

The Spectrum of Fungal Allergy

Birgit Simon-Nobbe Ursula Denk Verena Pöll Raphaela Rid
Michael Breitenbach

Department of Cell Biology, University of Salzburg, Salzburg, Austria

Key Words

Allergy · Cross-reactivity · Epitope · Fungi · IgE · Mold · Recombinant allergen

Abstract

Fungi can be found throughout the world. They may live as saprophytes, parasites or symbionts of animals and plants in indoor as well as outdoor environment. For decades, fungi belonging to the ascomycota as well as to the basidiomycota have been known to cause a broad panel of human disorders. In contrast to pollen, fungal spores and/or mycelial cells may not only cause type I allergy, the most prevalent disease caused by molds, but also a large number of other illnesses, including allergic bronchopulmonary mycoses, allergic sinusitis, hypersensitivity pneumonitis and atopic dermatitis; and, again in contrast to pollen-derived allergies, fungal allergies are frequently linked with allergic asthma. Sensitization to molds has been reported in up to 80% of asthmatic patients. Although research on fungal allergies

dates back to the 19th century, major improvements in the diagnosis and therapy of mold allergy have been hampered by the fact that fungal extracts are highly variable in their protein composition due to strain variabilities, batch-to-batch variations, and by the fact that extracts may be prepared from spores and/or mycelial cells. Nonetheless, about 150 individual fungal allergens from approximately 80 mold genera have been identified in the last 20 years. First clinical studies with recombinant mold allergens have demonstrated their potency in clinical diagnosis. This review aims to give an overview of the biology of molds and diseases caused by molds in humans, as well as a detailed summary of the latest results on recombinant fungal allergens.

Copyright © 2007 S. Karger AG, Basel

Introduction

Fungi are eukaryotic, non-chlorophyllous and heterotrophic organisms that depend on external nutrients and therefore live as saprophytes, parasites or symbionts of animals and plants under nearly all environmental conditions. The phenotype of molds ranges from a unicellular to a dimorphic or filamentous appearance. Out of over 100,000 fungal species reported, a few hundred occur as opportunists and about 100 are known to elicit mycoses in man and animals [1]. More than 80 mold genera have

ABPA = Allergic bronchopulmonary aspergillosis; ABPM = allergic bronchopulmonary mycosis; AD = atopic dermatitis; GST = glutathione-S-transferase; HSP = heat shock protein; MnSOD = manganese-dependent superoxide dismutase; RAST = radioallergosorbent test; SPT = skin prick test.

KARGER

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

© 2007 S. Karger AG, Basel
1018-2438/07/1451-0058\$23.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Birgit Simon-Nobbe
Department of Cell Biology, Division Genetics
University of Salzburg, Hellbrunnerstrasse 34
AT-5020 Salzburg (Austria)
Tel. +43 662 8044 5791, Fax +43 662 8044 144, E-Mail birgit.simon@sbg.ac.at

been shown to induce type I allergies in susceptible persons, whereas allergenic proteins have been identified in 23 fungal genera.

For decades, fungal spores and mycelial cells have been known to be a major health risk. In contrast to airborne pollen, fungal spores are not primarily associated with IgE-mediated type I allergies but also with a broad panel of other diseases, e.g. life-threatening primary and secondary infections in immunocompromised patients. Additionally, molds have been described to cause allergic bronchopulmonary mycosis (ABPM) and hypersensitivity pneumonitis, fungal sinusitis and toxic pneumonia, and a large number of mycotoxins have been listed [2, 3]. The broad panel of diseases results from the inhalation and ingestion of fungal spores and vegetative cells (hyphae) or the contact with fungal cells. In contrast to other allergenic sources, fungi are very common in the environment, and exposure to airborne spores is almost constant throughout the year. A major difference to other sources, e.g. house dust mite or pollen, is that fungi may colonize the human body, and they may damage airways by the production of toxins, proteases, enzymes [4] and volatile organic compounds [5]. Thus, molds have a far greater impact on the patients' immune system than pollen or other allergenic sources.

Biology of Molds

Fungi are eukaryotic, filamentous and mostly spore-bearing organisms representing a separate entity within living organisms. In general, a sexual generation is followed by an asexual generation during a life cycle. Each of these generations may propagate independently, exhibiting different morphologies (pleomorphism). The broad majority of allergy-causing molds belong to the divisions of ascomycota or basidiomycota. Ascomycota produce their ascospores in the course of sexual reproduction in the ascus, whereas basidiomycota produce their meiospores or basidiospores, respectively, in the basidium. About 30,000 species of ascomycota and 25,000 species of basidiomycota have been described. The size of fungal spores ranges from 2–3 μm (*Cladosporium*, *Aspergillus* and *Penicillium*) up to 160 μm (*Helminthosporium*). The average size lies between 2 and 10 μm , but spores of 500 μm (*Alternaria longissima*) [6] have also been found.

Although optimal growth conditions vary among molds, their optimal growth temperature ranges from 18 to 32°C. For growth, they require oxygen, water and a

carbohydrate source. Molds occur in outdoor and indoor environments, and they grow on virtually any substrate, including glass and plastic surfaces.

The outdoor spore concentration ranges from 230 to 10⁶ spores/m³ [7, 8]. Atmospheric fungal spore concentration exceeds mean pollen concentration 100–1,000 times [9]. Spore concentration in the air varies substantially depending on climatic factors such as temperature, wind and moisture. The majority of the fungal species grow in the outdoor environment. Examples are *Alternaria*, *Cladosporium*, *Epicoccum* and *Ganoderma*.

Indoor fungi are a mixture of those growing indoors and those that have entered from outdoors [10]. Their incidence is influenced by humidity, ventilation, the content of biologically degradable material, and the presence of pets, plants and carpets [11]. In general, indoor spore concentration is less than half of the outdoor count (unless there is indoor mold growth) varying from 100 to 1,000 spores/m³ [10, 12]. In a Danish study on 23 mold-infected buildings, the most frequent mold genera encountered were *Penicillium* (68%) and *Aspergillus* (56%), followed by *Chaetomium*, *Ulocladium*, *Stachybotrys* and *Cladosporium* (ranging from 22 to 15%) [13].

Fungal Type II, III and IV Allergies

The immunological mechanisms underlying mold allergies are hypersensitivity reactions of types I, II, III and IV. The spectrum of allergic symptoms caused by these hypersensitivity reactions is very broad, including rhinitis, asthma, atopic dermatitis (AD) and ABPM. Since this review has its main focus on IgE-mediated type I allergies, only a short overview about allergic diseases of types II, III and IV is given.

Clinical Manifestations of Fungal Type II, III and IV Allergies

Allergic Bronchopulmonary Mycoses

Most frequently, ABPM is caused by *Aspergillus fumigatus*, which may grow in the bronchial lumen, leading to a persistent bronchial inflammation inducing bronchiectasis in asthmatic patients. Seven to 22% of asthmatic patients suffer from allergic bronchopulmonary aspergillosis (ABPA) [14]. Besides *A. fumigatus*, ABPM is induced by *Candida albicans*, *Curvularia*, *Geotrichum* and *Helminthosporium* [14]. Allergic reactions involved include types I, III and IV.

Allergic Sinusitis

Molds (e.g. *Aspergillus*, *Curvularia*, *Alternaria* and *Bipolaris*) may cause allergic sinusitis and fungal ball production in the patients' sinuses [15]. In case of allergic sinusitis, multiple sinuses are affected, whereas tissue invasion does not occur. In the patients' mucus, fungal hyphae are detectable. Additionally, patients may show a cutaneous hypersensitivity to specific allergens along with specific IgE and IgG antibodies and an elevated total IgE level [14]. Immunologically, allergic sinusitis is a type I-, III- and IV-mediated allergic reaction.

Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis (also known as extrinsic allergic alveolitis) is based on type III/IV allergic reactions to repeated inhalation of allergens and may lead to a chronic disease with irreversible lung damage. It is characterized by the presence of precipitating antibodies and an antigen-induced lymphocyte stimulation. The following molds have been associated with hypersensitivity pneumonitis: *Aspergillus* and *Penicillium* species, and the basidiomycetes *Lentinus edodes*, *Merulius lacrymans* and *P. ostreatus* [16, 17].

Molds not only cause various allergic reactions but they may also produce mycotoxins which affect the immune system.

Mycotoxins

Mycotoxins – non-volatile, secondary metabolites of low molecular weight produced by fungi – impair the immune system and have neurotoxic, mutagenic, carcinogenic and teratogenic effects. Diseases caused by mycotoxins are called mycotoxicoses. The severity of toxic effects depends on the type of mycotoxin, the duration and dose of exposure and the age, health and nutritional status of the individual affected. Mycotoxins may occur in spores, mycelia, and the matrix in which fungi grow. They are a health risk for farm workers, for persons living in houses with excessive mold growth and for persons exposed to moldy material at the workplace. So far, approximately 300 mycotoxins have been identified. Chronic exposure to mycotoxins causes immunosuppression of varying extent. Prominent examples for mycotoxins are aflatoxin (*Aspergillus flavus* and *A. parasiticus*), ergot alkaloids (*Claviceps* spp., *A. fumigatus* and *Penicillium chermesinum*), ochratoxins (*A. ochraceus*, *A. alliaceus*, *A. terreus*, *P. niger* and *P. viridicatum*) and trichothecenes (*Fusarium sporotrichioides*, *Microdochium nivale* and *Stachybotrys atra*) [18, 19].

Fungal Type I Allergy

Type I allergy is induced by a large number of fungal genera. The majority of them are members of the ascomycota or the basidiomycota. The most important allergy-causing fungal genera belonging to the ascomycota are *Alternaria*, *Aspergillus*, *Bipolaris*, *Candida*, *Cladosporium*, *Epicoccum* and *Phoma*, whereas *Calvatia*, *Coprinus*, *Ganoderma*, *Pleurotus* and *Psilocybe* are the most prominent genera of the basidiomycota (table 1). In table 1, all allergy-causing fungal genera belonging to the ascomycota, the basidiomycota and the zygomycota along with their prevalence reported in the literature are listed.

The incidence of mold allergy ranges from 6 [20] to 24% [21] in the general population, up to 44% among atopics [22] and 80% among asthmatics [23]. The incidence of mold allergy within asthmatic children is 45% whereas it is 70% in asthmatic adults [24].

A high proportion of mold-allergic patients is polysensitized with specific IgE reactivity to various mold, pollen and even food allergens [10, 25].

Clinical Manifestations of Fungal Type I Allergy

Allergic Rhinitis

Allergic rhinitis is characterized by sneezing, rhinorrhea, pruritus and nasal obstructions. It is induced by a large number of fungal species, with *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Curvularia* and *Penicillium* being the most prominent.

Allergic Asthma

Comparing the size of pollen grains and fungal spores, it is obvious that fungal spores are smaller in general. Therefore, they may reach the alveolar surface of the lung inducing chronic inflammation of the lung tissue [26, 27].

In many studies, an apparent link between asthma and fungal sensitization was described [28]. In children, fungal allergy was shown to be associated with increased bronchial reactivity [29–31], whereas in adults severe asthma, intensive care unit admission and even death was observed [32, 33]. In an US study performed in asthmatic patients, up to 80% of the subjects showed sensitization to molds [23]. In a study on 981 4-year-old children from the Isle of Wight (UK), asthma was the most common disease in children sensitized to molds [20]. Reed [34] stated that fungi have been considered an important

cause of asthma for more than 60 years. In a Canadian study dealing with ‘thunderstorm asthma’, high spore (but not pollen) counts in the course of thunderstorms were strongly correlated with asthma exacerbations [35]. Additionally, a strong association between fungal sensitivity, exposure to fungal spores and life-threatening asthmatic episodes was described [28, 36]. Taken together, the molds *Alternaria*, *Aspergillus*, *Cladosporium*, *Helminthosporium*, *Epicoccum*, *Aureobasidium* and *Penicillium* have frequently been implicated in allergic asthma [27, 37–39].

Atopic Dermatitis

AD is a chronic inflammatory disease of the skin that is associated with high levels of total and allergen-specific IgE [40].

In recent years, *Malassezia furfur* has been implicated in the pathogenesis of AD whereas 40–65% of AD patients either have a positive skin test, atopy patch test or radioallergosorbent test (RAST) with *M. furfur* extract [41]. Sensitization to *Malassezia* allergens may be favored by impaired epidermal barriers, increased T-cell reactivity and distinctive features of antigen-presenting cells [42, 43]. Manganese-dependent superoxide dismutase (MnSOD) may be involved as an autoallergen in the pathogenesis of AD; 36% of patients with a positive *Malassezia sympodialis* skin test (n = 69) react with fungal and human MnSOD [44].

Saccharomyces cerevisiae is another yeast species showing a significant correlation between a positive skin prick test (SPT) and AD [45].

Fungal Allergens

Allergens from the Ascomycota

Alternaria alternata

Among molds associated with allergic disorders, *A. alternata* is one of the most frequently encountered species, predominantly occurring in the outdoor environment. The incidence of *A. alternata* sensitization within atopics varies between 3.6 and 39.4% (table 1) depending on the climatic zone and the population tested.

Mari et al. [46] showed that in a cohort of 4,962 patients with respiratory symptoms, 65% were SPT positive to at least one allergenic source, and 19% of these allergics reacted to at least one fungal extract, whereas the incidence of sensitization to *A. alternata* was 66%. Interestingly, within the group of patients being sensitized to a

single fungal species, *Alternaria*, *Candida* and *Trichophyton* were the most common.

In several studies, a strong association between an *A. alternata* sensitization and asthma severity was demonstrated [26, 27, 29, 31, 37, 38, 47]. In a cross-sectional study by Zureik et al. [26], asthma severity was not associated with sensitization to pollen and cats. According to a study by Halonen et al. [29], *Alternaria* sensitization at the age of 6 and 11 years, respectively, resulted in a statistically significantly increased risk to develop asthma in childhood. In a large scale study performed in the United States, 38.3% of 1,286 asthmatic children had positive skin test responses to *Alternaria* species [47].

Before 1990, little was known about the relevant allergens of *A. alternata*. Meanwhile 13 allergens of *A. alternata* have been identified (table 2). Most of these allergens are intracellular housekeeping proteins. Nine of these allergens, e.g. NADP-dependent mannitol dehy-

Table 1. Molds inducing type I allergy

	Prevalence, %	
	total population	atopics
ASCOMYCOTA		
Peizomyzota		
<i>Acremonium (Cephalosporium)</i>		16 ^a [267]
<i>Alternaria</i>	3.6–5.5 [20, 180]	66.1 ^b [46]
	12.6 [46]	39.4 [22]
		14.6 ^c [179]
		13.5 ^c [183]
		3–14.6 [181, 182]
<i>Aspergillus</i>	2.4 [46]	27.6 [22]
		21.3 ^c [179]
		15 [182]
		5 ^c [183]
<i>Aureobasidium</i>		20.5 ^d [22]
<i>Bipolaris</i>		36.8 [22]
(<i>Drechslera</i> , <i>Helminthosporium</i>)		18.8 ^c [179]
<i>Botrytis</i>		28.2 ^d [22]
<i>Chaetomium</i>		7.4 [268]
<i>Chrysosporium</i>		
<i>Cladosporium</i>	2.5 [46]	3–18.2 [181, 182]
	2.9 [20]	15.9 ^c [179]
		7.4 ^c [183]
<i>Claviceps</i>		
<i>Curvularia</i>		18.4 [22]
		28 [184]
<i>Cylindrocarpon</i>		

Table 1 (continued)

	Prevalence, %			Prevalence, %	
	total population	atopics		total population	atopics
<i>Daldinia</i>			<i>Boletus</i>		5.4 [123]
<i>Didymella</i>			<i>Calvatia</i>		7.8 [122]
<i>Embellisia</i>			<i>Cantharellus</i>		
<i>Epicoccum</i>		25.6 ^d [22]	<i>Chlorophyllum</i>		
<i>Epidermophyton</i>			<i>Coprinus</i>		5.4 [122]
<i>Eurotium</i>					6.2 [123]
<i>Fusarium</i>		3.4 ^c [183]	<i>Dacrymyces</i>		
		24.5 ^e [185]	<i>Ganoderma</i>		9.3 [122]
<i>Gliocladium</i>			<i>Gastrum</i>		6.4 [122]
<i>Leptosphaeria</i>			<i>Hypholoma</i>		
<i>Microsphaera</i>			<i>Inonotus</i>		
<i>Monilia</i>			<i>Lentinus</i>		
<i>Neurospora</i>			<i>Lycoperdon</i>		
<i>Nigrospora</i>			<i>Merulius</i>		
<i>Nimbya (Macrospora)</i>			<i>Pisolithus</i>		5.4 [122]
<i>Paecilomyces</i>		33 ^b [265]	<i>Podaxis</i>		
<i>Penicillium</i>	1.5 [46]	22 ^f [101]	<i>Polyporus</i>		
		13.9 ^c [179]	<i>Pleurotus</i>		10.6 [122]
		13.1 [22]			8.3 [123]
		7.3 [182]	<i>Psilocybe</i>		13.7 [122]
<i>Scopulariopsis</i>			<i>Schizophyllum</i>		
<i>Stemphylium (Pleospora)</i>		30.7 ^d [22]	<i>Scleroderma</i>		5.6 [122]
<i>Trichoderma</i>		23 ^d [22]	<i>Sporotrichum</i>		
<i>Trichophyton</i>	1.9 [46]	10.2 ^b [46]	<i>Stereum</i>		
		46.7 ^g [264]	<i>Trichosporon</i>		
<i>Ulocladium</i>			Urediniomycetes		
<i>Xylaria</i>			<i>Hemileia</i>		14.7 ^h [262]
Saccharomycotina			<i>Puccinia</i>		
<i>Candida</i>	8.5 [46]	44.3 ^d [46]	<i>Rhodotorula</i>		28 ^c [63]
		28.9 [22]	<i>Sporobolomyces</i>		
		23.1 ^c [179]	Ustilaginomycetes		
<i>Saccharomyces</i>	1.4 [46]	7.4 ^b [46]	<i>Malassezia (Pityrosporum)</i>		19.8 [186]
Mitosporic ascomycota					50.4 ^b [130]
<i>Phoma</i>		30.7 ^d [22]			66 ^b [41]
<i>Stachybotrys</i>	9.4 [263]		<i>Tilletia</i>		
<i>Thermomyces (Humicola)</i>			<i>Tilletiopsis</i>		
<i>Trichothecium</i>			<i>Ustilago</i>		14 [266]
<i>Wallemia</i>			ZYGOMYCOTA		
BASIDIOMYCOTA			Zygomycetes		
Hymenomycetes			<i>Absidia</i>		
<i>Agaricus (Amanita)</i>			<i>Mucor</i>		20.5 ^d [22]
<i>Armillaria</i>			<i>Rhizopus</i>		2.7 ^c [183]
<i>Boletinellus</i>					

The taxonomy is compiled according to the NCBI Taxonomy Browser (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/>) and the NEWT Taxonomy Browser of the European Bioinformatics Institute (<http://www.ebi.ac.uk/newt/display>). Synonymous genus names are given in parentheses. Allergy prevalence data of the fungal genera listed are given based on published data, whereas the numbers refer either to the percentage of prevalence within the total population or within atopics. The detailed specification of the

atopic test populations is as follows: ^a allergic asthmatics, ^b mold-allergic patients, ^c asthmatic, ^d atopic patients with strong suspicion of having mold allergy, ^e atopic individuals with symptoms of mold allergy, ^f asthmatic children, ^g patients with allergic asthma and tinea (fungal infection of the skin), and ^h atopic individuals residing in coffee-growing regions. If no superscript is given, then the test population is atopic with no further specification. No data are available for those fungi where no prevalence data are given.

Table 2. List of fungal allergens

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.
ASCOMYCOTA							
<i>Alternaria alternata</i>	Alt a 1	93 ^a (n = 43) [171] 47 ^b (n = 19) [171] 98 (n = 42) [54]		+	28	U82633	[164]
	Alt a 2	0 (n = 42) [54] 61 (n = 26) [59]		+	25	U62442	[59]
	Alt a 3		HSP70		70	U87807, U87808	[56]
	Alt a 4		protein disulfide isomerase		57	X84217	[48]
	Alt a 5		acid ribosomal protein P2	+	11	X78222, U87806	[48]
	Alt a 6	21.7 (n = 42) [52] 15 (n = 42) [54]	enolase	+	45	U82437	[52]
	Alt a 7		flavodoxin (YCP4 homologue)	+	22	X78225	[48]
	Alt a 8	41 (n = 22) [50]	mannitol dehydrogenase	+	29	AY191815	[50]
	Alt a 10		aldehyde dehydrogenase	+	53	X78227, P42041	[48]
	Alt a 12		acid ribosomal protein P1	+	11	X84216	[48]
	Alt a 13	82 (n = 17) [187]	GST	+	26	AY514673	[187]
	Alt a 70 kDa				70		[188]
	Alt a NTF2		nuclear transport factor 2	+	13.7	AJ493280	[189]
<i>Alternaria argyranthemis</i>	Alt arg 1		Alt a 1 related			AY563280	[190]
<i>Alternaria brassicicola</i>	Alt b 1		Alt a 1 related			AF499002	[191]
<i>Alternaria blumeae</i>	Alt bl 1		Alt a 1 related			AY563291	[190]
<i>Alternaria brassicae</i>	Alt br 1		Alt a 1 related			AY563309	[190]
<i>Alternaria capsici</i>	Alt c 1		Alt a 1 related			AY563298	[190]
<i>Alternaria carotiincultae</i>	Alt ca 1		Alt a 1 related			AY563287	[190]
<i>Alternaria cetera</i>	Alt ce 1		Alt a 1 related			AY563278	[190]
<i>Alternaria cheiranthi</i>	Alt ch 1		Alt a 1 related			AY563290	[190]
<i>Alternaria cinerariae</i>	Alt ci 1		Alt a 1 related			AY563308	[190]
<i>Alternaria conjuncta</i>	Alt co 1		Alt a 1 related			AY563281	[190]
<i>Alternaria crassa</i>	Alt cr 1		Alt a 1 related			AY563293	[190]
<i>Alternaria cucumerina</i>	Alt cu 1		Alt a 1 related			AY563300	[190]
<i>Alternaria dauci</i>	Alt d 1		Alt a 1 related			AY563292	[190]
<i>Alternaria dumosa</i>	Alt du 1		Alt a 1 related			AY563305	[190]
<i>Alternaria eryngii</i>	Alt e 1		Alt a 1 related			AY563313	[190]
<i>Alternaria ethzedia</i>	Alt et 1		Alt a 1 related			AY563284	[190]
<i>Alternaria euphorbiicola</i>	Alt eu 1		Alt a 1 related			AY563314	[190]
<i>Alternaria japonica</i>	Alt j 1		Alt a 1 related			AY563312	[190]
<i>Alternaria limoniasperae</i>	Alt l 1		Alt a 1 related			AY563306	[190]
<i>Alternaria longipes</i>	Alt lo 1		Alt a 1 related			AY563304	[190]
<i>Alternaria macrospora</i>	Alt m 1		Alt a 1 related			AY563294	[190]
<i>Alternaria metachromatica</i>	Alt me 1		Alt a 1 related			AY563285	[190]
<i>Alternaria mimicula</i>	Alt mi 1		Alt a 1 related			AY563310	[190]
<i>Alternaria mouchaccae</i>	Alt mo 1		Alt a 1 related			AY563279	[190]
<i>Alternaria oregonensis</i>	Alt o 1		Alt a 1 related			AY563279	[190]
<i>Alternaria petroselini</i>	Alt p 1		Alt a 1 related			AY563288	[190]
<i>Alternaria photistica</i>	Alt ph 1		Alt a 1 related			AY563282	[190]
<i>Alternaria porri</i>	Alt po 1		Alt a 1 related			AY563296	[190]
<i>Alternaria pseudorostrata</i>	Alt ps 1		Alt a 1 related			AY563295	[190]
<i>Alternaria radicina</i>	Alt r 1		Alt a 1 related			AY563286	[190]
<i>Alternaria solani</i>	Alt s 1		Alt a 1 related			AY563299	[190]
<i>Alternaria smyrnii</i>	Alt sm 1		Alt a 1 related			AY563289	[190]
<i>Alternaria sonchi</i>	Alt so 1		Alt a 1 related			AY563307	[190]
<i>Alternaria tagetica</i>	Alt t 1		Alt a 1 related			AY563297	[190]
<i>Alternaria tenuissima</i>	Alt te 1		Alt a 1 related			AY563302	[190]

Table 2 (continued)

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.
<i>Aspergillus flavus</i>	Asp fl 13	64 ^a (n = 14) [192]	alkaline serine protease	+	34	AF137272	[192, 193]
<i>Aspergillus fumigatus</i>	Asp fl 18		vacuolar serine protease				[194]
	Asp f 1	100 ^e (n = 20) [195] 75 ^g (n = 24) [90] 60 ^f (n = 20) [195] 46 ^c (n = 20) [73]	ribonuclease	+	18	M83781, S39330	[71, 196]
	Asp f 2	87 ^g (n = 24) [90]	fibrinogen binding protein	+	37	U56938	[72]
	Asp f 3	100 ^c (n = 11) [85] 100 ^d (n = 20) [195] 90 ^f (n = 20) [195] 62.5 ^d (n = 8) [85] 32 ^d (n = 16) [90]	peroxisomal membrane protein	+	19	U20722	[84, 85]
	Asp f 4	80 ^e (n = 20) [195] 77 ^g (n = 24) [90] 0 ^f (n = 20) [195]		+	30	AJ001732	[70]
	Asp f 5	74 ^c (n = 35) [73] 92.6 ^c (n = 54) [73]	metalloprotease	+	40	Z30424	[197]
	Asp f 6	70 ^e (n = 20) [195] 63 ^g (n = 24) [90] 56 ^d (n = 54) [73] 0 ^c (n = 35) [73] 0 ^f (n = 20) [195]	MnSOD	+	26.5	U53561	[88]
	Asp f 7	46 ^d (n = 54) [73] 29 ^c (n = 35) [73]		+	12	AJ223315	[73]
	Asp f 8		acid ribosomal protein P2	+	11	AJ224333	[91]
	Asp f 9	89 ^d (n = 54) [73] 31 ^c (n = 35) [73]		+	34	AJ223327	[73]
	Asp f 10	28 ^d (n = 54) [73] 3 ^c (n = 35) [73]	aspartic protease	+	34	X85092	[198]
	Asp f 11	90 (n = 30) [199]	peptidyl-prolyl isomerase	+	24	AJ006689	[200, 201]
	Asp f 12		HSP90	+	90	U92465	[92]
	Asp f 13		alkaline serine protease	+	34	Z11580	[202]
	(Asp f 15)		serine protease	+	16	AJ002026	[203]
	(Asp f 16)	70 ^g (n = 26) [204]		+	43	AF062651	[204]
	Asp f 17			+	27	AJ224865	[205]
	Asp f 18		vacuolar serine protease	+	34	Y13338	[93]
	Asp f 22		enolase		46	AF284645	[96]
	Asp f 23	26.7 ^g (n = 30) [206]	L3 ribosomal protein	+	44	AF464911	[206]
	Asp f 27		cyclophilin	+	18		[150]
	Asp f 28		thioredoxin	+	12		[152]
	Asp f 29		thioredoxin		12		
Asp f 34		PhiA cell wall protein		19.3	AM496018		
(Asp f 56 kDa)	75.5 ^g (n = 12) [207]	protease	+	56		[207]	
Asp f GST		GST		26		[187]	
<i>Aspergillus nidulans</i>	Aspe ni 2			+	29	Z50175	[208]
	Asp n 14	4 ^h (n = 171) [209]	β-xylosidase	+	105	AF108944	[209]
	Asp n 18		vacuolar serine protease	+	34	M96758	[210]
	Asp n 25	36.8 ⁱ (n = 38) [211]	3-phytase B (phosphatase)	+	84	P34754	[211, 212]
	Asp n glucoamylase	8 ^h (n = 171) [209] 19 ^j (n = 24) [213]	glucoamylase	+			[214]
<i>Aspergillus oryzae</i>	Asp n hemicellulase	43 ^j (n = 24) [213]	hemicellulase	+			[215]
	Asp o 13		alkaline serine protease	+	34	X17561	[210, 216]

Downloaded from <http://karger.com/iaa/article-pdf/145/1/58/2957681/000107573.pdf> by guest on 28 September 2023

Table 2 (continued)

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.
<i>Beauveria bassiana</i>	Asp o 21	67 ^j (n = 24) [213] 23 ^h (n = 171) [209] 0.9 ^k (n = 679) [217] 1 ⁿ (n = 529) [218] 6.2 ⁿ (n = 259) [219] 31.4 ⁿ (n = 207) [221] 28.7 ^o (n = 94) [222]	TAKA-amylase A	+	53	D00434, M33218	[220]
	Asp o lactase			+			[223]
	Asp o lipase			+			[224]
	Bb-Eno 1		enolase		47.4	DQ767719	[225]
	Bb-f2				28.6	DQ767720	[225]
<i>Candida albicans</i>	Bb-Ald		aldehyde dehydrogenase		53.9	DQ767721	[225]
	Bb-Hex		N-acetylhexosaminidase		72	DQ767722	[225]
	Cand a 1		alcohol dehydrogenase	+	40	X81694	[226]
	Cand a 3	56.25 ^l (n = 16) [227]	peroxisomal membrane protein	+	29	AY136739	[227]
	Cand a CAAP	36.7 ^p (n = 49) [111]	acid protease				[111]
<i>Candida boidinii</i>	Cand a CyP	>50 ^b (n = 21) [228]	cyclophilin (rotamase)	+	18		[148]
	Cand a enolase	37 (n = 54) [229]	enolase	+		L04943	[230, 231]
	Cand a HSP90		HSP90		90		[232]
	Cand b 2	100 ^m (n = 89) [84]	peroxisomal membrane protein	+	20	J04984, J04985	[84, 129]
	<i>Cladosporium herbarum</i>	Cand b FD		formate dehydrogenase		40.2	AJ011046
Cla h 1					13		[234]
Cla h 2					23		[234]
Cla h 5			acid ribosomal protein P2	+	11	X78223	[48, 235]
Cla h 6		22 [61]	enolase	+	46	X78226	[48]
Cla h 7			flavodoxin (YCP4 homolog)	+	22	X78224	[48]
Cla h 8		57.1 (n = 21) [49]	mannitol dehydrogenase	+	28.3	AY191816	[49]
Cla h 9		19.2 (n = 26) [143]	vacuolar serine protease	+	55	AY787775	
Cla h 10			aldehyde dehydrogenase	+	53	X78228	[48]
Cla h 12			acid ribosomal protein P1	+	11	X85180	[236]
Cla h 8 CSP			cold shock protein		8		[237]
Cla h GST			GST				[147]
Cla h HCh1			type I hydrophobin		10.5	AJ496190	[238]
Cla h HSP70			HSP70		70	X81860	[53]
Cla h NTF2			nuclear transport factor 2		14	AJ493279	[189]
<i>Cladosporium cladosporoides</i> <i>Curvularia lunata</i>	Cla c 9		vacuolar serine protease		36	EF407520	
	Cur l 1	80 (n = 15) [239]	serine protease			AY034826	[239]
	Cur l 2	100 ^q (n = 15) [240]	enolase	+	48	AY034826	[240]
	Cur l 3		cytochrome C		12	AY034827	
	Cur l ADH		alcohol dehydrogenase		37	A1YDT6	
	Cur l GST		GST				[147]
	Cur l oryzin				14.2	AY291575	
	Cur l SOD		SOD		21.4	AY291574	
	Cul l Trx		thioredoxin		12.3	AY291577	
	Cur l ZPS1				17.3	AY291573	
<i>Embellisia allii</i>	Emb a 1		Alt a 1 related			AY563322	[190]
<i>Embellisia indefessa</i>	Emb i 1		Alt a 1 related			AY563323	[190]
<i>Embellisia novae-zelandiae</i>	Emb nz 1		Alt a 1 related			AY563324	[190]
<i>Embellisia telluster</i>	Emb t 1		Alt a 1 related			AY563325	[190]
<i>Epicoccum purpurascens</i>	Epi p 1		serine protease		30	P83340	[241]
<i>Epicoccum nigrum</i>	Epi p GST		GST		26		[147]
<i>Fusarium culmorum</i>	Fus c 1	35 (n = 26) [242]	acid ribosomal protein P2	+	11	AY077706	[242]
	Fus c 2	50 (n = 26) [242]	thioredoxin-like protein	+	13	AY077707	[242]
	Fus c 3	15 (n = 26) [242]		+	49		[242]
<i>Fusarium solani</i>	Fus s 1				65	P81010	[243]
	Fus s 45 kDa		enolase		45		[244]

Downloaded from <http://karger.com/iaa/article-pdf/145/1/58/295768/1000107578.pdf> by guest on 28 September 2023

Table 2 (continued)

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.
<i>Nimbya caricis</i>	Nim c 1		Alt a 1 related			AY563321	[190]
<i>Penicillium brevicompactum</i>	Pen b 13		alkaline serine protease		33		[245]
	Pen b 26		acid ribosomal protein P1	+	11	AY786077	[109]
<i>Penicillium chrysogenum</i> (<i>Penicillium notatum</i>)	Pen ch 13	33 ^a (n = 212) [246]	alkaline serine protease	+	34	AF193420	[94, 105]
	Pen ch 18	76.9 ^f (n = 13) [106] 100 ^f (n = 8) [107]	vacuolar serine protease	+	32	AF263454	[94, 106]
	Pen ch 20		N-acetyl glucosaminidase	+	68	S77837	[247]
	Pen ch 31		calreticulin		61.6	AY850367	
<i>Penicillium citrinum</i>	Pen ch 33				16		
	Pen c 1		alkaline serine protease	+	33	AF084546	[248]
	Pen c 3	46.4 ^a (n = 28) [108]	peroxisomal membrane protein	+	18	AF144753	[108]
	Pen c 13		alkaline serine protease		33	AF084546	[248]
	Pen c 18		vacuolar serine protease	+	37.3	AF245168	[102]
	Pen c 19	41 (n = 34) [103]	HSP70	+	70	U64207	[103]
	Pen c 22	30.4 ^s (n = 23) [96]	enolase	+	46	AF254643	[96]
	Pen c 24	7.6 ^a (n = 92) [110]	elongation factor 1 β	+	25	AY363911	[110]
<i>Penicillium oxalicum</i>	Pen c 30		catalase		80.7	Q2V6Q5	
	Pen c 32		pectate lyases			EF159713	
	Pen o 18		vacuolar serine protease	+	34	AF243425	[94, 104]
<i>Pleospora herbarum</i>	Ple h 1		Alt a 1 related			AY563277	[190]
<i>Stemphylium botryosum</i>	Ste b 1		Alt a 1 related			AY563274	[190]
<i>Saccharomyces cerevisiae</i>	Sac c CyP		cyclophilin (rotamase)				[148]
	Sac c enolase	95 (n = 20) [249] 20 ^t (n = 20) [229]	enolase		46.8	J01322	[96, 250]
<i>Stachybotrys chartarum</i>	Sac c MnSOD		MnSOD		25.7	X02156	[148]
	Sta c cellulase		cellulase/glycosyl hydrolase				[145]
	Sta c hemolysin	38 (n = 21) [251]	hemolysin				[251]
	Sta c stachyrase-A	80.9 (n = 21) [251]					[251]
<i>Stemphylium callistephi</i>	Ste c 1		Alt a 1 related			AY563276	[190]
<i>Stemphylium vesicarium</i>	Ste v 1		Alt a 1 related			AY563275	[190]
<i>Thermomyces lanuginosus</i>	The l lipase		lipase			AF054513	[252]
<i>Trichophyton mentagrophytes</i>	Tri me 2		vacuolar serine protease			AJ430837, AJ430838, AJ430839, AJ430840, AJ430836	
	Tri me 4		alkaline serine protease				
	Tri r 2		vacuolar serine protease			AF082515	[253]
	Tri r 4		alkaline serine protease			AF082514	[253]
	Tri sc 2		vacuolar serine protease			AJ430841	
	Tri sc 4		alkaline serine protease			AJ430626	
<i>Trichophyton tonsurans</i>	Tri t 1				30		[254]
	Tri t 4		alkaline serine protease		83	P80514	[253]
<i>Ulocladium alternariae</i>	Ulo a 1		Alt a 1 related			AY563316	[190]
<i>Ulocladium atrum</i>	Ulo at 1		Alt a 1 related			AY563318	[190]
<i>Ulocladium botrytis</i>	Ulo b 1		Alt a 1 related			AY563317	[190]
<i>Ulocladium chartarum</i>	Ulo c 1		Alt a 1 related			AY563319	[190]
<i>Ulocladium cucurbitae</i>	Ulo cu 1		Alt a 1 related			AY563315	[190]
BASIDIOMYCOTA							
<i>Coprinus comatus</i>	Cop c 1	25 ^u (n = 92) [121]	transcription factor/leucine zipper motif	+	11	AJ132235	[121]
	Cop c 2		thioredoxin	+	11.7	AJ242791	
	Cop c 3					AJ242792	
	Cop c 4						
	Cop c 5				+	15.6	AJ242793

Table 2 (continued)

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.	
<i>Malassezia furfur</i>	Cop c 6							
	Cop c 7					AJ242794		
	Mal f 1	43–61 ^v (n = 95) [255] 17.5 ^v (n = 40) [256]	cell wall protein	+	35.9		[257]	
	Mala f 2	71.9 ^v (n = 64) [130]	peroxisomal membrane protein	+	21	AB011804	[130]	
	Mala f 3	70.3 ^v (n = 64) [130]	peroxisomal membrane protein	+	20	AB011805	[130]	
	Mala f 4	83.3 ^v (n = 36) [132]	mitochondrial malate dehydrogenase	+	35	AF084828	[132]	
	Mal f 5	48 (n = 25) [131] 35 ^v (n = 40) [256]	putative peroxisomal membrane protein	+	18.2	AJ011955	[131]	
	Mala f 6	92 (n = 48) [199] >50 ^b (n = 21) [228] 48 (n = 25) [131] 40 ^v (n = 40) [256]	putative cyclophilin	+	17.2	AJ011956	[131]	
	Mal f 7	48 (n = 25) [131] 40–60 ^v (n = 25) [258]		+	16.2	AJ011957	[131]	
	Mal f 8	24 (n = 25) [131] 10–18 ^v (n = 25) [258]		+	19.2	AJ011958	[131]	
	Mal f 9	24–36 ^v (n = 25) [258] 20 (n = 25) [131]		+	14.0	AJ011959	[131]	
	<i>Malassezia sympodialis</i>	Mala s 1	18.9 ^y (n = 127) [259] 46 ^x (n = 97) [260]	peroxisomal membrane protein	+		X96486	[257]
		Mala s 5	29.1 ^y (n = 127) [259] 19 ^x (n = 97) [260]	putative peroxisomal membrane protein	+	18.2	AJ011955	[131]
Mala s 6		25.2 ^y (n = 127) [259] 21 ^x (n = 97) [260]	cyclophilin (rotamase)	+	17	AJ011956	[131]	
Mala s 7		3 ^x (n = 97) [260]		+		AJ011957	[258]	
Mala s 8		8 ^x (n = 97) [260]		+	19	AJ011958	[258]	
Mala s 9		37.6 ^y (n = 125) [259] 24 ^x (n = 97) [260]		+	37	AJ011959	[258]	
Mala s 10		69 ^w (n = 28) [133]	HSP88	+	70	AJ428052	[97, 133]	
Mala s 11		75 ^w (n = 28) [133] 42 ^b (n = 67) [44]	MnSOD	+	23	AJ548421	[133]	
Mala s 12		62 ^x (n = 21) [261]	glucose-methanol-choline oxidoreductase	+	67	AJ871960		
Mala s 13			thioredoxin	+	12			
<i>Psilocybe cubensis</i>	Psi c 1		cyclophilin (rotamase)	+	16		[136]	
<i>Rhodotorula mucilaginosa</i>	Psi c 2		enolase	+	47	AF382946	[137]	
	Rho m 1	21.4 (n = 14) [137]	vacuolar serine protease	+	31	AY547285	[63]	

Allergens listed in the ‘official list of allergens’ of the International Union of Immunological Societies Allergen Nomenclature Subcommittee (<http://www.allergen.org/>) are shown in black, whereas allergens highlighted in grey are taken from other sources like the Allergome database (<http://www.allergome.org/>). Prevalence data are given based on published data, the specification of the respective test populations is as follows: ^a asthmatics, ^b patients with atopic dermatitis, ^c *A. fumigatus*-sensitized asthmatics with ABPA, ^d *A. fumigatus*-sensitized asthmatics without ABPA, ^e cystic fibrosis patients having ABPA, ^f *A. fumigatus*-sensitized cystic fibrosis patients, ^g ABPA patients, ^h bakers with workplace-related symptoms, ⁱ subjects occupationally exposed to powdered *A. niger* phytase having work-related respiratory symptoms, ^j subjects with baker’s asthma, ^k employees in flour milling and packing operations, ^l *C. albicans* CAP test-positive asthmatics, ^m *A. fumigatus*-sensitized asthmatics with no *C. albicans* infection, ⁿ workers formulating and packaging lactase, ^o pharmaceutical workers exposed to lactase, ^p asthmatic patients with positive immediate

skin response to crude *C. albicans* antigen, ^q *C. lunata* IgE-reactive patients suffering from allergic bronchial asthma and/or rhinitis, ^r *P. chrysogenum* IgE-reactive asthmatics, ^s *Penicillium* IgE-reactive asthmatics, ^t *C. albicans*-sensitized patients reactive with the *C. albicans* enolase, ^u basidiomycete-sensitized individuals, ^v *M. furfur* IgE-reactive atopic dermatitis patients, ^w *M. sympodialis* IgE-reactive patients with atopic eczema/dermatitis syndrome, ^x *M. sympodialis* IgE-reactive atopic eczema patients, ^y patients with atopic eczema.

If no superscript is given then the test population is allergic against the respective mold. Some *Aspergillus* allergens are given in parentheses since some inconsistencies have been identified when the coding sequences were compared with their genomic counterparts [97]. RA = Recombinant allergen, stating whether a given allergen has been cloned as a recombinant allergen. In the last column, the first publication of the respective fungal allergen is shown.

drogenase, enolase, aldehyde dehydrogenase, flavodoxin (YCP4 homolog), acid ribosomal protein P1 and P2, heat shock protein (HSP) 70, nuclear transport factor 2 and glutathione-S-transferase (GST), have not only been identified in *A. alternata* but also in the closely related mold *Cladosporium herbarum* [48–53].

Most of the *A. alternata* allergens cloned so far are minor allergens except for Alt a 1, which is recognized by up to 98% of *A. alternata*-sensitized patients [54]. Alt a 1 can be found as a predominant component in mycelial and culture filtrate extracts [55, 56]. A 20-mer peptide of Alt a 1 located at the N-terminal end showed weak binding of patients' IgE antibodies and induced antibody synthesis in Balb/c mice indicating that this peptide harbors a linear B-cell and a T-cell epitope [57].

Two clinical studies using recombinant allergens of *A. alternata* have been performed. Unger et al. [58] tested seven *A. alternata*-allergic patients with Alt a 1 and Alt a 6 (enolase), which is recognized by 15–22% of *A. alternata*-allergic patients [52, 54]. In this study, all seven *A. alternata*-allergic patients reacted to the two recombinant allergens whereas commercially available fungal extracts partially failed to correctly diagnose the patients' allergy. Asturias et al. [54] tested 42 *A. alternata*-allergic patients with natural and recombinant Alt a 1 (rAlt a 1), rAlt a 2 and rAlt a 6. Although the prevalence of Alt a 2 was previously determined to be 61% [59], none of the 42 patients reacted with rAlt a 2, but 41 of the 42 patients specifically reacted with rAlt a 6 (enolase) and rAlt a 1. Thus, the combination of Alt a 1 and Alt a 6 (maybe supplemented with one or two additional allergens) is a promising, molecule-based approach for the diagnosis and therapy of *A. alternata* allergy.

Alt a 1, the major allergen of *A. alternata*, was analyzed in respect to its B-cell epitopes. Kurup et al. [60] synthesized overlapping decapeptides (12 amino acids) spanning the entire Alt a 1 protein sequence and tested these peptides for their IgE reactivity with patient sera. They identified four linear IgE epitopes whereas two of them (K41-P50 and Y54-K63) showed strong IgE reactivity in all 4 *A. alternata*-sensitized patients tested.

Cladosporium herbarum

Airborne spores of *C. herbarum* are prominent causes of fungal allergy and can be found indoors as well as outdoors.

In a study by Tariq et al. [20], 2.9% of 981 4-year-old children reacted to *C. herbarum*. In their study, *C. herbarum* together with *A. alternata* were the third most common causes of sensitization after house dust mite

and grass pollen. Mari et al. [46] tested 4,962 patients having respiratory symptoms. The overall incidence of *C. herbarum* sensitization was 13%, but within the group of patients sensitized to more than two fungal sources, the prevalence of *C. herbarum* sensitization reached 84%. In other words, monosensitization to *C. herbarum* is rather seldom within mold-allergic patients.

So far, 14 allergens have been identified from *C. herbarum*, whereas seven of them have been cloned as recombinant proteins (table 2). Except for one, all of these allergens are minor allergens with a prevalence of about 20%. The only major allergen, Cla h 8, an NADP-dependent mannitol dehydrogenase, is recognized by 57% of the *C. herbarum*-allergic patients and represents a predominant component of the crude extract [49, 61, 62].

For some of the allergens (e.g. enolase and serine proteases), extensive cross-reactivity was demonstrated (see also Cross-Reactivity and Auto-Reactivity), making these proteins fungal pan-allergens [51, 52, 63].

IgE epitopes of *C. herbarum* enolase have been tested by a PCR-based approach. Ten different peptides spanning the entire protein sequence were tested for their IgE reactivity. Six peptides showed specific IgE reactivity in all patients tested ($n = 10$), whereas the smallest of them, with a length of 69 amino acids, corresponded to the overlapping region of the five other IgE-reactive peptides [52, 64].

Aspergillus Species

The saprophytic genus *Aspergillus* includes 132 different species. It is distributed ubiquitously in our natural environment and represents a dominant indoor pathogen [65–67]. *Aspergillus* grows outdoors on decaying vegetation or indoors (e.g. in air conditioning systems) and has the ability to release large quantities of small conidiospores of 2–3 μm . In case of inhalation, they either reach terminal airways or are deposited in large clusters in the upper respiratory tract [14, 65, 68, 69]. Human disorders caused by *Aspergillus* range from colonization of the respiratory tract, hypersensitivity pneumonitis (extrinsic allergic alveolitis), allergic rhinitis, sinusitis and asthma, to life-threatening systemic invasive aspergillosis and ABPA [66, 68]. Very often aspergillosis is favored by an impaired immune status of the patient either caused by immunosuppressive treatment after transplantation surgery, HIV infection, certain leukemias or hospitalization under intensive care.

The biological characteristics of *Aspergillus* are its small spore size, its thermo-tolerance allowing growth at

human body temperature, its resistance to oxidative killing and its ability to produce small metabolites and enzymes with proteolytic or even immunosuppressive activity [70–72].

Since *A. fumigatus* is implicated in about 80% of *Aspergillus*-related infections, a large number of allergens were cloned from cDNA and phage display libraries, and characterized and purified as recombinant proteins [70, 73–75]. The spectrum of the more than 40 IgE-binding components of *A. fumigatus* that account for the complex, variable and heterogeneous pattern obtained in Western blot experiments includes for example acid ribosomal proteins, enzymes such as proteases, toxins, HSPs as well as several unique proteins exhibiting no significant sequence homologies to structures already deposited in the databases [69, 76]. At molecular level, all these molecules differ in their allergenicity and can be subdivided into two separate categories, namely secreted and cytoplasmic proteins.

Among the most important *A. fumigatus* allergens identified through molecular approaches is Asp f 1, a non-glycosylated 18-kDa major allergen originally detected in the urine of patients suffering from invasive aspergillosis. It is related to ribotoxins, which are known to inhibit protein translation by cleaving a conserved region of the 28S acid ribosomal RNA [77]. Asp f 1, which was considered to be a kind of virulence factor promoting colonization as well as infection of human tissue, seems to be abundantly secreted after spore germination and during early phases of fungal growth [71, 78]. Although it is recognized by 85% of ABPA patients as well as *A. fumigatus* SPT-positive asthmatics, its effectiveness in diagnosis and therapy is still controversial because of its high toxicity [68, 69, 79]. Asp f 1 is one of the *A. fumigatus* allergens which have been analyzed regarding B- and T-cell epitopes. Kurup et al. [78] synthesized 13 linear decapeptides spanning the whole Asp f 1 molecule and tested them for their IgE reactivity and their potency to stimulate peripheral blood mononuclear cells from ABPA patients. They revealed several peptides harboring B- and T-cell epitopes, whereas the C-terminal region (aa 115–149) was shown to be involved in humoral as well as in cell-mediated immunoresponses in ABPA. Most of the Asp f 1-specific T-cell clones reacted with the peptides aa 46–65 and aa 106–125 restricted by HLA-DR2 and HLA-DR5 alleles [80].

Banerjee et al. [81] performed two studies on the B-cell epitopes of Asp f 2, identifying nine epitopes located in hydrophilic regions [81], with a putative major B-cell epitope at the N-terminus [82]. T-cell clones were generated

from ABPA patients using synthetic peptides from Asp f 2, identifying aa 54–74 as a major T-cell epitope [83].

The 19-kDa Asp f 3, which shares common IgE-binding epitopes with the peroxisomal membrane proteins A and B from *Candida boidinii*, can be regarded as the second major allergen of this fungus (94% IgE reactivity), with clinical relevance being already demonstrated in vivo by the provocation of mediator release [67, 73, 84, 85]. B-cell epitopes were analyzed using synthetic peptides and constructing Asp f 3 mutants. Ramachandran et al. [86] identified seven linear IgE-binding regions spanning the entire protein sequence. They identified 12 amino acids at the N-terminus and 8 amino acids at the C-terminus to be critical for IgE binding.

In case of Asp f 4, three cysteine deletion mutants were generated by selectively deleting cysteine residues. These mutants reacted differently with the IgE antibodies from ABPA patients. The authors concluded that the N-terminal IgE-epitope regions of the protein are crucial for the maintenance of the proper three-dimensional structure whereas the C-terminal cysteines play a significant supporting role in IgE binding [87].

Asp f 6, an MnSOD, represents a phylogenetically highly conserved protein belonging to the metalloenzyme superfamily, which is required for the conversion of superoxide radicals to hydrogen peroxide and oxygen [88]. Since Asp f 2, Asp f 4, whose biological function still is unresolved, and the MnSOD Asp f 6 are strictly intracellular proteins and thus very unlikely to be available as aeroallergens under normal conditions, sensitization against these two marker molecules seems to be sufficient to allow a precise diagnosis of ABPA [70, 89, 90]. ABPA is the result of fungal proliferation in the respiratory tract, exposing especially atopic asthmatics and patients suffering from cystic fibrosis to non-secreted *A. fumigatus* allergens due to cellular defense mechanisms and fungal damage [76].

A. fumigatus acid ribosomal protein P2, Asp f 8, shows a high degree of conservation among eukaryotic organisms and is characterized by the presence of cross-reactive epitopes shared with the homologous allergens from *C. herbarum* and *A. alternata* [48, 91].

Asp f 12, a HSP90 protein, may play a major role during stress response and possesses considerable homology to the HSP90 molecules from *C. albicans*, *S. cerevisiae*, *Trypanosoma*, housefly, mouse and homo sapiens. Asp f 12 is also thought to play a role in ABPA and other *Aspergillus*-induced diseases [92].

Furthermore, alkaline as well as vacuolar serine proteases have been identified to be major allergens in case

of *A. fumigatus* (Asp f 13 and Asp f 18), *A. flavus* (Asp f 13 and Asp f 18) and *A. oryzae* (Asp o 13) sharing IgE and IgG epitopes with each other as well as with fungal serine proteases from *Penicillium* spp. (Pen b 13, Pen c 13, Pen n 13, Pen n 18 and Pen o 18) [68, 93, 94]. In order to analyze the B-cell epitopes from Asp f 13, the protein was chemically and enzymatically cleaved and subsequently the N-terminal sequences were determined. At the end, 3 of 13 linear epitopes located at the C-terminus were proven to be immunodominant [95].

Another important *A. fumigatus* allergen is enolase (Asp f 22), a protein of 47 kDa, whose cross-reactivity with Pen c 22 (*Penicillium citrinum*), Alt a 6 (*A. alternata*) and Cla h 6 (*C. herbarum*) has been proven by inhibition immunoblotting [52, 96].

Recently, Bowyer and Denning [97] compared previously published *A. fumigatus* allergen sequences with *A. fumigatus* genomic sequences and revealed that Asp f 15 is identical to Asp f 13. Additionally, they observed partial homology between Asp f 16 and Asp f 9, whereas the Asp f 16 sequence, in contrast to the Asp f 9 sequence, could not be localized on two different *A. fumigatus* genomic sequences. Assuming either sequencing errors or the existence of an isoform, the authors concluded that the Asp f 9 sequence is more reliable and that Asp f 16 also should be termed Asp f 9. In case of the Asp f 56-kDa allergen, the authors could not find any corresponding genomic sequence. Since these new results have not been included into the WHO allergen list so far, the respective allergens were kept in the list of fungal allergens (table 2) but were parenthesized.

Additionally, *A. oryzae* α -amylase (Asp o 21) and *A. niger* β -xylosidase (Asp n 14), which are used as baking additives in the food industry as well as in the starch industry, show allergenic activity [65, 67].

Recombinant Asp f 1, rAsp f 4, rAsp f 6 (MnSOD) and rAsp f 8 (acid ribosomal protein P2), have been tested in several clinical studies [84, 85, 88, 91] involving patients suffering from asthma, ABPA and AD. In these studies, the diagnostic specificity was better in case of recombinant *A. fumigatus* allergens, and additionally no adverse reactions have been reported.

Penicillium Species

More than 150 *Penicillium* species exist, some of which have been described to be common indoor molds. Wei et al. [98] analyzed 88 homes in the Taipei area in order to isolate and identify the indoor *Penicillium* species. Their results showed that *P. citrinum* is the most common *Penicillium* species in this area. Muilenberg et al. [99] have

reported that *P. citrinum*, *P. oxalicum* and *Penicillium chrysogenum* (former *P. notatum*) are the five most frequently encountered species of *Penicillium* in Topeka (Kans., USA). *Penicillium* can cause atopic asthma in sensitive persons after inhalation of their spores [100]. In Taiwan, 22% of the asthmatic children showed a positive reaction in intracutaneous skin tests for *Penicillium* species [101]. Shen et al. [93] showed that IgE antibodies against components of *P. citrinum*, *P. notatum*, *P. oxalicum* and *P. brevicompactum* could be detected in the sera of 16–24% of asthmatic patients. In 100 patients, *P. chrysogenum* had the highest positive intradermal skin test reactivity (68%). Therefore, *P. chrysogenum* is the most frequent *Penicillium* species used for the clinical diagnosis of fungal allergy.

Results from Shen et al. [93] showed that 80–93% of asthmatics displayed IgE reactivity to the 32- to 34-kDa serine proteases from *P. citrinum*, *P. chrysogenum*, *P. oxalicum*, *P. brevicompactum*, *A. fumigatus*, *A. flavus*, *A. oryzae* and *A. niger*, suggesting a role as major allergens. Alkaline and vacuolar serine proteases from *Aspergillus* and *Penicillium* were termed group 13 and group 18 allergens, respectively, by the World Health Organization-International Union of Immunological Societies Allergen Nomenclature Subcommittee [93], whereby there also exist homologous and partially cross-reactive alkaline and serine proteases in other fungal species (table 3; see also Cross-Reactivity and Auto-Reactivity). Serine proteases are expressed as large precursor molecules which are posttranslationally cleaved forming the mature enzymes. Besides N-terminal cleavage of a pre-pro-sequence, which has been described for all serine proteases during maturation [94, 102–104], Pen c 18 and Pen o 18 also undergo C-terminal processing [104].

The alkaline serine protease Pen ch 13 was analyzed for linear IgE epitopes. Eleven peptide fragments spanning the whole molecule were generated and tested for their IgE reactivity in dot blot immunoassays. Determination of the IgE reactivity [105] revealed that peptide f-2n (aa 31–61) showed the highest frequency (77.1%, n = 35). Three further peptides were IgE reactive with incidences ranging from 31 to 51%. The B-cell epitope analysis was refined by narrowing down peptide f-2n and site-directed mutagenesis of Pen ch 13. Finally, one major linear B-cell epitope was identified to be located within aa 48–55.

In case of Pen ch 18, a dominant linear IgE epitope was mapped within aa 73–95 of the N-terminally processed allergen [106]. A similar result was observed by Yu et al. [107] who located nine different IgE-binding epitopes

Table 3. Cross- and/or auto-reactive fungal allergens

		Cross-reactivity		
		within 1 fungal phylum	between fungal phyla	with non-fungal species
Aldehyde dehydrogenase				
<i>Alternaria alternata</i>	Alt a 10	+		
<i>Beauveria bassiana</i>	Bb-Ald	+		
<i>Cladosporium herbarum</i>	Cla h 10	+		
<i>Harmonia axyridis</i>	Har a 2			+
Alt a 1 related				
<i>Alternaria argyranthemis</i>	Alt arg 1	+		
<i>Alternaria brassicicola</i>	Alt b 1	+		
<i>Alternaria blumeae</i>	Alt bl 1	+		
<i>Alternaria brassicae</i>	Alt br 1	+		
<i>Alternaria capsici</i>	Alt c 1	+		
<i>Alternaria carotiincultae</i>	Alt ca 1	+		
<i>Alternaria cetera</i>	Alt ce 1	+		
<i>Alternaria cheiranthi</i>	Alt ch 1	+		
<i>Alternaria cinerariae</i>	Alt ci 1	+		
<i>Alternaria conjuncta</i>	Alt co 1	+		
<i>Alternaria crassa</i>	Alt cr 1	+		
<i>Alternaria cucumerina</i>	Alt cu 1	+		
<i>Alternaria dauci</i>	Alt d 1	+		
<i>Alternaria dumosa</i>	Alt du 1	+		
<i>Alternaria eryngii</i>	Alt e 1	+		
<i>Alternaria ethzedia</i>	Alt et 1	+		
<i>Alternaria euphorbiicola</i>	Alt eu 1	+		
<i>Alternaria japonica</i>	Alt j 1	+		
<i>Alternaria limoniasperae</i>	Alt l 1	+		
<i>Alternaria longipes</i>	Alt lo 1	+		
<i>Alternaria macrospora</i>	Alt m 1	+		
<i>Alternaria metachromatica</i>	Alt me 1	+		
<i>Alternaria mimicula</i>	Alt mi 1	+		
<i>Alternaria mouchaccae</i>	Alt mo 1	+		
<i>Alternaria oregonensis</i>	Alt o 1	+		
<i>Alternaria petroselini</i>	Alt p 1	+		
<i>Alternaria photistica</i>	Alt ph 1	+		
<i>Alternaria porri</i>	Alt po 1	+		
<i>Alternaria pseudorostrata</i>	Alt ps 1	+		
<i>Alternaria radicina</i>	Alt r 1	+		
<i>Alternaria solani</i>	Alt s 1	+		
<i>Alternaria smyrnii</i>	Alt sm 1	+		
<i>Alternaria sonchi</i>	Alt so 1	+		
<i>Alternaria tagetica</i>	Alt t 1	+		
<i>Alternaria tenuissima</i>	Alt te 1	+		
<i>Embellisia allii</i>	Emb a 1	+		
<i>Embellisia indefessa</i>	Emb i 1	+		
<i>Embellisia novae-zelandiae</i>	Emb nz 1	+		
<i>Embellisia telluster</i>	Emb t 1	+		
<i>Nimbya caricis</i>	Nim c 1	+		
<i>Pleospora herbarum</i>	Ple h 1	+		
<i>Stemphylium botryosum</i>	Ste b 1	+		
<i>Stemphylium callistephi</i>	Ste c 1	+		
<i>Stemphylium vesicarium</i>	Ste v 1	+		
<i>Ulocladium alternariae</i>	Ulo a 1	+		
<i>Ulocladium atrum</i>	Ulo at 1	+		
<i>Ulocladium botrytis</i>	Ulo b 1	+		
<i>Ulocladium chartarum</i>	Ulo c 1	+		
<i>Ulocladium cucurbitae</i>	Ulo cu 1	+		

Table 3 (continued)

		Cross-reactivity		
		within 1 fungal phylum	between fungal phyla	with non-fungal species
Cyclophilin				
<i>Aspergillus fumigatus</i>	Asp f 11		+	
	Asp f 27		+	
<i>Betula verrucosa</i>	Bet v 7			+
<i>Candida albicans</i>	Cand a CyP		+	
<i>Catharanthus roseus</i>	Cat r 1			+
<i>Daucus carota</i>	Dauc c cyclophilin			+
<i>Homo sapiens</i>	Hom s CyP A			+
	Hom s CyP B			+
	Hom s CyP C			+
<i>Malassezia furfur</i>	Mala f 5		+	
<i>Malassezia sympodialis</i>	Mala s 6		+	
<i>Psilocybe cubensis</i>	Psi c 2		+	
<i>Saccharomyces cerevisiae</i>	Sac c Cyp		+	
Enolase				
<i>Alternaria alternata</i>	Alt a 6		+	
<i>Aspergillus fumigatus</i>	Asp f 22		+	
<i>Beauveria bassiana</i>	Bb-Eno1			+
<i>Candida albicans</i>	Cand a enolase		+	
<i>Cladosporium herbarum</i>	Cla h 6		+	
<i>Curvularia lunata</i>	Cur l 2		+	
<i>Cynodon dactylon</i>	Cyn d 22			+
<i>Hevea brasiliensis</i>	Hev b 9			+
<i>Penicillium citrinum</i>	Pen c 22		+	
<i>Rhodotorula mucilaginosa</i>	Rho m 1		+	
<i>Saccharomyces cerevisiae</i>	Sac c enolase		+	
Flavodoxin (YCP4 homolog)				
<i>Alternaria alternata</i>	Alt a 7	+		
<i>Cladosporium herbarum</i>	Cla h 7	+		
<i>Saccharomyces cerevisiae</i>	YCP4	+		
GST				
<i>Alternaria alternata</i>	Alt a 13		+	
<i>Aspergillus fumigatus</i>	Asp f GST		+	
<i>Blattella germanica</i>	Bla g 5			+
<i>Blomia tropicalis</i>	Blo t 8			+
<i>Cladosporium herbarum</i>	Cla h GST		+	
<i>Curvularia lunata</i>	Cur l GST		+	
<i>Dermatophagoides farinae</i>	Der f 8			+
<i>Dermatophagoides pteronyssinus</i>	Der p 8			+
<i>Epicoccum purpurascens</i>	Epi p GST		+	
<i>Sarcoptes scabiei</i>	Sar s GST			+
HSP70				
<i>Alternaria alternata</i>	Alt a 3		+	
<i>Blomia tropicalis</i>	Blo t HSP70			+
<i>Cladosporium herbarum</i>	Cla h HSP70		+	
<i>Dermatophagoides farinae</i>	Der f HSP70			+
<i>Penicillium citrinum</i>	Pen c 19		+	
<i>Toxoplasma gondii</i>	Tox g HSP70			+
Mannitol dehydrogenase				
<i>Alternaria alternata</i>	Alt a 8	+		
<i>Cladosporium herbarum</i>	Cla h 8	+		

Table 3 (continued)

		Cross-reactivity		
		within 1 fungal phylum	between fungal phyla	with non-fungal species
MnSOD				
<i>Aspergillus fumigatus</i>	Asp f 6		+	
<i>Curvularia lunata</i>	Cur l SOD		+	
<i>Drosophila melanogaster</i>	Dro m MnSOD			+
<i>Hevea brasiliensis</i>	Hev b 10			+
<i>Homo sapiens</i>	Hom s MnSOD			+
<i>Malassezia sympodialis</i>	Mala s 11		+	
<i>Olea europaea</i>	Ole e 5			+
<i>Saccharomyces cerevisiae</i>	Sac s MnSOD		+	
NTF2				
<i>Alternaria alternata</i>	Alt a NTF2	+		
<i>Cladosporium herbarum</i>	Cla h NTF2	+		
Peroxisomal membrane protein				
<i>Aspergillus fumigatus</i>	Asp f 3		+	
<i>Candida albicans</i>	Cand a 3		+	
<i>Candida boidinii</i>	Cand b 2		+	
<i>Malassezia furfur</i>	Mala f 2		+	
	Mala f 3		+	
	Mal f 5		+	
<i>Malassezia sympodialis</i>	Mala s 1		+	
<i>Penicillium citrinum</i>	Pen c 3		+	
Acid ribosomal protein P1				
<i>Alternaria alternata</i>	Alt a 12	+		
<i>Cladosporium herbarum</i>	Cla h 12	+		
<i>Penicillium brevicompactum</i>	Pen b 26	+		
Acid ribosomal protein P2				
<i>Alternaria alternata</i>	Alt a 5		+	
<i>Aspergillus fumigatus</i>	Asp f 8		+	
<i>Cladosporium herbarum</i>	Cla h 5		+	
<i>Fusarium culmorum</i>	Fus c 1		+	
<i>Homo sapiens</i>	Homo s P2			+
<i>Prunus dulcis</i>	Pru du 5			+
Serine protease				
<i>Apis mellifera</i>	Api m 7			+
<i>Cucumis melo</i>	Cuc m 1			+
<i>Curvularia lunata</i>	Cur l 1		+	
<i>Epicoccum purpurascens</i>	Epi p 1		+	
<i>Periplaneta americana</i>	Per a 10w			+
<i>Polistes dominulus</i>	Pol d 4			+
<i>Polistes exclamans</i>	Pol e 4			+
Alkaline serine protease				
<i>Aspergillus flavus</i>	Asp fl 13		+	
<i>Aspergillus fumigatus</i>	Asp f 13		+	
<i>Aspergillus oryzae</i>	Asp o 13		+	
<i>Bacillus lentus</i>	Bac l subtilisin			+
<i>Penicillium brevicompactum</i>	Pen b 13		+	
<i>Penicillium chrysogenum</i>	Pen ch 13		+	
<i>Penicillium citrinum</i>	Pen c 13		+	
<i>Trichophyton mentagrophytes</i>	Tri me 4		+	
<i>Trichophyton rubrum</i>	Tri r 4		+	
<i>Trichophyton schoenleinii</i>	Tri sc 4		+	
<i>Trichophyton tonsurans</i>	Tri t 4		+	

Table 3 (continued)

		Cross-reactivity		
		within 1 fungal phylum	between fungal phyla	with non-fungal species
Vacuolar serine protease				
<i>Aspergillus flavus</i>	Asp fl 18		+	
<i>Aspergillus fumigatus</i>	Asp f 18		+	
<i>Aspergillus niger</i>	Asp n 18		+	
<i>Cladosporium herbarum</i>	Cla h 9		+	
<i>Cladosporium cladosporioides</i>	Cla c 9		+	
<i>Penicillium chrysogenum</i>	Pen ch 18		+	
<i>Penicillium citrinum</i>	Pen c 18		+	
<i>Penicillium oxalicum</i>	Pen o 19		+	
<i>Rhodotorula mucilaginosa</i>	Rho m 2		+	
<i>Trichophyton mentagrophytes</i>	Tri me 2		+	
<i>Trichophyton rubrum</i>	Tri r 2		+	
<i>Trichophyton schoenleinii</i>	Tri sc 2		+	
Thioredoxin				
<i>Aspergillus fumigatus</i>	Asp f 28		+	
	Asp f 29		+	
<i>Coprinus comatus</i>	Cop c 2		+	
<i>Curvularia lunata</i>	Cur l Trx		+	
<i>Fusarium culmorum</i>	Fus c 2		+	
<i>Hevea brasiliensis</i>	Hev b Trx			+
<i>Homo sapiens</i>	Homo s Trx			+
<i>Malassezia sympodialis</i>	Mala s 13		+	
<i>Triticum aestivum</i>	Tri a 25			+
<i>Zea mays</i>	Zea m 25			+

For each cross- and/or auto-reactive allergen, a list of fungal species is given where the respective allergen has been identified. Additionally, the name of the allergen is listed along with the information whether cross-reactivity occurs within one fungal

phylum, within several fungal phyla or even within non-fungal species. Allergen names deposited in the official allergen list are shown in black, all others in grey. NTF2 = Nuclear transport factor 2.

distributed throughout the whole protein. One peptide, peptide C12 (V44-W62), was also located at the N-terminal end and was recognized by 75% (n = 8) of the patients tested.

Besides the highly cross-reactive serine proteases, several other *Penicillium* allergens have been identified. In case of *P. citrinum*, six allergens have been identified. One of them is Pen c 3, a peroxisomal membrane protein. Thirteen out of 28 (46.4%) sera of *Penicillium*-sensitized asthmatic patients demonstrated IgE binding to Pen c 3. Immunoblot inhibition experiments showed cross-reactivity between Pen c 3 and Asp f 3, which share 82.6% sequence identity [108].

Another *P. citrinum*-allergen was described to be HSP70. Members of the 70-kDa heat shock gene family are highly conserved across a wide range of organisms. They assist the proper folding of polypeptides, inhibit

protein aggregation and target misfolded proteins for degradation. The new allergen was designated Pen c 19, and 14 out of 34 (41%) allergic patients showed IgE binding to the recombinant and natural allergen [103].

A 47-kDa IgE-reactive component was shown to be an enolase (Pen c 22) being cross-reactive with enolases from *A. fumigatus* and *A. alternata*. Seven out of 23 (30.4%) sera of *Penicillium*-sensitized asthmatic patients reacted with a 47-kDa *P. citrinum* protein from the extract and the recombinant Pen c 22, respectively [96, 109].

Pen c 24, elongation factor 1 β (EF-1 β), shows a sequence identity of 53% with its yeast (*S. cerevisiae*) homolog [110]. The N-terminal (aa 1–118) half of the protein was recognized by 2 out of 7 Pen c 24-reactive patient sera, whereas 5 out of 7 sera reacted to the C-terminal half (aa 119–228) [110], indicating that on both halves B-cell epitopes are present.

An acid ribosomal protein, P1, was characterized to be an allergen of *P. brevicompactum* by Sevinc et al. [109]. It was designated Pen b 26, and only sera of individuals who were sensitized to this mold reacted with the protein. It is a polypeptide of 11 kDa, rich in acidic residues (> 20%) and its isoelectric point is 3.87.

Candida albicans

Although six *C. albicans* allergens have been described so far, it is still controversial whether the inhalation of this mold is causative for its allergenicity [111, 112].

Cand a enolase, for example, was isolated and analyzed for its B-cell epitopes by testing six proteolytic fragments for their IgE reactivity [113]. Ito et al. [113] identified a C-terminal fragment (F-171-I-399), which reacted to 90% IgE antibodies examined (n = 10). A similar result was obtained by Eroles et al. [114], who also demonstrated the high immunogenicity of the C-terminus.

Allergens from the Basidiomycota

Among fungi, the basidiomycota are a very large phylum comprising approximately 20,000 species including puffballs, bracket fungi, toad stools, jelly fungi, plant rusts, smuts and mushrooms like the edible *Boletus*, *Cantharellus* and *Coprinus*. Of the large number of basidiomycete species, about 25 species have been shown to be allergenic [115]. Basidiospores contribute most of all to the airborne fungal spore load ranging from 5 to 30% [8, 65, 116]. They particularly occur outdoors, but can also be found indoors, e.g. on wet decaying wood or as infiltrates from outdoors. In temperate zones, seasonal peaks of basidiospores are observed in spring and autumn [116]. The diameter of basidiospores ranges from 3 to 15 μm enabling them to reach the lower respiratory tract [117]. In contrast to ascomycota, basidiomycota do not have vegetative spore production. Since not only the spores but also the fruiting bodies of *Ganoderma*, *Coprinus* and *Pleurotus* contain allergens, they may induce food allergy in sensitized patients upon consumption of these mushrooms [118, 119]. Hence, basidiomycota as well as ascomycota are known to cause atopic asthma in susceptible persons [120]. The incidence of basidiomycota-caused allergy ranges from 3.5 [121] to 25.4% [122].

In a study performed in Europe and the USA [122], a total of 701 adults were tested for their reactivity to eight basidiomycete species. The majority (70%) of the individuals tested were classified to be atopic. Out of these 701 persons, 25.4% reacted to at least one basidiomycete ex-

tract, whereas *Psilocybe cubensis* elicited most of the positive skin reactions (13.7%) followed by *Pleurotus ostreatus* (10.6%), *Ganoderma meredithae* (9.3%) and *Coprinus quadrifidus* (5.4%). In a study by Helbling et al. [123], 9.8% of atopic subjects, who were not preselected with regard to mold allergy, were sensitized to at least one basidiomycete species. Within 457 atopic patients, 8.3% reacted to *Pleurotus pulmonalis*, 6.2% to *Coprinus comatus* and 5.4% to *Boletus edulis*. Moreover, they found that only 4% of the basidiomycete-sensitive subjects were exclusively skin test positive to basidiomycete extracts.

Up to now, the knowledge about basidiomycete allergens lags behind the information about ascomycete allergens. One of the reasons is the lack of source material since cultivation of basidiomycetes is much more complicated and in some cases even impossible.

Coprinus comatus

Among basidiomycota-sensitized patients, *C. comatus* shows a sensitization rate of 58% [123]. In 1999, Cop c 1 was cloned. It harbors two leucine zipper motifs. Its biologic function is unknown, and it represents a minor allergen being recognized by 25% of *C. comatus*-sensitized patients [121]. In sensitized individuals, Cop c 1 is skin test reactive in the picomolar range, making it a clinically relevant allergen [121]. Six further allergens with an open reading frame between 68 and 342 amino acids were isolated, whereas only in case of Cop c 2 (thioredoxin) any homology to previously isolated proteins was observed [124].

Malassezia furfur

M. furfur, previously also known as *Pityrosporum ovale* or *Pityrosporum orbiculare*, is a member of the normal cutaneous flora, preferentially colonizing the skin of the head-neck-face region as single-cell yeast, normally being non-pathogenic [125]. Nevertheless, this yeast can act as a pathogen causing pityriasis versicolor and seborrheic dermatitis [41, 126].

IgE reactivity to *M. furfur*, as shown in skin tests and radioallergosorbent tests, has frequently been observed in patients with AD [127]. *M. furfur* contains several IgE-reactive proteins ranging from 14 to 94 kDa [128].

Mala f 2 and Mala f 3 are peroxisomal proteins forming homodimers with an apparent molecular weight of 21 and 20 kDa, respectively, under reducing conditions in SDS-PAGE. They have a sequence identity of 51% and exhibit a high sequence similarity with Asp f 3 from *A. fumigatus* and two peroxisomal membrane proteins from *C. boidinii* [84, 129]. In a study of Yasueda et al. [130], 64

of 127 AD patients reacted with *M. furfur* extract, and 71.9 and 70.3% were IgE reactive to Mala f 2 and Mala f 3, respectively, making these proteins major allergens. Lindborg et al. [131] published the isolation of Mala f 5, which again has a high sequence identity with Mala f 2 (57%) and Mala f 3 (58%) and is recognized by 48% of *M. furfur* extract-reactive patients. Additionally, Mala f 6, a putative cyclophilin, was isolated, having an incidence of IgE reactivity of 48% [131].

Further allergens identified are Mala f 4, a mitochondrial malate dehydrogenase, with 83.3% of patients having elevated serum IgE levels to purified Mala f 4 [132].

Malassezia sympodialis

M. sympodialis as well as *M. furfur* are associated with AD. Several allergens were cloned, including MnSOD (Mala s 11) and HSP88 (Mala s 10) with IgE reactivities of 75 and 69%, respectively [97, 133]. First, Mala s 10 was published to be an HSP70 protein [133], but Nierman et al. [134] compared the published allergen sequences with the genomic sequences obtained recently and concluded that this allergen is actually an HSP88 protein.

Psilocybe cubensis

Skin test reactivity to *P. cubensis* spore extract is the highest (13.7%) among basidiomycetes in Europe and the USA [122]. More than ten allergens have been identified by SDS-PAGE immunoblots. Psi c 2, the first recombinant basidiomycete allergen (molecular weight: 16 kDa) shows high homology to cyclophilins and is recognized by 82%, representing a major allergen [135, 136].

Rhodotorula mucilaginosa

Rhodotorula mucilaginosa, also known as *R. rubra*, is one of the most frequently encountered yeast species in our environment. Chang et al. [137] published the isolation of an enolase (Rho m 1) which shows high sequence identity with other fungal IgE-reactive enolases. Rho m 1 is recognized by 21.4% of *R. mucilaginosa*-sensitized patients and cross-reacts with several fungal enolases. Rho m 2, a vacuolar serine protease, is the second cloned allergen, which also cross-reacts with other fungal vacuolar serine proteases [63].

Cross-Reactivity and Auto-Reactivity

Cross-reactivity can be seen when IgE antibodies originally directed against a given allergen also bind to a structurally related allergen from another allergen source

[138], thus it is the result of shared B-cell epitopes among homologous proteins. A sequence identity of more than 50% between homologous allergens seems to be necessary in order to exhibit cross-reactivity [139]. Cross-reactivity may be analyzed by various techniques, e.g. immunoblots, RAST and ELISA inhibition. Cross-reactivity between two allergens of different molds has to be distinguished from 'co-sensitization' of an allergic person to an allergen originating from another allergenic source. Co-sensitization and cross-reactivity may be differentiated by inhibition experiments between two extracts originating from distinct fungal species, where the degree of inhibition is determined. Cross-reactivity has been described for about 20 fungal allergens. Partly, the cross-reactivity observed may be ascribed to the close phylogenetic relationship of some fungal species. O'Neil et al. [140] performed skin tests with selected ascomycota and basidiomycota species demonstrating an association between *P. ostreatus*, *A. alternata*, *Fusarium solani* and *Epicoccum purpurascens*, as well as between *Calvatia cyathiformis*, *A. alternata* and *F. solani*. *C. quadrifidus* was associated with *F. solani* and *P. cubensis* with *A. fumigatus*. Thus, cross-reactivity is widespread within the two phyla and is one explanation for the clinical observation that the majority of mold-allergic patients react with several fungal species in vitro and/or in vivo [25]. Interestingly, very often cross-reactive fungal allergens represent intracellular proteins, whereas some species-specific mold allergens tend to be secreted, as it was shown for Asp f 1 [71] from *A. fumigatus* and Cop c 1 [121] from *C. comatus*.

Cross-reactive allergens may be subdivided according to the origin of their cross-reactive partners. In table 3, all cross-reactive fungal allergens are listed, along with the name of the allergen and whether or not the respective cross-reactive allergen can be found within one fungal phylum, all fungal phyla or even non-fungal species. In case of a few allergens, homologous human cross-reactive proteins have also been identified, which may give rise to auto-reactivity. The allergens showing only cross-reactivity within one fungal phylum are Alt a 1, flavodoxin (YCP4-homolog), mannitol dehydrogenase, nuclear transport factor 2 and the acid ribosomal protein P1. Cross-reactivity between fungal phyla in general has been obtained in case of peroxisomal proteins and vacuolar serine proteases. More than half of the cross-reactive fungal allergens (aldehyde dehydrogenase, alkaline serine protease, serine protease, enolase, GST and HSP70) have got homologous IgE-reactive proteins in non-fungal species. In four of them (thioredoxin, cyclophilin, MnSOD

and ribosomal protein P2), cross-reactivity with the human homolog has been observed. Taken together, it is obvious that within the last years the picture has changed in a way that meanwhile more than half of the cross-reactive fungal allergens show cross-reactivity to non-fungal species, raising the importance of fungal allergens in general.

Cross-Reactivity within One Fungal Phylum

Recently, several fungal species were tested for Alt a 1 homologues using a rabbit-anti-rAlt a 1 serum [141]. The authors could show that cross-reactive proteins were detectable in *Stemphylium botryosum*, *Ulocladium botrytis*, *Curvularia lunata* and *Alternaria tenuissima*, but not in *C. herbarum*, *P. chrysogenum* and *A. fumigatus*.

Cross-Reactivity within All Fungal Phyla

Vacuolar Serine Protease. Vacuolar serine proteases have been isolated from *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhodotorula* and *Trichophyton*. Lin et al. [142] generated monoclonal antibodies against culture medium and/or crude extract from *P. citrinum* and *A. fumigatus*. They obtained five monoclonal antibodies directed against serine proteases. Two of them (FUM20 and PCM39) were shown to be cross-reactive with the vacuolar serine proteases from *P. notatum*, *P. oxalicum* and *A. fumigatus*. From our work [143] we know that these mAbs are also cross-reactive with Cla h 9, the vacuolar serine protease from *C. herbarum*. Chou et al. [63] demonstrated cross-reactivity for the native and recombinant vacuolar serine proteases from *R. mucilaginosa* and *P. chrysogenum*.

Peroxisomal Membrane Protein. In a cross-inhibition study, Asp f 3 shared common IgE epitopes with Cand b 2, previously called peroxisomal membrane proteins A and B (PMPA and PMPB) [84].

Cross-Reactivity between Fungal and Non-Fungal Species

Enolase. Enolase represents an allergen in many fungal species, e.g. *C. herbarum*, *A. alternata*, *C. albicans*, *S. cerevisiae*, *A. fumigatus*, *F. solani*, *C. lunata*, *R. mucilaginosa*, *Beauveria bassiana* and *P. citrinum*. Preliminary data also indicate that *E. purpurascens* [144] and *Stachybotrys chartarum* [145] have got IgE-reactive enolases. *Cynodon dactylon* and *Hevea brasiliensis* are the non-fungal species where enolase has been described to be an allergen. The enolases of *C. herbarum*, *A. alternata*, *A. fumigatus* and *C. albicans* were shown to be cross-reactive by inhibition experiments [52]. Wagner et al. [146]

demonstrated cross-reactivity between *A. alternata*, *C. herbarum* and *Hevea brasiliensis* by pre-incubating a serum pool with rHev b 9 and testing this depleted serum with rCla h 6 and rAlt a 6, where there was no IgE-binding detectable.

Glutathione-S-Transferase. The crude extracts of *A. alternata*, *A. fumigatus*, *C. herbarum*, *C. lunata* and *E. purpurascens* were proven to have GST-enzymatic activity. Additionally, in all extracts a 26-kDa protein reacted with anti-GST antibodies. Using these anti-GST antibodies in ELISA inhibition experiments revealed inhibition in case of *C. herbarum*, *A. alternata*, *C. lunata*, *A. fumigatus* and *E. purpurascens* [147].

Auto-Reactivity

There is evidence that fungal sensitization also contributes to auto-reactivity against self-antigens due to shared epitopes between fungal and human proteins. The underlying mechanism seems to be molecular mimicry perpetuating severe chronic allergic diseases.

Cross-reactivity between fungal and human proteins has been demonstrated for MnSOD [148, 149], cyclophilin [150], acid ribosomal protein P2 [151] and thioredoxin [152]. Based on our own research on *C. herbarum* and *A. alternata* allergens, we could show that intracellular fungal proteins are presented to the immune system. Intracellular human proteins are normally not presented to the immune system. However, in case of chronic inflammation, tissue may be damaged and as a consequence these proteins may be accessible for the immune system. Thus human proteins like MnSOD or acid ribosomal protein 2 may sustain allergic symptoms. In a recent study on the pathogenesis of AD, 36% of the patients exhibiting *M. sympodialis* colonization of the skin had specific IgE antibodies against human MnSOD [44]. These patients were skin test positive to *M. sympodialis* extract, to human recombinant MnSOD and to structurally related MnSODs. In an atopy patch test with patients suffering from severe atopic eczema, the application of human recombinant MnSOD on healthy skin elicited an eczematous reaction [44]. The release of intracellular self-antigens as a consequence of inflammation processes causing tissue damage is also proposed to be involved in the pathogenesis of ABPA [88].

Asp f 8, the acid ribosomal protein P2 from *A. fumigatus*, cross-reacts with its human homologue P2. In skin tests, a humoral autoimmune response to the human P2 protein was seen in ABPA patients and patients with severe AD [91].

Diagnosis of Fungal Allergy

For decades, the diagnosis of mold allergy has based on the patient's history, and on in vivo (e.g. SPT, intradermal test or inhalation challenge) and in vitro tests (e.g. RAST, ELISA and Western blot). However, the accuracy and reliability of in vivo and in vitro assays is very highly dependent on the quality of the fungal extracts used. Unfortunately, the correlation of the results obtained with skin tests and serological tests is very poor. A direct comparison between in vitro and in vivo results is hampered by the fact that extracts immobilized on testing devices, e.g. ImmunoCAPs, are not available as SPT solution and vice versa.

The quality of crude extracts for diagnosis and therapy is very unsatisfactory in case of fungal extracts. Currently, the quality of mold extracts varies dramatically between commercial suppliers in Europe and the USA since no standardized extracts are available [46, 58, 153]. The reasons for the insufficient quality are manifold. On the one hand, crude extracts from ascomycota as well as basidiomycota were shown to vary considerably in their protein composition [154, 155]. These problems are caused by strain variabilities [156] and batch-to-batch variations [10, 74]. Additionally, mold extracts may be produced from mycelial cells and/or spores, which may vary in their protein pattern [157, 158]. On the other hand, growth conditions, protein extraction methods and storage conditions are critical with respect to the quantity and even existence of individual allergens [61, 65, 157]. Finally, degradation of the extracted proteins may occur, too [159]. In case of *A. alternata* [160], different allergens had different optimal extraction times, whereas the composition of the extraction buffer did not significantly affect the quantity of allergens extracted (with the exception that a low pH which resulted in a low protein yield). The diagnosis of mold allergy is also hampered by the fact that patients might not be aware of the mostly perennial fungal exposure, thus molds may not be taken into account for medical history. Moreover, the panel of allergy-causing molds exceeds by far the number of extracts that reasonably can be used in routine assessments [161].

To some extent, the problems with fungal extracts may be overcome by the use of recombinant allergens. The major advantages of recombinant proteins over crude fungal extracts are threefold. Firstly, the protein preparations are reproducible and can be standardized for biochemical and immunological tests, e.g. mass spectrometry, circular dichroism, inhibition ELISAs, determination of T-cell reactivity and histamine release assays, and

thus will give a batch-to-batch consistency. Secondly, the production of large quantities of pure proteins is possible. Thirdly, using recombinant allergens, it is possible to differentiate among co-exposure, co-sensitization and cross-reactivity. This differentiation is important since primary sensitizing molds have to be known for a successful immunotherapy. Although recombinant allergens have got major advantages, they also have some properties which have to be taken into account for their expression. A few allergens undergo secondary modifications such as glycosylation, phosphorylation, and N- and/or C-terminal processing. Although these modifications may not directly be involved in IgE binding, they nevertheless may have a large impact on the three-dimensional structure and thus on the formation of IgE epitopes of a given protein. Therefore, the choice of the expression system is very important. Routinely, bacterial systems such as *Escherichia coli* are employed, but since proteins may not be folded properly and eukaryotic posttranslational modifications are not accomplished, alternative eukaryotic systems like *Pichia pastoris*, *S. cerevisiae*, *Yarrowia lipolytica*, *Baculovirus* and tobacco plant may be used [162, 163]. The *P. pastoris* system, for example, has been used for the expression of Alt a 1, the major allergen of *A. alternata* [164].

In the last years, several diagnostic studies have proven the concept of a component-resolved allergy diagnosis instead of using crude extracts [165–169].

In order to use a high throughput test, an allergogram may be generated using a microarray format enabling a large number of allergens to be tested in duplicate or triplicate with a small amount of patient sera, in order to receive a profile of the patient's IgE reactivity pattern [170].

Since the total number of relevant IgE-reactive allergens in molds is mostly higher than in pollens or foodstuff, a panel of recombinant allergens may be necessary in order to cover the patients' allergen profile. Major allergens of all fungal phyla like Alt a 1 [48, 171], Cla h 8 [49], Asp f 1 [78], Pen n 18 [106], Mala f 6 [131], Mala s 11 [133] and Psi c 2 [136] have been described. These major allergens combined with minor allergens are promising candidate molecules for molecular-based, patient-tailored immunotherapy.

In the last years, the first diagnostic studies have compared recombinant fungal allergens and crude mold extracts with respect to their negative and positive predictability of mold sensitization. In case of *A. alternata* two clinical studies were performed [54, 58] in which two allergens (Alt a 6 and Alt a 1) were promising candidate

molecules. For *A. fumigatus*, a large number of allergens have been published. Since *A. fumigatus* is particularly known for its broad spectrum of human disorders, some groups aimed to find a link between a given disease and the patients' reactivity pattern to individual recombinant allergens. Hemmann et al. [89] and Kurup et al. [90] showed that individual recombinant allergens can be used to discriminate between ABPA (Asp f 2, Asp f 4 and Asp f 6) and fungal allergy (Asp f 1 and Asp f 3).

Therapy of Fungal Allergy

Specific immunotherapy is defined as the repeated administration of increasing doses of an allergen extract. For successful treatment, effective therapeutic doses are required, which often cannot be reached, especially in the case of mold allergy, since side effects due to a large number of non-allergenic components may occur. Several drawbacks have been ascribed to the use of crude protein extract. Since protein extracts contain a vast number of allergenic and non-allergenic components, in the course of immunotherapy a patient might develop IgE antibodies against additional components present in crude extracts, as was shown in case of specific immunotherapy of grass- and birch pollen-allergic patients using crude extracts [172, 173].

Immunotherapy with fungal extracts is possible, but in most countries not recommended because of problems with the standardization of extracts (see also Diagnosis of Fungal Allergy) [174] and the frequent occurrence of side effects [175]. Additionally, the use of fungal extracts for immunotherapy is hampered by the vast number of fungal species and the lack of knowledge on the degree of exposure to many molds. In the last years, only very few

studies reporting a moderate reduction in symptoms have been conducted [25, 175, 176]. In a high-dose (maximal dose of 100,000 biological units), placebo-controlled, double-blind study [177], 81% of the *C. herbarum*-allergic patients hyposensitized with *C. herbarum* extract improved their clinical symptoms, whereas 19% showed a deterioration in their symptoms. In a 3-year clinical study including 79 children with asthma and rhinitis showing *Alternaria* sensitization, Cantani et al. [178] reported a successful immunotherapy (doses were higher than 80,000 protein nitrogen units) in 80% of their children.

Using a defined panel of allergenic molecules instead of crude extracts, a patient-tailored immunotherapy may be a future aim.

Conclusions

Taken together, a large number of fungal allergens have been isolated and characterized in the last years. Some of them have already been tested in clinical trials, demonstrating their benefit in the diagnosis of mold allergy [54, 58] and other fungal diseases such as ABPA [89, 90]. It has been shown that the specificity of recombinant allergens in serology and skin tests is clearly superior to the specificity obtained with commercial extracts [165].

Nevertheless, there is still a long way to go until immunotherapy of mold allergy will be safe and successful.

Acknowledgement

This work was supported by project S8812-MED given to B. Simon-Nobbe and M. Breitenbach by the Austrian Science Fund (FWF).

References

- 1 De Hoog GS, Guarro J, Gene J, Figueras MJ: Atlas of Clinical Fungi, ed 2. Utrecht, Centraalbureau voor Schimmelcultures, 2000.
- 2 Kauffman HF, Tomee JF, van der Werf TS, de Monchy JG, Koeter GK: Review of fungus-induced asthmatic reactions. *Am J Respir Crit Care Med* 1995;151:2109–2115, discussion 2116.
- 3 Bush RK, Portnoy JM, Saxon A, Terr AI, Wood RA: The medical effects of mold exposure. *J Allergy Clin Immunol* 2006;117:326–333.
- 4 Kauffman HF, Tomee JF, van de Riet MA, Timmerman AJ, Borger P: Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol* 2000;105:1185–1193.
- 5 Fischer G, Schwalbe R, Moller M, Ostrowski R, Dott W: Species-specific production of microbial volatile organic compounds (MVOC) by airborne fungi from a compost facility. *Chemosphere* 1999;39:795–810.
- 6 Ingold CT, Hudson HJ: *The Biology of Fungi*, ed 6, revised. London, Chapman & Hall, 1993.
- 7 Lacey L: The aerobiology of conidial fungi; in Cole GT, Kendrick B (eds): *Biology of Conidial Fungi*. New York, Academic Press, 1981, pp 123–128.
- 8 D'Amato G, Spieksma FT: Aerobiologic and clinical aspects of mould allergy in Europe. *Allergy* 1995;50:870–877.
- 9 Burge HA: Airborne allergenic fungi classification, nomenclature, and distribution. *Immunol Allergy Clin North Am* 1989;9:307–319.
- 10 Burge HA: Fungus allergens. *Clin Rev Allergy* 1985;3:319–329.

- 11 Dharmage S, Bailey M, Raven J, Mitakakis T, Thien F, Forbes A, Guest D, Abramson M, Walters EH: Prevalence and residential determinants of fungi within homes in Melbourne, Australia. *Clin Exp Allergy* 1999;29:1481–1489.
- 12 Nevalainen A, Rautiala S, Hyvarinen A, Reponen T, Husman T, Kalliokoski P: Exposure to fungal spores in mouldy houses: effect of remedial work; in Agashe SN (ed): *Recent Trends in Aerobiology, Allergy and Immunology*. New Delhi, Oxford & IBH, 1994, pp 99–107.
- 13 Gravesen S, Nielsen PA, Iversen R, Nielsen KF: Microfungal contamination of damp buildings – examples of risk constructions and risk materials. *Environ Health Perspect* 1999;107(suppl 3):505–508.
- 14 Kurup VP: Fungal Allergy; in Arora N (ed): *Handbook of Fungal Biotechnology*. New York, Dekker, 2003, pp 515–525.
- 15 Brummund W, Kurup VP, Harris GJ, Duncavage JA, Arkins JA: Allergic sino-orbital mycosis. A clinical and immunologic study. *JAMA* 1986;256:3249–3253.
- 16 O'Brien IM, Bull J, Creamer B, Sepulveda R, Harries M, Burge PS, Pepys J: Asthma and extrinsic allergic alveolitis due to *Merulius lacrymans*. *Clin Allergy* 1978;8:535–542.
- 17 Sastre J, Ibanez MD, Lopez M, Lehrer SB: Respiratory and immunological reactions among Shiitake (*Lentinus edodes*) mushroom workers. *Clin Exp Allergy* 1990;20:13–19.
- 18 Bhatnagar D, Yu J, Ehrlich KC: Toxins of filamentous fungi. *Chem Immunol* 2002;81:167–206.
- 19 Samson RA: Mycotoxins: a mycologist's perspective. *J Med Vet Mycol* 1992;30(suppl 1):9–18.
- 20 Tariq SM, Matthews SM, Stevens M, Hakim EA: Sensitization to *Alternaria* and *Cladosporium* by the age of 4 years. *Clin Exp Allergy* 1996;26:794–798.
- 21 Salvaggio J, Aukrust L: Postgraduate course presentations. Mold-induced asthma. *J Allergy Clin Immunol* 1981;68:327–346.
- 22 Corey JP, Kaiseruddin S, Gungor A: Prevalence of mold-specific immunoglobulins in a Midwestern allergy practice. *Otolaryngol Head Neck Surg* 1997;117:516–520.
- 23 Lopez M, Salvaggio JE: Mold-sensitive asthma. *Clin Rev Allergy* 1985;3:183–196.
- 24 Hsieh KH, Shen JJ: Prevalence of childhood asthma in Taipei, Taiwan, and other Asian Pacific countries. *J Asthma* 1988;25:73–82.
- 25 Horst M, Hejjaoui A, Horst V, Michel FB, Bousquet J: Double-blind, placebo-controlled rush immunotherapy with a standardized *Alternaria* extract. *J Allergy Clin Immunol* 1990;85:460–472.
- 26 Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F: Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ* 2002;325:411–414.
- 27 Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM: The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006;27:615–626.
- 28 Targonski PV, Persky VW, Ramekrishnan V: Effect of environmental molds on risk of death from asthma during the pollen season. *J Allergy Clin Immunol* 1995;95:955–961.
- 29 Halonen M, Stern DA, Wright AL, Taussig LM, Martinez FD: *Alternaria* as a major allergen for asthma in children raised in a desert environment. *Am J Respir Crit Care Med* 1997;155:1356–1361.
- 30 Nelson HS, Szeffler SJ, Jacobs J, Huss K, Shapiro G, Sternberg AL: The relationships among environmental allergen sensitization, allergen exposure, pulmonary function, and bronchial hyperresponsiveness in the Childhood Asthma Management Program. *J Allergy Clin Immunol* 1999;104:775–785.
- 31 Peat JK, Tovey E, Mellis CM, Leeder SR, Woolcock AJ: Importance of house dust mite and *Alternaria* allergens in childhood asthma: an epidemiological study in two climatic regions of Australia. *Clin Exp Allergy* 1993;23:812–820.
- 32 Black PN, Udy AA, Brodie SM: Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy* 2000;55:501–504.
- 33 O'Driscoll BR, Hopkinson LC, Denning DW: Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pulm Med* 2005;5:4.
- 34 Reed CE: What we do and do not know about mold allergy and asthma. *J Allergy Clin Immunol* 1985;76:773–775.
- 35 Dales RE, Cakmak S, Judek S, Dann T, Coates F, Brook JR, Burnett RT: The role of fungal spores in thunderstorm asthma. *Chest* 2003;123:745–750.
- 36 O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, Sachs MI: Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991;324:359–363.
- 37 Bush RK, Prochnau JJ: *Alternaria*-induced asthma. *J Allergy Clin Immunol* 2004;113:227–234.
- 38 Neukirch C, Henry C, Leynaert B, Liard R, Bousquet J, Neukirch F: Is sensitization to *Alternaria alternata* a risk factor for severe asthma? A population-based study. *J Allergy Clin Immunol* 1999;103:709–711.
- 39 Niedoszytko M, Chelminska M, Jassem E, Czestochowska E: Association between sensitization to *Aureobasidium pullulans* (*Pulularia* sp) and severity of asthma. *Ann Allergy Asthma Immunol* 2007;98:153–156.
- 40 Leung DY: Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000;105:860–876.
- 41 Kieffer M, Bergbrant IM, Faergemann J, Jemec GB, Ottevanger V, Stahl Skov P, Svejgaard E: Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. *J Am Acad Dermatol* 1990;22:739–742.
- 42 Gfesser M, Abeck D, Rugemer J, Schreiner V, Stab F, Disch R, Ring J: The early phase of epidermal barrier regeneration is faster in patients with atopic eczema. *Dermatology* 1997;195:332–336.
- 43 Walker C, Kagi MK, Ingold P, Braun P, Blaser K, Bruijnzeel-Koomen CA, Wuthrich B: Atopic dermatitis: correlation of peripheral blood T cell activation, eosinophilia and serum factors with clinical severity. *Clin Exp Allergy* 1993;23:145–153.
- 44 Schmid-Grendelmeier P, Fluckiger S, Disch R, Trautmann A, Wuthrich B, Blaser K, Scheynius A, Cramer R: IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis. *J Allergy Clin Immunol* 2005;115:1068–1075.
- 45 Kortekangas-Savolainen O, Lammintausta K, Kalimo K: Skin prick test reactions to brewer's yeast (*Saccharomyces cerevisiae*) in adult atopic dermatitis patients. *Allergy* 1993;48:147–150.
- 46 Mari A, Schneider P, Wally V, Breitenbach M, Simon-Nobbe B: Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. *Clin Exp Allergy* 2003;33:1429–1438.
- 47 Eggleston PA, Rosenstreich D, Lynn H, Gergen P, Baker D, Kattan M, Mortimer KM, Mitchell H, Ownby D, Slavin R, Malveaux F: Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J Allergy Clin Immunol* 1998;102:563–570.
- 48 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.
- 49 Simon-Nobbe B, Denk U, Schneider PB, Radauer C, Teige M, Cramer R, Hawranek T, Lang R, Richter K, Schmid-Grendelmeier P, Nobbe S, Hartl A, Breitenbach M: NADP-dependent mannitol dehydrogenase, a major allergen of *Cladosporium herbarum*. *J Biol Chem* 2006;281:16354–16360.
- 50 Schneider PB, Denk U, Breitenbach M, Richter K, Schmid-Grendelmeier P, Nobbe S, Himly M, Mari A, Ebner C, Simon-Nobbe B: *Alternaria alternata* NADP-dependent mannitol dehydrogenase is an important fungal allergen. *Clin Exp Allergy* 2006;36:1513–1524.
- 51 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.

- 52 Simon-Nobbe B, Probst G, Kajava AV, Oberkofler H, Susani M, Cramer R, Ferreira F, Ebner C, Breitenbach M: IgE-binding epitopes of enolases, a class of highly conserved fungal allergens. *J Allergy Clin Immunol* 2000;106:887–895.
- 53 Zhang L, Muradia G, De Vouge MW, Rode H, Vijay HM: An allergenic polypeptide representing a variable region of hsp 70 cloned from a cDNA library of *Cladosporium herbarum*. *Clin Exp Allergy* 1996;26:88–95.
- 54 Asturias JA, Ibarrola I, Ferrer A, Andreu C, Lopez-Pascual E, Quiralte J, Florido F, Martinez A: Diagnosis of *Alternaria alternata* sensitization with natural and recombinant Alt a 1 allergens. *J Allergy Clin Immunol* 2005;115:1210–1217.
- 55 Curran IH, Young NM, Burton M, Vijay HM: Purification and characterization of Alt a-29 from *Alternaria alternata*. *Int Arch Allergy Immunol* 1993;102:267–275.
- 56 De Vouge 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.
- 57 Zhang L, Curran IH, Muradia G, De Vouge MW, Rode H, Vijay HM: N-terminus of a major allergen, Alt a 1, of *Alternaria alternata* defined to be an epitope. *Int Arch Allergy Immunol* 1995;108:254–259.
- 58 Unger A, Stoger P, Simon-Nobbe B, Susani M, Cramer R, Ebner C, Hintner H, Breitenbach M: Clinical testing of recombinant allergens of the mold *Alternaria alternata*. *Int Arch Allergy Immunol* 1999;118:220–221.
- 59 Bush RK, Sanchez H, Geisler D: Molecular cloning of a major *Alternaria alternata* allergen, rAlt a 2. *J Allergy Clin Immunol* 1999;104:665–671.
- 60 Kurup VP, Vijay HM, Kumar V, Castillo L, Elms N: IgE binding synthetic peptides of Alt a 1, a major allergen of *Alternaria alternata*. *Peptides* 2003;24:179–185.
- 61 Breitenbach M, Simon-Nobbe B: The allergens of *Cladosporium herbarum* and *Alternaria alternata*. *Chem Immunol* 2002;81:48–72.
- 62 Breitenbach M, Simon Nobbe B, Denk U, Schneider P, Richter K, Teige M, Radauer C, Ebner C, Nobbe S, Schmid-Grendelmeier P, Cramer R: Cloning, expression, characterization, and skin prick testing of NADPH-dependent mannitol dehydrogenase. A new major allergen of *Cladosporium herbarum*; in Lowenstein H, Bienenstock J, Ring J (eds): *From Genes to Phenotypes – The Basis of Future Allergy Management*. Cambridge, Hogrefe & Huber, 2005, pp 75–77.
- 63 Chou H, Tam MF, Lee SS, Tai HY, Chang CY, Chou CT, Shen HD: A vacuolar serine protease (Rho m 2) is a major allergen of *Rhodotorula mucilaginosa* and belongs to a class of highly conserved pan-fungal allergens. *Int Arch Allergy Immunol* 2005;138:134–141.
- 64 Simon-Nobbe B, Kodzius R, Kajava AV, Ferreira F, Kungl A, Achatz G, Cramer R, Ebner C, Breitenbach M: Structure of an IgE-binding peptide from fungal enolases. *Int Arch Allergy Immunol* 2001;124:93–94.
- 65 Horner WE, Helbling A, Salvaggio JE, Lehrer SB: Fungal allergens. *Clin Microbiol Rev* 1995;8:161–179.
- 66 Terr AI: Are indoor molds causing a new disease? *J Allergy Clin Immunol* 2004;113:221–226.
- 67 Kurup VP, Shen HD, Banerjee B: Respiratory fungal allergy. *Microbes Infect* 2000;2:1101–1110.
- 68 Kurup VP, Banerjee B: Fungal allergens and peptide epitopes. *Peptides* 2000;21:589–599.
- 69 Vijay HM, Kurup VP: Fungal allergens. *Clin Allergy Immunol* 2004;18:223–249.
- 70 Cramer R, Blaser K: Cloning *Aspergillus fumigatus* allergens by the pJFo filamentous phage display system. *Int Arch Allergy Immunol* 1996;110:41–45.
- 71 Arruda LK, Mann BJ, Chapman MD: Selective expression of a major allergen and cytotoxin, Asp f 1, in *Aspergillus fumigatus*. Implications for the immunopathogenesis of *Aspergillus*-related diseases. *J Immunol* 1992;149:3354–3359.
- 72 Banerjee B, Kurup VP, Greenberger PA, Hoffman DR, Nair DS, Fink JN: Purification of a major allergen, Asp f 2 binding to IgE in allergic bronchopulmonary aspergillosis, from culture filtrate of *Aspergillus fumigatus*. *J Allergy Clin Immunol* 1997;99:821–827.
- 73 Cramer R: Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications. *Int Arch Allergy Immunol* 1998;115:99–114.
- 74 Vailes L, Sridhara S, Cromwell O, Weber B, Breitenbach M, Chapman M: Quantitation of the major fungal allergens, Alt a 1 and Asp f 1, in commercial allergenic products. *J Allerg Clin Immunol* 2001;107:641–646.
- 75 Cramer R, Weichel M, Fluckiger S, Glaser AG, Rhyner C: Fungal allergies: a yet unsolved problem. *Chem Immunol Allergy* 2006;91:121–133.
- 76 Cramer R, Hemmann S, Mayer C, Appenzeller U, Blaser K: Molecular aspects and diagnostic value of fungal allergens (in German). *Mycoses* 1998;41:56–60.
- 77 Moser M, Cramer R, Menz G, Schneider T, Dudler T, Virchow C, Gmachl M, Blaser K, Suter 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.
- 78 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.
- 79 Moser M, Cramer R, Brust E, Suter M, Menz G: Diagnostic value of recombinant *Aspergillus fumigatus* allergen I/a for skin testing and serology. *J Allergy Clin Immunol* 1994;93:1–11.
- 80 Chauhan B, Santiago L, Kirschmann DA, Hauptfeld V, Knutsen AP, Hutcheson PS, Woulfe SL, Slavin RG, Schwartz HJ, Bellone CJ: The association of HLA-DR alleles and T cell activation with allergic bronchopulmonary aspergillosis. *J Immunol* 1997;159:4072–4076.
- 81 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.
- 82 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 Commun* 2000;270:1128–1135.
- 83 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.
- 84 Hemmann S, Blaser K, Cramer R: Allergens of *Aspergillus fumigatus* and *Candida boidinii* share IgE-binding epitopes. *Am J Respir Crit Care Med* 1997;156:1956–1962.
- 85 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* 1998;28:860–867.
- 86 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.
- 87 Ramachandran H, Banerjee B, Greenberger PA, Kelly KJ, Fink JN, Kurup VP: Role of C-terminal cysteine residues of *Aspergillus fumigatus* allergen Asp f 4 in immunoglobulin E binding. *Clin Diagn Lab Immunol* 2004;11:261–265.
- 88 Cramer R, Faith A, Hemmann S, Jaussi R, Ismail C, Menz G, Blaser K: Humoral and cell-mediated autoimmunity in allergy to *Aspergillus fumigatus*. *J Exp Med* 1996;184:265–270.
- 89 Hemmann S, Menz G, Ismail C, Blaser K, Cramer R: Skin test reactivity to 2 recombinant *Aspergillus fumigatus* allergens in A fumigatus-sensitized asthmatic subjects allows diagnostic separation of allergic bronchopulmonary aspergillosis from fungal sensitization. *J Allergy Clin Immunol* 1999;104:601–607.
- 90 Kurup VP, Banerjee B, Hemmann S, Greenberger PA, Blaser K, Cramer R: Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. *Clin Exp Allergy* 2000;30:988–993.

- 91 Mayer C, Appenzeller U, Seelbach H, Achatz G, Oberkofler H, Breitenbach M, Blaser K, Cramer R: Humoral and cell-mediated autoimmune reactions to human acidic ribosomal P2 protein in individuals sensitized to *Aspergillus fumigatus* P2 protein. *J Exp Med* 1999;189:1507–1512.
- 92 Kumar A, Reddy LV, Sochanik A, Kurup VP: Isolation and characterization of a recombinant heat shock protein of *Aspergillus fumigatus*. *J Allergy Clin Immunol* 1993;91:1024–1030.
- 93 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.
- 94 Shen HD, Lin WL, Tam MF, Wang SR, Tzuan SS, Huang MH, Han SH: Characterization of allergens from *Penicillium oxalicum* and *P. notatum* by immunoblotting and N-terminal amino acid sequence analysis. *Clin Exp Allergy* 1999;29:642–651.
- 95 Chow LP, Liu SL, Yu CJ, Liao HK, Tsai JJ, Tang TK: Identification and expression of an allergen Asp f 13 from *Aspergillus fumigatus* and epitope mapping using human IgE antibodies and rabbit polyclonal antibodies. *Biochem J* 2000;346:423–431.
- 96 Lai HYL, Tam MF, Tang RB, Chou H, Chang CY, Tsai JJ, Shen HS: 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.
- 97 Bowyer P, Denning DW: Genomic analysis of allergen genes in *Aspergillus* spp: the relevance of genomics to everyday research. *Med Mycol* 2007;45:17–26.
- 98 Wei DL, Jong SC, Shen HD: Indoor airborne *Penicillium* species in Taiwan. *Curr Microbiol* 1993;26:137–140.
- 99 Muilenberg M, Burge HA, Sweet T, Solomon W: *Penicillium* species in and out of doors in Topeka, KS. *J Allergy Clin Immunol* 1990;85:247.
- 100 Licorish K, Novey HS, Kozak P, Fairshater RD, Wilson AF: Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. *J Allergy Clin Immunol* 1985;76:819–825.
- 101 Hsieh KH: A study of intracutaneous skin tests and radioallergosorbent tests on 1,000 asthmatic children in Taiwan. *Asian Pac J Allergy Immunol* 1984;2:56–60.
- 102 Chow LP, Su NY, Yu CJ, Chiang BL, Shen HD: Identification and expression of Pen c 2, a novel allergen from *Penicillium citrinum*. *Biochem J* 1999;341:51–59.
- 103 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 crossreactivity to a hsp 70 human heat shock protein. *Clin Exp Allergy* 1997;27:682–690.
- 104 Shen HD, Wang CW, Lin WL, Lai HY, Tam MF, Chou H, Wang SR, Han SH: cDNA cloning and immunologic characterization of Pen o 18, the vacuolar serine protease major allergen of *Penicillium oxalicum*. *J Lab Clin Med* 2001;137:115–124.
- 105 Lai HY, Tam MF, Chou H, Lee SS, Tai HY, Shen HD: Molecular and structural analysis of immunoglobulin E-binding epitopes of Pen ch 13, an alkaline serine protease major allergen from *Penicillium chrysogenum*. *Clin Exp Allergy* 2004;34:1926–1933.
- 106 Shen HD, Chou H, Tam MF, Chang CY, Lai HY, Wang SR: Molecular and immunological characterization of Pen ch 18, the vacuolar serine protease major allergen of *Penicillium chrysogenum*. *Allergy* 2003;58:993–1002.
- 107 Yu CJ, Chen YM, Su SN, Forouhar F, Lee SH, Chow LP: Molecular and immunological characterization and IgE epitope mapping of Pen n 18, a major allergen of *Penicillium notatum*. *Biochem J* 2002;363:707–715.
- 108 Shen HD, Wang CW, Chou H, Lin WL, Tam MF, Huang MH, Kuo ML, Wang SR, Han SH: Complementary DNA cloning and immunologic characterization of a new *Penicillium citrinum* allergen (Pen c 3). *J Allergy Clin Immunol* 2000;105:827–833.
- 109 Sevinc MS, Kumar V, Abebe M, Casley WL, Vijay HM: Isolation and characterization of a cDNA clone encoding one IgE-binding fragment of *Penicillium brevicompactum*. *Int Arch Allergy Immunol* 2005;138:12–20.
- 110 Tang RB, Chen YS, Chou H, Lee SS, Tai HY, Shen HD: cDNA cloning and immunologic characterization of a novel EF-1 β allergen from *Penicillium citrinum*. *Allergy* 2005;60:366–371.
- 111 Akiyama K, Shida T, Yasueda H, Mita H, Yanagihara Y, Hasegawa M, Maeda Y, Yamamoto T, Takesako K, Yamaguchi H: Allergenicity of acid protease secreted by *Candida albicans*. *Allergy* 1996;51:887–892.
- 112 Martinez JP, Gil ML, Lopez-Ribot JL, Chaffin WL: Serologic response to cell wall mannoproteins and proteins of *Candida albicans*. *Clin Microbiol Rev* 1998;11:121–141.
- 113 Ito K, Ishiguro A, Kanbe T, Tanaka K, Torii S: Characterization of IgE-binding epitopes on *Candida albicans* enolase. *Clin Exp Allergy* 1995;25:529–535.
- 114 Eroles P, Sentandreu M, Elorza MV, Sentandreu R: The highly immunogenic enolase and Hsp70p are adventitious *Candida albicans* cell wall proteins. *Microbiology* 1997;143(Pt 2):313–320.
- 115 Burge HA, Solomon WR, Muilenberg ML: Evaluation of indoor plantings as allergen exposure sources. *J Allergy Clin Immunol* 1982;70:101–108.
- 116 Levetin E: Studies on airborne basidiomycete spores. *Aerobiologia* 1990;6:177–180.
- 117 Luo W: Deposition of large particles in the nose and mouth. *Grana* 1991;30:79–81.
- 118 Roncarolo D, Minalpe P, Mistrello G, Voltolini S, Falagiani P: Food allergy to *Boletus edulis*. *J Allergy Clin Immunol* 1998;101:850–851.
- 119 Levy AM, Kita H, Phillips SF, Schkade PA, Dyer PD, Gleich GJ, Dubravec VA: Eosinophilia and gastrointestinal symptoms after ingestion of shiitake mushrooms. *J Allergy Clin Immunol* 1998;101:613–620.
- 120 Epton MJ, Martin IR, Graham P, Healy PE, Smith H, Balasubramaniam R, Harvey IC, Fountain DW, Hedley J, Town GI: Climate and aeroallergen levels in asthma: a 12 month prospective study. *Thorax* 1997;52:528–534.
- 121 Brander KA, Borbely P, Cramer R, Pichler WJ, Helbling A: IgE-binding proliferative responses and skin test reactivity to Cop c 1, the first recombinant allergen from the basidiomycete *Coprinus comatus*. *J Allergy Clin Immunol* 1999;104:630–636.
- 122 Lehrer SB, Hughes JM, Altman LC, Bousquet J, Davies RJ, Gell L, Li J, Lopez M, Malling HJ, Mathison DA, et al: Prevalence of basidiomycete allergy in the USA and Europe and its relationship to allergic respiratory symptoms. *Allergy* 1994;49:460–465.
- 123 Helbling A, Gayer F, Pichler WJ, Brander KA: Mushroom (Basidiomycete) allergy: diagnosis established by skin test and nasal challenge. *J Allergy Clin Immunol* 1998;102:853–858.
- 124 Helbling A, Brander KA, Horner WE, Lehrer SB: Allergy to basidiomycetes. *Chem Immunol* 2002;81:28–47.
- 125 Faergemann J, Fredriksson T: Age incidence of *Pityrosporum orbiculare* on human skin. *Acta Derm Venereol* 1980;60:531–533.
- 126 Faergemann J, Aly R, Maibach HI: Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. *Acta Derm Venereol* 1983;63:346–348.
- 127 Lintu P, Savolainen J, Kalimo K: IgE antibodies to protein and mannan antigens of *Pityrosporum ovale* in atopic dermatitis patients. *Clin Exp Allergy* 1997;27:87–95.
- 128 Johansson S, Karlstrom K: IgE-binding components in *Pityrosporum orbiculare* identified by an immunoblotting technique. *Acta Derm Venereol* 1991;71:11–16.
- 129 Garrard LJ, Goodman JM: Two genes encode the major membrane-associated protein of methanol-induced peroxisomes from *Candida boidinii*. *J Biol Chem* 1989;264:13929–13937.

- 130 Yasueda H, Hashida-Okado T, Saito A, Uchida K, Kuroda M, Onishi Y, Takahashi K, Yamaguchi H, Takesako K, Akiyama K: Identification and cloning of two novel allergens from the lipophilic yeast, *Malassezia furfur*. *Biochem Biophys Res Commun* 1998;248:240–244.
- 131 Lindborg M, Magnusson CG, Zargari A, Schmidt M, Scheynius A, Cramer R, Whitley P: Selective cloning of allergens from the skin colonizing yeast *Malassezia furfur* by phage surface display technology. *J Invest Dermatol* 1999;113:156–161.
- 132 Onishi Y, Kuroda M, Yasueda H, Saito A, Sono-Koyama E, Tunasawa S, Hashida-Okado T, Yagihara T, Uchida K, Yamaguchi H, Akiyama K, Kato I, Takesako K: Two-dimensional electrophoresis of *Malassezia* allergens for atopic dermatitis and isolation of Mal f 4 homologs with mitochondrial malate dehydrogenase. *Eur J Biochem* 1999;261:148–154.
- 133 Andersson A, Rasool O, Schmidt M, Kodzius R, Fluckiger S, Zargari A, Cramer R, Scheynius A: Cloning, expression and characterization of two new IgE-binding proteins from the yeast *Malassezia sympodialis* with sequence similarities to heat shock proteins and manganese superoxide dismutase. *Eur J Biochem* 2004;271:1885–1894.
- 134 Nierman WC, Pain A, Anderson MJ, et al: Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* 2005;438:1151–1156.
- 135 Helbling A, Horner WE, Lehrer SB: Comparison of *Psilocybe cubensis* spore and mycelium allergens. *J Allergy Clin Immunol* 1993;91:1059–1066.
- 136 Horner WE, Reese G, Lehrer SB: Identification of the allergen Psi c 2 from the basidiomycete *Psilocybe cubensis* as a fungal cyclophilin. *Int Arch Allergy Immunol* 1995;107:298–300.
- 137 Chang CY, Chou H, Tam MF, Tang RB, Lai HY, Shen HD: Characterization of enolase allergen from *Rhodotorula mucilaginosa*. *J Biomed Sci* 2002;9:645–655.
- 138 Aalberse RC, Akkerdaas J, van Ree R: Cross-reactivity of IgE antibodies to allergens. *Allergy* 2001;56:478–490.
- 139 Aalberse RC: Structural biology of allergens. *J Allergy Clin Immunol* 2000;106:228–238.
- 140 O'Neil CE, Horner WE, Reed MA, Lopez M, Lehrer SB: Evaluation of Basidiomycete and Deuteromycete (Fungi Imperfecti) extracts for shared allergenic determinants. *Clin Exp Allergy* 1990;20:533–538.
- 141 Saenz-de-Santamaria M, Postigo I, Gutierrez-Rodriguez A, Cardona G, Guisantes JA, Asturias J, Martinez J: The major allergen of *Alternaria alternata* (Alt a 1) is expressed in other members of the Pleosporaceae family. *Mycoses* 2006;49:91–95.
- 142 Lin WL, Chou H, Tam MF, Huang MH, Han SH, Shen HD: Production and characterization of monoclonal antibodies to serine proteinase allergens in *Penicillium* and *Aspergillus* species. *Clin Exp Allergy* 2000;30:1653–1662.
- 143 Simon-Nobbe B, Pöll V, Wally V, Shen HD, Lottspeich F, Hawranek T, Lang R, Hemmer W, Jarisch R, Breitenbach M: Vacuolar serine proteases from *Cladosporium herbarum* and *Alternaria alternata*, submitted.
- 144 Gupta R, Singh BP, Sridhara S, Gaur SN, Kumar R, Chaudhary VK, Arora N: Allergenic cross-reactivity of *Curvularia lunata* with other airborne fungal species. *Allergy* 2002;57:636–640.
- 145 Karkkainen M, Raunio P, Rautiainen J, Auriola S, Hinke K, Pasanen AL: Partial amino acid sequence of a cellulase-like component with IgE-binding properties from *Stachybotrys chartarum*. *Int Arch Allergy Immunol* 2004;133:136–144.
- 146 Wagner S, Breiteneder H, Simon-Nobbe B, Susani M, Krebitz M, Niggemann B, Brehler R, Scheiner O, Hoffmann-Sommergruber K: Hev b 9, an enolase and a new cross-reactive allergen from *Hevea latex* and molds. Purification, characterization, cloning and expression. *Eur J Biochem* 2000;267:7006–7014.
- 147 Shankar J, Gupta PD, Sridhara S, Singh BP, Gaur SN, Arora N: Immunobiochemical analysis of cross-reactive glutathione-S-transferase allergen from different fungal sources. *Immunol Invest* 2005;34:37–51.
- 148 Fluckiger S, Scapozza L, Mayer C, Blaser K, Folkers G, Cramer R: Immunological and structural analysis of IgE-mediated cross-reactivity between manganese superoxide dismutases. *Int Arch Allergy Immunol* 2002;128:292–303.
- 149 Wagner S, Sowka S, Mayer C, Cramer R, Focke M, Kurup VP, Scheiner O, Breiteneder H: Identification of a *Hevea brasiliensis* latex manganese superoxide dismutase (Hev b 10) as a cross-reactive allergen. *Int Arch Allergy Immunol* 2001;125:120–127.
- 150 Glaser AG, Limacher A, Fluckiger S, Scheynius A, Scapozza L, Cramer R: Analysis of the cross-reactivity and of the 1.5 A crystal structure of the *Malassezia sympodialis* Mala s 6 allergen, a member of the cyclophilin pan-allergen family. *Biochem J* 2006;396:41–49.
- 151 Appenzeller U, Meyer C, Menz G, Blaser K, Cramer R: IgE-mediated reactions to autoantigens in allergic diseases. *Int Arch Allergy Immunol* 1999;118:193–196.
- 152 Weichel M, Glaser AG, Ballmer-Weber BK, Schmid-Grendelmeier P, Cramer R: Wheat and maize thioredoxins: a novel cross-reactive cereal allergen family related to baker's asthma. *J Allergy Clin Immunol* 2006;117:676–681.
- 153 Esch RE: Manufacturing and standardizing fungal allergen products. *J Allergy Clin Immunol* 2004;113:210–215.
- 154 Aas K, Leegaard J, Aukrust L, Grimmer O: Immediate type hypersensitivity to common moulds. Comparison of different diagnostic materials. *Allergy* 1980;35:443–451.
- 155 Portnoy J, Chapman J, Burge H, Muilenberg M, Solomon W: *Epicoccum* allergy: skin reaction patterns and spore/mycelium disparities recognized by IgG and IgE ELISA inhibition. *Ann Allergy* 1987;59:39–43.
- 156 Steringer I, Aukrust L, Einarsson R: Variability of antigenicity/allergenicity in different strains of *Alternaria alternata*. *Int Arch Allergy Appl Immunol* 1987;84:190–197.
- 157 Paris S, Fitting C, Ramirez E, Latge JP, David B: Comparison of different extraction methods of *Alternaria* allergens. *J Allergy Clin Immunol* 1990;85:941–948.
- 158 Hoffman DR, Kozak PP Jr, Gillman SA, Cummins LH, Gallup J: Isolation of spore specific allergens from *Alternaria*. *Ann Allergy* 1981;46:310–316.
- 159 Ferreira F, Wallner M, Thalhamer J: Customized antigens for desensitizing allergic patients. *Adv Immunol* 2004;84:79–129.
- 160 Portnoy J, Pacheco F, Ballam Y, Barnes C: The effect of time and extraction buffers on residual protein and allergen content of extracts derived from four strains of *Alternaria*. *J Allergy Clin Immunol* 1993;91:930–938.
- 161 Green BJ, Sercombe JK, Tovey ER: Fungal fragments and undocumented conidia function as new aeroallergen sources. *J Allergy Clin Immunol* 2005;115:1043–1048.
- 162 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.
- 163 Schmidt M, Hoffman DR: Expression systems for production of recombinant allergens. *Int Arch Allergy Immunol* 2002;128:264–270.
- 164 De Vouge MW, Thaker AJ, Curran IH, Zhang L, Muradia G, Rode H, Vijay HM: Isolation and expression of a cDNA clone encoding an *Alternaria alternata* Alt a 1 subunit. *Int Arch Allergy Immunol* 1996;111:385–395.
- 165 Schmid-Grendelmeier P, Cramer R: Recombinant allergens for skin testing. *Int Arch Allergy Immunol* 2001;125:96–111.
- 166 Ballmer-Weber BK, Scheurer S, Fritsche P, Enrique E, Cistero-Bahima A, Haase T, Wuthrich B: Component-resolved diagnosis with recombinant allergens in patients with cherry allergy. *J Allergy Clin Immunol* 2002;110:167–173.

- 167 Tresch S, Holzmann D, Baumann S, Blaser K, Wuthrich B, Cramer R, Schmid-Grendelmeier P: In vitro and in vivo allergenicity of recombinant Bet v 1 compared to the reactivity of natural birch pollen extract. *Clin Exp Allergy* 2003;33:1153–1158.
- 168 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Gronlund H: The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.
- 169 Mari A: Skin test with a timothy grass (*Phleum pratense*) pollen extract vs. IgE to a timothy extract vs. IgE to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12: epidemiological and diagnostic data. *Clin Exp Allergy* 2003;33:43–51.
- 170 Hiller R, Laffer S, Harwanegg C, et al: Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002;16:414–416.
- 171 Vailes LD, Perzanowski MS, Wheatley LM, Platts-Mills TA, Chapman MD: IgE and IgG antibody responses to recombinant Alt a 1 as a marker of sensitization to *Alternaria* in asthma and atopic dermatitis. *Clin Exp Allergy* 2001;31:1891–1895.
- 172 Ball T, Sperr WR, Valent P, Lidholm J, Spitzauer S, Ebner C, Kraft D, Valenta R: Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999;29:2026–2036.
- 173 Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T: Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. *Allergy* 2002;57:423–430.
- 174 Malling HJ: Immunotherapy for mold allergy. *Clin Rev Allergy* 1992;10:237–251.
- 175 Malling HJ, Dreborg S, Weeke B: Diagnosis and immunotherapy of mould allergy. V. Clinical efficacy and side effects of immunotherapy with *Cladosporium herbarum*. *Allergy* 1986;41:507–519.
- 176 Dreborg S, Agrell B, Foucard T, Kjellman NI, Koivikko A, Nilsson S: A double-blind, multicenter immunotherapy trial in children, using a purified and standardized *Cladosporium herbarum* preparation. I. Clinical results. *Allergy* 1986;41:131–140.
- 177 Malling HJ: Diagnosis and immunotherapy of mould allergy. With special reference to *Cladosporium herbarum*. *Dan Med Bull* 1990;37:12–22.
- 178 Cantani A, Businco E, Maglio A: *Alternaria* allergy: a three-year controlled study in children treated with immunotherapy. *Allergol Immunopathol (Madr)* 1988;16:1–4.
- 179 Ezeamuzie CI, Al-Ali S, Khan M, Hijazi Z, Dowaisan A, Thomson MS, Georgi J: IgE-mediated sensitization to mould allergens among patients with allergic respiratory diseases in a desert environment. *Int Arch Allergy Immunol* 2000;121:300–307.
- 180 Gergen PJ, Turkeltaub PC, Kovar MG: The prevalence of allergic skin test reactivity to eight common aeroallergens in the US population: results from the second National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 1987;80:669–679.
- 181 D'Amato G, Chatzigeorgiou G, Corsico R, Gioulekas D, Jager L, Jager S, Kontou-Fili K, Kouridakis S, Liccardi G, Meriggi A, Palma-Carlos A, Palma-Carlos ML, Pagan Aleman A, Parmiani S, Puccinelli P, Russo M, Spieksma FT, Torricelli R, Wuthrich B: Evaluation of the prevalence of skin prick test positivity to *Alternaria* and *Cladosporium* in patients with suspected respiratory allergy. A European multicenter study promoted by the Subcommittee on Aerobiology and Environmental Aspects of Inhalant Allergens of the European Academy of Allergology and Clinical Immunology. *Allergy* 1997;52:711–716.
- 182 Nolles G, Hoekstra MO, Schouten JP, Gerritsen J, Kauffman HF: Prevalence of immunoglobulin E for fungi in atopic children. *Clin Exp Allergy* 2001;31:1564–1570.
- 183 Gioulekas D, Damialis A, Papakosta D, Spieksma F, Giouleka P, Patakas D: Allergic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. *J Investig Allergol Clin Immunol* 2004;14:225–231.
- 184 Pumihiirun P, Towiwat P, Mahakit P: Aeroallergen sensitivity of Thai patients with allergic rhinitis. *Asian Pac J Allergy Immunol* 1997;15:183–185.
- 185 O'Neil CE, McCants ML, Salvaggio JE, Lehrer SB: *Fusarium solani*: prevalence of skin reactivity and antigenic allergenic analysis. *J Allergy Clin Immunol* 1986;77:842–849.
- 186 Nordvall SL, Johansson S: IgE antibodies to *Pityrosporum orbiculare* in children with atopic diseases. *Acta Paediatr Scand* 1990;79:343–348.
- 187 Shankar J, Singh BP, Gaur SN, Arora N: Recombinant glutathione-S-transferase a major allergen from *Alternaria alternata* for clinical use in allergy patients. *Mol Immunol* 2006;43:1927–1932.
- 188 Portnoy J, Olson I, Pacheco F, Barnes C: Affinity purification of a major *Alternaria* allergen using a monoclonal antibody. *Ann Allergy* 1990;65:109–114.
- 189 Weichel M, Schmid-Grendelmeier P, Fluckiger S, Breitenbach M, Blaser K, Cramer R: Nuclear transport factor 2 represents a novel cross-reactive fungal allergen. *Allergy* 2003;58:198–206.
- 190 Hong SG, Cramer RA, Lawrence CB, Pryor BM: Alt a 1 allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. *Fungal Genet Biol* 2005;42:119–129.
- 191 Cramer RA, Lawrence CB: Cloning of a gene encoding an Alt a 1 isoallergen differentially expressed by the necrotrophic fungus *Alternaria brassicicola* during *Arabidopsis* infection. *Appl Environ Microbiol* 2003;69:2361–2364.
- 192 Chou H, Lin W, Tam MF, Wang S, Han S, Shen H: 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.
- 193 Yu CJ, Chiou SH, Lai WY, Chiang BL, Chow LP: Characterization of a novel allergen, a major IgE-binding protein from *Aspergillus flavus*, as an alkaline serine protease. *Biochem Biophys Res Commun* 1999;261:669–675.
- 194 Stewart GA, Robinson C: The immunobiology of allergenic peptidases. *Clin Exp Allergy* 2003;33:3–6.
- 195 Hemmann S, Nikolaizik WH, Schoni MH, 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* 1998;28:1155–1160.
- 196 Arruda LK, Platts-Mills TA, Fox JW, Chapman MD: *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. *J Exp Med* 1990;172:1529–1532.
- 197 Jatou-Ogay K, Paris S, Huerre M, Quadroni M, Falchetto R, Togni G, Latge JP, Monod M: Cloning and disruption of the gene encoding an extracellular metalloprotease of *Aspergillus fumigatus*. *Mol Microbiol* 1994;14:917–928.
- 198 Reichard U, Monod M, Ruchel R: Molecular cloning and sequencing of the gene encoding an extracellular aspartic proteinase from *Aspergillus fumigatus*. *FEMS Microbiol Lett* 1995;130:69–74.
- 199 Fluckiger S, Fijten H, Whitley P, Blaser K, Cramer R: Cyclophilins, a new family of cross-reactive allergens. *Eur J Immunol* 2002;32:10–17.
- 200 Cramer R: Epidemiology and molecular basis of the involvement of *Aspergillus fumigatus* in allergic diseases. *Contrib Microbiol* 1999;2:44–56.
- 201 Limacher A, Kloer DP, Fluckiger S, Folkers G, Cramer R, Scapozza L: The crystal structure of *Aspergillus fumigatus* cyclophilin reveals 3D domain swapping of a central element. *Structure* 2006;14:185–195.

- 202 Jatón-Ogay K, Suter M, Cramer R, Falchetto R, Fatih A, Monod M: Nucleotide sequence of a genomic and a cDNA clone encoding an extracellular alkaline protease of *Aspergillus fumigatus*. *FEMS Microbiol Lett* 1992;71:163–168.
- 203 Arruda LK, Platts-Mills TA, Longbottom JL, el-Dahr JM, Chapman MD: *Aspergillus fumigatus*: identification of 16, 18, and 45 kd antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. *J Allergy Clin Immunol* 1992;89:1166–1176.
- 204 Banerjee B, Kurup VP, Greenberger PA, Johnson BD, Fink JN: Cloning and expression of *Aspergillus fumigatus* allergen Asp f 16 mediating both humoral and cell-mediated immunity in allergic bronchopulmonary aspergillosis (ABPA). *Clin Exp Allergy* 2001;31:761–770.
- 205 Hemmann S: Cloning, Characterization and Clinical Evaluation of Recombinant *Aspergillus fumigatus* Allergens; thesis, University of Zürich, 1998.
- 206 Saxena S, Madan T, Muralidhar K, Sarma PU: cDNA cloning, expression and characterization of an allergenic L3 ribosomal protein of *Aspergillus fumigatus*. *Clin Exp Immunol* 2003;134:86–91.
- 207 Nigam S, Ghosh PC, Sarma PU: A new glycoprotein allergen/antigen with the protease activity from *Aspergillus fumigatus*. *Int Arch Allergy Immunol* 2003;132:124–131.
- 208 Calera JA, Ovejero MC, Lopez-Medrano R, Segurado M, Puente P, Leal F: Characterization of the *Aspergillus nidulans* aspnd1 gene demonstrates that the ASPND1 antigen, which it encodes, and several *Aspergillus fumigatus* immunodominant antigens belong to the same family. *Infect Immun* 1997;65:1335–1344.
- 209 Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE, Baur X: Allergy to *Aspergillus*-derived enzymes in the baking industry: identification of beta-xylosidase from *Aspergillus niger* as a new allergen (Asp n 14). *J Allergy Clin Immunol* 1998;102:256–264.
- 210 Shen HD, Han SH: Characterization of allergens of *Penicillium* and *Aspergillus* species. *J Microbiol Immunol Infect* 1998;31:141–145.
- 211 Baur X, Melching-Kollmuss S, Koops F, Strassburger K, Zober A: IgE-mediated allergy to phytase – a new animal feed additive. *Allergy* 2002;57:943–945.
- 212 Doekes G, Kamminga N, Helwegen L, Heederik D: Occupational IgE sensitisation to phytase, a phosphatase derived from *Aspergillus niger*. *Occup Environ Med* 1999;56:454–459.
- 213 Quirce S, Fernandez-Nieto M, Escudero C, Cuesta J, de Las Heras M, Sastre J: Bronchial responsiveness to bakery-derived allergens is strongly dependent on specific skin sensitivity. *Allergy* 2006;61:1202–1208.
- 214 Quirce S, Fernandez-Nieto M, Bartolome B, Bombin C, Cuevas M, Sastre J: Glucoamylase: another fungal enzyme associated with baker's asthma. *Ann Allergy Asthma Immunol* 2002;89:197–202.
- 215 Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B, Taylor AJ: Clinical responses to ingested fungal alpha-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? *Allergy* 1997;52:346–349.
- 216 Tatsumi H, Ogawa Y, Murakami S, Ishida Y, Murakami K, Masaki A, Kawabe H, Arimura H, Nakano E, Motai H: A full length cDNA clone for the alkaline protease from *Aspergillus oryzae*: structural analysis and expression in *Saccharomyces cerevisiae*. *Mol Gen Genet* 1989;219:33–38.
- 217 Smith TA, Parker G, Hussain T: Respiratory symptoms and wheat flour exposure: a study of flour millers. *Occup Med* 2000;50:25–29.
- 218 Biagini RE, MacKenzie BA, Sammons DL, Smith JP, Striley CA, Robertson SK, Snawder JE: Evaluation of the prevalence of antiwheat-, anti-flour dust, and anti-alpha-amylase specific IgE antibodies in US blood donors. *Ann Allergy Asthma Immunol* 2004;92:649–653.
- 219 Moneo I, Alday E, Gonzalez-Munoz M, Maqueda J, Curiel G, Lucena R: α -Amylase hypersensitivity in non-exposed millers. *Occup Med (Lond)* 1994;44:91–94.
- 220 Tsukagoshi N, Furukawa M, Nagaba H, Kirita N, Tsuboi A, Udaka S: Isolation of a cDNA encoding *Aspergillus oryzae* Taka-amylase A: evidence for multiple related genes. *Gene* 1989;84:319–327.
- 221 Muir DC, Verrall AB, Julian JA, Millman JM, Beaudin MA, Dolovich J: Occupational sensitization to lactase. *Am J Ind Med* 1997;31:570–571.
- 222 Bernstein JA, Bernstein DI, Stauder T, Lumms Z, Bernstein IL: A cross-sectional survey of sensitization to *Aspergillus oryzae*-derived lactase in pharmaceutical workers. *J Allergy Clin Immunol* 1999;103:1153–1157.
- 223 Binkley KE: Allergy to supplemental lactase enzyme. *J Allergy Clin Immunol* 1996;97:1414–1416.
- 224 Lindstedt M, Schiott A, Johnsen CR, Roggen E, Johansson-Lindbom B, Borrebaeck CA: Individuals with occupational allergy to detergent enzymes display a differential transcriptional regulation and cellular immune response. *Clin Exp Allergy* 2005;35:199–206.
- 225 Westwood GS, Huang SW, Keyhani NO: Molecular and immunological characterization of allergens from the entomopathogenic fungus *Beauveria bassiana*. *Clin Mol Allergy* 2006;4:12.
- 226 Shen HD, Choo KB, Lee HH, Hsieh JC, Lin WL, Lee WR, Han SH: The 40-kilodalton allergen of *Candida albicans* is an alcohol dehydrogenase: molecular cloning and immunological analysis using monoclonal antibodies. *Clin Exp Allergy* 1991;21:675–681.
- 227 Chou H, Tam MF, Chang CY, Lai HY, Huang MH, Chou CT, Lee SS, Shen HD: Characterization of a novel *Candida albicans* 29 kDa IgE-binding protein – purification, cDNA isolation and heterologous expression of Cand a 3. *Allergy* 2003;58:1157–1164.
- 228 Asako Y, Saito A, Yasueda H, Kawaguchi H, Akiyama K, Endo M, Onishi Y, Takesako K: Analysis of IgE reactivities of purified allergens from *Candida albicans* and *Malassezia furfur* among patients with atopic dermatitis (in Japanese). *Arerugi* 2002;51:615–621.
- 229 Ito K, Ishiguro A, Kanbe T, Tanaka K, Torii S: Detection of IgE antibody against *Candida albicans* enolase and its crossreactivity to *Saccharomyces cerevisiae* enolase. *Clin Exp Allergy* 1995;25:522–528.
- 230 Mason AB, Buckley HR, Gorman JA: Molecular cloning and characterization of the *Candida albicans* enolase gene. *J Bacteriol* 1993;175:2632–2639.
- 231 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.
- 232 al-Dughaym AM, Matthews RC, Burnie JP: Epitope mapping human heat shock protein 90 with sera from infected patients. *FEMS Immunol Med Microbiol* 1994;8:43–48.
- 233 Muthuvel A, Rajamani R, Senthilvelan M, Manikandan S, Sheeladevi R: Modification of allergenicity and immunogenicity of formate dehydrogenase by conjugation with linear mono methoxy poly ethylene glycol: improvement in detoxification of formate in methanol poisoning. *Clin Chim Acta* 2006;374:122–128.
- 234 Aukrust L, Borch SM: Partial purification and characterization of two *Cladosporium herbarum* allergens. *Int Arch Allergy Appl Immunol* 1979;60:68–79.
- 235 Zhang L, Muradia G, Curran IH, 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;154:710–717.
- 236 Kurup VP, Shen HD, Vijay H: Immunobiology of fungal allergens. *Int Arch Allergy Immunol* 2002;129:181–188.
- 237 Falsone SF, Weichel M, Cramer R, Breitenbach M, Kungl AJ: Unfolding and double-stranded DNA binding of the cold shock protein homologue Cla h 8 from *Cladosporium herbarum*. *J Biol Chem* 2002;277:16512–16516.

- 238 Weichel M, Schmid-Grendelmeier P, Rhyner C, Achatz G, Blaser K, Cramer R: Immunoglobulin E-binding and skin test reactivity to hydrophobin HCh-1 from *Cladosporium herbarum*, the first allergenic cell wall component of fungi. *Clin Exp Allergy* 2003;33:72–77.
- 239 Gupta R, Sharma V, Sridhara S, Singh BP, Arora N: Identification of serine protease as a major allergen of *Curvularia lunata*. *Allergy* 2004;59:421–427.
- 240 Sharma V, Gupta R, Jhingran A, Singh BP, Sridhara S, Gaur SN, Arora N: Cloning, recombinant expression and activity studies of a major allergen ‘enolase’ from the fungus *Curvularia lunata*. *J Clin Immunol* 2006;26:360–369.
- 241 Bisht V, Arora N, Singh BP, Pasha S, Gaur SN, Sridhara S: Epi p 1, an allergenic glycoprotein of *Epicoecum purpurascens* is a serine protease. *FEMS Immunol Med Microbiol* 2004;42:205–211.
- 242 Hoff M, Ballmer-Weber BK, Niggemann B, Cistero-Bahima A, San Miguel-Moncin M, Conti A, Hausteiner D, Vieths S: Molecular cloning and immunological characterization of potential allergens from the mould *Fusarium culmorum*. *Mol Immunol* 2003;39:965–975.
- 243 Verma J, Pasha S, Gangal SV: Purification and characterization of Fus s13596*, a 65 kd allergen of *Fusarium solani*. *Mol Cell Biochem* 1994;131:157–166.
- 244 Verma J, Singh BP, Sridhara S, Gaur SN, Arora N: Purification and characterization of a cross-reactive 45-kD major allergen of *Fusarium solani*. *Int Arch Allergy Immunol* 2003;130:193–199.
- 245 Shen HD, Lin WL, Tsai JJ, Liaw SF, Han SH: Allergenic components in three different species of *Penicillium*: crossreactivity among major allergens. *Clin Exp Allergy* 1996;26:444–451.
- 246 Chou H, Chang CY, Tsai JJ, Tang RB, Lee SS, Wang SR, Peng HJ, Shen HD: The prevalence of IgE antibody reactivity against the alkaline serine protease major allergen of *Penicillium chrysogenum* increases with the age of asthmatic patients. *Ann Allergy Asthma Immunol* 2003;90:248–253.
- 247 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.
- 248 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.
- 249 Nittner-Marszalska M, Wojcicka-Kustrzeba I, Bogacka E, Patkowski J, Dobek R: Skin prick test response to enzyme enolase of the baker’s yeast (*Saccharomyces cerevisiae*) in diagnosis of respiratory allergy. *Med Sci Monit* 2001;7:121–124.
- 250 Holland MJ, Holland JP, Thill GP, Jackson KA: The primary structures of two yeast enolase genes. Homology between the 5’ noncoding flanking regions of yeast enolase and glyceraldehyde-3-phosphate dehydrogenase genes. *J Biol Chem* 1981;256:1385–1395.
- 251 Vojdani A: Antibodies against *Stachybotrys chartarum* extract and its antigenic components, Stachyhemolysin and Stachyrase-A: a new clinical biomarker. *Med Sci Monit* 2005;11:BR139–BR145.
- 252 Jakobsen CG, Bodtger U, Kristensen P, Poulsen LK, Roggen EL: Isolation of high-affinity human IgE and IgG antibodies recognising Bet v 1 and *Humicola lanuginosa* lipase from combinatorial phage libraries. *Mol Immunol* 2004;41:941–953.
- 253 Woodfolk JA, Wheatley LM, Piyasena RV, Benjamin DC, Platts-Mills TA: 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.
- 254 Deuell B, Arruda LK, Hayden ML, Chapman MD, Platts-Mills TA: Trichophyton tonsurans allergen. I. Characterization of a protein that causes immediate but not delayed hypersensitivity. *J Immunol* 1991;147:96–101.
- 255 Zargari A, Schmidt M, Lundberg M, Scheynius A, Whitley P: Immunologic characterization of natural and recombinant Mal f 1 yeast allergen. *J Allergy Clin Immunol* 1999;103:877–884.
- 256 Johansson C, Eshaghi H, Linder MT, Jakobson E, Scheynius A: Positive atopy patch test reaction to *Malassezia furfur* in atopic dermatitis correlates with a T helper 2-like peripheral blood mononuclear cells response. *J Invest Dermatol* 2002;118:1044–1051.
- 257 Schmidt M, Zargari A, Holt P, Lindbom L, Hellman U, Whitley P, van der Ploeg I, Harfast B, Scheynius A: The complete cDNA sequence and expression of the first major allergenic protein of *Malassezia furfur*, Mal f 1. *Eur J Biochem* 1997;246:181–185.
- 258 Rasool O, Zargari A, Almqvist J, Eshaghi H, Whitley P, Scheynius A: Cloning, characterization and expression of complete coding sequences of three IgE binding *Malassezia furfur* allergens, Mal f 7, Mal f 8 and Mal f 9. *Eur J Biochem* 2000;267:4355–4361.
- 259 Johansson C, Tengvall Linder M, Aalberse RC, Scheynius A: Elevated levels of IgG and IgG4 to *Malassezia* allergens in atopic eczema patients with IgE reactivity to *Malassezia*. *Int Arch Allergy Immunol* 2004;135:93–100.
- 260 Casagrande BF, Fluckiger S, Linder MT, Johansson C, Scheynius A, Cramer R, Schmid-Grendelmeier P: Sensitization to the yeast *Malassezia sympodialis* is specific for extrinsic and intrinsic atopic eczema. *J Invest Dermatol* 2006;126:2414–2421.
- 261 Zargari A, Selander C, Rasool O, Ghanem M, Gadda G, Cramer R, Scheynius A: Mala s 12 is a major allergen in patients with atopic eczema and has sequence similarities to the GMC oxidoreductase family. *Allergy* 2007;62:695–703.
- 262 Croce MA, da Costa Manso ER, Gambale W, Takayama L, Oliveira Andrade CE, Pereira Pinto JH, Morato Castro FF, Croce J: Sensitization to the fungus *Hemileia vastatrix* (coffee leaf rust). *Allergy* 2001;56:684–687.
- 263 Barnes C, Buckley S, Pacheco F, Portnoy J: IgE-reactive proteins from *Stachybotrys chartarum*. *Ann Allergy Asthma Immunol* 2002;89:29–33.
- 264 Mungan D, Baybek S, Peksari V, Celik G, Gugey E, Misirligil Z: Trichophyton sensitivity in allergic and nonallergic asthma. *Allergy* 2001;56:558–562.
- 265 Green B, Blachere F, Beezhold D, Weissman D, Hogan M, Wilson N, Schmechel D: IgE reactivity to *Paecilomyces variotii* antigens in fungal sensitized patients. *J Allergy Clin Immunol* 2007;119:S187.
- 266 Giannini EH, Northey WT, Leathers CR: The allergenic significance of certain fungi rarely reported as allergens. *Ann Allergy* 1975;35:372–376.
- 267 Martinez Ordaz VA, Rincon Castaneda CB, Lopez Campos C, Velasco Rodriguez VM: Cutaneous hypersensitivity in patients with bronchial asthma in La Comarca Lagunera (in Spanish). *Rev Alerg Mex* 1997;44:142–145.
- 268 Mohovic J, Gambale W, Croce J: Cutaneous positivity in patients with respiratory allergies to 42 allergenic extracts of airborne fungi isolated in São Paulo, Brazil. *Allergol Immunopathol (Madr)* 1988;16:397–402.