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Identification and purification of an important cross-reactive allergen from American (*Periplaneta americana*) and German (*Blattella germanica*) cockroach

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Aqueous whole body extracts from two major domiciliary cockroaches, the American, Periplaneta americana, and the German, Blattella germanica, were analyzed in crossed immunoelectrophoresis and immunoblotting. Forty-five antigens were found in P. americana and 29 in B. germanica. IgE-binding antigens were identified by crossed radioimmuno-electrophoresis with sera from 30 cockroach-allergic patients. Seven and three precipitates from P. americana and B. germanica bound significant amounts of IgE. A cross-reactive, apparently homologous allergen, from P. americana and B. germanica bound IgE from 100% and 70%, respectively, of the patients. These important allergens were tentatively named Per a I and Bla g I. The allergens were purified by sequential ion exchange, gel filtration, and isoelectric focusing. Both allergens had a molecular size of 33 to 37 kd in Sephadex G-75 gel filtration, and 28 kd in high-performance liquid chromatography gel filtration. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a minor band at approximately 25 kd, and most of the protein at 6 kd. The isoelectric point of both allergens was found below 3.5. In amino acid analysis, the allergens were highly similar. Skin test revealed the allergens to be important in vivo sensitizing agents. The allergens may be used for environmental assays for cockroach exposure in the homes of allergic subjects. (J ALLERGY CLIN IMMUNOL 1990;86:935-46.)

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Cockroaches have been demonstrated to represent a health problem to allergic subjects living in cockroach-infested environments.^{1, 2} Cockroaches cause direct cutaneous sensitivity^{3, 4} as well as inhalant allergy.⁵ Several studies have demonstrated that allergic subjects have a higher incidence of positive skin test reactions to cockroach extracts than

Abbreviations used

DEAE:	Diethylaminoethyl
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
FRIE:	Fused rocket immunoelectrophoresis
MW:	Molecular weight
RIE:	Rocket immunoelectrophoresis
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
CB:	Citrate buffer
WBE:	Whole body extract
IEF:	Isoelectric focusing
FE:	Fecal extract
k_{av} :	Distribution coefficient
HPLC:	High-performance liquid chromatography

normal control subjects.² This reaction pattern has been correlated to degree of exposure to cockroaches.

The aforementioned investigations have generally been performed with cockroach WBEs, and it has thus not been possible to study in detail the reactivity of each patient to individual allergens present in the test preparations. The nature of the allergenic material has received relatively little attention. The present study was undertaken to identify, purify, and characterize an important allergen from the two major domiciliary cockroaches of Europe and North America, that is, the American (*Periplaneta americana*) and the German (*Blattella germanica*). With the access to purified allergens, a standardization of commercial cockroach extracts will eventually become possible. Furthermore, studies of the relative importance of different allergens as sensitizing agents will benefit from unequivocally identified molecules.

MATERIAL AND METHODS

Raw materials

P. americana, *B. germanica*, and *Blatta orientalis* were obtained from cultures kept at the State Pest Infestation Laboratory, Lyngby, Denmark, and University of Copenhagen. Cultures contained all developmental stages. The insects were killed by freezing and cleaned by brushing before extraction. One gram of *B. germanica* contained about 42 individual cockroaches, and 6 gm of *P. americana* contained about 10 individual cockroaches. Fecal pellets of *P. americana* were collected by sieving through steel filters. Fecal pellets of *B. germanica* were collected manually under a dissecting microscope.

Defatting

Defatted cockroach bodies were produced by four consecutive treatments for 1 hour at 20° C with 6 vol of petrol-

ether. The defatted material was dried on filter paper before extraction.

Extraction

The insects were chopped in a hand-held onion chopper before extraction. Batch size was 20 gm. All extractions were performed at 10% wt/vol for 20 hours at 5° C in 0.125 mol/L of NH_4HCO_3 . The extract was centrifuged at 4° C for 60 minutes at 27,000 g. The supernatant was subsequently dialyzed (cutoff, 10,000 kd) at 5° C against 2000 vol of 5 mmol/L of NH_4HCO_3 for 40 hours, followed by dialysis against 50 vol of distilled water for 20 hours. The material was finally freeze-dried. Typical yield per batch extracted was 1.5 gm from *B. germanica* and 0.9 gm from *P. americana*.

Separation procedures

Freeze-dried extract was dissolved in 50 mmol/L of Tris HCl, pH 8.0, and applied to DEAE-Sepharose CL6B (Pharmacia, Ltd., Uppsala, Sweden) in the same buffer. The unbound fractions did not contain the allergens, *Per a* I or *Bla g* I, and was hence discarded. The bound material was subsequently eluted by sequential application of 50 mmol/L of Tris HCl, pH 8.0, containing 0.1, 0.15, 0.2, 0.3, 0.5, and 1.0 mol/L of NaCl. The fractions identified by FRIE and CIE to contain the allergen in question were pooled, dialyzed against 2000 vol of 5 mmol/L of NH_4HCO_3 as above, and freeze-dried.

The freeze-dried material was redissolved in 0.125 mol/L of NH_4HCO_3 and applied to a gel filtration column with Sephadex G-75 (Pharmacia Ltd.) in the same buffer. After chromatography, the fractions containing the allergen were identified by FRIE and CIE, pooled accordingly, and extensively dialyzed against distilled water. The pooled material was subjected to additional purification by IEF. IEF was performed in 50% (wt/vol) sucrose in water containing a total of 1% (vol/vol) of ampholyte (equal volumes of Ampholine, pH 2.5 to 4.0, and Ampholine, pH 4.0 to 6.0, LKB, Bromma, Sweden). The fractions eluted from the column after focusing were analyzed for the presence of allergen by FRIE and CIE, and pH was measured. Initial IEF covered the pH range from 2.5 to 11; *Per a* I and *Bla g* I focused at values <6; therefore, to obtain the best resolution, the preparative IEF range was chosen accordingly.

Protein concentration

Determination of protein concentration in the various allergen-containing fractions was performed according to the method of Lowry et al.⁶

HPLC gel filtration

The purified allergen samples were passed through a Superose 12 gel filtration column (Pharmacia Diagnostics) in 0.125 mol/L of NH_4HCO_3 on an HPLC system (LKB) equipped with a diode array detector covering 190 to 370 nm. Samples were run under nondenaturing conditions and after boiling for 2 minutes in 1% wt/vol SDS and 0.2 mol/L of dithiothreitol.

Patient population

The identification of cockroach allergens relied on serum from 30 patients that were skin test positive to cockroach WBE (B26, Greer Laboratories, Lenoir, N.C.). The patients were received at the Allergy and Immunology Clinics at the University of South Florida College of Medicine, Tampa, Fla. Blood was obtained from those patients who had a 2+, 3+, and 4+ skin test reactions. The following overall criteria for drawing blood were used: (1) patient was observed for the first time, (2) patient never received immunotherapy, (3) patient demonstrated clinical symptoms of allergic rhinitis and/or asthma, and (4) patient was resident of the Tampa Bay area.

To evaluate the allergenicity of the purified allergens, *Per a* I and *Bla g* I epicutaneous skin tests were performed with bifurcated needles (Allergy Laboratories of Ohio, Inc., Dayton, Ohio). Seventy-three consecutive patients attending an allergy clinic in Tampa, Fla., complaining of upper and lower respiratory symptoms, were skin tested with WBE of *B. germanica* and *P. americana*, and with purified allergens *Bla g* I and *Per a* I. The preparations of purified allergen were matched to the WBEs with respect to concentration of the two allergens.

A serum pool from 1000 healthy Danish blood donors was used as a negative control in CRIE and IgE immunoblotting experiments.

Immunoelectrophoresis

CIE and CRIE were performed essentially as previously described.⁷ Patient serum was incubated at 10% vol/vol overnight at room temperature. After plates were washed, they were incubated with iodinated antihuman IgE (a total of 125,000 cpm in our gamma counter) overnight. Washed and dried plates were autoradiographed for 30 days at -20° C with intensifying screens. Only CRIE scores⁸ of 4 or above were considered as positive reactions. FRIE was performed essentially as described by Svendsen.⁹ RIE was performed essentially as previously described.¹⁰

SDS-PAGE and autoradiography

Separation was performed on a discontinuous system; stacking gel, 4.5% T, 3% C, and separation gradient gel, 8% to 25% T, 2% C, in PhastSystem (Pharmacia Ltd.) at conditions according to manufacturers' instructions. Protein was revealed by silver-staining PhastSystem. When autoradiographs were required, the gels were fixed in 20% wt/vol of trichloroacetic acid for 5 minutes and washed for 2 minutes in 30% vol/vol of methanol and 10% vol/vol of formic acid in water. Gels were washed in distilled water and air-dried. Exposure of x-ray films (Kodak, Heidelberg, West Germany) was performed at room temperature or at -80° C.

Immunoblotting

SDS-PAGE gels used for immunoblotting had resolving gels of 16% acrylamide. Nitrocellulose used was GS filter from Millipore (Millipore GmbH, Eschborn, West Germany). After the blotting, residual binding sites were blocked in 0.5% Tween 20. Incubation was with *Ra Per a*

I and *Bla g* I overnight at 1:500 dilution. Incubation was with alkaline phosphatase-conjugated swine antirabbit immunoglobulin (code P217, DAKOPATTS, Glostrup, Denmark) 2 hours at 1:1000 dilution.

Radiolabeling

Iodination of the samples with ¹²⁵I was performed by the chloramine-T method. Unbound iodine was separated from protein by Sephadex G-25 gel filtration.

Amino acid analysis

Amino acid standard mixture was Beckman Instruments GmbH, Munich, West Germany, No. 338088; fluorescence detection, 338/450 nm; A buffer, citrate/nitrate, pH 3.10; B buffer, Na-borate, pH 10.10, both with 5% isopropanol; and MCI column P60, 0.46 × 12.5 cm.

Rabbit sera

Rabbit sera to WBEs of *P. americana* and *B. germanica* were raised by immunizing rabbits, subcutaneously, at monthly intervals with 1:1 (wt/wt) mixtures of defatted and nondefatted reconstituted freeze-dried extract of the species in question (dosage, 0.5 mg of protein in 0.05 ml plus 0.05 ml of Freund's incomplete adjuvant, three rabbits to each extract). Animals were bled monthly, and sera were pooled as *Ra Per a* and *Ra Bla g*. Rabbit serum to the *Per a* I allergen of *P. americana* was raised by immunizing three rabbits, subcutaneously, at monthly intervals with purified protein (dosage, 0.01 mg of protein in 0.05 ml plus 0.05 ml of Freund's incomplete adjuvant). Animals were bled monthly, and sera were pooled as *Ra Per a* I. Rabbit antibodies to determinants shared by allergens *Per a* I and *Bla g* I were immunopurified as outlined; 15 mg of purified *Bla g* I protein was coupled to 1.5 gm of CNBr-activated Sepharose 4B (Pharmacia Ltd.) as recommended by the manufacturer. Six milliliters of *Ra Per a* I was applied to the gel at room temperature. The gel was subjected to consecutive washes of 0.05 mol/L of sodium CB, pH 6.2, CB, pH 6.2, plus 0.5 mol/L of NaCl, and CB, pH 2.6 plus 1.0 mol/L of NaCl. The pH of eluted fractions was immediately brought to 7 with NaHCO₃. The eluted antibodies were revealed by FRIE with swine antirabbit immunoglobulin (code Z 196, DAKOPATTS) and pooled accordingly. After extensive dialysis against 0.005 mol/L of NH₄HCO₃ and, subsequently, against distilled water, the antibodies were freeze-dried and weighed. (Typically, 4.5 mg of immunoglobulin was purified from 6 ml of serum.) The ensuing preparation was called *Ra Per a* I and *Bla g* I. This antibody reacted with only *Per a* I and *Bla g* I when it was tested as peroxidase-labeled preparation in CIE overlay technique, as previously described.¹¹

The rabbit sera to cockroaches did not react in CIE with extracts of the food on which the insects were reared.

RESULTS

Identification

Aqueous extracts were produced from whole bodies of two major domiciliary cockroaches, that is, the American (*P. americana*) and the German (*B. ger-*

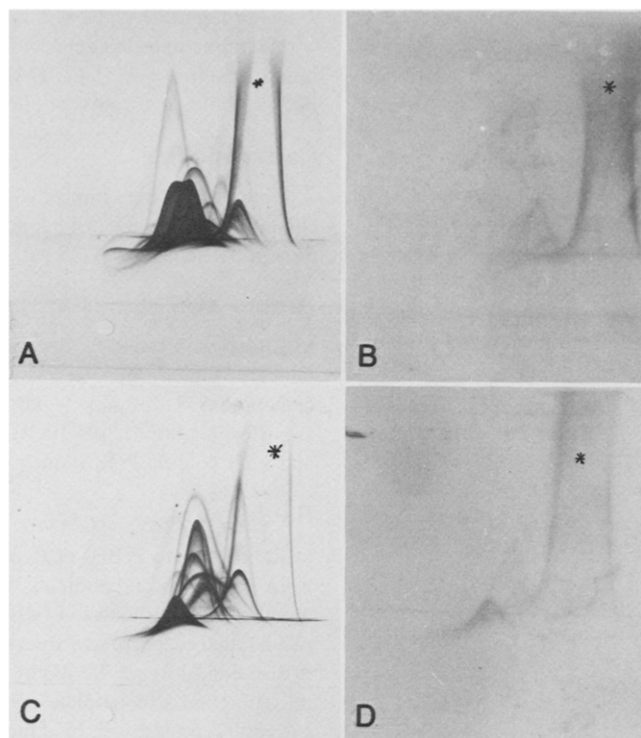


FIG. 1. A-B, CIE and CRIE analyses of extracts of whole cockroach bodies, *P. americana*, and **C-D,** *B. germanica*; 50 μ g of protein was applied to the first dimension. The second dimension gels contained rabbit antibody to the respective extracts. **A and C,** In CIE, it was possible to identify 45 antigen precipitates in the *P. americana* extract and 29 in that of *B. germanica*. **B and D,** CRIE gels were incubated with serum from one cockroach-allergic patient. The results presented are representative of results obtained with sera from a panel of 30 patients. Allergens *Per a* I and *Bla g* I are marked with an asterisk. Several other precipitates bound IgE but are, at the present time, considered to be less important allergens.

manica). These extracts were subjected to analysis in CIE with pooled rabbit sera raised against these antigen preparations. Protein-stained CIEs of the two cockroach extracts revealing 45 antigen precipitates in *P. americana* and 29 in *B. germanica* are illustrated in Fig. 1, A and C. None of the antigens revealed by this technique migrated toward the cathode. The extracts of defatted cockroaches contained fewer antigens than nondefatted preparations, as determined by CIE (data not presented), and all subsequent work was therefore performed on nondefatted material.

By means of CRIE, several allergens were identified in the extracts (Fig. 1, B and D). Individual sera from 30 cockroach-allergic patients were used to identify the allergens present. Seven IgE-binding precipitates were found in *P. americana* and three were found in *B. germanica*. With the sera selected for the present work, the CRIE analysis required 30 days of exposure to obtain reasonable staining intensities. The precipitate marked by asterisk in the CIEs and CRIEs of Fig. 1 was identified as an important allergen of both

species of cockroach. The allergen reacted with specific IgE from 21 of 30 patients in CRIE toward *B. germanica* and with 30 of 30 patients in CRIE toward *P. americana*. The allergens were tentatively named as *Bla g* I and *Per a* I. The allergens were present in commercial cockroach extracts from three American manufacturers tested, including B26 from Greer Laboratories (CIE data not presented).

IgE blot experiments were performed with WBEs of domiciliary cockroaches (Fig. 2). Several bands in the MW range from approximately 6 kd to 100 kd bound IgE from cockroach-allergic patients. A very prominent allergen was visible at 6 kd. Immunoblots with rabbit antibody to WBEs revealed numerous bands in the same MW range (data not presented).

As described below, the important allergen, *Per a* I from *P. americana*, was purified and used to raise rabbit serum with high titers against *Per a* I. Because of minor contaminants in the first immunogen preparations, the serum obtained was not completely monospecific, as revealed by additional precipitates

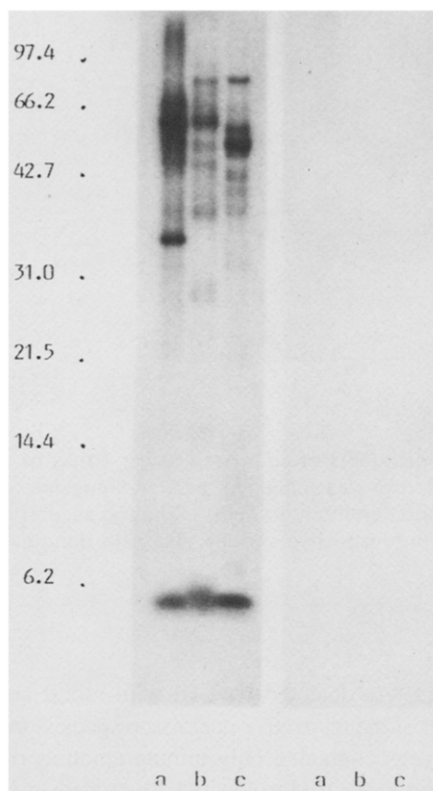


FIG. 2. IgE binding to WBEs of cockroaches; 5 μ g of protein of each extract was separated in SDS-PAGE before blotting to nitrocellulose membrane; lane a, *P. americana*; lane b, *B. germanica*; lane c, *Blattella orientalis*. The left blot was incubated with a pool of serum from cockroach-allergic subjects (UVA 89/01; a kind gift from Dr. Susan Pollart, Charlottesville, Va.). The right blot was incubated with serum from nonallergic subjects; MW in kilodaltons.

in Figs. 3 and 4, A. This antiserum has been used to reveal an extensive antigenic cross-reactivity between *Per a* I and the corresponding allergen from *B. germanica*, *Bla g* I. A CIE of *B. germanica* WBE that has been precipitated in the top second dimension gel by rabbit serum to *B. germanica* WBE is illustrated in Fig. 3. The intermediary second dimension gel, however, contained rabbit serum to *Per a* I and was observed to completely retain the *Bla g* I allergen. This finding was taken as evidence for an extensive homology between the two molecules at the antigenic level.

The allergens *Per a* I and *Bla g* I resisted boiling for up to 15 minutes before losing the ability to be precipitated by rabbit antibodies in CIE (data not presented).

As will be discussed later, other investigators have noted allergenic activity in cockroach feces. It was consequently relevant to the present work to determine

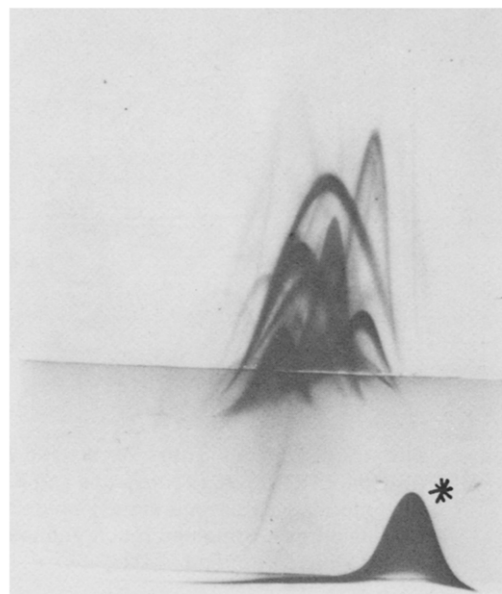


FIG. 3. CIE illustrating the cross-reaction between *Per a* I and *Bla g* I; 50 μ g of protein of *B. germanica* WBE was applied to the first dimension. The intermediary second dimension gel contained rabbit antibody to *Per a* I; the top second dimension gel contained rabbit antibody to *B. germanica* WBE. *Bla g* I was completely retained by the antibody to *Per a* I. *Bla g* I is marked with an asterisk.

if the two allergens were constituents of the fecal particles. CIEs of *P. americana* and *B. germanica* FEs that have been precipitated in the top second dimension gel by rabbit serum to the respective WBE are illustrated in Fig. 4. The intermediary second dimension gel contained rabbit serum to *Per a* I and was observed to completely retain both allergens from the fecal preparations. As measured by RIE with the purified allergens as reference (Table I), *Per a* I constituted up to 16.5% of the protein content in *P. americana* FEs (feces collected by sieving). *Bla g* I constituted about 5.5% of the protein content in *B. germanica* FEs (feces collected manually). Cockroaches are known to be cannibalistic,¹² and it is therefore, at the present time, not possible to determine if the two allergens originate in the digestive tract of the insects or if they come from other parts of the anatomy that are eaten and not fully digested.

Purification of allergens

The purification of allergens *Per a* I and *Bla g* I relied on sequential subjecting of the allergen-containing fraction to DEAE ion-exchange chromatography, Sephadex G-75 gel filtration, and preparative IEF. The initial purification of *Per a* I for immunization purposes was followed by CIE.

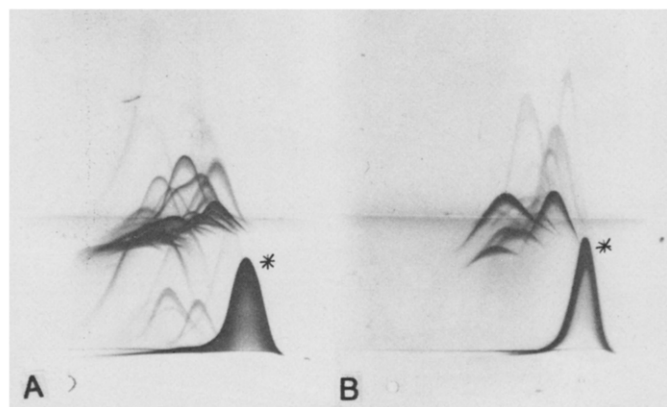


FIG. 4. CIE illustrating that *Per a* I and *Bla g* I are constituents of cockroach feces; 50 μ g of protein of FE from **A**, *P. americana* and **B**, from *B. germanica* was applied to the first dimension. The intermediary second dimension gels contained rabbit antibody to *Per a* I. The top second dimension gels contained rabbit antibody to WBE of the respective species. Asterisks denote **A**, *Per a* I and **B**, *Bla g* I.

TABLE I. *Per a* I/*Bla g* I content in cockroach extracts

	<i>P. americana</i> (%)	<i>B. germanica</i> (%)
WBE	8.3	11.0
FE	16.5	5.5
PF	>99.0	>99.0

PF, Purified allergen fraction.

Concentration of *Per a* I and *Bla g* I in *P. americana* and *B. germanica*, respectively; percentage of protein content according to the method of Lowry et al.⁶ in WBE, FE, and PF.

As described in MATERIAL AND METHODS, the identification of fractions containing *Per a* I and *Bla g* I was, at each purification step, performed with FRIE and CIE of the individual fractions against rabbit antibody to the relevant cockroach species. The two allergens had fractionation properties very much alike; that is, they eluted at similar conditions during the three purification steps.

The fractions containing the allergen in question eluted from DEAE-Sephacel at 0.3 mol/L of NaCl. Under the subsequent gel filtration on Sephadex G-75, the allergens eluted with k_{av} corresponding to an MW of 33,000 to 37,000. The allergens eluted from the IEF column at pH corresponding to an isoelectric point of 3.5.

The purification of each allergen has been performed five times with similar results.

The purified allergen from the two species was analyzed by CIE and SDS-PAGE to assess the purity. The purification procedure did not alter the electrophoretic mobility of *Per a* I and *Bla g* I. Both allergens had a mobility of about 1.9 times that of human serum al-

bumin. As detected by CIE with rabbit antibody to WBE of the respective cockroach species, the purified allergens contained only minute amounts of contaminating antigen (Fig. 5). As illustrated in Fig. 5, *E*, the contaminants were not allergens as determined by CRIE of the purified allergen fraction. Analyzed by SDS-PAGE under reducing (Fig. 6) or nonreducing conditions, the purified allergen preparations were found to contain no detectable impurities, as revealed by silver staining. The apparent MW of the main band was for both allergens, approximately 6 kd. This large divergence in MW, as determined by gel filtration and SDS-PAGE, called for additional analyses.

Although samples were extensively dialyzed, the purified allergen samples of both cockroach species were still contaminated with ampholytes. As illustrated in Fig. 7, the subsequent gel filtration on HPLC revealed, as detected by a diode array detector, two distinct peaks with k_{av} corresponding to MWs of approximately 28,000 (peak I) and 6000 (peak II). Fractions were pooled according to the chromatogram and were tested by CIE for the presence of allergen (data not presented). Peak I contained the allergen, *Per a* I or *Bla g* I, whereas peak II did not react with the rabbit antibody. Rerunning of peaks I and II did not reveal any signs of one peak converting to the other peak under the conditions of this analysis. Running dialyzed ampholytes on the column yielded one peak at an MW of 6000 with an absorption spectrum similar to that of peak II found in the allergen samples. The HPLC analyses were repeated on samples that were boiled in 1% wt/vol SDS and 0.2 mol/L of dithiothreitol before application. This treatment did not alter the k_{av} of peaks I and II, and we therefore concluded

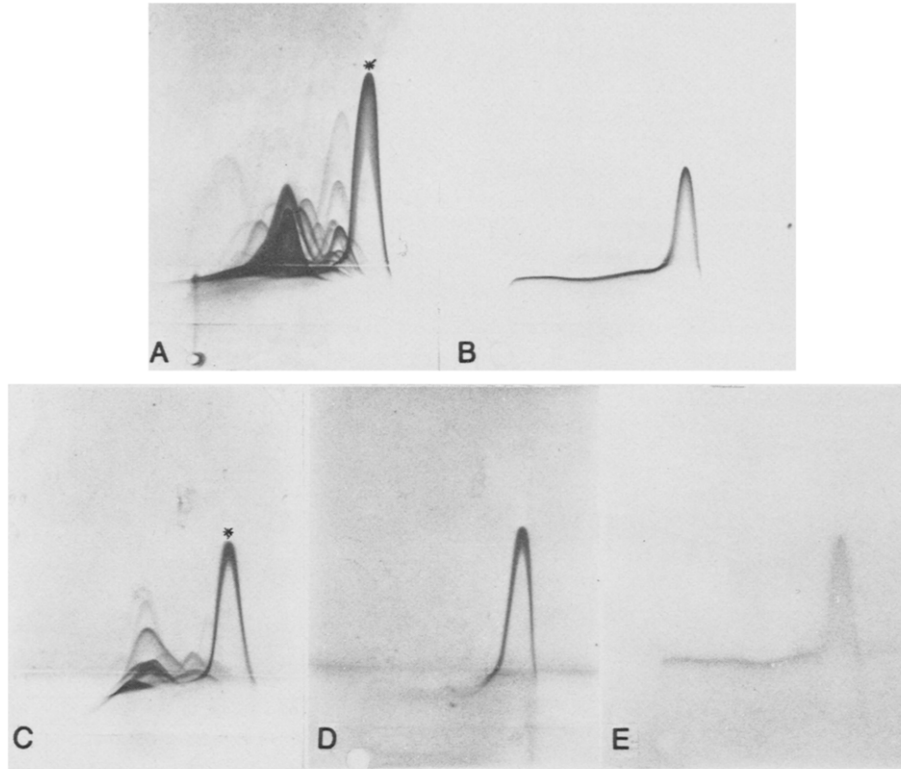


FIG. 5. CIE of WBEs of **A**, 20 μ g of *P. americana* and **C**, 15 μ g of *B. germanica*. **B** and **E**, 2 μ g of the purified allergens, *Per a* I and **D**, of *Bla g* I. The second-dimension gels contained rabbit antibody to WBEs of **A**, **B**, and **E**, *P. americana* and **C** and **D**, *B. germanica*. **E**, Electrophoresis incubated with a serum pool from cockroach-allergic patients. Asterisks denote **A**, *Per a* I and **C**, *Bla g* I.

that the allergens were not composed of disulfide-linked subunits.

To clarify further the relationship between the molecules of peaks I and II, the samples were iodinated individually and separated by SDS-PAGE before they were autoradiographed. As illustrated in Fig. 8, lane *b* and *d*, the main band in peak I of the allergen of both species was at an apparent MW of 6 kd. A minor stain was observed at approximately 25 kd, and a weak stain was observed in the migration front of the electrophoresis. Neither of the peaks II contained radioactivity except that observed in the migration front (lane *f* and *g*). It therefore appears that the allergens with an MW of 28 kd under the gel filtration conditions are prone to break down during SDS-PAGE into fragments with an MW of 6 kd. The fact that no bands at 25 kd were detected in silver-stained gels is likely to be due to lower sensitivity than obtained with the autoradiography.

Western blotting from SDS-PAGE of the WBE of cockroach and the purified allergens (Fig. 9) demonstrated bands at about 25 kd and 6 kd when they were analyzed with rabbit antibodies to cross-reactive determinants on *Per a* I and *Bla g* I. This further

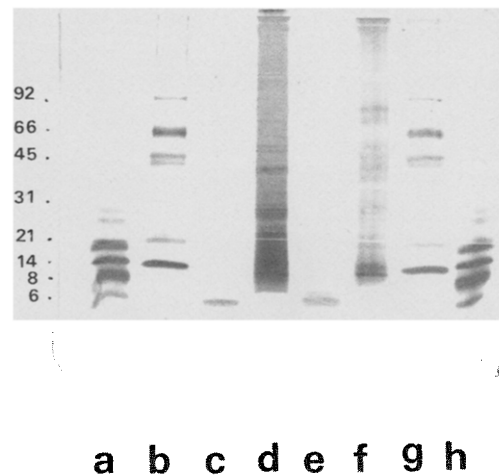


FIG. 6. SDS-PAGE of cockroach-antigen preparations. The separation gel was a gradient from 8% to 25% acrylamide. The gel was run under reducing conditions and, subsequently, silver stained; MW in kilodaltons; lanes *a* and *h*, low MW markers; lanes *b* and *g*, MW markers; lane *c*, 1 μ g of protein of purified allergen *Per a* I; lane *d*, 5 μ g of protein of *P. americana* WBE; lane *e*, 1 μ g of protein of purified allergen *Bla g* I; lane *f*, 5 μ g of protein of *B. germanica* WBE.

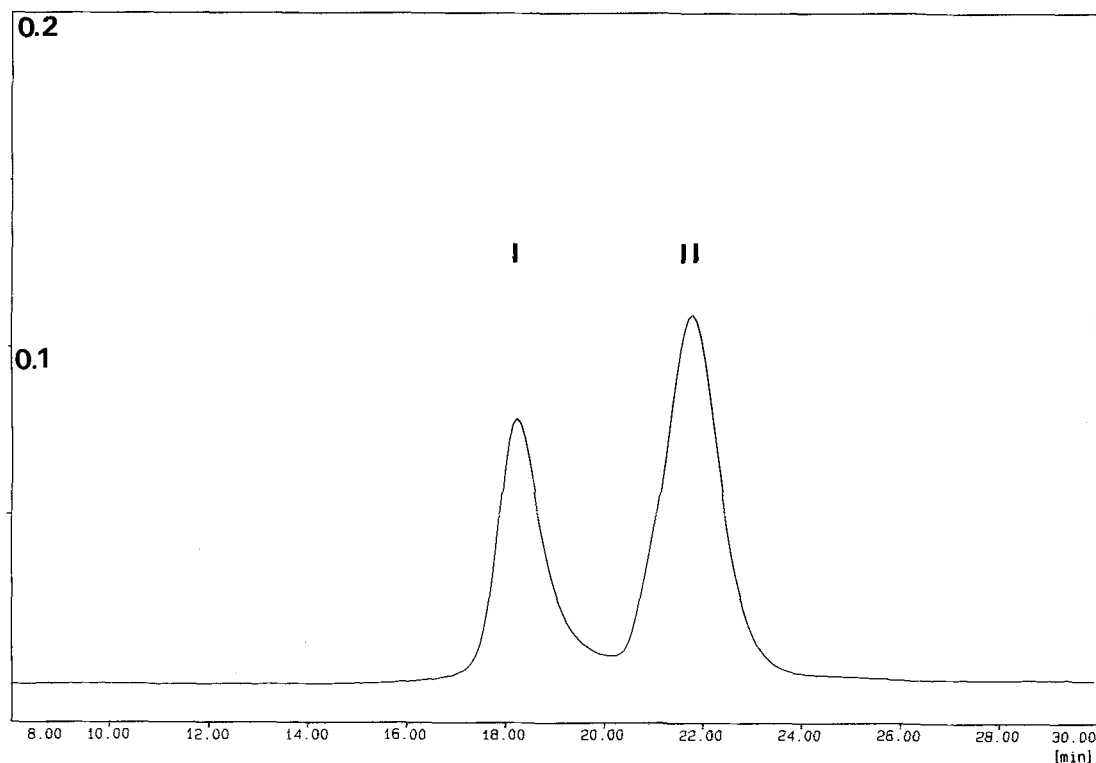


FIG. 7. HPLC gel filtration of the *Per a* I containing fraction from preparative IEF; x axis, minutes; y axis, absorbance at 225 nm; peak I (k_{av} corresponding to an MW of 28 kd) contains allergen *Per a* I; peak II (MW of 6 kd) contains ampholytes only.

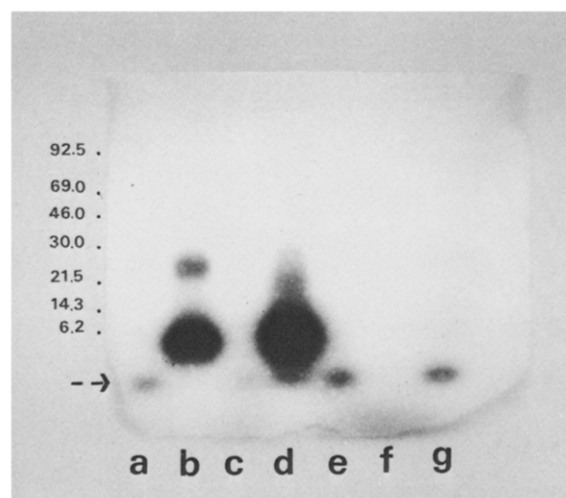


FIG. 8. Autoradiograph of SDS-PAGE of cockroach allergen. Purified allergen was separated by HPLC gel filtration (see text for description) and, subsequently, iodinated. Separation gel was a gradient from 8% to 25% acrylamide. Gel was run under reducing conditions. Exposure was for 16 hours at room temperature; MW in kilodaltons; lane a, ampholyte; lane b, peak I, HPLC, *Per a* I; lane c, free iodine; lane d, peak I, HPLC, *Bla g* I; lane e, free iodine; lane f, peak II, HPLC, *Per a* I; lane g, peak II, HPLC, *Bla g* I; arrow indicates migration front.

indicates that the allergens are likely to break down. Additional bands at MW of 80 to 100 kd may be aggregates formed during treatment involved in this technique, since they are present even in the purified preparation that does not contain any molecules at this MW, as judged by gel filtration chromatography.

The specific activity of the allergens in the extracts was calculated by means of RIE with the purified allergens as reference. In WBE of *P. americana*, *Per a* I constituted about 8.3% of the total protein content. *Bla g* I constituted about 11% of the *B. germanica* WBEs examined. This indicates that the allergens are major constituents of the extracts. With the purification procedures outlined above, the yield of purified allergen was typically (expressed as milligrams of protein, according to the method of Lowry et al.⁶; residual ampholytes were removed by HPLC gel filtration) 2.7 mg *Per a* I from 131.5 mg *P. americana* WBE and 7 mg *Bla g* I from 122 mg *B. germanica* WBE. The overall recovery of the allergens was thus about 25% for *Per a* I and about 52% for *Bla g* I.

It may be appropriate to note that the purification procedures presented here have been optimized for maximum purity and not for maximum yield.

The amino acid analysis of the purified allergens,

TABLE II. Amino acid analysis of *Per a* I and *Bla g* I (number of residues per 100 residues of amino acids)

Amino acids	<i>Per a</i> I	<i>Bla g</i> I
Asparagine and aspartic	14.1	16.2
Threonine	4.0	3.6
Serine	4.6	3.3
Glutamine	12.1	13.1
Proline	5.4	4.9
Glycine	6.3	5.4
Alanine	7.3	8.7
Valine	6.0	6.6
Methionine	1.9	0.8
Isoleucine	6.9	7.9
Leucine	12.3	15.6
Tyrosine	3.9	2.9
Phenylalanine	5.9	5.2
Histidine	3.6	1.6
Lysine	3.9	3.2
Arginine	2.7	1.4
Cysteine	ND	ND
Tryptophan	ND	ND

ND, Not determined.

as presented in Table II, suggests that the two allergens are very closely related. The most notable differences are found in methionine, histidine, and arginine.

The clinical significance of *Per a* I and *Bla g* I was investigated by skin tests (Table III); 73 consecutive patients attending an allergy clinic in Tampa, Fla., complaining of upper and/or lower respiratory symptoms were tested epicutaneously with WBEs and purified allergens. Twenty-six percent (19/73) of the patients were positive to at least one of the extracts; 79% (15/19) of the cockroach-sensitive patients were positive to German WBE, and 47% (7/15) of these patients reacted with purified *Bla g* I. Fifty-eight percent (11/19) of the cockroach-sensitive patients were positive to American WBE, and 55% (6/11) of these patients reacted with purified *Per a* I. This finding demonstrates that the allergens are important in vivo.

DISCUSSION

The present study was undertaken primarily to identify and purify an important allergen from WBEs of the major domiciliary cockroaches, that is, the American, *P. americana*, and the German, *B. germanica*.

With the material extracted for this work, it was possible by means of CIE to identify 45 antigen precipitates in extracts of *P. americana* and 29 from *B. germanica*. Very recently, other workers¹³ have counted 50 and 56 antigen precipitates, respectively,

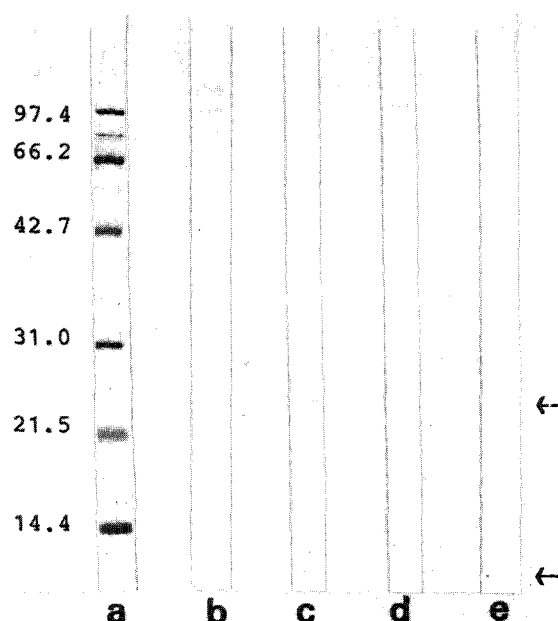


FIG. 9. Immunoblotting from SDS-PAGE of cockroach WBEs and purified allergens; 15 μ g of protein of *P. americana* extract (lane b) and *B. germanica* (lane d) and 1 μ g of *Per a* I (lane c) and *Bla g* I (lane e) were separated before blotting. Allergens were detected with rabbit antibodies to determinants shared by *Per a* I and *Bla g* I. Bound antibodies were revealed by alkaline phosphatase-conjugated swine antirabbit immunoglobulin; lane a, MW markers stained with colloidal gold; MW in kilodaltons; arrows indicate immunostained bands at approximately 25 and 6 kd.

in comparable extracts from the two species. This apparent discrepancy may be related to differences in extraction procedure as well as different electrophoresis conditions and rabbit antibodies. With sera from 30 cockroach-sensitive patients, we identified seven and three antigens from *P. americana* and *B. germanica*, respectively, that bound significant amounts of IgE. Stankus and O'Neil¹³ found five allergens from each cockroach species in their CRIE analyses. Other workers¹⁴ have reported 18 allergens in extract of *P. americana* as detected by CRIE. This lack of correspondence indicates differences in patient populations and may also be related to analysis conditions. These studies will, however, provide an immunochemical framework for the clinical studies that have pointed to cockroaches as a source of sensitizing material in allergic subjects.

The difficulties connected with comparing CRIE results based on different extracts, rabbit sera, and patient populations, stress the importance of the use of other means to assure proper identification of important allergens. Purified allergens will obviously

TABLE III. Skin test reactions

Pt	Area of reaction								Diagnosis
	<i>B. g.</i>		<i>Bla g I</i>		<i>P. a.</i>		<i>Per a I</i>		
	W	E	W	E	W	E	W	E	
1	6	1560	6	200	9	840	4	300	AR
2	6	825	1	140	66	2400	6	88	AR
3	2	36	1	—	4	56	1	—	AR
9	9	960	4	990	—	—	—	—	R
27	—	—	—	—	4	546	4	480	AR
31	—	—	—	—	4	875	4	100	AR
32	24	3320	20	1092	—	—	—	—	AR
33	4	780	—	—	—	—	—	—	R
36	40	560	—	—	200	900	—	—	R
37	39	800	—	—	9	108	—	—	AR
40	16	700	—	—	—	—	—	—	AR
42	—	—	—	—	9	100	—	—	R
52	4	100	—	—	—	—	—	—	ND
60	4	400	2	195	4	300	2	70	AR
61	4	300	4	225	—	—	—	—	ND
64	—	—	—	—	4	340	—	—	R
67	6	884	—	—	2	352	—	—	R
70	2	255	—	—	—	—	—	—	ND
71	15	140	—	—	—	—	—	—	AR

PT, Patient; *B. g.*, *Blattella germanica*, *P. a.*, *Periplaneta americana*; W, wheal; E, erythema; AR, asthma and rhinitis; R, rhinitis; —, no reaction; ND, no diagnosis.

Cockroach-allergic patients were tested epicutaneously for reaction with WBE of German and American cockroach and with purified allergens, *Bla g I* and *Per a I*. Concentration of *Bla g I*/*Per a I* in the purified preparations was identical to that in the WBEs. Area is in square millimeters.

make identification unequivocal and will therefore provide the best basis for comparison between different laboratories.

It may be premature to call the allergens we have labeled *Per a I* and *Bla g I* the "major allergens," but they bound IgE from 100% and 70%, respectively, of the patients used in the initial CRIE setup of the present study. The clinical significance of the allergens was evaluated in the skin test. About half the patients reactive with German or American cockroach WBEs were skin test positive to the purified allergens (47% and 55%, respectively). We therefore suspect that *Per a I* and *Bla g I* may be important as sensitizing agents in the natural environment of cockroach-allergic subjects.

A thorough investigation of the relationship between the severity of clinical symptoms and the magnitude of skin test reactions has not been performed at this time, but there was no correlation between the skin tests and diagnosis of the patients' asthma/rhinitis or just rhinitis.

The physicochemical procedures used to purify the two allergens were mild and nondenaturing, and they did not obliterate the binding of patients' IgE, as

judged by CRIE and skin test. The allergens were strongly cross-reactive at the antigenic level, even though a few patients (e.g., Nos. 9 and 27) apparently reacted with determinants that are not shared between the German and American cockroaches. The authors therefore suggest that *Per a I* and *Bla g I* are homologous molecules with an as yet undefined biologic function in the live insects. The resemblance between the amino acid compositions of the purified proteins also suggests that the allergens are closely related.

The presence of allergens in the fecal pellets of cockroaches has been noted from clinical studies of other investigators,¹⁵ but these authors did not identify the allergens of feces; therefore, it is not possible to tell which allergens were present in their preparation. It is, however, conceivable that *Bla g I* was a constituent of the extract used in that work. The skin test data presented here indicate that sensitizing allergens are present in the fecal material. Richman et al.¹⁶ reported low direct RAST activity of FEs of *B. germanica*. We have not performed RAST studies with our FEs, but we would expect some activity in that system, since *Bla g I* is an important allergen. The solution of this discrepancy will await further studies.

Steinberg et al.¹⁷ were able to demonstrate good direct RAST binding to *P. americana* FEs in sensitized laboratory workers. Since *Per a* I is a constituent of feces, this observation is compatible with the data presented here.

The apparent MW of *Per a* I and *Bla g* I was approximately 6 kd under both reducing and nonreducing conditions in silver-stained SDS-PAGE. Western blottings revealed strong IgE binding to a band in the WBEs with an MW of approximately 6 kd (Fig. 2). RIE quantification of *Per a* I and *Bla g* I in the WBEs demonstrated the allergens to constitute 8.3% and 11% of the protein content, and it was therefore expected that the molecules would stain heavily in silver-stained SDS-PAGE. However, the allergens did not stain very well (Fig. 6), and their concentration is thus likely to be underestimated by this technique. As revealed by immunoblotting with rabbit antibodies to determinants shared by *Per a* I and *Bla g* I, the purified allergens contained bands at about 22 to 24 kd as well as 6 kd. This observation is compatible with the bands at about 25 kd and 6 kd observed in autoradiographs of SDS-PAGE of the radioiodinated, purified allergens. The hydrodynamic volume of the allergens during gel filtration under nondenaturing conditions elicited an MW of 33 to 37 kd in Sephadex G-75 and of 28 kd in HPLC. These observations point to the allergens as polymers or molecules that are prone to degradation under the conditions experienced in SDS-PAGE. No reaction with rabbit antibody to *Per a* I was observed in the Sephadex G-75 and HPLC gel filtration fractions at MWs corresponding to 6 kd; therefore, the potential monomeric or degraded form was absent under the nondenaturing conditions used for these purification steps. Twarog et al.¹⁸ reported a cockroach allergen (CR III) with an apparent MW in SDS-PAGE of <10 kd; a direct comparison may demonstrate this allergen related to allergens purified in the present study. The CR I allergen reported by Twarog et al. shares with *Per a* I and *Bla g* I a low isoelectric point and a high electrophoretic mobility. The reported MW of 25.5 kd for CR I is in the same range as that observed for *Per a* I and *Bla g* I in gel filtration. The elucidation of a possible relationship between the allergens requires a direct comparison.

Helm et al.¹⁹ isolated an allergen from *B. germanica* with an MW of 36 kd in SDS-PAGE. The allergen described by Helm et al. is likely to be different from *Bla g* I, since the MWs are different and the authors do not mention that the allergen breaks down during SDS-PAGE. The American cockroach has been investigated by Wu and Lan¹⁴ who noticed several allergens, two of which were strong IgE binders. The MWs of 72 and 78 kd are compatible with our finding

(Fig. 2, lane a) that WBE of *P. americana* contains allergens in that MW range. Baldo and Panzani²⁰ found that six insect-allergic subjects had IgE to several bands in the 30 to 200 kd range of *B. germanica* extract. Their investigations suggest that several allergens are involved in allergy to cockroaches. The MW detected in IgE blotting is an informative property of an allergen, but it does not identify the molecule unequivocally. To understand better the relative importance of the different allergens responsible for the development of cockroach allergy, the molecules need to be purified.

The purified allergens presented in this study will provide a basis for developing environmental assays for cockroach-allergen exposure in the home of allergic patients. Because these allergens are important, they may serve as marker allergens in future standardization of cockroach allergenic extracts and may, as such, pave the way toward better commercial extracts.

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A double-blind comparison of intranasal budesonide with placebo for nasal polyposis

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Intranasal budesonide, 400 µg two times a day, was evaluated in 36 patients referred for treatment of nasal polyposis. The age range was 20 to 68 years. Polypectomy was done 5.6 (mean) times previously. After a 5-week, treatment-free, baseline period, patients were treated in a double-blind fashion with either budesonide or placebo during 4 weeks. After this treatment period, placebo-treated patients started receiving budesonide in an open trial for an additional 4 weeks. The patients rated their nasal symptoms daily. Nasal examinations and nasal inspiratory flow rate (IFR) measurements were done at clinic visits. After 3 and 4 weeks of treatment, the response to budesonide was significantly greater than response to placebo. The greater reduction in nasal blockage caused by polyps, observed on physical examination, $p = 0.005$, was mirrored by an increase in nasal IFR ($p = 0.0001$). Patient rating of the severity and frequency of nasal blockage were reduced more by budesonide than by placebo ($p \leq 0.0005$). Switching placebo-treated patients to budesonide treatment resulted in a reduction of nasal blockage ($p < 0.001$) and an increase in nasal IFR ($p < 0.001$). The results demonstrate that topical nasal budesonide, 400 µg two times a day, is an effective treatment of nasal polyps. (J ALLERGY CLIN IMMUNOL 1990;86:946-53.)

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Nasal polyposis is a disease of unknown cause. A hypothesis for the formation of nasal polyps involving nerve damage and mast cell degranulation has been suggested by Mygind.¹ Many signs of inflammation similar to signs observed in the damaged bronchial mucosa in asthma have also been found in nasal polyps.² Of particular interest is the elevated number of metachromatic cells in the epithelium of nasal polyps and the adjacent turbinate mucosa.³ Mast cells of