

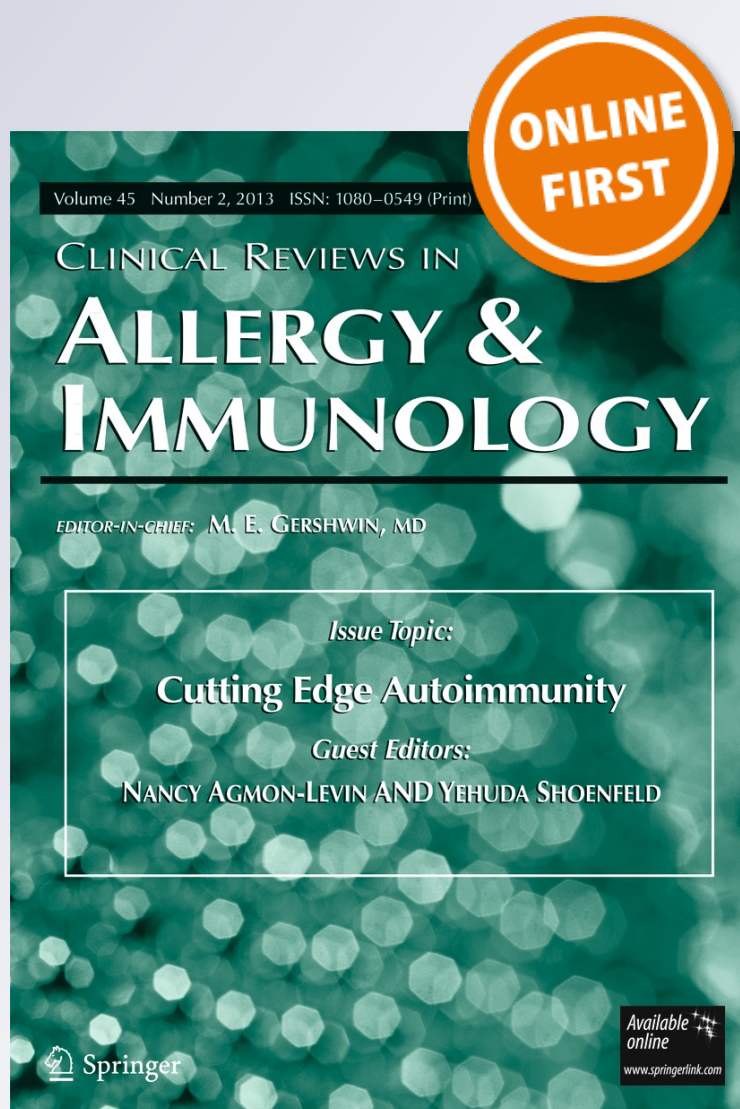
Immunotherapy for Mold Allergy

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Immunotherapy for Mold Allergy

Christopher A. Coop

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Abstract The objective of this article is to review the available studies regarding mold immunotherapy. A literature search was conducted in MEDLINE to identify peer-reviewed articles related to mold immunotherapy using the following keywords: mold, allergy, asthma, and immunotherapy. In addition, references cited within these articles were also reviewed. Articles were selected based on their relevance to the topic. Allergic responses to inhaled mold antigens are a recognized factor in allergic rhinitis and asthma. There are significant problems with respect to the production of relevant allergen material for the diagnosis and treatment of mold allergy with immunotherapy. Mold allergens contain proteases and should not be mixed with other allergens for immunotherapy. Most of the immunotherapy studies focus on two molds, *Alternaria* and *Cladosporium*. There is a lack of randomized placebo-controlled trials when evaluating the efficacy of mold immunotherapy with trials only focusing on immunotherapy to *Alternaria* and *Cladosporium*. Additional studies are needed regarding mold allergy and immunotherapy focusing on which molds are important for causing allergic disease.

Keywords Mold · Allergy · Asthma · Immunotherapy

Introduction

“The multitude of seeds produced by an Agaric or a Boletus is innumerable! It is astonishing! Yet not 1:10,000 answers the purpose of propagation. Is not

the air we breathe charged with them all the declining parts of the year? Do we not receive them into our lungs with every breath we draw? Whence proceed the quinsies, coughs and other complaints which prevail in autumn?” [1]

This statement was made in 1670 by James Bolton who postulated that there existed a relationship between mold spores and respiratory tract disorders. Charles Blackley [2] in 1873, Prince et al. [3] in 1934, and Feinberg [4] in 1935 also suggested the importance of molds in producing respiratory tract symptoms. Their evidence linking molds as a cause of hay fever and asthma was based primarily on anecdotal case reports of patients with symptoms after mold exposure. Since then stronger evidence showing a causal association between molds and respiratory tract diseases has gradually emerged, and clinicians have generally accepted a role of molds in allergy. Airborne fungal spores have been recognized as causing epidemic outbreaks [5] and life-threatening [6] episodes of asthma.

Molds are actually fungi, and literally tens of thousands of fungal species are thought to exist. Many genera of Hyphomycetes (*Fungi imperfecti*) class of fungi serve as sources of allergens. These include (1) *Cladosporium*, whose spores usually outnumber all other outdoor airborne biologic particles; (2) *Alternaria*, which is found throughout North America and can cause episodes of acute asphyxic asthma; (3) *Bipolaris*, which tends to be airborne in the highest concentration in warm humid regions; (4) *Epicoccum*, a mold found in many temperate regions and grasslands; (5) *Fusarium*, an important plant pathogen and considered to be the major wet mold because it is prominent in the air during damp, humid weather; and (6) *Aspergillus* and *Penicillium*, which are the main molds that colonize houses and other indoor areas [7]. Fungi of the basidiomycetes class (mushrooms, puffballs, and bracket fungi) have also been shown to be significant outdoor aeroallergens [8].

Numerous researchers have demonstrated that inhalation exposure to fungi induces the formation of specific IgE antibody which can be shown by in vivo skin testing and in vitro

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procedures such as the specific IgE testing (i.e., Immunocap). Because molds elicit IgE formation, immunotherapy has emerged as a treatment for mold-induced allergic rhinitis and asthma. From the standpoint of immunotherapy, fungi present several features that set them apart from the other inhalant antigens: (1) there are estimated to be hundreds of thousands of different species of fungi; (2) accurate information on exposure patterns to most fungal species is lacking; (3) fungal extracts are of variable quality [9], a characteristic that may relate in part to a high rate of somatic mutation and in part to their high content of proteolytic enzymes; and (4) for many of the genera of perfect fungi, extracts are lacking because they do not grow in artificial media.

Problems in Determining Mold Exposure Conditions

It is important to know if mold exposures are causing IgE-mediated allergies in patients and which molds are responsible for their allergic symptoms. Without such information, one cannot make logical decisions as to which materials should be used for skin testing and immunotherapy. Unfortunately, accurate information that defines either ambient or personal exposure patterns to fungal species is not available [10].

Most allergen material currently available as diagnostic and therapeutic agents have been chosen on the basis of conventional “settle-plate” or “settle-slide” sampling which provides only a rough estimate of the larger spore types present in the air. Smaller spores are always underrepresented in such samples, especially in outdoor air where significant air movement occurs during sampling [11].

Recognition and identification of spores on rods and slides adds additional bias [12]. In general, investigators trained to identify pollen grains use $\times 400$ magnification, which is reliable for identification of pollen and large spores such as *Alternaria*. However, this magnification is not ideal for many smaller fungal spores including those of *Penicillium* and *Aspergillus* species and basidiospores.

Some investigators rely only on culture sampling for evaluating certain environments, especially indoor areas. Unfortunately, many fungal spores do not germinate, grow, and produce new spores in artificial culture. In addition, viability does not equate to antigenicity and levels that are based strictly on numbers of viable spores may not be representative of allergen exposure.

Mold Sensitization and Allergic Disease

There have been several studies evaluating mold sensitization and allergic disease. Busse et al. [13] in July 2005 evaluated an inner-city adult population of 169 patients from New York with persistent asthma and found that 68.4 % of these people

were allergen sensitized to molds. Arbes et al. [14] published data in August 1995 from the National Health and Nutrition Examination Surveys looking at the prevalence of positive skin test responses to 10 common allergens in the US population. Of 10,508 patients skin-tested to *Alternaria alternata*, 12.9 % had a positive test showing that they had been sensitized to this mold. These patients may or may not have had allergic symptoms. In 2006, Calabria et al. [15] looked at the prevalence of positive skin test responses among military patients with rhinitis from San Antonio, Texas. Of the 1,137 patients who were skin tested to molds and other allergens, the sensitization rate of patients to *Alternaria tenuis* was 28.4 %, followed by *Helminthosporium* at 27.7 %, *Hormodendrum cladosporioides* at 21.6 %, *Fusarium* at 19.2 %, *Epicoccum* at 15.9 %, and *Aspergillus fumigatus* at 15.1 %. *Bipolaris* (13.1 %) and *Penicillium* (8.4 %) were found to be the molds with the least rate of sensitization among the rhinitis patients who were skin tested.

In 1985, Malling et al. [16] from Copenhagen, Denmark published a study evaluating the ability to screen for mold allergy using extracts of five common molds (*Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Mucor*). The *Cladosporium herbarum* extract was a partially purified, standardized preparation. The authors investigated 130 patients, 68 children and 62 adults, with asthma and/or rhinoconjunctivitis who had symptoms during mold season (June to September). The patients were screened by skin prick test and radioallergosorbent test (Phadebas RAST). The authors found that the skin prick test was more sensitive than the RAST as a diagnostic screening procedure for mold sensitization. In addition, the *Cladosporium* standardized extract was more sensitive than the crude mold extracts when used for skin testing. The conclusion made from this study was that most mold extracts are inadequate for diagnostic procedures. Also, the authors stated that this study strengthened the value of using standardized mold extracts.

Malling [17], in 1986, studied 24 adult asthmatic patients who were diagnosed with *Cladosporium* allergy based on positive bronchial provocation tests, skin tests, and RASTs to *Cladosporium*. These patients were diagnosed with autumnal asthma because most of their symptoms occurred in the fall season. They were followed by daily symptom scores for 11 weeks in the peak mold spore season (autumn).

Malling found a significant and positive association between fluctuation in *Cladosporium* spore counts and the weekly asthma symptom, peak flow, and medication scores. Of the 24 asthmatic patients, 18 patients were also allergic to *Alternaria*, but their asthma symptom scores were not associated with the *Alternaria* spore counts. The author concluded from this study that autumnal asthma is elicited by *Cladosporium* spores, and autumnal asthmatics had more symptoms when there were higher *Cladosporium* spore counts.

Problems in Standardization of Mold Allergens

Significant problems remain with respect to the production of relevant allergen material for the diagnosis and treatment of mold allergy with immunotherapy. These problems include the complexity, variability, and stability of fungal extracts.

In general, the quality of commercially available fungal extracts has been poor [9]. This point is illustrated by Yunginger et al. [18] and Aas et al. [19] who performed skin testing in 80 patients who had a suspected mold (*C. herbarum*) allergy. These patients were tested with a variety of commercial extracts, and the incidence of positive skin test reactivity with *C. herbarum* varied from 12 to 65 % depending on the commercial extract used. The same strains of a particular fungal species grown under identical culture conditions over similar time intervals can also produce extracts of varying potency.

Some of the problems involved with standardization of fungal allergens are related to the intrinsic variability of fungi. Most fungi have evolved as decay organisms and have developed the ability to adapt rapidly to changes in available food sources [20]. One mechanism for this phenomenon is a high rate of somatic mutation. For example, *Alternaria* is one of the fungi that will produce morphologically obvious somatic mutations in culture. Because such mutations are common in nature, each new strain of a specific fungus is likely to be slightly different than other strains. Sometimes these differences are so large that major differences in allergen content occur.

Variability can also result from methods of extract preparation. The processing of the fungal material can directly affect the allergen content of the resultant extracts. For example, some fungi produce cellulolytic enzymes that will digest dialysis tubing and other cellulosic dialysis devices, changing the pore size, and causing loss of specific allergens [21, 22]. In addition, fungi possess large amounts of carbohydrate-containing materials, some of which are allergens. Extraction and purification methods that focus on proteins alone can result in loss of important carbohydrate-rich allergens.

There have been attempts to standardize fungal allergens. One study used an enzyme-linked immunosorbent assay (ELISA) to evaluate the amount of Alt a 1 in *A. alternata* extracts [23]. Another study used a monoclonal antibody to Alt a 1 to evaluate the consistency of *Alternaria alternata* extracts [24]. In-house standardized extracts have been created for *A. alternata* and *Candida albicans* [25, 26]. In addition, a study from the University of Virginia showed that allergic extracts derived from *Alternaria* showed greater consistency when compared to *Aspergillus* by using ELISA-based assays to look for specific allergens (Alt a 1 and Asp f 1) [27].

Mold Immunotherapy Protease Activity

Fungal extracts along with dust mite and cockroach extracts contain proteases. These proteases are able to degrade proteins and glycoproteins, including their own fungal allergens. Multiple studies show that fungal enzymes will degrade other allergens when extracts are mixed together (see Table 1). Although glycerin helps to prevent fungal protease degradation, it is not 100 % effective, and most authorities do not recommend mixing fungal extracts with other types of allergen extracts [28, 29].

Knoll et al. [30] in 1991 from Appleton, WI, USA published an abstract showing that human serum albumin failed to protect the degradation of grass allergens by mold extracts. They studied the commercial allergenic extracts of grass, ragweed, cat, and birch by ELISA inhibition and by immunoblotting techniques when co-mixed individually with *Alternaria*, *Helminthosporium*, *Hormodendrum*, and dust mite (*Dermatophagoides farinae*).

Their studies demonstrated that co-incubation of grass with human serum albumin buffered *Alternaria* or *Helminthosporium* resulted in a tenfold loss of activity at 7 days and a 100-fold loss of activity by 50 days. In addition, they noted that ragweed, cat, and birch were unaffected by co-incubation with mold extracts.

Oliver et al. [31] in 1992 from Port Washington, NY, USA presented research evaluating the stability of the short ragweed extract major allergen Amb A 1 in the presence of *Alternaria* or dust mite (*Dermatophagoides pteronyssinus*) extract and 25 % glycerin. They mixed aqueous short ragweed with *D. pteronyssinus*, *Alternaria*, and a 50 % glycerinated saline control. The final glycerin concentration was 25 % volume per volume in each extract. The extracts were stored at 2–8 °C and Amb a 1 content was measured by radial immunodiffusion over 13 months.

There was no change in the Amb a 1 concentration in any of the samples after 6 months. After 13 months, the concentration of Amb a 1 mixed with dust mite dropped slightly (8 %), and the concentration of Amb a 1 mixed with *Alternaria* dropped significantly (20 %).

In 1993, Kordash et al. [32] from Spokane, WA, USA presented a study evaluating the effect of mixing allergenic extracts containing *Helminthosporium*, *D. farinae*, and cockroach with perennial ryegrass. These extracts also contained glycerin and buffered saline with phenol. The extracts were tested at 7, 30, 60, and 90 days after mixing, and the analysis included RAST inhibition and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting. In addition, to determine biologic potency for clinical relevancy, a quantitative intradermal skin test technique was performed.

All the tests performed showed significant decreases in ryegrass potency when it was mixed with *Helminthosporium*

Table 1 Mold immunotherapy protease activity affecting other allergen extracts

<i>Alternaria</i>	<i>Helminthosporium</i>	<i>Hormodendrum</i>	<i>Cladosporium</i>	<i>Penicillium</i>
Degradation of grass, ragweed, oak, box elder, and cat	Grass degradation	Grass degradation	Cat degradation	None

and cockroach. The decrease in potency was not seen with the ryegrass–*D. farinae* mix. The authors noted that glycerin seemed to have a protective effect on the potency of the mixed extracts. The authors also stated that even when the *Helminthosporium*–ryegrass mixture resulted in the most decrease in ryegrass potency, the resulting extract still contained an estimated biologic potency of 10,000 AU/ml (allergy units per milliliter). They felt that this remaining potency in the *Helminthosporium*–ryegrass mixture may explain why such extracts, which have been used in the past, appear to give adequate therapeutic results. Nevertheless, the authors concluded that until the potency of extract mixes can be routinely measured for clinical purposes, the allergist might be well advised to not mix extracts containing proteolytic enzymes, particularly *Helminthosporium*, with grass and perhaps other pollen extracts.

An abstract was published in 1994 by Kagen et al. [33] from Appleton, WI, USA looking at the stability of extracts used alone and when mixed together. The authors evaluated *Alternaria*, *Hormodendrum*, *Helminthosporium*, cat, ragweed, grass, and *D. farinae* extracts. Allergen stability and immunoreactivity of the extracts was ascertained by skin testing of patients, ELISA inhibition, SDS-PAGE immunoblotting, and Lol p1 (major grass pollen allergen) determinations.

The authors noted that grass allergens were destroyed by mold co-mixture within several days. Cat, mite, and ragweed allergens withstood mold co-incubation over 50 days. The authors concluded that patients receiving immunotherapy should not receive grass allergen immunotherapy which has been co-mixed with proteolytic enzyme-containing allergen extracts.

Nelson et al. [34] from Denver, CO, USA in 1996 published a study looking at the effects of dilution and mixing on allergy extract stability. They wanted to assess the effect of mixing pollen extracts of trees, grasses, weeds, dust mite, and cat with other extracts that contain proteases, specifically molds and cockroach. Pollen extracts of timothy grass, Bermuda grass, short ragweed, Russian thistle, white oak, box elder, *D. farinae*, and cat were stored alone or combined with one or more extracts of American cockroach, *Alternaria*, *Cladosporium*, *Penicillium*, and a house dust mite mix. The stock concentration of 1 ml of the allergen extract of interest was diluted in 9 ml of phenol–saline–human serum albumin (HSA) as a control. For comparison, 1 ml of the stock concentration of allergen extract being tested was combined with 1 ml of each of all four extracts (American cockroach,

Alternaria, *Cladosporium*, and *Penicillium*). The vials were stored at 4 °C. After 3 months, the vials were then analyzed by comparing the allergen extract diluted with phenol–saline–HSA to those that were mixed with the protease containing extracts. Extract potency was tested by inhibition ELISA using a pool of serum containing specific IgE for each allergen extract. The sample pool was obtained by combining 8–12 serum specimens from individuals with a strongly positive prick skin test response to that allergen extract.

In general, most of the extracts tested were susceptible to deterioration from the addition of one or more of the protease containing extracts. Only short ragweed appeared to be convincingly resistant to protease degradation. Focusing on the results of mixing as pertaining to the fungal extracts, *Alternaria* extract had the broadest activity. *Alternaria* reduced the potency of five of the target allergen extracts (timothy grass, Bermuda, white oak, box elder, and cat), *Cladosporium* affected one target extract (cat), and *Penicillium* did not affect the potency of any of the target extracts. In comparison, cockroach extract affected three target allergen extracts (timothy grass, Russian thistle, and box elder), and *D. pteronyssinus* did not affect the potency of any of the target extracts.

The effect of fungal and insect extracts on the potency of each other was also examined. There was no evidence of reduction in potency of cockroach, *Alternaria*, *Cladosporium*, or *Penicillium* extracts as a result of mixing. However, the cockroach extract did show a significant loss of potency at 3 months, compared with freshly diluted extract, whether stored alone or in mixture.

The authors reported from this study that the *Alternaria* extract was most frequently responsible for loss of potency, followed by the cockroach and the *Cladosporium* extracts. They advised to not combine *Alternaria*, *Cladosporium*, cockroach, and other fungal extracts with pollen extracts; however, their data suggested that 25 % glycerin may have some protective effect against protease degradation.

Mold Immunotherapy Studies in General

A number of studies have been accomplished in the study of immunotherapy for mold allergy. These studies include a rush immunotherapy protocol with a variety of molds given for immunotherapy and several controlled clinical trials with allergen extracts of two important outdoor fungi, *Cladosporium*, and *Alternaria* (Table 2).

Table 2 Mold immunotherapy studies

Mold	Number of patients	Dose of immunotherapy	Length of trial
<i>Alternaria</i> —Metzger et al. [36]	10 patients No controls	100,000 PNU	3 Months
<i>Alternaria</i> —Cantani et al. [37]	39 patients 40 controls	40,000–80,000 PNU	3 Years
<i>Alternaria</i> —Horst et al. [38]	13 patients 11 controls	100,000 PNU	1 Year
<i>Cladosporium</i> —Dreborg et al. [39]	16 patients 14 controls	100,000 BU	10 Months
<i>Cladosporium</i> —Malling et al. [16, 40, 41]	22 patients 11 controls	46,000–100,000 BU	5–7 Months
<i>Alternaria</i> , <i>Hormodendrum</i> , <i>Penicillium</i> , and <i>Aspergillus</i> —Goldstein and Chai [35]	13 patients No controls	3,000–14,000 PNU	4–5 Months

PNU protein nitrogen units, BU biological units

In 1981, Goldstein and Chai [35] from Kansas City and Chai from Denver, CO, USA published a study of rush immunotherapy among 13 children (eight boys and five girls) ages 8–16 years. This study did not look exclusively at mold immunotherapy, but many of the patients in the trial received immunotherapy from mold extracts. The patients had perennial asthma and were selected on the basis of positive skin tests to molds (*Alternaria*, *Hormodendrum*, *Penicillium*, and *Aspergillus*), mite dust, and oak/pecan tree. The patients also had positive bronchial provocation tests to the same antigens to which they were skin test positive. Their histories were compatible with the skin tests and the bronchial provocation challenges. The rush immunotherapy was performed with extracts of *Alternaria*, *Hormodendrum*, *Penicillium*, mite dust, and oak/pecan tree. The control patients were five children who had positive skin tests and bronchial provocation challenges to the same antigens but did not receive immunotherapy. The rush immunotherapy consisted of four antigen dilutions: 10, 100, 1,000, and 5,000 protein nitrogen units per milliliter. The maintenance dose of 0.5 ml of the 5,000 pnu/ml was achieved in 7–18 weeks (mean, 12 weeks). The total cumulative dose of antigen received from immunotherapy ranged from 3,000–14,000 protein nitrogen units with a mean dose of 7,200 protein nitrogen units.

Nine children completed the full course of immunotherapy. Four patients received immunotherapy with mite dust or house dust, and the other five patients received immunotherapy with mite dust and a mold antigen or mite dust and oak–pecan tree. These nine patients were rechallenged by bronchial provocation to a total of 14 antigens. Seven of the subjects had decreased bronchial sensitivity on 11 of the post-rush immunotherapy bronchial provocation tests. One patient had increased bronchial sensitivity to both antigens tested. The last patient had no change in bronchial sensitivity after rechallenge with bronchial provocation. The mean prerush immunotherapy bronchial provocation test was positive at 900 protein nitrogen units (range, 190–2,200 protein nitrogen units). The

mean postrush immunotherapy bronchial provocation test was positive at 2,600 protein nitrogen units (range, 20–7,200 protein nitrogen units).

From the study, the authors concluded that rush immunotherapy is a safe and effective treatment for asthma. Although this trial did not evaluate mold immunotherapy exclusively, the researchers felt that immunotherapy with mold antigens is effective. In addition, they stated that a marked change in bronchial sensitivity can be achieved in a short time (approximately 2–4 months) with a relatively small cumulative dose of antigen (3,000–14,000 protein nitrogen units).

***Alternaria* Mold Immunotherapy Studies**

In 1983, Metzger et al. [36] from Iowa City, IO, USA reported their findings in abstract form regarding the treatment of *Alternaria*-induced asthma with allergy immunotherapy. They enrolled 10 atopic asthma patients who received *Alternaria* immunotherapy and were symptomatic during mold season (autumn). These patients demonstrated a positive methacholine challenge and a dual early and late asthmatic response to *Alternaria* bronchoprovocation.

Of the 10 atopic asthmatic patients who had received *Alternaria* immunotherapy, six were rechallenged to *Alternaria* bronchoprovocation. These patients had received at least 100,000 protein nitrogen units of *Alternaria*. All of the subjects showed a decrement in their late asthmatic response following immunotherapy. The early asthmatic response was reduced significantly in only one subject. As a group, the late asthmatic response decreased by 67 %. Three subjects had a late asthmatic response which persisted for several days before receiving immunotherapy but completely resolved within a few hours after the course of allergy immunotherapy. One patient who was not treated with immunotherapy demonstrated a 52 % increase in the late asthmatic response. The authors concluded that high dose *Alternaria* immunotherapy may

preferentially reduce the severity of the late asthmatic response, and they recommended further studies.

In 1998, Cantani et al. [37] from Rome, Italy published a prospective controlled study of children with *Alternaria*-induced asthma and/or allergic rhinitis. Thirty-nine children aged 5–14 received immunotherapy with *Alternaria* alum precipitated pyridine extract over a 3-year period. The 40 control patients were treated conventionally with medications for 3 years without immunotherapy. The diagnosis of *Alternaria* allergy was established in each patient by clinical evaluation which included skin testing and the Phadebas RAST. All children tested were highly positive to *Alternaria tenuis*, while the intradermal skin tests to the other allergens were negative or weakly positive. In all patients, the Phadebas RAST confirmed the results of the skin tests.

The investigators found that *Alternaria* immunotherapy was successful. The results were based on a semiquantitative scale of 0 to 3 (0=worsening of symptoms, 1=poor response to treatment, 2=good response to treatment, and 3=excellent response to treatment) based on improvement of symptoms and a decreased need for bronchodilators, antihistamines, and nasal decongestants. Eighty percent of the children given the highest dose of *Alternaria* immunotherapy (80,000 protein nitrogen units) had excellent results, while 20 % had good results. At the lowest dose of *Alternaria* immunotherapy (40,000 protein nitrogen units) only 5 % of the patients had excellent results. At the medium dose of *Alternaria* immunotherapy (40,000–80,000 protein nitrogen units), 51 % of the patients had an excellent response. In regard to the control patients, 87.5 % had worsening of their symptoms, 10 % of the control patients had a poor response to treatment, and only 2.5 % of control patients had good results. There were no systemic reactions to the mold immunotherapy and only rare local reactions. The authors noted that sensitivity to *Alternaria* should be considered when children suffer from severe perennial respiratory symptoms. They showed that clinical improvement of their asthma and allergies was not in connection with the duration of the immunotherapy, but appeared to be dose-related. Those patients who had received the highest cumulative doses had a better response to *Alternaria* mold immunotherapy. They concluded by stating that *Alternaria* mold immunotherapy is beneficial in the treatment of *Alternaria* allergic patients and that patients should be given the highest tolerated dose.

In 1990, Horst et al. [38] from Montpellier, France reported on a 1-year study of immunotherapy in patients who were allergic to *Alternaria*. This double blind placebo-controlled study was performed in 24 patients who had a clinical history of rhinitis with or without asthma. The patients ranged in age from 5 to 56 years. The diagnosis of *Alternaria* allergy was made by a positive skin prick test with a standardized *Alt* extract. The positive prick skin test was confirmed by a positive Phadebas RAST. The patients were negative to *D.*

pteronysinus, *D. farinae*, animal danders, and other molds in skin prick test.

The *Alternaria* mold extract was standardized by isoelectric focusing, crossed immunoelectrophoresis, crossed radioimmunoelectrophoresis, RAST inhibition, and skin tests. The same *Alternaria* mold extract was used for both immunotherapy and prick skin testing.

Thirteen patients received the active treatment of *Alternaria* immunotherapy and 11 patients received the placebo which consisted of a preparation containing 0.9 % NaCl, 0.4 % phenol as a preservative, and 0.5–0.05 mg of histamine dihydrochloride. The placebo had a color similar to the color of the *Alternaria* extract. Immunotherapy was started by a 2-day rush protocol, and maintenance injections were administered for 1 year. Maintenance injections were administered for the first 6 weeks after the rush injections and then every 2 weeks for 1 year. Two of the 13 patients experienced a mild systemic reaction that responded to treatment.

Results of the 1-year *Alternaria* immunotherapy trial were based on a self-evaluation of the treatment by the patient, global symptom medication scores, nasal challenges with *Alternaria* extract, skin tests, and serum *Alternaria*-specific IgG and IgE measured before and after immunotherapy.

The patients receiving active treatment rated its efficacy significantly higher than the patients receiving placebo. Global symptom medication scores, including medications used to treat asthma and allergic rhinoconjunctivitis were significantly ($p < 0.005$) lower in the actively treated group. Nasal challenges with *Alternaria* extract performed before and after 1 year of immunotherapy treatment showed no difference in the placebo-treated group but showed a significantly ($p < 0.01$) increased mean provocative dose in the actively treated group. Skin tests were significantly reduced in the actively treated group. *Alternaria*-specific IgG increased significantly in the actively treated group and were stable in the placebo-treated group. There was a significant correlation between nasal challenges and nasal symptom medication scores ($p < 0.03$) or the patient's self-evaluation of efficacy ($p < 0.05$). Serum *Alternaria*-specific IgE titers did not change significantly in either treated group.

The authors concluded from the study that patients only sensitized to *Alternaria* benefit from specific immunotherapy with a standardized *Alternaria* extract. Specifically, the authors stated that specific immunotherapy with a standardized mold extract is clinically effective in alleviating nasal and bronchial symptoms.

***Cladosporium* Mold Immunotherapy Studies**

In 1986, Dreborg et al. [39] from Stockholm, Sweden reported the results of a double-blind histamine placebo-controlled immunotherapy trial using a standardized, purified *C.*

herbarum allergen preparation. The trial enrolled 30 children with a clinical history of mold-induced asthma and/or rhinoconjunctivitis. A typical history of the mold allergic patients included asthma symptoms that started or worsened in the *Cladosporium* season (June through October in Scandinavia). The diagnosis of mold allergy was confirmed by a positive skin prick test and a Phadebas RAST. In addition, the subjects underwent a positive bronchial and/or conjunctival provocation challenge to *C. herbarum*. The active treatment group which received *C. herbarum* immunotherapy consisted of 16 patients, and the histamine placebo group included 14 patients. Immunotherapy was given for 10 months in a double-blind manner to the randomized groups.

Of the 16 children treated with the *Cladosporium* preparation, only 5 reached the maintenance dose (100,000 biological units) because of asthmatic, urticarial, and large local reactions. All patients receiving the histamine placebo reached the maintenance dose with a few patients experiencing local reactions.

After 10 months of treatment, bronchial and conjunctival sensitivity were significantly reduced in the *Cladosporium* immunotherapy-treated group as compared with the histamine placebo group. The peak expiratory flow rates became slightly higher in the *Cladosporium*-treated group and slightly lower in the histamine placebo group, but there was no significant difference between the groups. In addition, skin sensitivity decreased in 10/16 (62 %) of the actively treated patients, whereas skin sensitivity decreased in 6/14 (43 %) patients in the placebo group. The difference between the groups was not significant.

The authors stated that this trial showed the clinical importance of *Cladosporium* allergy in patients with severe asthma and mold allergy. They noted that the ideal trial for the evaluation of immunotherapy for *Cladosporium* allergic patients would include only patients that were monosensitized to *C. herbarum* and that such patients are rare. The authors concluded that this is the first double-blind histamine placebo-controlled trial showing the clinical efficacy of immunotherapy using a mold preparation in asthmatic children.

Also, in 1986, Malling et al. [40] published a placebo-controlled, double blind study treating *C. herbarum* allergic adult asthmatic patients with a standardized *Cladosporium* immunotherapy extract. Twenty-two patients completed the first year of immunotherapy (11 patients on *Cladosporium* immunotherapy and 11 patients on placebo immunotherapy). Immunotherapy was given in a cluster method with patients receiving five injections on each clinic visit. Clinic visits were spaced 14 days apart. Molds were considered the clinically most important allergens for these patients, as patients were either symptom-free or had only minimal asthma outside the *Cladosporium* season (August to mid October). The diagnosis of *Cladosporium* allergy was based on bronchial provocation test results, positive skin

tests to *Cladosporium*, and daily symptom scores during the *Cladosporium* season.

Only one patient was able to reach the scheduled top dose (100,000 biological units) of the *Cladosporium* immunotherapy extract due to asthma and anaphylactic side effects (angioedema and urticaria). The mean top dose in the *Cladosporium* group was 46,000 biological units.

After 5–7 months of immunotherapy, the clinical efficacy was evaluated by a combination of symptoms (asthma symptom score and peak flow results) and consumption of anti-asthmatic medication over a 10-week period during peak *Cladosporium* season in 1982. These results were compared to the results from the previous peak *Cladosporium* season in 1981 when patients had not received *Cladosporium* immunotherapy. Patients were asked to rate their asthma as “improved,” “unchanged,” or “deteriorated.” A statistically significant difference was not found in either symptom or medication score, but the combined evaluation with respect to clinical efficacy showed a significant difference in favor of *Cladosporium* immunotherapy. Patients noted improvement in their asthma in 45 % of the *Cladosporium* group versus 9 % improvement in the placebo treated group. Side effects were observed frequently in the *Cladosporium*-treated group; 70 % of patients had large local reactions and 100 % of patients had exacerbations of their asthma during the dose-increase phase.

The authors stated that based on the clinical efficacy shown by the improvement of asthmatics with *Cladosporium* immunotherapy in this trial, it is feasible to administer *Cladosporium* immunotherapy in highly specialized clinics. The authors also noted that they had a 100 % incidence of systemic side effects in the *Cladosporium*-treated group, and this was a consequence of the high doses of potent allergen extract and the clustered dose increase regimen.

IgG Subclass Response to *Cladosporium* Immunotherapy

IgG blocking antibodies that attenuate the IgE response is theorized to be a mechanism of action for allergen immunotherapy. Malling et al. [41] in 1988 published an article looking at the IgG subclass response, and its relationship to the clinical efficacy of *Cladosporium* immunotherapy. The authors used a sensitive allergen and subclass-specific solid phase immunoradiometric assay to measure the IgG subclass response of 22 adult asthmatic patients. These patients were randomized and participated in a 1-year placebo-controlled, double-blind *C. herbarum* immunotherapy study. Eleven patients received *C. herbarum* immunotherapy, and 11 patients received placebo immunotherapy.

The authors found that the IgG response was mainly restricted to the IgG subclasses 1 and 4, although there were a few patients that responded to immunotherapy with IgG₂ and IgG₃. During the first clusters of injection immunotherapy,

there was an intense and early IgG₁ response that gradually leveled down. The IgG₄ response had a later onset and slowly increased during the 12 months of immunotherapy. Surprisingly, little or no increase of IgG₁ and IgG₄ were associated with improvement of allergy symptom–medication scores. In fact, the authors noted that the magnitude of the IgG₁ response during the dose increase phase was directly correlated to the number of systemic side effects from allergy immunotherapy. They found no relation of IgG₁ and IgG₄ to the changes in their IgE parameters which included skin prick test, bronchial challenge, and circulating specific IgE.

The authors concluded that the data obtained from their research trial did not support the hypothesis of IgG acting as a blocking antibody as a mechanism of immunotherapy. They noted that this trial was based on few patients and looked only at *Cladosporium* immunotherapy for a 1-year period of time.

Inhibiting the Allergenicity of Mold with Sodium Hypochlorite (Bleach)

In mold-contaminated buildings that may pose an exposure risk to mold allergic individuals, Martyny et al. [42] showed that aerosolized sodium hypochlorite inhibited the viability and allergenicity of mold on building materials. They performed this study by growing *A. fumigatus* on three common building construction materials (oriented strand board, gypsum drywall, and plywood) and in conventional laboratory solution. Next, they sprayed two sodium hypochlorite-containing household products (diluted bleach and Tilex) on the mold-contaminated materials and in solution. Then, the researchers removed the surface mold from the treated and the untreated building materials and analyzed the mold by light microscopy, electron microscopy, and culture. *A. fumigatus* allergen was evaluated by skin prick testing using extracts prepared from both the boards and the cultured materials. The mold extracts were also tested for *A. fumigatus* antigen by ELISA.

The authors noted that both sodium hypochlorite disinfectants killed *A. fumigatus* in solution and on mold-contaminated building materials. Also, both dilute bleach and Tilex inhibited *A. fumigatus* recognition by ELISA. Skin testing supported the results of the ELISAs and demonstrated loss of skin test reactivity to the sodium hypochlorite-treated mold solutions in most of the subjects. Of the four individuals who had a positive skin test to mold grown on oriented strand board building material, three no longer reacted to extracts from the bleach-treated boards. The authors concluded that aerosolized application of sodium hypochlorite onto mold-contaminated building materials kills *A. fumigatus*, reduces the recognition of *A. fumigatus* mold by ELISA, and results in a loss of skin test reactivity to the treated mold in individuals allergic to *A. fumigatus*.

Table 3 Recommended dosing for mold immunotherapy

All molds (nonstandardized)

1:10 to 1:40 weight/volume or 10,000–40,000 PNU—increase to highest tolerated dose

Cox et al. [29]

Mold Immunotherapy for Allergic Fungal Sinusitis

Immunotherapy for allergic fungal sinusitis has been shown to be effective. Mabry et al. treated patients with histologically proven allergic fungal sinusitis with surgery followed by immunotherapy. The immunotherapy was based on skin testing showing sensitivity to both fungal and nonfungal antigens. Of 11 patients who received immunotherapy (mean time on immunotherapy was 29 months), none required regular treatment with systemic steroids and only three were using topical nasal steroids. No repeat surgeries were required as well for these patients [43, 44].

More recently, Greenhaw et al. evaluated the safety of fungal immunotherapy in patients with allergic fungal sinusitis. Fourteen patients with allergic fungal sinusitis who were receiving high dose fungal immunotherapy for at least 6 months were examined. These patients were compared with a control group of 14 patients who were receiving immunotherapy but did not have allergic fungal sinusitis. The conclusion from the study was that subcutaneous fungal immunotherapy is unlikely to cause adverse reactions other than those reactions normally seen with immunotherapy for pollen allergy (Table 3) [45].

Conclusion

In summary, we know that inhaled mold antigens play a role in asthma. There is less evidence to support the idea that mold exposure, either indoor or outdoor, contributes as a trigger to allergic rhinitis. It is difficult to ascertain which molds are responsible for causing allergic symptoms because accurate information that defines ambient and personal exposure patterns to fungal species is not available. Significant problems remain with the production of relevant allergen material for the diagnosis and treatment of mold allergy with immunotherapy. Most mold immunotherapy extracts are not standardized.

Regarding immunotherapy, molds contain significant amounts of proteases which degrade pollens. Grass pollen seems to be the most sensitive to protease degradation, while weed allergens, especially ragweed, are the most resistant. Because of the mold protease activity, experts do not recommend combining mold extracts with the pollen extracts of trees, grasses, and weeds, unless the diluent contains 25–50 % glycerin.

Generally, there is a lack of randomized placebo-controlled trials when evaluating the efficacy of mold immunotherapy. Although several controlled trials on the effectiveness of mold immunotherapy have been published, these trials have focused on only two molds, *Alternaria* and *Cladosporium*.

The indications for mold immunotherapy include patients who have asthma and/or allergic rhinitis and are sensitized to molds. Mold immunotherapy is also effective for allergic fungal sinusitis and this is another indication for mold immunotherapy.

Finally for those patients who have allergic sensitization to *A. fumigatus* and have indoor *Aspergillus* contamination, household bleach (sodium hypochlorite) is effective for killing *A. fumigatus* and eliminating its allergenicity. We can most likely generalize this study to include all types of mold that contaminate indoor environments.

Future studies regarding mold allergy and immunotherapy should focus on which molds are clinically important for causing allergic rhinitis and asthma. Research should concentrate on the standardization of molds for allergy testing and immunotherapy, and there should be additional randomized controlled trials evaluating the efficacy of mold immunotherapy.

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