

31387

Shared Allergenic Activity in Asian (*Blattella asahinai*), German (*Blattella germanica*), American (*Periplaneta americana*), and Oriental (*Blatta orientalis*) Cockroach Species

Ricki M. Helm^a, Diane L. Squillace^a, Richard T. Jones^a, Richard J. Brenner^b

^a Department of Immunology, Allergic Diseases Research Laboratory, Mayo Medical School, Mayo Clinic and Foundation, Rochester, Minn.; ^b Agricultural Research Service, US Department of Agriculture, Gainesville, Fla., USA

Abstract. Whole-body extracts of the feral and peridomestic Asian cockroach (*Blattella asahinai*) and the three domestic cockroach species, German (*Blattella germanica*), American (*Periplaneta americana*), and Oriental (*Blatta orientalis*), were compared allergenically using an IgE serum pool from 4 German cockroach sensitive individuals. In crossover radioallergosorbent inhibition analysis, the Asian cockroach shared allergenic activity primarily with the German cockroach polymer and to a lesser extent with either the American or Oriental cockroach polymers. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and thin-layer isoelectric focusing analysis of the extracts showed similar although varying intensities of Coomassie blue stained banding patterns among five extracts analyzed. Electroblotting analysis with 12.5% SDS-PAGE of the whole-body German cockroach extract and IgE serum from individuals sensitive to German cockroach revealed eight allergenic components with apparent molecular weights of 92, 80, 67, 48, 36, 27, 25 and 18 kD. Five components could be identified in the whole-body extract of the Asian cockroach corresponding to apparent molecular weights of 92, 67, 48, 40, and 32 kD. Analysis of individual serum by immunoblot analysis with each of the cockroach extracts showed considerable heterogeneity in the IgE-binding pattern. Although the Asian cockroach demonstrated considerable cross-reacting allergenic components to German and relatively fewer cross-reacting allergenic components to either the Oriental or American, it is too early to establish genus- or species-specific cockroach allergens. It is important to point out that German cockroach sensitive individuals should be made aware of the potential exposure of Asian cockroach aeroallergens both indoors and outdoors in areas with high infestations of Asian cockroaches.

Introduction

Although over 4,000 species of cockroaches have been described worldwide, most are feral and are rarely encountered by man. Only about 50 species are pestiferous, and only 8 or 10 substantially affect our health and welfare [1]. Generally, these species originated in Africa and Asia and have infested most continents via commerce routes [2]. Most are peridomestic, such as the American (*Periplaneta americana*) and oriental cockroaches (*Blatta orientalis*). These occur commonly in households throughout most of the United States, but also dwell in sewers and other habitats adjacent to homes. In contrast, the German cockroach, *Blattella germanica*, is strictly domestic; populations rarely survive outside of buildings. Today it is the most common household pest in all 50 states. In

recent years, several studies have documented respiratory sensitization to cockroach allergens of these species [3-9]. A clear demonstration of both an immediate and late-asthmatic reaction has been demonstrated by bronchoprovocation testing with cockroach antigen among patients with extrinsic asthma [10, 11].

During the early 1980s, a new cockroach species was introduced inadvertently into Florida [12]. Called the Asian cockroach (*Blattella asahinai*), it differs behaviorally from other pest species. Asian cockroaches are feral and peridomestic with populations in lawns and leaf litter commonly exceeding 100,000 per acre [13, 14]. Adults are strong fliers and are attracted to light-colored walls or brightly lit areas at sunset. Consequently, the species readily enters buildings and will infest homes, warehouses, stores, and restaurants.

Some involvement with agriculture also has been reported [15]. Since 1986, Asian cockroaches have spread from three counties in Florida to about 17. One infestation was confirmed in Michigan, resulting from passive transport in a recreational vehicle. Thus, distribution of this species, and problems associated with it, likely will expand with time.

Asian cockroaches are so closely related morphologically and phylogenetically to German cockroaches that hybridization can occur [16, 17]. Consequently, we elected to study the allergenic cross-reactivity of the prominent allergens from these species using the serum of persons hypersensitive to the German cockroach allergens. Our aim in the present study was to determine if allergens present in whole-body extracts of German, American, and Oriental cockroaches shared cross-reactive allergens with allergens in whole-body extracts of the recently identified Asian cockroach [12].

Materials and Methods

Lyophilized (powdered, defatted) American, German, and Oriental cockroaches were supplied by Dome/Hollister-Stier Laboratories (Spokane, Wash., USA). Frozen whole-body Asian and German (Orlando strain) cockroaches (male and female) were obtained from research colonies maintained at Insects Affecting Man and Animals Laboratory (USDA-ARS, Gainesville, Fla., USA). These colonies were fed a diet of Purina Rodent Laboratory Chow.

To prepare extracts from the commercially prepared lyophilized powdered cockroach preparations, phosphate-buffered saline (PBS) was added (20 ml/g) and extraction performed at 4°C for 48 h with end-over-end tumbling. The extracts were clarified by centrifugation and the supernatant collected and stored at -20°C or as a 50% (v/v) glycerinated extract at 10°C. Frozen whole-body Asian and German cockroaches were separately crushed in a mortar and pestle, defatted for 3 h with petroleum ether in a Soxhlet extractor, and dried at room temperature. Defatted cockroaches were weighed and extracted (1:10, w/v) in PBS as described above. Protein was determined by the BCA procedure (Pierce Chemical Co., Rockford, Illinois) using bovine serum albumin as a standard.

Human IgE antibodies: A serum pool containing elevated IgE to German cockroach allergens was prepared using sera from 4 urban patients from Chicago who were cockroach sensitive as determined by clinical history, skin test, and radioallergosorbent testing (RAST; sera kindly provided by Dr. Bann Kang, University of Kentucky, Lexington, Ky., USA). None of the patients was receiving cockroach immunotherapy.

Preparation of Solid-Phase Allergens

Solid-phase allergens were prepared using conventional procedures [18]. The three Dome/Hollister-Stier (D/H-S) cockroach preparations (American = D/H-S Am, German = D/H-S Ger, and Oriental = D/H-S Or) were coupled to polyacrylamide beads (Ma-

trex Pel 102; Amicon, Lexington, Mass., USA), and the two extract preparations from the USDA (Asian and Orlando German) were coupled to Avicel microcrystalline cellulose (Brinkman Instruments, Des Plaines, Ill., USA). The Orlando German cockroach is the WHO insecticide-susceptible reference strain. Fifty microliters of the respective extract (1:10, w/v) was reacted with 500 mg of support in 0.05 M borate buffer, pH 8.0 (total reaction volume 7.5 ml). Mixtures were tumbled end-over-end overnight at 4°C and washed with borate buffer until the optical absorbance at 277 was less than 0.05. Greater than 90% of the material applied was coupled to the respective solid support. Solid-phase supports were diluted to 1 mg/ml with RAST diluent and stored at 4°C.

Measurement of Allergenic Activity

The allergenic activity was determined by RAST inhibition assays with various solid-phase allergens and a pool of German cockroach-reactive sera as described [18]. Briefly, in stage I, 500 µl of support, 50 µl of pooled serum, and varying amounts of free allergen (inhibitor) in a total volume of 1.0 ml (sufficient quantity with RAST diluent) in a 5-ml test tube were mixed with constant agitation overnight at room temperature. Mixtures were washed three times with RAST wash the following day and in stage II 100 µl of radiolabeled sheep antihuman IgE (200 ng/ml) and 900 µl of RAST diluent were mixed as above. The mixtures were washed three times and counted in a gamma scintillation counter. Assay results were analyzed by regression analyses (percent inhibition vs. log dilution inhibitor) using a Hewlett-Packard 9845 B computer (Hewlett-Packard, Palo Alto, Calif., USA).

Thin-Layer Isoelectric Focusing (TLIEF) and Blotted Radioisoelectric focusing (BRIEF)

TLIEF was performed using LKB PAG plates (pH 3.5-9.5) and the LKB Multiphor system (LKB Pharmacia, Piscataway, N.J., USA). Replicate samples were applied at the cathode margin and run at constant current for 45 min at 10°C; wicks containing the extracts were removed and electrofocusing continued at 900 V for 60 min at 10°C. After electrofocusing, one replicate pattern was removed and stained with Coomassie blue following the LKB staining procedure. BRIEF was carried out with the remaining unstained portion of the gel capillary transfer to a nitrocellulose membrane for 3 h [19]. The remaining active sites on the nitrocellulose blot were blocked with PBS containing 0.5% Tween 20 for 60 min at room temperature. The nitrocellulose print was incubated with the human IgE pools (1:3 v/v RAST diluent) overnight at room temperature, washed three times (30 min each in PBS), and incubated with 10 ml radiolabeled sheep antihuman IgE (20 ng/ml) overnight at room temperature. The immunoprint was washed three times as above and exposed to X-Omat X-ray film with intensifying screens (Kodak) at -70°C. X-ray film was developed in a Kodak X-Omatic developer following different storage periods (1-5 days).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Immunoblotting

SDS-PAGE was performed using a 12.5% resolving gel following the method of Laemmli [20]. Protein samples were heated at 100°C in the presence of 0.5% SDS and 5% 2-mercaptoethanol for 3 min prior to electrophoresis. Replicate samples were applied in a 3% stacking gel and electrophoresed at 20 mA/gel until the tracking dye reached the end of the gel. The gel was divided and one portion stained with Coomassie brilliant blue (Bio-Rad Laboratories), or

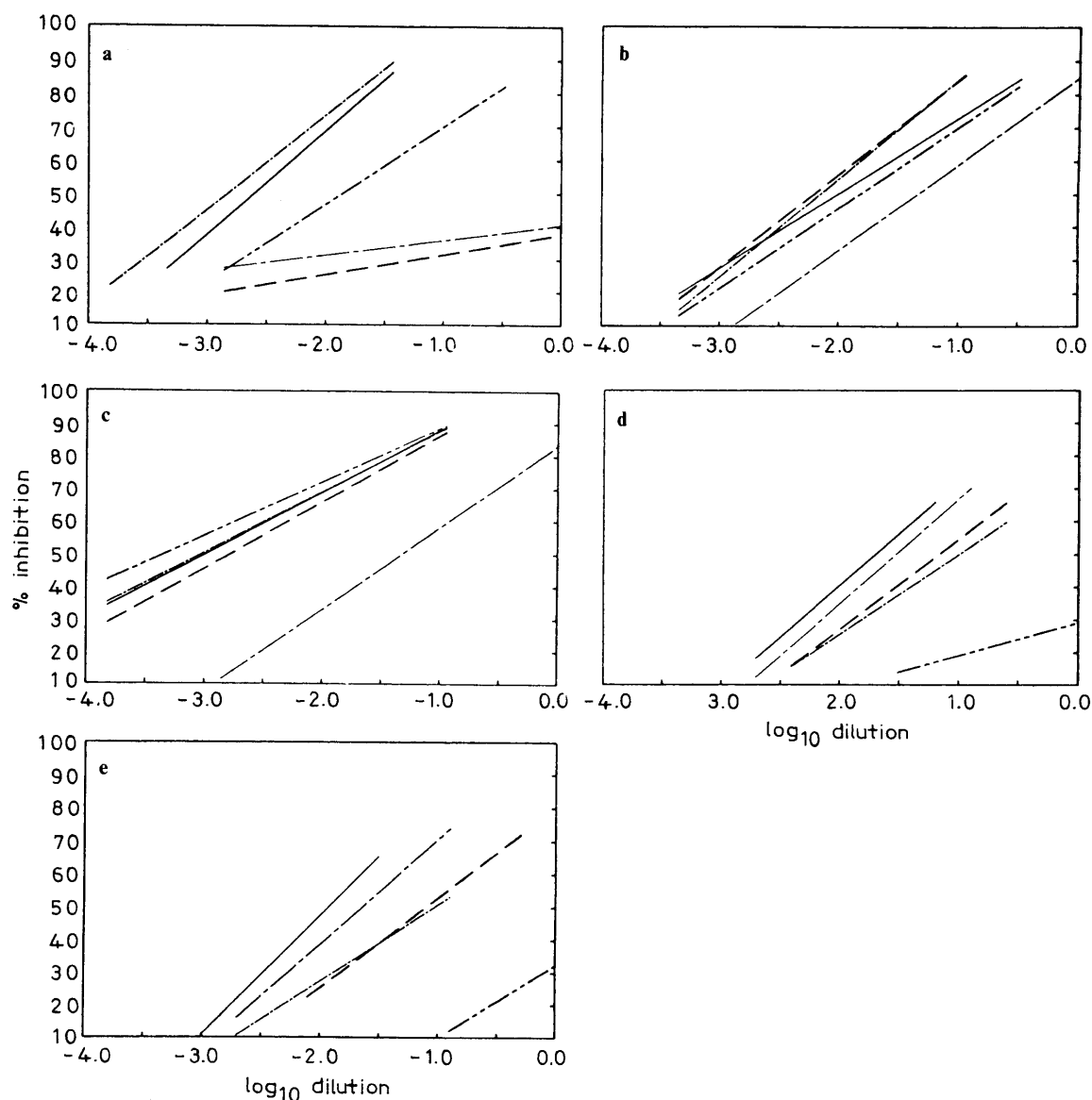


Fig. 1. RAST inhibition results using D/H-S Ger cockroach polymer (a), Asian cockroach polymer (b), Orlando German cockroach polymer (c), D/H-S Am cockroach polymer (d), and D/H-S Or cockroach polymer as solid-phase allergens (e). German cockroach-reactive human IgE antibody pool and various fluid-phase inhibitors: — = D/H-S Ger extract; - - - = D/H-S Am extract; — · — = D/H-S Or extract; - - - - = Asian extract; — · — · — = Orlando German extract. In the absence of inhibitors this serum pool produced binding of 8.24% (a), 3.95 (b), 3.34 (c), 3.54 (d), and 2.93% (e) of total counts, respectively. Normal serum used as controls produced binding of 0.07% (a), 0.21 (b), 0.12 (c), 0.16 (d), 0.15 (e) of total counts, respectively.

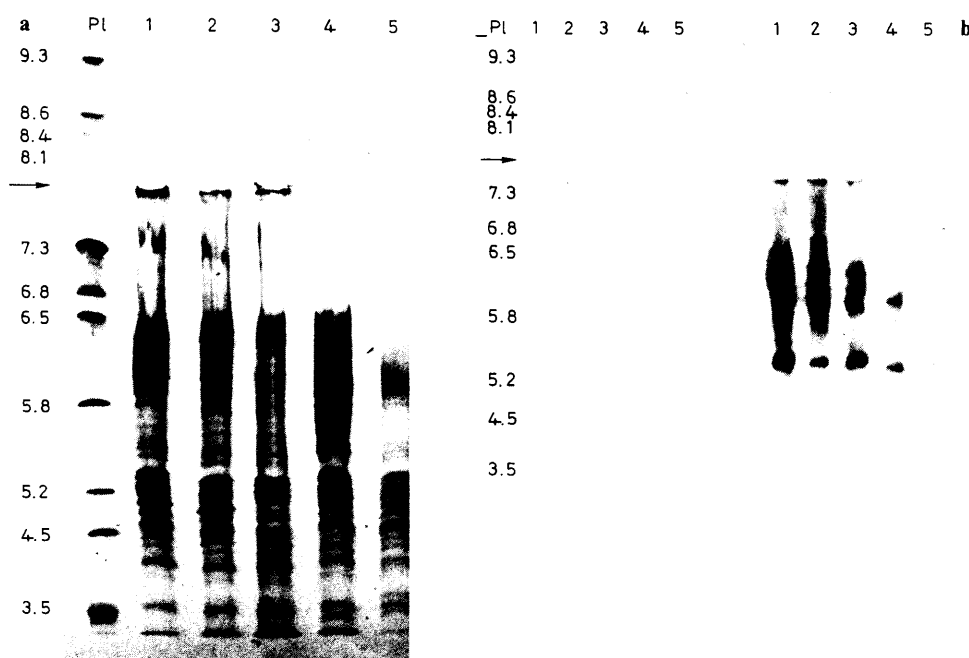
protein bands were transferred to nitrocellulose membranes [21]. Transfer was effected using the Bio-Rad transblot apparatus at a constant voltage of 15 V overnight. Following transfer, nitrocellulose membranes were washed in PBS containing 0.5% Tween 20 for 60 min to block the remaining active sites. Nitrocellulose membranes were then prepared for immunoprint analysis as outlined for BRIEF analysis.

Results

RAST Inhibition

Cockroach extracts covalently linked to solid supports were used to analyze the cross-reactivity of fluid-phase extracts in RAST inhibition experiments

Fig. 2. **a** Coomassie blue stained TLIEF gel (pH 3.5–9.5). **b** Autoradiography of TLIEF gel blot using serum from a nonallergic control (left) and German cockroach-reactive human IgE serum pool (right) and radiolabeled anti-IgE. Lanes from left to right represent isoelectric point (pI) markers: lane 1 = D/H-S Ger cockroach extract; lane 2 = Orlando German cockroach extract; lane 3 = Asian cockroach extract; lane 4 = D/H-S Am cockroach extract; lane 5 = D/H-S Or cockroach extract. Approximately 200 µg of each extract was applied at the application site indicated by the arrow.



(fig. 1). Each of the extracts was tested for its ability to inhibit binding of IgE from pooled German cockroach sensitive individuals to the solid-phase preparations. In all of the solid-phase systems tested, the Orlando German and D/H-S German cockroach extracts showed extensive cross-reactivity with the Asian cockroach polymer, all cockroach extracts showed appreciable inhibition. With either the D/H-S Am or D/H-S Or cockroach polymers, less than 40% inhibition was shown with the Asian cockroach extract, even at the highest inhibitor concentration tested. Only with the D/H-S Am and D/H-S Or cockroach polymer system was the rank order at the 50% inhibition level the same (D/H-S Ger > D/H-S Or > D/H-S Am > Orlando German > Asian) irrespective of the slope of the curve. Although the D/H-S Or and D/H-S Am cockroach extracts failed to inhibit more than 40% in the D/H-S Ger cockroach polymer system, these two extracts showed good inhibitory capabilities in the other solid-phase system. We concluded that our IgE serum pool used in these assays had antibodies which recognized allergens presented in each of the polymers, and an appreciable degree of cross-reactivity was demonstrated.

However, the results suggested that considerable

heterogeneity exists with respect to epitope density and expression of these allergens on the solid-phase systems analyzed.

TLIEF/BRIEF

Analysis of the five extracts by TLIEF showed similar staining patterns between pH 3.5 and 7.0 with no cathodal bands evident (fig. 2a). A remarkable similarity in the extracts are evident in the pH 3.5–5.5 region of the gel, with less similar banding patterns evident in the pH 5.5–7.0 region of the gel. Intense staining was seen at approximately pH 6.0 for Orlando German and D/H-S Ger cockroach extracts, whereas less intense staining was noted at pH 5.8 for the D/H-S Am cockroach extract and even less intense with the Asian and D/H-S Or extracts. The complexity of the staining pattern was also evident in BRIEF analysis (fig. 2b). Prominent radiostaining was seen in the pH 6.0, 6.2, and 5.4 regions of the autoradiograph for D/H-S Ger, Orlando German, Asian, and D/H-S Am extracts.

SDS-PAGE/Immunoblot

Approximately 200 µg of the cockroach extracts was applied to a 12.5% SDS-PAGE gel. Coomassie blue stain-

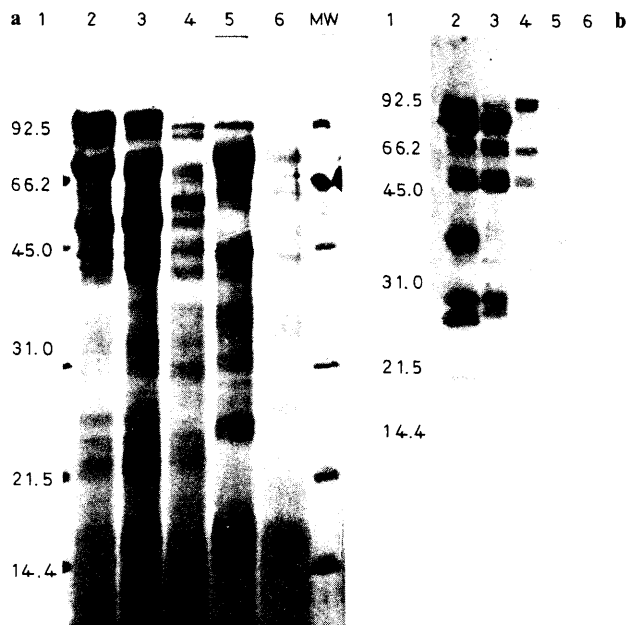


Fig. 3. a Coomassie blue stain of SDS-PAGE (12.5%) gel using cockroach extract. **b** Autoradiography of SDS-PAGE gel blot using German cockroach reactive human IgE serum pool and radiolabeled anti-IgE. Lanes from left to right: lane 1 = molecular weight (MW) markers; lane 2 = D/H-S Ger cockroach extract; lane 3 = Orlando German cockroach extract; lane 4 = Asian cockroach extract; lane 5 = D/H-S Am cockroach extract; lane 6 = D/H-S Or cockroach extract. Approximately 200 μ g of each extract was applied.

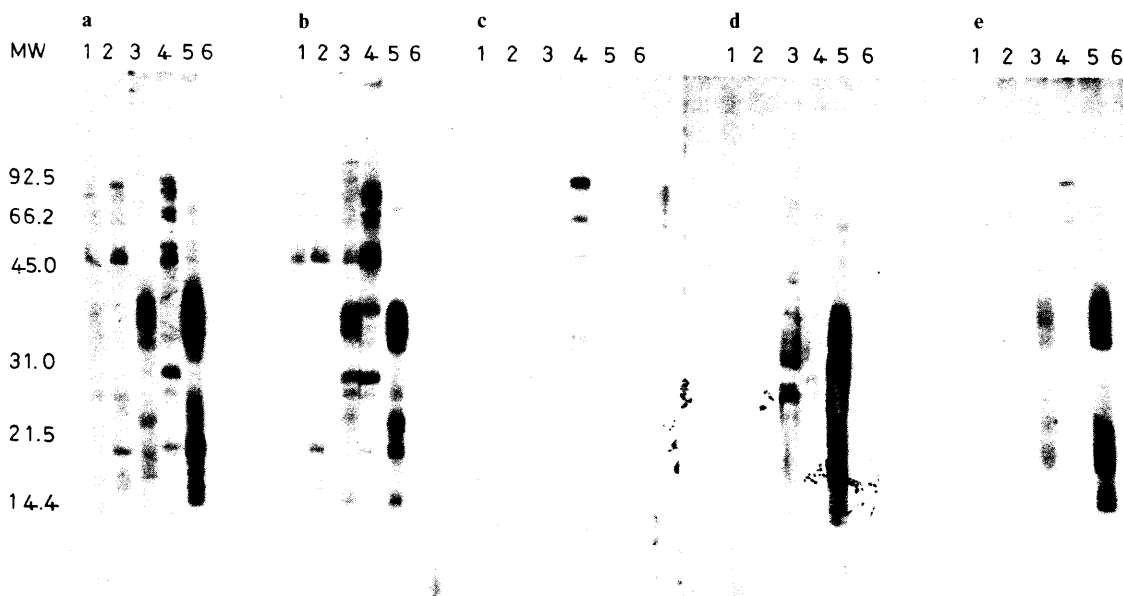


Fig. 4. Autoradiography of SDS-PAGE gel blot using German cockroach reactive human IgE from individuals used to prepare the IgE serum pool and radiolabeled anti-IgE. **a** Asian cockroach extract blots (350 μ g). **b** D/H-S Ger cockroach extract blots (353 μ g). **c** Orlando German cockroach extract blots (353 μ g). **d** D/H-S Am cockroach extract blots (382 μ g). **e** D/H-S Or cockroach blots (349 μ g). In each panel, lane 1 = German cockroach reactive human IgE serum pool; lanes 2-5 = German cockroach reactive human IgE from 4 individuals (patients 1-4) used to prepare the serum pool; lane 6 = control serum from a nonallergic individual. In a direct RAST analysis using a German cockroach polymer, each serum bound 4.0, 5.4, 9.7, 13.1, 8.5, and 0.2 total counts, respectively. MW = Molecular weight.

ing patterns of the gel demonstrated heterogeneity in both the staining intensity among the extracts and within each extract (fig. 3a). The D/H-S Or cockroach extract showed the least intense staining, even though 200 μ g of protein equivalent was applied. The Orlando German and D/H-S Ger cockroach extracts were very similar with the darkest staining bands having apparent molecular weights of 43, 48, 67, and 92 kD. Two distinct bands at approximately 92 kD in the Asian cockroach extract can be identified in the Orlando German cockroach extract and as only one band in the same region in the D/H-S Am cockroach extract. Several bands can be identified below the 45-kD region in each of the extracts; however, they are less distinct. The immunochemical characteristics of the crude extracts are shown in the autoradiograph from immunoblot analysis using cockroach-reactive human IgE and radiolabeled anti-IgE (fig. 3b). In the Orlando German and D/H-S Ger cockroach lanes, eight radiostained bands can be clearly identified with apparent molecular weights of 18, 25, 27, 36, 48, 67, 80, and 92 kD. Four bands at 32, 40, 48, and 67 kD and two additional bands at approximately 92 kD are prominent in the Asian cockroach extract. The D/H-S Am cockroach extract showed bands at 36, 67, and 92 kD, and only the 36-kD band was evident in the D/H-S Or cockroach extract. In both the Orlando German and Asian cockroach extracts the 36-kD band is less prominent and show bands on either side of this major staining band when compared to the three commercial preparations.

We further analyzed the extent of IgE-binding components in the various extracts by using the individual sera used to prepare our serum pool and a negative control serum against each of the cockroach extracts (fig. 4). The heterogeneity of the IgE-binding patterns demonstrated by individual serum to each cockroach extract is apparent. Sera 1 and 2 contained IgE directed to only a few components, and serum No. 6 (negative control) failed to bind any component in the extracts tested. Serum No. 5 in the Orlando German immunoblot strip was also negative, suggesting a technical problem with this immunoblot, because serum No. 5 reacted extremely well in the remaining blots tested. Consistent throughout the individual immunoblots is the radiostaining of bands with apparent molecular weights of 36, 48, 67, and 92 kD. Although all cockroach-reactive sera tested contained IgE antibodies in the immunoblots, each sera demonstrated unique binding patterns, showing the heterogeneity in individual responses to cockroach allergens.

Discussion

Using a serum pool from individuals clinically defined as German cockroach sensitive we performed RAST inhibition, TLIEF/BRIEF, and SDS-PAGE/immunoblot analysis with crude whole-body extracts of five cockroach preparations. We were able to demonstrate cross-reactive allergens by RAST inhibition among all cockroach species tested. We further identified acidic components (pH 4–6) and separated up to eight allergens by SDS-PAGE/immunoblot analysis with apparent molecular weights of 18, 25, 27, 36, 48, 67, 80, and 92 kD. Although 200- μ g protein equivalents of D/H-S Am and D/H-S Or whole-body extracts were analyzed by SDS-PAGE/immunoblot analysis, an insufficient or total lack of antigens would account for the diminished number of bands identified by our serum pool. Alternately, the total protein content could be subject to the limitations applied to allergenic extracts in which complex mixtures of proteins, carbohydrates, stabilizing agents, and preservatives may interfere with the detection of their complex mixtures.

The extent of cross-reactivity to American and German whole-body extracts to whole-body German cockroach polymers in our studies is in agreement with the prior studies by Richman et al. [4]. Data obtained by RAST inhibition in their studies indicated that allergens of German and American cockroach extract preparations demonstrated little cross-reactivity. Our cross-inhibition studies demonstrated similar low levels of cross-reactivity using either D/H-S Am or D/H-S Or cockroach extracts as inhibitors with D/H-S Ger cockroach extract solid-phase polymer and a German cockroach sensitive IgE antibody serum pool. However, all fluid-phase extracts showed a good RAST inhibition using the Asian cockroach extract solid-phase polymer and the IgE serum pool. Conversely, the Asian extract showed little inhibitory capacity with either D/H-S Am or D/H-S Or cockroach extract solid-phase polymers and IgE serum pool. Although direct RAST indicated that extracts of whole bodies, cast skins, and egg cases of the German cockroach contained the clinically relevant allergens [4], RAST inhibition studies demonstrated cross-inhibition to American hemolymph by American, German, and Brown-banded cockroach whole-body extracts [22]. Collectively, these results suggest that the allergen concentration and/or presentation exposed on the polymers may vary significantly and lead to an

oversimplification or misinterpretation of the results. Shared epitopes on allergens may be present among all cockroach species and could be dependent on differences in the respective allergen concentration. A simple variation in the qualitative/quantitative display of antigens/allergens on solid-phase polymers, respectively, or in extracts would account for the reduced inhibitory capacity of one extract with respect to other extracts. Moreover, the heterogeneous IgE antibody response seen when individual sera are reacted with the respective extracts and the pooling of these sera may account for the diverse inhibition demonstrated in analyses using pooled IgE serum.

Although it has been regarded that the German cockroach is the most widespread pest and, therefore, has the highest immunogenic potential, environmental exposure to any species of cockroach will play an important role in cockroach sensitivity. Environmental surveys conducted by Stankus and O'Neil [23] confirmed the presence of both American and German cockroach species in households studied in New Orleans. Skin test analysis with American and German whole-body extracts demonstrated reactivity to German whole-body extract alone in only 2 subjects and reactivity to both in 52 of 100 atopic subjects tested. Skin prick test, direct RAST, and RAST inhibition supported their theory on the occurrence of species-specific and shared interspecies allergens existing in cockroach extracts. Our data using RAST inhibition and SDS-PAGE/immunoblot analysis would be in direct agreement with their hypothesis.

Twarog et al. [8] were able to show concordance of skin test reactivity to crude American and German whole-body extracts. They also identified three major allergens with apparent molecular weights of <10, 25, and 63–65 kD using sequential purification steps on Sephadex G-75, DEAE-cellulose, and agarose gel electrophoresis. However, no concordance was shown among individuals having positive skin tests with their purified components, suggesting to them that sensitization to each was independent and that their proteins did not merely represent complexes of smaller subunits. Although we have not isolated similar components, our gel electrophoresis-immunoblot analysis suggests that allergenic components with relative molecular weights of 25 and 67 kD in both Asian and German whole-body extracts may be similar to the components described in their studies. Wu and Lan [24] recently identified nine allergenic components of molecular weights ranging from 26 to 120 kD

which bound IgE in electroblotting analysis, two of which (72 kD and 78 kD) bound 100% of the sera from atopic patients sensitive to the American cockroach. Taken together, although variable molecular weights have been assigned to allergenic components, it would appear that major cross-reactive allergens exist and further suggest that common interspecies and possibly species-specific allergens may exist.

In summary, RAST inhibition and SDS-PAGE/immunoblot analysis have revealed common IgE-binding components in crude whole-body extracts from at least four cockroach species including *B. asahinai*, the Asian cockroach. Clearly the introduction of the Asian cockroach into the environment of Florida and the extensive cross-reactivity of allergens shared with the German cockroach will increase the potential allergen burden to cockroach-sensitive individuals. It is premature to assess the clinical importance of this potential based on the present studies; however, cockroach allergens with apparent molecular weights of 25, 36, 67, and 92 kD may be considered as principal cockroach allergens. Further isolation and characterization of genus and species-specific cockroach allergens are needed to confirm the molecular identity of these allergens. Studies are in progress to purify and characterize the major IgE-binding components of both the Asian and the German cockroach.

Acknowledgements

This work was supported in part by the Mayo Foundation. The authors wish to thank Dr. Bann Kang for the contribution of sera from cockroach-sensitive individuals and Ms. Janette Breeding for secretarial assistance. We also thank Dr. John W. Yunginger for his helpful suggestions and editorial comments during the course of this study.

References

- 1 Cornwell PB: The Cockroach. London, Hutchinson, 1968, vol 1.
- 2 Rehn JA: Man's uninvited traveler: The cockroach. *Sci Monthly* 1945;61:265–276.
- 3 Fraser BN: Cockroaches in relation to bronchial asthma in the Durban area. *S Afr Med J* 1979;55:637–638.
- 4 Richman P, Khan HA, Turkeltaub PC, Malveaux FJ, Baer H: The important sources of German cockroach allergens as determined by RAST analyses. *J Allergy Clin Immunol* 1984;73: 590–595.
- 5 Chang JL, Ryo UY, Kang B: Changes in free histamine in pe-

- ripheral circulation following cockroach antigen challenge in asthmatics. *Mt Sinai J Med (NY)* 1984;51:197-202.
- 6 Kang B, Sulit N: A comparative study of prevalence of skin hypersensitivity to cockroach and house dust antigens. *Ann Allergy* 1978;41:333-336.
- 7 Hulett A, Dockhorn R: House dust mite (*D. farinae*) and cockroach allergy in a midwest population. *Ann Allergy* 1979;42:160-165.
- 8 Twarog FJ, Picone FJ, Strunk RS, So J, Colten HR: Immediate hypersensitivity to cockroach: Isolation and purification of major antigens. *J Allergy Clin Immunol* 1977;59:154-160.
- 9 Helm RM, Bandele EO, Swanson MC, Campbell AR, Wynn SR: Identification of a German cockroach-specific allergen by human IgE and rabbit IgG. *Int Arch Allergy Appl Immunol* 1988;87:230-238.
- 10 Kang B, Vellody D, Homburger H, Yunginger J: Cockroach cause of allergic asthma: Its specificity and immunologic profile. *J Allergy Clin Immunol* 1979;63:80-86.
- 11 Kang B: Study on cockroach antigen as a probable causative agent in bronchial asthma. *J Allergy Clin Immunol* 1976;58:357-365.
- 12 Roth LM: *Blattella asahinai* introduced into Florida (Blattaria: Blattellidae). *Psyche* 1986;93:371-374.
- 13 Brenner RJ, Patterson RS, Koehler PG: Ecology, behavior, and distribution of *Blattella asahinai* (Orthoptera: Blattellidae) in central Florida. *Ann Entomol Soc Am* 1988;81:432-436.
- 14 Brenner RJ: Activity and distribution of cockroaches with implications to developing control strategies. *Proc Natl Conf on Urban Entomology*, held at College Park, Maryland, February, 1988, pp 33-40.
- 15 Brenner RJ: Asian cockroaches: Implications to the food industry and complexities of management strategies. *FDA Tech Bull*, in press, 1990.
- 16 Roth LM: A taxonomic revision of the genus *Blattella*: Caudell (Dictyoptera, Blattaria: Blattellidae). *Entomol Scand* 1985;22 (suppl):8.
- 17 Carlson DA, Brenner RJ: Hydrocarbon-based discrimination of three North American *Blattella* cockroach species (Orthoptera: Blattellidae) using gas chromatography. *Ann Entomol Soc Am* 1988;81:711-723.
- 18 Anderson MC, Baer H: RAST-Inhibition Procedure; Technical Report. Bethesda, Office of Biologies, Food and Drug Administration, 1981.
- 19 Reinhart MP, Malamud D: Protein transfer from isoelectric focusing gels: The native blot. *Anal Biochem* 1982;123:229-235.
- 20 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-685.
- 21 Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350-4354.
- 22 Steinberg DR, Bernstein DI, Gallagher JS, Arlian L, Bernstein IL: Cockroach sensitization in laboratory workers. *J Allergy Clin Immunol* 1987;80:586-590.
- 23 Stankus RP, O'Neil CE: Antigenic/allergenic characterization of American and German cockroach extracts. *J Allergy Clin Immunol* 1988;81:563-570.
- 24 Wu CH, Lan JL: Cockroach hypersensitivity: Isolation and partial characterization of major allergens. *J Allergy Clin Immunol* 1988;82:727-735.

Received: January 22, 1990

Accepted after revision: May 23, 1990

Correspondence to: Dr. Ricki M. Helm
Department of Pediatrics
Arkansas Children's Hospital
800 Marshall St.
Little Rock, AR 72202-3591 (USA)