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Review article

Fungal allergens and peptide epitopes☆

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Abstract

Fungal allergens represent a major cause of atopic disorders. Immunochemical and molecular characterization of fungal allergens has been hampered by the lack of pure proteins and to inherent variation among fungal proteins and in their poor yields. With the advent of molecular biology techniques, a number of allergens have been cloned, sequenced, and expressed from a variety of fungal species. The knowledge of the primary, secondary, and tertiary structures of these allergens, the immunodominant regions of these proteins, and their interaction with T and B-cell epitopes, results in better understanding of the molecular mechanisms of allergy and may provide avenues of immunologic intervention to treat patients. The present review deals with the current understanding of fungal allergen epitopes. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

In industrialized nations, atopic allergy affects up to 20% of the population. The allergic conditions include allergic rhinitis, conjunctivitis, and bronchial asthma. Clinically, the presenting symptoms are sneezing, nasal discharge, coughing, wheezing, and shortness of breath with evidence of reversible airway obstruction, urticaria, angiedema, and anaphylaxis. Allergy is characterized as a hyper response of IgE to environmental allergens such as pollen, food, house dust mite, animal dander, insect venom, and fungal spore [1,68]. Allergen is defined as an antigen that induces IgE antibody synthesis in atopic patients in response to the allergen, leading to release of histamine and other pharmocological mediators of immediate hypersensitivity from mast cells and basophils.

The inflammatory responses in the sensitized patients result from adverse immune reaction involving responses of both cell mediated and humoral immunities. The genetical predilection may have a direct link to the pathophysiology of the disease. In atopic patients, the sensitization lead to elevated serum IgE levels and peripheral blood and lung eosinophilia. On re-exposure to the allergens, interaction of specific IgE on mast cells lead to degranulation and release of mediators such as histamine and results in acute allergic responses including, sneezing, wheezing, and urticaria. However, chronic symptoms of bronchial hyperreactivity may be explained on the basis of eosinophil mediated tissue damages. Both IgE and eosinophils are induced by factors secreted by stimulated T-lymphocytes of $CD4^+$ Th2 type (Th2 cells) and are important in the pathophysiology of allergy [39]. These cells produce a variety of cytokines, of which interleukin-4 (IL-4), IL-5, and IL-13 are the most significant in the allergic asthma. IL-4 and IL-13 are responsible for signaling the B-cells and stimulating the production of IgE, whereas IL-5 is responsible for eosinophil differentiation, recruitment, and activation [39,40,53].

2. Environmental allergens

A number of allergens have been reported from both indoor and outdoor environments [14,19,68]. Plant pollens and fungal spores are the two major groups of outdoor allergens. Indoor allergens are mainly from house dust mites, cats, dogs, mice, cockroaches, and fungi. The knowl-

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edge of the allergens and their concentration in the environment may be helpful in reducing or avoiding the allergen exposure, thereby lessening or eliminating the hypersensitivity reactions.

Allergens are proteins, glycoproteins, or carbohydrates capable of stimulating the immune system and bind specifically to IgE antibody produced in animals in response to stimulation. The antigen exposure may be via inhalation, injection, and contact or by parenteral introduction. The term allergen has been used to define those antigens capable of producing IgE antibody in animals and bind specifically to IgE antibody [39,72,74]. There are over 300 plant and animal allergens reported and these allergens comprise multiple molecules of different physical, chemical, and functional characteristics. Although strong cross reactivity exists within a number of allergens, frequently up to 50% or more of the total IgE in a patient is directed toward a single plant, animal, or mold allergen. A number of allergens from pollens, house dust mites, and cockroaches have been well characterized, the same is not true with fungal allergens [59].

Our knowledge of the allergens and their structure, function, and interactions with the immune system has increased greatly in recent years [59,72,73]. This information is the result of sophisticated techniques available to study protein structures. A large number of allergens have been identified and their role in allergic reaction has been established. The most striking development in recent years has been the availability of pure allergens. The advancement in protein chemistry and the introduction of molecular biology techniques resulted in the rapid purification of these proteins in quantities [45]. Furthermore, the knowledge of amino acid sequences facilitated the understanding of the structure-function properties of the allergens. Presently, the complete amino acid sequences of nearly 80 allergens are known and the number of well-characterized allergens are rapidly increasing, although a large number of clinically relevant allergens still have not been properly identified, purified, or characterized [68,72,73]. Nevertheless, the knowledge of biochemical and physicochemical characteristics of the known allergens are not sufficient enough to differentiate allergens from the non-allergic proteins or to predict the potential nature of antigenic proteins eliciting allergy in susceptible individuals. Based on the currently available information, allergens have been classified into 1) hydrolytic enzymes such as proteases, 2) nonhydrolytic enzymes such as enolases, 3) inhibitors such as Trypsin Amylase, 4) transport proteins such as Lipocalins, and 5) regulatory proteins such as heat shock proteins. However, proteins with unknown biochemical activities still constitute the majority of the allergens [68].

In allergy, there seems to be a breakdown of tolerance of the host to the proteins resulting in the stimulation of immune system leading to the development of high serum IgE levels to the allergens. Clinical and experimental observations suggest that IgE antibody response to allergen is under the influence of a number of genes, which probably operate at the level of T-cell mediated immune regulatory system. In atopic patients, this results in the manifestation of clinical symptoms described above and is associated with high serum levels of allergen-specific IgE, eosinophils, and are induced by a spectrum of cytokines produced by allergen specific $CD4^+$ Th2 cells. Respiratory mucosa is the primary site of host aeroallergen interaction involving allergens and immunocompetent cells such as MHC Class II positive dendritic cells that enhances IgE production in atopic individuals [28,53,68]. Reports are also available on non-IgE mediated mast cell degranulation and cytokine production by respiratory epithelium resulting in the protease mediated disruption of respiratory epithelium [57]. To develop the full spectrum of the disease, other factors such as the size of the allergen molecules, concentration of the allergens, the presence of antigen presenting cells, the processing of the antigens, and the specificity of receptors may contribute substantially. The post translation modification of the allergens also plays a major role in the activation of the immune system.

3. Fungal antigen

Although fungi are outdoor allergens, they have been reported from indoors with varying frequencies. Usually fungi grow in vegetable and decomposing organic matters and liberate spores and respirable mycelial fragments in large numbers. There are certain indoor conditions, which also help the growth of the fungi in large quantities. 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 *Alternaria, Cladosporium*, *Aspergillus*, *Penicillium*, and *Candida* groups [1,6,36,37,49,55,58,61, 63–66,80]. Proteins capable of producing allergic responses from mushrooms and yeasts have also been reported recently [30,32]. The immunologic and inflammatory responses in fungal asthma, particularly the fungal induced early and late asthmatic reactions follow the same pattern as that of other inhalant allergens such as pollen and house dust mite [1,31,39,41]. The fungal allergens purified by conventional methods or cloned and expressed by molecular biology techniques are given in Tables 1 and 2.

Alternaria alternata, a member of the Deuteromycetes, is one of the most important allergenic fungi [1,15,80]. 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 was found to be the most frequently recognized allergen demonstrating IgE binding in more than 80% of asthmatic patients with allergies to *Alternaria*. Paris and coworkers reported the heat stable glycoprotein of 31 kDa containing 20% carbohydrate located in the cytoplasm of both hyphae and spores [55]. Various isoelectric variants and isoforms of Alt a 1

Table 1
Major allergens of *Alternaria, Cladosporium,* and *Aspergillus*

Alternaria				*Cladosporium*				*Aspergillus*			
Allergen*	kD	Nature of allergen	Binding of **patients' IgE	Allergen	kD	Nature of allergen	Binding of **patients' IgE	Allergen	kD	Nature of allergen	Binding of **patients' IgE
Alt a 1	30	—	80***	Cla h 1	30	—	61***	Asp f 1	17	Robotoxin	83***
Alt a 2	45	—	47	Cla h 2	45	—	43	Asp f 2	37	Fibrinogen binding (?)	90
Alt a 3	85	Heat shock protein 70	42	Cla h 3	53	Aldehyde dehydrogenase	36	Asp f 3	18	Peroxisomal protein	94
Alt a 4	63	—	37	Cla h 4	11	Ribosomal protein	22	Asp f 4	30	—	78
Alt a 5	30	—	33	Cla h 5	22	YCP4 protein	22	Asp f 5	42	Metalloproteinase	93
Alt a 6	11	Ribosomal protein	8	Cla h 6	48	Enolase	20	Asp f 6	23	Mn superoxide dismutase	56
Alt a 7	22	YCP4 protein	7	Cla h 7	42	—	17	Asp f 7	12	—	46
Alt a 8	39	—	7	Cla h 8	110	—	11	Asp f 8	11	Ribosomal protein-2	—
Alt a 9	42	—	5	Cla h 9	100	—	6	Asp f 9	34	—	89
Alt a 10	53	Aldehyde dehydrogenase	2					Asp f 10	34	Aspartic proteinase	28
								Asp f 11	24	Peptidyl-prolyl isomerase	—
								Asp f 12	47	Heat shock protein-90	—
								Asp fl 13	34	Alkaline serine protease	—
								Asp o 13	34	Alkaline serine protease	—
								Asp f 13	34	Alkaline serine protease	—
								Asp n 14	105	Beta xyloxidase	4
								Asp f 15	—	—	—
								Asp f 16	43	—	70
								Asp f 17	—	—	—
								Asp f 18	34	Vacuolar serine proteinase	—
								Asp n 18	34	Vacuolar serine proteinase	—

* Allergens are designated according to the taxonomic name of their sources 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 (42).

** Patients allergic to *Alternaria, Cladosporium,* or *Aspergillus.*

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

Table 2
Allergens from other fungi

Allergen*	kD	Nature of allergen	Allergen	kD	Nature of allergen
Penicillium			*Candida albicans*		
Pen c 1	70	Heat shock protein	CAMP	43	Phosphomanno protein
Pen c 3	18	Peroxisomal membrane proteins (PMP)			
Pen c 13	33	Alkaline serine proteinase	CAAP	44	Acid protease
Pen b 13	33	Alkaline serine proteinase	Cand alb 1	40	ADH DH
Pen n 13	34	Alkaline serine proteinase		46	Enolase
Pen n 18	32	Vacuolar serine proteinase		43	Phosphoglycerate
Pen o 18	34	Vacuolar serine proteinase			Kinase
Pen n 20	68	Glycoprotein		37	Aldolase
Pen c 21	70	—		70	HSP70
			Cand b 2	20	PMP
Trichophyton			*Malassezia furfur*		
Tri t 1	30	—	Mal f 1	36	
Tri t 4	83	Serine protease	Mal f 2	20	Peroxisomal membrane (PMP)
Tri r 2	29	Subtilase D	Mal f 3	20	Protein (PMP)
Tri r 4	83	Serine protease	Mal f 4	35	—
		(prolyloligopeptidase)	Mal f 5	18	—
			Mal f 6	17	Cyclophilin

* Allergen Nomenclature (942).

have been reported recently [1,55]. This protein with two subunits of 15 kDa each, interact with IgE antibody only when present in unreduced form, indicating the presence of conformational epitopes specific for IgE antibodies to Alt a 1. In a recent study, Achatz and associates described three additional cDNA clones encoding functionally active *A. alternata* allergens [1]. The 53-kDa aldehyde dehydrogenase (ALDH), 22 kDa allergen with 70% sequence homology with the YCP4 protein of *Saccharomyces cerevisiae* and 11-kDa ribosomal P2 protein exhibited diverse binding with IgE in the sera from *Alternaria*-sensitized patients. All the three allergens have also been purified from expressed cDNA clones of *Cladosporium herbarium* [1,82]. Two allergens, 13 kDa Cla h 1 and 20 kDa Cla h 2 were purified by gel filtration [6]. Cla h 2 is a glycoprotein composed of approximately 80% carbohydrate and 20% protein (Table 1). Highly conserved allergens, the enolases from *Alternata* and *Cladosporium* have been cloned and characterized, and the recombinant enolases from both the fungi showed IgE binding, with about 50% of the sera from patients sensitized to *Alternaria* and *Cladosporium* [13].

Aspergillus species are saprophytic fungi widely distributed in nature and are associated with a number of diseases [45]. A number of recombinant allergens from *A. fumigatus* have been identified and purified from cDNA and phage display library of *A. fumigatus* (Table 1). Majority of these proteins showed specific binding to IgE from asthmatic and ABPA patients and some of these allergens also exhibited high sequence homologies with the known functional proteins and enzymes [9,10,18,45,51].

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 [65]. Similar serine proteinases Pen b 13, Pen c 13, and Pen n 13 with sequence homology have also been identified from various species of *Penicillium* [61,63,64,66]. The allergens Asp o 2 with amylase A activity and Asp n 14 with β-xylosidase activity were recent addition to the list of fungal allergens with enzyme activity [11,58]. Recently, a group of another homologous vacuolar serine proteinases Asp f 18, Asp n 18, Pen n 18, and Pen o 18 from *Aspergillus* and *Penicillium* species have been reported (personal communication, Dr Wayne Thomas, Chairman, IUIS Committee on Allergens). Hence, the available information on fungal allergens indicate that majority of these allergens are highly conserved proteins.

Penicillium species have been reported as important indoor allergens [31,64,66]. Major allergens purified and characterized from various species of *Penicillium* are listed in Table 2. Of the several proteins, the 64-kDa and 68-kDa *Pencillium* antigens from *P. notatum* showed 40–56% IgE binding with asthmatic [31,63]. Another 33-kDa major allergen from *P. citrinum*, an alkaline serine proteinase exhibited homology with 33-kDa group of major allergens of *P. notatum* and *P. brevicompactum* [64,66]. Recently, an allergen demonstrating sequence homology and antigenic cross-reactivity to a human heat shock protein hsp 70 has been isolated from *P. citrinum* [61].

4. *Trichophyton* species

Proteins from the dermatophyte, *Trichophyton* that infect keratinized skin, nail, and hair, show immediate hypersensitivity (IH) and cell-mediated or delayed-type hypersensitivity (DTH) in allergic patients [31,76–78]. Furthermore, an association between chronic dermatophytosis and atopic symptoms including urticaria and asthma is now well es-

tablished [76]. The allergens purified and characterized from *Trichophyton* species are shown in Table 2 [77,78]. Tri r 4 exhibited 41–58% sequence homology with the prolyl oligopeptidase family of serine proteinases. Allergens with IgE binding specificity have also been reported from *T. tonsurans* [78].

5. *Candida albicans*

Although IgE reactivity to *C. albicans* has been reported, its role as a major inhalant allergen remains controversial [2,49]. In a recent study, a 44-kDa aspartic protease from *C. albicans* (CAAP) has been reported as an important allergen inducing IgE mediated mucosal allergy in asthmatic patients [2]. In another study, the epitopes binding to IgE antibodies are identified at the carbohydrate portion of the *C. albicans* phosphomannoprotein (CAMP) molecule [49]. The molecular cloning and characterization of a 40-kDa allergen from *C. albicans* with structural homology to alcohol dehydrogenase was reported by Shen et al. [62]. Ishiguru and associates investigated the IgE binding components of *C. albicans* by Western blot analysis and reported the frequent presence of IgE antibodies specific to enolase (46 kDa), phosphoglycerate kinase (43 kDa), and aldolase (36 kDa) in the sera from patients who showed IgE antibodies to *C. albicans* [36]. The characterization of candidal enolase antigenic motifs revealed that the C-terminal portion of the protein was highly immunogenic [20].

6. Basidiomycetes

A recombinant allergen of 16 kDa, Psi c2 from the mushroom *Psilocybe cubensis* with 78% homology to the enzyme cyclophilin (a cytosolic isomerase) from the fission yeast *Schizosaccharomyces pombe* has been identified [32]. Extract from *Boletus sp* another Basidiomycetes exhibited several protein bands in the range of 34–50-kDa reacting with IgE antibody of patients with asthma and allergy [30]. Immunoprints with a panel of sera from subjects showing SPT positivity to fungal allergens revealed at least 21 different allergens in *Calvatia* [30]. Allergenicity of extract from spores, caps, and stipes of *C. quadrifidus* has been reported. Two recombinant allergens (Cop c 1 and Cop c 2) were identified and characterized by screening conventional expression library and pJUFO library of *Coprinus* with sera from sensitized patients [30]. Table 2 shows the recombinant allergens purified and characterized from different species of Basidiomycetes.

7. *Malassezia furfur*

M. furfur extracts induced positive skin tests and leukocyte histamine release in subjects with atopic dermatitis [31]. Recently, three allergens from *M. furfur* have been cloned and characterized [60,81]. The 35.9-kDa Mal f 1 expressed as a fusion protein exhibited specific IgE binding with sera from patients with atopic dermatitis [60,81]. Recombinant allergens Mal f 2 and Mal f 3 showed sequence homology with peroxisomal proteins from *Candida boidinii* as well as with a major allergen Asp f 3 from *A. fumigatus* [79]. Recent evidence strongly suggests the involvement of the low molecular weight protein (9–15 kDa) from *M. furfur* in allergic reactions [31].

8. Cross reactivity

Although many of the known fungal allergens exhibit homology with allergens from more than one fungal species, only very few homologies have been reported with non-fungal allergens. Majority of the mold allergens reported so far are highly conserved proteins with diverse enzymatic activities such as enolase, aldehyde dehydrogenase (ALDH), aldolase, and acidic ribosomal proteins. The two allergens from *Alternaria* and *Cladosporium* Alt a 10 and Cla h 3 showed 77% identity and shared a homology of 86% [1]. There is also sequence homology between ALDH from *A. fumigatus* and Alt a 10 and Cla h 3 [1]. The allergens Alt a 6 and Cla h 4 and the ribosomal P2 proteins exhibit low levels of IgE antibody binding with sera from patients with mold allergy, although they demonstrate over 75% identity and 85% homology [1]. The minor allergens, Alt a 7 and Cla h 5 share high amino acid sequence homology (70%) with YCP4 proteins of *S. cerevisiae*, whereas enolase from *C. herbarum* (Cla h 6) showed high sequence homology with the enolases from *S. cerevisiae*, *A. alternata*, and *C. albicans* [13]. Recent studies have indicated that mannans are responsible for the immunologic cross-reactivity of *C. albicans* with other fungal species [49]. Enolase of *C. albicans* share common IgE epitope with the enolase of baker's yeast *S. cerevisiae* [37]. Similarly, the sequence homology at the protein level between enolases from *S. cerevisiae* and Cla h 6 from *C. herbarum* is reported as 83% [37].

In a cross inhibition study, the recombinant allergen Asp f 3 from *A. fumigatus* shared common IgE epitopes with two peroxisomal membrane proteins (PMPA and PMPB) of *C. boidinii* [27]. However, at the molecular level Asp f 3 showed 58% similarity and 36% identity with the two *Candida* proteins. Recently, two of the recombinant allergens Mal f 2 and Mal f 3 from the lipophilic yeast, *M. furfur* have been shown to share sequence homology with the peroxisomal proteins from *C. boidinii* and *A. fumigatus* allergen Asp f 3. On the other hand, aspartic protease from *C. albicans* shared structural homology to the acid protease Bla g 2 from cockroach, a nonfungal allergen [5]. Besides Asp f 1, nonfungal allergens such as grass pollens (Timothy grass pollen and a few other grass species) also demonstrated ribonuclease activity. However, functional homol-

Table 3
Fungal allergens with Cross-reactivity

Allergens and nature	Cross-reactivity
Peroxiaomal membrane protein	Asp f 3, Cand b, Pen c 3, Mal f 2, Mal f 3
Aldehyde dehydrogensi	Alt a 10, Cla h 3
Ribosomal protein P2	Alt a 6, Cla h 4, Asp f 8
Enolase	Alt a, Cand a, S. cerevisiae, Cla h 6
YCP4	Alt a 7, Cla h 5, S. cerevisiae
Heat shock protein	Alt a 3, Cla h, Asp f 12, Cand a, Pen c 1
Alkaline serine protease	Asp f 13, Asp fi 13, Asp o 13
	Pen b 13, Pen c 13, Pen n 13
Cyclophilin	Mal f 1, Psi c 2
Fibrinigen binding protein	Asp f 2, Asp nd 1, Cand a
Vacuolar serine Proteinase	Asp f 18, Asp n 18
	Pen n 18, Pen o 18

Cand b = *Candida boidinii*; Cand a = *Candida albicans*; Cla h = *Cladosporium herbarum*.

ogy between the fungal and pollen ribonucleases is yet to be defined [68].

Similarly, 68-kDa glycoprotein allergen from *P. notatum* demonstrated a 54% sequence homology with β-N-acetyl-glucosaminase from *C. albicans* [63]. The 40-kDa allergen, the alcohol dehydrogenase (ADH) from *C. albicans* share a 70% amino acid sequence homology with ADH isozyme 1 of *S. cerevisiae* [62]. The 34 kDa major allergen, alkaline serine proteinase from *A. flavus* (Asp fl 13) reported to share IgE cross-reactivity with serine proteinase from *A. oryzae* (Asp o 13) and from *P. citrinum* (Pen c 13) [64,65]. Major IgE cross-reactivity was demonstrated by immunoblot among the 33-kDa allergens of *P. citrinum, P. notatum,* and *P. brevicompactum* [66]. A cDNA clone of *P. citrinum* representing a partial sequence homology to human Hsp 70 protein also exhibited allergenic properties [61]. Cross-reactive fungal allergens with various functional properties are shown in Table 3. The IgE inhibition studies have also demonstrated cross-reactive epitopes among the conserved allergens from these fungi, although this may be due to co-sensitization of different allergens by allergic patients.

9. Allergen epitopes

Epitope is defined as the sites or regions of an allergen molecule that interact with T- and B-cells of the immune system. The allergens interact with the immunoglobulin E as the B-cell epitope, while it is presented to T-cells after ingestion or processing by antigen presenting cells as peptide fragments linked to major histocompatibility (MHC) molecule as the T-cell epitopes. Epitope mapping studies have been carried out using T-cell clones and peripheral blood mononuclear cell (PBMC) lines from patients with pollen, mite, cat, and bee venom induced allergies. The results indicate that T-cell epitopes are dispersed throughout the allergen molecules [69]. By studying the T-cells from a large panel of patients it would be possible to identify the major immunodominant regions of the allergens.

10. T-cell epitope prediction

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 and binding of the MHC antigen complex to the TCR triggers the activation of T-cell. T-cell epitopes presentation to the TCR involves MHC class II molecules and is presented frequently by single HLA molecule such as DR, DQ, or DP [16,17]. In vitro studies conducted using overlapping synthetic peptides demonstrated that T-cell lines of some of the patients recognize several epitopes within the same allergen. 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 [35]. Based on the physicochemical studies of peptides and their functional activities, a number of T-cell epitopes have been identified. From these results, algorithms have been developed to predict the presence of peptide sequences with T-cell stimulating activity from the primary structure of the protein [23,69]. Such predictors include α-helical periodicity and presence of amphipathic segments in addition to the primary structure of the protein [23]. The successful prediction of T-epitopes, therefore, includes high-affinity binding features, antigen processing, presentation, and competitive activation [35,38]. Hence, only a combined application of prediction methods with empiric approaches of assessing immunogenicity of T-cells can provide the most satisfactory results.

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* [17,43,44]. The Asp f 1 specific T-cell lines established from ABPA have been characterized as $CD4^+$ Th2 like in their cytokine secretion pattern [40]. 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 46–65 and 106–125 and restricted by HLA-DR2 and HLA-DR5 alleles [16,17]. The major T-cell and B-cell epitopes of the *A. fumigatus* allergens are shown in Table 4. In a mouse model of ABPA, epitopes of Asp f 1 demonstrated differential induction of $CD4^+$ Th2 and $CD4^+$ Th1 cells based on the cytokine patterns [44]. T-cell lines established from subjects with IH to protein IV of Trichophyton showed a cytokine profile of Th2/Th0 cells, whereas cell lines from individuals with DTH to the same protein generally exhibited a Th1 profile [76]. The results confirmed that a single defined Trichophyton protein can elicit distinct

Table 4
Human T-cell and B-cell epitopes of *A. fumigatus* allergens, Asp f 1, and Asp f 2

Allergen sources/ allergen	Size	T-cell epitopes	T-cell sources	IgE binding epitopes	Reference
Aspergillus fumigatus					
Asp f 1	16.9 kD 148 amino acids	1–20, 48–65, 61–80 91–110, 106–125,	*TCC	—	[17]
Asp f 1	—	36–51, 53–68, 69–84, 115–130, 135–146	PBMCs	—	[43]
Asp f 1	—	—	—	40–48, 76–102, 116–130, 140–149	[43]
Asp f 2	37 kD 268 amino acids	—	—	16–22, 51–55, 96–98, 113–117, 139–143, 147–150, 158–162, 189–194, 254–264	[8]

* T-cell clones.

T-cell cytokine profiles in patients depending on their immunologic status. 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.

11. B-cell epitopes

B-cell epitopes of the allergens may be sequential (continuous or linear) and topographical (discontinuous) [56]. 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 are linearly placed in the sequence of the protein [12,56]. The identification of sequential epitopes is readily achieved by the systematic synthesis of all the possible short peptides homologous with 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 [12]. In several studies, molecular biology approaches such as expression of allergen portions fused to β-galactosidase or other expression vectors as well as random expression of cDNA fragments were used to identify the conformational B-cell epitopes of the allergens [7,71].

The identification of B-cell epitopes should be approached in several different ways as the majority of the B-cell epitopes are structurally three-dimensional (3-D) in their native and functional forms. When the three dimensional structure is disturbed, the antibody binding reactivity disappears or weakens because of the altered conformational changes in the molecule. Hence, for the delineation of B-cell epitope, several integrated approaches including physicochemical methods, statistical approaches, and application of information techniques are essential [29,47,75]. From the available 3-D structures, statistical models have been developed to identify the location of epitopes [21,24, 46,48]. These techniques examine the frequencies with which an amino acid residue occurs within a known epitope region or secondary structure or surface exposed region of the protein. The physicochemical methods used for the identification of polar or nonpolar amino acids in the molecule may provide a better understanding on the antigen binding to the antibody. Other predictors including hydrophilic, flexible, surface exposed (accessible) protein regions, and localization of secondary structures (β-turns) are postulated as essential for antibody binding [4,70]. Thus, successful prediction of B-cell epitopes needs complex algorithms combining all these predictors [12,26].

Three-dimensional structure of fungal allergens is not known until now. However, considerable progress has been made in the structural analysis of pollen allergens [52]. The NMR spectroscopy of major ragweed allergen Amb a 5 revealed a C-terminal α-helix with two to three β-strands, whereas birch pollen allergen Bet v 1 consists of seven stranded anti-parallel β-sheet that wrapped around a C-terminal α-helix [25,52]. The highly conserved birch pollen allergen profilin demonstrated β- and α-helicity comparable with mammalian profilin [22]. However, three-dimensional allergen structures obtained so far do not reveal conserved structural motifs among unrelated allergens. As only certain portions of a protein are exposed on its surface, it has been assumed that IgE epitopes are less diverse than T-cell epitopes. Of particular interests are the ongoing attempts to

Table 5
Sequence homology of identified IgE epitopes of *Aspergillus* and *Candida albicans*

Epitope	Sequence			
	Asp f 2 (*A. fumigatus*)	Asp n d1 (*A. nidulans*)	Fibrinogen binding protein (*C. albicans*)	pH-regulated protein (*C. albicans*)
1	ATQRRQI	ATEQRQL	ATQYNQL	ATQYNQL
2	RKYFG	RKYFG	RKYFG	RKYFG
3	HWR	HWR	YWR	YWR
4	YTTRR	YTRR	FVTRR	FVTRR
5	ASDLM	AGDLL	AGDLL	AGDLL
6	YHVP	YHMP	WHLK	WHLK
7	DHFAD	EHYAD	EHYAD	EHYAD
8	ALEAYA	ALEVYA	ALDVYA	ALDVYA
9	THEGGQ	THEGGE	THADGE	THADGE

identify precisely the allergen surface area and IgE interaction sites by cocrystallization of allergens and specific IgE-Fabs, obtained from combinatorial libraries of patient's lymphocytes [67].

We reported the presence of both sequential and conformational epitopes in Asp f 2, a major allergen from *A. fumigatus* [8]. The epitope mapping of linear peptides synthesized on derivatized cellulose membranes demonstrated distinct IgE binding epitope of three to seven amino acids throughout the molecule (Table 4). 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 *A. fumigatus* is also conserved in the proteins from *Candida* (Table 5). Two immunodominant epitopes of Asp f 1, another major allergen from *A. fumigatus* 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 [43].

Epitopes associated with protective immunity in candidasis has been elucidated by the IgG antibody binding to epitopes of candidal HSP-90. The role of these epitopes in providing protective immunity in experimental models of candidasis have been studied [3]. Auto-reactive antibodies to shared epitopes on *C. albicans* and human HSP 90 have been reported in patients recovering from systemic candidasis. Epitope mapping of human heat shock protein 90 with sera from a range of infected patients have shown that patients with malaria also recognized similar epitopes as those with invasive aspergillosis and candidasis. Monoclonal antibody raised against one of the immunodominant epitopes of *C. albicans* HSP 90 (aa 406–411; LKVIRK) conferred a degree of protection in a mouse model of disseminated candidasis [50].

12. Application of recombinant allergens and their epitopes

Recombinant allergens are valuable tools to investigate the T-cell and B-cell involvement in allergic process as well as to study the pathogenesis of the disease [33,38]. A number of recombinant fungal allergens from various sources with structural and biologic properties comparable to the native counter parts have been reported [71,72,74]. The primary structure determination at the amino acid sequence level demonstrated extensive homology among the allergens and hence, it seems that the number of epitopes needed for diagnosis and specific therapy is less diverse than originally anticipated. Allergen specific therapy may aim for the prophylaxis of atopy, the induction of tolerance or to the modification of ongoing immune responses [34]. In an approach to induce T-cell nonresponsiveness in patients, attempts have been directed to develop strategies where peptides representing major T-cell epitopes are administered to induce T-cell tolerance and anergy [54,59,71,72].

Specific immunotherapy of Type I allergy should be based on reducing allergic reactions to those allergens which are recognized by the patients' IgE antibodies. The establishment of patients' IgE reactivity profile (allergogram) with recombinant allergens will be of value in selecting the components against which a substantial IgE response is mounted [71,73]. Selection of allergens according to the patients' allergogram might improve therapeutic specificity by avoiding de novo sensitization against unwanted components. On the other hand, immunodominant IgE binding hapten, as has been identified for grass pollen allergen Phl p 1, can be used for local saturation of IgE-producing cells to prevent subsequent activation by exposure to the complete allergen [7]. Another potentially significant report where human IgE Fabs with specificity for the grass pollen allergen Phl p 5 have been obtained from a combinatorial IgE library from an allergic patient [67]. Using recombinant DNA technology, we expressed a polypeptide fragment of a major allergen Asp f 2 without IgE antibody binding to sera from *A. fumigatus*-sensitized patients [8]. This allergen fragment with disrupted conformational IgE epitopes, but intact T-cell epitopes might be explored further for immunotherapy in patients sensitized to *A. fumigatus*. Isoforms of major allergens were also identified, which showed very low IgE binding capacity, but possessed strong T-cell stimulating sequences [73]. Such isoforms may be explored for inducing immune deviation from a Th2 to Th1 cytokine pattern to reduce or alleviate the allergic response.

13. Conclusions

In this review, we have presented the available informations on allergenic epitopes of mold proteins and elucidated

the contribution of molecular biology technology in understanding the structure-function relationship of fungal allergens. Majority of the clinically important allergens have been identified and the physicochemical properties of these allergens have been studied. These well-characterized allergens contributed in the improved diagnosis and may provide novel immunotherapeutic approaches to control fungal allergic diseases. The extensive sequence homology as well as functional similarities among allergens from different molds may facilitate diagnosis of mold allergy by use of a few representative cross-reactive allergens. 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 by using either synthetic peptides, recombinant allergens and mutated and other modified allergens.

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