Review

The Spectrum of Fungal Allergy

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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.

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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.

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

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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 10^6 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		
Pezizomycota		
Acremonium (Cephalosporium)		16[a] [267]
Alternaria	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]
Aspergillus	2.4 [46]	27.6 [22]
		21.3[c] [179]
		15 [182]
		5[c] [183]
Aureobasidium		20.5[d] [22]
Bipolaris		36.8 [22]
(Drechslera, Helminthosporium)		18.8[c] [179]
Botrytis		28.2[d] [22]
Chaetomium		7.4 [268]
Chrysosporium		
Cladosporium	2.5 [46]	3–18.2 [181, 182]
	2.9 [20]	15.9[c] [179]
		7.4[c] [183]
Claviceps		
Curvularia		18.4 [22]
		28 [184]
Cylindrocarpon		

Table 1 (continued)

	Prevalence, %	
	total population	atopics
Daldinia		
Didymella		
Embellisia		
Epicoccum		25.6[d] [22]
Epidermophyton		
Eurotium		
Fusarium		3.4[c] [183]
		24.5[e] [185]
Gliocladium		
Leptosphaeria		
Microsphaera		
Monilia		
Neurospora		
Nigrospora		
Nimbya (Macrospora)		
Paecilomyces		33[b] [265]
Penicillium	1.5 [46]	22[f] [101]
		13.9[c] [179]
		13.1 [22]
		7.3 [182]
Scopulariopsis		
Stemphylium (Pleospora)		30.7[d] [22]
Trichoderma		23[d] [22]
Trichophyton	1.9 [46]	10.2[b] [46]
		46.7[g] [264]
Ulocladium		
Xylaria		
Saccharomycotina		
Candida	8.5 [46]	44.3[d] [46]
		28.9 [22]
		23.1[c] [179]
Saccharomyces	1.4 [46]	7.4[b] [46]
Mitosporic ascomycota		
Phoma		30.7[d] [22]
Stachybotrys	9.4 [263]	
Thermomyces (Humicola)		
Trichothecium		
Wallemia		
BASIDIOMYCOTA		
Hymenomycetes		
Agaricus (Amanita)		
Armillaria		
Boletinellus		

	Prevalence, %	
	total population	atopics
Boletus		5.4 [123]
Calvatia		7.8 [122]
Cantharellus		
Chlorophyllum		
Coprinus		5.4 [122]
		6.2 [123]
Dacrymyces		
Ganoderma		9.3 [122]
Geastrum		6.4 [122]
Hypholoma		
Inonotus		
Lentinus		
Lycoperdon		
Merulius		
Pisolithus		5.4 [122]
Podaxis		
Polyporus		
Pleurotus		10.6 [122]
		8.3 [123]
Psilocybe		13.7 [122]
Schizophyllum		
Scleroderma		5.6 [122]
Sporotrichum		
Stereum		
Trichosporon		
Urediniomycetes		
Hemileia		14.7[h] [262]
Puccinia		
Rhodotorula		28[c] [63]
Sporobolomyces		
Ustilaginomycetes		
Malassezia (Pityrosporum)		19.8 [186]
		50.4[b] [130]
		66[b] [41]
Tilletia		
Tilletiopsis		
Ustilago		14 [266]
ZYGOMYCOTA		
Zygomycetes		
Absidia		
Mucor		20.5[d] [22]
Rhizopus		2.7[c] [183]

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							
Alternaria alternata	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]
Alternaria argyranthemi	Alt arg 1		Alt a 1 related			AY563280	[190]
Alternaria brassicicola	Alt b 1		Alt a 1 related			AF499002	[191]
Alternaria blumeae	Alt bl 1		Alt a 1 related			AY563291	[190]
Alternaria brassicae	Alt br 1		Alt a 1 related			AY563309	[190]
Alternaria capsici	Alt c 1		Alt a 1 related			AY563298	[190]
Alternaria carotiincultae	Alt ca 1		Alt a 1 related			AY563287	[190]
Alternaria cetera	Alt ce 1		Alt a 1 related			AY563278	[190]
Alternaria cheiranthi	Alt ch 1		Alt a 1 related			AY563290	[190]
Alternaria cinerariae	Alt ci 1		Alt a 1 related			AY563308	[190]
Alternaria conjuncta	Alt co 1		Alt a 1 related			AY563281	[190]
Alternaria crassa	Alt cr 1		Alt a 1 related			AY563293	[190]
Alternaria cucumerina	Alt cu 1		Alt a 1 related			AY563300	[190]
Alternaria dauci	Alt d 1		Alt a 1 related			AY563292	[190]
Alternaria dumosa	Alt du 1		Alt a 1 related			AY563305	[190]
Alternaria eryngii	Alt e 1		Alt a 1 related			AY563313	[190]
Alternaria ethzedia	Alt et 1		Alt a 1 related			AY563284	[190]
Alternaria euphorbiicola	Alt eu 1		Alt a 1 related			AY563314	[190]
Alternaria japonica	Alt j 1		Alt a 1 related			AY563312	[190]
Alternaria limoniasperae	Alt l 1		Alt a 1 related			AY563306	[190]
Alternaria longipes	Alt lo 1		Alt a 1 related			AY563304	[190]
Alternaria macrospora	Alt m 1		Alt a 1 related			AY563294	[190]
Alternaria metachromatica	Alt me 1		Alt a 1 related			AY563285	[190]
Alternaria mimicula	Alt mi 1		Alt a 1 related			AY563310	[190]
Alternaria mouchaccae	Alt mo 1		Alt a 1 related			AY563279	[190]
Alternaria oregonensis	Alt o 1		Alt a 1 related			AY563279	[190]
Alternaria petroselini	Alt p 1		Alt a 1 related			AY563288	[190]
Alternaria photistica	Alt ph 1		Alt a 1 related			AY563282	[190]
Alternaria porri	Alt po 1		Alt a 1 related			AY563296	[190]
Alternaria pseudorostrata	Alt ps 1		Alt a 1 related			AY563295	[190]
Alternaria radicina	Alt r 1		Alt a 1 related			AY563286	[190]
Alternaria solani	Alt s 1		Alt a 1 related			AY563299	[190]
Alternaria smyrnii	Alt sm 1		Alt a 1 related			AY563289	[190]
Alternaria sonchi	Alt so 1		Alt a 1 related			AY563307	[190]
Alternaria tagetica	Alt t 1		Alt a 1 related			AY563297	[190]
Alternaria tenuissima	Alt te 1		Alt a 1 related			AY563302	[190]

Table 2 (continued)

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.
Aspergillus flavus	Asp fl 13	64[a] (n = 14) [192]	alkaline serine protease	+	34	AF137272	[192, 193]
	Asp fl 18		vacuolar serine protease				[194]
Aspergillus fumigatus	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]
Aspergillus nidulans	Aspe ni 2			+	29	Z50175	[208]
Aspergillus niger	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]
	Asp n hemicellulase	43[j] (n = 24) [213]	hemicellulase	+			[215]
Aspergillus oryzae	Asp o 13		alkaline serine protease	+	34	X17561	[210, 216]

Simon-Nobbe/Denk/Pöll/Rid/
Breitenbach

Table 2 (continued)

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.
	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]	TAKA-amylase A	+	53	D00434, M33218	[220]
	Asp o lactase	28.7[o] (n = 94) [222]		+			[223]
	Asp o lipase			+			[224]
Beauveria bassiana	Bb-Eno 1		enolase		47.4	DQ767719	[225]
	Bb-f2				28.6	DQ767720	[225]
	Bb-Ald		aldehyde dehydrogenase		53.9	DQ767721	[225]
	Bb-Hex		N-acetylhexosaminidase		72	DQ767722	[225]
Candida albicans	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]
	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]
Candida boidinii	Cand b 2	100[m] (n = 89) [84]	peroxisomal membrane protein	+	20	J04984, J04985	[84, 129]
	Cand b FD		formate dehydrogenase		40.2	AJ011046	[233]
Cladosporium herbarum	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]
Cladosporium cladosporoides	Cla c 9		vacuolar serine protease		36	EF407520	
Curvularia lunata	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	
Embellisia allii	Emb a 1		Alt a 1 related			AY563322	[190]
Embellisia indefessa	Emb i 1		Alt a 1 related			AY563323	[190]
Embellisia novae-zelandiae	Emb nz 1		Alt a 1 related			AY563324	[190]
Embellisia telluster	Emb t 1		Alt a 1 related			AY563325	[190]
Epicoccum purpurascens	Epi p 1		serine protease		30	P83340	[241]
(*Epicoccum nigrum*)	Epi p GST		GST		26		[147]
Fusarium culmorum	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]
Fusarium solani	Fus s 1				65	P81010	[243]
	Fus s 45 kDa		enolase		45		[244]

Table 2 (continued)

Species	Allergen	Prevalence %	Biochemical name	RA	MW kDa	GenBank Accession No.	Ref.
Nimbya caricis	Nim c 1		Alt a 1 related			AY563321	[190]
Penicillium brevicompactum	Pen b 13		alkaline serine protease		33		[245]
	Pen b 26		acid ribosomal protein P1	+	11	AY786077	[109]
Penicillium chrysogenum (*Penicillium notatum*)	Pen ch 13	33[a] (n = 212) [246]	alkaline serine protease	+	34	AF193420	[94, 105]
	Pen ch 18	76.9[r] (n = 13) [106] 100[r] (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	
	Pen ch 33				16		
Penicillium citrinum	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 1β	+	25	AY363911	[110]
	Pen c 30		catalase		80.7	Q2V6Q5	
	Pen c 32		pectate lyases			EF159713	
Penicillium oxalicum	Pen o 18		vacuolar serine protease	+	34	AF243425	[94, 104]
Pleospora herbarum	Ple h 1		Alt a 1 related			AY563277	[190]
Stemphylium botryosum	Ste b 1		Alt a 1 related			AY563274	[190]
Saccharomyces cerevisiae	Sac c CyP		cyclophilin (rotamase)				[148]
	Sac c enolase	95 (n = 20) [249] 20[t] (n = 20) [229]	enolase		46.8	J01322	[96, 250]
	Sac c MnSOD		MnSOD		25.7	X02156	[148]
Stachybotrys chartarum	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]
Stemphylium callistephi	Ste c 1		Alt a 1 related			AY563276	[190]
Stemphylium vesicarium	Ste v 1		Alt a 1 related			AY563275	[190]
Thermomyces lanuginosus	The l lipase		lipase			AF054513	[252]
Trichophyton mentagrophytes	Tri me 2		vacuolar serine protease			AJ430837, AJ430838, AJ430839, AJ430840	
	Tri me 4		alkaline serine protease			AJ430836	
Trichophyton rubrum	Tri r 2		vacuolar serine protease			AF082515	[253]
	Tri r 4		alkaline serine protease			AF082514	[253]
Trichophyton schoenleinii	Tri sc 2		vacuolar serine protease			AJ430841	
	Tri sc 4		alkaline serine protease			AJ430626	
Trichophyton tonsurans	Tri t 1				30		[254]
	Tri t 4		alkaline serine protease		83	P80514	[253]
Ulocladium alternariae	Ulo a 1		Alt a 1 related			AY563316	[190]
Ulocladium atrum	Ulo at 1		Alt a 1 related			AY563318	[190]
Ulocladium botrytis	Ulo b 1		Alt a 1 related			AY563317	[190]
Ulocladium chartarum	Ulo c 1		Alt a 1 related			AY563319	[190]
Ulocladium cucurbitae	Ulo cu 1		Alt a 1 related			AY563315	[190]
BASIDIOMYCOTA							
Coprinus comatus	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.
	Cop c 6						
	Cop c 7					AJ242794	
Malassezia furfur	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]
Malassezia sympodialis	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		
Psilocybe cubensis	Psi c 1						
	Psi c 2		cyclophilin (rotamase)	+	16		[136]
Rhodotorula mucilaginosa	Rho m 1	21.4 (n = 14) [137]	enolase	+	47	AF382946	[137]
	Rho m 2		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 fl 13 and Asp fl 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-prosequence, 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

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

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

Table 3 (continued)

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

Table 3 (continued)

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

Table 3 (continued)

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

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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

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