

Fungal Allergens

Viswanath P. Kurup, PhD

Address

Medical College of Wisconsin, VA Medical Center, Research Service
151-I, 5000 West National Avenue, Milwaukee, WI 53295, USA.
E-mail: vkurup@mcw.edu

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Many fungi are capable of causing IgE-mediated hypersensitivity in humans. However, the most predominant fungi implicated in allergy belong to the genera *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium*. Pure and relevant allergens are essential for diagnosis as well as for understanding the immunopathogenesis of the disease. Until recently, pure and standardizable antigens from fungi were not available. In recent years, many recombinant allergens have been produced by molecular cloning. Using these allergens, novel methods are being developed to improve diagnosis of mold-induced allergy. By understanding the immunopathogenesis of allergens, new avenues might open up leading to improved patient care, including immunotherapy and vaccination. This review covers the current status of fungal allergens, their role in reliable immunodiagnosis, and their probable use in immunotherapy and vaccination.

Introduction

Fungi have interminably affected humans in various ways, including plant and animal diseases, and superficial and systemic mycoses in humans. Fungi are also involved in many hypersensitivity diseases and toxin-induced health hazards in humans. Diseases associated with inhalation of fungal spores include toxic pneumonia, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, and kidney failure.

Allergy is one of the most common ailments, affecting more than 20% of the population of the industrialized world. Allergy develops upon sensitization with extraneous proteins from various sources [1-3]. The allergens constitute foreign proteins from animals and plant sources. Exposure to allergens results in the induction of IgE antibody, which is thought to be the central player in the induction of allergy. The exposure to allergens is by inhalation, contact, ingestion, or injection. The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis [4-6]. Clinically, the presenting symptoms are sneezing, nasal discharge, coughing, wheezing, and shortness of breath, with evidence of reversible airway obstruction, urticaria, angioedema, and anaphylaxis [7].

Mold allergy constitutes 25% to 30% of all allergic asthma cases [8,9]. Patients sensitized to the allergens demonstrate immunologic and inflammatory responses. The immunologic responses include both humoral and cell-mediated responses. Lung inflammation resulting from antigen exposure leads to airway remodeling and hyper-reactivity. The mechanism involved in this disease is complex and is frequently dependent on the nature of allergens, underlying diseases, concentration of allergens, duration of exposure, and genetic predisposition of the host.

Fungi and Their Allergens

Fungi are eukaryotic, nonchlorophyllous, unicellular to multicellular organisms that are present in all types of environments. Although most fungi are saprophytic, a few of them are parasitic in humans and animals. The concentration of fungal spores in the environment depends on many factors, including climate and vegetation [10]. The types of fungi present indoors and their prevalence depends on moisture, ventilation, presence or absence of carpets, pets, and houseplants. The fungal spore count indoors and outdoors varies considerably depending on these factors [11]. The indoor fungi constitute transient spores from outdoor air and those growing indoors. *Alternaria* and *Drechslera* are major outdoor molds, but have been reported in some exclusive indoor environments. Similarly, *Aspergillus* and *Penicillium* are found indoors and outdoors, albeit less commonly reported outdoors. Other fungi, such as *Cladosporium*, *Epicoccium*, and *Fusarium*, have been reported in both indoor and outdoor environments. Aerobiologic studies of indoor and outdoor fungal flora are necessary for evaluating the allergic status of patients. The role of fungi in allergy has been established based on clinical histories of patients and in vivo and in vitro diagnostic tests using specific fungal antigens. A number of allergenic fungi have been identified. Those most frequently encountered are listed in Table 1. The most important among these are *Alternaria*, *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium*. The extracts from these fungi have been used in skin testing patients and for demonstrating IgE antibody by in vitro assays [7,12].

Fungal allergens are usually proteins, polysaccharides, or glycoproteins [13]. These molecules, on interaction with the immune system, produce IgE antibody in atopic individuals. More than 300 allergens from plants, animals, and fungi have been reported, and some show cross-reactivity resulting from the chemical nature and sequence similarities they share [14]. Based on the physicochemical char-

Table 1. Fungi associated with IgE-mediated allergy

Zygomycetes	Ascomycetes	Basidiomycetes	Deuteromycetes (fungi imperfecti)	Yeasts	
Mucor	Chaetomium	Agaricus	Acremonium	Helminthosporium	Saccharomyces
Rhizopus	Claviceps	Boletus	Alternaria	Neurospora	Candida
	Daldinia	Calvatia	Aspergillus	Nigrospora	Rhodotorula
	Didymella	Coprinus	Aureobasidium	Paecilomyces	
	Erysiphe	Ganoderma	Botrytis	Penicillium	
	Eurotium	Lentinus	Cephalosporium	Phoma	
	Microsphaera	Merulius	Chrysosporium	Scopulariopsis	
		Pleurotus	Cladosporium	Stachybotrys	
		Psilocybe	Coniosporium	Stemphylium	
		Puccinia	Curvularia	Torula	
		Tilletia	Cylindrocarpon	Trichoderma	
		Urocystis	Drechslera	Trichophyton	
		Ustilago	Epicoccum	Trichothecium	
			Fusarium	Ulocladium	
			Gliocladium	Wallemia	

acteristics, the allergens associated with disease have been classified as: 1) hydrolytic enzymes, such as proteases; 2) nonhydrolytic enzymes, such as enolases; 3) inhibitors, such as trypsin and amylase inhibitors; 4) transport proteins, such as lipocalins; and 5) regulatory proteins, such as heat-shock proteins. In addition, allergens with diverse chemical nature and unknown biochemical function and activities constitute the majority of the allergens.

Recombinant Allergens

The crude extracts from mycelia, spores, and culture filtrates of fungi contain considerable amounts of nonantigenic components, including potent toxins. For effective diagnosis of fungal allergy, well-characterized, pure, and standardized antigens are essential. The complexity and the variability of the fungal allergens have complicated the process of obtaining purified and standardized antigens. Considerable research has been directed to purify relevant antigens from fungi. These include fractionation methods, including size exclusion chromatography, affinity chromatography using monoclonal antibodies, partition chromatography, electrophoresis, isoelectric focusing, and chromatofocusing [15,16]. None of these methods has yielded relevant allergens useful in the diagnosis of mold allergy. However, recently, using molecular biology to clone and express relevant standardizable antigens from mold has gained significant progress [17•]. Currently, many allergens have been cloned, and the proteins expressed. Such allergens are currently being evaluated for standardization. Many techniques have been used to clone complementary DNA (cDNA), encoding allergens and antigens. Approximately 70 such allergens have been reported from various fungi and approved by the International Allergen Nomenclature Committee [14]. Most of them have been expressed by cloned genes from the various fungi, and their amino acid sequences have been ascertained. The T- and B-cell epitopes of some of these allergens have been investigated

and mapped. Currently, cloned allergens are available for immunologic assays from *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, yeast, and a few *Basidiomycetes* [14,17•,18].

The cloning of allergens consists of the isolation of messenger RNA (mRNA) from 72- to 96-hour-old actively growing cultures. The total RNA from the mycelium can be extracted with guanidium isothiocyanate or any of the new commercial preparations available [19,20]. The mRNA can be purified using oligo (dT) cellulose chromatography. The purified mRNA was then reverse transcribed to obtain cDNA [20,21]. Of the several methods available to clone the gene from the cDNA library, the amplification of the desired DNA fragments by polymerase chain reaction (PCR) is the most efficient and frequently used method [22•,23••]. Although this method is faster than other cloning methods, it has the inherent limitation that the sequence of the allergen must be known. Hence, the strategy is to construct an expression library that can be screened using sera-containing, allergen-specific IgE antibody from patients. Libraries constructed on bacteriophage lambda vectors must be used to immobilize the particles to a solid phase before testing the reactivity with the sera. The allergens encoding genes will be processed using lambda coding, and the protein will be expressed. Recently, phage-display libraries were also constructed to aid in the understanding of the immune responses of the allergens expressed [24,25••].

Among the various vectors used for expression of fungal allergens, the pET (pET expression protocol; Novagen, Madison, WI) has been the most favored prokaryotic expression system [22•]. This system utilizes the T7 polymerase-based expression of the proteins in *Escherichia coli*. The expression of the target protein is induced by a T7 RNA polymerase in the bacterial host, *E. coli*. The allergen expressed can be purified using a histidine tag at the N- or C-terminal end of the protein. The expressed allergen can be purified using single-step Ni-agarose affinity chromatography. Most of the allergens from *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium*

have been cloned in high-expression prokaryotic systems to obtain recombinant allergens for in vitro immunoassays and skin tests [26–29]. Most of these allergens showed similar skin-test reactivity and in vitro IgE binding with native allergens. The IgE binding of these allergens is frequently directed to the protein parts of the molecules, and, hence, posttranslational modification of the molecules is not a significant factor in the IgE binding.

For antigens with posttranslational modification, it is possible to express the clones in eukaryotic systems. One of the expression systems that has been used for high-level expression of recombinant proteins is the yeast *Pichia pastoris*. The target gene in *Pichia* is under the control of an alcohol oxidase promoter. Many *Aspergillus fumigatus* proteins and glycoproteins have been expressed using this system [22•]. Recently, a phage display library was developed in which the gene products were displayed on the surface of filamentous phages [24,25••]. Compared with screening of lambda libraries, the screening of cDNA libraries displayed on phage surfaces has several advantages. The phages can multiply in a suitable host, and, hence, can be used to infect *E. coli*, and thereby amplify the phages with affinity to the ligand. Phages with protein having specific binding to the desired ligand can be enriched by successive rounds of phage growth and selection. Such concentrated phages can be used in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot for analysis and evaluation. The pLuo phagemid is a useful cloning vector for phage display library [24,25••]. Numerous *Aspergillus*, *Alternaria*, and *Cladosporium* allergens displayed on phages have been constructed, and their usefulness in IgE recognition has been ascertained [25••].

Allergens from Fungi

Although there are many allergenic molds capable of causing type 1 hypersensitivity, the most frequently involved fungi are *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria*. Significant allergens from these and other fungi have been cloned and the protein expressed. Because these four genera represent the most common fungal allergens, they are considered here in detail.

Aspergillus species

Aspergillus species are saprophytic fungi widely distributed in nature and are associated with a number of human diseases [16,30]. Several recombinant allergens from *A. fumigatus* have been identified and purified from cDNA and phage display libraries of *A. fumigatus* [14]. Most of these proteins showed specific binding to IgE from asthmatic and allergic bronchopulmonary aspergillosis (ABPA) patients. The molecular structures cover a wide range of functional proteins, including toxins, enzymes, heat shock proteins, and several unique proteins lacking homology to any of the known proteins. Asp f 1, a ribotoxin that inhibits protein translation, was found to

be toxic to Epstein-Barr virus (EBV)-transformed and phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) [31,32]. This allergen showed positive skin test reactivity in 80% of ABPA patients and 50% of asthmatic patients [31,32]. However, because of the high toxicity and reactivity with skin test positive asthmatics and some normals, the usefulness of this allergen in diagnosis is questioned. This allergen demonstrated 13 linear epitopes binding to IgE [33]. Asp f 1 also showed Th1- and Th2-specific epitopes when studied in a murine model of allergic aspergillosis [34].

Aspergillus fumigatus major allergen Asp f 2 is a 310 amino acid protein that has a fibrinogen-binding property [35]. The gene encoding Asp f 2 has two introns of 83 and 52 nucleotides and four potential glycosylation sites. There was strong sequence homology shown with Asp nd 1, a fibrinogen-binding protein from *Aspergillus nidulans* [35]. Asp f 2 also showed homology with pH-regulatory proteins from *Candida albicans*. The native protein purified using monoclonal antibody measured 37 kDa, whereas the *E. coli* expressed proteins measuring only 32 to 35 kDa. However, when the protein was expressed in the eukaryotic host, *P. pastoris*, it measured 70 to 75 kDa. Asp f 2 reacted strongly with IgE antibody from ABPA patients. Approximately 87% of ABPA patients from the United States and Switzerland carried IgE antibody to Asp f 2 in their sera, whereas only very few skin test positive asthmatics and normal controls carried the IgE antibody [36••]. The *Pichia*-expressed proteins showed more reactivity with IgG antibody in the sera of patients with ABPA and asthma [22•]. Deletion mutants of Asp f 2 varied in their binding to IgE antibody from ABPA and non-ABPA controls. IgE-binding epitope analysis demonstrated nine linear IgE-binding regions spanning the entire sequence [37]. By selective deletion of amino acids, a few conformational IgE-binding epitopes also have been identified [22•,37]. Recent studies confirm that one of these epitopes also functions as a T-cell epitope [38]. Of the eight cysteine residues in the molecule, the two cysteines at 204 and 267 positions were found to be essential for IgE binding [23••].

Asp f 3, a 18.5-kDa protein with homology to *Candida boidinii* peroxisomal protein showed skin test reactivity and in vitro reactivity with serum IgE of ABPA patients [39]. Recently it was found that this allergen is valuable in the serodiagnosis of ABPA [39,40]. More than 80% of ABPA patients carried IgE to Asp f 3, whereas only a few asthmatics carried IgE [36••]. The epitope and structure analysis indicate that the 10 N-terminal amino acids and 143 to 150 C-terminal amino acids of Asp f 3 are important in binding IgE in the sera of ABPA patients [41•]. Epitope analysis demonstrated seven IgE-binding regions spanning the entire sequence of Asp f 3.

Asp f 4, an intracellular protein allergen with unknown biochemical function, showed strong reactivity with IgE from sera of cystic fibrosis (CF) patients with asthma or ABPA [24,42]. Asp f 5, a metalloproteinase, demonstrated skin test reactivity in more than 70% of allergic asthmatics without

ABPA, whereas Asp f 5 showed reactivity in more than 90% of ABPA patients [24,36••]. Although its diagnostic significance is not clear, Asp f 5 might be used as an indicator for sensitization to *Aspergillus*. Asp f 6, manganese superoxide dismutase (MNSOD), has a strong homology to MNSODs of human, bovine, fruit fly, gum tree, yeast, and *E. coli*. Asp f 6 reacted positively in skin tests and bound to IgE in the sera of ABPA patients. Recently its usefulness as a significant allergen for detecting IgE antibody in the sera of ABPA patients has been reported [36••,40,43].

Asp f 8 has homology with acidic P2 ribosomal proteins isolated from *Alternaria* and *Cladosporium*. This allergen reacted with IgE antibody in the sera of patients and showed skin test reactivity in ABPA patients. Asp f 10, an aspartic protease, showed only low prevalence of IgE binding in ABPA patients. Asp f 11, a cyclophilin that has homology with similar proteins from *Malassezia furfur*, reacted with IgE from 50% of ABPA patients [24]. The heat shock protein Asp f 12 has homologous counterparts in *C. albicans*, *Saccharomyces*, *Trypanosoma*, housefly, mouse, and humans because of the extremely conserved HSP gene [20].

Asp f 16, which has no known biologic function, showed strong binding to IgG from ABPA patients [44]. This antigen showed sequence homology with Asp f 9 and a membrane protein from *Saccharomyces*. Other minor allergens isolated from *A. fumigatus* and related *Aspergillus* spp. also bound to the IgE antibody from ABPA and allergic asthma patients [14]. Several of these *Aspergillus fumigatus* allergens also exhibited high sequence homologies with known, functional proteins and enzymes [24,27,36••]. Alkaline serine proteinases with allergenic properties such as Asp f 13, Asp f 13, and Asp o 13 from *A. fumigatus*, *Aspergillus flavus*, and *Aspergillus oryzae*, respectively, have been reported [45,46]. Similar serine proteinases, Pen b 13, Pen c 13, and Pen ch 13, with sequence homology to *Aspergillus* proteinases, have also been identified from various species of *Penicillium* [47,48•]. Recently, another group of homologous vacuolar serine proteinases from *Aspergillus* and *Penicillium*, Asp f 18, Asp n 18, Pen ch 18, and Pen o 18, with conserved sequence, have been reported [46,49]. *A. flavus* extracts demonstrated IgE-antibody binding in 44% of asthmatic patients studied by immunoblotting [50]. Additionally, a 34-kDa alkaline serine proteinase, Asp f 13, which demonstrates significant IgE antibody binding, was purified, and its enzyme activity was ascertained [50].

Penicillium species

Species belonging to the genus *Penicillium* are prevalent indoor fungi [2]. Inhalation of *Penicillium* spores in quantities comparable with those encountered by natural exposure can induce both immediate and late asthma in sensitive persons [11]. Among more than 100 different *Penicillium* species, *Penicillium citrinum*, together with *Penicillium chrysogenum* (*Penicillium notatum*), *Penicillium oxalicum*, *Penicillium brevicompactum*, and *Penicillium spinulosum*, were the five most frequently recovered species of *Penicillium* in the United States, whereas *P. citrinum* was the most prevalent *Penicillium* species reported from Taiwan [51,52].

Approximately 12 antigens from *P. citrinum* and 11 antigens from *P. chrysogenum* have been shown to react with IgE from patients' sera by immunoblotting. Recently, several *Penicillium* allergens have also been characterized at the molecular level [14]. Among the *Penicillium* allergens, the 32 to 34 kDa alkaline and/or vacuolar serine proteases were identified as the major allergens of *P. citrinum*, *P. brevicompactum*, *P. chrysogenum*, and *P. oxalicum* [44,46]. Immunoblotting data showed that IgE antibodies against components of these prevalent *Penicillium* species could be detected in the sera of approximately 16% to 26% of the asthmatic patients [46]. The alkaline serine protease allergens with strong IgE binding properties have been isolated from *P. citrinum* (Pen c 13) and *P. chrysogenum* (Pen ch 13), and the vacuolar serine proteases from *P. citrinum* (Pen c 18), *P. oxalicum* (Pen o 18), and *P. chrysogenum* (Pen ch 18) [47,48•]. The mature Pen ch 13 allergen is formed by the removal of the preprosequence of the precursor molecule [48•]. Besides N-terminal cleavage, the mature Pen c 18 and Pen o 18 also undergo C-terminal processing [49]. The IgE cross-reactivity among the allergens in *Penicillium* and *Aspergillus* spp. has been detected [45,47,50,52]. In addition to the reactivity with IgE antibody, serine proteases (Pen ch 13) also demonstrated histamine-releasing activity from peripheral blood leukocytes of asthmatic patients [48•].

Besides the serine protease allergens, a 68-kDa allergen N-acetylglucosaminidase and an allergenic heat shock protein belonging to the hsp 70 family have also been identified from *P. chrysogenum* and *P. citrinum*, respectively [53,54]. They have been designated by the Allergen Nomenclature Subcommittee as Pen ch 20 and Pen c 19, respectively [14]. An 18 kDa peroxisomal membrane protein (Pen c 3), similar to Asp f 3, and enolase (Pen c 22), similar to Asp f 22, were also identified from *P. citrinum* [55,56]. Cross-reacting IgE antibodies have been reported against these allergens [55,56].

Alternaria alternata

Alternaria alternata, a member of the imperfect fungi, is one of the most important among all allergenic fungi [57–59]. The spores produced by imperfect fungi vary in shape, size, texture, color, number of cells, and thickness of the cell wall. *Alternaria* has dark "dictyospores," with both horizontal and vertical septa. Airborne fungal spores of *Alternaria* and *Cladosporium* are found throughout the world. The airborne spores of these two fungi are generally considered to be important causes of both allergic rhinitis and allergic asthma.

Several allergens from *A. alternata* have been purified and characterized either by conventional fractionation or by employing molecular biology techniques [14]. Alt a 1, a major allergen of *Alternaria*, binds to the IgE of more than 80% of asthmatic patients with allergy to this fungus [60••]. Several variants and isoforms of Alt a 1 have been reported [57,58]. A reduced form of Alt a 1 produces a doublet pattern on SDS-PAGE with molecular weight of 14.5 and 16.0 kDa. In immunoblots with human atopic serum, this doublet demonstrated reactivity with IgE of patients sensitized to *Alternaria*.

Achatz *et al.* [57] described three additional cDNA clones encoding functionally active *A. alternata* allergens. Alt a 7, the 22 kDa allergen, demonstrated more than 70% sequence homology with the YCP4 protein of *Saccharomyces cerevisiae*, whereas Alt a 6, the 11 kDa protein, demonstrated homology with ribosomal P2 protein. However, these allergens reacted diversely with IgE from sera of allergic patients.

Cladosporium herbarum

Cladosporium herbarum is distributed widely in our environment and is a major source of inhalant allergens. *A. alternata* is the predominant fungus demonstrated in warm and humid climates, whereas *Cladosporium* is the leading allergenic mold observed in cooler climates. Approximately 60 antigens from *Cladosporium herbarum* have been identified by crossed immunoelectrophoresis, and approximately 36 of them have been shown to react with IgE from patients' sera [61]. Three major *C. herbarum* allergens have been purified and characterized [14]. Cla h 1 is a small, 13-kDa acidic allergen composed of five isoallergens (isoelectric point [pI] 3.4–4.4), and Cla h 2, a slightly larger molecule (23 kDa), is a glycoprotein (pI 5.0) with more than 80% carbohydrates. The protein part retained the IgE-binding property even after removing the carbohydrate from the allergen. Cla h 4, the ribosomal P2 protein, is a low-molecular weight (11-kDa) acidic allergen (pI 3.94) with high alanine and serine content. It shares 60% sequence homology with ribosomal P2 protein [62]. Achatz *et al.* [57] reported that purified recombinant *C. herbarum* (Cla h 6; 48 kDa) bound strongly to IgE antibodies in 20% of *Alternaria* allergic patients, as measured by immunoblots. Enolase has been found to be a highly conserved major allergen in most fungi, and might contribute to allergen cross-reactivity in mold allergy. Approximately 20% of the serum IgE from patients sensitized to *Alternaria* and *Cladosporium* showed binding to enolase. An allergenic HSP 70 has also been isolated from this organism [62].

Other Fungi

There are several other fungi also involved in allergy and asthma (Table 1). These fungi might be seasonal or might be present throughout the year. Some of these fungi are predominantly saprophytic, whereas others are parasites of humans, animals, or birds. In addition, the prevalence of several of these molds is seasonal or dependent on the vegetation. However, because of their lesser prevalence, not much attention has been paid to isolate and purify the relevant allergens. The pathogenic fungi such as *Trichophyton* and *Candida* also can elicit allergic responses in humans. Antigens from most of these fungi are species specific, but they also can have cross-reactive epitopes.

Many of the *Basidiomycetes* spp. have been implicated in seasonal allergy and are listed in Table 1. Although *Basidiomycetes* spores are predominantly outdoors, indoor air, especially in damp conditions, show the presence of *Basidiomycetes*

antigen incorporated with dust and are a major cause of respiratory allergy. Studies conducted in Europe and the United States demonstrate that 25% to 30% of patients with respiratory allergy have been sensitized to *Basidiomycetes*. The predominant species involved in allergy are *Boletus*, *Coprinus*, and *Pleurotus*. Other mushrooms such as *Ganoderma*, *Clavatis*, *Psilocybe*, *Schizophyllum*, and *Lentinus* have been reported as causing allergy [63].

Epitopes

Epitopes are sites or regions of an allergen molecule that interact with T and/or B cells of the immune system. The allergens that interact with IgE are the B-cell epitope, whereas those presented to T cells after processing by antigen presenting cells (APC) as peptide fragments linked to major histocompatibility (MHC) molecules are the T-cell epitopes.

T-cell epitopes

T cells recognize small peptide fragments derived by processing the antigen by APC and presented as MHC complexes on the surface to T-cell receptors (TCR). The TCRs rearrange themselves on the surface, resulting in only one receptor on each T cell. The binding of the MHC antigen complex to the TCR triggers the activation of the T cell. T-cell epitope presentation to the TCR involves MHC class II molecules and is presented frequently by single human leukocyte antigen (HLA) molecules such as DR, DQ, or DP [64]. The minimum peptide length that can bind and initiate a T-cell response is between 7 and 10 residues, although peptides with more than 10 residues are also presented efficiently. Based on the physicochemical studies of peptides and their functional activities, several T-cell epitopes have been identified.

The only fungal allergen studied extensively so far with regard to cell-mediated immune response and T-cell epitope mapping is Asp f 1 from *A. fumigatus* [33,64]. The Asp f 1-specific T-cell lines established from ABPA have been characterized as CD4⁺ Th2 cells based on their cytokine secretion pattern [65]. Most of the Asp f 1-specific T-cell clones isolated from PBMCs of ABPA responded to two peptide fragments of Asp f 1 representing amino acid (aa) residues aa 46 to 65 and aa 106 to 125 and restricted by HLA-DR2 and HLA-DR5 alleles [64]. In a mouse model of ABPA, epitopes of Asp f 1 demonstrated differential induction of CD4⁺ Th2 and CD4⁺ Th1 cells as indicated by the cytokine patterns [34]. T-cell clones were generated from ABPA patients against Asp f 2. Stimulation of these T-cell clones and PBMCs from ABPA patients using synthetic peptides from Asp f 2 sequence identified aa 54 to 74 as a major T-cell epitope. This epitope was further defined, and it was found that T-cell clones stimulated with aa 60–74 secreted interleukin (IL)-5, but not interferon (IFN)- γ [38].

B-cell epitopes

B-cell epitopes of the allergens might be sequential (linear) and topographic (conformational). Linear epitopes are short

sequences of amino acids with up to eight residues located and exposed on protein surfaces where the adjacent hydrophilic amino acids contribute to the interaction with antibody. The identification of sequential epitopes is readily achieved by the systematic synthesis of overlapping short peptides spanning the sequence of the protein allergen. The second type of epitope is discontinuous, in which the amino acid residues of the antigenic determinants are not sequentially contiguous, but brought close together in space by folding of the polypeptide chain.

Both sequential and conformational epitopes have been identified in Asp f 2, from *A. fumigatus* [37]. The epitope mapping of linear peptides synthesized on derivative cellulose membranes demonstrated distinct IgE-binding epitopes of three to seven amino acids long and were distributed throughout the whole molecule. However, IgE antibody binding of recombinant polypeptide fragments representing various identified epitopes suggest the possible involvement of other structural constraints in the proper folding and three-dimensional structure of Asp f 2. B-cell epitope mapping of Asp f 2 revealed conserved B-cell epitope sequences present in other *Aspergillus* species as well as in the proteins from *Candida*. Two immunodominant epitopes of Asp f 1, from the C-terminal end of the protein (aa 115–149) with strong T-cell proliferative responses as well as IgE antibody binding with sera from ABPA patients, have been reported [33].

Structure Function Properties of Allergens and Allergic Responses

The molecular structure of allergens and their interaction with the immune system of the host is not fully understood. Recently, because of the availability of purified recombinant allergens, limited attempts have been made in this direction. The sequence comparison and homology search indicates the distribution of unique and shared epitopes in some pollen allergens. However, only very few structure function studies have been conducted with mold allergens. Structure function responses of one of the major allergens of *Aspergillus*, Asp f 2, were studied after engineering the allergen using molecular techniques. Deletion of N, C, and N' and C' terminals showed diverse binding reactivity with IgE antibody. N- or C-terminal end is essential for the conformation of the molecule as both N and C deleted mutants failed to react with IgE. Postglycosylation of the protein demonstrated less IgE binding, but more IgG binding compared with nonglycosylated Asp f 2 [22•]. The role of cysteine residues in Asp f 2 was studied, and the results indicate that cysteines at positions C264 and C204 are essential for the IgE binding [23••].

Very little is known about the three-dimensional structure of the mold allergens. The three-dimensional structure is suggested for restrictocin, a closely related ribotoxin belonging to Asp f 1 family. It has three alpha helical turns and five stranded antiparallel beta sheets. Recently, Asp f 6 (MNSOD) from *A. fumigatus* was studied, and the 3-D structure has been elucidated [66••]. It has a homotetrameric structure

with four conserved manganese ions and exposed amino acids with potential cross-reactivity with other MNSODs. Therefore, 3-D structure of allergens will be valuable in determining the cross-reactivity and specificity of the allergen.

Specific Immunotherapy

The use of specific immunotherapy for fungal allergy is debatable. Specific immunotherapy for type I allergy should be based on reducing allergic reactions to those allergens that are recognized by the patients' IgE antibodies. The establishment of patients' IgE reactivity profile (allergogram) with recombinant allergens will be valuable in selecting the components against which a substantial IgE response is mounted. Selection of allergens according to the patients' allergogram might, therefore, improve both diagnostic and therapeutic specificity of the allergens [18,67••]. Allergograms might also help in monitoring the effectiveness of immunotherapy. With the availability of recombinant allergens, structure function studies might be carried out, resulting in valuable information in our understanding of the immunopathogenesis of mold allergy. This information might contribute to more focused and refined immunomodulatory therapy for controlling the disease.

Conclusions

Fungus-induced allergy comprises a major group of diseases. Until recently, the diagnosis of allergy was cumbersome, owing to the lack of defined purified fungal antigens. Recently, interest in obtaining purified mold allergens through molecular cloning has been enhanced. The emergence of purified allergens led to better diagnosis and to our understanding of the immunopathogenesis of the disease. Cross-reactivity among allergens and epitope sharing have attracted considerable attention recently. This knowledge might add to renewed patient care and better diagnosis. The results of the structure function studies and epitope analysis will also contribute to our understanding of these diseases and in improved patient care.

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