Specific IgE and IgG antibody-binding patterns to recombinant cockroach allergens

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Background: The specificity of serum antibody responses to different cockroach allergens has not been studied.
Objective: We sought to quantitate serum IgE and IgG antibodies to a panel of purified cockroach allergens among cockroach-sensitized subjects.
Methods: IgE antibodies to recombinant cockroach allergens (rBla g 1, rBla g 2, rBla g 4, rBla g 5, and rPer a 7) were measured in sera containing IgE antibodies to *Blattella germanica* extract (n = 118) by using a streptavidin CAP assay and a multiplex flow cytometric assay. Specific IgG antibodies were determined by using radioimmunoprecipitation techniques.
Results: Specific IgE antibodies measured by means of CAP assay and multiplex assay were strongly correlated ($r = 0.8$, $P < .001$). The sum of IgE antibodies (in international units per milliliter) against all 5 allergens equated to IgE antibodies to cockroach extract. Although the prevalence of IgE antibodies was highest for rBla g 2 (54.4%) and rBla g 5 (37.4%), patterns of IgE antibody binding were unique to each subject. Surprisingly, only 16% of cockroach-sensitized subjects with IgE antibodies to house dust mite exhibited IgE antibody binding to cockroach tropomyosin (rPer a 7). Specific IgE antibodies were associated with increased IgG antibody levels, although detection of IgG in the absence of IgE was not uncommon.
Conclusion: The techniques described offer a new approach for defining the hierarchy of purified allergens. IgE antibodies directed against 5 allergens constitute the majority of the IgE antibody repertoire for cockroach. Such distinct patterns of IgE-IgG responsiveness to different cockroach allergens highlight the complexity of B-cell responses to environmental allergens. (J Allergy Clin Immunol 2005;115:803-9.)

Key words: *Cockroach, asthma, allergens, tropomyosin, IgE antibody, IgG antibody, multiplex*

Abbreviations used
HDM: House dust mite
RIA: Radioimmunoprecipitation assay

Sensitization to cockroach allergens is an established risk factor for asthma among inner-city populations.[1,2] This has been attributed to the high levels of cockroach allergens found in the urban environment.[1,3] However, sensitization to cockroach resulting from low-level exposure might extend to suburban or more rural areas, pointing to a widespread problem.[4] Interestingly, individuals who are sensitized to cockroach are frequently cosensitized to house dust mite (HDM) allergens.[5-7] A possible explanation is that tropomyosins derived from mite (Der p 10 and Der f 10) and cockroach (Bla g 7 and Per a 7) species are cross-reactive.[7,8] Rapid advances in the molecular characterization of cockroach allergens have resulted in the production of multiple recombinant allergens from German *(Blattella germanica)* and American *(Periplaneta americana)* cockroach species with comparable immunoreactivity to natural allergens.[7,9-23] However, a direct comparison of IgE and IgG antibody binding to different cockroach allergens has not been carried out among individuals with cockroach allergy, largely as a result of the lack of a reliable technique that could be applied to all allergens. Recent development of an assay that incorporates allergen bound to a high-capacity solid phase (streptavidin CAP[24,25]) has facilitated definition of a hierarchy of cockroach allergens on the basis of IgE antibody binding. Serum antibody levels were measured in samples obtained from subjects with cockroach allergy living in both inner-city (Atlanta, Georgia, and Wilmington, Delaware) and rural areas (Charlottesville, Virginia).[2,26] The objectives of this study were 3-fold: (1) to determine the characteristics of IgG and IgE antibody profiles to a panel of cockroach allergens, (2) to examine tropomyosin cross-reactivity in the context of co-sensitization to cockroach and mite allergens, and (3) to assess the validity of a novel cytometric bead assay for simultaneous quantitation of IgE antibodies to multiple allergens. This latter technique could have broad-based applications within the allergy field.

METHODS

Serum samples

Pre-existing serum samples from subjects living in inner-city Atlanta, Georgia (age, 5-16 years; n = 106)[26]; inner-city Wilmington,

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Delaware (age, 15-55 years; n = 127)[2]; and Charlottesville, Virginia (age 18-75 years; n = 143) were screened for IgE antibodies to *B germanica* extract by means of CAP assay. Detailed serologic studies were carried out on 118 sera with measurable IgE antibodies, and 70% of these sera were from asthmatic subjects. Sera with no measurable IgE antibodies to *B germanica* (n = 15) provided negative controls for IgE and IgG antibody assays. Studies were approved by the University of Virginia Human Investigation Committee.

Cockroach and dust mite allergens

Recombinant allergens from German cockroach were produced in *Pichia pastoris* (Bla g 1, Bla g 2, and Bla g 4) or *Escherichia coli* (Bla g 5) (all from Indoor Biotechnologies, Inc, Charlottesville, Va).[9-11,14,22,23] Tropomyosins derived from American cockroach (Per a 7, a generous gift of Dr Karla Arruda[7]) and dust mite (Der p 10, a generous gift of Dr Wayne Thomas[27]) were produced in *E coli*.

Measurement of serum IgG and IgE antibodies to cockroach and HDM allergens

Total IgE and IgE antibodies to extracts of *B germanica*, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae* were measured by means of CAP assay (Pharmacia Biotech, Uppsala, Sweden). IgG antibodies to rBla g 1, rBla g 4, rBla g 5, rPer a 7, and rDer p 10 were measured by means of antigen-binding radioimmunoprecipitation assay (RIA).[28] Briefly, serum samples were diluted 1:12.5 and 1:50 and incubated with iodine 125–labeled allergen (approximately 100,000 cpm added). Immune complexes were precipitated with anti-human IgG (Strategic Biosolutions, Ramona, Calif), and precipitates were washed and counted in a gamma counter. For each allergen, IgG antibodies were quantitated by using standard curves established with pooled sera from patients with cockroach allergy who exhibited the highest IgG antibody binding (in counts per minute). Each control curve was arbitrarily assigned to contain 2000 U of allergen-specific IgG antibody/mL. Assay cutoff points were 40 U/mL (rBla g 1), 90 U/mL (rBla g 4), 25 U/mL (rBla g 5), and 10 U/mL (rPer a 7 and rDer p 10). The streptavidin CAP assay was used to quantitate specific IgE antibodies to rBla g 1, rBla g 2, rBla g 4, rBla g 5, rPer a 7, and rDer p 10.[24,25] Briefly, antigens were biotin labeled, diluted at 1:8 (rBla g 1, rBla g 2, rBla g 4, and rBla g 5) or 1:15 (rPer a 7 and rDer p 10), and applied to streptavidin CAPs (Pharmacia Biotech). The quantity of allergen bound to each CAP was estimated to be 6.25 μg for rBla g 1, rBla g 2, rBla g 4, and rBla g 5 and 3.33 μg for rPer a 7 and rDer p 10. Sera were incubated with allergen-bound streptavidin CAPs at room temperature (30 minutes), and CAPS were washed. CAPs were then incubated with β-galactosidase–labeled rabbit anti-IgE antibody for 2.5 hours and developed by washing and addition of 0.01% 4-methylumbellifery-β-D-galactoside. IgE antibodies were detected by using a Fluorocount 96 fluorometer (Pharmacia CAP system).

Suspension array assay for measurement of cockroach-specific IgE antibodies

Coupling of allergens to microspheres. IgE antibodies to rBla g 1, rBla g 2, rBla g 4, and rBla g 5 were assayed in parallel by using the Bio-Plex System (BioRad, Hercules, Calif). Methods were carried out with reagents and buffers supplied by the manufacturer (BioRad).[29-31] Fluorescent microspheres (bead sets 24, 28, 42, and 46; 1.25×10^6 each) were washed in PBS, pH 7.2, containing 0.05% Tween-20, and washing was carried out between each step of the allergen-coupling procedure. Beads were resuspended in 100 μL of Bead Activation Buffer and mixed with 0.5 mg of N-hydroxysulfosuccinimide (Sulfo-NHS), followed by 0.5 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl (Sigma-Aldrich, St Louis, Mo) for 20 minutes at room temperature in the dark. Each bead set was coupled to a single allergen (10 μg of rBla g 1 and rBla g 2 and 5 μg of rBla g 4 and r Bla g 5) in 500 μL of PBS at room temperature for 2 hours. Beads were then blocked with 250 μL of Blocking Buffer for 30 minutes before resuspending in 150 μL of Storage Buffer, counting with a hemocytometer, and storing at 4°C.

Suspension array assay. Assays were performed in a Bio-Plex 96-well assay plate. All incubations were carried out at room temperature in the dark, and washing was incorporated between addition of each reagent to the wells. Briefly, a mixture of allergen-coupled beads was dispensed at 4×10^4 beads per well. After removal of Storage Buffer by means of filtration with a vacuum manifold (Millipore, Watford, United Kingdom), beads were washed, and 50 μL of diluted serum was added to each well (1:4 and 1:20 in Human Diluent [BioRad]). Wells were then incubated with 25 μL of biotinylated goat anti-human IgE (2 μg/mL) for 30 minutes, followed by phycoerythrin-coupled streptavidin (50 μL for 10 minutes). Fluorescence was detected after resuspending beads in 125 μL of Assay Buffer (BioRad) by using the Bio-Plex System. Values were interpolated from a standard curve established with a serum pool derived from individuals with cockroach allergy with high titers of IgE antibodies to rBla g 1, rBla g 2, rBla g 4, and rBla g 5 measured by means of streptavidin CAP assay. This standard serum was arbitrarily assigned to contain 400 U/mL specific IgE antibodies to each cockroach allergen tested. The limit of detection of each assay was obtained by using a value corresponding to the mean + 2 SDs for 6 blank wells.

Statistical analysis

The nonparametric Mann-Whitney *U* test was used for comparisons of IgE and IgG antibody titers. The relationship between variables was analyzed by using the Spearman rank correlation. All statistical tests were 2-tailed, and *P* values of less than .05 were considered statistically significant. Data were analyzed with SPSS for Windows (version 10.0, SPSS Inc, Chicago, Ill).

RESULTS

Bla g 2 and Bla g 5 dominate the IgE antibody response to cockroach

IgE antibodies to rBla g 1, rBla g 2, rBla g 4, and rBla g 5 were assayed in 118 sera from cockroach-sensitized subjects (mean IgE antibody to *B germanica* extract, 5.15 IU/mL; range, 0.37-97.1 IU/mL). Because tropomyosins from different cockroach species exhibit high amino acid sequence identity (>95%), rPer a 7 was also included (Table I).[7,9-21] Previously, measurement of specific IgE antibodies to Bla g 2 by means of RIA was hindered by an inability to label this molecule with iodine 125. Given that titers of IgE antibodies to other cockroach allergens measured by means of RIA and streptavidin CAP assay were strongly correlated in initial studies ($r > 0.8$, $P < .001$), subsequent experiments used the streptavidin CAP assay for assessing patterns of IgE antibody responsiveness to all 5 cockroach allergens. The prevalence of IgE antibodies was highest for rBla g 2 (54.4%), rBla g 5 (37.4%), and rBla g 1 (26.1%) and lowest for rBla g 4 (17.4%) and rPer a 7 (12.7%). Among sera with high IgE to cockroach extract (3.5-100 IU/mL), the prevalence of IgE antibodies to rBla g 2 and rBla g 5 was 71.4% and 57.8%, respectively. Mean IgE antibody titers were highest for rBla g 5 (7.61 IU/mL) and lowest for rBla g 1 (2.24