

Association of sensitization to *Alternaria* allergens with asthma among school-age children

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Background: Molds in the *Alternaria* genus, normally found on outdoor vegetation, produce some of the most common fungal allergens to elicit a skin test response.

Objectives: The objectives of this study were to evaluate a serum assay for IgE antibodies to *Alternaria* allergens and to establish the prevalence of sensitization to *Alternaria* allergens among children and adults enrolled in epidemiologic studies of asthma. In addition, the significance of sensitization to *Alternaria* allergens as a risk factor for asthma was compared with that of sensitization to indoor allergens or pollens.

Methods: Using the Pharmacia Capsulated Hydrophobic Carrier Polymer (CAP) system, we have evaluated the significance of *Alternaria* allergens by using sera from several epidemiologic studies of asthma.

Results: Comparisons between serum assays and skin test results suggest that this in vitro assay yields results similar to those for traditional RASTs and is as sensitive as skin prick testing. In each of the groups studied, sensitization to *Alternaria* allergens was more common among asthmatic than control subjects, and in two studies the relationship was highly significant. *Alternaria* allergens were significantly associated with asthma in middle schools in Charlottesville, Virginia and Los Alamos, New Mexico but not in Albemarle County, Virginia. Logistic regression analysis of the results for the three schools identified an association between sensitization to *Alternaria* allergens and asthma independent of, but not as strong as, that found between sensitization to indoor allergens and asthma ($p < 0.001$).

Conclusions: The Pharmacia CAP system is a useful tool for measuring specific IgE to *Alternaria* allergens. Although not as important as sensitization to dominant local indoor allergens, sensitization to *Alternaria* allergens appears to be a significant independent risk factor for asthma in children in

some locations of the United States. (J Allergy Clin Immunol 1998;101:626-32.)

Key words: *Alternaria*, asthma, indoor allergens, Cladosporium, outdoor allergens, children, fungal allergens, atopic dermatitis, serum IgE assays

Molds in the *Alternaria* genus, normally found on outdoor vegetation, produce some of the most common fungal allergens to elicit a skin test response. Sensitization to fungi have been shown to be a risk factor for allergic disease.^{1,2} Found in temperate regions worldwide, these molds are most common inland, especially in grain growing areas. Their spores are significantly larger than spores of most other common fungi (e.g., *Penicillium*, *Cladosporium*, and *Aspergillus* species). Spore release is triggered by an increase in light and a decrease in relative humidity from near-saturation conditions, a change that most often occurs on summer and autumn mornings.³ Outdoor airborne *Alternaria* spore concentrations vary seasonally, with spore levels rising in the summer months and peaking in autumn because of increased growth of these fungi on rotting leaves and other biomaterial.⁴ This is similar to the reports of other outdoor fungal allergens such as *Aspergillus* organisms.⁵ Although most often thought of as an outdoor allergen, the spores can come indoors.⁶ Evaporative coolers, used in hot climates with low relative humidity, have been shown to provide a good environment for growth of *Alternaria* organisms and other fungi. These coolers can increase indoor spore counts by two- to fivefold relative to outdoor spore counts.³ Damp housing conditions and the presence of indoor molds have both been linked to asthma.^{7,8} In addition, sensitization to *Alternaria* allergens has been reported to be significantly associated with asthma in the Eastern and Midwestern United States,^{9,10} a dry inland region of Southern Australia,¹¹ and, most recently, in Arizona.¹² In most cases sensitization has been reported in patients with a high potential for being sensitized to other inhaled allergens.¹³ Exposure to this airborne allergen has also been reported to be a risk factor for respiratory arrest in children and young adults.¹⁰ Molecular research that could lead to the development of a useful method for assessing exposure to *Alternaria* allergens in homes has included the characterization and cloning of major *Al-*

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Supported by grants AI-30840, AI-20565, RR-00847, and 1U01-AI-34607 from the National Institutes of Health and a grant from the Center for Indoor Air Research.

Received for publication July 22, 1997; accepted for publication Jan. 26, 1998.

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J Allergy Clin Immunol 1998;101:626-32.

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0091-6749/98 \$5.00 + 0 1/1/89257

Abbreviations used

AD: Atopic dermatitis
BHR: Bronchial hyperreactivity

ternaria allergens combined with the development of monoclonal assays.¹⁴⁻¹⁶ In these experiments sensitization to *Alternaria* allergens was assessed by using the ImmunoCAP system (Pharmacia Upjohn, Uppsala, Sweden). With this system, serum antibodies bind to antigens covalently linked to a cellulose matrix. This assay has been reported to have greater specificity than that of modified RASTs.¹⁷

We have taken advantage of the availability of sera from schoolchildren to assess the relationship between sensitization to *Alternaria* allergens and symptoms with or without bronchial hyperreactivity (BHR) in a population-based study of two different areas of the country. The objectives of this study were to evaluate the specificity and sensitivity of a serum assay for IgE antibodies to *Alternaria* allergens, to establish the prevalence of sensitization to *Alternaria* allergens among children and adults enrolled in epidemiologic studies on asthma, and to compare the risk for asthma related to sensitization to *Alternaria* allergens with that for indoor allergens and pollens.

METHODS

Study patients

Populations of children and adults in which data was already available on indoor allergen exposure and sensitization were selected. We wished to evaluate the relevance of *Alternaria* allergens in different clinical and socioeconomic groups. The serum used for this study had been obtained as part of several published studies.¹⁸⁻²³ Serum assay results were used in conjunction with previously obtained data, including symptoms, demographic information, sensitization to other allergens (assessed by lancet skin tests and RASTs), BHR to histamine, and total IgE levels. The following seven patient groups were included in the evaluation: middle-school children (age range, 11 to 13 years) from Los Alamos, New Mexico involved in a study of the relevance of allergen exposure to asthma symptoms in a dry climate (group A)¹⁸; middle-school children from Charlottesville, Virginia in whom indoor allergen exposure was investigated as a risk factor for asthma (group B)²³; middle-school children from Albemarle, the county that surrounds Charlottesville, who were also studied for allergen exposure as a risk factor for asthma (group C)²³; asthmatic and control children (age range, 3 to 15 years) from a study in Atlanta, Georgia first seen at Grady Memorial Hospital pediatric emergency department (group D)²¹; children from a second study in Atlanta, including inner-city asthmatic children (age range, 5 to 16 years) enrolled in an allergen-avoidance study (group E)²⁰; asthmatic and control adult patients seen in an emergency department in Wilmington, Delaware (group F)²²; and, for the inhibition study, a group of seven patients with atopic dermatitis (AD) with IgE antibodies to *Alternaria* allergens who were selected from patients first seen at the University of Virginia Allergy Clinic (group G).

The children from groups A, B, and C were divided into three categories. Those with asthma symptoms (determined by ques-

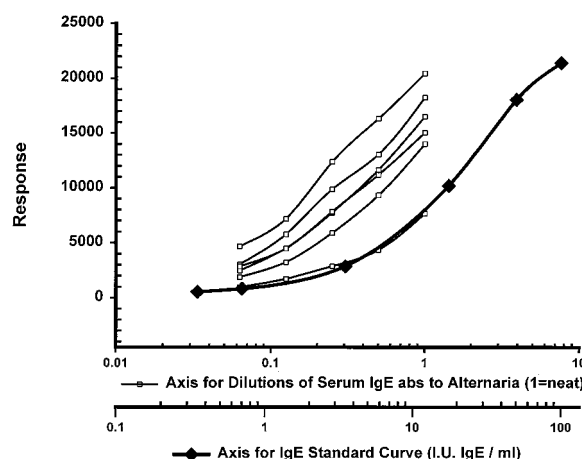


FIG. 1. Graph of serial dilutions of sera with positive results to *Alternaria* allergens measured for specific IgE to *Alternaria* allergens and IgE curve used to quantitate results. Abs, Antibodies.

tionnaire) and BHR to histamine, those with asthma symptoms but no BHR to histamine, and control subjects with no reported asthma symptoms. For groups D, E, and F, asthma was determined by physician diagnosis.

Alternaria allergen serum assay

For this study, sensitization to *Alternaria alternata* was assessed by the CAP assay for IgE antibodies. With this assay, antigens are prebound to a cellulose sponge-like matrix. Serum samples are incubated with the CAP assay for 30 minutes, a conjugate enzyme anti-IgE binds to the IgE bound to the CAP, and the detecting antibody reacts with a substrate to form a fluorescent product. Measurement of fluorescence is used to quantitate specific IgE antibodies by reference to a control curve run in parallel with each experiment.

Results from the CAP system are reported in international units of IgE per milliliter of serum. Patients can then be classified on a scale from 1 to 6 on the basis of the concentration of specific IgE. A class 2 or greater response corresponds to 0.7 IU or more of IgE/ml (i.e., ≥ 1.7 ng IgE/ml). For the statistical analyses, patients with a CAP score of 2+ or greater were considered to be sensitized to *Alternaria* allergens. The 2+ cut-off point was chosen because the quantity of IgE antibody is similar to that used as a cut-off point in the RAST assay. For its standard curve, this assay system uses dilutions of IgE and solid-phase bound anti-IgE. Several experiments were done to validate the *Alternaria* allergen serum assay. Serum samples from six asthmatic patients previously shown to have high titers of specific IgE antibodies to *Alternaria* allergens were serially diluted. Dilution curves of the IgE antibodies to *Alternaria* allergens were compared with dilution curves of the IgE standard (Fig. 1).

The specificity of the assay was further evaluated by inhibition assays with fungal and other allergen extracts. Sera from seven patients with asthma and seven patients with AD containing IgE antibodies to *Alternaria* allergens were diluted to identify a concentration of serum that gave approximately 60% of the maximum binding for that sample. Inhibition was carried out by preincubating 25 μ l of serum with 25 μ l of serial dilutions of the allergen extract (from 1:2 to 1:20,000). After a 3-hour incubation at room temperature with the allergens, the

TABLE I. Inhibition assay of IgE antibodies to *Alternaria* allergens with fungal and dust mite extracts: Sera from patients with asthma*

Allergen	Patient nos.						
	1	2	3	4	5	6	7
<i>Alternaria</i>	0.050 μ l†	0.015 μ l	0.015 μ l	0.025 μ l	0.015 μ l	0.075 μ l	0.13 μ l
<i>Cladosporium</i>	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l
<i>Aspergillus</i>	>25 μ l	>25 μ l	>25 μ l	15 μ l	>25 μ l	>25 μ l	>25 μ l
<i>Candida</i>	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l
<i>Trichophyton</i>	>25 μ l	>25 μ l	>25 μ l	24 μ l	>25 μ l	>25 μ l	>25 μ l
<i>D. pteronyssinus</i>	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l	>25 μ l

By using the dilution of sera giving 60% of maximum binding, serial dilutions of extracts were incubated with sera for 3 hours before remaining IgE antibodies to *Alternaria* allergens were assayed.

*Seven sera from school-age children with high levels of IgE antibodies to *Alternaria* allergens.

†Values are the volume of extract that gives 50% inhibition.

TABLE II. Inhibition assay of IgE antibodies to *Alternaria* allergens with fungal and dust mite extracts: Sera from patients with AD*

Allergen	Patient nos.						
	1	2	3	4	5	6	7
<i>Alternaria</i>	0.050 μ l	0.075 μ l	0.050 μ l	0.075 μ l	0.33 μ l	0.50 μ l	2.0 μ l
<i>Cladosporium</i>	>25 μ l	0.02 μ l	>25 μ l	0.015 μ l	2.9 μ l	>25 μ l	>25 μ l
<i>Aspergillus</i>	>25 μ l	1.5 μ l	>25 μ l	0.045 μ l	0.05 μ l	>25 μ l	>25 μ l
<i>Candida</i>	>25 μ l	0.45 μ l	>25 μ l	0.0075 μ l	7.5 μ l	>25 μ l	>25 μ l
<i>Trichophyton</i>	>25 μ l	0.75 μ l	>25 μ l	0.013 μ l	7.5 μ l	>25 μ l	>25 μ l
<i>D. pteronyssinus</i>	>25 μ l	0.025 μ l	>25 μ l	>25 μ l	2.5 μ l	>25 μ l	>25 μ l

*Seven sera from patients with AD first seen at the University of Virginia Allergy Clinic.

serum was tested for unbound IgE antibodies.²⁴ The following allergens were used: *Alternaria tenuis* (Bayer 1:10 wt/vol, #5009GF), *Hormodendrum cladosporioides* (*Cladosporium* Bayer 1:10 wt/vol, #5129GF), *Aspergillus fumigatus* (partially purified extract containing 6 mg/ml Asp f 1; from K. Arruda, University of Virginia), *Trichophyton tonsurans* (purified extract, 3 mg/ml protein; from J. Woodfolk, University of Virginia), *Candida albicans* (Hollister-Steir, lot#244336), and *Dermatophagoides pteronyssinus* (Miles 1:10 wt/vol, #6692UL, glycerin removed by dialysis). The concentration of allergen that reduced the measured IgE concentrations by 50% of the uninhibited response was recorded (Tables I and II).

The correlation between the serum assay and lancet skin test results with the Bayer *Alternaria tenuis* skin prick extract was assessed in 83 children with asthma from Atlanta (group E). Overall, there was a good correlation between the two methods. CAP scores of 2+ or greater and skin tests of a 4 $\times$ 4 mm or larger wheal were considered positive. Of the 83 patients, 13 had positive results with both skin tests and CAP, four had positive results with skin tests only, and six had positive results with the CAP assay only. The remaining 60 had negative results with both tests.

Comparison of *Alternaria* species RAST data with *Alternaria* species CAP results for 215 adult asthmatic and control patients from Wilmington, Delaware showed an overall correlation of $r = 0.64$ ($p < 0.001$) between the same serum samples (group F). For RAST results greater than 100 U/ml, 15 of 17 patients had CAP scores of 2+ or greater. For RAST values between 40 and 100 U/ml, 17 of 28 serum samples had CAP scores of 2+ or greater. For serum samples with RAST values less than 40 U/ml, only 13 of 170 patients had CAP scores of 2 or greater.

Cladosporium allergens

To examine the uniqueness of sensitization to *Alternaria* allergens, sensitization to another type of fungal allergen, *Cladosporium* species, was also examined. To verify the specificity of the *Cladosporium* allergens, CAP inhibition assays were done with five asthmatic subjects and four patients with AD with the same *Alternaria* and *Cladosporium* allergen extracts that were used in the *Alternaria* allergen inhibition experiments. The assay demonstrated good specificity in that only one serum was inhibited 50% by the *Alternaria* species extract. By using the *Cladosporium* species extract to inhibit IgE binding to the *Cladosporium* species solid phase, 50% inhibition was achieved in only six of the 11 sera tested. Serum from middle-school children in Los Alamos, Charlottesville, and Albemarle (groups A, B, and C) were assayed by the CAP system for specific IgE to *Cladosporium* allergens.

Statistics

Statistical analysis on the data from the middle-school children from Los Alamos, Charlottesville, and Albemarle was carried out in two phases. The data were first analyzed as two sets of nominal groups by Cramer's V test. The correlation between the dependant variable (patient classification) and the independent variable (sensitization to dust mite, cat, dog, cockroach, ragweed, rye grass, Russian Thistle, and *Alternaria* allergens) was examined. Total IgE above 40 IU/ml was also considered as an independent variable.

To further examine the significance of sensitization to *Alternaria* allergens as a risk factor for asthma relative to indoor allergens, the results from the middle-school studies were next analyzed by logistic regression. Data from the three schools

TABLE III. Prevalence of IgE antibodies to *Alternaria* allergens in sera from middle-school children: Analysis by asthma status and titer of IgE antibody (CAP class)*

	Symptomatic patients†									<i>p</i> Value‡
	BHR ⁺			BHR [−]			Control patients			
	≥1+	≥2+	≥3+	≥1+	≥2+	≥3+	≥1+	≥2+	≥3+	
Los Alamos	61%	56%	39%	20%	15%	13%	21%	17%	10%	0.037
Charlottesville	44%	(<i>n</i> = 18)	24%	13%	(<i>n</i> = 40)	4%	6%	(<i>n</i> = 48)	2%	<0.002
		(<i>n</i> = 25)			(<i>n</i> = 23)			(<i>n</i> = 64)		
Albemarle	19%	19%	13%	20%	20%	20%	18%	9%	3%	0.15
Combined	42%	(<i>n</i> = 16)	25%	18%	(<i>n</i> = 10)	11%	14%	(<i>n</i> = 34)	5%	<0.001§
		(<i>n</i> = 59)			(<i>n</i> = 73)			(<i>n</i> = 146)		

*Sensitization was judged by *Alternaria* CAP score of ≥1+, ≥2+, or ≥3+ equivalent to 0.84, 1.7, and 8.4 ng of IgE antibody/ml, respectively.

†Classification of disease status on the basis of a respiratory questionnaire and provocation with histamine (BHR⁺).

‡Probability (p) values obtained by chi square test for trend for ≥2+ anti-*Alternaria* IgE score.

§With the combined middle-school results, chi square test for trend for ≥1+ was $p = <0.001$ and for ≥3+ was $p = <0.001$.

were combined. Sensitization to any indoor allergen (dust mite, cat, dog, or cockroach allergens), sensitization to any outdoor allergen (ragweed, rye grass, Russian Thistle, or mountain cedar allergens), and sensitization to *Alternaria* allergens and total IgE above 40 IU/ml were all considered as possible variables. Patients sensitized to indoor allergens were combined in the analysis because different allergens dominate different areas of the country. Patients sensitized to outdoor allergens were also combined. Two sets of dependant variables were assessed in separate analyses, symptomatic versus nonsymptomatic subjects (as judged by questionnaire) and asthmatic versus nonasthmatic subjects (children were considered to have asthma if they had both symptoms and BHR to histamine). For both tests, correlation coefficients and p values are given without adjustment for multiple comparisons. Therefore p values for analysis of the individual schools (Tables III and IV) may be overestimated.

RESULTS

The CAP assay was found to be a useful serum assay for measuring IgE antibodies to *Alternaria* allergens. Dilution curves of serum with positive results to *Alternaria* allergens matched well with the IgE curve used to quantitate results (Fig. 1). The inhibition experiments with asthmatic patients demonstrated that the assay for *Alternaria* allergens had good specificity for *Alternaria alternata* (Table I). The *Alternaria* allergen extract inhibited all of the sera by 50% at volumes of allergen 100 times less than the maximum volume used for other allergens. Only one serum demonstrated 50% inhibition with any of the other allergens. The IgE binding was inhibited in patient 4 with both *Aspergillus* and *Trichophyton* allergens; however, 50% inhibition with these allergens required 500 times or more the volume of *Alternaria* extract giving 50% inhibition. For the patients with AD, the inhibition results were not as definitive (Table II). In four of seven of the patients with AD, only *Alternaria* allergens inhibited the assay. For the remaining three sera, inhibition was seen with several of the

allergens tested. Inhibition volumes for *Alternaria* allergens were not consistently less than those for other allergens.

Comparison between the prevalence of sensitization to *Alternaria* and *Cladosporium* allergens revealed the former to be more common in the children from each of the schools studied (i.e., Los Alamos, Charlottesville, and Albemarle County). The measured quantity of IgE antibody to *Alternaria* allergens was almost twice as high as that for *Cladosporium* allergens. There was, however, a high degree of correlation between the two allergens (paired t test correlation = 0.698, $p < 0.001$). In general, the patients who were sensitized to *Cladosporium* allergens were also sensitized to *Alternaria* allergens, and they had a higher concentration of IgE antibodies to *Alternaria* than to *Cladosporium* allergens. Of the 249 middle-school children (groups A, B, and C) studied, 20% were sensitized to *Alternaria* allergens, and 8% were sensitized to *Cladosporium* allergens. Only three of the 21 children who had IgE antibodies to *Cladosporium* allergens did not also have IgE antibodies to *Alternaria* allergens. None of these three patients had asthma. Therefore the addition of the *Cladosporium* allergen serum assay to the allergen test panel for these patients would not contribute to the statistical analysis of the relevance of inhalant fungi in this population.

Sera from all of the patient groups were assayed for IgE antibodies to *Alternaria* allergens. The results from the middle-school children are listed in Table III. Sensitization levels with CAP scores of 1+ or more, 2+ or more, and 3+ or more are displayed. A CAP score of 2+ or more was considered to be the cut-off point for sensitization to *Alternaria* allergens. For each of these locations, *Alternaria* allergens seemed to be associated with different groups. In Los Alamos, sensitization was largely associated with the asthma group. In Charlottesville, the asthma group had the highest degree of sensitization, followed by the symptomatic group. The

TABLE IV. Correlation between sensitization and patient classification (symptomatic BHR⁺*, symptomatic BHR⁻, and control subjects) among middle-school children (age range, 11 to 13 years)

Specific sensitization	Charlottesville (n = 111)	Albemarle (n = 59)	Los Alamos (n = 101)
Dust mite†	0.427 (<0.001)†	0.428 (<0.01)	0.174 (NS)
Cat	0.091 (NS)	0.390 (0.01)	0.338 (<0.01)
Dog	0.161 (NS)	—	0.377 (0.001)
Cockroach	0.279 (0.012)	0.00 (NS)	0.109 (NS)
Ragweed	0.041 (NS)	0.230 (NS)	0.218 (0.085)
Rye grass	0.139 (NS)	0.177 (NS)	0.071 (NS)
Russian Thistle	—	—	0.239 (0.05)
Mountain cedar	0.094 (NS)	—	0.171 (NS)
<i>Alternaria</i> allergens	0.303 (<0.01)	0.152 (NS)	0.355 (0.001)
Total IgE (>40 I.U./ml)§	0.462 (<0.001)	0.382 (0.012)	0.276 (0.018)
Total IgE (>200 I.U./ml)	0.349 (0.001)	0.274 (0.101)	0.322 (0.004)

NS, Not significant.

*BHR to histamine.

†Sensitization to allergens judged by a positive skin test response (4 × 4 mm wheal by lancet prick), positive RAST result (>20 RAST units), or positive Pharmacia CAP result (>1.7 ng IgE/ml).

‡Statistical correlation by Cramer's V nominal-by-nominal analysis. Probability (*p*) values are in parentheses. Statistically significant correlations are boldface.

§Total IgE measured by Pharmacia CAP (International Units IgE per milliliter of serum sample).

control subjects had the least amount of sensitization. In Albemarle, those who had reported symptoms, which include both the asthma and symptomatic groups, had the highest levels of sensitization. Chi square test for trend indicated a significant association between sensitization to *Alternaria* allergens and asthma classification in Los Alamos and Charlottesville but not in Albemarle.

In the serum samples from the children seen at the emergency department in Atlanta (group D), 13 of 33 asthmatic subjects and four of 33 control subjects had a CAP score of 2+ or more to *Alternaria* allergens; however, this did not reach statistical significance (chi square, *p* = 0.09). In contrast to some of the pediatric groups, *Alternaria* allergens were not a significant risk factor for asthma in adult patients from the emergency-department study in Wilmington, Delaware (IgE to *Alternaria* allergens: asthmatic subjects, 26 of 102; control subjects, 20 of 113). Asthma, presence of a smoker in the home, insurance, race, or urban residence had no significant association with sensitization to *Alternaria* allergens in these adult patients.

The significance of sensitization to *Alternaria* allergens was further investigated among the children in the three middle schools in Los Alamos, Charlottesville, and Albemarle. Skin test and RAST data were included to assess sensitization to other common outdoor and indoor allergens. The Cramer's V test, a nominal-by-nominal statistical analysis, was used to evaluate sensitization to the various allergens as a risk factor for classification into the asthmatic, symptomatic, or control groups (Table IV). In Charlottesville, an urban environment in central Virginia, dust-mite sensitization was the strongest risk factor for asthma. *Alternaria* allergens were the second strongest sensitization risk factor. In Albemarle, the rural county that surrounds Charlottesville, sensitization to dust mite and cat allergen were

found to be the greatest sensitization risk factors for asthma. *Alternaria* allergens were not significantly associated with asthma in this population. In Los Alamos, a city that does not have dust mites because of its elevation (7200 feet) and climate, sensitization to dog allergen was the strongest risk factor for asthma. For all three locations, total IgE level greater than 40 IU/ml was a statistically significant risk factor for asthma as determined by this univariate analysis.

Logistic regression analysis of the results from the three middle schools indicated that sensitization to indoor allergens was the strongest predictor of both asthma symptoms and BHR (Table V). Sensitization to *Alternaria* allergens was an independent risk factor for symptomatic BHR but not for asthma symptoms without BHR. Sensitization to outdoor allergens was not a significant predictor of symptoms. For asthma, however, the analysis indicated a significant negative correlation with outdoor allergens. This analysis also indicated that total IgE greater than 40 IU/ml was an independent risk factor for symptoms but not for asthma (Table V).

DISCUSSION

Evaluating the role of different allergens in asthma or other allergic diseases requires a reliable method for evaluating sensitization. In epidemiologic studies it is possible to carry out skin prick tests or serum assays, but intradermal tests are generally not possible. This study evaluates the use of the CAP system for assessing sensitization to *Alternaria* allergens. The results show that the serum assay was not more sensitive than the skin prick tests. The inhibition experiments suggest that the IgE antibodies detected in the sera of patients with asthma are specific for *Alternaria* allergens. By contrast, sera from patients with AD showed that the binding of IgE antibodies to the solid phase could be inhibited by

several other extracts. Although this experiment did not involve a large number of fungal antigens, the results for *Cladosporium* allergens were less common, and titers were generally lower than those for *Alternaria* allergens. Taken together, the preliminary experiments suggest that the in vitro assay of IgE antibodies to *Alternaria* allergens is a good method for evaluating sensitization to a fungus that is generally considered the dominant inhalant fungus.

Using sera from epidemiologic studies that have been published elsewhere, we were able to evaluate the relevance of sensitization to *Alternaria* allergens to asthma. The results for the middle-school studies are striking because there was a significant association with symptomatic subjects who had BHR to histamine both in Los Alamos and Charlottesville (i.e., two areas with dramatically different climates). By contrast, there was not a significant association in Albemarle County or among children first seen at the emergency department in Atlanta. The results for sensitization to *Alternaria* allergens in the adult emergency-department study in Wilmington, Delaware have been reported previously.²² By using the current assay system, the prevalence of positive results was higher than that determined with RASTs, but there was still no significant association with asthma.

Skin tests are routinely used in evaluating patients with asthma to identify relevant allergens. It is clear, however, that a large number of asymptomatic individuals also have positive skin test results. Thus it is necessary to compare sensitization among symptomatic individuals with sensitization among control subjects. Atopic individuals tend to become allergic to multiple allergens. This multicollinearity in sensitization makes interpretation of the prevalence of sensitization difficult. In current studies a typical example is seen with the logistic regression analysis of sensitization to domestic animals. In Los Alamos, sensitization to dog allergens was more significant than that to cat allergens, but there was a close correlation between the two. Thus sensitization to cat allergens was strongly associated with asthma but was not judged to be an independent factor by logistic analysis. Attempting to answer whether sensitization to *Alternaria* allergens is an independent risk factor for asthma may depend on which other allergens are included in the analysis. We have chosen to compare *Alternaria* allergens to indoor allergens as a group. The results for the middle-school children show that sensitization to *Alternaria* allergens is strongly associated with asthma by univariate analysis ($p < 0.001$, Table IV). With multivariate analysis, sensitization to this fungus contributed to the association seen with indoor allergen sensitization, but overall the strength of the association was not as strong as that for indoor allergens (Table V). It is clear that further studies are needed to understand the results in different age groups, geographic areas, and socioeconomic classes. In our view the priority is to be able to measure *Alternaria* antigens in the dust and air of homes.

TABLE V. Logistic regression analysis of sensitization and asthma among middle-school children*

	Symptomatic versus control subjects	Asthmatic versus Nonasthmatic subjects
Indoor allergens†	0.19 (0.001)§	0.37 (<0.001)
Outdoor allergens‡	NS	-0.17 (<0.01)
<i>Alternaria</i> allergens	NS	0.19 (<0.001)
Total IgE (>40 IU/ml)	0.11 (0.010)	NS

NS, Not significant.

*Logistic analysis of sensitization and asthma among middle-school children from Los Alamos ($n = 101$), Charlottesville ($n = 59$), and Albemarle ($n = 111$).

†Sensitization to any indoor allergen tested (dust mite, cat, dog, or cockroach).

‡Sensitization to any outdoor allergen tested (ragweed, rye grass, Russian Thistle, or mountain cedar).

§Probability (p) values are in parentheses. Significant positive correlations are boldface.

Previous results have consistently shown an association between sensitization to indoor allergens and asthma. In addition, there are now results from New Zealand, Australia, the United Kingdom, and the United States showing that sensitization to outdoor allergens does not contribute to the association.²⁵⁻²⁷ Indeed, our own data shows a negative association between sensitization to pollens and asthma in the multiple-regression analysis (Table V). We have assumed that the negative association reflects the fact that exposure to allergens outdoors is not a significant cause of BHR. The data in relation to *Alternaria* allergens could therefore indicate either that a season that is as prolonged as that for *Alternaria* allergens can contribute to asthma or that *Alternaria* allergens come indoors sufficiently to behave as an indoor perennial allergen. Clearly this question is difficult to resolve without measuring *Alternaria* allergens indoors. Despite progress in understanding the immunochemistry of *Alternaria* allergens and cloning allergens, a simple sensitive assay for a representative protein is not generally available.^{1, 14-16} Similarly, it is currently difficult to make sense of the differences reported between the groups studied. Thus these results do not provide a simple explanation for the difference between the data in Albemarle County and the city of Charlottesville.

Development of an allergen-assay assessment would allow for detailed examination of homes and could help resolve the question of indoor sensitization. Analyzing the role of *Alternaria* allergens could be of major theoretic importance. At present, the practical significance may be limited because there is no convincing evidence for proposing either immunotherapy or avoidance measures for *Alternaria* allergens. However, this could change rapidly with the availability of recombinant allergens or full understanding of the exposure to these allergens. The important conclusions are that sensitiza-

tion to *Alternaria* allergens appears to be significantly associated with asthma, and in vitro assays of IgE antibodies can provide a consistent method for assessing sensitization.

We thank the staff at the emergency departments at the Medical Center of Delaware and the Grady Memorial Hospital; the school boards in Los Alamos, Albemarle County, and the city of Charlottesville; Pharmacia for supplying CAP reagents; and Bayer Pharmaceuticals for supplying skin test reagents for the school studies.

REFERENCES

- Verhoeff AP, Burge HA. Health risk assessment of fungi in home environments. *Ann Allergy Asthma Immunol* 1997;78:544-56.
- Wickman M, Gravesen S, Nordvall SL, Pershagen G, Sundell J. Indoor viable dust-bound microfungi in relation to residual characteristics, living habits, and symptoms in atopic and control children. *J Allergy Clin Immunol* 1992;89:752-9.
- Al-Doory Y, Domson JF. Mould allergy. Philadelphia: Lea & Febiger; 1984. p. 244-66.
- Platts-Mills TAE, Solomon WR. Aerobiology and inhalant allergens in allergy in principle and practice. Fourth Ed. Philadelphia: Mosby; 1993. p. 498-513.
- Sporik RB, Arruda LK, Woodfolk J, Chapman MD, Platts-Mills TA. Environmental exposure to *Aspergillus fumigatus* allergen (Asp f 1). *Clin Exp Allergy* 1993;23:326-31.
- Solomon WR. Assessing fungus prevalence in domestic interiors. *J Allergy Clin Immunol* 1975;56:235-47.
- Dales RE, Zwanenburg H, Burnett R, Franklin CA. Respiratory health effects of home dampness and molds among Canadian children. *Am J Epidemiol* 1991;134:196-203.
- Strachan DP. Damp housing and childhood asthma: validation of reporting of symptoms. *BMJ* 1988;297:1223-6.
- Henderson FW, Henry MM, Ivins SS, Morris R, Neebe EC, Leu S, et al. Correlates of recurrent wheezing in school-age children. *Am J Respir Crit Care Med* 1995;151:1786-93.
- O'Hollaren MT, Yunginger JW, Offord KP, Somer MJ, O'Connell EJ, Ballerd DJ, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991;324:359-63.
- Peat JK, Woolcock AJ. Sensitization to common allergens: relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas of Australia. *Clin Exp Allergy* 1991;21:573-81.
- Halonen M, Stern DA, Wright AL, Taussig LM, Martinez FD. *Alternaria* as a major allergen in children raised in a desert environment. *Am J Respir Crit Care Med* 1997;155:1356-61.
- Iversen M, Dahl R. Characteristics of mold allergy. *J Investig Allergol Clin Immunol* 1995;5:205-8.
- Portnoy J, Pacheco F. A double monoclonal antibody assay for the *Alternaria* allergen GP70. *Ann Allergy* 1993;71:401-7.
- DeVouge MW, Thaker AJ, Curren IH, Zhang L, Muradia G, Rode H, et al. Isolation and expression of a cDNA clone encoding an *Alternaria alternata* Alt a 1 subunit. *Int Arch Allergy Immunol* 1996;111:385-95.
- Bush RK, Sanchez H. *In vitro* synthesis of *Alternaria* allergens and their recognition by murine monoclonal and human IgE antibodies. *Ann Allergy Asthma Immunol* 1997;78:287-92.
- Williams PB, Dolen WK, Koepke JW, Selner JC. Comparison of skin testing and three in vitro assays for specific IgE in the clinical evaluation of immediate hypersensitivity. *Ann Allergy* 1992;68:35-45.
- Sporik R, Ingram JM, Price W, Sussman JH, Honsinger RW, Platts-Mills TAE. Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude. *Am J Respir Crit Care Med* 1995;151:1388-92.
- Ingram JM, Sporik R, Rose G, Honsinger R, Chapman MD, Platts-Mills TAE. Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relation to sensitization and asthma among children living in Los Alamos, New Mexico. *J Allergy Clin Immunol* 1995;96:449-56.
- Carter M, Perzanowski M, Raymond A, Platts-Mills TAE. Sensitization and exposure of inner city children enrolled in a controlled trial of allergen avoidance for asthma [abstract]. *J Allergy Clin Immunol* 1997;99:S86.
- Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TAE. Risk factors for asthma in inner city children. *J Pediatr* 1992;121:862-6.
- Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TAE. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 1993;147:573-8.
- Squillace SP, Sporik RB, Rakes G, Couture N, Lawrence A, Merriam S, et al. Sensitization to dust as a dominant risk factor for asthma among adolescents living in central Virginia. *Am J Respir Crit Care Med* 1997;156:1760-4.
- Gleich GJ, Larson JB, Jones RT, Baer H. Measurement of the potency of allergy extracts by their inhibitory capacities in the radioallergosorbent test. *J Allergy Clin Immunol* 1974;53:158-69.
- Sears MR, Herson GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risk of sensitivity to grass pollen, house dust mite, and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989;19:419-24.
- Peat JK, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmic A, et al. House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 1996;153:141-6.
- Meldrum JR, O'Rourke MK, Pleskett PR, Stetzenbach LD. *Proc Indoor Air* 1993;4:189.