Review

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2002;129:181–188
DOI: 10.1159/000066780

Immunobiology of Fungal Allergens

Viswanath P. Kurup[a, b] Horng-Der Shen[c] Hari Vijay[d]

[a]Allergy-Immunology Division, Medical College of Wisconsin, and [b]Research Service, VA Medical Center, Milwaukee, Wisc., USA; [c]Department of Medical Research, Veterans General Hospital, Taipei, Taiwan/ROC and [d]Environmental Health Directorate, Health Canada, Ottawa, Ont., Canada

Abstract

In recent years, considerable attention has been paid in obtaining purified relevant allergens from fungi associated with allergy. Using molecular biology techniques, a number of mold allergens have been obtained by cloning the genes encoding the allergens. Currently, about 70 fungal allergens have been approved by the International Allergen Nomenclature Committee. In this review, we have presented major allergens from *Aspergillus, Penicillium, Cladosporium*, and *Alternaria* and discussed their immunochemical characteristics and their role in the diagnosis of allergy and possible usefulness in understanding the pathogenesis of the disease. The structure-function properties and the potential role of these recombinant allergens in the immunomodulatory therapy also are presented.

Copyright © 2002 S. Karger AG, Basel

Introduction

Atopic allergy affects over 25% of the population in the industrialized world. Allergens associated with the disease include environmental allergens such as pollen, food, house dust mites, animal dander, insect venom, and various fungal spores. The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis [1–5]. Clinically the presenting symptoms are sneezing, nasal discharge, coughing, wheezing, and shortness of breath with evidence of reversible airway obstruction, urticaria, angioedema, and anaphylaxis. Mold allergy constitutes 25–30% of all allergic asthma cases [6–8]. Allergy develops upon sensitization with allergens from various sources [9]. Patients sensitized to the allergens demonstrate immunological and inflammatory responses involving both arms of immunity, namely T- and B-cell-mediated responses. The mechanism involved in the disease is extremely complex and is dependent on the nature of allergens, host genetical makeup, underlying diseases, and to duration of exposure to allergens [10].

Mold Allergens

Fungi are eukaryotic non-chlorophyllus organisms present both indoor and outdoor air. Fungi are predominantly saprophytic but rarely parasitize humans, animals, and plants and their concentration outdoors depends on climatic and other environmental factors including vegetation. The types of fungi present indoors and their prevalence depend on the moisture content, ventilation, and the presence and absence of carpets, pets, and houseplants [11]. Fungi grow on most substrates even in adverse conditions. The fungal spores outdoors exist throughout the year and at times exceed that of pollens.

The spore counts in outdoor and indoor air vary considerably depending on various environmental and other factors [12]. The indoor fungi constitute a mixture of spores which have entered from outdoors and those grow

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2002 S. Karger AG, Basel
1018–2438/02/1293–0181$18.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Viswanath P. Kurup
VA Medical Center, Research Service 151-I
5000 West National Ave., Milwaukee, WI 53295 (USA)
Tel. +1 414 384 2000/ext. 41459/41510, Fax +1 414 382 5374
E-Mail vkurup@mcw.edu

and multiply indoors [13, 14]. *Aspergillus* and *Penicillium* are less common outdoors and are usually considered the major indoor fungi. Although *Alternaria* is a major outdoor mold, it has been reported from exclusive indoor habitats. Other fungi like *Cladosporium, Epicoccum, Fusarium*, etc. have been isolated from both indoor and outdoor environments [9]. Not much information is available on the effect of indoor or outdoor spore concentrations and allergic symptoms, although excess spore concentration may result in the increased prevalence of respiratory symptoms such as bronchitis and asthma [6].

Aerobiological assessment of outdoor and indoor fungal spores and their identification are necessary to determine their role in causing allergy. Surveys conducted in different parts of the world and in vitro and in vivo diagnostic tests such as antibody assays and skin tests identify some of the most predominant fungi associated with allergy. The important fungi include *Alternaria, Aspergillus, Penicillium, Cladosporium, Fusarium*, and *Epicoccum* with a number of other fungi with a definite role in allergy. Antigens from these predominant fungi have been used for antibody assays as well as for immunotherapy [6, 9].

Nature of Mold Allergens

Allergens are proteins, glycoproteins or carbohydrates capable of stimulating the immune system and bind specifically to IgE antibody produced in atopic individuals in response to stimulation [15, 16]. The antigen exposure may be via inhalation, skin contact, enteral or parenteral introduction. There are over 300 plant and animal allergens reported and these allergens comprise multiple molecules of diverse physical, chemical and functional characteristics. Although strong cross-reactivity exists among some of these allergens, over 50% of the total IgE in the patients are directed towards a single plant, animal or mold allergen. In recent years, a number of allergens from pollens, house dust mites, cockroaches, and fungi have been cloned and expressed [17, 18].

Our knowledge of the allergens and their structure, function, and interactions with the immune system has increased greatly in recent years [17, 19]. This information has been the result of sophisticated techniques available for the study of protein structures. 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 [20]. Furthermore, the knowledge on amino acid sequences facilitated the understanding of the structure-function properties of the allergens. At present, the complete amino acid sequences of about 70 mold allergens have been known. This number of well-characterized allergens is rapidly increasing, although a large number of clinically relevant allergens still have not been properly identified, purified or characterized [17, 19, 21]. Nevertheless, the knowledge of biochemical and physicochemical characteristics of the known allergens are not sufficient enough to differentiate allergens from the nonallergic 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 (a) hydrolytic enzymes such as proteases, (b) non-hydrolytic enzymes such as enolases, (c) inhibitors such as trypsin amylase inhibitors, (d) transport proteins such as lipocalins, and (e) regulatory proteins such as heat-shock proteins. However, proteins with unknown biochemical activities still constitute the majority of the allergens [21].

Characterization of Fungal Allergens

Purified, natural, and recombinant allergens are valuable tools in understanding the T- and B-cell involvement in the allergic process as well as in the elucidation of the pathogenesis of the disease [17]. A number of recombinant fungal allergens with structural and biological properties comparable to the native counterparts have been reported [22]. The primary structure at the amino acid sequence level demonstrates extensive homology among the allergens and hence, it appears that the number of epitopes needed for diagnosis and specific therapy is less diverse than originally anticipated. The various allergens identified from the major fungi associated with allergy and approved by the International Allergen Nomenclature Committee of the International Union of Immunological Societies are shown in table 1 [23].

Alternaria alternata

Alternaria alternata, a member of the imperfect fungi, is one of the most important among all allergenic fungi [24–26]. Most of 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 spectra [27]. Airborne fungal spores of *Alternaria* and *Cladosporium* are found throughout the world. The airborne spores of the fungi are generally considered to be important causes

of both allergic rhinitis and allergic asthma. Most fungi including *A. alternata* and *Cladosporium herbarum* have a seasonal spore-releasing pattern [7]. Recently, *Alternaria*, a predominantly outdoor fungi, has been reported in house dust samples in the absence of environmental mold spores [28]. *Cladosporium* has also been found both indoors and outdoors, in winter, and late spring [29]. The fungal spore count in outdoor air is usually about 230/m^3 while indoor count may vary from 100 to 1,000/m^3. A spore count of 10–100/m^3 is a substantially high antigen load to exposed individuals.

Several allergens from *A. alternata* have been purified and characterized either by conventional fractionation or by employing molecular biology techniques (table 1). Alt a 1, a major allergen of *Alternaria*, binds to IgE of more than 80% of asthmatic patients with allergy to this fungus. A number of variants and isoforms of Alt a 1 have been reported [24, 25]. A reduced form of Alt a 1 produces a doublet pattern on SDS-PAGE with a molecular weight of 14.5 and 16.0 kD. In immunoblots with human atopic serum, this doublet was confirmed as IgE-binding [30, 31].

Achatz et al. [24] described three additional cDNA clones encoding functionally active *A. alternata* allergens. Alt a 7, the 22-kD allergen, demonstrated over 70% sequence homology with the YCP4 protein of *Saccharomyces cerevisiae*, while Alt a 6, the 11-kD protein, demonstrated homology with ribosomal P2 protein. However, these allergens reacted diversely with IgE from sera of allergic patients.

Cladosporium herbarum

C. 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 climate, while *Cladosporium* is the leading allergenic mold observed in cooler climates. About 60 antigens from *C. herbarum* have been identified by crossed immunoelectrophoresis and about 36 of them have been shown to react with IgE from patients' sera by crossed radioimmunoelectrophoresis [32]. Three major *C. herbarum* allergens have been purified and characterized (table 1). Cla h 1 is a small 13-kD acidic allergen composed of five isoallergens (pI 3.4–4.4), and Cla h 2, a slightly larger molecule with a size of 23-kD, is a glycoprotein (pI 5.0) with over 80% carbohdydrates [33, 34]. The protein part retained the IgE binding property even after removing the carbohydrate from this allergen. Cla h 4, the ribosomal P2 protein, is a low-molecular-weight (11-kD) acidic allergen (pI 3.94) with high alanine and serine content, and shares 60% sequence homology with ribosomal P2 protein [35].

Table 1. Mold allergens approved by the Allergen Nomenclatural Committee*

Fungus	Molecular size, kD	Biological activity	Sequence accession No.
Alternaria alternata			
Alt a 1	28		U82633
Alt a 2	25		U62442
Alt a 3		Heat-shock protein 70	U87807
			U87808
Alt a 4	57	Protein disulfidisomerase	X84217
Alt a 6	11	Acidic ribosomal protein P2	X-78222
			U-87806
Alt a 7	22	YCP4 protein	X-78225
Alt a 10	53	Aldehyde dehydrogenase	X-78277
			P-42041
Alt a 11	45	Enolase	U82437
Alt a 12	11	Acidic ribosomal protein P1	X84216
Cladosporium herbarum			
Cla h 1	13		*
Cla h 2	23		*
Cla h 3	53	Aldehyde dehydrogenase	X-78228
Cla h 4	11	Acidic ribosomal protein P2	X-78223
Cla h 5	22	YCP4 protein	X-78224
Cla h 6	46	Enolase	X-78226
Cla h 12	11	Acidic ribosomal protein P1	X85180
Aspergillus fumigatus			
Asp f 1	18	Ribonuclease	M-83781
			S-39330
Asp f 2	37		U-56938
Asp f 3	19	Peroxisomal protein	U20722
Asp f 4	30		AJ001732
Asp f 5	42	Metalloproteinase	Z-30424
Asp f 6	26.5	Mn superoxide dismutase	U53561
Asp f 7	12		AJ-223315
Asp f 8	11	Ribosomal protein P2	AJ224333
Asp f 9	34		AJ223327
Asp f 10	34	Aspartic proteinase	X85092
Asp f 11	24	Peptidyl-prolyl isomerase	*
Asp f 12	90	Heat-shock protein P90	*
Asp f 13	34	Alkaline serine proteinase	*
Asp f 15	16		AJ002026
Asp f 16	43		g3643813
Asp f 17			AJ224865
Asp f 18	34	Vacuolar serine proteinase	*
Asp f 22w	46	Enolase	AF284645
Penicillium chrysogenum			
Pen ch 13	34	Alkaline serine proteinase	AF321100
Pen ch 18	32	Vacuolar serine proteinase	AF263454
Pen ch 20	68	N-acetylglucosaminidase	AF144753
Penicillium citrinum			
Pen c 3	18	Peroxisomal membrane protein	AF084546
Pen c 13	33	Alkaline serine proteinase	*
Pen c 19	70	Heat-shock protein P70	U64207
Pen c 22w	46	Enolase	AF254643

*http://www.allergen.org (IUIS Allergen List).

Breitenbach et al. [36] recently reported purified recombinant *Cladosporium* enolase (Cla h 6; 48 kD) having strong binding to IgE antibodies in 20% of *Alternaria*-allergic patients by immunoblots. Enolase has been found to be a highly conserved major allergen in most fungi, and may contribute to allergen cross-reactivity in mold allergy. About 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 [35].

Aspergillus *Species*

Aspergillus species are saprophytic fungi widely distributed in nature and are associated with a number of human diseases [37, 38]. Several recombinant allergens from *A. fumigatus* have been identified and purified from cDNA and phage display library of *A. fumigatus* (table 1). The majority of these proteins showed specific binding to IgE from asthmatic and 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, which inhibits protein translation, was shown to be toxic to EBV-transformed or PHA-stimulated PBMCs [39]. This allergen showed positive skin test reactivity in 80% of ABPA and 50% asthmatic patients. This allergen also demonstrated IgE antibody in 68–83% of patients with skin test positivity to *Aspergillus* allergens [40]. However, because of the high toxicity and reactivity with skin-test-positive asthmatics and some normals, the usefulness of this allergen in the diagnosis is questioned. This allergen demonstrated 13 linear epitopes binding to IgE. Asp f 1 also showed Th1- and Th2-specific epitopes when studied in a murine model of allergic aspergillosis. Asp f 3, a 18.5-kD peroxisomal protein with homology to *Candida boidinii* peroxisomal protein showed skin test reactivity and bound to serum IgE of ABPA patients. Recently it was found that this allergen is valuable in serodiagnosis of ABPA. The epitope and structure analysis indicates that the 10 'N' terminal amino acids and 'C' terminal 143–150 amino acids of ASP f 3 are important in the binding to IgE in the sera of ABPA patients [41].

Asp f 4 is an allergen with unknown biochemical function and is an intracellular protein that showed strong reactivity with IgE from the sera of both asthmatics and cystic fibrosis patients with ABPA [40, 42]. Asp f 5, a metalloproteinase, demonstrated skin test reactivity in over 70% of allergic asthmatics without ABPA while it showed reactivity in over 90% of ABPA patients. Although its diagnostic significance is not clear, the potential as an indicator for exposure to *Aspergillus* is well recognized. Asp f 6, manganese superoxide dismutase (MNSOD), has strong homology to MNSOD of human, bovine, fruit fly, gum tree, yeast, and *Escherichia coli* enzymes. Asp f 6 reacted positively in skin test 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 [40].

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 is an aspartic protease that showed only low prevalence of IgE binding in ABPA patients. Asp f 11, a cyclophilin, has homology with similar proteins from *Malassezia furfur* that showed reactivity with IgE from 50% of ABPA patients. The heat-shock protein Asp f 12 has homologous counterparts in *Candida albicans*, Saccharomyces, Trypanosoma, housefly, mouse, and humans because of the extremely conserved HSP gene.

Asp f 16 has no known biological functions that showed strong binding to IgG from ABPA patients. This antigen showed sequence homology with Asp f 9 and a membrane protein from Saccharomyces. There are few other minor allergens isolated from *A. fumigatus* and related *Aspergillus* species which demonstrated binding to IgE antibody from ABPA and allergic asthma patients (table 1). Several of these *A. fumigatus* allergens also exhibited high-sequence homologies with the known functional proteins and enzymes [40, 42, 43]. Alkaline serine proteinases with allergenic properties such as Asp f 13, Asp fl 13, and Asp o 13 from *A. fumigatus, A. flavus*, and *A. oryzae* respectively have been reported [44, 45]. 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* [46, 47]. Recently, another group of homologous vacuolar serine proteinases Asp f 18, Asp n 18, Pen ch 18, and Pen o 18 with conserved sequence have been reported from *Aspergillus* and *Penicillium* [45, 48]. *A. flavus* extracts demonstrated IgE antibody binding in 44% asthmatic patients studied by immunoblotting [49]. Recently, a 34-kD alkaline serine proteinase, Asp fl 13 with significant IgE antibody binding, was purified and its enzyme activity ascertained [49].

Penicillium *Species*

Species belonging to the genus *Penicillium* are prevalent indoor fungi [1, 2]. Inhalation of *Penicillium* spores in

quantities comparable with those encountered by natural exposure can induce both immediate and late asthma in sensitive persons [14]. Among more than a hundred different *Penicillium* species, *P. citrinum*, together with *P. chrysogenum (P. notatum), P. oxalicum, P. brevicompactum*, and *P. spinulosum*, were the five most frequently recovered species of *Penicillium* in the USA, while *P. citrinum* was the most prevalent *Penicillium* species reported from Taiwan [50, 51].

About 12 antigens from *P. citrinum* and 11 antigens from *P. chrysogenum* have been shown to react with IgE from patients' sera by immunoblotting [52]. Recently, several *Penicillium* allergens have also been characterized at the molecular level (table 1). Among the *Penicillium* allergens, the 32–34 kD alkaline and/or vacuolar serine proteases were identified as the major allergens of *P. citrinum, P. brevicompactum, P. chrysogenum*, and *P. oxalicum* [45, 46]. They have been designated as group 13 for alkaline serine protease and group 18 for vacuolar serine protease allergens as recommended by the Allergen Nomenclature Subcommittee [23, 45]. Immunoblotting data showed that IgE antibodies against components of these prevalent *Penicillium* species could be detected in the sera of about 16–26% of the asthmatic patients [45]. The majority of the positive serum samples tested showed IgE binding to the 32–34 kD-serine proteinase(s) with a frequency >80% in different fungal species tested. The cDNAs of the alkaline serine protease allergens 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) have recently been isolated [47, 48, 53]. The mature Pen ch 13 allergen is formed by the removal of the preprosequence of the precursor molecule [47]. Besides N-terminal cleavage, the mature Pen c 18 and Pen o 18 also undergo C-terminal processing [48]. The IgE cross-reactivity between the allergens in *Penicillium* and *Aspergillus* species has been detected [44, 46–49, 52, 54]. 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 [47].

Besides the serine protease allergens, a 68-kD allergen N-acetylglucosaminidase and an allergenic heat-shock protein belonging to the HSP-70 family have also been identified from *P. chrysogenum* and in *P. citrinum*, respectively [55, 56]. They have been designated by the Allergen Nomenclature Subcommittee as Pen ch 20 and Pen c 19, respectively [23]. An 18-kD peroxisomal membrane protein (Pen c 3), similar to Asp f 9, and enolase (Pen c 22), similar to Asp f 22, were also identified from *P. citrinum* [57, 58]. Cross-reacting IgE antibodies have been reported against these allergens [57, 58].

Other Fungi

An aerobiological study performed in different countries demonstrated the presence of *Botrytis, Phoma, Helminthosporium, Fusarium* and *Epicoccum*. In addition to these fungi, *Saccharomyces, M. furfur, C. albicans* and *Trichophyton* species that invade the skin have been implicated in allergic diseases [6, 9, 59–63]. Several mushrooms and other *Basidiomycetes* spores have also been implicated in fungal allergy [59].

Epitopes

Epitopes are sites or regions of an allergen molecule that interact with T and/or B cells of the immune system. The allergens interacting with immunoglobulin E are the B-cell epitope, while 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 type 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 a single HLA molecule such as DR, DQ or DP [64, 65]. The minimum peptide length that can bind and initiate a T-cell response is between 7 and 15 residues, although peptides with >15 residues are also presented efficiently [66]. 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* [65–67]. The Asp f 1-specific T-cell lines established from ABPA have been characterized as $CD4^+$ Th2 cells based on their cytokine secretion pattern [68]. 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 [64, 65]. 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 [69]. T-cell clones were generated from ABPA patients against Asp f 2. Stimulation of these T-cell clones and PBMCs using synthetic peptides from Asp f 2 sequence identified aa 54–74 as a major T-cell-binding site. These epitopes were further defined and it was found that T-cell clones stimulated with aa 60–74 secreted IL-5, but not IFN-γ [70].

B-Cell Epitopes

B-cell epitopes of the allergens may be sequential (linear) and topographical (conformational). Linear epitopes are short sequences of amino acids up to eight residues located and exposed on protein surfaces where the adjacent hydrophilic amino acids contribute to the interaction with antibody [71]. 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.

Both sequential and conformational epitopes have been identified in Asp f 2, a major allergen from *A. fumigatus* [72]. The epitope mapping of linear peptides synthesized on derivatized cellulose membranes demonstrated distinct IgE-binding epitope of 3–7 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 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 [67].

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. In recent years, 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 [73, 74]. However, only very few studies have been conducted with mold allergens. The structure-function response of one of the major allergens of *Aspergillus*, Asp f 2, was studied after engineering the allergen using molecular techniques. Deletion of 'N', 'C', and 'NC' terminal 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. Post-glycosylation of the protein demonstrated less IgE binding, but more IgG binding compared to non-glycosylated Asp f 2 [75]. The role of cysteine residues in Asp f 2 was studied and the results show that cysteines at positions C264 and C204 are essential for the IgE binding [76].

Very little is known about the three-dimensional (3-D) structure of the mold allergens. The 3-D structure is suggested for restrictocin, a closely related ribotoxin belonging to the Asp f 1 family [77]. It has three α helical turns and five stranded anti-parallel β sheets. Recently, Asp f 6 (MNSOD) from *A. fumigatus* was studied and the 3-D structure was elucidated [78]. It has a homotetrameic structure with four manganese ions and 745 water molecules and has conserved exposed amino acids with potential cross-reactivity with other MNSODs. Thus, the 3-D structure of allergens will be of value in determining the cross-reactivity and specificity of the allergen.

Specific Immunotherapy

Specific immunotherapy for fungal allergy is questionable due to several reasons and concerns. Specific immunotherapy for 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. Selection of allergens according to the patients' allergogram might, thus, improve both diagnostic and therapeutic specificity of the allergens [79]. Allergograms may also help in monitoring the effctiveness of immunotherapy. With the availability of recombinant allergens, structure-function studies may yield valuable information in understanding immunopathogenesis, which may contribute towards immunomodulatory therapy.

References

1 Kurup VP, Fink JN: Fungal allergy; in Murphy JW, Friedman H, Bendinelli M (eds): Fungal Infections and Immune Responses. New York, Plenum Press, 1993, pp 393–404.
2 Burge HA: Airborne-allergenic fungi. Immunol Allergy Clin North Am 1989;9:307–319.
3 Greenberger PA: Allergic bronchopulmonary aspergillosis and fungoses. Clin Chest Med 1988;9:599–608.
4 Katzenstein AA, Sale SR, Greenberger PA: Allergic *Aspergillus* sinusitis: A newly recognized form of sinusitis. J Allergy Clin Immunol 1983; 72:89–98.
5 Kurup VP: Hypersensitivity pneumonitis due to sensitization with thermophilic actinomycetes; in Solomon WR (ed): Airborne Allergens. Immunology and Allergy Clinics of North America. Philadelphia, Saunders, 1989, pp 285–306.
6 Vijay HM, Thaker AJ, Banerjee B, Kurup VP: Mold allergens; in Lockey RF, Bukantz SC (eds): Allergens and Allergen Immunotherapy. New York, Dekker, 1998, pp 133–154.
7 Portnoy J, Chapman J, Burge H, Muielenberg M, Solomon W: Epicoccum allergy. Skin reaction patterns and spore/mycelium disparities recognized by IgG and IgE ELISA inhibition. Ann Allgery 1987;59:39–43.
8 Wuethrich B: Epidemiology of allergic diseases: Are they really on the increase. Int Arch Allergy Appl Immunol 1989;90:3–10.
9 Kurup VP, Shen HD, Banerjee B: Respiratory fungal allergy. Microbes Infect 2000;2:1101–1110.
10 Kurup VP: Immunopathogenesis of fungal induced asthma and allergy. Proceedings of International Symposium on Recent Advances in Molecular Biology, Allergy, Immunology, Baroda, India, Sept 3–5, 2000, pp 81–94.
11 Gravesen S: Microfungal contamination of damp buildings: Biological aspects; in Johanning E (ed): Bioaerosols, Fungi and Mycotoxins. New York, Eastern New York Occupational and Environmental Health Center, 1999, pp 505–511.
12 Kozak PP Jr, Gallup J, Cummins LH, Gilman SA: Factors of importance in determining the prevalence of indoor molds. Ann Allergy 1979;43:88–94.
13 Burge HA: Fungus allergens. Clin Rev Allergy 1985;3:319–329.
14 Licorish K, Novey HS, Kozak P, Fairshter RD, Wilson AF: Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. J Allergy Clin Immunol 1985;76:819–825.
15 Bierman WC, Van Arsdel PP Jr: Clinical evaluation of the patient with allergic and immunologic disease; in Lockey RF, Bukantz SC (eds): Principles of Immunology and Allergy. Philadelphia, Saunders, 1987, pp 1–26.
16 Blumenthal MN, Rosenberg A: Definitions of an allergen (immunobiology); in Lockey RF, Bukantz SC (eds): Allergens and Allergen Immunotherapy. New York, Dekker, 1999, pp 39–51.
17 Scheiner O, Kraft D: Basic and practical aspects of recombinant allergens (review). Allergy 1995;50:384–391.
18 Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A: Recombinant allergens for diagnosis and therapy of allergic disease. J Allergy Clin Immunol 2000;106:409–418.
19 Valenta R, Steinberger P, Duchene M, Kraft D: Immunological and structural similarities among allergens: Prerequisite for a specific and component-based therapy of allergy. Immunol Cell Biol 1996;74:187–194.
20 Wilson IA, Stanfield RL: Antibody-antigen interactions: New structures and new conformational changes. Curr Opin Struct Biol 1994;4: 857–867.
21 Stewart GA, Thompson PJ: The biochemistry of common aeroallergens. Clin Exp Allergy 1996;26:1020–1044.
22 Valenta R, Flicker S, Eibensteiner PB, Steinberger P, Laffer S, Doleck C, Kraft D: Recombinant allergen-specific antibody fragments: Tools for diagnosis, prevention and therapy of type I allergy. Biol Chem 1997;378:745–749.
23 Official list of allergens: IUIS Allergen Nomenclature Subcommittee, http://www.allergen.org.
24 Achatz G, Oberkofler H, Lechenauer E, Simon B, Unger A, Kandler D, Ebner C, Prillinger H, Kraft D, Breitenbach M: Molecular cloning of major and minor allergens of *Alternaria alternata* and *Cladosporium herbarum*. Mol Immunol 1995;32:213–227.
25 DeVouge MW, Thaker AJ, Curran IHA, Zhang L, Muradia G, Rode H, Vijay HM: Isolation and expression of cDNA clone encoding *Alternaria alternata* Alt a 1 subunit. Int Arch Allergy Immunol 1996;111:385–395.
26 DeVouge MW, Thaker AJ, Zhang L, Muradia G, Rode H, Vijay HM: Molecular cloning of IgE binding fragments of *Alternaria alternata* allergens. Int Arch Allergy Immunol 1998;116: 261–268.
27 Kendrick B: The history of conidial fungi; in Cole GT, Kendrick B (eds): Biology of Conidial Fungi. New York, Academic Press, 1981.
28 Becker AB, Muradia G, Vijay HM: Immunoreactive *Alternaria* allergens in house dust in the absence of environmental mold (abstract). J Allergy Clin Immunol 1996;97:220.
29 Garrett MH, Hooper BM, Cole FM, Hooper MA: Airborne fungal spores in 80 homes in Latrobe Valley, Australia: Levels, seasonality and indoor-outdoor relationship. Aerobiologia (Ireland) 1997;13:121–126.
30 Curran IHA, Young NM, Burton M, Vijay HM: Purification and characterization of Alt a 29 from *Alternaria alternata*. Int Arch Allergy Immunol 1993;102:267–275.
31 Matthiesen F, Olsen M, Lowenstein H: Purification and partial sequenization of the major allergen of *Alternaria alternata* Alt a 1 (abstract). J Allergy Clin Immunol 1992;89:241.
32 Akurst L, Borch SM: Partial purification and characterization of two *Cladosporium herbarum* allergens. Int Arch Allergy Appl Immunol 1979;60:68–79.
33 Sward-Nordmo M, Wold JK, Paulsen BS, Aukrust L: Purification and partial characterization of the allergen Ag54 from *Cladosporium herbarum*. Int Arch Allergy Appl Immunol 1985;78:249–255.
34 Sward-Nordmo M, Paulsen BS, Wold JK: Immunological studies of glycoprotein allergen Ag-54 (Cla h II) in *Cladosporium herbarum* with special attention to the carbohydrate and protein moieties. Int Arch Allergy Appl Immunol 1989;90:155–161.
35 Zhang L, Muradia G, Curran IHA, Rode H, Vijay HM: A cDNA clone coding for a novel allergen, Cla h III, of *Cladosporium herbarum* identified as a ribosomal P2 protein. J Immunol 1995;154:710–716.
36 Breitenbach M, Simon B, Probst G, Oberkofler H, Ferreira F, Briza P, Achatz G, Unger A, Ebner C, Kraft D, Hirschwehr R: Enolases are highly conserved fungal allergens. Int Arch Allergy Immunol 1997;113:114–117.
37 Kurup VP, Kumar A: Immunodiagnosis of aspergillosis. Clin Microbiol Rev 1991;4:439–459.
38 Kurup VP, Apter A: Immunology and Allergy Clinics of North America. Philadelphia, Saunders, 1998, vol 18.
39 Moser M, Crameri R, Menz G, Schneider T, Dudler T, Virchow C, Gmachl M, Blaser K, Surer M: Cloning and expression of recombinant *Aspergillus fumigatus* allergen I/a (rAsp f I/a) with IgE binding and type I skin test activity. J Immunol 1992;149:454–460.
40 Kurup VP, Banerjee B, Hemmann S, Greenberger PA, Blaser K, Crameri R: Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. Clin Exp Allergy 2000;30:988–993.
41 Ramachandran H, Jayaraman V, Banerjee B, Greenberger PA, Kelly KJ, Fink JN, Kurup VP: IgE binding conformational epitopes of Asp f 3, a major allergen of *Aspergillus fumigatus*. Clin Immunol 2002;103:324–333.
42 Crameri R: Recombinant *Aspergillus fumigatus* allergens: From the nucleotide sequences to clinical applications. Int Arch Allergy Immunol 1998;115:99–114.
43 Banerjee B, Kurup VP: Molecular biology of *Aspergillus* allergens; in Kurup VP, Apter AJ (eds): Immunology and Allergy Clinics of North America. Philadelphia, Saunders, 1998, vol 18, pp 601–618.
44 Shen HD, Lin WL, Tam MF, Wang SR, Tsai JJ, Chou H, Han SH: Alkaline serine proteinase: A major allergen of *Aspergillus oryzae* and its cross-reactivity with *Penicillium citrinum*. Int Arch Allergy Immunol 1998;116:29–35.
45 Shen HD, Tam MF, Chou H, Han SH: The importance of serine proteinases as aeroallergens associated with asthma. Int Arch Allergy Immunol 1999;119:259–264.

46 Shen HD, Lin WL, Liaw SF, Tam MF, Han SH: Characterization of the 33-kilodalton major allergen of *Penicillium citrinum* by using MoAbs and N-terminal amino acid sequencing. Clin Exp Allergy 1997;27:79–86.
47 Chou H, Lai HY, Tam MF, Chou MY, Wang SR, Han SH, Shen HD: cDNA cloning, biological and immunological characterization of the alkaline serine protease major allergen from *Penicillium chrysognum*. Int Arch Allergy Immunol 2002;127:15–26.
48 Shen HD, Wang CW, Lin WL, Lai HY, Tam MF, Chou H, Wang SR, Han SH: cDNA cloning and immunological characterization of Pen o 18, the vacuolar serine protease major allergen of *Penicillium oxalicum*. J Lab Clin Med 2001;137:115–124.
49 Chou H, Lin WL, Tam MF, Wang SR, Han SH, Shen HD: Alkaline serine proteinase is a major allergen of *Aspergillus flavus* – A prevalent airborne *Aspergillus* species in the Taipei area. Int Arch Allergy Immunol 1999;119:282–290.
50 Muilenberg M, Burge H, Sweet T, Solomon W: *Penicillium* species in and out of doors in Topeka KS. J Allergy Clin Immunol 1990;85:247.
51 Wie DL, Chen JH, Jong SC, Shen HD: Indoor airborne *Penicillium* species in Taiwan. Curr Microbiol 1993;26:137–140.
52 Shen HD, Lin WL, Tsai JJ, Liaw SF, Han SH: Allergenic components in three different species of *Penicillium*: Cross-reactivity among major allergens. Clin Exp Allergy 1996;26:444–451.
53 Su NY, Yu CJ, Shen HD, Pan FM, Chow LP: Pen c 1, a novel enzymic allergen protein from *Penicillium citrinum*. Purification, characterization, cloning and expression. Eur J Biochem 1999;261:115–123.
54 Shen HD, Lin WL, Tam MF, Chou H, Wang CW, Tsai JJ, Wang SR, Han SH: Identification of vacuolar serine proteinase as a major allergen of *Aspergillus fumigatus* by immunoblotting and N-terminal amino acid sequence analysis. Clin Exp Allergy 2001;31:295–302.
55 Shen HD, Liaw SF, Lin WL, Ro LH, Yang HL, Han SH: Molecular cloning of cDNA coding for the 68 kDa allergen of *Penicillium notatum* using MoAbs. Clin Exp Allergy 1995;25:350–356.
56 Shen HD, Au LC, Lin WL, Liaw SF, Tsai JJ, Han SH: Molecular cloning and expression of a *Penicillium citrinum* allergen with sequence homology and antigenic cross-reactivity to a hsp 70 human heat shock protein. Clin Exp Allergy 1997;27:682–690.
57 Shen HD, Wang CW, Chou H, Lin WL, Tam MF, Huang MH, Kuo ML, Wang SR, Han SH: cDNA cloning and immunological characterization of a new *Penicillium citrinum* allergen (Pen c 3). J Allergy Clin Immunol 2000;105:827–833.
58 Lai HY, Tam MF, Tang RB, Chou H, Chang CY, Tsai JJ, Shen HD: cDNA cloning and immunological characterization of a newly identified enolase allergen from *Penicillium citrinum* and *Aspergillus fumigatus*. Int Arch Allergy Immunol 2002;127:181–190.
59 Horner WE, Helbling A, Salvaggio JE, Lehrer SB: Fungal allergens. Clin Microbiol Rev 1995;8:161–179.
60 Ishiguro A, Homma M, Torii S, Tanaka K: Identification of *Candida albicans* antigens reactive with immunoglobulin E antibody of human sera. Infect Immun 1992;60:1550–1557.
61 Baldo BA, Baker RS: Inhalant allergies to fungi: Reactions to bakers' yeast *(Saccharomyces cerevisiae)* and identification of bakers' yeast enolase as an important allergen. Int Arch Allergy Appl Immunol 1988;86:201–208.
62 Woodfolk JA, Wheatley LM, Piyasena RN, Benjamin DC, Platts-Mills TAE: Trichophyton antigens associated with IgE antibodies and delayed-type hypersensitivity. Sequence homology to two families of serine proteinases. J Biol Chem 1998;273:29489–29496.
63 Verma J, Gangal SV: *Fusarium solani*: Immunochemical characterization of allergens. Int Arch Allergy Immunol 1994;104:175–183.
64 Chauhan B, Santiago L, Kirschmann DA: The association of HLA-DR alleles and T-cell activation with allergic bronchopulmonary aspergillosis. J Immunol 1997;159:4072–4076.
65 Chauhan B, Knutsen AP, Hutcheson PS, Slavin RG, Bellone CJ: T-cell subsets, epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. J Clin Invest 1996;97:2324–2331.
66 Hudeez F: Prediction of B- and T-cell epitopes; in Raynavolgyi E (ed): Synthetic Peptides in the Search for B- and T-Cell Epitopes. Austin, RG Landes, 1994, pp 19–30.
67 Kurup VP, Banerjee B, Murali PS, Greenberger PA, Krishnan M, Hari V, Fink JN: Immunodominant peptide epitopes of allergen, Asp f 1 from the fungus *Aspergillus fumigatus*. Peptides 1998;19:1469–1477.
68 Knutsen AP, Mueller KR, Levine AD, Chauhan B, Hutcheson PS, Slavin RG: Asp f 1 $CD4^+$ T cell lines in allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1994;92:215–221.
69 Kurup VP, Hari V, Guo J, Murali PS, Krishnan M, Fink JN: *Aspergillus fumigatus* peptides differentially express Th1 and Th2 cytokines. Peptides 1996;17:183–190.
70 Rathore VB, Johnson B, Fink JN, Kelly KJ, Greenberger PA, Kurup VP: T-cell proliferation and cytokine secretion to T-cell epitopes of Asp f 2 in ABPA patients. Clin Immunol 2001;100:228–235.
71 Pettersson I: Methods of epitope mapping (review). Mol Biol Rep 1992;16:149–153.
72 Banerjee B, Greenberger PA, Fink JN, Kurup VP: Conformational and linear B-cell epitopes of Asp f 2, a major allergen of *Aspergillus fumigatus*, bind differently to immunoglobulin E antibody in the sera of allergic bronchopulmonary aspergillosis patients. Infect Immun 1999;67:2284–2291.
73 Vailes LD, Li Y, Bao Y, DeGroot H, Aalberse RC, Chapman MD: Fine specificity of B cell epitopes on *Felis domesticus* allergen (Fel d 1): Effect of reduction and alkylation or deglycosylation on Fel d 1 structure and antibody binding. J Allergy Clin Immunol 1994;93:22–33.
74 Ikagawa S, Matsushita S, Chen YZ, Ishikawa T, Nishimura T: Single amino acid substitutions on a Japanese cedar pollen allergen (Cry j 1)-derived peptide induced alterations in human T-cell responses and T-cell receptor antagonism. J Allergy Clin Immunol 1996;97:53–64.
75 Tang B, Banerjee B, Greenberger PA, Fink JN, Kelly KJ, Kurup VP: Antibody binding of deletion mutants of Asp f 2, the major *Aspergillus fumigatus* allergen. Biochem Biophys Res Comm 2000;270:1128–1135.
76 Banerjee B, Kurup VP, Greenberger PA, Kelly KJ, Fink JN: C-terminal cysteine residues determine the IgE binding of *Aspergillus fumigatus* allergen Asp f 2. J Immunol 2002, in press.
77 Brandhorst T, Yang R, Knealy WR: Heterologous expression of the cytotoxin restrictocin in *Aspergillus nidulans* and *Aspergillus niger*. Protein Expr Purif 1994;5:486–497.
78 Flückiger S, Mittl PRE, Scappozza L, Fijten H, Folkers G, Grütter MG, Blaser K, Crameri R: Comparison of the crystal structures of the human manganese superoxide dismutase and the homologous *Aspergillus fumigatus* allergen at 2-Å resolution. J Immunol 2002;168:1267–1272.
79 Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, Barletta B, Becker WM, Blaser K, Breiteneder H, Chapman M, Crameri R, Duchene M, Ferreira F, Fiebig H, Hoffmann-Sommergruber K, King TP, Kleber-Janke T, Kurup VP, Lehrer SB, Lidholm J, Müller U, Pini C, Reese G, Scheiner O, Scheynius A, Shen HD, Spitzauer S, Suck R, Swoboda I, Thomas W, Tinghino R, Van Hage-Hamsten M, Virtanen T, Kraft D, Müller MW, Valenta R: Microarrayed allergen molecules: Diagnostic gatekeepers for allergy treatment. 2002 http://www.fasebj.org/cgi/doi/10.1096/fj.01–0711fje