Environmental exposure to cockroach allergens: Analysis with monoclonal antibody–based enzyme immunoassays

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Quantitative two-site monoclonal antibody (MAb)-based enzyme-linked immunosorbent assays for two cockroach (CR) allergens, Bla g 1 and Bla g II, have been developed and used to measure allergen levels in house-dust samples. Dust collected from CR-infested homes of two patients with asthma from Charlottesville, Va., demonstrated wide variation in the levels of Bla g 1, depending on the location of dust collection. Dust from kitchen floors and cabinets contained 50-fold more allergen (mean, 10.755 U/gm of dust) than dust from bedrooms and unpolished furniture (mean, 204 U/gm). One hundred forty-five dust samples were collected from the bedrooms and living rooms of 22 children with asthma and 16 control subjects without asthma living in Atlanta, Ga. Twenty-seven of the 38 homes (21) with asthma; 10/16 control subjects) had detectable Bla g I (4 to 1340 U/gm of dust). Bla g II levels were assayed in 40 kitchen, bedroom, and living room samples from homes in Wilmington, Del. Highest levels of Bla g II were detected in kitchen-floor dust (300 U/gm of dust). Additionally, 20% of homes with no visual evidence of CR infestation had significant levels of Bla g II in at least one dust sample (>4 U/gm of dust). Our results demonstrate that CR may be an occult allergen in homes. The kitchen appears to be the primary site of CR-allergen accumulation, but significant CR-allergen levels can also be found at other sites in the home. The MAb-based assays can be used for quantitation of environmental exposure to CR allergens. MAb should be very useful in epidemiologic studies to establish levels of CR-allergen exposure that should be considered as a risk factor for the development of allergic diseases, particularly asthma. (J ALLERGY CLIN IMMUNOL. 1991;87:503-9.)

The role of allergens in asthma has been studied by comparing the prevalence of allergen-specific IgE antibodies in surveys of clinics or random populations (e.g., skin testing or in vitro assays) by comparing changes in lung function and symptoms after allergen avoidance and, more recently, by comparing the prevalence of allergen-specific IgE antibodies in patients with asthma or control patients admitted to ERs. The epidemiologic studies suggest that the development of IgE antibody responses and exposure to common allergens are significant risk factors for asthma, both in adults and children. Elevated levels of IgE antibody to routinely encountered inhalant allergens, such as grass pollen, dust mite, cat and CR, clearly increase an individual's risk of developing asthma. However, the presence of allergen-specific IgE alone is not sufficient to induce acute

Abbreviations used
MAb: Monoclonal antibody
RIA: Radioimmunoassay
Bla g I: Blattella germanica allergen I, a 25 to 30
    kd acidic cross-reacting allergen
Bla g II: Blattella germanica allergen II, a 36 kd
    Blattella-specific allergen
PBS-T: Phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20
UNA: University of Virginia
ER: Emergency room
CR: Cockroach

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attacks of asthma; allergen exposure is a dependent risk factor whose presence is also necessary for exacerbations of allergic disease. Thus, having IgE antibody to ryegrass pollen is only a risk factor for asthma during the ryegrass-pollen season, and similarly, patients with IgE antibody to dust mites are at increased risk of developing exacerbations of symptoms when allergen levels in their homes are high.8,9 The availability of sensitive MAb-based assays has significantly improved the assessment of environmental exposure to mite and cat allergens and made it possible to investigate further the role that these allergens play in the etiology of asthma.10-12 CRs are an important cause of urban and inner-city asthma,13 and yet there have been very few previous studies on CR allergen levels in houses, primarily because specific assays for these allergens have not been available.

In the accompanying article,14 we report the production of a panel of CR-specific MAb. The present article describes the development of MAb-based ELISA for two CR allergens (Bla g 1 and Bla g II) and the use of these assays to measure CR allergens in dust samples obtained from different geographic locations in the United States (Charlottesville, Va.; Atlanta, Ga.; Wilmington, Del.; and Fairfield County, Calif.) and from several different locations within the home.

MATERIAL AND METHODS
Measurement of CR allergens by ELISA
MAbs to Blatella germanica were produced as previously described.15 Two MAb were used in the present studies: MAb 10A6, which is directed against the 25 kd cross-reacting allergen, Bla g 1, and 8F4, which is directed against the 36 kd Blatella-specific allergen, Bla g II.16 The ELISA assay for Bla g 1 and Bla g II was modified from the solid-phase radioimmunoassay (RIA) described previously by use of polyclonal rabbit anti-MAb antibodies instead of human IgE antibodies as secondary antibodies. Microtiter plates coated with MAb (1 µg per well) were incubated with dilutions of dust extracts, commercial CR extracts, or CR standard for 90 minutes, washed five times with PBS, pH 7.4, and 0.05% Tween 20 (PBS-T), and incubated for 90 minutes with 100 µl of polyclonal rabbit anti-CR serum diluted either 1/2000 or 1/10,000 for the Bla g 1 and Bla g II extracts, respectively. The plates were washed five times with PBS-T and incubated for 1 hour with 100 µl 1/1000 dilution of peroxidase-conjugated goat (Fab') anti-rabbit IgG (Tago, Inc., Burlingame, Calif.). After plates were washed again, they were developed with 100 µl of 0.01 mol/L of 2,2'-azino-bis-(3-ethylbenzthiazoline sulphonate acid) in 0.07 mol/L of citrate-phosphate buffer, pH 4.2, containing 0.03% H2O2, and the color was read at 405 nm with a Titertek Multiscan Plus Mk II plate reader (Flow Laboratories, McLean, Va.). The ELISA was initially quantitated with doubling dilutions of German-CR reference extract, UVA 86/04 (which contained 5000 U/ml of Bla g 1 and Bla g II) to form a control curve, from 0.01 to 5.0 units of allergen per milliliter.17 Recently, a new reference was prepared from ether-extracted B. germanica extract. This reference (UVA 89/01) contained 5000 U/ml of Bla g 1 and 3000 U/ml of Bla g II, relative to the UVA 86/04 extract, and the quantitation of both allergens is now based on the UVA 89/01 reference. The intra-assay variability for the Bla g 1 ELISA was 5.9%, and the coefficient of variation between assays was 4.5%. The intra-assay variability for the Bla g II ELISA was 4.2%; the interassay variability was 4.8%.

Dust samples were also assayed for the group I dust-mite allergens (Der p 1 and Der f 1) and the major cat allergen (Fel d 1) with two-site MAb-based ELISA, as previously described.18,19

House-dust extracts
Fifteen dust samples were obtained from the homes of two CR-allergic patients with asthma (each home sampled twice) in Charlottesville, Va., during the spring of 1988. In addition to sampling bedding, sofas, and bedroom and living room floors, dust samples were collected from kitchen floors and kitchen cabinets. The kitchen samples were obtained by vacuuming the entire surface of the kitchen cabinets above, below, and/or adjoining the kitchen sink and the surface of the kitchen floor, which was against the kitchen cabinets. The other samples were collected by vacuuming a 1 m² area for 2 minutes with a modified hand-held vacuum cleaner.20,21

Dust was also collected from 38 homes in Atlanta, Ga., and from 40 houses in Wilmington, Del. The Atlanta samples (from sofas, bedding, and bedroom and living room floors) were obtained from the homes of 22 children who presented to the ER of Grady Memorial Hospital with acute asthma from July 1985 to June 1986. Dust samples were also obtained from the homes of 16 nonatopic pediatric control subjects presenting to the ER for diagnoses other than asthma during the same period. The samples from Wilmington were collected from September through December of 1988 from the homes of subjects enrolled in an ongoing study of asthma requiring ER treatment. Dust samples from 15 homes in Fairfield County, Calif., were collected as part of a previous ER asthma study from May to June 1986.5

Dust extracts were prepared by extracting 100 mg of sieved dust (170 µm sieve mesh, 300 µm in 2 ml of borate-buffered saline, pH 8.0, for 4 hours on an orbital rotator.14,15 Extracts were stored at -20°C until they were tested.

RAST analysis
IgE antibodies to CR were measured by quantitative RAST, as described previously.17 RAST values were quantitated in units per milliliter relative to a D. farinae control curve, with values of >40 U/ml considered a significant positive result.

Statistical analysis
The RIA and ELISA assays for CR allergens were compared by linear regression. The mean levels of CR allergens in the homes of children with asthma and control children in Atlanta were compared by Student's t test.
RESULTS
Comparison of CR, mite, and cat allergen levels in central Virginia

Comparison of the Bla g 1 and Bla g II allergen levels in 15 dust samples collected in Charlottesville, Va., demonstrated a very good quantitative correlation between the results obtained by RIA and ELISA, with correlation coefficients of 0.80 and 0.95, respectively (p < 0.01 for both allergens). There was also a good correlation between Bla g 1 and Bla g II allergen levels (as measured by ELISA) in these extracts (r = 0.92; p < 0.01).

The Charlottesville, Va., dust samples were collected from the houses of two CR-allergic patients with asthma who had obvious CR infestation in their homes. These samples were obtained from several sites to compare the distribution of CR, mite, and cat allergens. The results demonstrated that the levels of CR allergen, Bla g 1, found on kitchen floors and cabinets (mean, 10.755 U/gm of dust) were up to 50-fold higher than the levels found in sofa, bedding, and bedroom-floor dust (mean, 204 U/gm of dust) (Fig. 1, A). In contrast, the levels of the dust-mite allergens (Der f 1 and Der p 1) and cat allergen (Fel d 1) were highest in bedding, bedroom floors, and upholstered furniture, with insignificant levels on kitchen floors or cabinets (Fig. 1, B and C). Nine of the dust samples (one set from each home) were also assayed for Bla g 1 by Dr. Carsten Schou (ALK Laboratories, Horsholm, Denmark) with affinity-purified, polyclonal rabbit antibodies in an ELISA, and the results demonstrated an excellent quantitative correlation with the ELISA with MAH IOA6 (r = 0.96; p < 0.001).16

CR-allergen levels in Georgia, Delaware, and California

One hundred forty-five samples were assayed from the homes of 22 children with acute asthma and 16 children without asthma who presented to the ER of Grady Memorial Hospital, Atlanta, Ga. Dust samples (three to five from each home) were obtained from bedding, bedroom floors, upholstered furniture, and living room floors and were assayed for Bla g 1. Fifty-eight samples from 27 homes had detectable Bla g 1, and the levels were very similar to levels found in comparable sites in Charlottesville (range, 4 to 1340...
TABLE I. Comparison of the prevalence of IgE anti-CR antibodies and household CR-allergen exposure in children presenting to an ER in Atlanta

<table>
<thead>
<tr>
<th>No. of dust samples containing Bla g 1</th>
<th>Range of IgE anti-CR antibodies (RAST U/ml)</th>
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<tbody>
<tr>
<td></td>
<td>&lt;50</td>
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<tr>
<td>A: Patients with asthma (N = 22)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
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<tr>
<td>1</td>
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<td>2</td>
<td>1</td>
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<tr>
<td>&gt;2</td>
<td>4</td>
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<tr>
<td>B: Control subjects (N = 16)</td>
<td></td>
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<tr>
<td>0</td>
<td>5</td>
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<td>1</td>
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*Serum from patients with asthma or control subjects without asthma were analyzed for IgE antibodies to CR by quantitative RAST, as described previously. Dust samples were assayed for Bla g 1 allergen by ELISA. Each patient had four dust samples collected from bedroom floor, bedding, living room floor, and sofa or other upholstered furniture.

†Number of samples from each home with >4 units of Bla g 1 per gram of dust (assay background, 0.2 U/gm of dust).

U/gm of dust) (Fig. 2). Only two of the 38 Atlanta samples contained allergen levels that were comparable to levels found in the Charlottesville kitchen samples. Dust extracts from the homes of 11 subjects (six with asthma and five control subjects) had no detectable Bla g 1. In general, in houses in which CR allergen was detected, several sites were positive. Thus, three or more positive samples were obtained from 17/27 homes.

The results were analyzed by comparing the number of sites from each home that contained detectable Bla g 1 with the levels of CR allergen-specific IgE antibody in the patients’ serum (Table I, A and B). Eleven patients from the 17 homes with more than two positive sites had asthma. Among the 10 patients with asthma with detectable IgE anti-CR antibody (>50 RAST units per milliliter), nine lived in homes with two or more CR-positive sites, and one in the 16 control sera contained >50 RAST units per milliliter of anti-CR IgE, and only one of these patients had more than two household sites with detectable Bla g 1. One control patient had very high levels of CR-specific IgE antibody (1305 RAST units per milliliter) but had no detectable Bla g 1 at any site (Table I, B). The subject with the highest level of CR allergen in his home had 1340 units of Bla g 1 per gram of bedroom floor dust; 150 U/gm of bed dust; 38 U/gm of sofa dust; and 244 U/gm of living room floor dust. This subject had 702 RAST units per milliliter of CR-specific IgE and had asthma. Thus, 9/22 subjects with asthma had both IgE antibody >50 units and exposure in two or more sites, whereas 1/16 control subjects had similar risk factors (p < 0.05).

Dust samples from subjects enrolled in an ER study in Wilmington were collected from kitchens, bedroom, upholstered furniture, and bedroom and living room floors and then were analyzed for Bla g 2. The results demonstrate the highest levels of Bla g 2 in kitchen floor dust (up to 300 U/gm of dust) and lower levels at other sites, particularly in bedding and sofa dust (Fig. 3). All homes with direct visual evidence of CR infestation had at least one dust sample with >2 units of allergen per gram of dust. An additional 146 homes have been sampled, and we have found that ~20% of homes without visual evidence of CR infestation have >2 units of Bla g 2 per gram of dust in at least one dust sample; 15% of these homes had >10 units of Bla g 2 per gram of dust.

As negative controls for these studies, Bla g 1 was measured in dust samples from 15 homes in Fairfield County, Calif. These homes were studied during the spring of 1986 during the “grass-pollen asthma epidemic” that occurs each year, concurrent with hay grass-pollen season. The samples had previously revealed no detectable German-CR allergen by RAST inhibition, and 45/45 samples contained <0.2 U/gm of Bla g 1.

DISCUSSION

The advent of MAbs has greatly improved our ability to understand the role that allergens play in allergic disease. MAb-based assays have made allergen quantitation in dust samples and allergen extracts efficient and reproducible. In the past few years, these techniques have been applied to epidemiologic and aerobiologic studies of mite and cat allergens. Our results confirm the advantages of the use of MAb-
based assays for measuring allergens in the indoor environment. Although only one MAb for CR was available for measuring each allergen, sensitive ELISA assays could be developed with polyclonal second antibodies. The assay was modified to use rabbit IgG antibodies instead of human IgE antibodies because of the difficulty in obtaining sufficient quantities of serum from CR-allergic patients. Both the sensitivity and specificity of the ELISA were comparable to the RIA with IgE antibodies.

In Charlottesville, we investigated two houses for sites of CR-allergen accumulation. These preliminary studies suggested, not surprisingly, that kitchens are a major source of CR allergens. In the houses that we examined, there was gross visible CR infestation of the kitchen walls and cabinets, but little evidence of CRs in the bedrooms or living rooms. The allergen levels may be present in furnishings and other rooms because they have few CRs or because the allergen is distributed through the household. Allergen would then reach these areas from the kitchen during domestic activity or when it becomes airborne. Assays of dust from both Charlottesville and Atlanta demonstrate that CR allergen can be detected at sites that are usually sampled when dust-mite or cat allergens are being investigated. The results confirm that CRs are an important source of indoor allergens in Atlanta. However, it is likely that the levels of CR exposure in the Atlanta homes may have been underestimated because kitchen sites were not sampled.

As a result of these studies, we have included samples of kitchen sites in a study of asthma requiring ER treatment recently carried out in Wilmington. Data from 40 homes confirmed that the kitchen is the primary location for CR infestation, although significant levels of Bla g II were found on other floor surfaces throughout the house. Additionally, sampling of homes without visual evidence of CR infestation found that significant levels of CR allergen could be found in ~20% of homes in which there was no visual evidence of CRs observed by the inhabitants and the investigators. The results of the Charlottesville, Atlanta, and Wilmington studies suggest that assays for Bla g I or Bla g II can be used to assess exposure to B. burgdorferi. Additional studies are needed to establish the quantitative relationship between exposure to these two allergens and whether or not there are a significant number of houses in which Bla g I is detected in the absence of Bla g II (possibly reflecting infestation by Periplaneta and CR genera other than Blattella). These issues are currently being investigated as part of more detailed studies on sensitization and exposure to CRs in Delaware.

With the use of MAb immunoassays for specific allergens, it has become possible to propose levels of indoor allergens that should be considered as risk factors for allergic disease. For dust-mite allergens (Der p 1 and Der f 1), levels of 2 μg/gm and 10 μg/gm have been proposed as levels that are likely to induce sensitization or cause exacerbations of allergic disease, respectively. Similarly, several studies have defined the level of cat allergen (Fel d 1) found in houses with cats (often >50 μg/gm), the quantities that become airborne under undisturbed or disturbed conditions, and the time necessary to reduce cat-allergen exposure to levels observed in houses without cats. In the present study, we have demonstrated statistically significant differences in CR-allergen exposure between patients presenting with asthma and nonatopic control subjects. However, these findings clearly need to be confirmed, and additional studies are required to determine whether it is possible to define a specific level of CR allergens that is associated with exacerbations of symptoms.

In conclusion, we have demonstrated that environmental exposure to CR allergens can be assessed with MAb-based immunoassays for two antigenically distinct allergens. These assays provide the tools for establishing the clinical significance of CR-allergen exposure and the effectiveness of methods used to reduce or eradicate CRs from the houses of allergic patients. Although total extermination of CRs from heavily infested houses is difficult, it may be possible to develop avoidance regimens that reduce the likelihood of sensitization and control the development of symptoms. The MAb assays could also be used to monitor other environments for CRs and would make it possible to...
set public health standards for CR exposure in areas such as restaurants, schools, and day-care centers. We are grateful to Dr. Don Mullins for providing supplies of CR frass and to Dr. Carsten Schou for confirming the Bla g 1 levels in several dust extracts. We also thank Madeleine Watkins for drawing the figures and Nancy Malone for preparing the manuscript.

REFERENCES