MOLECULAR BIOLOGY AND IMMUNOLOGY OF FUNGAL ALLERGENS

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ABSTRACT

Fungi are non-chlorophyllous microorganisms, which constitutes the main source of outdoor and indoor allergens. The antigens present in the spores and fragments of hyphae induce allergic responses in sensitized patients. The frequently recognized fungi associated with asthma include *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium*. With the advent of molecular biology techniques a number of fungal genes encoding relevant allergens have been cloned and the expressed allergens purified and characterized. In this review, we have presented the recent developments, where recombinant allergens have been used in the precise diagnosis of fungal allergy. We have also discussed the role played by these allergens and the T- and B-cell epitopes in the immune mechanism in fungal allergy.

KEY WORDS : Fungal allergy; Allergens; Epitopes; Immunoassays

FUNGAL ALLERGY

Allergy is defined as the exaggerated response of the immune system to foreign proteins [allergens] (1,2). Since only select individuals develop allergy in spite of the extensive exposure to allergens, evidence for genetic predisposition for development of atopic disease in these patients is likely. The most frequently recognized allergic conditions caused by fungi are allergic rhinitis, conjunctivitis, and bronchial asthma. Presenting clinical symptoms are rhinorrhea, nasal congestive discharge, coughing, wheezing, and shortness of breath. Reversible pulmonary airway obstruction, angioedema, urticaria, and even anaphylaxis may occur in these patients (2).

Over 20% of the population is affected by allergy of one form or other. A number of allergens

associated with the different forms of allergy have been reported from all over the world (2,3). The concentration of allergens in the environment varies depending on multiple factors, which include climate, vegetation, and air quality. The outdoor allergens predominantly constitute plant pollens and fungal spores. The indoor allergens, on the other hand, are represented by proteins from dust mite, cockroach, dog, cat, and mice. Fungal spores may contribute to the indoor environment (4). The concentration and prevalence of the indoor allergens depend on moisture content, ventilation, and the presence and absence of pets, carpets, and houseplants (5). Avoidance of allergens or reducing the exposure may be beneficial in preventing the development of allergy to these indoor and outdoor allergens.

Fungi are eukaryotic, filamentous, and mostly spore bearing organisms. They exist as saprophytes or as parasites of animals and plants. Allergy to fungi follow the same biological phenomena as allergy to other environmental allergens. Some genera of airborne fungal spores such as *Alternaria*, *Aspergillus*, and *Cladosporium* are found throughout the world (6). The airborne spores of these fungi are generally considered to be important cause of both allergic rhinitis and allergic asthma (7).

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The prevalence of respiratory allergy to fungi is estimated at 20 to 30% among atopic individuals and up to 6% in general population (3,8,9). The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis (4,10). These diseases can result from exposure to spores, vegetative cells, or metabolites of the fungi. In addition, as the fungal spores are small [usually less than 10 μm] a majority of them are capable of penetrating the lower airways of the lung and mediate allergic reactions. The conidia and fungal spores associated with immediate type of hypersensitivity are usually larger than 5 μm (4,5,11). The site of deposition of spores also depends on whether spores enter the respiratory tract as individual propagules or as aggregates. The clusters of small conidia of *Aspergillus fumigatus* are usually deposited in the upper respiratory tract, while the smaller individual spores reach the lower airways. Bronchial provocation tests with spores and fungal extracts cause both early and late phase respiratory reactions in patients. More than 80 genera of the major fungal groups have been associated with symptoms of respiratory tract allergy (4,9). Most of the allergenic fungi are classified under Ascomycetes and Deuteromycetes with a few in Basidiomycetes. However, only a few species such as *Aspergillus fumigatus*, *Alternaria alternata* and *Cladosporium herbarum* have been investigated systematically for their role in causing allergy (6,7).

MECHANISM OF ALLERGY

The immune response in allergy is mediated by both T- and B-cells (2). Although seemingly simple, the mechanisms involved in the manifestations of allergy are multiple and a number of regulatory cells and factors are implicated (12). In allergy, the activated T-cells follow a Th2 pathway with secretion of IL-4, IL-5, IL-6, IL-10, and IL-13, which favor an allergic humoral immune response (2,13). The IgE synthesis involves a number of signals following T- and B-cell interaction. The interactions between the receptors on B-cells and IL-4 and/or IL-13 constitute one of the signals associated with antibody class switching. The other signals are CD40 on B-cells and its interaction with CD40L on T cells and the Fc ϵ RII mediated B-cell activation signal leads to the production of IgE antibody (14). Both these

signals require prior interaction with processed peptides presented to T-cell receptor [TCR] by antigen presenting cell [APC] in a MHC Class II restricted fashion. These APC can be macrophage, B-cell or dendritic cell. A number of transcription factors [STAT 6, NF κ B and BSAP] that bind to the promoter regions of various genes associated with allergic reaction have been identified and characterized (15-17). Among the STATs, STAT 4 and 6 are important in the CD4⁺ Th1 and Th2 pathways. STAT 6 is essential for IL-4 response and for the upregulation Th2 pathway, while STAT 4 induce IFN- γ and upregulate Th1 response (16). The transcription factors NF κ B and BSAP mediate both IL-4 and CD-40 delivered signals (13,15-17). Genetic factors are suggested as major components in the development of allergy. Convincing evidence indicates that specific immune response is the result of antigenic epitopes binding to MHC Class II molecules. The HLA DR region of the MHC molecule is involved in antigen presentation and subsequent events of cell/cell and cell/factor interaction is important in the pathogenesis of allergy.

IgE antibody plays an important role in allergic diseases. However, additional factors such as eosinophils and T-cells have been identified as important in the development of allergy. Primary exposure to allergens leads to sensitization and immune-recognition, while subsequent exposures lead to interaction of allergens with receptor bound IgE antibodies on various effector cells. The Fc portion of IgE binds to basophils, mast cells and antigen presenting cells through the high affinity receptor Fc ϵ R1. The low affinity receptors of IgE [Fc ϵ RII/CD 23] are expressed by B cells, monocytes/macrophages, dendritic cells and eosinophils (2). The allergen interaction with IgE/Fc ϵ R1 on mast cells and basophils results in cross linking of the receptors, and this triggers the release of a number of pharmacologic mediators and cytokines, which enhance immediate and late hypersensitivity responses. In addition, interaction of allergens with IgE bound to the high and low affinity receptors on antigen presenting cells may facilitate receptor mediated endocytosis and finally the presentation of processed antigen to specific T-cells. This results in the production of cytokines, particularly IL-4, IL-5 or IFN- γ , and IL-4 promotes further secretion of IgE molecules. IL-5 leads to

eosinophil recruitment, and activation and continued upregulation of the Th2 type immune responses and pulmonary airway hyperreactivity. However, IFN- γ secretion leads to down regulation of Th2 response and enhancement of the Th1 response. The acute symptoms of allergy such as sneezing, wheezing, and urticaria are due to the release of histamine by mast cells, while chronic symptoms such as bronchial hyperreactivity could be explained by eosinophil mediated tissue damage caused by the release of various eosinophil proteins (12-19).

ALLERGENS IN FUNGI

Relevant fungal allergens are important in the diagnosis of allergy due to fungi by demonstrating IgE antibody in the sera of patients or immediate wheal and flare skin test reactivity. The diagnosis of allergic disease is mainly based on clinical symptoms of the patients, skin test reactions, detection of allergen specific serum IgE antibodies [RAST, ELISA], and in some cases inhalation challenge using fungal antigens (8,9,11). However, the effective in vivo and in vitro diagnosis of fungal allergies is based on the availability of well-characterized allergen preparations. Fungi are highly variable both in their morphology and in their antigenic makeup. The wide spread antigenic cross reactivity among related fungi is a major concern. With some fungi it is almost impossible to grow two consecutive cultures with similar antigenic profiles (11,20,21). Factors contributing to the variability of commercial and laboratory made extracts are due to the variability of stock cultures used to prepare allergenic extracts, the use of mycelial rich material as the source of allergens, the conditions under which molds are grown and extracts prepared, and finally to the stability of the proteins present in the extracts. Uniform quality control measures are also not available to ascertain comparability of extracts. However, it is now possible to grow allergenic fungi in synthetically defined medium with less variability compared to complex media containing macro-molecules (21,22). Two to three weeks old cultures are usually a rich source of culture filtrate antigens while reliable mycelial antigens for immunoassay can be obtained from short-term aerated fungal culture (21).

The allergenicity of an extract or fraction can be evaluated by prick testing or intradermal testing

of allergic subjects. The most common in vitro tests for assay of allergen activity are RAST and ELISA (23-25). The radioactive counts and optical density values directly correlate with the allergen specific IgE in the sera of patients. However, RAST and ELISA can be adapted to obtain quantitative and semiquantitative estimations of antibody in the sera of patients. The allergenic activity and specificity of the allergen can also be compared by competitive inhibition assays (25). The stability of allergenic extracts depends on the chemical nature and quality of the allergen, the storage temperature, and the presence of preservatives and other non-allergic materials in the mixture. For most extracts, lyophilization is the best method to maintain the allergenic potency, but some allergens may be permanently altered and inactivated by this process. Moreover, reconstituted extract must contain a stabilizer such as human serum albumin, glycerol, phenol, or epsilon-aminocaproic acid to preserve the integrity of allergenic extracts (26,27).

FUNGI ASSOCIATED WITH ALLERGY

The most important group of air disseminated fungi that cause respiratory allergic diseases in humans are the conidial fungi, comprising the form class Deuteromycetes. The spores produced by the imperfect fungi vary in shape, size, texture, color, number of cells, thickness of the cell wall, and methods by which they are attached to each other and to their conidiophores. The identification of the common fungi is difficult as the fungal colony characteristics and even microscopic characteristics vary according to the medium on which the fungus is grown, temperature of incubation, and variation among strains and pleomorphic nature of the spores.

Fungal identification is based frequently on spore morphology such as color, septation, and different methods of spore production. In addition, the nature and morphology of sexual spores and the different spore types involved in the sexual and asexual life cycles are also used for fungal identification. The distinct chemical compositions of the cell walls of different fungi are also helpful in their classification and determining their role in causing allergic responses in patients. Other aspects of vegetative morphology commonly used for identification purposes are color of colony,

diffusible pigments, metabolites, and mycelial structures.

The antigens present in the spores and fragments of hyphae sensitize patients leading to allergic responses. Fungi that are commonly involved in asthmatic reaction in atopic individuals belong to the genera *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium*. (4,9,27,28,29,30). Proteins capable of causing allergy in patients have been reported also from mushrooms and yeasts (31). The common fungal allergens are either purified by conventional methods or cloned and expressed by molecular biology techniques. Based on the primary structure of the fungal allergens, it appears that majority of the identified allergens are proteins associated with essential basic metabolism like glycolysis or protein synthesis. Presence of cross-reactivity among allergens may result in reduced specificity and necessitate the use of secondary testing to determine the specific sensitizing mold. On the other hand, cross-reactive antigens are more advantageous for immunotherapy due to the broad-spectrum effect with fewer numbers of allergens. The approved allergens from *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium* by the WHO/IUIS committee on allergen nomenclature are given in Tables 1-4.

RECOMBINANT ALLERGENS FROM FUNGI

ALTERNARIA ALTERNATA

Alternaria alternata, a member of the imperfect fungi, is one of the most important among all allergenic fungi (7,30,32,33). A number of allergens from *A. alternata* have been purified and characterized either by conventional fractionation or molecular biology techniques [Table 1]. Alt a 1, a major recognized allergen, binds to IgE of more than 80% of asthmatic patients with *Alternaria* allergy. Various isoelectric variants and isoforms of Alt a 1 have been reported recently (7,33). Alt a 1 with two subunits of 15 KD each, interact with IgE antibody only when present in unreduced form indicating the presence of conformational epitopes in Alt a 1 specific for IgE antibodies. Achatz and associates recently described three additional cDNA clones encoding functionally active *A. alternata* allergens (7). The 53 KD aldehyde dehydrogenase [ALDH], 22 KD allergen with 70% sequence homology with the YCP4 protein of *Saccharomyces cerevisiae* and 11 KD ribosomal P2 protein exhibited diverse binding with IgE in the sera from *Alternaria* sensitized patients (7).

Table 1. Allergens of *Alternaria*

Allergen*	kD	Nature of Allergen	Binding of Patients' IgE
Alt a 1	30	-	80***
Alt a 2	45	-	47
Alt a 3	85	Heat shock protein 70	42
Alt a 4	63	-	37
Alt a 5	30	-	33
Alt a 6	11	Ribosomal protein P2	7
Alt a 7	22	YcP4	7
Alt a 8	39	-	7
Alt a 9	42	-	5
Alt a 10	53	Aldehyde dehydrogenase	2

*Allergens are designated according to the taxonomic name as follows: the first three letters of the genus name, space, the first letter of the species name, space, and an Arabic numeral. The number has been assigned to the allergens in the order of their identification and the same number has been assigned to designate homologous allergens from the related species.

**Patients allergic to *Alternaria*.

***% of patients' serum IgE showing binding to the allergen.

Table 2. Allergens of *Cladosporium*

Allergen*	kD	Nature of Allergen	Binding of Patients' IgE
Cla h 1	30	-	61***
Cla h 2	45	-	43
Cla h 3	53	Aldehyde dehydrogenase	36
Cla h 4	11	Ribosomal protein P2	22
Cla h 5	22	YCP4 protein	22
Cla h 6	48	Enolase	20
Cla h 7	42	-	17
Cla h 8	110	-	11
Cla h 9	100	-	6

*Same as in Table 1

**Patients allergic to *Cladosporium*.

***% of patients' serum IgE showing binding to the allergen.

CLADOSPORIUM HERBARUM

Cladosporium herbarum is the most abundant fungus found in our environment and is a major source of inhalant allergens. The fungus *A. alternata* is the predominant organism demonstrated in warm and humid climate, while *Cladosporium* is the leading allergenic mold observed in cooler climate. About 60 antigens from *C. herbarum* have been identified by crossed immunoelectrophoresis [CIE] and about 36 of them have been shown to be allergenic by crossed radioimmuno-electrophoresis [CRIE] (20). Three major *C. herbarum* allergens have been purified and characterized. Cla h 1 is a small 13-KD acidic allergen composed of five isoallergens [pI 3.4-4.4], and Cla h 2 is a slightly larger 23-KD glycoprotein [pI of 5.0] with over 80% carbohydrates (34,35). The protein part retained the IgE binding property even after removing the carbohydrate from this allergen, and the binding to IgE antibody was much stronger than shown by the native Cla h 2. Cla h 4, the ribosomal P2 protein, is a low-molecular weight [11-KD] acidic allergen [pI 3.94], and this alanine and serine rich allergen shares about 60% sequence homology with ribosomal P2 proteins present in other organisms including humans. Brietenbach and his coworkers recently reported purified recombinant *Cladosporium* enolase [Cla h 6; 48 KD] having binding to IgE antibodies in 20% of *Alternaria* allergic patients by immunoblots (36). Enolase has been found to be a highly conserved major allergen in fungi, and has been reported in

both *Alternaria* and *Cladosporium*. About 50% of the serum IgE from patients sensitized to *Alternaria* and *Cladosporium* showed binding to enolase. A list of the currently recognized allergens from *Cladosporium* is shown in Table 2.

ASPERGILLUS SPECIES

Aspergillus species are saprophytic fungi widely distributed in nature and are associated with a number of human diseases (11,18). Several recombinant allergens from *A. fumigatus* have been identified and purified from cDNA and phage display library of *A. fumigatus* [Table 3]. The majority of these proteins showed specific binding to IgE from asthmatic and allergic bronchopulmonary aspergillosis [ABPA] patients. Some of these *A. fumigatus* allergens also exhibited high sequence homologies with the known functional proteins and enzymes (37-39). Alkaline serine proteinases with allergenic properties such as Asp f 13, Asp f l 13, and Asp o 13 from *A. fumigatus*, *A. flavus*, and *A. oryzae* respectively have been reported (40). Similar serine proteinases Pen b 13, Pen c 13, and Pen n 13 with sequence homology to *Aspergillus* proteinase have also been identified from various species of *Penicillium* (28,29,41). Recently, another group of homologous vacuolar serine proteinases Asp f 18, Asp n 18, Pen n 18, and Pen o 18 with conserved sequence have been reported from *Aspergillus* and *Penicillium*.

Table 3. Allergens of *Aspergillus*

Allergen*	kD	Nature of Allergen	Binding of Patients' IgE
Asp f 1	17	Ribotoxin	83***
Asp f 2	37	Fibrinogen binding (?)	90
Asp f 3	18	Peroxisomal protein	94
Asp f 4	30	-	78
Asp f 5	42	Metalloproteinase	93
Asp f 6	23	Mn superoxide dismutase	56
Asp f 7	12	-	46
Asp f 8	11	Ribosomal protein-2	-
Asp f 9	34	-	89
Asp f 10	34	Aspartic proteinase	28
Asp f 11	24	Peptidyl-prolyl isomerase	-
Asp f 12	47	Heat shock protein-90	-
Asp f 13	34	Alkaline serine protease	-
Asp f 14	105	Beta xylooxidase	4
Asp f 15	-	-	-
Asp f 16	43	-	70
Asp f 17	-	-	-
Asp f 18	34	Vacuolar serine proteinase	-

*Same as in Table 1

**Patients allergic to *Aspergillus*.

***% of patients' serum IgE showing binding to the allergen.

A. flavus extracts demonstrated IgE antibody binding in 44% asthmatic patients studied by immunoblotting (42). Recently a 34 KD alkaline serine proteinase, Asp f 13 with significant IgE antibody binding was cloned and the allergen

expressed. The cDNA of this allergen Asp f 13, showed 100% sequence identity to the 34 KD alkaline serine proteinase, major allergen [Asp o

Table 5. Fungal Allergens from Various Species Showing Cross-reactivity**Table 4. Allergens of *Penicillium***

Allergen*	kD	Nature of Allergen
<i>Penicillium</i>		
Pen c 1	70	heat shock protein
Pen c 3	18	peroxisomal membrane proteins (PMP)
Pen c 13	33	alkaline serine proteinase
Pen b 13	33	alkaline serine proteinase
Pen n 13	34	alkaline serine proteinase
Pen n 18	32	vacuolar serine proteinase
Pen o 18	34	vacuolar serine proteinase
Pen n 20	68	glycoprotein
Pen c 21	70	-

*Same as in Table 1

Allergens and Nature	Cross-reactivity
Peroxisomal membrane protein	Asp f 3, Pen c 3
Aldehyde dehydrogenase	Alt a 10, Cla h 3
Ribosomal protein P2	Alt a 6, Cla h 4, Asp f 8
Enolase	Alt a, Cla h 6
YCP4	Alt a 7, Cla h 5
Heat shock protein	Alt a 3, Cla h, Asp f 12, Pen c 1
Alkaline serine protease	Asp f 13, Asp f 13, Asp o 13
	Pen b 13, Pen c 13, Pen n 13
Fibrinogen binding protein	Asp f 2, Asp nd 1
Vacuolar serine Proteinase	Asp f 18, Asp n 18
	Pen n 18, Pen o 18

13] of *A. oryzae* (43).

Glucoamylase, cellulase and hemicellulase from *A. niger* were shown to be allergenic by skin prick tests and by in vitro specific IgE detection assays (44,45). In addition, α β -xylosidase [Asp n 14] from *A. niger* was also identified as an occupational allergen present in baking additives, which causes sensitization in symptomatic bakers (45). The vacuolar serine proteinase [Asp n 18] has also been identified as a major allergen of *A. niger* with significant IgE binding to sera from asthmatic patients.

α -amylase of *A. oryzae* has been recognized as an important occupational allergen in baker's asthma (44,45). Results reported show that of the 48% of work-related respiratory symptoms among bakers, 32% was due to sensitization with α -amylase [Asp o 2] from *A. oryzae* used as a baking additive (45). In addition, the 34 KD alkaline serine proteinase was also identified as a major allergen in *A. oryzae* [Asp o 13] (40).

Penicillium species

Among the fungi, *Penicillium* and *Aspergillus* are the two dominant fungal genera frequently demonstrated indoors (4,27). Intradermal skin reactivity in atopic subjects using different mold

extracts demonstrated 68% skin test reactivity to *P. notatum*. The frequencies of positive Phadebas RAST in asthmatic children for *Penicillium* and *Aspergillus* were 21% and 14%, respectively (46). The prevalence of *Penicillium* species showed considerable variations in different places. Among the allergens of *Penicillium* species, the 32-34 KD alkaline serine proteinases were the major allergens of *P. citrinum*, *P. brevicompactum*, *P. notatum*, and *P. oxalicum* [Table 4] (47). These allergens showed cross-reactivity with other major allergens from different *Penicillium* and *Aspergillus* species [Table 5] (29,40,42). In addition, the purified Pen n 13 demonstrated positive immediate skin test reactivity in asthmatic patients and showed histamine release from the peripheral leukocytes of asthmatic patients. Other allergens, particularly the 68 KD β -N-acetylglucosaminidase and a heat shock protein in the hsp 70 family have been identified from *P. notatum* and *P. citrinum*, respectively (28,29). Furthermore, an 18 KD peroxisomal membrane protein [Pen c 3] having IgE cross-reactivity with recombinant Asp f 3 has also been reported (48).

Allergen Epitopes

Epitope is defined as the site or region of an allergen molecule that interact with T- and/or B-cells of the immune system. The allergens interact with immunoglobulin E as the B-cell epitope, while they

Table 6. T-cell and B-cell Epitopes of *A. fumigatus* Allergens, Asp f 1 and Asp f 2

Allergen Sources Allergen	Size	T-cell Epitopes (residues amino acid)	T-cell Sources	IgE Binding Epitopes (residues amino acid)
<i>Aspergillus fumigatus</i>				
Asp f 1	16.9 kD 148 amino acids	1-20, 48-65, 61-80 91-110, 106-125	*TCC	
Asp f 1	-	36-51, 53-68, 69-84, 115-130, 135-146	PBMCs	
Asp f 1	-	-	-	40-48, 76-102, 116-130, 140-149
Asp f 2	37kD 268 amino acids	-	-	16-22, 51-55, 96-98, 147-150, 158-162, 189-194, 254-264
			113-117, 139-143,	

*T-cell clones

PBMCs-Peripheral blood mononuclear cells

are presented to T-cells after ingestion or processing by antigen presenting cells [APC] as peptide fragments linked to major histocompatibility [MHC] molecule as 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 the 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 HLA molecule such as DR, DQ or DP (49,50). The minimum peptide length that can bind and initiate a T-cell response is between seven and eight residues although peptides with greater than 10 residues are also presented efficiently (51). Based on the physicochemical studies of peptides and their functional activities, a number of 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* (50,52). The Asp f 1 specific T-cell lines established from ABPA have been characterized as CD4+ Th2 like in their cytokine secretion pattern (53). The majority 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 residues 46 to 65 and 106 to 125 and restricted by HLA-DR2 and HLA-DR5 alleles (49,50). The major T-cell and B-cell epitopes of the *A. fumigatus* allergens are shown in Table 6. 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 (54). The preferential expression of one type of T-helper cell [either Th1 or Th2] during the course of immune responses to an allergen depends also on dose and route of administration of the antigen and the presence or absence of cofactors. Intrinsic factors from the host that are critical include the

nature of antigen presenting cells, cytokine milieu at the site of antigen exposure and the immune status of the host.

B-cell epitopes

B-cell epitopes of the allergens may be sequential [continuous or linear] and topographical [conformational]. Continuous epitopes are short linear sequences of amino acids up to eight residues located and exposed on protein surfaces where the adjacent hydrophilic amino acids contributing to the interaction with antibody (55,56). 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 epitopes 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. Because of this specific feature, monoclonal antibody specific for such epitopes will react only with the protein or fragment of proteins or synthetic peptides in which the native configuration of the amino acid residues are preserved. Hence, no linear synthetic peptides can bind to such monoclonal antibodies (56).

We reported the presence of both sequential and conformational epitopes in Asp f 2, a major allergen from *A. fumigatus* (57). The epitope mapping of linear peptides synthesized on derivatized cellulose membranes demonstrated distinct IgE binding epitope of three to seven amino acids long and were distributed throughout the whole molecule. On the other hand, IgE antibody binding of recombinant polypeptide fragments representing various identified epitopes suggested 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 *Aspergillus* as well as in the proteins from *Candida*. Two immuno-dominant 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 (55) [Table 6].

Assays for Mold Specific IgE Antibody

Demonstration of circulating antibodies against different allergens in the sera of sensitized subjects is a valuable aid in the diagnosis of fungal allergy. A variety of tests with varied sensitivities have been developed to detect circulating antibodies to allergens from various sources. In vivo measurement of allergen specific IgE consists of skin prick test and intracutaneous test and both involved the production of an immediate wheal and erythema reaction, the characteristic skin reaction of atopic sensitization. On the other hand, in vitro procedures such as the Radioallergosorbent test [RAST], Enzyme linked immunosorbent assay [ELISA], and Western blot detect circulating antibody against specific allergens in sera from allergic patients.

ELISA has been demonstrated to be a sensitive, reliable, and semi-quantitative diagnostic tool for allergy. Although the sensitivity of the conventional ELISA system depends on many variables, the nature and type of antigens used and their ability to bind to polystyrene plates are the most important decisive factors (23). Western blot [WB] involves the separation of antigens in polyacrylamide gel followed by transfer of separated proteins onto nitrocellulose membranes. The reactivity of the particular protein to the antibody in the sera of patients can be visualized by this assay and was shown to be specific and sensitive.

Automated specific IgE assay [Pharmacia immunoCAP system]

The Pharmacia immunoCAP system is a fully automated system for the measurement of circulating IgE antibodies (58). This assay is calibrated against the World Health Organization [WHO] standard for IgE and allows quantitative expression of total or allergen specific IgE. The results are expressed as kilo units of allergen specific IgE per liter [KUA/l].

Conclusions

We have presented information on fungal allergens and mold induced allergies. A number of clinically important fungal allergens have been identified, purified, and characterized. These well-characterized allergens contributed in the improved diagnosis and may also provide better insight into the pathogenesis of fungal allergic diseases. The increasing knowledge of the three dimensional structures and the T-cell and B-cell epitopes of the allergens may be of further value in developing therapeutic strategies including immunotherapy by employing either synthetic peptides, recombinant deletion mutants, and other modified allergens.

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