The Role of Fungi as Aeroallergens in Asthma and Allergies

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Summary: Allergies and asthma are worldwide diseases causing various health problems. Their symptoms include episodic wheeziness, breathlessness, dry cough, etc. Sensitivity to a variety of fungi is known to be a major factor in the occurrence of allergic rhinitis and asthma. Fungal spores act as a potent indoor as well as outdoor allergens. The quantity and diversity of the fungi vary from location to location depending upon the environmental factors. Apart from fungal allergens, a range of other variables also contribute in the prevalence of allergic rhinitis and bronchial asthma, including proportion of native community and chemical irritants from air. This article sheds light on the correlation of various airborne fungal spores to asthma and associated allergies.

Introduction

People are exposed to aeroallergens in their surroundings or neighborhoods either directly or through penetration of the allergen-bearing particles. Certain biological particles in the air trigger seasonally allergic symptoms in the skin, eyes, nose and chest of an hypersensitive person [1]. Fungi are among the earliest recognized allergens and today fungal allergies stands as a worldwide reported diseases. They are among the most common bioaerosols that humans inhale. Airborne fungi are widely spread and highly variable in occurrence [2]. These fungi, which are ubiquitous in distribution, cause upper and lower respiratory tract diseases [3]. Fungi are of fundamental importance from the aerobiological standpoint because they:

a) grow easily in moist environment
b) produce toxins which can be both acute and/or chronic in terms of its mode of action related to sick building syndrome
c) distribute spores which become part of the air we inhale and all of these spores synergistically make the situation much worse in our lungs [4].

More than 80 genera of fungi are reportedly associated with respiratory tract allergy [5] and almost 100 species of fungi are involved in serious human and animal infections [6]. Sensitization to fungal allergens is sometimes associated with life-threatening asthma and death [7]. Allergic reactions associated with fungi affect the lower respiratory tract more frequently than do the pollen allergies [5]. Therefore, it is extremely important to characterize allergenic proteins in the spores [8], in order to develop new treatment options thereby to avoid damages caused by molds [9]. Along with different factors and conditions in asthma, atopy stands as the principal predisposition factor. It is also attributed to different genetic mutations that increase bronchial reactivity and modify host immune response, which stands as the basis of allergic disease.

Sensitivity to a variety of fungi is known to be a major factor in allergic rhinitis and asthma. In recent times, a keen interest in measuring the exposure to airborne fungi has increased due to the complex and diverse associations suspected to occur between such exposures and the adverse health effects, notably allergic rhinitis and asthma [10]. Contact of aeroallergens is the causative factor for sensitization, which occurs either through inhalation or as a consequence of direct contact through the conjunctival mucosa [11]. It has been demonstrated that the immune system weakens in allergic patients [8].

Recent advances in medical sciences have increased our understanding of diseases and immunity with respect to the emergence of new

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concepts of allergy vaccine development. The technique currently being employed in allergic immunotherapy is the injection of the small doses of allergens [12]. The allergists utilize recombinant proteins, peptides, immuno-modulatory therapy and DNA vaccines. Furthermore, new concepts based on patient specific immunotherapy with recombinant allergens and anti-IgE immunotherapies are being used for treatment purposes [13].

Allergy

Allergy is an undesirable exaggerated immune response to a molecule called allergen (containing proteins) which is present in our environment and is generally harmless to the non-allergic persons [14, 15]. Along with genetics, environment is also a very important factor in developing allergic diseases such as asthma. Now environment is treated as the major controlling factor in the prevention of allergic diseases and problems [16]. Fig. 1 explains the general immunological mechanisms underlying allergy symptoms, which entail hypersensitivity reactions of type I, II, III (based on antibody) and IV (cell-mediated) [14].

This flowchart shows allergic sensitization phenomena, in which allergen-specific T helper 2 (T_H2) cells are activated in response to exposure with allergen by professional antigen-presenting cells (APCs) and IgE synthesis. These allergens bind with B cells with the help of T-Cells leading to the B-Cells proliferation. B lymphocytes, are responsible for making IgE antibodies [17]. Type I allergy is the IgE associated hypersensitivity disease, which affects 25% of the population [18]. In hypersensitive patients, allergens are recognized by the IgE present in the serum, through which biologically active compounds like different types of Interleukins, tumor necrosis factors etc. are released which are associated with the symptoms of allergy [19].

Allergenic Molds

Fungi are important pathogenic microorganisms [20]. In addition to known allergens, fugal proteins have also the ability to act as allergens and to cross-react with other allergens [21]. The combined effect of mold and its released mycotoxins increases the chances of an exposed person's susceptibility to infectious diseases [22].

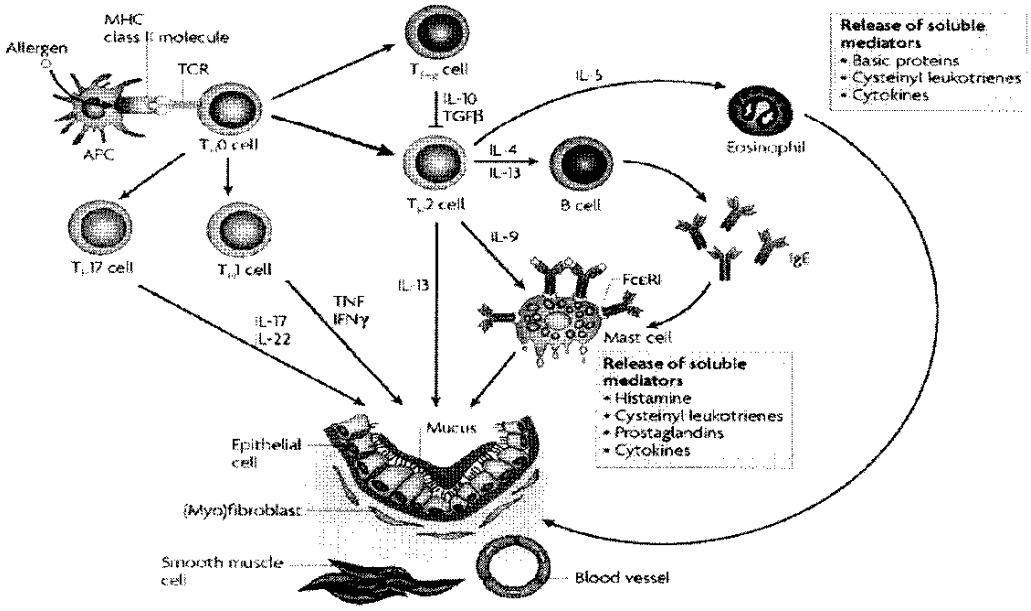

Fig. 1: Mechanism of immune cell function in allergy [15].

Alternaria and *Cladosporium* (*Hormodendrum*) are the allergenic molds which are most commonly found throughout the world in both indoor and outdoor environments [23]. *Aspergillus, Penicillium, Helminthosporium, Epicoccum, Fusarium, Mucor, Rhizopus and Aureobasidium (Pullularia)* are also commonly occurring species [23, 24]. The clinically important saprophytic seasonal molds include *Alternaria, Hormodendrum* and *Helminthosporium.* These molds are unable to sporulate during winter when they are covered with snow, while other occasional spores of *Aspergillus, Penicillium, Monillia, Rhizopus, Mucor and* yeast cells blossom during the winter season [25]. Localized fungal flora are also significant in ranging from signs and symptoms of diseases to forensic investigation of cases related to particular areas and fields [26].

Climatic factors are very important in the growth of fungi. Humid climate conditions offer greater opportunities for the growth of various allergen producing molds, *e.g.* costal areas have high humidity levels, which help in the widespread growth of fungi [27]. Naturally, outdoor humidity affects indoor humidity levels, which creates a perfect environment for mold growth. During the summer season, the concentration of *Alternaria, Epicoccum, Cladosporium, Fusarium, Curvularia, Acremonium and Penicillium* fungal spores rapidly increases, as compared to the winter season, whereas *Aspergillus* and *yeast* spore concentration levels do not show any significant difference between the two seasons [28].

Compared to other common allergens [29] mold allergens are known to produce severe diseases because they are widely distributed in our surroundings. In fact, airborne spore levels may be up to 1,000 times higher than pollen levels [30], while the fungus *Candida* is probably universally present in human gut. Excess mucous in asthmatic patients allows the allergen producing fungi to germinate and produce allergenic proteins, fungal proteases and glycosidases, which can additionally scratch the mucosa [31]. 10 to 50 KDa allergenic proteins are known to be present on the fungal surface [32]. The deposition of fungal spores in the lungs and their effect on human health depends on spore concentration and size [33], as well as on the sensitivity level of the airway passages. Large spores (>10 μm) are deposited in the upper airway (nose and pharynx) since hairs in the nose serve as a filter by removing particles larger than 10 μm, which can result in hay fever symptoms. However, smaller spores (<10 μm, especially <5μm in size) can penetrate the lower airways and may lead to asthma or other allergic conditions. Fungal allergens, with sizes from ultra fine (<0.1 μm) to sub-micrometer level, have the potential of attacking the deepest part of the respiratory tract and may penetrate deep down into the bronchioles [32, 34].

Fungi are associated with numerous allergic diseases in humans. The prevalence of respiratory allergy caused by fungi is about 20-30% in atopic individuals, while the general population shows prevalence levels of up to 6%. In the industrialized world, 25% of the population is affected by atopic allergies, while mold allergy constitutes 25-30% of all allergic asthma cases [35]. One study explained the mechanism of airborne fungi effecting essential items, such as the quality of drinking water. This investigation included the following species: *Acremonium, Alternaria, Aspergillus, Chaetomium, Cladosporium, Penicillium, Phalophora, Rhizopus.* Some of these produce patulin and geosmin, which generate a peculiar odor and earthy smell and can cause water toxicity [36, 37].

Alternaria

Alternaria varies in width and length according to species, with the usual length ranging from 8 to 75 micrometers (μm) [38]. The *Alternaria* spores are multi-septate (Fig. 2) in both the transverse and longitudinal dimensions [38, 39] and contain a higher concentration of allergens as compared to mycelia [40].

Alternaria is a strong allergenic fungus and plays a significant role in allergic asthma pathogenesis. An association between *Alternaria* sensitization and life-threatening asthma has also been identified [1, 3, 41, 42]. A study of allergic rhinitis showed the greatest degree of serum immunoglobulin (IgE) sensitization to *Alternaria and* reported the most severe cases of rhinitis [41-45]. Cross-reactivity of *Alternaria* among other fungal allergens shared species including *A. tenuis, Stemphylium sp., Curvularia sp. and Aspergillus fumigatus* [46]. Through the use of Immuno gold

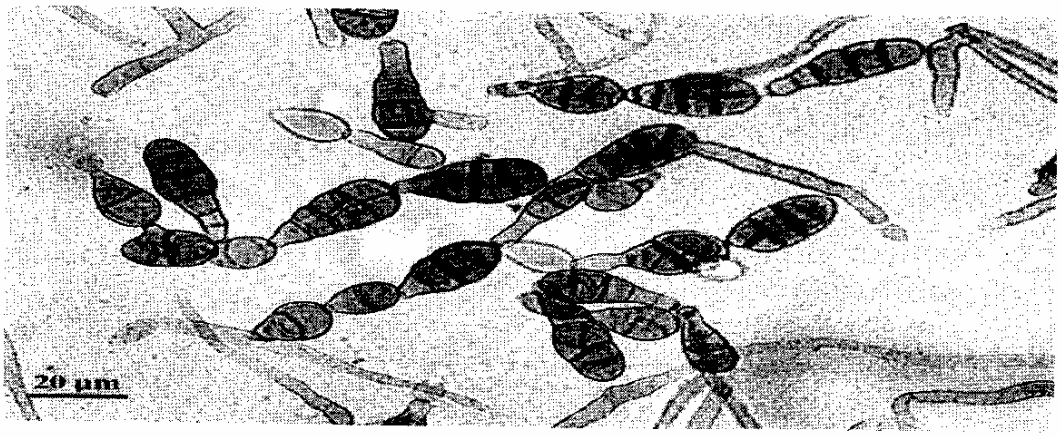

Fig. 2: Microscopic view of the Alternaria mold.

Electron Microscopy Endow, it was proved that an allergen Alt a 1 is released from both the spores and mycelia of *Alternaria* [47]. Furthermore, studies of spores and mycelia sensitization record demonstrate that out of all fungal allergies in the United States, 70% comprises of *Alternaria* sensitization among patients at the same time, as 3. 6% were positive to the skin prick test (SPT) [48].

Although not all spores release allergens, a large number of *Alternaria* spores release allergens, including the major allergen Alt a 1 which has been confirmed by Alt a 1–specific antibodies[49]. A study focused on a comparison of enzyme immunoassay with environmental *Alternaria* demonstrates that in 40% atopic individuals *Alternaria alternata* showed positive skin reactions [43, 50]. *Alternaria alternata* and *Cladosporium herbarum* are important sensitization factors, as a study has revealed skin test reactivity in 6% of atopic children (4 years of age) [51]. It is also reported that in humid and warm climatic areas, *Alternaria* is known to be a causative agent of type I hypersensitivity reactions [52]. Certain species of *Alternaria* produce alt 1 related fungal allergen proteins, which includes *A argyranthemi, A. brassicicola, A. blumeae, A. brassicae, A. capsici A. carotiincultae, A. cetera, A. cheiranthi, A. cinerariae, A. conjuncta, A. crassa, A. cucumerina, A. dauci, A. dumosa, A. eryngii, A. ethzedia, A. euphorbiicola, A. japonica A. limoniasperae, A. longipes, A. macrospora A. metachromatic, A. mimicula, A. mouchaccae, A. oregonensis, A. petroselini, A. photistica, A. porri, A. pseudorostrata, A. radicina, A. solani, A. smyrnii, A. sonchi, A. tagetica* and *A. tenuissima* [53]. Three major and three minor allergens found in *Alternaria* are shown in Table-1 and Table-2, respectively.

Table-1: Major allergens found in Alternaria [54, 55].

Allergen	Characteristics
1-Alt a 1	Major allergen with a molecular weight of approximately 30 kDa
2-Alt a 2	Conserved protein found in six different stains of Alternaria Molecular weight approximately 25-kDa
3- Enolase	Also highly conserved fungal protein present in Cladosporium and Candida along with Alternaria.

Table-2: Minor allergens found in Alternaria [54].

Allergen	Characteristics
Alt a6	A ribosomal protein with molecular weight 53 kDa
Alt a7	A 22 kDa protien that is homologous to YCP4 yeast protein
Alt a10	An aldehyde dehydrogenase protein with 11 kDa molecular weight

Aspergillus

The size of *Aspergillus* spores ranges from 2 to 10 micrometres (microns) [38, 56]. The spores of some of its species are hyaline (colourless), while other species such as *Aspergillus niger* have spores that are dark brown in colour (as shown by the stained spores in Fig. 3 and Tables-9, 10). *Aspergillus* is found on stored cereal grains and greater concentration of its spores is commonly observed in the outdoor air around the vicinity of compost masses and dead vegetation [57-59].

Aspergillus bas of high sporulating power, that stands as the main factor in the ubiquitous

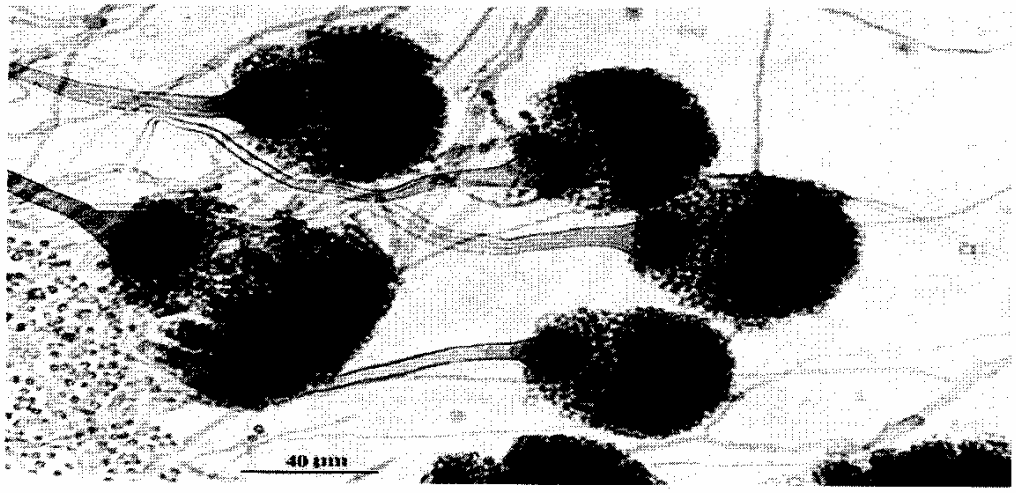

Fig. 3: Microscopic view of the Aspergillus spores.

presence of high concentrations of conidia (1–100 conidia/ m^3) both in the indoor and outdoor air [60]. In addition to the host's immune responses, the mode and rate of recurrence of organism determines the allergy or hypersensitivity to *Aspergillus* antigens [61,62]. It is mainly widespread in the air of some tropical countries, since the climatic circumstances control its prevalence in outdoor air [63]. Interestingly, the predominance among humans of *A. fumigatus* over other *Aspergilli* may be due to its ability to grow at human body temperature, smaller spore size and comparative resistance to oxidative killing [63]. Table-3 highlights the recombinantly expressed allergens and antigens in *A. fumigates* [64].

Table-3: Proteins, allergen source and molecular weight of Aspergillus fumigatus allergens [55, 64].

Proteins	Allergen	Mol. Wt. (kDa)
Mitogilin family	Asp f 1	18
Enolase	Asp f 22	46
Peroxysomal protein	Asp f 3	19
Metalloprotease	ASP f 5	40
Mn superoxide dismutase	Asp f 6	26
Aspartate protease	Asp f 10	34
Peptidyl prolyl isomerase	Asp f 11	24
Alkaline serine protease	Asp f 13, Asp o 13	34
Vacuolar serine protease	Asp f 18,Asp n 18	34
Heat shock protein	Asp f 12	90

Asp f 1 is a member of the mitogillin family of cytotoxins (a nonglycosylated IgE-binding protein), which is 99% homologous to the mitogillin family of cytotoxins from *Aspergillus restrictus*. It is also a major allergen in *A. fumigatus* and has a molecular weight of 18-kDa (0. 87 to 12. 1 micrograms/ml). Northern analysis has confirmed that Asp f I was only detected in *A. fumigatus* and *A. restrictus*, as this allergen was not detected in *Aspergillus flavus, Aspergillus niger, Aspergillus terreus and Aspergillus nidulans* cultures [65-67].

Bipolaris

Bipolaris conidia are 39-65 µm long, tapered with septation and segments (Fig. 4). It is found in those areas that predominantly have hot and humid climatic conditions. *Bipolaris* spores are large in size and elongated with septation [38].

The spores of *Bipolaris* spp. contain allergenic proteins which bring out IgE-mediated reactions. A study conducted for *Bipolaris* allergenic protein identification and isolation showed that a *Bipolaris* spore extract contained 4 IgE binding proteins, ranging from molecular weight 11-36 (protein 1, MW 11-13 kDa, protein 2,16-17 kDa, protein 3, 20-22 kDa and protein 4,36 kDa) [68]. The U. S. Government's Occupational Safety and Health Administration (OSHA) has demonstrated allergen, irritant, hypersensitivity pneumonitis and dermatitis as being the possible health effects of *Bipolaris* mold spores [69]. The *Bipolaris* species have been classified as plant pathogens, while three species (*B. australiensis, B. spicifera and B. Hawaiiensis*) have also been associated with human problems, such as allergic

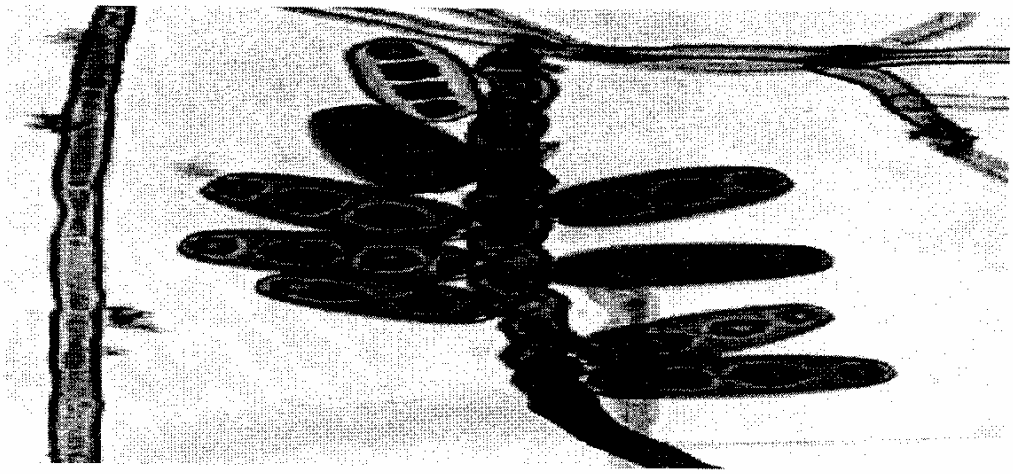

Fig. 4: Microscopic view of the Bipolaris conidia.

fungal sinusitis, allergic broncho-pulmonary mycosis (ABPM) and other infections [70].

Table-4: Allergenic protein from Bipolaris and their molecular weight range [68].

Allergens	Mol.Wt (kDa)
protein 1	11-13
protein 2	16-17
protein 3	20-22
protein 4	36

Cladosporium

Cladosporium have single celled spores that contain either a single transverse septum or several transverse septa (Fig. 5, Table-9, 10). Their size ranges from 4 to 20 micrometers (μm) in length and are found mostly in bunches [38].

As compared to any other fungal spore type, *Cladosporium* spores are often found in higher concentrations in the air, particularly during the summer season and attain a peak level of up to 10^5 spore/m^3 in agricultural outdoor environments [41,44,46,71]. Both *Alternaria* and *Cladosporium* species are commonly found in outdoor environments worldwide. The airborne spores of these two genera are present during the spring and summer seasons, while their concentration is much

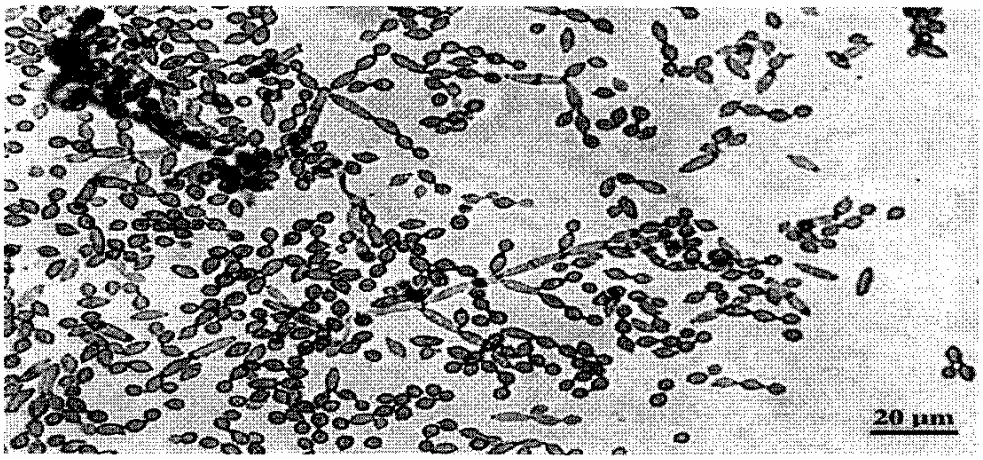

Fig. 5: A microscopic view of Cladosporium.

higher in autumn because of the degradation of leaves and other biomaterial [31]. In industrial areas, *Cladosporium* spores were recorded at the highest levels in winter, especially during the morning hours [72]. Spore counts for *Cladosporium* changes from day to day depending on environmental factors and an unexpected and dramatic increase in its spores can bring about severe and acute attacks of asthma in those individuals who are sensitized to these spores [73]. A study has shown that *Cladosporium herbarum* is an important allergenic fungal species, since 5–30% of the allergic population has IgE antibodies and it has been reported in all types of climatic conditions [74]. A study conducted in Argentina revealed that *Cladosporium* was very common in environment and its spores were present at highest concentration in indoor air [75]. This led to the conclusion that *Cladosporium* has some effect on asthma patients. In 1979, two *Cladosporium* allergens (Ag 54, Ag 32) were purified and had an estimated molecular weight of 25 KDa and 13 KDa Daltons respectively [76]. Some of the allergens that were identified from *Cladosporium* are illustrated in Table-5.

Table-5: Allergens from Cladosporium [74, 77, 78]

Allergens	Molecular weight (KDa)
Cla h 1	30
Cla h 2	45
Cla h 3	53
Cla h 4	11
Cla h 5	22
Cla h 6	48
Cla h 7	42
CLA H 8	110
Cla h 9	100

Curvularia

The fungi *Curvularia,* formerly classified as a fungi imperfecti, is now recognized as being in the anamorph stage of the ascomycete Cochliobolus intermedius [79]. Its spores range in length from 18 to 43 micrometers. Macroconidia is large, dark and curved due to swelling of the central segment (Fig. 6), which also is its identification symbol (3-5 segments) [38].

Curvularia is an important fungus with respect to respiratory allergic disorders, as some airborne inhalant allergens indicated cross-reactivity of *Curvularia* with other fungi [79]. However, the cross-reactive allergenic component of these fungi has still not been identified [79]. The proportion of those spores which release allergenic proteins vary markedly among the species, as 5% to 40% of *Curvularia* spores release allergens [24]. Sera from 80% of the patients, sensitive to *Curvularia lunata* showed positive reactions to cur l 1. It was identified as serine protease allergen which binded with IgE antibodies, thereby indicating that *C. lunata* is an important allergen-producing fungus throughout the world and is closely related to asthma and allergy problems [80]. Table-5 highlights the allergens and associated characteristics of *Curvularia.*

Penicillium

Penicillium is known as an airborne contaminant with septate hyphae and chains of

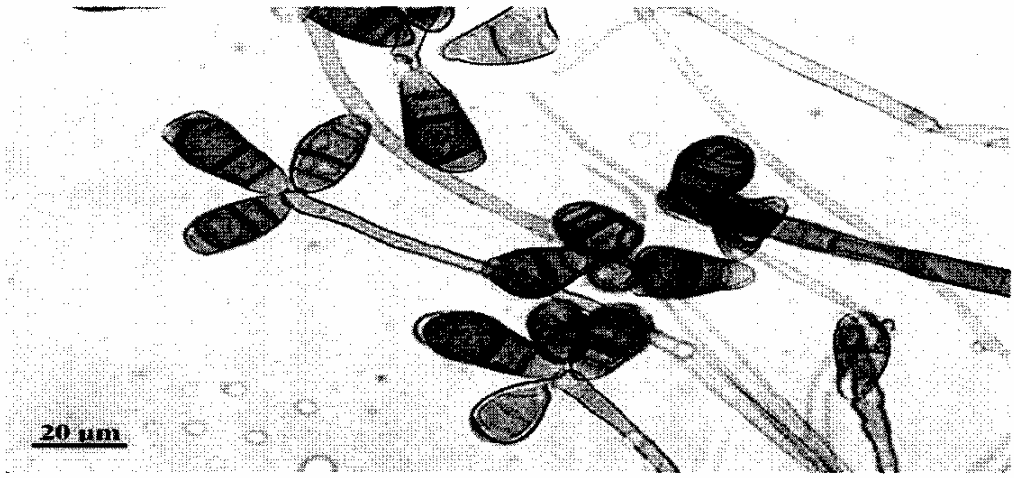

Fig. 6: A microscopic view of Curvularia.

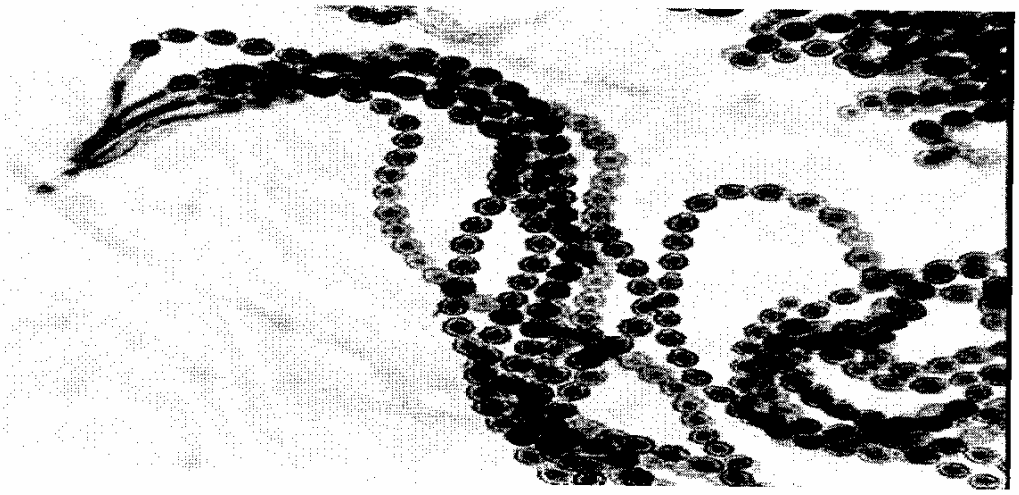

Fig. 7: Microscopic view of Penicillium.

Table-6: Allergens of Curvularia [81]

Allergen	Characteristics
Cur l 1	Serine protease
Cur l 2	Enolase mol. wt.48kDa
Cur l 3	Cytochrome C mol. wt.12 kDa
Cur l 1 ADH	Alcohol dehydrogenase mol. wt 37 kDa
Cur l 1GST	GST
Cur l 1 SOD	SOD mol. wt 21.4 kDa
Cur l 1 TRX	Thioredoxin mol. wt 12.3 kDa

[81]

unbranched 3-5μm conidia [38] (Fig. 7). *Penicillium* species do not have annual patterns of occurrence. It is generally present within indoor environments and is characterized by less seasonal changes [82]. The spores of *Penicillium* have been described as one of the main causes in the development of asthma symptoms [83]. Few allergens from *penicillium* sp. have been isolated, such as *Penicillium citrinum* allergen which is 33 to 34-kda (alkaline serine proteinase) [84]. It has been suggested that the degree of urbanization and environmental pollution affects fungal spore concentration, especially in the case of *Penicillium,* which does not differ in either rainy or dry seasons [85]. During winter season, fungal allergies and respiratory symptoms are marginally more common among children exposed to *Cladosporium or Penicillium* [86]. Allergenic fungi such as *Alternaria alternata* and *Penicillium crysogenum* have also shown that allergens with low molecular weight (<30kDa) exhibit higher IgE activity [87]. Furthermore, *P. citrinum* produces pen c 13, which is an allergenic serine protease responsible for the release of IL-8 in the airway epithelial cells of asthmatic patients [88].

Pen n 18 is an unusual yet important allergen produced by *Penicillium, Candida, Cladosporium, Alternaria and Aspergillus.* Certain significant allergens from *Penicillium citrinum* like Pen c 13 have been identified through the use of molecular cloning. Pen c 13 was found to binds with IgE from sera of 95% of patients suffering from allergy and this allergen bears significant resemblance to the alkaline serine protease [89]. Some of these allergenic proteins are represented in Table-7.

Stachybotrys

Stachybotrys conidium are 4. 5- 9 μm in length, oval in shape and hyaline or pigmented in appearance (Fig. 8). They are unicellular and are found in clusters [38,81] *Stachybotrys* has strong cellulolytic activity, colonize in water-damaged buildings and produce mycotoxin [90].

Stachybotrys chartarum is a toxigenic mold [91], which produces trichothecenes and atranones [92] and is believed to be the reason for the presence of mycotoxicoses in both animals and humans [94]. *S. chartarum* also produces satratoxin H and G [94]. It is an important environmental fungus and inhalation of mycelial fragments, spores

Table-7: Allergens produced by different Penicillium species [55].

Protein	Allergen	Source	Mol Wt. (KDa)
Peroxysomal membrane protien	Pen c 3	P. citrinum	18
Alkaline serine protease	Pen c 13 Pen b 13 Pen ch 13	P.citrinum	33
		P.brevicompactum	33
		P.crysogenum	34
Vacuolar serine protease	Pen o 18	P.oxalicum P.crysogenum	34
	Pen ch 18		32
Enolase	Pen c 22	P. citrinum	46
Heat shock protein	Pen c 19	P. citrinum	70
catalase	Pen c 30	P. citrinum	97
Pectate lysate	Pen c 32	P. citrinum	40
N- acetyl glucosaminidase	Pen ch 20	P.crysogenum	68
Acidic ribosomal protein P1	Pen b 26	P.brevicompactum	11

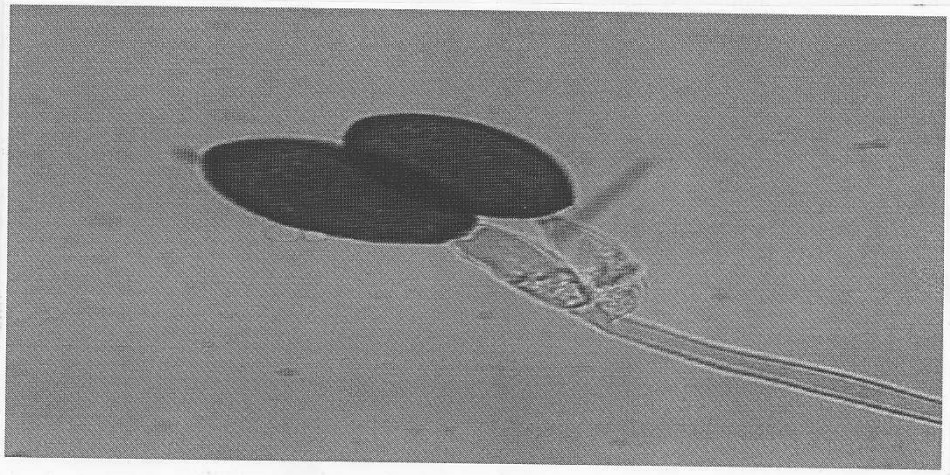

Fig. 8: A microscopic view of Stachybotrys conidium.

and building dust can lead to serious health problems [93]. *Stachybotrys chartarum* mycotoxins can potentially make the condition of patients worse, since these mycotoxins are biologically active molecules capable of bringing about a range of physiological and pathologic changes in humans and animals depending on the modulation of inflammation and changed alveolar surfactant phospholipid concentrations [95].

The potential health risks coupled with exposure to *Stachybotrys chartarum* are the subject of intense research. However, data regarding the detection of these specific species through the use of immunoassays is yet to be documented [96].

Allergenic protein from *Stachybotrys chartarum* are Sta c cellulase,Sta c hemolyascin,Sta c stachyrase A (Table-10).

Ulocladium

Ulocladium is a dematiaceous (brown pigmented) filamentous fungus. Its conidia are 13-30 μm in size, egg-shaped, with a smooth or rough texture and have a narrow base compared to its apex [97] (Fig. 9). The dark brown conidial morphology and pigmentation of *U. chartarum* changes with any variations in the environmental temperature [98]. Ulocladium is strongly related to Alternaria primarily based upon its morphological factors, as Ulocladium is considered to be cosmopolitan in the environment and is most often isolated from dead and decaying vegetation, soil, paper, dung, grasses, textiles and air samples. Colonies of this fungus grow very well on all general fungal media. It comprises of dry spores that are generally scattered through the wind dispersal mechanism [99,100].

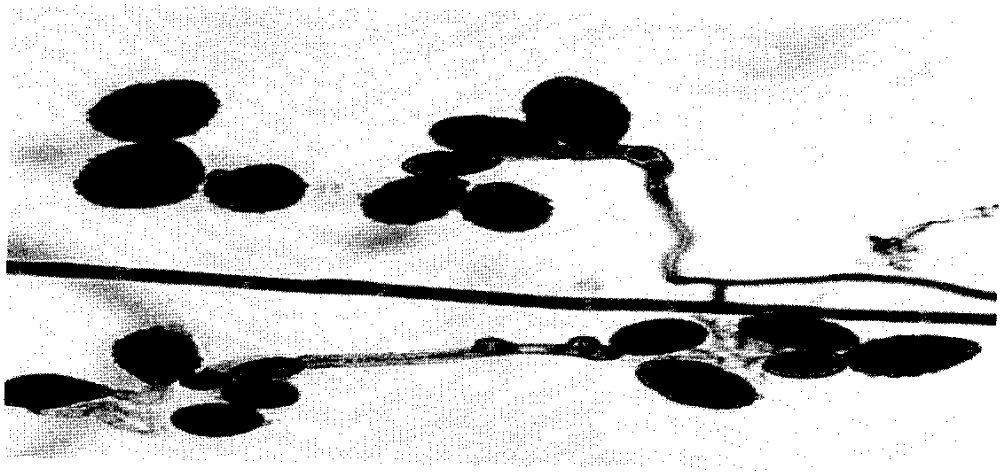

Fig. 9: A microscopic view of *Ulocladium.*

Table-8: Allergens from Ulocladium genera [81].

Allergen	Fungal species
Ulo a 1 (Alt a 1 related)	Ulocladium alternarriae
Ulo at 1(Alt a 1 related)	Ulocladium atrum
Ulo b 1(Alt a 1 related)	Ulocladium botrytis
Ulo c 1(Alt a 1 related)	Ulocladium chartarum
Ulo cu 1(Alt a 1 related)	Ulocladium cucurbitae

Ulocladium chartarum and *Stemphilium floridanum* species are affected by variations in temperature and the shape, size and color of their spores and ornamentation are the basics of mycological identification, especially in the case of fungi imperfect. Environmental factors such as light, temperature, nutrition and pH can change the expression of fungal morphology characteristics [98].

Wallemia and *Fusarium*

Wallemia sebi and *Fusarium* species are commonly found in agricultural environments [101,102]. *W. sebi* conidia are 2.5-3.5 μm in diameter (Fig. 10). It is believed to be an allergen producer and, owing to its small size and rough surfaced spherical shape, it reaches the respiratory bronchioles to potentially cause asthma and farmer's lung disease [102]. Macroconidia of Fusarium (3-8 x 11-70 μm) are produced from phialides on unbranched or branched conidiophores. They are 2 or more celled, thick-walled, smooth and cylindrical or sickle shaped (Fig. 10).

Fusarium sp. is recognized as an important pathogen associated with human, animal and plant seed quality [103,104]. In Central America, Africa and Asia (1992-1995), *Fusarium* spp. was identified as the largest group of fungi present in maize samples, from which *Fusarium moniliforme* specific species were primarily isolated [104]. A significant concentration of *Fusarium* can act as

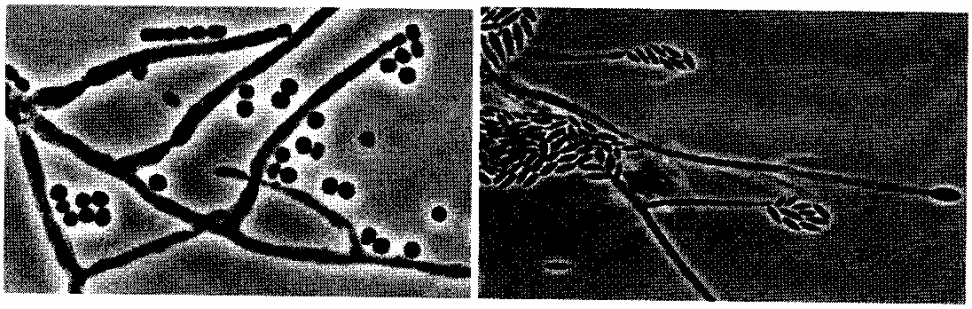

Fig. 10: *Microscopic view of Wallemia sebi* and *Fusarium.*

source of aeroallergen, as the evaluation of 52 sera indicated that 50% of the patients were sensitized to *Fusarium* [105]. *Fusarium solani* is a significant source of fungal allergen, as a 45-kD major allergen showed reactivity with the sera of those patients that are susceptible to numerous fungi [106]. *Fusarium* is also considered to be a source of mycoprotein and sensitization to mold allergens in the respiratory tract and subsequent oral ingestion of cross-reactive proteins may lead to severe food-allergic reaction [107]. The *F. culmorum* allergen Fus c 1 is cross-reactive with 60S acidic ribosomal protein P2 of *F. venenatum* Quorn-mycoprotein [108]. Perhaps, *F. culmorum* has been characterized as an underestimated source of aeroallergens, since the new recombinant allergens have provided a more sTable-allergenic reference material; for instance, 81% of *F. culmorum* patients have shown

Table-9: Allergens from Fusarium [81].

Allergen	Fungal species	Characteristics
Fus c 1	Fusarium culmorum	60S acidic ribosomal protein P2(mol. wt 11)
Fus c 2	Fusarium culmorum	Thioredoxin-like protein (mol. wt 13)
Fus c 3	Fusarium culmorum	mol. wt 49kDa
Fus s 45kDa	Fusarium solani	Enolase, mol. wt45kDa

Table-10: Major fungal spore morphology, allergenicity, distribution and known allergens

FUNGUS	SPORE MORPHOLOGY	ALLERGENICITY	DISTRIBU-TION	ALLERGENS	REFRENCES
ALTERNARIA	8-75µm,club like in shape with transverse and longitudinal multi septation	Highly allergenic IgE/IgG (in ABPS) mediated.	Both in Indoor and Outdoor.	Alta1,2,3,4,5,6, 7,8,10,12,13, Alt a NTF 2	[38-41,46-48, 81, 99]
ASPERGILLUS	2-10 µm small rounded hyaline to dark brown	Highly allergenic IgE/IgG (in ABPS) Mediated	Both in Indoor and Outdoor.	Asp fl1,18.Asp f 1,2,3,4,5,6,7,8,9, 10,11,12,13,15,16, 17,18,22,23,27,28, 29,34.	[55,58,64,81,111]
BIPOLARIS	39-65 µm Long tapered With septation and segments	Allergenic	Outdoor.	Protein 1,2,3,4.	[3,68,69,99]
CLADOSPORIUM	4-20 µm single celled one or more transverse septa	Highly allergenic IgE Mediated	Both in Indoor and Outdoor.	Cla h1,2,5,6,7,8,9, 10,12,8CSP,GST, HCh1,HSP70, NTF2.	[38,,71-78,81, 99, 109, 112]
CURVULARIA	18-43 µm macroconidia Large,dark curved due to swelling of central segment (3-5 segments)	Allergenic	Outdoor.	Curl 1,2,3,ADH, GST, oryzin,OSD, Trx, ZPS 1.	[38,78,79,99]
PENICILLIUM	3-5µm small round chain of conidia	Allergenic	Outdoor.	Pen b 13,26, Pen ch 13,18, 20,31,33.Pen c 1, 3,13,18,19,22,24,3 0,32,Pen o 18	[35,75,83-86,99]
FUSARIUM	3-8 x 11-70 µm µm large sickle shaped with multiseptate macroconidia	Allergenic	Outdoor.	Fus c 1,Fus c 2, Fus c 3,Fus s 1,45 kDa	[35,81,99,101,102, 105]
ULOCLADIUM	13-30 µm egg-shaped, smooth or rough and have a narrower base compared to its apex	Allergenic	Outdoor.	Ulo at 1,Ulo b 1,Ulo c1,Ulo cu 1	[81,97-100]
STACHYBOTRYS	4.5- 9 µm,conidia are oval, hyaline or pigmented, 1-celled and in clusters	Allergenic	Both in Indoor and Outdoor	Sta c cellulase, Sta c hemolycin, Sta c stachyrase A	[75,81,86-95,99]
PLEOSPORA	15-20 µm with transverse and longitudinal multi septation	Allergenic	Outdoor.	Ple h 1	[38,81,99]
COPRINUS	7-11µm no septation in spores	Allergenic	Outdoor.	Cop c 1,2,3,4,5,6,7.	[38,81,99,109]

sensitization to a combination of rFus c 1 and rFus c 2 [105].

Among basidiomycetes, the most important source contributing to the total spore burden in the outdoor air is *Coprinus comatus*. rCop c 1 is the earliest cloned allergen from the genus Coprinus, with specific proliferative responses been observed in PBMCs of *C comatus*-sensitized individuals [109].

Since *Fusarium* species are difficult to culture and *Wallemia sebi* have only recently been discovered, tremendous opportunities exist in terms of evaluating the exposure of these two fungal species for the advancement of allergic symptoms identification [110].

Prevalence of Allergenic Fungal Species

Allergic diseases are not only common in many parts of the world, but their incidence is continuously on the rise as airborne fungi are everywhere in nature and sensitivity to a variety of fungi is known to be a major factor in allergic rhinitis and asthma Table-11 shows prevalence of fungal spores in different countries.

Clinical Manifestations of Mold Allergy

Allergic Rhinitis and/or Allergic Conjunctivitis

These are general problems among both children and adults in those areas where humidity and temperature is generally high. Fungal allergen-induced asthma can occur in both children and adults [133]. Asthma has IgE antibodies against fungal spores such as *Alternaria* and *Cephalosporium* species. Along with asthma, chronic rhinosinitus is a persistent condition in certain individuals. An increase in IL- 5 and IL-13 production and IgG levels is observed when contact takes place with *Alternaria* and *Cladosporium* [134].

Allergic Fungal Sinusitis (AFS)

AFS appears to be a "sinonasal" allergic bronchopulmonary aspergillosis (ABPA) like syndrome [75]. AFS is provoked with exposure to fungi, including *Bipolaris, Curvularia, Alternaria, Exserohilum, Helminthosporium, Aspergillus* and *Rhizopus* species. This condition is relatively uncommon among newborns.

Allergic BronchoPulmonary Aspergillosis (ABPA):

ABPA commonly occurs in the presence of clinical asthma. Children with CF (Cystic fibrosis) are susceptible to ABPA, along with mucoid impaction of *Aspergillus* species. *A fumigatus* is the most recurrent *Aspergillus* species that infects humans. The main factor of this condition is spore size (almost 2-3. 5 μm), which can penetrate into smaller airways [23,75,133].

Table-11: Allergenic fungal species reported in different regions of the world.

Fungi Type	Reported countries
Alternaria	China [112,113], India [114-118], Indonesia [119], Iran [120,121], Italy [122], Kuwait [123], Malaysia [124], Saudi Arabia [125], Spain [126-128], Taiwan [129,130], Thailand [131], Turkey [132]
Aspergillus	China [112,113], India [114-117], Indonesia [119], Iran [120,121], Italy [122], Kuwait [123],Malaysia [124], Saudi Arabia [125], Spain [126-128], Taiwan [129,130], Thailand [131], Turkey [132]
Aureobasidium	China [112]
Beauveria	Italy [122]
Bipolaris	China [113], India [116,117], Spain [127]
Chaetomium	India [115], Kuwait [123]
Cladosporium	China [112], India [114-116], Iran [121], Italy [122], Kuwait [123], Malaysia [124], Saudi Arabia [125], Spain [126-128], Taiwan[129,130] Thailand [131] Turkey [132].
Curvularia	India [117,118,], Indonesia [122], Iran [120], Kuwait [123], Malaysia [124]
Exserohilum	Indonesia [119]
Fusarium	India [114,117], Italy [122], Saudi Arabia [125], Thailand [131], Turkey [132].
Ganoderma	India [114], Spain [126]
Hormodendrum	Thailand [131]
Penicillium	China [112,113], India [115, 116], Indonesia [119], Iran [121], Italy [122], Kuwait [120], Saudi Arabia [125], Taiwan [129,130], Turkey [132].
Pleospora	Spain [128]
Rhizopus and Mucor	India [115-118] Saudi Arabia [125], Taiwan [131]
Stachybotrytis	Saudi Arabia [125]
Smut	India [115], Thailand [131]
Trichoderma	Taiwan [129]
Ulocladium	Kuwait [123], Saudi Arabia [125]
Wallemia	India [117]

Non Aspergillus Allergic Broncho Pulmonary Mycosis (ABPM)

As the name suggests, ABPM is not related with *Aspergillus* and its most common causes are the *C. albicans, Cladosporium* and *Curvularia* species [75,112].

Extrinsic Allergic Alveolitis (EAA)

EAA is caused by repeated exposure to various molds including wood pulp worker's lung (*Alternaria* species), malt worker's lung (*Aspergillus clavatus*), farmer's lung (*A fumigatus*), maple bark stripper's lung (*Cryptostroma corticale*) and sewage worker's lung (*Cephalosporium* species). It is a broad spectrum of pulmonary interstitial and alveolar diseases caused by repeated (occupational) contact to a wide variety of organic dusts, microbes and chemicals reactions [75,133].

Treatment Options for Fungal Allergies

According to the flowchart in Fig. 11, three treatment choices are currently in practice for the treatment of fungal allergic diseases, which are stated as under:

A. Preventing the development of disease-promoting T helper 2 (T_H2) responses towards allergens by blocking the initiation of the human immune response, e. g. intervention at an early stage of life
B. Allergen immunotherapy or treatment with anti-inflammatory drugs, such as glucocorticoids, by blocking the activation of allergen-specific T_H2 cells, thereby affecting the antigen-presenting cells
C. Treatment with either antihistamines and leukotriene antagonists, or neutralizing antibodies specific to T_H2 cytokines, IgE, IL, interleukin, TCR and T-cell receptors that obstruct those effector molecules which bring about the clinical symptoms of allergic diseases [111].

The presence of fungal spores in the environment plays an important role in the development of allergenic responses and diseases. This rather small group of fungi has the potential of affecting the health and wellbeing of millions of people worldwide, ultimately resulting in enormous economic losses in the form of agricultural yield and medical care costs.

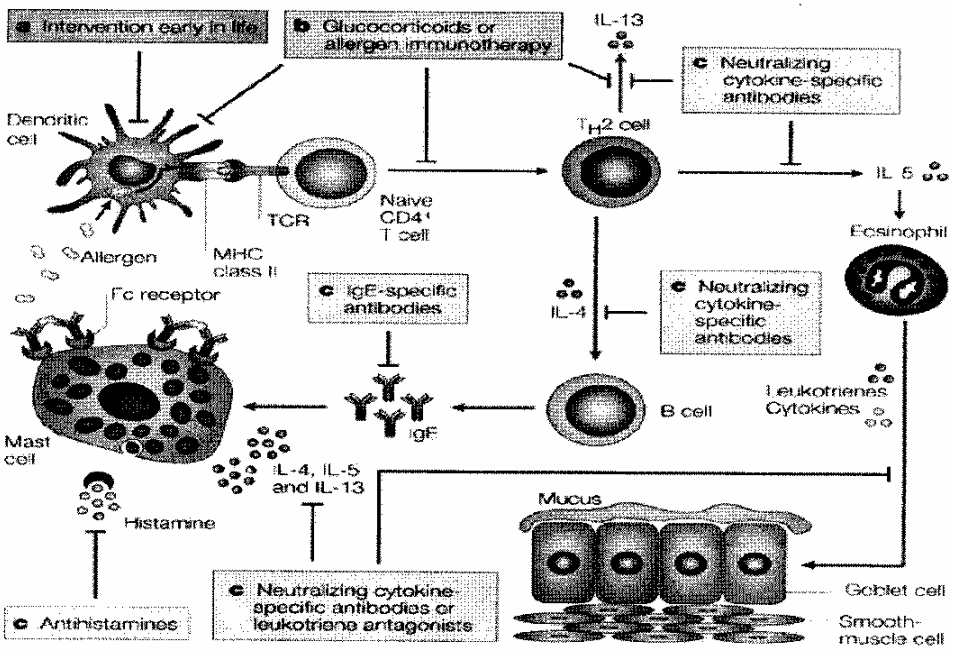

Fig. 11: Methods of treating fungal allergy symptoms [111].

Since it is impossible to completely eradicate fungal spores from the air and environment, it is absolutely imperative to adopt preventive measures in order to avoid allergic manifestations among susceptible individuals. A comprehensive evaluation of positive reactions to all types of fungi is the need of the hour, since it will greatly enhance our knowledge with respect to the variations of airborne spores. Sincere efforts are required to reduce the burden of fungal allergens from both indoor and outdoor air, which could be made possible to some extent by keeping our environment and living areas clean. More importantly, particular attention needs to be paid on the specific conditions that discourage the growth of fungi, with special emphasis on the spore formation and dispersion patterns. Moreover, development of potent allergy vaccines is required for the treatment of fungal allergies.

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References

1. A.W. Frankland, *Grana*, **30**, 19 (1991).
2. S. M. Hasnain, The 6th Asia pacific congress of *Allergology Clinical Immunology*, p. 33 (2004).
3. H. A. Burge and C. A. Rogers, *Environmental Health Perspective*, **108**, 653 (2000).
4. F. E. Leon-S, *Journal of Clinical Epidemiology*, **48**, 1183 (1995).
5. W. E. Horner, A. Helbling, J. E. Salvaggio and S.B. Lehrer, *Clinical Microbiology Review*, **8**, 161 (1995).
6. C. Z. Pepeljnjak, *Atmospheric Environment*, **31**, 491 (1997).
7. B. J. Green and R. Tovey, *Medical Mycology*, **44**, 245 (2006).
8. C. Beisswenger and R. Bals, *Journal of Occupational Medicine and Toxicology*, **3**, S3 (2008).
9. B. Flannigan, E. M. McCabe, F. McGarry, *Journal of Applied Bacterial*, **70**, 61 (1991).
10. S. H. Downs, T. Z. Mitakakis, G. B. Marks, N. G. Car, E. G. Belousova, J. D. Leuppi, W. Xuan, S. R. Downie, A. Tobias and J. K. Peat, *American Journal of Respiratory and Critical Care Medicine*, **164**, 455 (2001).
11. F. Munoz-lopez, Allergologia et immuno-pathologia, **35**, 41 (2007).
12. J. M. Rolland, J. Douglass and R. E. O'Hehir, *Expert opinion on investigational Drugs*, **9**, 515 (2000).
13. R. Crameri and C. Rhyner. *Current Opinion in Immunology*, **18**, 761 (2006).
14. W. E. Paul, *Fundamental Immunology, 6th edition*, p. 1127 (1991).
15. I. Roitt, J. Brostoff and D. Male. *Immunology*, 6th Edition, Mosby Publishers, p. 324 (2001).
16. A. B. Becker, *Clinical Reviews in Allergy and Immunology*, **28**, 5(2005).
17. S. T. Holgate and R. Polosa. *Nature Reviews Immunology*, **3**, 218 (2008).
18. S. Vrtala, *Allergy*, **63**, 299 (2008).
19. K. Deinhofer, H. Sevcik, N. Balic, C. Harwanegg, R. Hiller, H. Rumpold, M. W. Mueller and S. Spitzauer., *Methods*, **32**, 249 (2004).
20. Maria de la Luz, Z. Basilico, C. Chiericatti, E. E. Aringoli, R. L. Althaus and J. C. Basilico, *Science of The Total Environment*, **376**, 143 (2007).
21. P. Bowyer, M. Fraczek & D. Denning, W*BMC Genomics.*, 7, 251 (2006).
22. D. W. Denning, B. R. O. Driscoll, C. M. Hogaboam, P. Bowyer and R. M. Niven, *European Respiratory Journal*, **27**, 615 (2006).
23. B. J. Green, T. Z. Mitakakis and E. R. Tovey, *Journal of Allergy and Clinical Immunology*, **111**, 285 (2003).
24. A. W. Campbell, J. D. Thrasher, M. R. Gray and A. Vojdani, *Advances in Applied Microbiology*, **55**, 406 (2004).
25. H. A. Charles Walton, *Canadian Medical Association Journal*, **60**, 272 (1949).
26. D. C. Mildenhall, *Forensic Science International*, **163**, 236 (2006).
27. A. T. Donnell, L. C. Grammer,. *Allergy and Asthma Proceedings*, **25** (4), 2 (2004).
28. E. Aríngoli, B. María de la Luz Z, R. L. Altahus, J. C. Basílico, *International Journal of Biodeterioration & Biodegradation*, **62**, 281 (2008).
29. T. J. Kindt, R. A. Goldsby, B. A. Osborne, J. Kuby, *Immunology*, sixth edition 2007, W.H.. Freeman and company.
30. J. Salvaggio, J. Seabury, E. Schoenhardt,

Journal of Allergy and Clinical Immunology, **48**, 96 (1971
31. D. W. Denning, *European Respiratory disease*, p. 28 (2006).
32. M. Hargreaves, S. Parappukkaran, L. Morawska. J. Hitchins, H. Congrong and D. Gilbert, *The Science of The Total Environment*, **312**, 89 (2003).
33. T. Reponen, S. Grishpun and V. Ulevicius, *Grana.*, **40** (3), 119 (2001).
34. S. Das and S. Gupta-Bhattacharya, *Annals of agricultural and environmental medicine*, **15**, 105 (2008)
35. V. P. Kurpp, H. D. Shen and H. Vijay, *International archives of Allergy Immunology,* **129**, 181 (2002).
36. A. B. Gonçalves, R. R. M. Paterson and N. Lima, *International Journal of Hygiene and Environmental Health*, **209**, 257 (2006).
37. S. Bavbek, F.O. Erkekol, T. Ceter, D. Mungan, F. Ozer, M. Pinar and Z. Misirligil, *Journal of Asthma,* **43**, 421 (2006).
38. D. H. Laronge, Medically important fungi: *A guide to identification* Published by Harper and Row publishers, Hagerstown. Maryland, 60 (1975).
39. Q. A. Mandeel, *Mycopathologia,* **159**, 291 (2005).
40. H. Bouziane, J. P. Latge, C. Fitting, S. Mecheri, M. Lelong and B. David, *Allergologia et Immunopathol,* **33**, 125 (2005).
41. L. M.Wijnads, W. D. C. Desiz and F. M.van Leusden, *Allergy,* **55** (9), 856 (2000).
42. P. M. Salo, S. J. Arbes Jr, M. Sever, R. Jaramillo, R. D. Cohn, S. J. London and D. C. Zeldin, *Journal of Allergy and Clinical Immunology*, **118**, 892 (2006).
43. C. Barnes, J. Portnoy, M. Sever, S. Arbes Jr, B. Vaughn and D. C. Zeldin, *Annals of Allergy, Asthma and Immunology*, **97**, 350 (2006).
44. M. T. Lizaso, A. Martínez, J. A. Asturias, J. Algorta, B. Madariaga, N. Labarta and A.I. Tabar, *Journal of Allergy and Clinical Immunology,* **16**, 94 (2006).
45. A. Morga, D. Sara, M. Teresa, L. Jörg, M.Guy, *Pediatric Allergy and Immunology*: **14**, 100 (2003).
46. M. J. Schumacher, R. S. Farr, J. K. McMlatchy, *Journal of Allergy and Clinical Immunology,* **56**, 54 (1975).
47. I. Ignacio, S.C. María, M. C. Arilla, M. Alberto1, M. Javier and C. José, *Annals of Allergy Asthma and Immunology*, **93**, 589 (2004).
48. P. B. Schneider, U. Denk, M. Breitenbach, K. Richter, P. Schmid-Grendelmeierw, S. Nobbew, M. Himlyz, A. Mari, C. Ebnerz and B. Simon Nobbe, *Clinical &Experimental Allergy*, **36**, 1513 (2006).
49. T. Z. Mitakakis, C. Barnes and E. R.Tovey, *Journal of Allergy and Clinical Immunology,* **107**, 388 (2001).
50. J. Portnoy, D. Brothers, F. Pacheco, J. Landuyt and C. Barnes, *Annals of Allergy, Asthma and Immunology*, **81**, 59 (1998)
51. S. M. Tariq, S. M. Matthews, M. Stevens and E. A. Hakim, *Clinical & Experimental Allergy,* **26** , 794 (2006).
52. J. M. Corden, W. M. Millington, Aerobiologia **17**, 1573(2001)
53. B. Simon-Nobbe, U. Denk, V. Pöll, R. Rid and M. Breitenbach, *International Archives of Allergy Immunology,* **145**, 58 (2008).
54. H. Sanchez and R. K. Bush, *Reviata Iberoamericana de Micologia,* **18**, 56 (2001).
55. www.allergen.org/allergen.aspx
56. A. Rementeria, N. López-Molina, A. Ludwig, A. Belén Vivanco, J. Bikandi, J. Pontón and J. Garaizar, *Reviata Iberoamericana de Micologia,* **22**, 1 (2005).
57. B. Banerjee and V. P. Kurup, *Immunol Allergy Clinics in North America,* **18**, 601 (1998).
58. M. T. Hedayati, A. C. Pasqualotto, P. A. Warn, P. Bowyer and D. W. Denning, *Microbiology,* **153**, 1677 (2007).
59. H. J. Stark, J. T. Randell, M-R. Hirvonen, M. K. Purokivi, M. H. Roponen and H. O. Tukiainen, *European Respiratory Journal*, **26**, 887 (2005).
60. Jean-Paul Latgé, *Trends in Microbiology,* **9**, 382 (2001).
61. Shih-Wen Huang, Mold Allergy, www.emedicine.com/ped/TOPIC1471.htm (2007)
62. C. I. Ezeamuzie, S. Al-Ali, M. Khan, Z. Hijazi, A. Dowaisan, M. S. Thomson, J. Georgi, *International Archives of Allergy and Immunology,* **121**, 300 (2000).
63. A. Amchentsev, N. Kurugundla, A. G. Saleh, *Respiratory Medicine,* **1**, 205 (2008)
64. www.uniprot.org/ (UniProtKB/Swiss-Prot).
65. L. K. Arruda, B. J. Mann and M. D. Chapman, *Journal of Immunol*ogy, **149**, 3354 (1992).

66. M. Moser, R. Crameri, G. Menz, T. Schneider, T. Dudler, C. Virchow, M. Gmachl, K. Blaser and M. Suter, *Journal of Immunology*, **149**, 454 (1992).
67. V. P. Kurup, B. Banerjee, P. S. Murali, P. A. Greenberger, M. Krishnan, V. Hari and J. N. Fink, *Peptides*, **19**, 1469 (1998).
68. S. H. Lim, F. T. Chew, S. M. Sim, Y. T. Huang and D. Y. Goh, *Asian Pacific Journal of Allergy and Immunology*, **13**, 101 (1995).
69. W. Buzina, H. Braun, K. Schimpl and H. Stammberger, *Journal of Clinical Microbiology*, **41**, 4885 (2003)
70. Z. A. Dyer, R. S. Wright, I. H. Rong and A. Jacobs, *Medical Mycology*, **46**, 589 (2008).
71. Z. Qing-Yin, W. Sven-Olof, A. Rasmuson-Lestanderb and W. Xiao-Ru, *Journal of Environmental Monitoring*, **8**, 153 (2006).
72. B. K. Nayak, A. Nanda and N. Behera, *Aerobiologia*, **14**, 59 (1998).
73. P. N. Black, A. A. Udy and S. M. Brodie, *Allergy*, **55**, 501 (2000).
74. B. S. Nobbe, U. Denk, P. B. Schneider, C. Radauer, M. Teige, R. Crameri, T. Hawranek, R. Lang, K. Richter, P. Schmid-Grendelmeier, S. Nobbe, A. Hartl and M. Breitenbach, *Journal of Biological Chemistry*, **281**, 16354 (2006).
75. J. J. Braun, G. Pauli, P. Schultz, A. Molard, A. Gentine and F. de. Blay, *Revue Française d'Allergologie et d'Immunologie Clinique*, **47**, 298 (2007).
76. L. Aukrust , S. M. Borch, *International Archives of Allergy Applied Immunology*, **60**, 68 (1979).
77. F. Hong Chou a Ming, L. Tam c Lin-Hau, C. Chi-Huei, T. Hsiao-Yun, C. Raphael, H. D. Shen, *International Achieves of Allergy and Immunology*, **146**, 277 (2008).
78. V. P. Kurup and B. Banerjee, *Peptides*, **21**, 589 (2000).
79. R. Gupta, B. P. Singh, S. Sridhara, Gaur, S. N. Kumar, V. K. Chaudhary and N. Arora. *Allergy*, **57**, 636 (2002).
80. R. Gupta, V. Sharma, S. Sridhara, B. P. Singh, N. Arora, *Allergy*, **59**, 421 (2004).
81. B. S. Nobbe, U. Denk, V. Pöllm, R.Rid and M. Breitenbach, *International Achieves of Allergy and Immunology*, **145**, 58 (2008).
82. H. S. Goodridge and D. M. Underhill, *Drug Discovery Today: Disease Mechanism*, **4**, 247 (2007).
83. M. B. Morrow and E. P. Lowe, *Mycologia*, **35**, 638 (1943).
84. R. K. Bush and J. M. Portnoy, *Journal of Allergy and Clinical Immunology*, **107**, S430 (2001).
85. I. Rosas, C. Calderón, M. Ulloa and J. Lacey, *Applied Environmental Microbiology*, **59**, 2648 (1993).
86. M. H. Garrett, P. R. Rayment, M. A. Hooper, M. J. Abramson and B. M. Hooper, *Clinical and Experimental Allergy*, **28**, 459 (1998).
87. A. Cruz, M. Saenz de Santamaría, J. Martínez, A. Martínez, J. Guisantes and R. Palacios, *Allergologia et immunopathologia*, **25**, 153 (1997).
88. C. Li-Li, Diahn-Warng Perng, Y. Chia-Hsien, S. Song-Nan and C. Lu-Ping, *The Journal of Immunology*, **178**, 5237 (2007).
89. C. J. Yu, Y. M. Chen, S. N. Su, F. Forouhar, S. H. Lee and L. P. Chow, *Biochemical Journal,*, **363**, 707 (2002).
90. B. Andersen and A. T. Nissen, *International Journal of Biodeterioration & Biodegradation*, **46**, 111 (2000).
91. B. J. Green, D. Schmechel, L. Millecchia and F. Blachere, *Immunoassay*, **2**, 31 (2006).
92. I. Yike, T. G. Rand and D. G. Dearborn, *Toxicological Sciences*, **84**, 408 (2005).
93. S. Gravesen, P. A. Nielsen, R. Iversen and K. F. Nielsen, *Environmental Health Perspective*, **107**, (1999).
94. B. Andersen, K. F. Nielsen and B. B. Jarvis, *Mycologia*, **94**, 392 (2002).
95. T. G. Rand, M. Mahoney, K. White and M. Oulton, *Toxicological Sciences*, **65**, 239 (2002).
96. M. Mahmoudi, and M. E. Gershwin, *Journal of Asthma*, **37**, 191 (2000).
97. P. R. Badenoch, C. L. Halliday, D. H. Ellis, K. J. Billing and R. A. D. Mills, *Journal of Clinical Microbiology*, **44**, 1190 (2006).
98. C. M. Leach and M. Aragaki, *Mycologia*, **62**, 1071 (1970).
99. T. S. Mohan, L. Sigler, I. Witterick, R. Summerbell, I. Campbell and T. Mazzulli. *Journal of Clinical Microbiology*, November; **41**, 5333 (2003)
100. Srivandana Kilambi info@emlab.com *Environmental. Microbiology Laboratory*, Fungus of the month 2, 7 (2004)..
101. G. Reboux, R. Piarroux, F. Mauny, A. Madroszyk, L. Millon, K. Bardonnet and Jean-

Charles Dalphin, *American Journal of Respiratory and Critical Care Medicine*, **163**, 1534 (2001).
102. Z. Qing-Yin, W. Sven-Olof, R. Åsa and W. Xiao-Ru, *Applied Environmental Microbiology*, **70**, 7295 (2004).
103. T. J. Walsh, A. Groll, J. Hiemenz, R. Fleming, E. Roilides and E. Anaissie *Clinical Microbiology and Infection*, **10**, 48 (2004).
104. M. V. Macdonald and R. Chapman, *Plant Pathology*, **46**, 112 (1997).
105. M. Hoff, B. K. Ballmer-Weber, B. Niggemann, A. Cistero-Bahima, M. San Miguel- Monc´ın, A. Conti, D. Haustein and S. Vieths, *Molecular Immunology*, **39**, 965 (2003).
106. J. Verma, B. P. Singh, S. Susheela, S. N. Gaur and N. Arora, *International Archives of Allergy and Immunology*, **130**, 193 (2003).
107. M. Hoff, R. M. Troeb, B. K. Ballmer-Weber, S. Vieths and B. Wuethrich, *Journal of Allergy and Clinical Immunology*, **111**,1106 (2003).
108. David Ellis 2006.*www.mycology on line*. The University of Adelaide.04.07.2006.
109. K. A. Brander, P. Borbély, R. Crameri, W. J. Pichler and A. Helbling, *Journal of Allergy and Clinical Immunology*, **104**, 630 (1999).
110. S. Lappalainen, A. L. Pasanen, M. Reiman, P. Kalliokoski, *Annales of Allergy, Asthma and Immunology*, **81**, 585 (1998).
111. C. M. Hawrylowicz and A. O'Garra. *Nature Reviews Immunology*, **5**, 271 (2005).
112. J. P. Yang, Q. Z. Yin, M. J. Ye, C. W. Zhang and Q. A. Yu, *Hua Xi Yi Ke Da Xue Xue Bao.*, **20**, 448 (1989).
113. L. F. Hu, Z. G. Fang, Z. Y. Ouyang, X. L. Liao, X. Q. Lin and X. K. Wang, *Huan Jing Ke Xue.*, **26** (5), 22 (2005).
114. A. B. Singh and P.Kumar, *Indian Journal of Pediatrics*, **69**, 245 (2002).
115. R. N. Tripathi, *Water, Air and Soil Pollution*, **34**, 125 (1987).
116. V. Raju, B. P. R.Vittal, U. Prakash, T. Janaki and S. Bhuvaneswari, *Indian Journal of Allergy, Asthma and Immunology*, **16**, 55 (2002).
117. A. Adhikari, T. Reponen, Shu-An Lee and S. A. Grinshpun, *Annals of Agriculture and Environmental Medicine*, **11**, 269 (2004).
118. L. Kumar and M. Singh, *Indian Journal of Pediatrics*, **69**, 237 (2002).
119. I. R. Baratawidjaja, P. P. Baratawidjaja, A. Darwis, L. Soo-Hwee, C. Fook-Tim, L. Bee-Wah and K. G. Baratawidjaja, *Asian Pacific Journal of Allergy and Immunology*, **17**, 9 (1999).
120. A. R. Hariri , A. Ghahary , M. Naderinasab and C. Kimberlin, *Iran Annals of Allergy*, **40**, 349 (1978).
121. A. A. Nourian, H. Badalil, M. Khodaverdi, H. Hamzehei and S. Mohseni, *International Journal of Agriculture and Biology*, **9**, 628 (2007).
122. S. Nardoni, F. Mancianti, M. Sgorbini, F. Taccini and M. Corazza, *Mycopathologia*, **160**, 29 (2005).
123. A. F. Moustafa and S. M. Kamel, *Mycopathologia*, **59**, 29 (1976).
124. T. M. Ho, B. H. Tan, S. Ismail and M .K. Bujang, *Asian Pacific Journal of Allergy and Immunology*, **13**, 17 (1995).
125. A. S. Al-Suwaine, A. H. Bahkali and S. M. Hasnain, *Mycopathologia*, **145**, 15 (1999).
126. J. Morales, F. J. Gonza´lez-Minero, M. Carrasco, V. M. Ogalla and P. Candau, *Aerobiologia*, **22**, 127 (2006).
127. M. Venturini, T. Lobera, A. Blasco, Del Pozo, I. González and B. Bartolomé, *Journal of Investional Allergology and Clinical Immunology*, **15**, 219 (2005).
128. A. Dı´ez Herrero, S. Sabariego Ruiz, M. Gutie´rrez Bustillo and P. Cervigo´n Morales, *Aerobiologia*, **22**, 135 (2006).
129. J. H. Su, A. Rotnitzky, H. A. Burge and J. D. Spengler, *Applied and Environemental Microbiology*, **58**, 181 (1992).
130. C. S. Li, L. Y. Hsu, C. C. Chou and K. H. Hsieh, *Archives of Environmental Health*, **50**, 38 (1995).
131. M. Tuchinda and Y. Theptaranon, *Annals of Allergy*, **37**, 47 (1976).
132. M. Topbas, L. Tosun, G. Can, N. Kaklikkaya and F. Aydin, *Turkish Journal of Medical Sciences*, **36**, 31 (2006).
133. S. W. Huang and J. W. Kimbrough, *Clinical Pediatrics*, **36**, 695(1997).
134. S. J. Ponikau, D. Sherris, D. Congdon, E. Frigas, H. Homburger, M. Swanson, G. Gleich, H . Kita. *Journal of Allergy and Clinical Immunology*, **114**, 1369 (2004).