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

Shama M. Satinover, MS,[a] Amanda J. Reefer, BS,[a] Anna Pomes, PhD,[b] Martin D. Chapman, PhD,[b] Thomas A. E. Platts-Mills, MD, PhD,[a] and Judith A. Woodfolk, MBChB, PhD[a] *Charlottesville, Va*

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,

From [a]Asthma and Allergic Diseases Center, University of Virginia Health System, and [b]Indoor Biotechnologies Inc.
Supported by National Institutes of Health grant AI-20565, and grants from Indoor Biotechnologies, Inc, and from Philip Morris Inc (to A. Pomés and M. D. Chapman).
Received for publication November 5, 2004; revised January 12, 2005; accepted for publication January 13, 2005.
Reprint requests: Judith A. Woodfolk, MBChB, PhD, Allergy Division, PO Box 801355, University of Virginia Health System, Charlottesville, VA 22908-1355. E-mail: jaw4m@virginia.edu.
0091-6749/$30.00

doi:10.1016/j.jaci.2005.01.018

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

TABLE I. Cockroach allergens

Species	Allergen*	Sequence homology or obsolete name	MW (kd)	Sequence derivation	Gene expression system	Reference
Blattella germanica	**Bla g 1**	Bla g Bd90k	90 (Variable)	cDNA	*Pichia pastoris*	Helm et al[13] and Pomes et al[14]
	Bla g 2	Aspartic protease	36	cDNA	*P pastoris*	Arruda et al[9]
	Bla g 4	Calycin	21	cDNA	*P pastoris*	Arruda et al[10]
	Bla g 5	Glutathione *S*-transferase	22	cDNA	*Escherichia coli*	Arruda et al[11]
	Bla g 6	Troponin C	27	cDNA	*P pastoris*	Arruda et al[12]
	Bla g 7	Tropomyosin	42	cDNA	*E coli*	Jeong et al[19]
Periplaneta americana	Per a 1	Cr-PII	26	cDNA	*E coli*, mammalian cells	Wu et al,[15] Melen et al,[18] and Wu et al[20]
	Per a 3	Cr-PI	47-80	cDNA	*E coli*	Wu et al[16] and Wu et al[21]
	Per a 7	Tropomyosin	37	cDNA	*E coli*	Santos et al[7] and Asturias et al[17]

MW, Molecular weight.
*Allergens shown in bold were analyzed in the present study.

IU/mL, $P = .002$, Fig 1). No specific IgE antibody was detected in sera without IgE antibodies to *B germanica* extract. Analysis of the relationship between IgE antibodies to cockroach extract and specific IgE showed a correlation for rBla g 2 and rBla g 5 only ($r = 0.56$ and $r = 0.68$, respectively; $P < .01$). Adding the titers of specific IgE antibodies for all 5 allergens strengthened the correlation with IgE antibodies to cockroach extract ($r = 0.77$, $P < .001$) and yielded titers of IgE antibodies equivalent to those for cockroach extract (Fig 2). The sum of specific IgE antibodies was not related to total IgE ($r = 0.16$, $P = .187$), and IgE antibody levels to cockroach extract were only weakly correlated with total IgE ($r = 0.36$, $P < .001$).

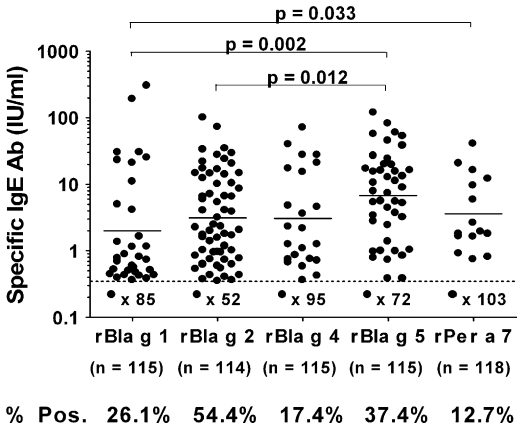

FIG 1. Titers of specific IgE antibodies to purified recombinant cockroach allergens in samples with IgE antibodies to *B germanica* extract. *Horizontal bars* represent geometric means for sera with measurable specific IgE levels, and *dotted line* represents the limit of detection of the assay.

Unique serum IgE antibody profiles to cockroach allergens are a feature of cockroach sensitization

Measurement of IgE antibodies to all 5 cockroach allergens was carried out on 114 samples with IgE antibodies to cockroach extract. Forty-two (36%) sera showed no IgE antibody binding to any allergen, and 75% of these sera had low IgE (0.35-3.49 IU/mL, equivalent to CAP classes I and II). By contrast, all sera (except one) with IgE antibody levels to cockroach extract of greater than 10 IU/mL (32%) had specific IgE antibodies (Fig 2). Mono-sensitization was common (17.5%), with rBla g 2 (9%) and rBla g 5 (6%) accounting for the majority of cases. Only 4% of sera had IgE antibodies to all 5 allergens tested. The pattern of IgE antibody responsiveness was unique to each subject, irrespective of geographic region. However, the prevalence of IgE antibodies to rBla g 1 and rPer a 7 was significantly decreased in sera from Wilmington compared with that from other regions ($P < .05$). Levels of specific IgE antibodies to cockroach allergens measured simultaneously by means of cytometric bead assay and individually by means of streptavidin CAP assay were highly correlated for samples with IgE antibodies detected by using both methods ($r > 0.83$, $P < .001$, n = 114). Detection of IgE antibodies with streptavidin CAP assay but not with bead assay or vice versa was restricted to samples with IgE antibody levels close to the limit of detection for each assay (Fig 3, *A*). The allergen-specific IgE antibody profiles obtained by means of cytometric bead assay paralleled those obtained by means of streptavidin CAP assay (Fig 3, *B*).

Specific IgE antibodies to cockroach allergens are associated with high-titer IgG antibodies

Specific IgG antibodies to rBla g 1, rBla g 4, rBla g 5, and rPer a 7 were measured by means of antigen-binding RIA. Among sera with measurable specific IgE antibody levels, titers of IgE and IgG antibodies were strongly correlated for rBla g 1 and rBla g 5 only ($r = 0.65$ and $r = 0.69$, respectively; $P < .001$; Fig 4, *A*, and data not

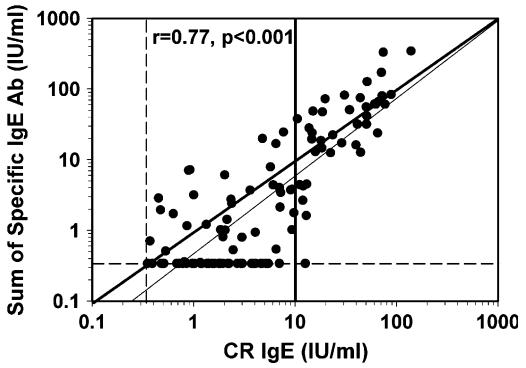

FIG 2. The relationship between IgE antibody titers to cockroach *(CR)* extract and the sum of cockroach-specific IgE antibodies. A line passing through the point of origin and a line corresponding to 10 IU/mL IgE antibody are shown in bold. *Dotted lines* represent the limit of detection of the assay.

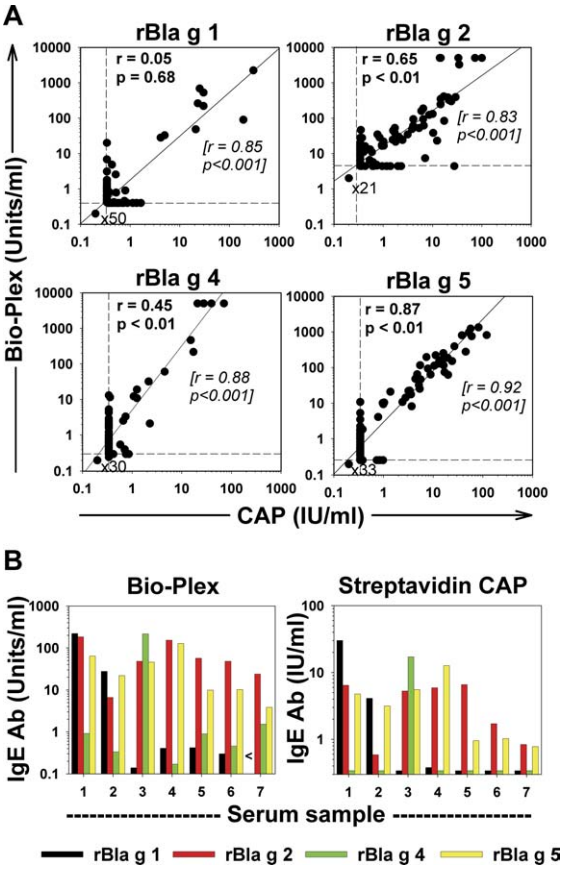

FIG 3. Comparison of streptavidin CAP assay and cytometric bead assay for quantitating cockroach-specific IgE antibodies. **A**, Coefficient values are shown for all data points, excluding double-negatives (regular font), and for double-positive data points (italics). **B**, Serum IgE antibody profiles of 7 representative sera. Bead assay values are arbitrary units above background.

shown). Moreover, levels of IgG antibodies were significantly increased among subjects with specific IgE antibodies compared with those without IgE antibodies for rBla g 4 (median, 150 vs 90 U/mL), rBla g 5 (median, 454 vs 25 U/mL), and rPer a 7 (median, 305 vs 72.5 U/mL; $P \leq .001$; Fig 4, *B*). The presence of IgE antibodies without IgG antibodies varied considerably for different allergens among cockroach-sensitized subjects (ranging from 0% for rPer a 7 to 45% for rBla g 1). Similarly, the presence of IgG antibodies without specific IgE antibodies was also highly variable (13% for rBla g 5 vs >95% for rPer a 7; Fig 4, *B*).

Co-sensitization to cockroach and mite and relation to tropomyosin cross-reactivity

A high prevalence of IgE antibodies to *D farinae* (90/118 [76.3%]) or *D pteronyssinus* (93/118 [78.8%]) was identified among sera with IgE antibodies to *B germanica* extract. Among subjects with high-titer (>3.5 IU/mL, n = 67) versus low-titer (0.35-3.49 IU/mL, n = 51) IgE antibodies to cockroach, there was no difference in the prevalence of IgE antibodies to *D farinae* (79.1% vs 72.6%) or *D pteronyssinus* (76.1% vs 82.4%) nor in the mean titer of IgE antibodies to *D farinae* (21.6 vs 33.6 IU/mL) or *D pteronyssinus* (29.7 vs 25.5 IU/mL, $P > .4$). As expected, levels of IgE antibodies to *D farinae* and *D pteronyssinus* were strongly correlated ($r = 0.97$, $P < .001$). By contrast, there was no quantitative correlation between IgE antibodies to cockroach extract and to either species of dust mite ($r < 0.03$, $P > .05$). Cross-reactivity of cockroach (rPer a 7) and HDM (rDer p 10) tropomyosins was confirmed in IgE antibody inhibition assays (data not shown). However, only 15 (16%) of 93 sera with IgE antibodies to either species of dust mite had specific IgE antibodies to rPer a 7. Levels of both specific IgE antibodies ($r = 0.90$, $P < .001$) and specific IgG antibodies ($r = 0.46$, $P < .001$) were strongly correlated for rPer a 7 and rDer p 10 (Fig 5).

DISCUSSION

Our findings yield insight into cockroach allergens which are relevant to the generation of IgE and IgG antibody responses and the development of asthma among cockroach-sensitized subjects. Analysis of the sum of specific IgE antibodies indicated that antibodies directed against 5 allergens constituted the majority of the IgE antibody repertoire for cockroach. Dominance of IgE antibody responses to cockroach by Bla g 2 and Bla g 5 is consistent with reports of these proteins as major allergens.[9,11,12] Indeed, the majority of sera with high IgE levels to cockroach extract (3.5-100 IU/mL) exhibited IgE antibody binding to one or both of these allergens. The prevalence of IgE antibody binding to rBla g 2 and rBla g 5 was lower among low-IgE sera (33% for rBla g 2 and 12% for rBla g 5). One possible interpretation of this is that IgE antibodies to cockroach extract in low-IgE samples (ie, IgE antibody of <3.5 IU/mL) reflect cross-reactivity with non-cockroach allergens, such as those derived from HDMs; however, the lack of a difference in the prevalence

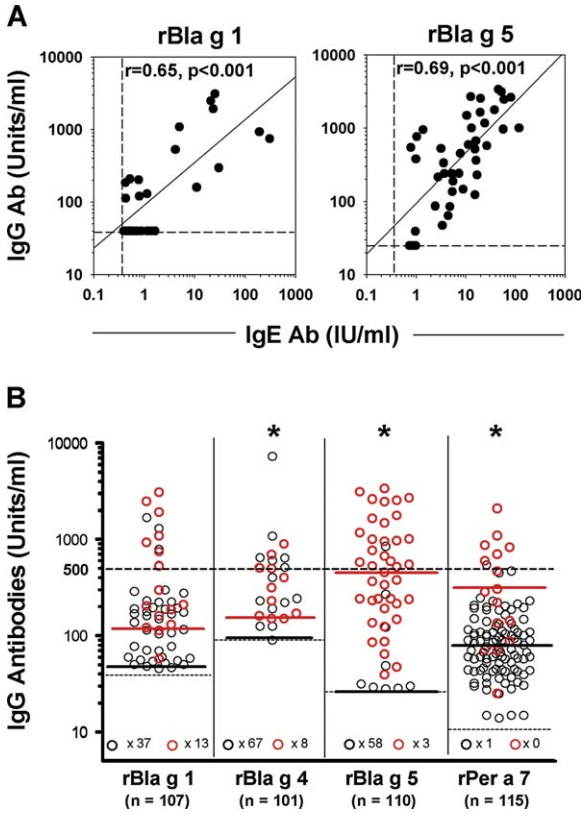

FIG 4. Relationship between specific IgE and IgG antibodies to cockroach allergens. Analysis of sera with specific IgE antibodies to rBla g 1 and rBla g 5 **(A)** and with IgE antibodies to cockroach extract **(B)** is shown. In Fig 4, *B, dotted line* represents the limit of detection of the assay, and *horizontal bars* represent median values for sera with (red) and without (black) specific IgE antibodies. *$P \leq .001$ for IgG antibody titers among IgE-positive versus IgE-negative sera.

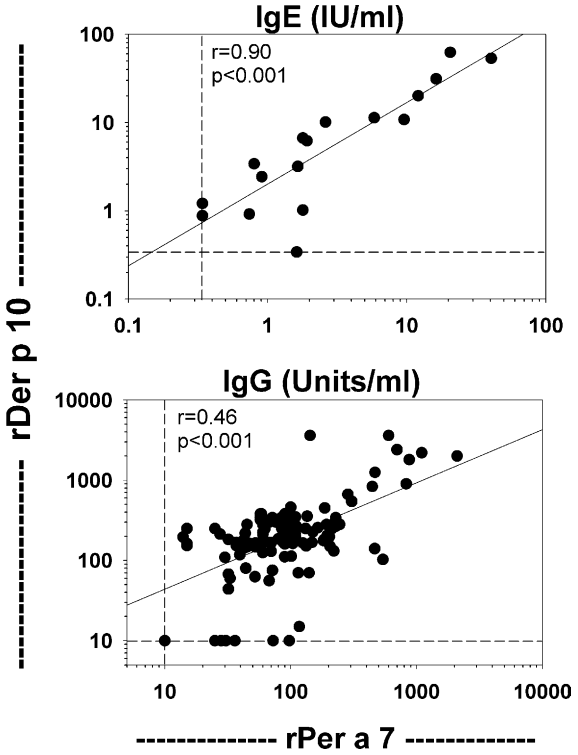

FIG 5. Tropomyosin cross-reactivity among sera with IgE antibodies to *B germanica*. Correlation between IgE and IgG antibody titers to dust mite (rDer p 10) and American cockroach (rPer a 7) tropomyosins are shown. *Dotted lines* represent the limit of detection of the assay.

of sensitization to HDM allergens in low-IgE versus high-IgE sera coupled with a failure for tropomyosin cross-reactivity to segregate with low IgE to cockroach refutes this theory. Cross-reactivity with other muscle-associated allergens, such as mite-derived paramyosins, cannot be definitively excluded.[32] However, we argue that low IgE to cockroach reflects sensitization to minor cockroach allergens, which remain to be identified. Consistent with this, 36% of sera with IgE antibodies to cockroach extract had no detectable IgE antibodies to any of the purified allergens tested, and the majority of these sera had IgE antibody levels to cockroach of less than 3.5 IU/mL.

The strong correlation between specific IgE antibody titers detected by using the streptavidin CAP and multiplex assays provided evidence of assay specificity. For rBla g 4 and rBla g 5, several sera exhibited IgE antibody binding with the multiplex assay but not with the streptavidin CAP assay, and this was restricted to sera with low specific IgE levels ($\leq$10 U/mL, Fig 3). Thus sensitivity of the multiplex assay might be increased relative to that of other assay techniques, similar to previous reports.[29,30] In a small number of samples (approximately 9%), low-titer IgE antibodies to rBla g 1 or rBla g 2 were detected by using one assay but not by using the other, suggesting that the nature of the allergen could also contribute to interassay discrepancies. In the streptavidin CAP assay allergen is biotin-labeled before binding to the solid phase. By contrast, in the bead assay binding of allergen to microspheres occurs through covalent linkage. Thus the potential for loss of B-cell epitopes during coupling of allergen to the solid phase exists for both techniques. Initial studies that compared the fluorescence intensity generated by monoplex (ie, single allergen) versus tetraplex (ie, 4 allergens) assays for rBla g 5 excluded a role for interference between bead sets. Advantages of the multiplex assay over existing methodologies include the requirement for only a small volume of sample (15 μL for detection of IgE antibodies to $\geq$10 allergens) and reduced time and labor. These features are especially applicable to studies in pediatric subjects and large-scale epidemiology studies, which focus on identifying relevant allergens in at-risk populations.

The present study analyzed samples obtained previously from subjects living in the inner city, where exposure to cockroach allergens is high.[1,2,26,33,34] Indeed, 41% of the sera from Atlanta and Wilmington had detectable IgE antibody levels to *B germanica* extract. This represents a very high prevalence of cockroach sensitization, even when considering that samples were

obtained from a selected population (ie, patients attending a hospital emergency department or an outpatient clinic).[2,26] Nevertheless, the profile of specific IgE antibodies to all 5 allergens tested was unique for each individual. The majority of sera (70%) with IgE antibodies to *B germanica* were from asthmatic subjects, indicating that the allergens tested are appropriate targets for the treatment of allergic disease. The design of the present study precluded a detailed comparison of the patterns of IgE responsiveness to cockroach allergens between asthmatic and nonasthmatic subjects. However, preliminary studies suggest that both the prevalence and titer of cockroach-specific IgE antibodies are increased in asthmatic individuals. This is consistent with increased titers of IgE antibodies to cockroach extract among asthmatic subjects living in the inner city.[2] Surprisingly, there was no relationship between the titer of specific IgE antibodies and mono-sensitization versus multi-sensitization. Studies on how the pattern of IgE responsiveness relates to both genetics and exposure to specific allergens, particularly among inner-city populations, could provide further insight into these observations.

Despite the high prevalence of IgE antibodies to *D farinae* and *D pteronyssinus* among sera with IgE antibodies to cockroach, only a minority of these sera exhibited tropomyosin cross-reactivity. Our findings suggest that concomitant exposure to HDM and cockroach allergens, as opposed to antigenic cross-reactivity, explains co-sensitization among subjects with cockroach allergy living in the inner city. In support of this, the high prevalence of IgG antibodies to rPer a 7 in the absence of IgE antibodies might result from co-exposure to HDM tropomyosin, rather than high exposure to cockroach tropomyosin per se. In Brazil, IgE antibodies to Per a 7 were detected by means of plaque immunoassay in 50% of cockroach-sensitized patients with asthma or rhinitis.[7] Those results could reflect the type of assay used or cross-reactivity with tropomyosins from other sources, such as shrimp.

Both the prevalence and titer of specific IgG antibodies was increased among sera with specific IgE antibodies to the relevant allergen. Thus high-titer specific IgG antibodies were a marker for IgE. This observation coupled with the strong correlation between specific IgE and IgG antibodies for rBla g 1 and rBla g 5 suggests that increased exposure to cockroach allergens favors the induction of specific IgE. Obviously, this is based on the assumption that the titer of specific IgG antibodies is a direct corollary of exposure; however, this requires further study. Surprisingly, the presence of specific IgG antibodies without IgE antibodies was relatively common for rBla g 1, rBla g 4, and rPer a 7 but not for rBla g 5. However, the presence of high-titer IgG antibodies ($\geq$500 U/mL) in the absence of IgE antibodies was uncommon (10 samples). The high prevalence of low-titer IgG antibodies without IgE antibodies for rPer a 7 might reflect tropomyosin cross-reactivity in a population co-exposed to HDM. Unfortunately, IgG antibody binding to rBla g 2 was not examined because of an inability to radiolabel the allergen. Nevertheless, our results support a linear dose-response relationship for exposure versus sensitization to cockroach allergens.

In summary, the use of recombinant allergens in conjunction with the multiplex assay has the potential to broaden the scope of allergen-specific antibodies analyzed in large-scale studies. Such an approach offers much promise for advancing our knowledge of allergens that are relevant to the development of asthma in well-defined populations, thereby identifying new targets for therapy.

REFERENCES

1. Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TA. Risk factors for asthma in inner city children. J Pediatr 1992;121:862-6.
2. 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.
3. Eggleston PA, Rosenstreich D, Lynn H, Gergen P, Baker D, Kattan M, et al. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. J Allergy Clin Immunol 1998;102:563-70.
4. Matsui EC, Wood RA, Rand C, Kanchanaraksa S, Swartz L, Curtin-Brosnan J, et al. Cockroach allergen exposure and sensitization in suburban middle-class children with asthma. J Allergy Clin Immunol 2003;112:87-92.
5. Hulett AC, Dockhorn RJ. House dust, mite *(D. farinae)* and cockroach allergy in a midwestern population. Ann Allergy 1979;42:160-5.
6. van Wijnen JH, Verhoeff AP, Mulder-Folkerts DK, Brachel HJ, Schou C. Cockroach allergen in house dust. Allergy 1997;52:460-4.
7. Santos AB, Chapman MD, Aalberse RC, Vailes LD, Ferriani VP, Oliver C, et al. Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. J Allergy Clin Immunol 1999;104:329-37.
8. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez A, Martinez J, Palacios R. Sequencing and high level expression in *Escherichia coli* of the tropomyosin allergen (Der p 10) from *Dermatophagoides pteronyssinus*. Biochim Biophys Acta 1998;1397:27-30.
9. Arruda LK, Vailes LD, Mann BJ, Shannon J, Fox JW, Vedvick TS, et al. Molecular cloning of a major cockroach *(Blattella germanica)* allergen, Bla g 2. Sequence homology to the aspartic proteases. J Biol Chem 1995;270:19563-8.
10. Arruda LK, Vailes LD, Hayden ML, Benjamin DC, Chapman MD. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses. J Biol Chem 1995;270:31196-201.
11. Arruda LK, Vailes LD, Platts-Mills TA, Hayden ML, Chapman MD. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach *(Blattella germanica)*. J Biol Chem 1997;272:20907-12.
12. Arruda LK, Ferriani VP, Vailes LD, Pomes A, Chapman MD. Cockroach allergens: environmental distribution and relationship to disease. Curr Allergy Asthma Rep 2001;1:466-73.
13. Helm R, Cockrell G, Stanley JS, Brenner RJ, Burks W, Bannon GA. Isolation and characterization of a clone encoding a major allergen (Bla g Bd90K) involved in IgE-mediated cockroach hypersensitivity. J Allergy Clin Immunol 1996;98:172-80.
14. Pomes A, Melen E, Vailes LD, Retief JD, Arruda LK, Chapman MD. Novel allergen structures with tandem amino acid repeats derived from German and American cockroach. J Biol Chem 1998;273:30801-7.
15. Wu CH, Wang NM, Lee MF, Kao CY, Luo SF. Cloning of the American cockroach Cr-PII allergens: evidence for the existence of cross-reactive allergens between species. J Allergy Clin Immunol 1998;101:832-40.
16. Wu CH, Lee MF, Liao SC, Luo SF. Sequencing analysis of cDNA clones encoding the American cockroach Cr-PI allergens. Homology with insect hemolymph proteins. J Biol Chem 1996;271:17937-43.
17. Asturias JA, Gomez-Bayon N, Arilla MC, Martinez A, Palacios R, Sanchez-Gascon F, et al. Molecular characterization of American

cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. J Immunol 1999;162:4342-8.
18. Melen E, Pomes A, Vailes LD, Arruda LK, Chapman MD. Molecular cloning of Per a 1 and definition of the cross-reactive Group 1 cockroach allergens. J Allergy Clin Immunol 1999;103:859-64.
19. Jeong KY, Lee J, Lee IY, Ree HI, Hong CS, Yong TS. Allergenicity of recombinant Bla g 7, German cockroach tropomyosin. Allergy 2003;58: 1059-63.
20. Wu CH, Lee MF, Wang NM. Expression of the American cockroach Per a 1 allergen in mammalian cells. Allergy 2000;55:1179-83.
21. Wu CH, Lee MF, Wang NM, Luo SF. Sequencing and immunochemical characterization of the American cockroach per a 3 (CR-PI) isoallergenic variants. Mol Immunol 1997;34:1-8.
22. Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A. Recombinant allergens for diagnosis and therapy of allergic disease. J Allergy Clin Immunol 2000;106:409-18.
23. Pomes A, Vailes LD, Helm RM, Chapman MD. IgE reactivity of tandem repeats derived from cockroach allergen, Bla g 1. Eur J Biochem 2002; 269:3086-92.
24. Erwin E, Wickens K, Custis NJ, Siebers R, Woodfolk J, Barry D, et al. Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. J Allergy Clin Immunol 2005;115:74-9.
25. Erwin EA, Custis NJ, Perzanowski MS, Woodfolk JA, Crane J, Wickens K, et al. Quantitative measurement of IgE antibodies to purified allergens using streptavidin linked to a high capacity solid phase. J Allergy Clin Immunol. In press 2005.
26. Carter MC, Perzanowski MS, Raymond A, Platts-Mills TA. Home intervention in the treatment of asthma among inner-city children. J Allergy Clin Immunol 2001;108:732-7.
27. Pittner G, Vrtala S, Thomas WR, Weghover M, Kundi M, Horak F, et al. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. Clin Exp Allergy 2004;34: 597-603.
28. Rowntree S, Platts-Mills TA, Cogswell JJ, Mitchell EB. A subclass IgG4-specific antigen-binding radioimmunoassay (RIA) for comparison between IgG and IgG4 antibodies to food and inhaled antigens in adult atopic dermatitis after desensitization treatment and during development of antibody responses in children. J Allergy Clin Immunol 1987;80: 622-30.
29. Lal G, Balmer P, Joseph H, Dawson J, Borrow R. Development and evaluation of a tetraplex flow cytometric assay for quantitation of serum antibodies to *Neisseria meningitidis* serogroups A, C, Y and W-135. Clin Diagn Lab Immunol 2004;11:272-9.
30. Pickering JW, Martins TB, Schroder MC, Hill HR. Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for quantitation of antibodies to tetanus, diphtheria, and *Haemophilus influenzae* type b. Clin Diagn Lab Immunol 2002;9:872-6.
31. de Jager W, te Velthuis H, Prakken BJ, Kuis W, Rijkers GT. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol 2003;10:133-9.
32. Lee C-S, Tsai L-C, Chao P-L, Lin C-Y, Hung M-W, Chien A-I, et al. Protein sequence analysis of a novel 103-kDa *Dermatophagoides pteronyssinus* mite allergen and prevalence of serum immunoglobulin E reactivity to rDer p 11 in allergic adult patients. Clin Exp Allergy 2004; 34:354-62.
33. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997;336:1356-63.
34. Arbes SJ, Sever M, Archer J, Long EH, Gore JC, Schal C, et al. Abatement of cockroach allergen (Bla g 1) in low-income, urban housing: a randomized controlled trial. J Allergy Clin Immunol 2003;112:339-45.