Allergenic materials in the house dust of allergy clinic patients

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Introduction: Environmental agents including animal, fungal, tree, and weed antigens are known to cause allergic rhinitis and asthma. The following study was performed to measure the antigen concentration of several of these in house dust of children seen in an allergy clinic. Comparisons are made between household allergen levels of children seen for asthma and children seen for other reasons.

Methods: Dust samples were solicited from patients in a pediatric allergy specialty clinic and other individuals associated with the clinic. Persons submitting dust were asked to complete a questionnaire describing their house. Samples were extracted, centrifuged, and filtered for sterility. Samples were stored in 50% glycerol at -20° C. Specific antigens for *Alternaria*, *Cladosporium*, *Aspergillus*, *Candida*, *Dermatophagoides farinae*, cat, dog, oak, fescue, ragweed, plantain, and cockroach were measured using inhibition assays developed with whole antigen extract. Allergens Der p1, Der f 1, Alt a 1, and Alt a 70 kD were measured using double monoclonal antibody assays.

Results: Significant concentrations of whole antigen from cat, dog, oak, *Alternaria*, and *Cladosporium* were detected. Between 0.1 and 18 µg of Der f1 and Der p1 per gram of dust were also measured. Alt a 1 and Alt a 70 kD levels varied between 3.0 and 1000 U/g of dust. Significant positive correlations were observed in levels of dust mite and *Alternaria* allergen for patients with an evaluation of asthma.

Conclusions: We found measurable levels of fungal antigens (*Alternaria*, *Cladosporium*), mite antigens, and animal antigens (dog and cat) in the majority of dust samples in this self-selected set of allergy clinic patients. Specific allergens Alt a 1, Alt a 70kD, and Der p 1 were significantly higher in the homes of asthmatic patients when compared with patients seen for reasons other than asthma. These studies support the hypothesis that fungal allergen exposure is an important component in the pathogenesis of the clinical condition known as asthma.

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INTRODUCTION

Asthma is a major health problem in the United States and around the world. Although this respiratory disease is seen in all age groups, children are particularly affected. [1] Data from The US National Health Interview Survey in 1981 showed that 3.2% of children younger than 18 years of age had asthma. By 1988 that number had increased to 4.3% of children. [2] The overall asthma rate for the United States in 1998 was estimated to be 5.8% to 7.2% within a 95% confidence interval. [3] There is considerable evidence linking exposure to environmental allergens with clinical sensitization and the development and/or persistence of asthma. [4,5] Strong correlations have been found between asthma and exposure to dust mite, cockroach, cat [6,7,8] and tobacco smoke. [9] The direct link between allergen exposure and asthma is strongly supported in that avoidance measures including environmental controls can be an effective treatment for asthma. [10,11,12,13,14] The critical tool in establishing the link between allergens and asthma and also between avoidance and improved clinical outcome is the ability to measure the allergen.

Despite the evidence linking mold exposure and sensitivity to the development and/or severity of asthma, the specific role of fungal allergen exposure and the development of asthma has received little attention. Previous reports of the relationship of allergen exposure parameters and asthmatic symptoms have not included fungal exposure to any great extent. [15] One possible reason for the small number of reported studies is that few assays for fungal allergens are available. Monoclonal antibody (MAB)-based assays specific for two *Alternaria* allergens as well as polyclonal antibody-based assays for several species of fungi have been developed. [16,17] Using this analytical capability in conjunction with other, well established analytical methods for assessing allergen exposure, we test the hypothesis that there is an association between the presence of fungal allergens in the environment and asthmatic disease in children.

MATERIALS AND METHODS

Patient Recruiting and House Dust Collection

Dust samples for this study were solicited from patients recruited from the population seen in the allergy/immunology clinic at Children's Mercy Hospital (CMH), from the staff of CMH, and from a private allergy office. Individuals were instructed to bring a sample of dust taken from their vacuum cleaner. We requested that the size of the sample be approximately 1 cup and that it be brought in a plastic bag or similar container. Patients who did not have a vacuum were supplied with a Dirt Devil (Dirt Devil Co, Highland Heights, OH) handheld cleaner with an ultrafine collection bag. Individuals who submitted dust samples were asked to complete a short questionnaire concerning the source of the dust and the general environment. The questionnaire included the relative dampness of the home, the presence of pets, the presence of a dehumidifier or air conditioning system, and the nature of the heating

system in the home. We did not exclude patients who were renting. We also did not impose any minimum length of residency requirement for inclusion in the study.

Processing Dust Samples
Upon its arrival, the sample was immediately transported to the laboratory and frozen. It remained frozen until it was processed for analysis. After thawing at room temperature for 30 minutes, the dust sample including any loose material was separated by size through a 50 mesh (300-μm) brass screen to remove gross debris. The total weight of the sieved sample was measured and samples yielding at least 500 mg of sieved material were processed further. A 500 mg-sample of the dust was suspended in 2.5 mL of pH 7.5, 0.01 M ammonium bicarbonate and extracted for 3 hours at 48° C. The resulting extract was cleaned with a 5-μ filter, brought to a total volume of 2.5 mL with water and then brought to 50% glycerol extract by the addition of 2.5 mL of glycerol. The extracts then represented 0.1 g of dust/mL. Extracts were stored at -20° C until analysis.

Immunoassays
The dust was analyzed for fungal antigens specific to *Alternaria alternata* (*Alternaria* mold), *Cladosporium herbarum* (*Cladosporium* mold) and *Aspergillus fumigatus* (*Aspergillus* mold). Also, antigens unique to *Candida albicans* (yeast), *Dermatophagoides farinae* (dust mite), *Canis familaris* (dog), *Felis domesticus* (cat), *Quercus alba* (oak), *Festuca eleator* (fescue grass), *Ambrosia artemisiifolia* (ragweed), *Plantago lanceolata* (plantain weed), and *Paraplataria americana* (American cockroach) were measured. Two methods, inhibition enzyme immunoassay (EIA) and antigen capture sandwich EIA, are used for allergen analysis. These assays have either been constructed in our laboratory using antigen-specific polyclonal rabbit antibodies (Greer Laboratories, Lenoir, NC) and antigen-specific MABs or they have been obtained commercially (Indoor Biotechnologies, Charlottesville, VA). The assays include two double MAB EIAs for specific *Alternaria* allergens Alt a 1 and Alt a 70 kD. These assays are sensitive to 10 units of allergen protein/mL and specific for allergens from *Alternaria* and a few related molds. Allergen levels of Alt a 1 and GP70 are reported in U/g of dust. They have been described previously. [16,17] Reagents for Der p1 and Der f 1 assay by double MAB EIAs were obtained from Indoor Biotechnologies.

For the inhibition immunoassays, allergen preparations of known protein content and rabbit antiserum directed against these allergen preparations were obtained commercially (Greer Laboratories). Microtiter plates (Immulon 2, Dynatec, Chantilly, VA) are coated with 100 μL of whole antigen (Greer Laboratories) at a concentration of 0.01 mg/mL of 0.1 M bicarbonate buffer, pH 9.6. The plate is coated at 4° C overnight. The plate is blocked with 0.5% gelatin for 30 minutes at 37° C and then washed with PTA. After blocking, specific unlabeled rabbit anti-allergen antibody is added to the antigen-coated wells as well as with serial

dilutions of competitive antigen. The proper ration of allergen to inhibitor was determined empirically for each assay. The concentrations are generally 1:1,000 dilution of antibody containing rabbit sera and 10 µg/mL antigen titered 2-fold down the plate for 8 concentration points. Unknown samples are routinely applied to the plate at 1:10 dilution of the 0.1 g of dust/mL initial extract concentration. After incubation, to allow free rabbit antibody to bind to the antigen-coated wells, unbound antibody is washed away. Next, alkaline phosphatase labeled goat anti-rabbit antibody (Sigma, St. Louis, MO) is added and allowed to bind to the rabbit antibody-antigen complex. Unbound labeled antibody is washed away, and alkaline phosphatase substrate (Sigma) is added. After a suitable time for color development, the reaction is stopped, and the plate is read on a plate reader at 405 nM. The system has been optimized for each antigen. Specific parameters for many of these assays have been published previously. [18]

Clinical Methods and Subject Groups

All clinical studies were performed after obtaining approval from the pediatrics Institutional Review Board of the University of Missouri-Kansas City. Written consent was obtained before any experimental procedure was performed. Either the single needle technique or a Dermapik device (Greer Laboratories) was used for skin testing. Skin tests were divided into five categories: mites, molds, cat, roach, and pollen. The subjects in the study are divided into asthmatic and nonasthmatic categories on the basis of the NIH criteria. A physician determined the diagnosis at the time the patient was seen. The diagnosis was then recovered in a retrospective chart review. The person performing the diagnosis was blinded to the allergen evaluations and the person performing the analysis was blinded as to the diagnosis. The diagnosis category is correlated with the concentrations of specific allergens in the home. Statistical analysis was performed using the SPSS Statistical Software Package (SPSS Inc., Chicago, IL).

RESULTS

Summary of Samples Collected

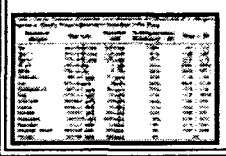
We received 69 dust samples from October 1, 1995 to October 1, 1996. Samples were received from the CMH allergy clinic (45) two private allergists in the community (21) and from CMH staff (3). Twelve samples did not contain sufficient material for analysis. We were able to collect retrospectively clinical data, including diagnoses for 47 samples.

The collected dust was from a variety of types of vacuum cleaners and represented a general collection from the entire house. Because most samples were taken from household vacuum cleaners, they could represent the total amount collected since the filter was last changed. The collection period ranged over the entire year and samples were received evenly throughout the year.

Immunoassays

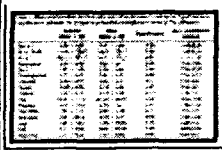
Range and general limit of detection of the EIA determinations performed are listed in Table 1. The detection limit of the individual polyclonal tests ranged from <50 ng/mL (*Aspergillus*, cat) to 2.5 µg/mL (American cockroach). For many of the assays, the ambient allergen level was lower than the detection level of the assay. For example, fewer than 10% of samples tested had detectable levels of *Aspergillus*, American cockroach, plantain, and ragweed. Fewer than 20% of samples tested had detectable levels of *Candida* and fescue. In contrast, 100% of homes tested had detectable levels of cat and more than 50% of samples contained measurable levels of *Alternaria*, *Cladosporium*, dog, and oak. More than 80% of the samples tested by EIA performed using MABs contained sufficient amounts of allergen to be detected.

Table 1. Range, Frequency of Detection, Limit of Detection, and Mean Level of 17 Allergenic Species or Specific Allergens Detected in House Dust in this Study



Patients were classified by history and physician diagnosis during retrospective chart review as either asthmatic or other. We were able to retrieve sufficient data on 47 individuals to make this designation. Of the 47 subjects, 21 were identified as asthmatic and 26 were identified as other. The other diagnosis category included viral rhinitis, otitis media, allergic rhinitis, psoriasis, sinusitis, and rhinitis. Averages and standard deviations for the asthmatic and other group were calculated. These values are presented in Table 2. For both polyclonal and monoclonal determinations, the values for the asthmatic group and the other group were compared using Student's *t* test. Significant *P* values and the associated confidence intervals are reported in Table 2. Three of the specific allergens tested (both *Alternaria* allergens and the one of the dust mite allergens) were found at significantly higher levels in dust from homes of asthmatic patients. Skin test data were recovered on 10 of 21 in the asthmatic group and 4 of 26 in the control group. Of the 10 subjects in the asthmatic group skin tested, only 2 were negative to all categories (mites, molds, cat, roach, and pollen). Of the subjects in the asthma group tested, 4 of 10 were positive for mites; 4 of 10 for molds; 4 of 10 for cat; 2 of 10 for roach; and 2 of 10 for pollen.

Table 2. Mean and Deviation for Asthmatic and Other Groups Including the Significance of the Difference between the Groups and the 95% Confidence Interval of the Difference



As all data that involve quantitative measurement of material that may or may not be present, these data are restricted by the assay detection limits. It is often informative to examine such data in a logarithmic format. Box plots of selected allergen data using a log normal format in Figures 1 and 2 illustrate the distribution of the data and the relative difference between the asthma and control groups. Simple inspection of these figures indicates that in some cases the domain of the data for the asthmatic group is clearly higher than the domain of the data for the other group. This observation is confirmed by a significant *P* value (Table 2) for Der p 1 and the two MAB-based *Alternaria* assays.

Figure 1. Data for the allergens measured using MAB methods. The data have been transformed into a log format. The line in the center of the shaded box represents the mean of the data. The shaded area represents the domain of the 25th percentile and the whiskers represent the 95th percentile of the data. Individual circles represent outlying data points. For all of the figures the group of patients with the diagnosis of asthma is represented on the right and the group of patients with other diagnoses is represented on the left.

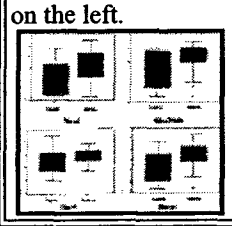
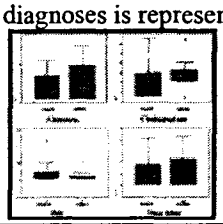


Figure 2. Data for four of the species measured using polyclonal antibody methods. The data have been transformed into a log format. The line in the center of the shaded box represents the mean of the data. The shaded area represents the domain of the 25th percentile and the whiskers represent the 95th percentile of the data. Individual circles represent outlying data points. For all of the figures the group of patients with the diagnosis of asthma is represented on the right and the group of patients with other diagnoses is represented on the left.



DISCUSSION

This study contributes to the growing body of evidence that exposure to fungal allergens is associated with development of asthma in susceptible populations. These findings support the results of numerous investigations into the allergens found in house dust. It should be

understood that these patients are self-selected in that they were seeking care in an allergy specialty clinic. The control population includes patients seen for reasons other than asthma. These patients cover a broad range of diagnoses including rhinitis and DiGeorge syndrome. Dust from the family vacuum was used because it was thought to represent exposure in the whole house as opposed to one isolated area. Once these conditions are understood we can still state that this study strengthens the growing body of evidence that links asthma and allergen exposure.

Cat allergen is present in most house dust especially when cats are present in the home. The cat allergen, Fel d 1, becomes airborne on smaller particles that can remain airborne for long periods of time. [19] The levels of cat allergen in this study are in accordance with cat allergen measured with a two-site specific MAB assay that varies from 10 to100 μg/g of dust in homes with a cat present. [20] Finding cat allergen in homes without a cat has also been reported previously. [21] Likewise our results for dog allergen levels approaching130 μg/g agree with previous dog allergen measurements. Reported mean Can f 1 in homes with dogs is 158 μg/g and in homes without dogs it is 3 μg/g. [22] Although we are aware that allergen concentrations may vary by time of day, location in the house, and season of the year no attempt was made to evaluate our data in light of these confounding variables. It is known that levels of mite allergen generally rise in response to seasonal increases in humidity. Mite exposure also differs substantially in the microclimate within the home. [23] Since fungi often respond favorably to the same general conditions as dust mites, a similar seasonal pattern would be expected for fungal allergens. Specific measurement of dust mite allergen content in house dust has proven to be a reliable indicator of the number of house dust mites present. [24] Peak Der p1 and Der f 1 values <25 μg/g reported in this study were lower than the reported range of up to 250 μg/g of dust. [25] These lower levels may be the reason that Der f 1 levels were not significant between the asthmatic and nonasthmatic groups.

Although several of the allergens measured were greater in the houses of asthmatic patients on average, the deviation in the measured values is great enough that the differences are not significant. Two of these allergens (polyclonal *Alternaria* and *Cladosporium*) tended to significance and might well become significant if there were more patients in the study. There are also several allergens in which the average values for asthmatic patients are lower. These are notably cat, dog, and oak. Perhaps one reason cat and dog allergen tend to be higher in the nonasthmatic group is that asthmatic patients might tend to avoid exposure and thus not own a pet. None of the antigens measured by polyclonal assay methods was significantly different between the asthmatic and control group, whereas 3 of the 4 monoclonal assays were different. The reasons for this are unclear. Perhaps, the polyclonal assays measure several cross-reacting substances and therefore produce higher deviations than MAB assays. The other unique aspect in the relationship between the polyclonal antigen assays and monoclonal allergen assays is

the failure of the units for each to add up. It is not uncommon for the sum of the monoclonal allergen assays to exceed the total antigen level measured by the polyclonal assay. The answer to this disparity most likely lies in the biologic nature of the assays themselves. The polyclonal assays measure a large repertoire of primary, secondary, and tertiary structural epitopes. Monoclonal assays, depending on the specific antibody, measure only two epitopes per molecule. Therefore, a standard used for a polyclonal antibody based assay may contain a larger number of potential epitopes than the same standard used for MAB-based assays. However, polyclonal assays may be more susceptible to steric hindrance (one antibody crowding out the binding site of another antibody), especially as they approach antibody excess.

The skin testing data associated with these patients is unremarkable. Since this is a retrospective study, skin testing was not indicated for many of the patients in the nonasthmatic category. The skin testing data set is therefore too small to draw more extensive conclusions from. Many studies have demonstrated that approximately 45% of atopic individuals are allergic to molds and approximately 40% are sensitive to dust mites. [26,27]

The box plots best illustrate the differences between the asthmatic and control groups Figures 1 and 2. These give not only an indication of the differences in the data sets but also an indication of the overall distributions of the data. *Alternaria* fungi that we found to be increased in the homes of asthmatic patients is one of the dominant species identified by skin testing in the NHANES II study to be associated with asthma. [2] *Alternaria* fungal exposure was also considered to be a factor in childhood asthma in other studies. [27]

In any survey of this type, there is the potential for one of the many parameters tested to be statistically significant by chance alone. We cannot rule out that occurrence in this study. Also, because the study is retrospective and the population is self-selected for persons needing to be seen in an allergy clinic, the power of the data is somewhat diminished. The data does add to the ever-increasing body of evidence that environmental factors and especially exposure to aeroallergens is an important contributor to allergic disease. Based on an overwhelming amount of evidence that mite and cat allergens negatively affect human health, standards for safe indoor exposure to allergens have been suggested. [28] Many of these patients may have taken control measures that could change the significance of cat and dust mite allergens in this study. Further, there is increasing evidence that exposure control is capable of resolving allergic problems in many patients. [29]

CONCLUSION

We found measurable levels of fungal antigens (*Alternaria, Cladosporium*), mite antigens, and animal antigens (dog and cat) in the majority of dust samples in this self-selected set of allergy clinic patients. Specific allergens Alt a 1, Alt a 70 kD and Der p 1 were significantly higher in the homes of asthmatic patients when compared with patients seen for reasons other than asthma. Our data from these studies support the hypothesis that fungal allergen exposure is an important component in the pathogenesis of the clinical condition known as asthma, and these data demonstrate the need for more extensive investigation.

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