

Review

Respiratory fungal allergy

Viswanath P. Kurup^{a, b, *}, Horng-Der Shen^c, Banani Banerjee^{a, b}

^aDepartment of Pediatrics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin, USA

^bVA Medical Center, 5000 West National Avenue, Milwaukee, Wisconsin, USA

^cDepartment of Medical Research and Education, Veterans General Hospital-Taipei, No. 201, Sec. 2, Shih-pai Road, Shih-pai, Taipei 11217, Taiwan

ABSTRACT – Fungal allergy including allergic rhinitis, conjunctivitis, bronchial asthma, and allergic bronchopulmonary mycoses results from exposure to spores. In this review we have dealt with the common allergenic fungi and allergens, immunopathogenesis, diagnostic assays, and the possible control of allergy in the future based on epitope-specific immunotherapy and vaccination.

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1. Introduction

Allergy in one form or other affects about 20% of the population. A number of allergens associated with various forms of allergy have been reported from all over the world [1, 2]. The concentration of allergens in the environment varies, depending on various factors including climate, vegetation, and air quality. The outdoor allergens are predominantly constituted of plant pollens and fungal spores. The indoor allergens, on the other hand, are represented by allergens from dust mites, cockroaches, dogs, cats, and mice. Fungal spores also have been reported from the indoor environment [3]. The concentration and prevalence of the indoor allergens vary substantially and are dependent on moisture content, ventilation, and the presence or absence of pets, carpets, and houseplants [4]. Avoidance of allergens or reducing exposure may be beneficial in preventing allergy to these indoor and outdoor allergens.

Allergy is defined as the exaggerated response of the immune system to foreign proteins [5]. Not all individuals develop allergy even after extensive exposure to allergens, suggesting strong evidence of genetic predisposition. The allergic conditions most frequently recognized are allergic rhinitis, conjunctivitis, and bronchial asthma. Clinically the presenting symptoms are sneezing, nasal discharge, coughing, wheezing, and shortness of breath. Reversible pulmonary airway obstruction, angioedema, urticaria, and even anaphylaxis may be present in these patients.

2. Mechanism of allergic reaction

Patients sensitized to the allergens demonstrate immunological and inflammatory responses involving both arms

of immunity, namely T-cell-mediated and B-cell- or antibody-mediated responses. Although seemingly simple, the mechanisms involved in the manifestations of allergy are multiple and a number of regulatory and effector cells and factors are involved.

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 a humoral immune response [6]. The interaction between the cytokines IL-4, IL-13 and their receptors on B cells is the first signal for antibody class switching. The second signal involves the interaction of CD40 on B cells and CD40L on T cells that leads to the production of IgE antibody [7]. 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 [8–10]. STAT 6 is considered essential for IL-4 response and for the upregulation of the Th2 pathway, while STAT 4 is essential for the IFN- γ production and upregulation of Th1 response [9]. The transcription factors NF- κ B and BSAP mediate both IL-4 and CD-40-delivered signals [6–10]. Genetic or host specific factors are frequently suggested as major components in the development of allergy. There is convincing evidence that specific immune response is associated with the MHC class II molecules, particularly the HLA DR region of the molecule in the antigen presentation and the subsequent events of cell/cell and cell/factor interactions.

Allergy develops upon sensitization with allergens from various sources [2–4]. Although IgE antibody is believed to play an important role in allergic diseases, other factors such as eosinophils and T cells also have been identified. The primary exposure to allergens leads to sensitization and immunorecognition, and subsequent exposures lead to interaction of allergens with receptor-bound IgE antibodies on various cells. IgE binds to basophils, mast cells

* Correspondence and reprints

^aAddress for correspondence

and antigen-presenting cells through high-affinity receptor, FcεR1. The low-affinity receptors of IgE (FcεRII/ CD 23) are expressed by B cells, monocytes/macrophages, dendritic cells and eosinophils. The interaction of allergens with IgE/FcεR1 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 in turn mediate 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, phagocytosis, and pinocytosis and finally, the presentation of processed antigen to specific T cells. This event promotes further release of IgE molecules, eosinophil recruitment, and activation by enhancing the secretion of IL-4 and IL-5. The acute symptoms of allergy such as sneezing, wheezing, and urticaria may be due to the release of histamine by mast cells, while chronic symptoms such as bronchial hyperreactivity may be explained by eosinophil-mediated tissue damage caused by the release of various eosinophil proteins [11–13].

3. Fungal allergy

Fungi are eukaryotic, filamentous, and mostly spore-bearing organisms, which exist as saprophytes or as parasites of animals and plants. The development of allergies to fungi follows the same biological phenomena as allergies to other environmental allergens. Fungal spores are ubiquitous and the number of fungal species present in the environment is estimated to be at least one million. Some genera of airborne fungal spores such as *Alternaria*, *Aspergillus*, and *Cladosporium* are found throughout the world [14]. The airborne spores of these fungi are generally considered to be important causes of both allergic rhinitis and allergic asthma [14].

Fungi are associated with a number of allergic diseases in humans. The prevalence of respiratory allergy to fungi is estimated at 20 to 30% among atopic individuals and up to 6% in the general population [2, 15, 16]. The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis [3, 17]. 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, while those associated with delayed type of hypersensitivity are considerably smaller (approximately 1 µm) and can penetrate the smaller airways [3, 17, 18]. 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. On bronchial provocation tests spores and fungal extracts cause both early- and late-phase reactions in patients. More than 80 genera of the major fungal groups

Table I. Genera of fungi frequently associated with allergy.

<i>Alternaria</i>	<i>Fusarium</i>	<i>Trichoderma</i>
<i>Aspergillus</i>	<i>Gliocladium</i>	<i>Trichophyton</i>
<i>Aureobasidium</i>	<i>Helminthosporium</i>	<i>Trichothecium</i>
<i>Botrytis</i>	<i>Paecilomyces</i>	<i>Ulocladium</i>
<i>Cephalosporium</i>	<i>Penicillium</i>	<i>Saccharomyces</i>
<i>Cladosporium</i>	<i>Phoma</i>	<i>Candida</i>
<i>Curvularia</i>	<i>Scopulariopsis</i>	<i>Epicoccum</i>
<i>Drechslera</i>	<i>Stachybotrys</i>	<i>Stemphylium</i>

have been associated with symptoms of respiratory tract allergy [3, 17]. The frequently encountered fungi in allergy are listed in *table I*

Most of these fungi are classified under *Ascomycetes* and *Deuteromycetes* with a few in *Basidiomycetes*. However, only a few species such as *A. fumigatus*, *Alternaria alternata* and *Cladosporium herbarum* have been investigated systematically for their role in causing allergy [14, 15, 19, 20].

4. Distribution of indoor and outdoor mold allergens

Fungi grow on most substrates if enough moisture is available. Large numbers of airborne spores are usually present in outdoor air throughout the year and frequently exceeding pollen concentration by 100- to 1000-fold, depending on environmental factors such as water, nutrients, temperature, and wind [3]. Most fungi commonly considered allergenic such as *Alternaria*, *Cladosporium*, and *Epicoccum* have a seasonal spore releasing pattern [16, 20–22].

Indoor fungi are a mixture of those which have entered from outdoors and those which grow and multiply indoors [22, 23]. *Aspergillus* and *Penicillium* are less common outdoors and are usually considered the major indoor fungi. Recently, *Alternaria* has been reported in house dust samples in the absence of environmental mold spores [24]. There is not much information available on the effect of indoor spore concentration and allergic symptoms [21, 25]. Dampness, excess moisture and mold growth in buildings is associated with the increased prevalence of respiratory symptoms such as asthma and bronchitis. The indoor air fungal flora may differ from that of outdoor air both quantitatively and qualitatively. The ratio of indoor/outdoor concentration of spores is usually less than one and is of concern when this ratio reverses. The intramural sources of fungi alter the composition of indoor airborne fungi compared to outdoor air [26].

Once fungi are detected growing in the building, other types of exposure-induced diseases also may be considered. Moist conditions in buildings seem to favor the growth of toxigenic fungi. An example of this is *Stachybotrys atra*, a toxigenic fungus that grows on moist surface materials containing cellulose. Mycotoxins produced by the fungi, which may reach high concentration in spores,

could cause severe symptoms [27]. The concentrations of both spores and their volatile metabolites may become significantly higher in indoor, than in outdoor environments. Since people spend most of their time indoors, they are in continuous contact with the airborne spores and toxins, and persistent exposure may become significant even if the toxin concentrations are low.

The fungal spores in both indoor and outdoor environments can be studied quantitatively or qualitatively using samplers. Most mold spore samplings in indoor air have been performed with discontinuous, viable samplers, while outdoor mold spores have been sampled with continuous non-viable sampling techniques. The spectrum of indoor airborne mold spores, such as in homes, offices, and other workplaces, differs from place to place due to the influx of spores from outdoor air through ventilations and air exchangers. Hence, it is difficult to arrive at any significant conclusion on the role of the indoor mold spore in the allergic response. Again, it is not always the quantity but allergenicity of the mold, which determines the overall development of clinical allergy.

The fungal spore counts in outdoor and indoor air vary considerably depending on various environmental and other factors [27]. A study conducted in southeastern Australian homes for the airborne fungal spores demonstrated *Cladosporium*, *Penicillium*, and yeasts, both indoors and outdoors in winter and late spring [28]. The spore number and disease activity in most instances cannot be correlated [21]. Outdoor levels were higher than indoor throughout the year, and significant seasonal variation in spore levels was seen both indoors and outdoors, with overall maximum in summer. Contrary to this trend, the levels of *Aspergillus*, yeasts, *Cephalosporium*, and *Glocladium* were higher in winter. *Penicillium* spores were more than common indoors. Data on the airborne spore load and the associated allergen levels in both indoor and outdoor environments remain incompletely characterized. Similarly no concrete guidelines on the fungal spore load in different environments are available or those available are not reliable [29].

5. Taxonomy and identification of fungi associated with allergy

The most important group of air-disseminated fungi that causes respiratory allergic diseases in humans are the conidial fungi, which comprise the form-class *Deuteromycetes*. Most of 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 in which the fungus is grown, temperature of incubation, and variation in 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. The cell walls of yeasts are mostly composed of a chitin-glucan combination, in contrast to the predominantly chitin cell walls in mycelial fungi. Other aspects of vegetative morphology commonly used for identification purposes are color of colony, diffusible pigments, metabolites, and mycelial structures. The colony characteristics and spore morphology of a few common allergenic fungi are shown in *figure 1*.

6. Allergenic extracts and allergen content in molds

Diagnosis of allergic disease is mainly based on clinical symptoms of the patients, skin test reaction, detection of allergen-specific serum IgE antibodies (radioallergosorbent test (RAST), ELISA), and in some cases provocative inhalation challenge test [17, 19]. 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 prevalent 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 [20, 30, 31]. Factors contributing to the variability of commercial and laboratory-made extracts are (a) variability of stock cultures used to prepare allergenic extracts and their proper identification (b) the use of mycelial-rich material as the source of allergens (c) conditions under which molds are grown and extracts prepared (d) the stability of the extracts and (e) the quality control measures employed to ascertain comparability of extracts. It is now possible to grow allergenic fungi in synthetically defined media rather than in complex media containing no macromolecules. These allergenic extracts show less variability and demonstrate specific reactivity with patients [31, 32]. Two- to three-week-old cultures are a rich source of culture filtrate antigens, while reliable mycelial antigens for immunoassay can be obtained from short-term fungal growth of aerated culture [32].

The allergenicity of an extract or fraction can be evaluated by skin-testing allergic subjects. Either prick testing or intradermal testing can be used. The most common *In vitro* tests for assay of allergen activity are RAST and ELISA [33, 34]. 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 estimation of antibody in the sera of patients. Activities of allergens and their specificity can also be compared by competitive inhibition assays [34].

The stability of allergenic extracts depends on the chemical nature and quality of the allergens, 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 aller-

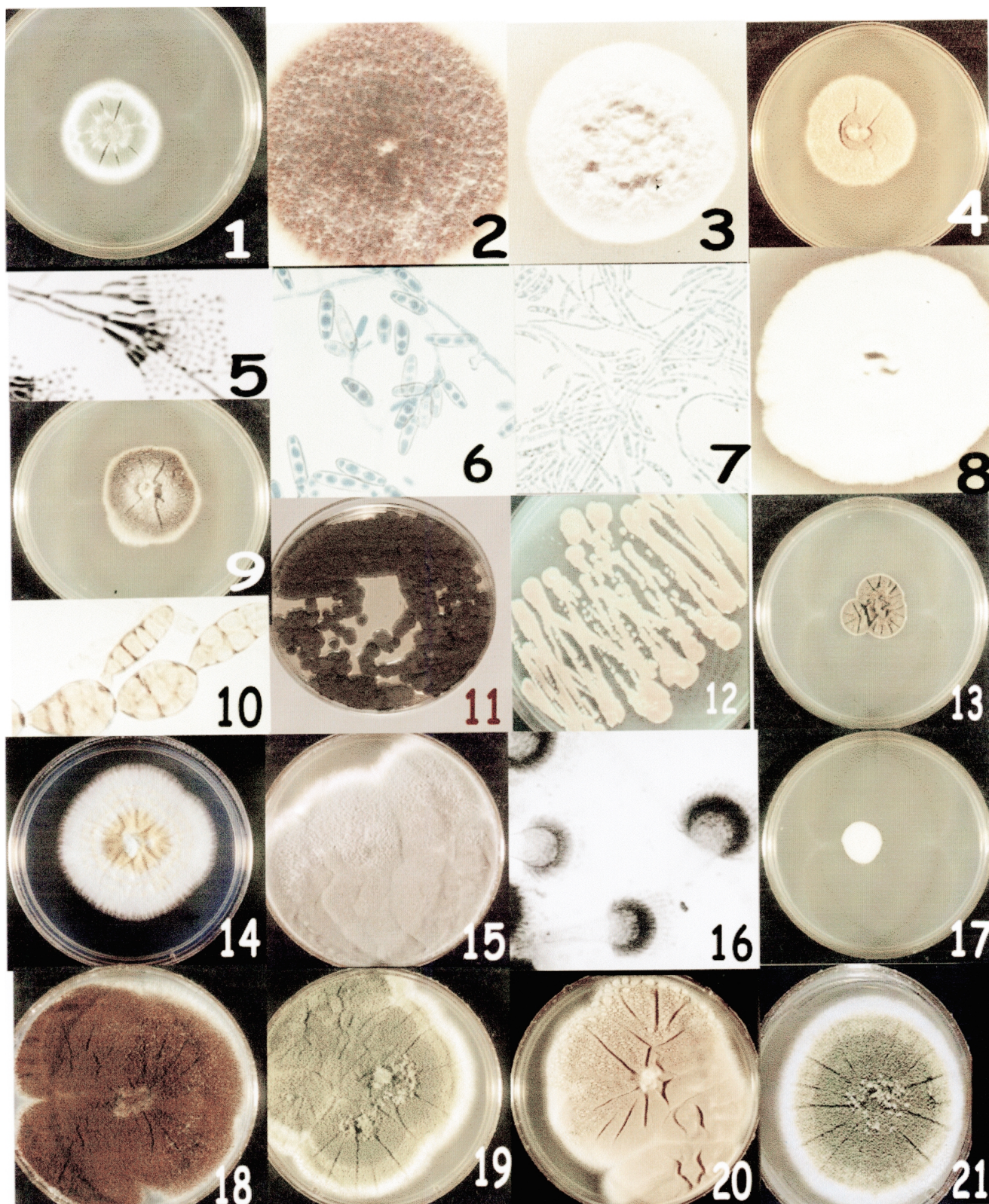


Figure 1. Morphology of fungal cultures in artificial medium (Sabourand's dextrose agar) and microscopic morphology of the spores: (1) colony of *Penicillium* sp. (2) *Helminthosporium* (3) *Verticillium* (4) *Stenphyllum* (5) *Penicillium* conidia and Phialides (6) *Helminthosporium* conidia (7) *Fusarium* conidia (8) *Fusarium* (9) *Alternaria* (10) *Alternaria* conidia (11) *Aureobasidium* (12) colony of yeast, *Saccharomyces* (13) *Cladosporium* (14) *A. oryzae* (15) *A. fumigatus* (16) *A. fumigatus* conidial head and conidia (17) *C. albicans* (colony) (18) *A. niger* (19) *A. flavus* (20) *A. terreus* and (21) *A. nidulans*.

genic 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 ϵ -aminocaproic acid to preserve the integrity of allergenic extracts [35].

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* species (table I) [3, 21, 36]. Proteins from mushrooms and yeasts capable of producing allergic responses in patients have also been reported recently [37]. The major fungal allergens purified by conventional methods or cloned and expressed by molecular biology techniques are given in table II.

6.1. *Alternaria* species

A. alternata, a member of the *Deuteromycetes*, is one of the most important allergenic fungi [14, 36, 38, 39]. A number of allergens from *A. alternata* have been purified and characterized either by conventional fractionation or molecular biology techniques (table II). Alt a 1 was the most frequently recognized allergen binding to IgE of more than 80% of asthmatic patients with *Alternaria* allergy. Paris and coworkers reported a heat-stable glycoprotein of 31 kDa, containing 20% carbohydrate located in the cytoplasm of both hyphae and spores [39]. Various isoelectric variants and isoforms of Alt a 1 have been reported recently [14, 39]. Alt a 1 with two subunits of 15 kDa 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 [14]. 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 showed different binding properties with IgE in the sera from *Alternaria* sensitized patients.

6.2. *C. herbarum*

C. herbarum is the most abundant fungus found in our environment and is a major source of inhalant fungal allergens. *A. alternata* has been reported as the predominant organism in warm, humid, and dry climates, while *Cladosporium* is frequently found in cooler climates [14].

At least 60 antigens from *C. herbarum* have been detected by CIE and 36 of them have been shown to be allergenic by crossed radioimmunoelectrophoresis (CIE) [30]. Three major *C. herbarum* allergens have been purified and characterized. Cla h1 (Ag 32) is a small (13-kDa) acidic allergen composed of five isoallergens (pI 3.4–4.4), and Cla h2 (Ag 54) is a slightly larger (23-kDa, with a pI of 5.0) glycoprotein, which contains 80% carbohydrates [40, 41]. The protein part retained the IgE binding properties even after the carbohydrate was removed and the binding was equal to or stronger than that shown by the native allergen, Cla h2. Brietenbach and his coworkers recently reported purified recombinant *Cladosporium* enolase (Cla h6; 48 kDa) having binding affinity to IgE antibodies in the sera of allergic patients by immunoblots [42]. Enolase has

been found to be a highly conserved major allergen in most fungi. The enolases from *Alternaria* and *Cladosporium* showed IgE binding with about 50% of the sera from patients sensitized to *Alternaria* and *Cladosporium*.

6.3. *A. fumigatus*

Aspergillus species are saprophytic fungi widely distributed in nature and are associated with a number of human diseases [19]. Several recombinant allergens from *A. fumigatus* have been identified and purified from cDNA and phage display library of *A. fumigatus* (table II). The majority of these proteins showed specific binding to IgE from asthmatic and allergic bronchopulmonary aspergillosis (ABPA) patients. Some of these allergens also exhibited high sequence homologies with the known functional proteins and enzymes [43–45]. Alkaline serine proteinases with allergenic properties such as Asp f 13, Asp f I 13, and Asp o 13 from *A. fumigatus*, *A. flavus*, and *A. oryzae*, respectively, have been reported [46]. 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* [47].

6.4. *A. flavus*

A. flavus antigens on immunoblotting demonstrated IgE antibody in 44% of the 68 asthmatic sera studied [48]. The 34-kDa alkaline serine proteinase, Asp fl 13, with the highest binding was considered a major allergen of *A. flavus*. The cDNA of Asp fl 13 has recently been cloned and sequenced, which showed 100% sequence identity to the 34-kDa alkaline serine proteinase, a major allergen (Asp o 13) of *A. oryzae* [49].

6.5. *Aspergillus niger*

Glucosylase, cellulase and hemicellulase from *A. niger* were shown to be allergenic by skin-prick tests, specific serum IgE response, and reactivity in immunoblots. In addition, β -xylosidase (Asp n 14) from *A. niger* was also identified as an occupational allergen present in baking additives, which causes sensitization in 4% of symptomatic bakers [50]. The vacuolar serine proteinase (Asp n 18) has also been identified as a major allergen in *A. niger* analyzed by immunoblotting using sera from asthmatic patients (Shen et al., unpublished).

6.6. *A. oryzae*

α -amylase of *A. oryzae* has been recognized as an important occupational allergen in baker's asthma [51]. Results from Baur et al. showed that 43 (48%) of 89 bakers had work-related respiratory symptoms, and 14 (32%) of them were sensitized to the isolated baking additive α -amylase from *A. oryzae* [51]. In addition, the 34-kDa alkaline serine proteinase (Asp o 13) was also identified as a major allergen in *A. oryzae* [46].

6.7. *Penicillium* species

Penicillium and *Aspergillus* are the dominant fungal genera indoors [3, 22]. Intradermal skin reactivity in atopic subjects using different mold extracts demonstrated 68% skin test reactivity to *Penicillium notatum*. The frequencies of positive Phadebas RAST in asthmatic children for *Peni-*

Table II. Mold allergens approved by the allergen nomenclatural committee.

<i>Fungus</i>	<i>Mol. Size (kDa)</i>	<i>Biological activity</i>	<i>Sequence data and number</i>
<i>A. alternata</i>			
Alt a 1			U82633
Alt a 2	25		
Alt a 3		Heat shock	U87807
		Protein 70	U87808
Alt a 6	11	Ribosomal	X-78222
		Protein P2	U-87806
Alt a 7	22	YCP4 protein	X-78225
Alt a 10	53	Aldehyde dehydrogenase	X-78227 P-42041
<i>C. herbarum</i>			
Cla h 1	13		
Cla h 2	23		
Cla h 3	53	Aldehyde dehydrogenase	X-78228
Cla h 4	11	Ribosomal P ₂	
Cla h 5	22	YCP4 protein	X-78224
Cla h 6	46	Enolase	X-78226
Cla h		Heat shock protein 70	
Cla h	42		
Cla h	110		
Cla h	100		
<i>A. fumigatus</i>			
Asp f 1	18	Ribotoxin	M-83781 S-39330
Asp f 2	37	Laminin binding	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	11.6		AJ-223315
Asp f 8	11	Ribosomal protein P2	AJ224333
Asp f 9	33.7		AJ223327
Asp f 10	34	Aspartic proteinase	X-85092
Asp f 11	24	Peptidyl prolyl isomerase	
Asp f 12	65	Heat shock protein 90	U-92465
Asp f 13	34	Alkaline serine proteinase	
			AJ002026 g-3643813 AJ224865
Asp f 15			
Asp f 16	43		
Asp f 17			
Asp f 18	34	Vacuolar serine protease	
<i>A. flavus</i>			
Asp fl 13	34	Alkline serine protease	AF137272
<i>A. niger</i>			
Asp n 14	105	Beta-xylosidase	
Asp n 18	34	Vacuolar serine proteinase	
<i>A. oryzae</i>			
Asp o 13	34	Alkaline serine proteinase	
Asp o 21	53	TAKA-Amylase A	
<i>P. citrinum</i>			
Pen c 3	18	Peroxisomal membrane protein	AF144753
Pen c 13	33	Alkaline serine proteinase	AF084546
Pen c 19	70	Hsp 70	U64207
<i>P. brevicompactum</i>			
Pen b 13	33	Alkaline serine proteinase	
<i>P. notatum</i>			
Pen n 13	34	Alkaline serine proteinase	
Pen n 18	32	Vacuolar serine proteinase	
Pen n 20	68	N-acetylglucosaminidase	
<i>P. oxalicum</i>			
Pen o 18	34	Vacuolar serine proteinase	

cillium and *Aspergillus* were 21 and 14%, respectively [52]. The prevalence of *Penicillium* species showed considerable variations in different places.

Among the allergens of *Penicillium* species, Shen et al. showed that the 32–34-kDa alkaline and/or vacuolar serine proteinases were the major allergens of *P. citrinum*, *P. brevicompactum*, *P. notatum*, and *P. oxalicum* (table II) [53]. These allergens showed cross-reactivity with other major allergens from different *Penicillium* and *Aspergillus* species [46, 48]. 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. Furthermore, an 18-kDa peroxisomal membrane protein (Pen c 3) having IgE cross-reactivity with recombinant Asp f 3 has also been reported [54].

6.8. *Botrytis* species

An aerobiological study performed in the Netherlands showed that *Botrytis* may be found in considerable numbers both outside and inside the dwellings of patients with asthma [55]. It was found that *Botrytis* played an important role, ranking second, after *Cladosporium*, in aerobiological sampling studies in Northeast Netherlands and about 7% of suspected patients showed positive skin tests with *Botrytis*. The presence of *Botrytis*-specific IgE antibody was detected in 55 patients with suspected mold allergy [56]. The results also indicate that some mold genera, e.g., *Botrytis*, *Phoma*, *Helminthosporium*, and *Epicoccum* are also as important as *Alternaria* and *Cladosporium*.

6.9. *Fusarium* species

Fusarium solani has been considered as one of the most prevalent molds responsible for allergic diseases [57]. *F. solani* extract elicited positive skin-prick test reactivity in about 25% of the atopic individuals tested. A 65-kDa protein was purified and identified as a major allergen in several species of *Fusarium* [57].

6.10. *Candida albicans*

C. albicans colonizes the gastrointestinal tract and the nasopharynx and is an opportunistic pathogen of man and animals. A role of allergy to *C. albicans* has been proposed where bronchial asthma, allergic rhinitis, chronic urticaria and atopic dermatitis have been demonstrable [58, 59]. Ishiguro and associates investigated various components of *C. albicans* by Western blot analysis and reported the specific IgE-binding to enolase (46 kDa), phosphoglycerate kinase (43 kDa) and aldolase (36 kDa) in the sera of patients who showed IgE to *C. albicans* [60]. The localization of *Candida* enolase in a cell wall fraction and in the culture supernatant suggests that the enolase could be important for the sensitization of patients. *C. albicans* enolase shares cross-reacting epitopes with *S. cerevisiae* enolase. The alcohol dehydrogenase and mannan polysaccharide have also been identified as allergens from *C. albicans*.

6.11. *S. cerevisiae*

S. cerevisiae (bakers' yeast) is widely used in foods and has been considered to be a major triggering factor in

bakers' asthma. The most important protein allergen of *S. cerevisiae* appears to be the enolase enzyme with a molecular weight of 48–52 kDa [61]. Interestingly, sera from a patient reactive to *Cladosporium* enolase also reacted equally well with the enolase of *Alternaria*, *Saccharomyces*, and *Candida* [42].

7. Assays for mold-specific IgE antibody

Demonstration of circulating antibodies against different allergens in the sera of sensitized subjects is a valuable criterion 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 the skin-prick test and intracutaneous test and both involved the production of wheal and erythema reaction, the characteristics of atopic sensitization. On the other hand, In vitro procedures such as RAST, ELISA, and Western blot detect circulating antibody against specific allergens in sera from allergic patients. In vitro testing may be of use under special circumstances, such as when patients are under medications which interfere with skin testing and in some cases to avoid the possibility of anaphylaxis and uncomfortable local reaction. In addition, for patients with dermatographism and widespread skin disease, In vitro IgE determination may be the better option for immunodiagnosis.

ELISA has been demonstrated to be a sensitive, reliable, and semiquantitative 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 [62].

Western blot involves the separation of antigens in polyacrylamide gel followed by transfer of separated proteins onto nitrocellulose membranes. The reactivity of these proteins with antibody in the sera of patients can be visualized on the membrane. Western blot has been used with great success in detecting specific antibodies to fungal antigens. Analysis of sera from patients suffering from allergic asthma, hypersensitivity pneumonitis due to *A. fumigatus* and ABPA by Western blot showed heterogeneous IgG subclasses and IgE responses [63].

Radioimmunoassay was developed to measure antibodies to radiolabelled acid-soluble antigenic fractions of various allergenic extracts. RAST, the most commonly used radioimmunoassay, has extensive application in the detection of IgE class to various allergens [64].

7.1. Automated specific IgE assay (Pharmacia immunoCAP system)

The Pharmacia CAP system is a fully automated system for the measurement of circulating IgE antibodies [65]. This assay is calibrated against the World Health Organization 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 (KU_A/l). In an effort to establish recombinant allergens as standardized reagents for the diagnosis of ABPA, the CAP system is

being investigated using recombinant allergens from *A. fumigatus* [65]. Sera from patients with ABPA, asthmatics with *A. fumigatus* allergy and control subjects were studied using this system. The results when compared with specific ELISA for IgE showed high correlation and were in perfect agreement with the skin test results. Although recombinant allergens from *A. fumigatus* have shown great promise as suitable allergens for the development of a fully automated diagnosis of aspergillosis, a panel of other fungal allergens must be individually evaluated using the immunoCAP system to demonstrate that IgE binding is not influenced by the chosen immobilization procedures [66, 67].

7.2. Skin test

Two types of skin reactions can be observed in allergic patients. Type I (immediate) reactivity consists of a 'wheal and flare' within five to 20 minutes of antigen challenge. The type III reaction (Arthus reaction) is an induration usually developed within four to eight hours after skin testing. The reaction usually subsides after 24 hours, but occasionally a delayed reaction persists for 24 hours or more. Patients are regarded as being sensitive to a particular antigen preparation if the immediately developing wheal diameter is greater than 4 mm. Until recently crude fungal antigens have been used for skin testing patients, however, the results of these reactions are not always satisfactory. In recent years, purified recombinant allergens from *Aspergillus*, *Alternaria*, and *Cladosporium* have been used with considerable success [21, 68]. The significance of the late skin response is not yet fully understood, but may be associated with infiltrate pulmonary diseases such as ABPA.

8. Immunotherapy and vaccination in mold allergy

Allergen-specific therapy appears feasible in the future because of the availability of an increasing number of functional recombinant fungal allergens. Allergen-specific therapy may aim at the prophylaxis of atopy through induction of tolerance or modification of ongoing immune reactions. Such attempts may be based on the application of T-cell epitopes as multiple peptides, pure recombinant allergens or as IgE binding haptens. More recent approaches such as modification of allergens by manipulating allergen encoding cDNAs (i.e., site directed mutagenesis) or gene immunotherapy using cDNA encoding relevant allergens (naked DNA therapy) and modulation by bacterial CpG DNA may also find application in immune intervention of allergic diseases.

In addition, isoforms of major allergens with very low IgE binding capacity, but possessing strong T-cell-stimulating sequences may be explored for inducing immune deviation from a Th2 to Th1 cytokine pattern to reduce or alleviate the allergic response.

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References

- [1] Blumenthal M.N., Rosenberg A., Allergens and Allergen Immunotherapy, in: Lockey R.F., Bukantz S.C. (Eds.), Marcel Dekker, Inc., New York, 1999, pp. 39–51.
- [2] Wuethrich B., Epidemiology of allergic diseases: Are they really on the increase, *Int. Arch. Allergy Appl. Immunol.* 90 (1989) 3–10.
- [3] Burge H.A., Airborne-allergenic fungi, *Immunol. Allergy Clin. North Am.* 9 (1989) 307–319.
- [4] Gravesen S., Indoor airborne mould spores, *Allergy* 40 (1985) 21–23.
- [5] Bierman W.C., Van Arsdel Jr P.P., Allergens and Allergen Immunotherapy, in: Lockey R.F., Bukantz S.C. (Eds.), Marcel Dekker, Inc., New York, 1999, pp. 1–26.
- [6] Mosmann T.R., Coffman R.L., Th1 and Th2 cells: Different pattern of lymphokine secretion lead to different functional properties, *Annu. Rev. Immunol.* 7 (1989) 143–173.
- [7] Renshaw B.R., Fanslow W.C., Armitage R.J., Campbell K.A., Liggitt D., Wright B., Davison B.L., Maliszewski C.R., Humoral immune response in CD40 ligand-deficient mice, *J. Exp. Med.* 180 (1994) 1889–1900.
- [8] Abbas A.K., Murphy K.M., Sher A., Functional diversity of helper T lymphocytes, *Nature* 383 (1996) 787–793.
- [9] Del Prete G., Maggi E., Romagnani S., Human Th1 and Th2 cells: functional properties, mechanisms of regulation, and role in disease, *Lab. Invest.* 70 (1994) 299–306.
- [10] Bacharier L.B., Jabara H., Geha R.S., Molecular mechanisms of immunoglobulin E regulation, *Int. Arch. Allergy Immunol.* 115 (1998) 257–269.
- [11] Kurup V.P., Grunig G., Knutsen A.P., Murali P.S., Cytokines in allergic bronchopulmonary aspergillosis, 73rd Forum in, *Immunology* (1998) 466–477.
- [12] Grunig G., Corry D.B., Coffman R.L., Rennick D.M., Kurup V.P., Immunology and Allergy Clinics of North America, in: Kurup V.P., Apter A.J. (Eds.), W. B. Saunders, Philadelphia, 1998, pp. 661–679.
- [13] Murali P.S., Greenberger P.A., Kurup V.P., Immunology and Allergy Clinics of North America, in: Kurup V.P., Apter A.J. (Eds.), W. B. Saunders, Philadelphia, 1998, pp. 681–694.
- [14] 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.* 32 (1995) 213–227.
- [15] Latge J.P., Paris S., The fungal spore and disease initiation in plants and animals, in: Cole G.T., Moch H.C. (Eds.), Plenum Press, New York, 1991, pp. 379–401.
- [16] Portnoy J., Chapman J., Burge H., Muieleberg M., Solomon W., Epicoccum allergy. Skin reaction patterns and spore/mycelium disparities recognized by IgG and IgE ELISA inhibition, *Ann. Allergy* 59 (1987) 39–43.

- [17] Kurup V.P., Fink J.N., in: Murphy J.W., Friedman H., Bendinelli M. (Eds.), *Fungal Infection and Immunity Responses*, Plenum Press, New York, 1993, pp. 393–404.
- [18] Kurup V.P., Hypersensitivity pneumonitis due to sensitization with thermophilic actinomycetes, *Immunol. Allergy Clin. North Am.* 9 (1989) 285–306.
- [19] Kurup V.P., Kumar A., Immunodiagnosis of aspergillosis, *Clin. Microbiol. Rev.* 4 (1991) 439–459.
- [20] Vijay H.M., Thaker A.J., Banerjee B., Kurup V.P., Allergens and Allergen Immunotherapy, in: Lockey R.F., Bukantz S.C. (Eds.), Marcel Dekker, Inc., New York, 1999, pp. 133–154.
- [21] Beaumont F., Kauffman H.F., Sluiter H.J., De Vries K., Sequential sampling of fungal air spores inside and outside the homes of mold sensitive, asthmatic patients: a search for a relationship to obstructive reactions, *Ann. Allergy* 55 (1985) 740–746.
- [22] Burge H.A., Fungus allergens, *Clin. Rev. Allergy* 3 (1985) 319–329.
- [23] Licorish K., Novey H.S., Kozak P., Fairshier R.D., Wilson A.F., Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma, *J. Allergy Clin. Immunol.* 76 (1985) 819–825.
- [24] Becker A.B., Muradia G., Vijay H.M., Immunoreactive *Alternaria* allergens in house dust in the absence of environmental mold, *J. Allergy Clin. Immunol.* 97 (suppl 1) (1996) 151, abstr.
- [25] Malling H.J., Diagnosis and immunotherapy of mold allergy. IV. Relation between asthma symptoms, spore counts and diagnostic tests, *Allergy* 41 (1986) 342–350.
- [26] Miller J.D., Fungi as contaminants in indoor air, *Atmospheric Environment* 26A (1992) 2163–2172.
- [27] Kozak Jr P.P., Gallup J., Cummins L.H., Gillman S.A., Factors of importance in determining the prevalence of indoor molds, *Ann. Allergy* 43 (1979) 88–94.
- [28] Garrett M.H., Hooper B.M., Cole F.M., Hooper M.A., Airborne fungal spores in 80 homes in Latrobe Valley, Australia: levels, seasonality and indoor-outdoor relationship, *Aerobiologia (Ireland)* 13 (1997) 121–126.
- [29] Lippalainen S., Lindoors O., Reijula K., Bioaerosols, Fungi and Mycotoxins: Health Effects Assessment, Prevention and Control, Eastern New York Occupational and Environmental Health Center, in: Johanning E. (Ed.), Albany, New York, 1999, pp. 616–619.
- [30] Aukrust L., Cross radioimmuno-electrophoretic studies of distinct allergens in two extracts of *Cladosporium herbarum*, *Int. Arch. Allergy Appl. Immunol.* 58 (1979) 375–390.
- [31] Kim S.J., Chaparas S.D., Characterization of antigens from *Aspergillus fumigatus*. I. Preparation of antigens from organism grown in completely synthetic medium, *Am. Rev. Respir. Dis.* 118 (1978) 547–551.
- [32] Kurup V.P., Fink J.N., Scribner G.H., Falk J., Antigenic variability of *Aspergillus fumigatus* strains, *Microbios* 19 (1977) 191–204.
- [33] Wisdom G.B., Enzyme-immunoassay, *Clin. Chem.* 22 (1976) 1243–1255.
- [34] Turner K.J., Stewart G.A., Sharp A.H., Czarny D., Standardization of allergen extracts by inhibition of RAST, skin test and chemical composition, *Clin. Allergy* 10 (1980) 441–450.
- [35] Weber R.W., Allergen immunotherapy and standardization and stability of allergen extracts, *J. Allergy Clin. Immunol.* 84 (1989) 1093–1095.
- [36] Yunginger J.W., Jones R.T., Nesheim M.E., Geller M., Studies on *Alternaria* allergens. III. Isolation of a major allergenic fraction (ALT-1), *J. Allergy Clin. Immunol.* 66 (1980) 138–147.
- [37] Horner W.E., Helbling A., Lehrer S.B., Basidiomycete allergens, *Allergy* 53 (1998) 1114–1121.
- [38] Bush R.K., Sanchez H., *In vitro* synthesis of *Alternaria* allergens and their recognition by murine monoclonal and human IgE antibodies, *Ann. Allergy Asthma Immunol.* 78 (1997) 287–292.
- [39] Paris S., Debeaupuis J.P., Prevost M.C., Casotto M., Latge J.P., The 31 kD major allergen Alt a I (1563) of *Alternaria alternata*, *J. Allergy Clin. Immunol.* 88 (1991) 902–908.
- [40] Sward-Nordmo M., Wold J.K., Paulsen B.S., Aukrust L., Purification and partial characterization of the allergen Ag54 from *Cladosporium herbarum*, *Int. Arch. Allergy Appl. Immunol.* 78 (1985) 249–255.
- [41] Sward-Nordmo M., Paulsen B.S., Wold J.K., 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.* 90 (1989) 155–161.
- [42] 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.* 113 (1997) 114–117.
- [43] Banerjee B., Greenberger P.A., Fink J.N., Kurup V.P., Immunological characterization of Asp f 2, a major allergen from *Aspergillus fumigatus* associated with allergic bronchopulmonary aspergillosis, *Infect. Immun.* 66 (1998) 5175–5182.
- [44] Banerjee B., Kurup V.P., Immunology and Allergy Clinics of North America, in: Kurup V.P., Apter A.J. (Eds.), W. B. Saunders, Philadelphia, 1998, pp. 601–618.
- [45] Crameri R., Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications, *Int. Arch. Allergy Immunol.* 115 (1998) 99–114.
- [46] Shen H.D., Lin W.L., Tam M.F., Wang S.R., Tsai J.J., Chou H., Han S.H., Alkaline serine proteinase: a major allergen of *Aspergillus oryzae* and its cross-reactivity with *Penicillium citrinum*, *Int. Arch. Allergy Immunol.* 116 (1998) 29–35.
- [47] Shen H.D., Lin W.L., Liaw S.F., Tam M.F., Han S.H., Characterization of the 33-kilodalton major allergen of *Penicillium citrinum* by using MoAbs and N-terminal amino acid sequencing, *Clin. Exp. Allergy* 27 (1997) 79–86.
- [48] Chou H., Lin W.L., Tam M.F., Wang S.R., Han S.H., Shen H.D., Alkaline serine proteinase is a major allergen of *Aspergillus flavus* – a prevalent airborne *Aspergillus* species in the Taipei area, *Int. Arch. Allergy Immunol.* 119 (1999) 282–290.
- [49] Yu C.J., Chiou S.H., Lai W.Y., Chiang B.L., Chow L.P., Characterization of a novel allergen, a major IgE-binding protein from *Aspergillus flavus*, as an alkaline serine protease, *BioChem. Biophys. Res. Comm.* 261 (1999) 669–675.

- [50] Sander I., Raulf-Heimsoth M., Siethoff C., Louhaus C., Meyer H.E., Bau X., Allergy to *Aspergillus*-derived enzymes in the baking industry: identification of β -xylosidase from *Aspergillus niger* as a new allergen (Asp n 14), J. Allergy Clin. Immunol. 102 (1998) 256–264.
- [51] Baur X., Chen Z., Sander I., Isolation and denomination of an important allergen in baking additives of α -amylase from *Aspergillus oryzae* (Asp o 11), Clin. Exp. Allergy 24 (1994) 465–470.
- [52] Koivikko A., Viander M., Lanner A., Use of the extended Phadebas RAST panel in the diagnosis of mould allergy in asthmatic children, Allergy 46 (1991) 85–91.
- [53] Shen H.D., Lin W.L., Tam M.F., Wang S.R., Tzean S.S., Huang M.H., Han S.H., Characterization of allergens from *Penicillium oxalicum* and *Penicillium notatum* by immunoblotting and N-terminal amino acid sequence analysis, Clin. Exp. Allergy 29 (1999) 642–651.
- [54] Shen H.D., Wang C.W., Chou H., Ling W.L., Huang M.H., Kuo M.L., Wang S.R., Han S.H., Molecular cloning and expression of a *Penicillium citrinum* allergen with sequence homology to the peroxisomal membrane protein major allergen (Asp f 3) of *Aspergillus fumigatus*, Allergy 54 (1999) 120.
- [55] Beaumont F., Kauffman H.F., Sluiter H.J., Vries K.D.E., A volumetric-aerobiological study of seasonal fungus prevalence inside and outside dwellings of asthmatic patients living in Northeast Netherlands, Ann. Allergy 53 (1984) 486–492.
- [56] Karlsson-Borga A., Jonsson P., Rolfsen W., Specific IgE antibodies to 16 widespread mold genera in patients with suspected mold allergy, Ann. Allergy 63 (1989) 521–526.
- [57] Verma J., Gangal S.V., *Fusarium solani*: immunochemical characterization of allergens, Int. Arch. Allergy Immunol. 104 (1994) 175–183.
- [58] Itkin I.H., Dennis M., Bronchial hypersensitivity to extract of *Candida albicans*, J. Allergy 37 (1966) 187–194.
- [59] Keeney E.L., *Candida* asthma, Ann. Intern. Med. 34 (1951) 223–226.
- [60] Ishiguro A., Homma M., Torii S., Tanaka K., Identification of *Candida albicans* antigens reactive with immunoglobulin E antibody of human sera, Infect. Immun. 60 (1992) 1550–1557.
- [61] Baldo B.A., Baker R.S., Inhalant allergies to fungi: reactions to bakers'yeast (*Saccharomyces cerevisiae*) and identification of bakers'yeast enolase as an important allergen, Int. Archs Allergy Appl. Immun. 86 (1988) 201–208.
- [62] Kurup V.P., Enzyme-linked immunosorbent assay in the detection of specific antibodies against *Aspergillus* in patient sera, Zentral Bakteriell. Microbiol. Hyg. 261 (1986) 509–516.
- [63] Kurup V.P., Kumar A., Kenealy W.R., Greenberger P.A., *Aspergillus* ribotoxins react with IgE and IgG antibodies of patients with allergic bronchopulmonary aspergillosis, J. Lab Clin. Med. 123 (1994) 749–756.
- [64] Yunginger J.W., Jones R.T., Gleich G.J., Studies on Alternaria allergens. II. Measurement of the relative potency of commercial Alternaria extracts by the direct RAST and by RAST inhibition, J. Allergy Clin. Immunol. 58 (1976) 405–413.
- [65] Sarma P.U., Banerjee B., Bir N., Kurup V.P., Immunology and Allergy Clinics of North America, in: Kurup V.P., Apter A.J. (Eds.), W. B. Saunders, Philadelphia, 1998, pp. 525–547.
- [66] Hemmann S., Ismail C., Blaser K., Menz G., Cramer R., Skin test reactivity and isotype specific immune responses to recombinant Asp f 3, a major allergen of *Aspergillus fumigatus*, Clin. Exp. Allergy 28 (1998) 860–867.
- [67] Hemmann S., Nikolaizik W.H., Schoni M.H., Blaser K., Cramer R., Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or *Aspergillus* allergy, Eur. J. Immunol. 28 (1998) 1155–1160.
- [68] Horner W.E., Helbling A., Salvaggio J.E., Lehrer S.B., Fungal allergens, Clin. Microbiol. Rev. 8 (1995) 161–179.