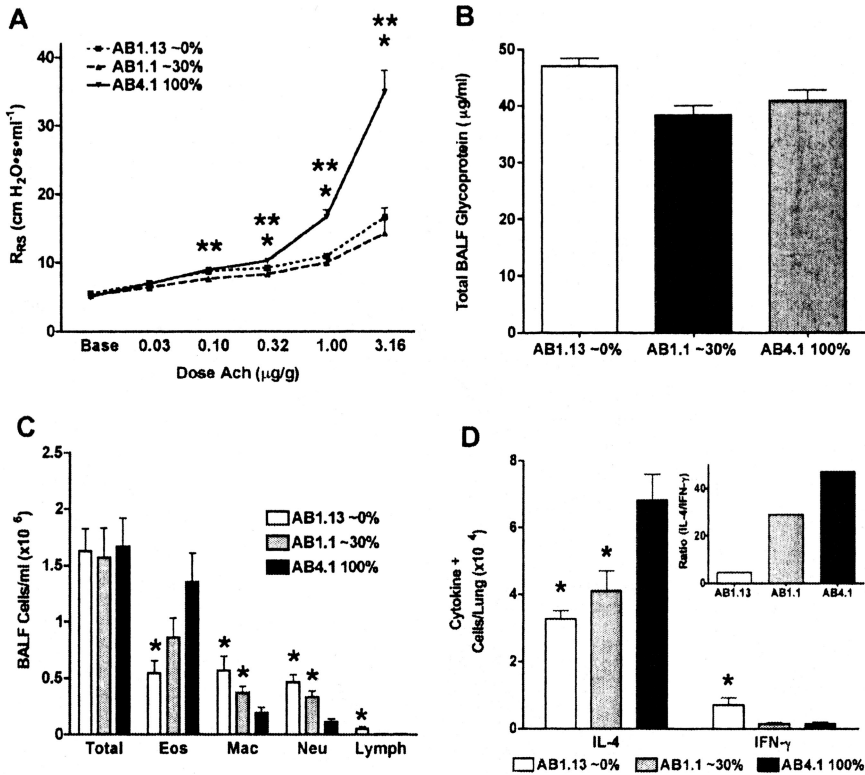


FIG. 3. Proteinase production is required for *A. niger*-dependent allergic lung disease. Mice received intranasally 400×10^3 *A. niger* conidia derived from wild type (AB4.1), aspergillopepsin I-deficient (AB1.1) or multiple proteinase-deficient (AB1.13) strains every other day for a total of 8 challenges. Data were collected 24hr after the final challenge. Airway responsiveness as assessed by increases in respiratory system resistance (R_{RS}) following serial intravenous acetylcholine challenge (A), total secreted BAL fluid (BALF) glycoproteins (B), total BAL fluid inflammatory cells (C) and total lung IL-4 and IFN- γ -secreting cells as assessed from whole lung (D; inset: IL-4/IFN- γ ratio) were determined. Data are from one of two comparable experiments with $n = 5$ animals each. Percentages refer to the % proteinase activity present in hyphae relative to wild type (AB4.1) *: $P < 0.05$ comparing AB4.1 to AB1.13; **: $P < 0.05$ comparing AB4.1 to AB1.1.

expression. Moreover, we have demonstrated in mice that fungi are capable of infecting the airway and inducing allergic lung disease that is indistinguishable from that induced in mice challenged with proteinase alone. Together with our observation that eosinophils possess potent anti-fungal activity, these findings clarify the likely pathogenesis of allergic lung diseases and suggest the possibility that for many subjects, asthma may be caused by a low-grade fungal infection.

For a fungal infectious etiology of asthma to be credible, many

criteria must be satisfied. Because asthma is a very common condition, the infecting organism must be common if not ubiquitous in human environments, as *A. niger* and similar fungi are. Moreover, the organism must be readily capable of infecting the lower respiratory tract, as we have shown to be true for *A. niger* using an experimental mouse model. However, an additional requirement for human infectivity is that viable fungi must have ready access to the lower respiratory tract. In this regard, fungi are, relative to other allergenic organisms, uniquely suited. Conidia, the reproductive structures of many fungi, are produced in abundance especially during times of nutrient deprivation and are resistant to the sterilizing effects of desiccation, temperature extremes, ultraviolet radiation and scavenging microbes. Conidia, therefore, persist and accumulate in stable and relatively protected human environments in a viable form. Equally important, conidia are readily aerosolized by even minor disturbances and possess an optimal aerodynamic size to persist as aerosolized particles and travel deep into the lungs.

Particle deposition in the airway is determined by many factors, but particularly by size, which for airborne particles is measured in terms of the mass median aerodynamic diameter (MMAD). Inhaled larger particles (MMAD > 20 microns) tend to be deposited in the upper airways (nose, sinuses and trachea) whereas smaller particles are deposited in progressively more distal airways down to an extremely small size (MMAD < 0.1 micron) at which most particles are inhaled deep into the lung and never deposited, but simply exhaled. The conidia of *A. niger* are of precisely the ideal MMAD (3–5 microns) to ensure that when inhaled they travel to and are deposited largely in the most distal airways⁽³⁷⁾. For many reasons, therefore, the fungal conidia of *A. niger*, but also other fungi, are ideally suited to deposition in and induction of infection of the human lower respiratory tract.

In addition to the evidence presented here, proof that ubiquitous fungi are responsible for asthma in at least a subset of patients will require additional detailed analyses of asthma patients themselves⁽³⁸⁾. The usual criteria for establishing an infectious etiology for human disease, which include the repeated demonstration of the organism in involved tissues, is extremely difficult in asthma as the fungal infectious burden is expected to be extremely low. Sampling of deep airways using bronchoscopy, a technique useful for acquiring microbiology samples free of contaminating upper airway secretions, is invasive and potentially hazardous. Intensive analysis of induced sputum might overcome the hazard-related limitations of bronchoscopy. However,

demonstration of fungi from respiratory samples irrespective of the technique used is still not sufficient for proving infection.

Respiratory tract cultures frequently reveal the presence of common fungi, even when obtained from subjects with normal immune function. As such, culture results are often unexpected and of often no clinical significance; they are typically interpreted as representing environmental contamination, a conclusion that is reinforced by the ubiquitous nature of fungi (³⁹). Conversely, even in the setting of known invasive respiratory tract fungal disease, respiratory cultures frequently fail to reveal the pathogen (³⁹). Our studies suggest that, in contrast, such positive fungal cultures are in many cases not the result of contamination, but are indicative of true, low-grade airway infection. Moreover we propose that, although such infections may, indeed, be inconsequential for many subjects, depending on the actual fungal burden in combination with as yet unidentified genetic predisposing factors, such infection could lead directly to symptomatic allergic respiratory tract disease.

Given the unique difficulties of linking airway fungi to disease, a fully convincing demonstration that airway fungi participate etiologically in respiratory tract allergic disease will require unique approaches, most likely used in combination. In addition to revealing fungi from respiratory tract specimens, disease palliation following the institution of antifungal therapy would constitute important supporting evidence of infection (^{40, 41}). Such results would further need to be combined with quantitative immunological evidence of fungal infection that modulates in response to antifungal therapy. Further studies are needed especially to develop suitable immunodiagnostic methods that reliably reveal and quantitate the degree of airway fungal infection, especially with very low infectious burdens.

Fungi are ancient, predominantly terrestrial organisms, predating the first appearance of land animals by hundreds of millions of years. Consequently, in order to evolve, mammals have had to develop sophisticated mechanisms to cope with a world literally filled with fungi. As often the first point of contact with aerosolized conidia, the respiratory tract especially has had to develop sophisticated means of defense against potentially invasive fungi. Thus, rather than an aberrant immune response, our data indicate that asthma is in many cases a specific response against fungal airway infection that limits the organism to the epithelial surface and prevents potentially lethal invasion into the lung parenchyma. Current asthma therapy is centered on broad-based immunosuppression using topical or systemic corticosteroids. This approach is clearly effective at inhibiting the

inflammation that leads to airway obstruction, but may simultaneously lead to fungal overgrowth and persistent infection-masking without eradicating the ultimate cause of disease. Clarification of the relationship between common environmental fungi and asthma is, therefore, essential for designing more effective preventative and therapeutic approaches for asthma and related allergic lung diseases.

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DISCUSSION

Bray, Houston: Are there colonized people with a certain genetic predisposition or can you comment on the gene environment story?

Corry, Houston: Excellent question and very appropriate. Of course gene environment interactions are absolutely important here. If you think about it, what I'm saying is true. If a lot of asthma is infectious, and obviously we have to prove that, but clearly we have shown that these fungi are prevalent, and yet only about six to 10% of kids in the Houston area anyway get asthma. So there must be gene environment issues that dictate how susceptible one is to fungal colonization infection and the development of the full spectrum of the allergic lung disease that ultimately leads to asthma. This sort of work opens up new vistas for looking at new genes to consider, including those involved in fungal host defense.

Johnson, Ponte Vedra: Excellent paper. I enjoyed it. I wonder if you would comment on the similarity or dissimilarity with the so called organic pneumoconioses due to organisms apparently related to thermophilic actinomycetes in farmers in the group in which the reaction appears to be much more of a granulomatous pneumonitis.

Corry, Houston: Another excellent question. I can comment on that. We have tried to establish a model of hypersensitivity pneumonitis, which is the disease that you are referring to. It turns out the whole reason we got into fungi was precisely for this issue. We were trying to develop a model of hypersensitivity pneumonitis. We chose *Trichosporon asahii*, which is the major cause of summer-type hypersensitivity pneumonitis in Southeast Asia. It turns out, when we gave the spores of this organism, all we got was asthma, and we couldn't understand why. Digging a little bit deeper, it turns out that if you take any of these fungi or if you just take the proteinase to induce allergic lung disease, simultaneously you add extracts of the cell walls, for example of the thermo-

phylic actinomyces as you referred to, then you get the world's best model of hypersensitivity pneumonitis. Quite honestly, I think that HP is a combination of organisms or parts of organisms, including most importantly fungi but mixed with some of the cell wall products of bacteria or bacteria-like organisms. That's a complicated answer but, hopefully, begins to address your question.

Hochberg, Baltimore: So, since inhaled glucocorticoids are now the mainstay of therapy for asthma and a lot of these allergic lung disease and work as anti-inflammatory agents, what are the effects of inhaled glucocorticoids on the growth of these fungal spores?

Corry, Houston: Fantastic question! We are setting up those studies now in this model, now that we have an infectious model that we can play with. Our guess is that as corticosteroids are known to do in other infectious processes, it is probably going to inhibit clearance. So ultimately, yes, corticosteroids do help in asthma. They do reduce the inflammation that ultimately leads to the airway's obstruction, but probably what is going to happen in the long run is you are going to be harmed, because you are going to be decreasing the clearance of this organism; and so again, in the future, it may be appropriate to use corticosteroids if we prove our point, that this is infectious at least in many cases, due to fungi; but you are going to have to add anti-fungals to those corticosteroids.

Berl, Denver: In Denver there is lack of interest in asthma. So this trickles down to me. Have you looked at other coexisting infections? There is a group at National Jewish that has been very interested in other infectious, viral and such as triggers, that perhaps interact with your fungi and select the people who do or don't develop asthma—besides the genetic factors. Do you look for other infectious agents?

Corry, Houston: Indirectly. Again, that is a great question. So we don't think of proteinases as being linked to viruses but, in fact, there is a very important link, and rhinovirus, in particular, is the most common association between exacerbations of childhood asthma compared to all other possible triggers. It turns out that 30% of all viral proteins that are made during an active upper respiratory tract infection are proteinases. We have a collaboration with Jim Gern in Wisconsin. He is shipping to us those recombinant proteinases from rhinoviruses. If you actually give those recombinant rhinovirus proteinases, you get a fantastic model of asthma. One possible link between rhinoviruses and asthma may be that those proteinases trickle down into the lower airway to help provoke ongoing allergic disease.

Billings, Baton Rouge: Why is it that the chronic exposure to an allergen like cat dander or dog dander or these infections that you are eluding to do not result in a desensitization the same way as taking allergy shots do? Why is it that with the chronic exposure to what clearly doesn't go away in these households, why doesn't their asthma and their immune situation go away?

Corry, Houston: Great question! There is a lot of insight now into the immunological mechanisms that mediate desensitization or the emergence of tolerance to these chronic things in the environment that we are always being exposed to. One thing that has emerged from that is that tolerance will only emerge in the absence of additional adjuvant factors, danger signals if you will, that promote the ongoing inflammatory component. So what we're positing, and it is possible to test in this model, is that ongoing IgE responses, TH2 responses, etc. to cat danders, is because there is ongoing exposure to proteinases, particularly those that are fungal derived. So we would posit that the ongoing *in situ* proteinase production is going to abrogate any tolerogenic response that might come along, but again, if you get rid of fungi by giving anti-fungals or whatever, you will get rid of that pro-inflammatory stimulus, and hopefully, you would be able to lead to tolerance.

Baum, New York: Along the lines of other potential pathogens, there seems to be increasing evidence that *Mycoplasma pneumoniae* has some predisposing activity in causing asthma. Any comment on that as an agent?

Corry, Houston: Yes, thank you. Mycoplasma, Chlamydia, very small bacteria have been linked in some studies to asthma. We have not studied them. So I can't really comment on how they might fit in this model, but clearly worthwhile for further study.