

Ricki M. Helm^a
Wesley Burks^a
Larry W. Williams^b
David E. Milne^c
Richard J. Brenner^c

^a Department of Pediatrics,
University of Arkansas for Medical
Sciences, and Arkansas Children's Hospital,
Little Rock, Ark.;

^b Duke University Medical School,
Durham, N.C.;

^c United States Department of Agriculture,
Medical and Veterinary Entomology
Research Laboratory,
Gainesville, Fla., USA

Identification of Cockroach Aeroallergens from Living Cultures of German or American Cockroaches

Abstract

The Air Sentinel and polytetrafluoroethylene (PTFE) membranes were used to capture airborne particles over living colonies of German or American cockroaches. Silver-stained SDS-PAGE gels revealed protein bands at 80, 55, 36, and several bands below the 33-kD marker. SDS-PAGE/immunoblots of PTFE eluates from German cockroach colonies incubated with serum from cockroach-sensitive individuals revealed IgE-binding bands with apparent molecular weights of 36 and 80 kD. Only the 36-kD allergen and allergens below the 33-kD marker were evident in the American PTFE eluate. ELISA analysis with a monoclonal antibody assay identified the presence of both Bla g I and Bla g II in the German PTFE eluate. No Bla g I or Bla g II could be identified in the American PTFE eluate. These studies demonstrate that in addition to Bla g I and Bla g II, several other aerosolized allergens become airborne over cockroach colonies and may be important in the environment where cockroaches are abundant.

Key Words

German cockroach
American cockroach
Aeroallergens

Introduction

Cockroaches, present in many households, food establishments and certain occupational settings, represent a serious health problem to susceptible individuals in cockroach-infested environments [1, 2]. Whole body cockroach extracts have been shown to cause direct cutaneous sensitivity [3, 4] as well as inhalant allergy [5]. A significant proportion (ca. 60%) of dust allergic patients have been shown to have positive skin tests, positive bronchial challenge tests, and serum IgE antibodies to proteins in crude extracts prepared from whole body cockroaches [6-11].

Major allergens ranging in molecular weight from 6,000 to 100,000 daltons have been identified in several species of cockroach including the German (*Blattella germanica*), Asian (*Blattella asahinai*) and American (*Periplaneta americana*) cockroach species [12-15]. A recent study suggests that both bodies and feces of American and German cockroaches are important sensitizing agents in atopic asthma [16].

Although the prevalence of asthma is increasing, triggers of the process are often unrecognized by the susceptible individual. Cockroaches are not easily enumerated in household environments; moreover, the amorphous na-

ture of cockroach particles and fecal material reduce their recognition as part of household dust. Environmental assays have been designed to directly measure the concentration of major allergens in extracts of cockroaches or house dust [17, 18]. To our knowledge, no assessment of cockroach aeroallergens has been made. We, therefore, undertook an investigation in which the physicochemical and immunological characteristics of American and German cockroach aeroallergens could be evaluated. Air samplings of living cockroach colonies were taken and the captured material tested for allergens using human serum with elevated IgE levels to cockroach.

Materials and Methods

Cockroach Cultures

The rearing colony for American cockroaches consisted of 50 females with oothecae placed in a 4 cubic foot chamber. Each ootheca was estimated to produce 14 adults resulting in a population estimate of 700 (50 adults $\times$ 14/ootheca = 700). American cockroach colonies reach maturity in 6–8 months with an estimated mortality rate of 5%. Relative size: 3.56 cm in length; 1,320 mg in average weight. Air sampling was performed on colonies at 5.5–6 months.

The rearing colony for German cockroaches consisted of 360 females with oothecae in a chamber as above. Each ootheca was estimated to produce 30 adults resulting in a population estimate of 10,800 (360 adults $\times$ 30/ootheca). German cockroach colonies reach maturity in 7–8 weeks with an estimated mortality rate of 20%. Relative size: 1.6 cm in length; 60 mg in average weight. Air sampling was performed on colonies at 9–10 weeks with food (Purina laboratory rat chow) and water provided as needed.

Air Samplings

The 'Air Sentinel' (Quan-Tec Air, Inc., Rochester, Minn., USA) was modified to collect aerosolized cockroach material onto polytetrafluoroethylene (PTFE) membranes. Continuous air samplings ranged from 24 to 140 h and were stored frozen until being shipped on ice to ACH. The PTFE membranes were delaminated from their nylon backings and extracted in 250–500 μ l PBS and clarified by centrifugation at 1,500 g for 10 min. Alternatively, five PTFE membranes were delaminated, extracted in 1.0 ml PBS overnight at 4 °C, dialyzed against 100 mM NH_4HCO_3 , and lyophilized. Dried material was solubilized in Laemmli sample buffer with 5% mercaptoethanol and clarified by centrifugation as above. The total contents were then analyzed by SDS-PAGE/immunoblot analysis.

Protein Content

The BCA technique (Pierce Chemical Co, Rockford, Ill., USA) was used for protein determinations following the manufacturer's instructions with BSA as a standard.

IgE Serum Pool

A serum pool containing elevated IgE levels to German cockroach allergens was prepared using sera from 4 urban asthmatic 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.

SDS-PAGE/Immunoblot Analysis

SDS-PAGE was performed using 12.5% resolving gels following the method of Laemmli [19]. PTFE membranes were extracted directly using 0.5 ml Laemmli sample buffer containing 5% 2-mercaptoethanol. Replicate samples were applied in a 3% stacking gel and electrophoresed at 20 mA/gel. The gel was divided into individual replicate patterns; one was silver stained and the protein bands in the other were transferred to an Immobilon membrane following the procedure of Towbin et al. [20]. Following electrophoresis, membranes were washed in PBS containing 0.5% Tween 20/PBS for 20 min to block the remaining active sites. Membranes were incubated with human IgE pool (1:4 v/v in PBS) overnight at 4 °C, washed three times with PBS, incubated with alkaline phosphatase labeled goat anti-human IgE (1:2,000 v/v PBS) (KPL) for 2 h at room temperature, washed three times and developed using the BioRad alkaline phosphatase substrate kit (BioRad). Alternatively, we used radiolabelled anti-IgE (Sonofia, Minneapolis, Minn., USA) for the detection of the bands which bound IgE in our PTFE membrane eluates.

ELISA

The two-site monoclonal-based enzyme-linked immunoassay for Bla g I and Bla g II was a gift from Drs. Pollart and Chapman [17]. In the place of peroxidase-conjugated goat antirabbit IgG, we used alkaline phosphatase conjugated goat antirabbit serum (BioRad) diluted 1:2,000 (v/v) and subsequently developed with an alkaline phosphatase substrate kit (BioRad). The plates were read at 490 nm.

Results

Air Sampling

The results of air samples collected for 48 and 96 h are shown in table 1. All colonies were in their latter stages of maturation. Concentrations (μ g/ml) were consistent with respect to the American colony collection (600 and 594 μ g/ml) and converted to 3.2 and 1.6 μ g/m³ air for the 48- and 96-hour collection periods, respectively. In the German cockroach colonies we found increased protein content when comparing the 48-hour versus the 96-hour collection period (mean = 696 μ g/ml at 48 h; mean = 1,166 μ g/ml at 96 h). When converted to protein/m³ air, the results were consistent (3.7 and 3.1 μ g/m³ air) for the 48- and 96-hour sampling times, respectively.

SDS-PAGE Analysis

SDS-PAGE analysis was performed on 120-hour collection periods for both the American and German PTFE membrane extracts. Each filter was extracted in 25 μ l of Laemmli sample buffer (10% SDS, 5% 2-mercaptoethanol) and 7- μ l aliquots applied to 12.5% SDS-PAGE gels. The results are shown in figure 1. The silver stained pattern (panel A) of the American PTFE extract (lane 1) and

Table 1. Protein/allergen evaluation of PTFE membranes

American cockroach ^a			German cockroach ^b			
Time h	protein µg/ml	protein µg/m ³ air ^c	protein µg/ml	protein µg/m ³ air	Blag I (U)	Blag II (U)
48	600	3.2	696	3.7	n.d.	n.d.
96	594	1.6	1,165	3.1	1.63	0.26

^a American cockroach-rearing colony consisted of 50 females with ootheca (14/ootheca) placed in a 4-cubic foot chamber. Population estimate ($50 \times 14 = 700$). Colony reached maturity in 6–8 months with an estimated mortality rate and 50 adults removed for colony renewal.

^b German cockroach-rearing colony consisted of 360 females with ootheca (30/ootheca) placed in a 4-cubic foot chamber. Population estimate ($360 \times 30 = 10,800$). Colony reached maturity in 7–8 weeks with an estimated mortality rate and 20% and 500 adults removed for colony renewal. Food and water ad libitum.

^c Mean airflow was 100 ft/min during the 48-hour collection period representing approximately 190 m³ air.

German PTFE (lane 2) revealed prominent stained proteins at 80, 55, 36, and several minor bands less than 30 kD. The PTFE extract immunostained profiles (panel B) clearly identified IgE-binding proteins at the 36-kD region with minor bands at lower molecular ranges (lanes 1 and 2). At higher concentrations of the eluates which showed smearing with silver staining, we could identify a band at 80 kD in the German PTFE eluate that was not present in the American PTFE [data not shown]. It was apparent that the IgE-binding pattern of the PTFE membrane extracts corresponded with IgE-binding profiles in the whole body German cockroach extracts in a least two identifiable regions – 36 and 80 kD – with minor bands appearing below the 30-kD marker. A whole-body German cockroach extract and the results of five PTFE membrane eluates from German PTFE cockroach colony collections is shown in panel C for comparison.

In related experiments, we used radiolabelled anti-IgE to detect allergen bands in the PTFE membrane eluates. The results are shown in figure 2. The German PTFE (panel B, lane 1) shows a prominent band at the 36-kD molecular weight position and a faint band at 80 kD. Only the 36-kD allergen can be identified in the American PTFE extract (panel B, lane 2). Panel A represents an immunoblot with nonatopic serum in which no bands can be detected, clearly demonstrating that the 36- and 80-kD bands are IgE-binding proteins.

ELISA Analysis

Serial 10-fold dilutions in PBS of four 96-hour German cockroach PTFE membrane eluates were analyzed using the monoclonal assay system described by Pollart et al.

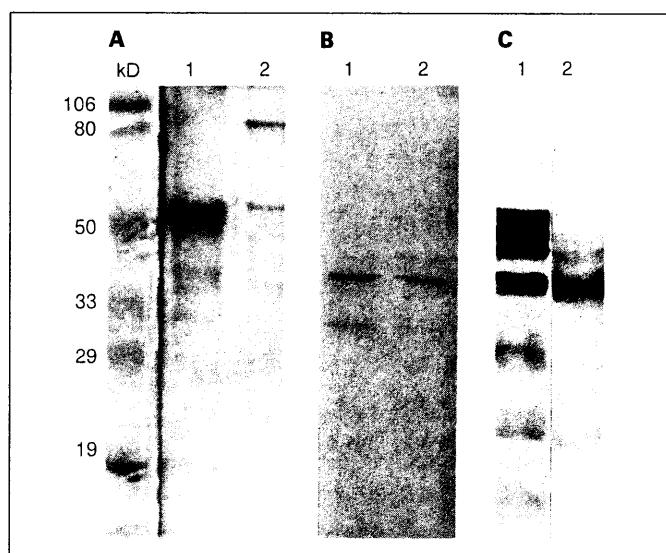


Fig. 1. SDS-PAGE/Immunoblot analysis of American and German cockroach PTFE extracts. Panel A = silver-stained pattern of a 12.5% SDS-PAGE gel; panel B = IgE immunoblot analysis of electrophoretically transferred protein from 12.5% SDS-PAGE gel to an Immulon nitrocellulose membrane. Bound IgE was detected by enzyme-labeled anti-IgE. Lanes 1 and 2 = American and German PTFE extracts respectively. Panel C = IgE immunoblot analysis of whole body German cockroach extract (lane 1) and German cockroach PTFE extract (lane 2) from a 10% SDS-PAGE gel.

[15]. The results are shown in table 1. The Bla g I assay showed 1.63U in the undiluted German PTFE eluate and fell off rapidly at a 1:10 dilution. The German whole body extract did show appreciable quantities of Bla g I at the dilutions tested [data not included]. The whole body cock-

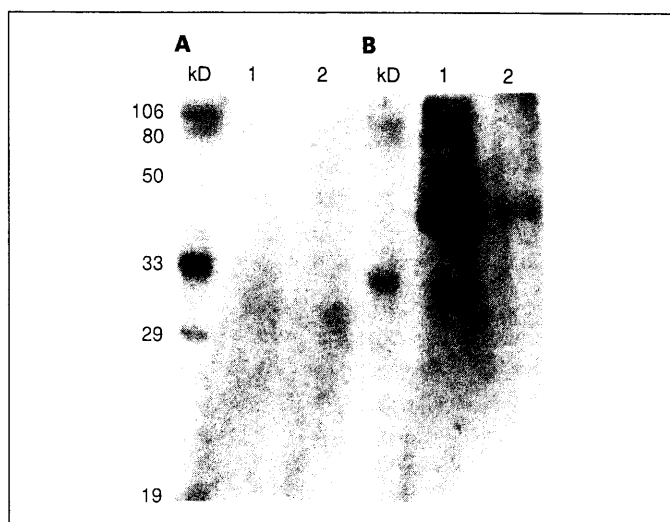


Fig. 2. SDS-PAGE/Immunoblot analysis of German and American cockroach PTFE extracts. Panel A = Immunoblot analysis using serum from a nonatopic individual; panel B = immunoblot analysis using serum from cockroach-sensitive individuals. Lane 1 = American PTFE; lane 2 = German PTFE. Bound IgE was detected by radiolabelled anti-IgE.

roach eluate (1:10 dilution) reacted with Bla g II at approximately a twofold higher optical density reading than the PTFE extracts and could be serially diluted to background optical readings. We were unable to determine either Bla g I or Bla g II in the American PTFE membrane eluates following 100 h of air sampling.

Discussion

Considerable information is available regarding the characterization of cockroach extracts. Environmental assays have been developed to quantitate the major allergen concentration in extracts prepared from various cockroach sources and dust samples. Although the precise source of cockroach allergenic material is unknown, studies with RAST analysis suggested that whole body cockroaches were the main source of cockroach allergens [21, 22]. Menon et al. [23] and Lehrer et al. [16] recently demonstrated allergenic activity in fecal extracts by skin testing and RAST inhibition.

For their environmental monoclonal antibody assay the material used by Pollart et al. [15] was identified as frass and included the material which accumulated at the bottom of cockroach culture jars consisting mainly of feces (frass), secretions, egg cases, and body parts, but relatively few whole bodies. Body washes of live cockroaches also

have been shown to be potent sources of both the Bla g I and Bla g II allergens [24].

Dust samples have been used routinely to document the presence of cockroach allergens. Data from 40 homes in Wilmington, Del., demonstrated that the kitchen was the primary source for cockroach infestation [17]. Samplings of homes without visual evidence of cockroach infestation revealed that significant levels of cockroach allergen could be found in 20% of homes in which no visible evidence of cockroaches was found by either the homeowner or the investigators.

Although the environmental assay for major cockroach allergens in dust is clinically important, we must be certain that the relevant allergens are being identified and quantitated. In the present study, we chose to characterize the allergens released into the air above colonies of living cockroaches. Our attempt was to assess the conditions which may apply to cockroach infested structures where they may be present.

It has been shown that German cockroach infestations commonly exceed 13,000–15,000 insects in low-income apartments [25]. The majority of these occurring in the kitchen. The volume of air and the time required for air sampling will be quite lengthy based on our initial observations. However, persistence of some allergens, and the accumulation of allergens resulting from chronic infestations may reduce necessary sampling times. The protein concentration released into the air by 10,000 German cockroaches in four cubic feet of air was approximately 700 and 1,000 $\mu\text{g}/\text{ml}$ for 48- and 96-hour collection periods, respectively. This converts to approximately 3.2 and 1.6 μg protein/ m^3 of air for the American cockroach PTFE extract and 3.7 and 3.1 $\mu\text{g}/\text{m}^3$ of air for the German PTFE cockroach extract for the 48- and 96-hour collection periods, respectively. For IgE-binding studies, 100 or more hours of air sampling was required to identify specific German cockroach allergens. If we make conversions for Bla g I Units of allergen, we find that in the 96-hour German PTFE membrane extract that 4×10^{-3} U of Bla g I/ m^3 of air can be determined. This may be relevant if one considers that prolonged exposure to low levels of allergen are responsible for exposure, sensitization and reaction to the allergen. No risk levels of cockroach allergen have been established to indicate the levels of cockroach allergen that will sensitize or exacerbate allergic symptoms.

Silver-stained SDS-PAGE gels of the German and American PTFE extracts revealed similar banding patterns with proteins identified at 80, 55, and 36 kD with several minor bands below the 33-kD marker. It is difficult to assess the degree of denaturation or degradation of the

proteins in these experiments because of the prolonged collection periods. Although every effort was made to keep the filters dry during the collection periods and to keep the filters frozen prior to extraction, we cannot eliminate the possibility that degradation of larger molecular weight proteins did not take place. IgE-binding bands at the 36-kD position in the American PTFE extract and failure to identify Bla g I or II in the same extract suggest that there may be different proteins in the American extract compared to the German PTFE extract. A critical evaluation of the IgE-binding proteins in the American and German extracts will be necessary to determine if cross-reactive allergens (epitopes) are shared between the two genera. In the ELISA assay and IgE-immunoblot analysis of the German extracts (whole body or PTFE membranes), we were able to demonstrate both Bla g I and Bla g II. Although we have clearly shown by these techniques that IgE-binding proteins can be identified in the PTFE

extracts taken from living cockroach colonies, the exact nature of these allergens awaits elucidation.

Studies are underway to identify and quantify the cockroach allergens released in the natural environment using similar technology. These studies will be used to determine relative risk levels of aerosolized cockroach allergens in the home which exacerbate the symptoms of the exposed allergic individual. Identification of aerosolized allergens will also be useful in the control of allergen levels in the environment of the sensitive individual.

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