
Allergenicity of the Madagascar hissing cockroach

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Background: The Madagascar hissing cockroach (*Gromphadorhina portentosa*) has become popular as a pet and as an educational tool in classrooms, zoos, and museums.

Objective: To determine whether proteins in *G portentosa* are allergens and whether these allergens cross-react with those of other cockroach species.

Methods: Sera from cockroach-sensitive individuals and control subjects were used to probe immunoblots for the presence of circulating IgE that bound to proteins present in extracts of 4 cockroach species.

Results: Serum from an individual sensitized to *G portentosa* had circulating IgE that bound to proteins in extracts of all 4 cockroach species. Eight of 15 *Blatella germanica*-sensitized patients had IgE that bound to proteins in extracts prepared from *G portentosa*. Rabbit antiserum to *Periplaneta americana* and to Bla g 1 also contained antibody that bound to proteins in *G portentosa* extract, demonstrating antigenic cross-reactivity among these cockroach species.

Conclusions: Allergists, teachers, parents, and patients should be aware that sensitization and allergic reactions to the Madagascar hissing cockroach can occur. Caution should be taken if these exotic cockroaches are used in educational exercises in schools, museums, and zoos to protect predisposed individuals.

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INTRODUCTION

Several species of cockroaches that inhabit homes worldwide are sources of potent allergens. The cockroach species best known to be sources of indoor allergens include *Blatella germanica* (German cockroach), *Periplaneta americana* (American cockroach), and *Blatta orientalis* (Oriental cockroach), although others, such as *Supella longipalpa* (brown banded), *Parcoblatta pennsylvanica* (Pennsylvania wood), *Periplaneta fuliginosa* (smoky brown), and *Blaberus giganteus* (palmetto bug) commonly inhabit or migrate into dwellings, schools, and commercial establishments (eg, restaurants and grocery stores).¹ The allergenicity of these latter species has not been determined. Allergens from cockroaches are associated with feces, saliva, secretions, bodies (living and dead), and exoskeletons and are contained in washes (extracts) of these materials.

The Madagascar hissing cockroach (*Gromphadorhina portentosa*) is usually confined to the island of Madagascar off the southeast coast of Africa. Recently, however, this insect has become popular as a pet and as an educational tool in classrooms, zoos, and museums. These cockroaches are large (5–10 cm), wingless, gentle, and docile, which makes them easy to handle. They are available from pet stores and bio-

logical suppliers, and numerous Web sites describe their care and outline educational activities for students. Children are exposed to these cockroaches and their by-products as a result of handling and caring for them. Therefore, they could become sensitized to any of the allergenic proteins they produce, or previously sensitized children could experience allergic symptoms on reexposure. The objective of this study was to determine whether proteins in *G portentosa* are allergens and whether these allergens cross-react with those of other cockroach species.

MATERIALS AND METHODS

Serum Samples

Serum was collected from a 38-year-old man who had conducted research on *G portentosa* for approximately 11 years under a protocol approved by the Wright State University institutional review board. The researcher reported allergic symptoms consisting of itchy watery eyes and nose, sneezing, and an eczematous rash after handling the cockroaches or cleaning their cages. Allergic symptoms developed after approximately 1 year of exposure. The symptoms appeared within minutes of exposure to the cockroach, and the resultant skin irritation and swollen eyes persisted for approximately 4 days. *Gromphadorhina portentosa* was the only cockroach species used by the investigator during his 11 years of research. In addition, the researcher worked with several other arthropods, such as ticks, mites, ants, wasps, and flies, none of which elicited any type of hypersensitivity reaction.

Sera from 15 patients with positive skin test reactions to *B germanica* were provided under an exempt protocol approved

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by the institutional review board of the University of Cincinnati. Four control serum samples were obtained from atopic individuals who were not sensitized to cockroaches (radioallergosorbent test, 0 or 0/1) but who had high circulating IgE levels to other allergens. These serum samples and the cockroach radioallergosorbent test data were provided to us without personal identifiers by the Laboratory Corporation of America (Burlington, NC). Two other control serum samples were obtained from nonatopic donors. All control serum samples were obtained under Wright State University institutional review board–approved protocols.

Cockroach Extracts

Midguts and associated contents were dissected from *G portentosa*, *B giganteus*, *B germanica*, and *P americana* specimens and placed in 40-mmol/L Tris, 20-mmol/L sodium acetate, and 1-mmol/L EDTA (pH 8.3) and stored at -80°C . Fecal pellets were also collected from *G portentosa*. Before homogenization, HALT Protease Inhibitor Cocktail (Pierce Biotechnology, Rockford, IL) was added to each sample according to the manufacturer's directions. Each tissue sample was ground in a TenBroeck homogenizer and centrifuged for 10 minutes at 14,000g, and the supernatants were collected.

Extracts of *B germanica* and *P americana* were also purchased from 2 different commercial suppliers. Both were glycerinated extracts prepared at 1:10 or 1:20 (W:V) and were reported by their manufacturers to have been made from ground whole cockroach bodies. The protein contents of all the samples were estimated using the method of Bradford,² with bovine serum albumin as the standard.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblot Analyses

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot analyses of cockroach extracts were performed as previously described.³ Briefly, extracts (4 μg of protein per lane) were fractionated under nonreducing conditions on 12% mini-gels (Bio-Rad, Hercules, CA). Proteins were electrophoretically transferred to ProBlott membranes (Applied Biosystems, Foster City, CA), and blots were probed using a 1:7.5 dilution of human serum (2 mL of serum + 13 mL of buffer). IgE-binding allergens were visualized using ^{125}I -labeled anti-human IgE (0.8 μCi per blot) (Dia-Med, Windham, ME) and autoradiography for varying exposure times from 1 to 14 days. After autoradiography, blots were developed for the detection of IgG binding using peroxidase-labeled anti-IgG as previously described.³

Similar blots were also probed using rabbit antiserum to various cockroach antigens. Rabbit antisera to *P americana*, Bla g 1, Bla g 2, and Bla g 5 were purchased from Indoor Biotechnologies (Charlottesville, VA) and to chicken gizzard tropomyosin from Sigma-Aldrich Corp (St Louis, MO). Blots were probed at a dilution of 1:500, and binding was detected using peroxidase-labeled anti-rabbit immunoglobulin as before.³ Some of the blots that had been probed using human

serum were also probed using antiserum to Bla g 5 or tropomyosin. In these experiments, binding of rabbit antibody was detected using alkaline phosphatase–labeled anti-rabbit IgG and was developed using AP blue membrane substrate solution (Sigma-Aldrich Corp). This produced blue bands in response to rabbit antibody binding that could be clearly distinguished from the red-brown bands associated with peroxidase-identified human IgG binding.

RESULTS

A blot of extracts of *G portentosa* gut and fecal particles alongside extracts of 3 more common cockroach species was probed using the serum of an individual who reported sensitization to *G portentosa*. This blot showed strong IgE binding to 4 or more proteins in the 2 *G portentosa* extracts (Fig 1). This individual also had circulating IgE that bound to proteins in extracts of the 3 other cockroach species with IgE binding to at least 7 proteins in extracts of *P americana* and *B germanica* (Fig 1). Almost all of the proteins to which this individual's IgE bound were greater than 40 kDa. This individual also had some detectable IgG binding to proteins in each of the cockroach extracts tested (data not shown).

In light of the observations made using the serum of the *G portentosa*-sensitive individual and the possibility of cross-reactivity among the various cockroach species, we used sera from 15 patients who had positive skin test reactions to *B germanica* and from 6 control subjects to continue this investigation. Blots of the various cockroach extracts were probed using these sera, and IgE binding was again detected by means of autoradiography (Fig 1). Four of the 15 *B germanica*-sensitive individuals showed no IgE binding to any cockroach extract, and a fifth showed very faint IgE binding to only 1 protein of 31 kDa in the *G portentosa* extract. Eight of the *B germanica*-sensitive patients had circulating IgE that bound to 1 or more proteins in the *G portentosa* gut extract, and 6 showed binding to the fecal extract, although with 1 exception the intensity of radiostaining was much less than that produced using the *G portentosa*-sensitive individual's serum. Three participants showed IgE binding to low-molecular-weight *G portentosa* proteins that were not observed with the *G portentosa*-sensitive individual's serum. The IgE binding patterns to the various cockroach extracts varied considerably among these participants, with most showing strong binding to a limited set of proteins in a given extract (representative blots are shown in Fig 1). Seven of these patients had IgE that bound to proteins of lower molecular weights than those bound by IgE from the *G portentosa*-sensitive individual's serum. All 15 participants had circulating IgG that bound to proteins in extracts from the various cockroach species (data not shown).

All of the control subjects showed little or no IgE binding on the 5-day autoradiograms and only faint IgE binding on the 14-day films, with all except for control 3 having patterns identical to those of control 2 (Fig 1). Control 3 was identical to the others except that there was strong binding to a few

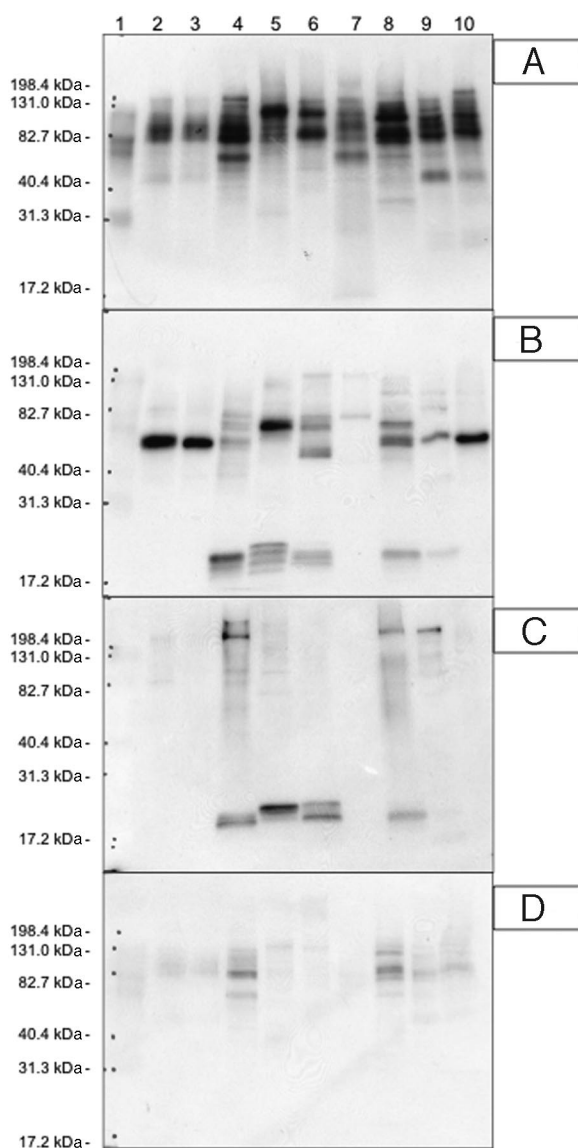


Figure 1. Representative 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis blots probed using sera from *Gromphadorhina portentosa*-sensitive (A), *Blatella germanica*-sensitive (B and C), and control (D) subjects. IgE binding was detected by means of autoradiography for 14 days. Lane 1 shows the molecular weight standard; lanes 2 to 4, *Periplaneta americana*; lane 5, *Blaberus giganteus*; lane 6, *G. portentosa* gut; lane 7, *G. portentosa* feces; and lanes 8 to 10, *B. germanica*. Extracts in lanes 2, 3, 9, and 10 were obtained from commercial suppliers.

proteins in each *P. americana* extract, perhaps suggesting previous exposure to this species of cockroach.

Blots with the various cockroach extracts were also probed using rabbit antiserum to *P. americana*, Bla g 1, Bla g 2, and Bla g 5. No antibody binding to any cockroach extract was observed using antiserum to Bla g 1 or Bla g 2 (data not shown). Antiserum to *P. americana* showed antibody binding

to several proteins in extracts from each of the 4 cockroach species, indicating cross-antigenicity among all of these species (Fig 2). Notable antibody binding was observed to a band of 77 kDa in all the extracts, with especially strong binding to the *G. portentosa* samples; to a band of 92 kDa in extracts from all the species except *G. portentosa*; and to a 45-kDa band in the *G. portentosa* fecal extracts. Antiserum to Bla g 5 did not bind to any proteins in the *P. americana* extract but did react with a protein of 31 kDa in the gut extracts from all other species and to a band of 84 kDa in the *B. germanica* and *B. giganteus* extracts (Fig 2). In addition, none of the participants had IgE directed at Bla g 5 because none had a band on any autoradiogram that coincided with the Bla g 5 band identified by binding of rabbit antibody to this protein (data not shown).

The possibility that some of the observed cross-reactivity was due to the presence of the panallergen tropomyosin was also investigated. Several blots were probed using rabbit antiserum raised against tropomyosin from chicken gizzard because tropomyosin from *B. germanica*, Bla g 7, is a known allergen.^{4,5} This antibody bound to proteins of 65 and 120 kDa in 1 of the commercial *B. germanica* extracts. Four of the study participants (1 sensitized to *G. portentosa* and 3 sensitized to *B. germanica*) showed weak IgE and possibly faint IgG binding to these proteins (data not shown).

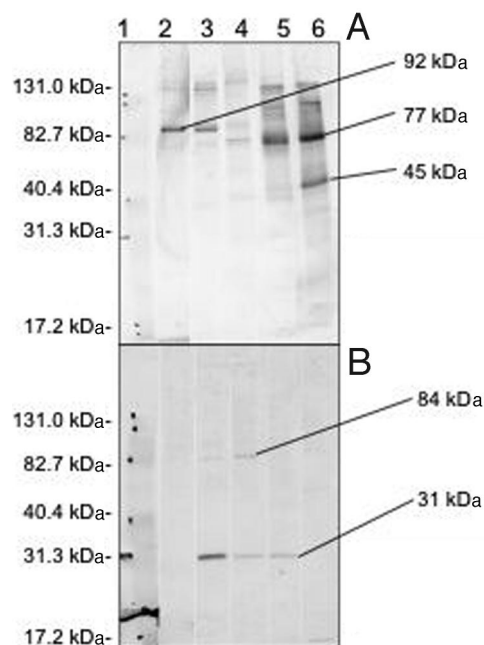


Figure 2. Blots of extracts of 4 cockroach species probed using rabbit antiserum to *Periplaneta americana* (A) and Bla g 5 (B). Lane 1 shows the molecular weight standard; lane 2, *P. americana*; lane 3, *Blatella germanica*; lane 4, *Blaberus giganteus*; lane 5, *Gromphadorhina portentosa* gut; and lane 6, *G. portentosa* feces.

DISCUSSION

This study reveals that gut and fecal extracts of the cockroach *G portentosa* contained several proteins that bound IgE from the serum of an individual who reported allergic symptoms on exposure to this cockroach. This individual also exhibited IgE binding to proteins in extracts of *P americana*, *B giganteus*, and *B germanica*, cockroaches to which the individual had no known exposure, indicating the possibility of the presence of cross-reactive allergens among these 4 cockroach species. More important, half (8 of 15) of the *B germanica*-sensitive patients tested had circulating IgE that bound to proteins in the *G portentosa* extracts, and 3 showed binding to proteins not detected using the *G portentosa*-sensitive individual's serum. We do not know whether these patients had any exposure to *G portentosa*, but previous exposure seems unlikely, suggesting that *G portentosa* contains several allergens that cross-react with allergens from the more common cockroaches. However, based on the intensity of radiostaining, the *B germanica*-sensitive participants had lower levels of IgE binding to *G portentosa* proteins than did the *G portentosa*-sensitive individual.

We did not directly investigate the cross-allergenicity of these *G portentosa* allergens with those of other cockroach species because we did not have sufficient serum from the *G portentosa*-sensitized individual to continue the studies. However, rabbit antibody raised against *P americana* and against Bla g 5 bound to proteins in extracts of other cockroach species, indicating substantial antigenic cross-reactivity among all the species tested, including *G portentosa*. These data do not support the panallergen tropomyosin as a contributor to the observed cross-reactivity because little IgE binding to this protein was detected.

We undertook this study on learning of the allergic symptoms exhibited by the *G portentosa*-sensitized researcher. An extensive literature search did not uncover any previous reports of allergic reaction to this cockroach species. However,

the fact that this cockroach is the source of multiple allergens, some of which seem to be cross-reactive with allergens from the more common American and German cockroaches, could be problematic given that a high percentage of patients with asthma or rhinitis (atopy) are sensitive to other species of cockroaches.¹ Surveys conducted in many US cities show that in selected populations up to 60% of patients with asthma are sensitive to cockroach allergens. Allergists, teachers, parents, and patients should be aware that sensitization and allergic reactions to the Madagascar hissing cockroach can occur. Caution should be taken if these exotic cockroaches are used in educational exercises in schools, museums, and zoos to protect predisposed individuals.

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