

Allergenic structures in cockroach hypersensitivity

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The tissues from which cockroach allergens were derived were identified by use of serum IgE of five patients with allergic rhinitis and bronchial asthma as a result of hypersensitivity to German cockroach. RAST and skin test results demonstrated four of five patients to be positive to cockroach only, and one patient was tested positive to house dust mite as well. Allergen-specific IgE binding to German cockroach was investigated by cryostat sections by means of immunofluorescent test. In all patients with cockroach hypersensitivity, we found IgE bound to the gastrointestinal epithelium and contents of the intestinal tract. In four cases, IgE was also bound to the Malpighian vessels (equivalent in function to kidneys). In three cases, IgE was also bound to the ovarian cells. All cases revealed cytoplasmic staining. (J ALLERGY CLIN IMMUNOL 1991;87:626-30.)

The role of cockroaches in the provocation of allergic respiratory reactions has long been recognized.¹⁻³ The most important cockroach species in civilized countries are the German cockroach (*Blattella germanica*) and the American cockroach (*Periplaneta americana*).³⁻⁶ Rapid surges of cockroach populations are a phenomenon encountered in urban environments, allowing cockroach to become an increasingly important source of allergens.^{4, 6-12} Respiratory sensitization to cockroach WBEs has been reported a number of times. In these studies the highest allergenic activity was found in WBEs of the German cockroach, but cast skins, egg shells, and feces were also found to contain allergens.¹³ Allergenic proteins and major allergens have previously been investigated by researches with different immunologic methods.^{5, 14} In those studies working with RAST inhibition, the ultimate organic sources of allergens, however, remained unexplored.^{6, 13}

The most suitable materials for producing extracts for the diagnosis and therapy of cockroach hypersensitivity have been subject to speculation; therefore, in

Abbreviations used

PRIST: Paper radioimmunosorbent test
PBS: Phosphate-buffered saline
FITC: Fluorescein isothiocyanate
WBE: Whole body extract

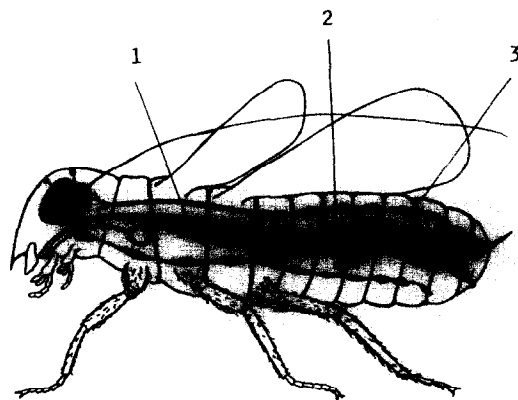


FIG. 1. Schematic representation of anatomic structures of a cockroach: 1, intestinal tract; 2, Malpighian vessel; and 3, ovarian cells.

the present study, we searched for allergenic structures of the German cockroach by means of micromorphologic methods.

MATERIAL AND METHODS

Study subjects

The study population was five patients, all of whom were suffering from allergic rhinitis and bronchial asthma caused by hypersensitivity to cockroach. All patients related their

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TABLE I. Patients' data

Patient No.	Total IgE (kU/L)	Specific IgE*	Wheal diameter of skin prick test to cockroach (mm)
1	112	cr (3)	6
2	363	cr (2)	4
3	580	cr (3)	5
4	106	cr (1)	5
5	338	cr (2), Dpt (2), Df (2)	5

cr, Cockroach; Dpt, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*.
*Phadezym RAST classes are listed in parentheses.

TABLE II. Allergenic structures of cockroaches

Patient No.	Epithelium of intestine	Feces	Malpighian vessel	Ovaries
1	Pos	Pos	Pos	Pos
2	Pos	Pos	Pos	Pos
3	Pos	Pos	Pos	Neg
4	Pos	Pos	Pos	Neg
5	Pos	Pos	Neg	Pos

pos. Positive; neg. negative.
The positive IgE immunofluorescent reactions of the patients' sera (dilution 1:20) in different tissues of the cockroaches are listed.

allergic complaints to their homes or workplaces in which they had been exposed to cockroaches. Positive nasal-challenge test results confirmed allergic sensitization to cockroach in two patients without definite anamnesis (patients 2 and 5). Four subjects from the laboratory staff with no record of cockroach exposure served as control subjects (two of these subjects were nonallergic [total IgE, 42 and 36 kU/L], and the remaining two subjects were allergic to house dust [total IgE, 156 and 635 kU/L]).

In all study subjects, total IgE was measured by Phadezym PRIST and allergen-specific IgE by Phadezym RAST (Pharmacia, Uppsala, Sweden), respectively.¹⁵ We measured allergen-specific IgE to cockroach (i6), house dust mites (d1 and d2), animal danders (e1, e5, and e6), birch pollens (t3), grass pollens (g3), mugwort pollens (w6), *Alternaria* (m6), *Cladosporium* (m2), and *Penicillium* (m1).

For the skin prick test we used a 1:20 (wt/vol) cockroach WBE containing 0.4% of phenol.⁴ Isotonic saline containing 0.4% of phenol served as negative control for the skin prick test, and it produced wheals of <2 mm in diameter in all study subjects. The skin prick test was considered positive when wheals that developed were 2 mm larger than wheals of the negative control. The skin test and Phadezym RAST results demonstrated all patients, but none of the control subjects, to be positive to cockroach allergens.¹⁵

Immunofluorescent test

Serum from the study subjects stored at -30° C for no longer than 6 months was used for immunofluorescent tests. Live German cockroaches (male and female) (*B. germanica*) were frozen at -20° C and embedded in Tissue Tek (Miles

Scientific, Naperville, Ill.). Serial cryostat sectioning of the entire body was performed on a sagittal plane, and the sections were then mounted on glass slides (Fig. 1). The study subjects' sera were diluted with PBS (pH 7.3) 1:20. The serial sections of the entire male and female cockroaches were incubated with the serum of each patient for 30 minutes at room temperature, rinsed three times in PBS, and thereafter incubated with mouse antihuman IgE FITC, antihuman IgG FITC, antihuman IgM FITC, or antihuman IgA FITC conjugates (Atlantic Antibodies, Scarborough, Maine; dilution 1:500 in PBS) for another 30 minutes. The slides were rinsed another three times in PBS, covered by PBS-glycerin (1:1), and investigated under an epifluorescence microscope.¹⁶ For investigating the intensity of immunofluorescent staining, we used cryostat sections that contained intestinal epithelium, Malpighian vessels, or ovarian cells and processed them in the above fashion with the patients' sera diluted 1:80. Negative controls were performed either without serum incubation or with serum of four separately investigated control subjects.

RESULTS

The patients' data are presented in Table I. All patients except for patient 5 proved sensitive to cockroach only. The results of immunofluorescent tests of serial cryostat-section investigations may be summarized as follows (Table II): Up to a serum dilution of 1:80, all patients revealed the strongest positive immunofluorescent staining with antihuman IgE conjugates on epithelial cells from the whole intestinal



FIG. 2. **A**, Immunofluorescent test on a cryostat section of a German cockroach. The cytoplasm of intestinal cells reveals a specific immunofluorescent staining. The lumen is marked with *L* and the basement membrane of the intestine with an *arrow*, respectively. **B**, Immunofluorescent test on the same anatomic structures and with the serum of a control subject. Under the same photomicrographic conditions, these structures reveal no specific staining. (Approximate original magnification $\times 60$.)



FIG. 3. **A**, The positive immunofluorescent test result of cytoplasmic structures of Malpighian vessel of a cockroach is marked with an *arrow*. **B**, The immunofluorescent test on Malpighian vessel and with the serum of a control subject. Under the same photomicrographic conditions, these structures reveal no specific staining. (Approximate original magnification $\times 250$.)

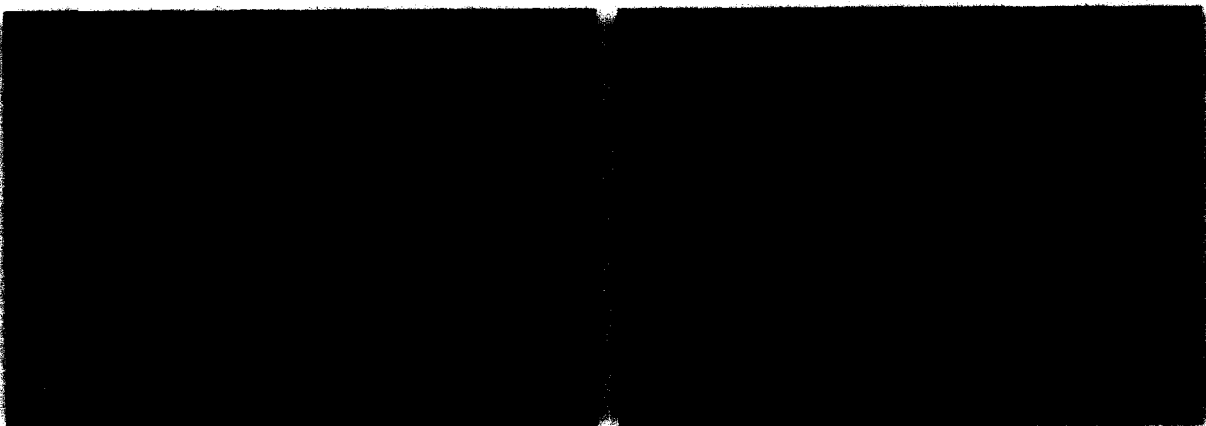


FIG. 4. **A**, The positive immunofluorescent test results of cytoplasmic structures of ovarian cells of a female cockroach is marked with an *arrow*. **B**, Immunofluorescent test on ovarian cells and with the serum of a control subject. These structures reveal no specific staining under the same photomicrographic conditions. (Approximate original magnification $\times 300$.)

tract (Fig. 2). In four patients we detected specific IgE binding to the Malpighian vessels; in three patients the specific IgE binding to ovarian cells was discovered (Figs. 3 and 4). Only at a serum dilution of 1:20 was the staining of these structures definitely positive. In all structures we observed exclusively cytoplasmic staining. The contents of the intestinal tract also produced IgE-specific immunofluorescent staining, which, however, was less intensive than that of the other structures. Because of strong autofluorescence, a definite assessment of the reactions to cast skins was impossible. All other organs and cellular structures demonstrated no IgE-specific immunofluorescent signal. The immunofluorescent staining of all structures was limited only to IgE. The negative controls, in which the sera of nonallergic subjects or subjects allergic to house dust mite were used separately, produced no positive reactions in any subject.

DISCUSSION

Since the first descriptions of cockroach allergy, several investigations have been performed to characterize the allergenicity of cockroach, whereas the organic structures of allergic reactions are still subject to speculation. In this allergomicromorphologic investigation, we searched for the source of allergenic materials in cockroach-sensitized patients. Serial cryostat sections in connection with the immunofluorescent test enabled us to assess all anatomical structures of the German cockroach. Thus, we were able to demonstrate that any binding to various organs was limited to IgE. Unspecific IgE binding has been precluded by different controls. In all our study subjects, both the sensitivity and the specificity of the immunofluorescent test, skin prick test, and Phadezym RAST were the same.

Immunofluorescent tests are a very sensitive means for the identification of immunoglobulins. Therefore, the fact that there was no IgG, IgM, or IgA reactivity in the study subject's sera is reason to attach great importance.^{16, 17} In our patients, the immunofluorescent reactivity limited to IgE therefore indicates an isolated hypersensitivity reaction.

As far as the different organic structures are concerned, all patients demonstrated strong reactions to cytoplasmic structures of epithelial cells from the intestinal tract. The contents of the intestinal tract, as well as the Malpighian vessels, equivalent in function to kidneys, and also ovarian cells, proved allergenic. These results reveal a better understanding of the high allergenicity of whole body extracts reported in former studies.^{6, 13} Proteins of all structures discussed are excreted by cockroaches, or may be found in the house dust containing putrefaction products of cockroaches, and may therefore account for allergic reactions. The

results of our study suggest the tissues from which allergenic proteins of WBEs and feces originate. Although individual differences in sensitization to cockroach may occur, the proteins derived from epithelial cells of the intestinal tract and the Malpighian vessels appear to be from the most important anatomic structures because they are found in feces as well as in whole bodies of dead cockroaches. Unlike other researchers,¹³ we therefore believe that WBEs are the most appropriate material for diagnosis and therapy of cockroach hypersensitivity.

Further immunoblotting analysis of the allergenic structures of cockroaches may reveal the origin of various major allergens previously described.^{5, 14, 18, 19} Together with the identification of these cockroach allergens in air samples,²⁰ the role of clinically relevant major allergens derived from the various organic structures might be elucidated.

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REFERENCES

1. Perlman F. Insects as inhalent allergens. *J ALLERGY* 1958; 29:302-28.
2. Perlman F. Insect allergens: their interrelationship and differences. *J ALLERGY* 1961;32:93-101.
3. Bernton HS, Brown H. Insect allergy—preliminary studies of the cockroach. *J ALLERGY* 1964;35:506-13.
4. Mendoza J, Snyder RD. Cockroach sensitivity in children with bronchial asthma. *Ann Allergy* 1970;28:159-63.
5. Twarog FJ, Picone FJ, Strunk RS, So J, Colten HR. Immediate hypersensitivity to cockroach: isolation and purification of the major antigens. *J ALLERGY CLIN IMMUNOL* 1977;59:154-60.
6. Steinberg DR, Bernstein DI, Gallagher JS, Arlian L, Bernstein IL. Cockroach sensitization in laboratory workers. *J ALLERGY CLIN IMMUNOL* 1987;80:586-90.
7. Bernton HS, Brown H. Cockroach allergy: age of onset of skin reactivity. *Ann Allergy* 1970;28:420-2.
8. Kang B. Study on cockroach antigen as a probable causative agent in bronchial asthma. *J ALLERGY CLIN IMMUNOL* 1976; 58:357-65.
9. Kang B, Sulit N. A comparative study of prevalence of skin hypersensitivity to cockroach and house dust antigens. *Ann Allergy* 1978;41:333-6.
10. Hulett A, Dockhorn R. House dust, mite (*D. farinae*), and cockroach allergy in a midwestern population. *Ann Allergy* 1979;42:160-5.
11. Kang B, Vellody D, Homburger H, Yunginger J. Cockroach cause of allergic asthma: its specificity and immunologic profile. *J ALLERGY CLIN IMMUNOL* 1979;63:80-6.
12. Lan JL, Lee DT, Wu CH, Chang CP, Yeh CL. Cockroach hypersensitivity: preliminary study of allergic cockroach asthma in Taiwan. *J ALLERGY CLIN IMMUNOL* 1988;82:736-40.
13. Richman PC, Khan HA, Turkeltaub PC, Malveaux FJ, Baer H. The important sources of German cockroaches as determined by RAST analyses. *J ALLERGY CLIN IMMUNOL* 1984; 73:590-5.
14. Wu CH, Lan JL. Cockroach hypersensitivity: isolation and partial characterization of major allergens. *J ALLERGY CLIN IMMUNOL* 1988;82:727-35.

15. Johansson SGO, Bennich H, Berg T. In vitro diagnosis of atopic allergy. III. Quantitative estimation of circulating IgE antibodies by the radioallergosorbent test. *Int Arch Allergy Appl Immunol* 1971;41:443-51.
16. Vorlaender KO. *Immunologie*. Stuttgart: Thieme Verlag, 1983.
17. Popp W, Zwick H, Rauscher H. Indirect immunofluorescent test on spore sampling preparations: a technique for diagnosis of individual mold allergies. *Stain Technol* 1988;63:249-53.
18. 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;87:230-8.
19. Stankus RP, O'Neil CE. Antigenic/allergenic characterization of American and German cockroach extracts. *J ALLERGY CLIN IMMUNOL* 1988;81:563-70.
20. Swanson MC, Agarwal MK, Reed CE. An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. *J ALLERGY CLIN IMMUNOL* 1985;76:724-9.

Reassessment of the temporal patterns of bronchial obstruction after exposure to occupational sensitizing agents

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*Typical asthmatic reactions after exposure to common or occupational allergens have been classified as isolated immediate, early late, late, and dual (Pepys and Hutchcroft, 1975⁶). Atypical reactions can also occur, but their temporal behavior and frequency are unknown. We analyzed 69 bronchospastic reactions after exposure to three types of occupational sensitizers in the laboratory: isocyanates, western red cedar, and IgE sensitizing agents. Graphs of the reactions were presented to three observers in a blind, randomized way. Reactions were defined as follows: (1) typical patterns, that is, isolated immediate, early late, late, and dual, as put forward by Pepys and Hutchcroft,⁶ and (2) atypical patterns including progressive (onset, during, or minutes after exposure, progressing to a maximum reaction 5 to 6 hours later) and square waved (similar to a dual reaction but with only partial recovery [$<10\%$] between the immediate and late falls in FEV₁). Some curves were also analyzed by polynomial regression. The three observers agreed in 59/69 cases (86%), and a consensus was reached after discussion for the remaining subjects. Fifteen of 63 (22%) reactions were atypical. IgE-mediated reactions and reactions to red cedar were, respectively, mainly of the immediate and late types. The distribution of reactions to isocyanates, however, was significantly different (chi-square, 6.1; $p = 0.01$), the "progressive" pattern occurring in 7/23 instances (30%). There was a satisfactory concordance between visual assessment and polynomial fit analysis in distinguishing dual from square-waved reactions. We conclude that isocyanates frequently cause atypical progressive bronchospastic reactions that are rarely observed after exposure to IgE agents and western red cedar. (*J ALLERGY CLIN IMMUNOL* 1991;87:630-9.)*

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