
IgE-mediated allergy to fungal allergens in Finland with special reference to *Alternaria alternata* and *Cladosporium herbarum*

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Background: *Alternaria alternata* and *Cladosporium herbarum* are common fungi in outdoor environments, but their clinical significance has not been elucidated in Finland.

Objective: To evaluate the prevalence of IgE-mediated allergy and clinical outcomes caused by sensitization to fungal allergens in patients with suspected allergy.

Methods: Skin prick tests (SPTs) were performed with *C. herbarum* in 6,376 patients and also with *A. alternata* in 1,504 of these patients. SPTs were repeated in 40 patients who showed a positive reaction to either allergen using commercial and in-house extracts. The association of SPT with allergen-specific IgE antibodies in serum was evaluated. Seven patients also underwent a conjunctival challenge test with these fungal allergens.

Results: The prevalence of positive SPT results to *A. alternata* and *C. herbarum* was low (2.8% and 2.7%, respectively). Among the 40 patients, atopic eczema/dermatitis syndrome was found in 58%, asthma in 44%, and rhinitis in 31%. Most of the patients displayed SPT reactions also to several other fungal allergens, and 75% to 80% showed a positive SPT reaction to allergens of pet animals or pollens. Four patients had a positive reaction to *A. alternata* and 6 to *C. herbarum* in the conjunctival challenge test.

Conclusion: In the Finnish population with allergic symptoms, IgE-mediated sensitization to 2 common fungal allergens was rare and of minor clinical importance. SPT reactions to fungi are mostly observed in patients with multiple sensitivity to various allergens.

Ann Allergy Asthma Immunol. 2003;91:280–287.

INTRODUCTION

Exposure to fungal allergens may lead to respiratory, dermal, and conjunctival disorders in sensitized persons.^{1,2} The fungi may originate outdoors, indoors, or even from occupational settings. Exposure occurs most often by inhalation when spores or fragments of fungi enter the respiratory tract as inhalant aeroallergens. The fungi may also be involved in skin or conjunctival contact.

Hypersensitivity to fungal allergens caused by IgE mast cell-mediated reactions manifests as allergic rhinoconjunctivitis and asthma.³ Heavy exposure to fungal antigens may also lead to extrinsic allergic alveolitis (hypersensitivity pneumonitis).⁴ In allergic bronchopulmonary aspergillosis, *Aspergillus fumigatus* increases IgE and IgG antibody levels in the serum and the number of eosinophils in the lungs.⁵

Alternaria alternata and *Cladosporium herbarum* are the 2 most common fungal species that appear in outdoor environments and lead to sensitization and immediate-type allergic

reactions.¹ *A. alternata* has been associated with IgE-mediated allergy (rhinoconjunctivitis and asthma), both in adults and children, primarily in outdoor environments.^{1,6–11} *C. herbarum* is another important and ubiquitous allergenic fungus in indoor and outdoor environments, which often appears in cooler climates and causes rhinoconjunctivitis and asthma.^{1,12–14}

The epidemiology of allergic diseases such as asthma and allergic rhinitis due to mold exposure has not been fully elucidated. It has been estimated that the incidence of sensitization to fungal allergens is approximately 10% in the atopic population in Scandinavia¹⁴ and even 30% in the southern United States.¹⁵ The present investigation was performed to evaluate the prevalence of IgE-mediated sensitization to fungal allergens in patients residing in Finland with suspected allergy.

METHODS

All patients gave their informed consent to participate in the study, which was approved by the ethical committee of the Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland.

Patient Screening

The patients were from the Helsinki area and were examined at the Skin and Allergy Hospital, Helsinki University Central Hospital. All 6,376 patients who were referred to the hospital

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Received for publication January 8, 2003

Accepted for publication in revised form March 24, 2003.

because of symptoms that suggested allergy and who underwent skin prick tests (SPTs) during January 1, 1996, to May 31, 1997, were eligible for further studies. These patients were tested to 11 common inhalant allergens (cat, dog, horse, and cow epithelium; birch, timothy, mugwort, and meadow grass pollen; *Dermatophagoides pteronyssinus*; natural rubber latex) (10 HEP; Soluprick-SQ; ALK, Horsholm, Denmark), including *C. herbarum* (1/20 wt/vol). Altogether, 1,504 of these patients were also tested with *A. alternata* (1/20 wt/vol) in a special series of fungal allergens (*A. fumigatus*, *Aureobasidium pullulans*, *Fusarium roseum*, *Mucor racemosus*, *Penicillium notatum*, *Phoma herbarum*, *Rhizobus niger*, and *Rhodotorula rubra* [10 HEP] and *Acremonium kiliense* [5 mg/mL]) because they had a history of indoor air problems at home or work (Fig 1).

Inclusion Criteria

Patients who had SPT results that showed a skin wheal reaction of at least 4 mm in diameter to *A. alternata* were included in the study. This limit was chosen to exclude doubtful reactions. A total of 33 patients (2.2%) of 1,504 fulfilled the predefined criteria and were invited for further

testing. A total of 24 (73%) of them attended. Because of the small number of patients who tested positive to *A. alternata*, those showing a skin wheal reaction of at least 4 mm in diameter to *C. herbarum* in the basic testing were also included. A total of 84 patients (1.3%) of 6,376 fulfilled the criteria. Twenty-seven of these, living in southern and western Helsinki near the hospital, were invited for further testing and 17 (63%) attended. Altogether, 40 (0.7%) of 6,376 patients (23 females and 17 males; mean age, 34 years; range, 14–60 years) were selected to participate in the present study using these criteria.

Procedures

SPTs were performed on the volar forearm, and the wheal reaction was measured after 15 minutes. Generally, the test result was considered positive if the allergen caused a wheal reaction of at least 3 mm in diameter and the control solutions gave expected results.¹⁶ SPTs were repeated in the selected 40 patients with a previous positive reaction to *A. alternata* or *C. herbarum* using commercial extracts (ALK) and a specially prepared in-house *A. alternata* extract. For skin tests, the in-house SPT extract from chromatography-purified *A. alternata* strain LS14/2 was diluted at a concentration of 100 mg/mL in phosphate-buffered saline (pH 7.4). The skin test was also performed on 20 healthy persons who showed at least 2 positive SPT results to 11 common inhalant allergens (referred to as “atopic”) (14 females and 6 males; age, 16–57 years) and on 20 healthy persons with negative SPT results (referred to as “nonatopic”) (16 females and 4 males; age, 25–57 years).¹⁷ To investigate the association of SPT with allergen-specific IgE-antibodies in serum using *A. alternata* or *C. herbarum* as allergens, blood samples were obtained from the 40 selected patients (20 atopic and 20 nonatopic persons).

IgE antibodies to *A. alternata* and *C. herbarum* and total IgE concentrations were measured by the Pharmacia CAP FEIA (fluorescent enzyme immunoassay) method (Pharmacia AB, Uppsala, Sweden). For allergen-specific IgE, the positive cutoff limit was 0.35 kU/L. IgE antibodies were also measured by an enzyme-linked immunosorbent assay (ELISA) method,¹⁸ using both commercial extracts (ALK) and in-house test preparations (Finnish Institute of Occupational Health, Helsinki, Finland). Commercial and in-house preparations were diluted in 50 mmol/L carbonate buffer, pH 9.6, to a concentration of 15 µg/mL and applied to polystyrene microtiter plates. The optical density (OD) was read at 405 nm with an automated ELISA reader (Titertek Multiscan, Eflab, Turku, Finland). A positive cutoff for the test was the mean OD value plus 3 SDs of the control preparations.

Preparation of In-House Fungal Extracts

A. alternata, strain LS14/2, isolated from the wallpaper of a water-damaged library in Helsinki was inoculated (4×10^5 spores) to 100 mL of mycological peptone liquid medium (2% malt extract, 1% peptone, and 4% glucose). *C. herbarum* (strain HRA MI), isolated from water-damaged building ma-

Figure 1. Flow of participants through the trial. SPT indicates skin prick test; ELISA, enzyme-linked immunosorbent assay; FEIA, fluorescent enzyme immunoassay.

terial, was inoculated (9×10^5 spores) to 100 mL of mycological peptone liquid medium. The strains were grown for 3 weeks at 22° C.

The cultures of fungi were homogenized from 15 minutes on ice with a homogenizer (IKA Ultra-Turrax; Labortechnik, Staufen, Germany). They were centrifuged at 3600g for 30 minutes at 4° C (Minifuge RF; Heraeus, Hanau, Germany) after which the supernatants were preparatively (5×1.8 mL) chromatographed in a 3-mL, reversed-phase column (packed with source RPC15 material, Pharmacia) by using a linear acetonitrile gradient from 15% to 100% (containing 0.075% trifluoro acetic acid) for 15 minutes. The eluting protein fractions were pooled and concentrated 10-fold under vacuum. Protein concentrations of these concentrates were determined by the Lowry assay (Bio-Rad, Hercules, CA). Protein concentrations of commercial antigen extracts were also determined.

Conjunctival Challenge Test

Conjunctival challenge¹⁹ was performed in 7 patients with a positive SPT result (wheal at least 4 mm) to *A. alternata* or *C. herbarum*. All of these patients had rhinoconjunctivitis as their main symptom, and they volunteered for the challenge test. Two healthy persons with negative SPT results also participated in the challenge test.

Water-soluble extracts of *A. alternata* and *C. herbarum* (1/100 wt/vol, ALK) were used. The prechallenge status was examined by an ophthalmologist (O.K.) using a biomicroscope. The test was performed in a double-blinded fashion. The allergen was inoculated into one eye by a syringe connected to a needle used for lacrimal irrigation giving a constant small drop (8 μ L). The diluent was inoculated to the other eye. Immediately after the inoculation the patient closed the eyes, and the nasal canthus was compressed for 30 seconds with the fingertip. Both eyes were re-examined 5 minutes after the inoculation or when a clear reaction occurred.

The following 3 signs were recorded: redness of the limbal or tarsal conjunctiva, conjunctival chemosis, and lid swelling. The intensity of possible itch was also asked. Each of the 4 variables was estimated on a scale from 0 to 3. The reaction time for itching and conjunctival reaction was calculated as follows: less than 1 minute, score 3; less than 5 minutes, score 2; and more than 5 minutes but less than 6 hours, score 1. The ophthalmologist followed up the patient for an immediate reaction after which the patient was asked to report the symptoms. If no symptoms or signs could be registered within 6 hours, the result was negative. The allergen challenge test result was considered positive if the sum score was at least 5 or the difference between the 2 eyes was at least 5.

Statistical Analysis

The results of antibody measurements and SPTs were compared with each other, and their correlation was calculated with the χ^2 test and the Pearson test. Statistical analyses were performed using the SAS statistical software package (SAS Institute Inc, Cary, NC).

RESULTS

Fungal Allergen SPTs

The results of fungal allergen SPTs are given in Table 1. Altogether, 42 (2.8%) of 1,504 patients showed a positive SPT result (wheal ≥ 3 mm) to *A. alternata*, whereas 175 (2.7%) of 6,376 patients tested positive to *C. herbarum*. Positive SPT results were detected in 2.1% of the patients to *F. roseum*, 1.7% to *P. herbarum*, 1.6% to *M. racemosus*, and 0.9% to *A. fumigatus*.

In the 40 patients with a positive SPT result (wheal ≥ 4 mm) to *A. alternata* or *C. herbarum*, positive SPT results to dog were detected in 32 (80%), to cat in 32 (80%), to birch pollen in 31 (78%), and to timothy or meadow grass in 30 (75%; Table 2). Atopic eczema/dermatitis syndrome (AEDS) was diagnosed in 21 (58%), asthma in 16 (44%), rhinitis in 11

Table 1. Skin Prick Test Reactivity to Fungal Allergens among 1,504 Patients Examined between January 1, 1996, and May 31, 1997, at the Skin and Allergy Hospital, Helsinki University Central Hospital*

Allergen	Females, no. (%)		Males, no. (%)		Total, no. (%)	
	Positive result	All	Positive result	All	Positive result	All
<i>Alternaria alternata</i> †	25 (2.3)	1097	17 (4.2)	407	42 (2.8)	1504
<i>Aspergillus fumigatus</i> †	8 (0.7)	1114	6 (1.5)	415	14 (0.9)	1529
<i>Aureobasidium pullulans</i> †	18 (1.6)	1094	5 (1.2)	406	23 (1.5)	1500
<i>Acremonium kiliense</i> ‡	5 (1.2)	420	1 (0.7)	141	6 (1.1)	561
<i>Cladosporium herbarum</i> *	107 (2.6)	4096	68 (3.0)	2280	175 (2.7)	6376
<i>Fusarium roseum</i> †	25 (2.3)	1094	7 (1.7)	406	32 (2.1)	1500
<i>Mucor racemosus</i> †	18 (1.7)	1094	6 (1.5)	406	24 (1.6)	1500
<i>Penicillium notatum</i> †	9 (0.8)	1096	4 (1.0)	406	13 (0.9)	1499
<i>Phoma herbarum</i> †	20 (1.8)	1093	5 (1.2)	406	25 (1.7)	1499
<i>Rhizobus niger</i> †	12 (1.1)	1093	2 (0.5)	406	14 (0.9)	1499
<i>Rhodotorula rubra</i> †	7 (0.6)	1090	0	405	7 (0.5)	1495

* A total of 6,376 patients were tested to *Cladosporium herbarum*. Skin wheal diameter of 3 mm or more was regarded as a positive reaction.

† 10 HEP.

‡ 5 mg/mL.

§ 1/20 wt/vol.

Table 2. Positive Skin Prick Test Reactions (Wheal ≥ 3 mm) to Other Inhalant Allergens in 40 Patients Showing at Least a 4-mm Wheal Reaction to *Alternaria alternata*

Inhalant Allergen	No. (%)
Dog	32 (80)
Cat	32 (80)
Birch	31 (78)
Timothy or meadow grass	30 (75)
<i>Cladosporium herbarum</i>	21 (58)
Horse	16 (40)
Mugwort	10 (25)
<i>Dermatophagoides pteronyssinus</i>	8 (20)
Cow	6 (15)
Natural rubber latex	4 (10)

(31%), rhinoconjunctivitis in 7 (19%), and conjunctivitis in 5 (14%) of these patients. The results of SPT using commercial (ALK) *A. alternata* extract and an in-house *A. alternata* extract showed a good correlation ($r = 0.63$).

IgE Measurements

The mean of the total IgE value in the serum samples was 1632 kU/L (SD, 4204 kU/L) in the 40 patients, 196 kU/L (SD, 252 kU/L) in the atopic control subjects, and 61 kU/L (SD, 55 kU/L) in the nonatopic control subjects. The results of IgE ELISA showed a good correlation when in-house *A. alternata* ($r = 0.99$) and *C. herbarum* ($r = 0.92$) extracts were compared with the relevant commercial allergen extracts (Table 3). Good agreement was also observed when CAP FEIA results were compared with IgE ELISA results using commercial ($r = 0.91$ with *A. alternata* and $r = 0.88$ with *C. herbarum*) and in-house extracts ($r = 0.90$ and $r = 0.96$, respectively).

Figure 2 shows IgE binding of patient and control serum samples to antigens extracted from commercial *A. alternata* and *C. herbarum* and in-house *A. alternata* and *C. herbarum*. In ELISA, serum samples from 5 patients showed IgE binding (were positive) to both in-house *A. alternata* and commercial *A. alternata* extracts. Serum samples from 20 patients tested positive to both in-house *C. herbarum* and commercial

C. herbarum extracts in ELISA. In addition, serum samples from 4 patients tested slightly positive to in-house *C. herbarum* extract but not to commercial extract.

Correlation of SPTs and IgE Measurements

No correlation was observed between SPTs performed by commercial *A. alternata* and IgE ELISA performed by commercial ($r = 0.12$) or in-house *A. alternata* extracts ($r = 0.12$) (Table 3). The correlations were better when SPTs were performed by commercial *C. herbarum* antigen extract compared with IgE ELISA performed by commercial ($r = 0.47$) or in-house *C. herbarum* extracts ($r = 0.63$).

Conjunctival Challenge Test

The conjunctival challenge test result was positive in 4 of 7 patients with *A. alternata* and in 6 of 7 patients with *C. herbarum*, whereas the challenge result was negative in the 2 control subjects (Table 4).

DISCUSSION

The prevalence of IgE-mediated allergy to fungal allergens has varied in previous investigations from 1% to 2% in the adult population to 5% to 30% in adolescents and patients with allergic rhinitis and asthma.^{14,15} In the present study, consecutive patients referred to the university outpatient clinic were investigated to find IgE responses to common fungi appearing in both outdoor and indoor environments. *A. alternata* and *C. herbarum* were then used as allergens in antibody measurements to evaluate the possible differences in ELISA and CAP FEIA tests compared with skin test reactivity. Furthermore, the conjunctival challenge test was performed to confirm clinical diagnosis of fungal allergy among some patients with a positive SPT result to the relevant microbes and with symptoms.

In the present study, the prevalence of positive SPT results to fungal allergens among patients with suspected allergy was 2.8% with *A. alternata* and 2.7% with *C. herbarum*. The low prevalence of positive reactions to fungal allergens in skin tests most likely reflects the real situation of fungal sensitization in Finland, a country with a relatively cold climate.

Table 3. Correlations of Fungal Antigen Extracts of In-house and Commercial *Alternaria alternata* and *Cladosporium herbarum* in Skin Prick Testing (SPT), Enzyme-Linked Immunosorbent Assay (ELISA), and CAP FEIA (Fluorescent Enzyme Immunoassay) Tests in Serum Samples of 40 Patients*

Test	<i>r</i>	
	<i>A. alternata</i>	<i>C. herbarum</i>
SPT Commercial and in-house extracts	0.63	ND
SPT commercial and IgE ELISA (commercial extract as antigen)	0.12	0.47
SPT commercial and IgE ELISA (in-house extract as antigen)	0.12	0.63
IgE ELISA (commercial extract as antigen) and total IgE	0.19	0.36
IgE ELISA (in-house extract as antigen) and total IgE	0.12	0.13
CAP FEIA and IgE ELISA (commercial extract as antigen)	0.91	0.88
CAP FEIA and IgE ELISA (in-house extract as antigen)	0.90	0.96
IgE ELISA (in-house extract and commercial extract)	0.99	0.92

Abbreviation: ND, not done.

* CAP FEIA is manufactured by Pharmacia AB, Uppsala, Sweden.

Figure 2. IgE binding to antigen extracts of commercial (ALK, Horsholm, Denmark) and in-house (Finnish Institute of Occupational Health, Helsinki, Finland) *Alternaria alternata* and *Cladosporium herbarum* in enzyme-linked immunosorbent assay in patients and atopic and nonatopic control subjects. Five serum samples from patients show IgE binding to both *A. alternata* extracts. Twenty serum samples from patients show IgE binding to both *C. herbarum* extracts. Four serum samples show IgE binding to the in-house *C. herbarum* extract but not the commercial extract. One to 2 control serum samples from atopic patients show slight IgE binding to antigen extracts. The threshold value for positivity (mean optical density [OD] value plus 3 SDs of controls) is indicated by the line. OD was read at 405 nm.

Table 4. Results of Conjunctival Challenge Test (CC) Compared with Total IgE, Specific IgE Antibodies, and Skin Prick Test (SPT) Result

Patient No.	Total IgE, kU/L	IgE/ <i>Alternaria alternata</i> , kU/L	IgE/ <i>Cladosporium herbarum</i> , kU/L	SPT/ <i>Alternaria alternata</i> , mm	SPT/ <i>Cladosporium herbarum</i> , mm	CC/ <i>Alternaria alternata</i> score*	CC/ <i>Cladosporium herbarum</i> score*
1	106	6.2	2.3	5	5	10	7
2	481	8.8	18	5	4	9	8
3	360	5.2	16	6	6	8	12
4	21,670	26	43	4	6	5	11
5	3523	2.2	46	4	7	4	10
6	218	0.1	15	<3	6	2	7
7	2430	1.5	2.9	5	4	1	0

* A score of 5 or more was considered positive; see the Methods section.

The prevalence of a positive skin test result to *A. alternata* was 3.6% among 17,000 US citizens.²⁰ In a large multicenter study conducted in 35 medical centers in 16 countries in Europe (13,883 serum samples), the median prevalence of a positive IgE test result (>0.35 kU/L) to *C. herbarum* was 2.4% (minimum, 0.3%; maximum, 13.6%).²¹

In the study patients with conjunctival, dermal, and respiratory symptoms, sensitivity to 2 fungal allergens was confirmed in 42 (2.8%) of the patients with *A. alternata* and in 175 (2.7%) of the patients with *C. herbarum* by showing an immediate response in SPTs. The confirmation of clinical allergy due to these fungi would have needed provocation tests. In 40 of the patients, however, AEDS was present in 21, asthma in 16, rhinitis in 11, rhinoconjunctivitis in 7, and conjunctivitis in 5. In 7 of the 40 patients with conjunctival symptoms, 6 showed a positive provocation test result with either *A. alternata* or *C. herbarum*.

Patients with IgE-mediated allergy to molds often show positive SPT results and the presence of antibodies to other inhalant allergens.^{21–23} In the present study, most patients with a positive SPT result to *A. alternata* or *C. herbarum* also showed a positive SPT result to other inhalant allergens, such as animal dander, birch, and grass pollen. Simultaneous positive reactions to different allergens in allergy tests are due to a common disposition to produce IgE antibodies against ubiquitous inhalant allergens and sometimes cross-reactivity between the antigens. High total IgE levels (mean, 1,632 kU/L) detected in the present patients, 58% of whom had AEDS, may also have caused polyclonal nonspecific allergen binding. This often makes it difficult to interpret the findings and to determine the causative factors of symptoms in an atopic patient.

Most patients with fungal sensitization had respiratory, dermal, or conjunctival symptoms. Elevation of IgE antibodies to *Alternaria* species has previously been associated with allergic asthma and rhinitis.²⁴ On the other hand, IgE antibodies to *A. alternata* have been detected significantly more often among patients with AEDS than among patients with asthma or asymptomatic controls; of 73 patients with AEDS, 43 showed a positive IgE reaction to *A. alternata*.²² In another study, 10 (25%) of 40 children with AEDS and 4.3% of 1762 children without AEDS had specific IgE antibodies to *C. herbarum*.²³

The characterization of fungal allergens has been difficult.^{25–27} The allergenic extracts used in the clinical diagnosis of fungal allergy are unpredictable in their biological activity. Without the use of uniform fungal extracts for diagnostic testing, it is extremely difficult to relate symptoms of respiratory allergy with the exposure.^{28,29} Lack of proper test reagents has often led to overestimation of mold-induced allergies. The present study, however, shows that, at least with *A. alternata* and *C. herbarum*, commercial IgE antibody measurements work well with atopic patients. In antibody measurements with serum samples of 40 patients, commercial and in-house test reagents of *A. alternata* correlated well when the same allergen was used in CAP FEIA and ELISA

tests. Also when the 2 test methods (CAP FEIA and IgE ELISA) were compared using either commercial test reagents or in-house preparations as antigens ($r = 0.90$ to 0.96), the correlation was good.

On the other hand, the correlation between serum antibody measurements and SPTs with relevant allergens was not satisfactory, which causes clinical concern. The correlation with *A. alternata* was especially poor ($r = 0.12$). One possibility for this discrepancy may be that specific IgE is mainly bound to its receptors (Fc δ RI) on the surface of mast cells and basophils, and therefore only trace amounts of such antibodies are present as soluble form in the circulation. On the other hand, we cannot exclude the possibility that some components in uncharacterized SPT extracts might also induce histamine release that is not mediated by IgE antibodies.

The major allergen of *A. alternata* is a 29-kDa protein (Alt a-29).³⁰ Several researchers have purified similar antigens from *Alternaria* either as 29-kDa (Alt a Bd29K)³¹ or 31-kDa (Alt a I₁₅₆₃)³² proteins. Curran et al³³ described the purification and N-terminal sequence of a 29-kDa protein. Alt a I is the most completely characterized *Alternaria* allergen. Extracts of *Alternaria* have as many as 32 antigens and 19 different allergens identified by crossed radioimmuno-electrophoresis and immunoblotting.³⁴ The allergenic composition of the extracts from this organism is complex and difficult to characterize due to the limited amount of protein in the extracts and batch-to-batch variation of the allergens in the culture.^{25,35}

Cla h I and Cla h II allergens were partially purified by gel filtration, showing that the first is a group of allergens with a molecular mass of 13 kDa and a isoelectric point (pI) that ranges from 3.4 to 4.4,³⁶ and the second is a 20-kDa glycoprotein.³⁷ In a recent study, *C. herbarum* showed altogether 17 allergens, with molecular masses that ranged from 15 to 143 kDa using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, 2-dimensional gel electrophoresis, and immunoblot.²⁵

Achatz and coworkers²⁶ cloned and sequenced complementary DNAs (cDNAs) coding for allergens of both *A. alternata* and *C. herbarum*. Zhang et al³⁸ established a cDNA library from which Cla h III allergen was cloned and sequenced. Further analysis of Cla h III revealed that the allergen belongs to the family of ribosomal P2 proteins.³⁸ Zhang et al³⁹ also cloned another new allergen, which according to sequencing was determined as a C-terminal fragment of a protein belonging to the heat shock protein 70 family. Simon-Nobbe et al⁴⁰ showed that glycolytic enzyme enolase could be found in *C. herbarum* (Cla h 6 allergen) and in *A. alternata* (Alt a 5), which exhibit high cross-reactivity with each other and other enolases from other fungal species.

In the present study, the conjunctival challenge test with a relevant fungal allergen was performed in 7 of 40 patients with conjunctivitis and a positive SPT result to *A. alternata* or *C. herbarum*. The challenge test is occasionally needed to confirm the clinical diagnosis of a sensitized patient, especially if the symptoms are work related. Six patients of 7 had a positive test result, whereas the 2 healthy subjects did not show reactions.

Altogether, 6 (0.10%) of the 6,376 patients were confirmed to have exposure to *A. alternata* or *C. herbarum*. This may somewhat underestimate the true prevalence because not all the 40 patients were challenged. It seems, however, that the occurrence of clinically relevant fungal allergy in Finland is rare and does not exceed 0.5% to 1% in patients with symptoms that suggest allergy.

Although IgE-mediated allergy was rare among the present patients, the exposure to airborne molds may increase the incidence of respiratory symptoms and diseases with other mechanisms both in outdoor and indoor environments. This has been shown to occur in water-damaged buildings.^{41,42}

CONCLUSION

IgE-mediated sensitivity to common fungal allergens was rare in patients residing in Finland who presented with suspected allergic symptoms. Commercial allergen test reagents and in-house preparations detected IgE antibodies to *A. alternata* and *C. herbarum* in the patient serum samples. Conjunctival challenge with *A. alternata* and *C. herbarum* was successfully used in sensitized patients.

ACKNOWLEDGMENTS

This study was financially supported by the Finnish Work and Environment Fund, the Ministry of Social Affairs and Health, and Helsinki University Central Hospital (EVO research grant), Helsinki, Finland. Mrs. Leena Petman is kindly thanked for arranging clinical checkups of the patients.

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