

Seeking common pathophysiology in asthma, atopy and sinusitis

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Asthma and chronic sinusitis are inexplicably common airway diseases that are linked to atopy and allergic inflammation. T helper type 2 (Th2) cells and the associated cytokines are believed to play crucial pathogenic roles in asthma, but the environmental factors that instigate allergic airway disease remain poorly understood. Environmental proteinases are highly allergenic and are candidate inducers of airway Th2 responses. Determining the proteinases and their sources that are relevant to airway disease, however, remains challenging. In this Opinion, we summarize the evidence that implicates fungi as both a relevant source of allergenic proteinases and a potential cause of asthma, atopy and chronic sinusitis through airway infection. Clarification of the extrinsic causes of these processes will markedly improve diagnosis, prognosis and therapy.

Allergic inflammation in asthma: aberrant or appropriate?

Allergic asthma and related respiratory tract allergic disorders such as chronic sinusitis are among the commonest of all human medical conditions and afflict up to 10% of adults in the USA. These disorders cause facial pain, breathing difficulty, cough and, in the severest forms of asthma, death owing to profound airway obstruction. Asthma and chronic sinusitis are associated with diverse inflammatory markers including eosinophilia, immunoglobulin E (IgE) and lung T helper type 2 (Th2) cells that secrete the signature cytokines interleukin(IL)-4, IL-5 and IL-13 [1–4].

Studies conducted in several laboratories have demonstrated that airway obstruction is intimately tied to Th2 cells, the cytokines IL-4 and IL-13, and numerous related effector molecules [5–11]. Two fundamentally distinct but related processes can be conceived for how asthma arises: an environmental exposure and the immune response to that exposure that perpetuates the pathological inflammation. A widely held perception of asthma pathogenesis is that airway allergic responses arise aberrantly following inhalation of putatively benign environmental agents, including fungal antigens. This view powerfully influences clinical practice in asthma in which the principle objective

is to reduce allergic inflammation through immunosuppressive medications. In this Opinion, we summarize the growing literature that challenges the prevailing view of asthma as an aberrant inflammatory condition and propose an alternative model in which inflammation is appropriately directed against an insidious fungal infection of either the upper or lower airway. Fungi are known to cause airway allergic diseases, but entities such as allergic bronchopulmonary aspergillosis (ABPA) are currently viewed as clinically distinct from asthma. Our recent analyses of mice experimentally infected with household fungi and of human subjects undergoing surgery for fungal-related sinus disease emphasize why this distinction might be misplaced and how further studies have the potential to radically alter how asthma and sinusitis are diagnosed and treated.

Exogenous causes of asthma: what triggers Th2 responses?

Numerous hypotheses have been put forward to explain the proximate causes of asthma and related disorders, most of which have not survived close scrutiny [12]. Nonetheless, a fruitful approach to understanding the environmental causes of asthma has been to consider cases that have been definitively assigned a specific etiology. Examples of such natural human experiments are rare, but have the potential to be etiologically informative. Perhaps the most interesting of such investigations involved workers in a detergent factory who were exposed to proteinases of bacterial origin, an additive that greatly improves the performance of many cleaning products. Asthma with clear allergic features was documented exclusively in the workers that handled the proteinases and not other chemicals, such as the detergent itself [13–16]. Similar cases of occupational asthma have been reported with workers handling proteinases of plant origin [17].

These reports established that proteinase exposure can cause human asthma, but such cases are confined to industrial settings and are less informative of the exposures that are typical of most asthma subjects. The strongest risk factor for asthma is atopy, which is defined as the exaggerated tendency to mount symptomatic immune reactions (immediate hypersensitivity) against common environmental antigens. Atopy is usually quantified in

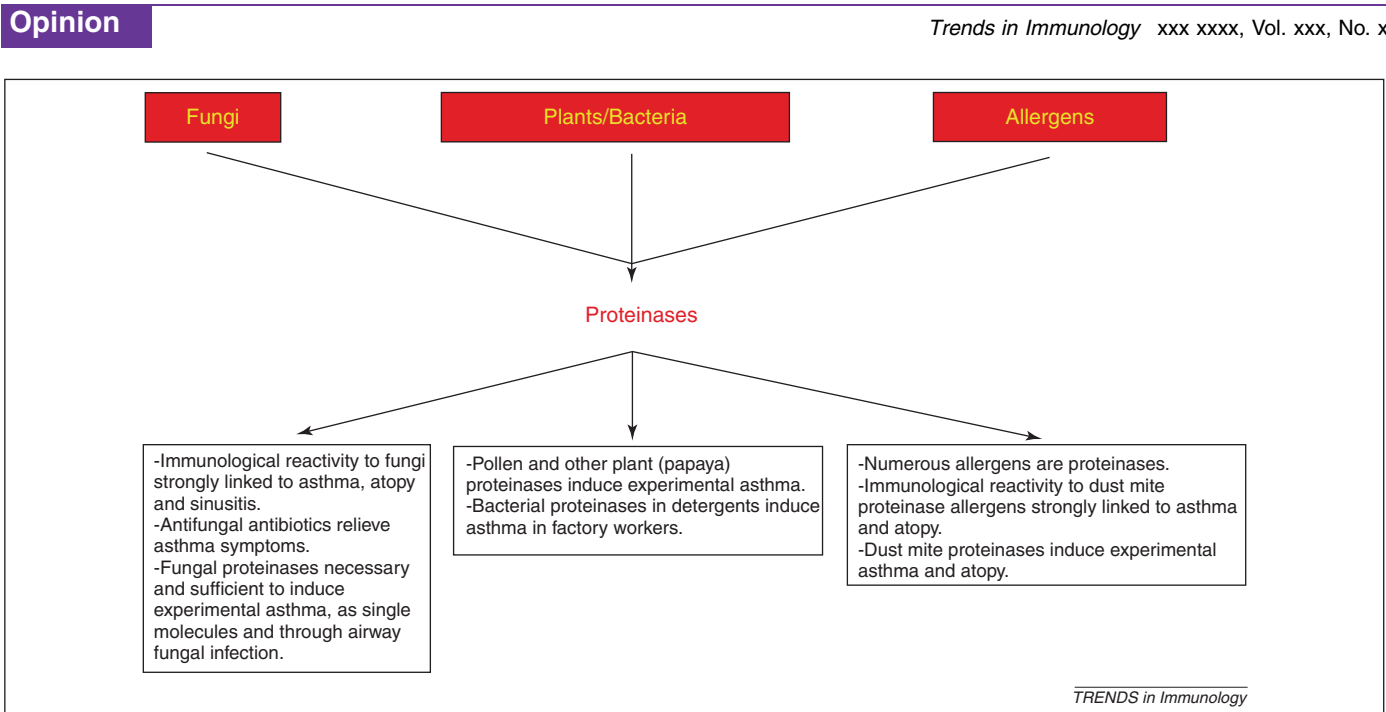


Figure 1. Evidence linking environmental sources of proteinases to allergic airway disease. Virtually all organisms produce proteinases, but three major sources are most likely to be inhaled, releasing proteinase activity in the respiratory tract and eliciting allergic disease. These proteinase sources include filamentous fungi inhaled as spores, seasonally prevalent plant pollens, and perennially present allergens derived from common household organisms, such as dust mites. Some of the experimental and human data linking these proteinase sources and allergic airway disease are summarized in the boxes.

terms of total and antigen-specific serum IgE levels and immediate cutaneous reactions to panels of antigens (commonly called allergen skin testing) [18]. A surprising feature of the many human allergens that have been defined structurally and biochemically is that proteinase activity is a common feature (Figure 1). For example, the allergen most commonly associated with atopy in asthma is Der p 1, a common dust mite allergen [19], which is a cysteine proteinase that is capable of cleaving numerous host proteins with immune relevance [20–23]. Other proteinases that have been linked to human atopy and allergic disease include the major allergens from cat dander (Fel d 1), honeybee venom (Api m 7) and numerous other allergens from fungi and dust mites (Table 1; and see SDAP All Allergens http://fermi.utmb.edu/cgi-bin/SDAP/sdap_07?dB_Type=0&Code=10).

All organisms produce proteinases, so it is perhaps not surprising that many allergens are proteolytically active, an association that therefore might arise merely by chance. However, the organisms that have been most strongly linked to asthma-like diseases and atopy (fungi, pollens) are unique in that they must secrete highly durable proteinases into their environments and become airborne as a

matter of survival. Thus, abundant circumstantial evidence associates exogenous proteinases with human allergic disease and suggests that at least some organismal proteinases are intrinsically allergenic (Figure 1). However, in order to prove the allergenic nature of proteinases, we must turn again to experimental models.

Distinct mechanisms for Th2 cell generation *in vivo*

The apparent requirement for proteolytic activity in some environmental allergens must be reconciled with the well-known ability of chicken egg ovalbumin, which possesses no biochemical activity, to induce allergic disease in rodents. Many non-proteolytic human allergens are also known. Local lung immune mechanisms consisting of immunosuppressive dendritic cells that preferentially induce regulatory T cells (Tregs) powerfully suppress effector immune responses against such innocuous antigens in the absence of immunization [24,25]. Experimental allergic lung disease caused by exposure to ovalbumin can be induced only if mice are initially immunized against this protein through a route that is remote from the lung, such as peritoneum or skin, before lung delivery is able to elicit allergic inflammation [26]. It is possible that sensitization

Table 1. Exemplary proteolytically active allergens.

Allergen	Source	Allergen	Source
Api m 7	<i>Apis mellifera</i> (honeybee) venom	Der m 1	<i>Dermatophagoides 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>Aspergillus fumigatus</i> (fungus)	Pen b 13	<i>Penicillium citrinum</i> (fungus)
Asp f 18	<i>Aspergillus fumigatus</i> (fungus)	Pen c 13	<i>Penicillium brevicompactum</i> (fungus)
Asp fl 13	<i>Aspergillus flavus</i> (fungus)	Pen c 2	<i>Penicillium citrinum</i> (fungus)
Asp n 18	<i>Aspergillus niger</i> (fungus)	Pen ch 13	<i>Penicillium chrysogenum</i> (fungus)
Asp o 13	<i>Aspergillus oryzae</i> (fungus)	Pen ch 18	<i>Penicillium chrysogenum</i> (fungus)
Der f 2	<i>Dermatophagoides farinae</i> (dust mite)	Pen o 18	<i>Penicillium oxalicum</i> (fungus)
Der p 1	<i>Dermatophagoides pteronyssinus</i> (dust mite)	Tri t 4	<i>Trichophyton tonsurans</i> (fungus)

to non-enzymatically active antigens implicated in atopy and asthma occurs through routes remote from the lung; i.e. through skin and gut. However, a more likely possibility is that some allergens contain an adjuvant substance that is perceived by the airway immune system as a danger signal and overcomes normally tolerogenic mechanisms to induce inflammatory responses [27].

The molecular basis for immune adjuvants is now well understood, at least for Th1 and Th17 responses, and involves recognition of structural features of microbial cell wall components, nucleic acids and some proteins, termed pathogen-associated molecular patterns (PAMPs), by Toll-like receptors (TLRs) and related immune receptors [28–33]. There is evidence that TLRs participate in experimental Th2 and allergic responses as well as asthma under some conditions [34–37], whereas other studies refute the requirement of TLRs for allergic disease [38–41]. By contrast, our laboratory demonstrated that allergic lung disease and lung Th2 responses can be induced by proteinases of fungal and plant origin acting as Th2 adjuvant factors [42]. This observation, subsequently confirmed and extended to proteinases from other plants, animals and microbes [43–48], is important because it experimentally validates the circumstantial link between proteinases of diverse sources and asthma (Figure 1). Moreover, in contrast to ovalbumin, proteinases need only be delivered through the respiratory tract to induce allergic disease and thus are presumably more physiologically relevant [42]. The adjuvant activity of proteinases lies in their enzymatic activity, not the proteinase itself, thus allergenic proteinases are manifestly not PAMPs. Nonetheless, a link between allergenic proteinases and TLRs remains possible and deserves further study.

Common fungi induce allergic lung disease through active infection and secretion of proteinases

A major challenge of mucosal immunology research is to understand how agents as diverse as ovalbumin, proteinases and TLR ligands relate to Th2 cell-dependent human diseases such as asthma and to other important disease features such as atopy. We began this undertaking by first determining that proteinase activity can be found in the dust of virtually all homes in the Houston area and that fungi were a major source of the proteinases [43]. Although we have identified more than 25 distinct fungal species from these samples, by far the most commonly isolated organisms were *Aspergillus* spp. Surprisingly, although Der p 1 protein could be detected in the majority of households by immunoassay, its enzymatic activity could not be detected by zymography, leaving fungi as the major, if not sole, source of active proteinases in most house dust samples [43].

To understand the relationship between fungi and their secreted proteinases to allergic disease, we challenged mice with relatively low doses (2000–400,000 conidia/day) of the *Aspergillus niger* conidia to determine if this organism could grow in the airway and induce allergic lung disease. In a dose-dependent manner, the viable conidia of *A. niger* readily induced many salient aspects of asthma-like disease, including airway hyperreactivity, goblet cell metaplasia, highly polarized lung Th2 responses and

serum fungus-specific antibodies. Active fungal infection was the underlying cause of the allergic lung phenotype because these responses were all either attenuated or abrogated in mice that received irradiated conidia that were incapable of producing hyphae [49–51]. Further, we showed that the conidia convert to filamentous hyphae in the airway, proving that the fungi were actively growing in the airway. Thus, *A. niger* readily infects the mouse airway and induces a disease phenotype closely resembling allergic asthma.

Fungi secrete or contain numerous potentially allergenic substances in addition to proteinases, including chitin [52], β -glucans [53,54] and many proteins linked to atopy. To specifically address the importance of proteinases, we challenged mice with induced mutant strains of *A. niger* in which either the aspergillopepsin I gene was specifically deleted or all detectable proteinase activity was deleted [55]. These proteinase mutants were capable of growing well *in vitro* and in the mouse airway *in vivo* and the aspergillopepsin I mutant was more difficult to clear from the lungs of mice relative to animals infected with wild type strains. More importantly, neither mutant *A. niger* strain was capable of inducing robust allergic lung disease in mice. These studies are remarkable because they demonstrate that proteinases are necessary for allergic lung disease in response to airway infection with a fungus that possesses many distinct allergy-inducing factors. Combined with our earlier finding that proteinases alone are sufficient for the induction of experimental asthma, the observations indicate that fungal proteinases secreted during active airway infection are crucial factors in allergic disease pathogenesis [42,43].

Additional studies are required to determine what are the essential host genes that recognize the presence of foreign proteinase and trigger allergic responses. Two general models by which fungal proteinases might interact with host proteins are depicted in Figure 2. Cell-associated proteins that might be targeted directly or indirectly by proteinases potentially include tight junctions of airway epithelial cells [20] and various immune receptors, including CD23 and CD25 [56], proteinase-activated receptor 2 (PAR2) [57], and the C3a anaphylatoxin receptor [58]. One soluble factor that has been implicated as a proteinase substrate in allergic disease is complement protein 3 (C3) [59]. Moreover, our recent discovery that matrix metalloproteinase 7 (MMP7) cleaves and activates IL-25 to facilitate induction of allergic lung disease suggests that exogenous proteinases might activate IL-25 or conversely deactivate Th1-inducing cytokines (e.g. IL-12 [60]) to induce allergic disease [61].

Experimental atopy, human chronic sinusitis and antimicrobial clinical trials further connect asthma to fungal infection

Additional studies from humans and mice provide further evidence for a link between fungi and asthma. Atopy is currently believed to represent a primary, genetically controlled hypersensitivity to antigens that predisposes to secondary immune-related disorders such as asthma [18,62–64]. However, we showed recently that atopy can, like allergic lung disease, be secondary to a primary fungal

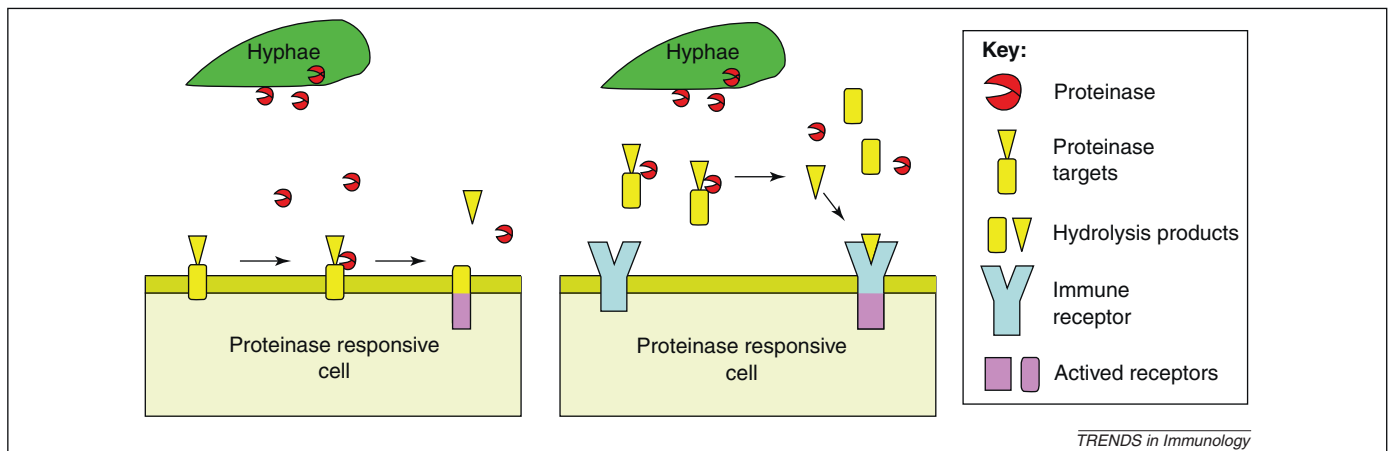


Figure 2. Models for fungal proteinase-induced activation of allergic airway inflammation. Left, fungal proteinase cleaves a cell-associated target, thereby activating or modifying intracellular signaling pathways to favor Th2 responses. Right, proteinase cleaves a soluble target to release a ligand capable of activating an immune receptor controlling Th2 responses. Although depicted as membrane bound, this putative receptor could equally be intracellular or soluble. Candidate cell-associated proteins pertinent to this model include tight junctions of airway epithelial cells, CD23, CD25, proteinase activated receptor 2 (PAR2) and the C3a anaphylatoxin receptor. Soluble factors potentially include complement protein 3 (C3) and IL-25 (see the text).

airway infection [43]. Ovalbumin alone behaves as a typical innocuous antigen and fails to elicit inflammation when delivered to the respiratory tract; however, atopy to ovalbumin is induced when it is combined with *A. niger* conidia. Strangely, although Th2 responses to low-dose *A. niger* exposures were seen in these studies, atopy to the fungus was not detectable, indicating that antibody responses to fungi are an inefficient means of revealing active infection. The combined immune response to both low-dose *A. niger* infection and ovalbumin was, however, sufficient to induce the full spectrum of allergic lung disease, whereas either antigen administered alone was insufficient. Thus, the combination of a bystander antigen such as ovalbumin and viable conidia is synergistic and establishes the possibility that both atopy and asthma could represent secondary manifestations of the same primary fungal airway infection (Figure 3).

In addition to atopy, asthma is linked to upper respiratory tract inflammatory disorders such as rhinitis and chronic rhinosinusitis (CRS) [65–69]. CRS exists in several clinical variants, including CRS with and without nasal polyposis and, when associated with biopsy-proven fungi and fungal atopy, is termed allergic fungal rhinosinusitis (AFRS). In addition to the mucosal inflammation, mucopurulent sinus drainage, nasal obstruction and facial pain that are typical of this syndrome, most (> 60%) CRS patients are highly atopic and exhibit immunologic features that are characteristic of AFRS, including nasal eosinophilia and Th2 cell polarization [70–72]. Moreover, using specialized methods, virtually all CRS patients can be shown to have fungal culture-positive nasal secretions or possess hyphae in sinus specimens even in the absence of fungal atopy [70], a finding with remarkable parallel with our inability to demonstrate fungal atopy to low-grade

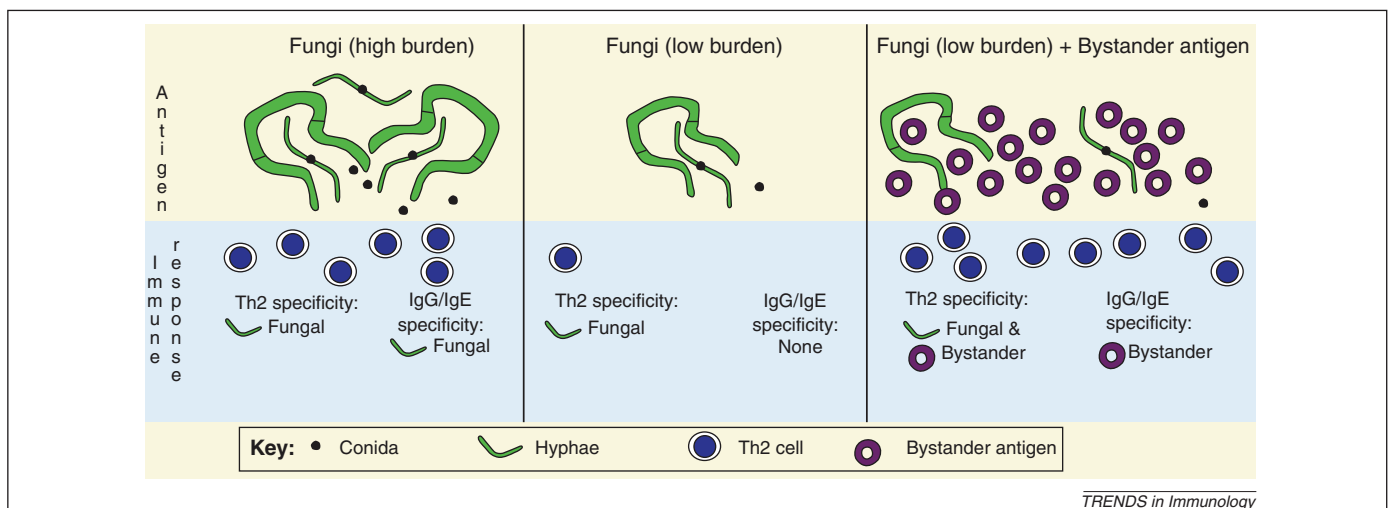


Figure 3. Paradoxical immune responses during fungal airway infection. As assessed in mouse studies, the nature of airway immune responses to inhaled antigens is strongly dependent on concomitant airway fungal infection. Left-hand panel: high airway fungal burdens induce robust fungal-specific Th2 and atopic (IgG or IgE) responses. Middle panel: low-grade fungal burdens induce only Th2 responses; atopic responses are not detectable. Right-hand panel: inert bystander antigens if inhaled alone induce no inflammation or detectable immune response (not shown). By contrast, low-grade fungal airway infection induces specific Th2 responses against the bystander antigen, but not the fungus itself. This example, which is most representative of antigen encounters likely to be seen by humans, potentially explains the lack of fungal atopy in many asthma and CRS patients.

fungal lung infection in mice (Figure 3). Thus, analyses of mice and humans with respiratory tract allergic disease suggest that the degree of fungal-related disease, including atopy, is strongly related to the fungal burden. In addition to simply correlating fungal isolation with allergic disease, future clinical studies are likely to benefit from quantifying the degree of fungal infection, although the techniques required for this remain at the experimental stage [73].

Despite the strong association between the growth of fungi in the upper respiratory tract, allergic inflammation and CRS, many investigators view the link to fungi in terms of mucosal colonization and not true infection [74]. However, the fungi are present in close association with profoundly abnormal, highly inflamed sinus tissue that causes airway obstruction. Moreover, unlike healthy control subjects that are largely non-reactive, the majority of AFRS patients manifest fungal antigen-specific Th2 responses when peripheral blood cells are stimulated with fungal antigens [75]. Together, we interpret these findings as indicative of true fungal infection and not mucosal colonization. These observations further suggest that peripheral blood T cell-based immunodiagnosis might prove useful for the future diagnosis of fungal-related allergic airway disease.

A final clue suggesting fungal infection in asthma and CRS is the salutary effect of anti-fungal therapy on asthma severity. Two randomized clinical trials performed on fungus-sensitized asthmatic subjects have demonstrated that standard antibiotics (fluconazole, itraconazole) ameliorate disease overall and, for itraconazole, radically improve the quality of life of more than 50% of patients with severe disease [76,77]. These and related studies [78,79] support the concept that active fungal infection, in part, drives the expression of asthma and related respiratory tract allergic diseases.

Concluding remarks

The heterogeneity in clinical expression for which asthma is notorious suggests to many investigators that asthma is not a specific disease, but rather a syndrome consisting of etiologically distinct entities with shared clinical features [80]. Viewed from an immune perspective, however, even clinically diverse asthma cases demonstrate a far more homogeneous nature in which Th2 cell-driven allergic inflammation is a consistent finding [18,81]. Experimental studies further validate that diverse antigens administered to the respiratory tract of widely divergent species and animal strains provoke a stereotypical immune and physiological reaction that strongly resembles allergic asthma. Thus, a substantially similar pathophysiology nonetheless appears to underlie the clinical heterogeneity of asthma and CRS, giving hope that relatively few, highly specific therapies might be developed that far exceed the utility of current treatment approaches.

For such a fortuitous outcome to be realized, a much greater understanding of the exogenous factors that initiate allergic airway inflammation is required. We have reviewed the growing literature that indicates a prominent role for common environmental fungi in the pathophysiology of both upper and lower respiratory tract allergic diseases. Our research has further uncovered the essential

molecular basis by which fungi elicit allergic inflammation through secreted proteinases. Despite these insights, asthma and chronic sinusitis remain strongly tied to atopy and the knowledge that many non-fungal environmental antigens are likely to play prominent roles in disease expression. Here again, however, the discovery that primary fungal airway infection can lead to atopic responses against bystander antigens provides a remarkably parsimonious explanation for these phenomena.

We have emphasized here the role of fungal proteinases during airway infection, but viruses such as human rhinovirus are also linked to both asthma and sinusitis and proteinases of this organism can elicit allergic lung disease [44]. Thus, in addition to delineating the molecular basis by which diverse proteinases elicit allergic inflammation, future studies should focus on confirming whether active airway infection by fungi and viruses in fact underlies concomitant respiratory tract allergic disease.

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