

Cross-reactivity of IgE-binding components between boiled Atlantic shrimp and German cockroach

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IgE-antibody reactivity to boiled Atlantic shrimp (*Pandalus borealis*) and German cockroach (*Blattella germanica*) of sera from 89 patients, sensitive to one or the other, was investigated with an enzymatic immunoassay for specific IgE detection (CAP-FEIA System, Pharmacia, Sweden). IgE serum levels to both antishrimp and antickroach allergens were found to be positive in 76 of the 89 (85.4%) tested sera. A positive antickroach IgE was very rare in the absence of detectable antishrimp IgE (five of 89 sera). Linear regression analysis on antishrimp and anti-German cockroach IgE levels-log plot revealed a correlation coefficient (r) of 0.73. Inhibition experiments showed that boiled Atlantic shrimp extract inhibited CAP with German cockroach, and vice versa. Immunoblotting showed the strongest IgE binding for both allergenic extracts between 30 and 43 kDa. By blot inhibition, the binding capacity of German cockroach was totally abolished by Atlantic shrimp extract, while German cockroach extract only partially IgE binding to Atlantic shrimp. Cross-reactivity exists between shrimp, an important food allergen, and German cockroach, which has an increasing role in allergic asthma. It could be important to determine the clinical significance of cross-allergy to both allergens, in which exposures occur in different ways.

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Seafood is among the foods most frequently implicated as inducing adverse reactions in food-sensitive adults (21). Previously reported studies of adverse reactions to shrimp in shrimp-sensitive, food-allergic subjects have demonstrated predominantly type I, IgE-mediated, immediate-hypersensitivity reactions (30). Seafood consists of a wide variety of mollusks (snails, mussels, oysters, scallops, clams, squid, and octopus) and crustaceans (lobster, crabs, prawns, and shrimp). In Spain, the prevalence of IgE-mediated reactions to seafood has been reported to be up to 5.4% of all IgE-mediated food hypersensitivity reactions in children (3). Shrimp allergens have been most extensively studied (10, 11, 14, 16). Considerable cross-reactivity among crustaceans has been demonstrated through skin-test and RAST analyses (4, 12). However, no oral challenge studies have been performed to determine the extent of symptomatic cross-reactivity among the various crustaceans.

Cross-reactions between aeroallergens and food

allergens have been reported mainly in pollen hypersensitivity associated with fruit and vegetable allergy. This suggested the importance, in some cases, of the taxonomic relations among different allergens in cross-reactivity. Cross-reactivity has been demonstrated among diverse grass pollens and vegetable foods (carrot, celery, and potato); birch pollen and fruits (apple, pear, cherry, peach); and ragweed pollen with fruits (melon, banana) (21). The advent of molecular biology as a tool for studying allergens has revealed a new group of panallergens and interrelations among allergens (27). Proteins such as the profilins, characterized as the birch allergen Bet v 2, exist in many other vegetable and animal sources, and it has been suggested that they may be one of the causes of the multiple sensitization to vegetable foods in some patients (27). There are other proteins, such as plant to pathogenesis-related proteins (PRP), which are related to the plant stress conditions, that are common to many vegetable plants (29). However, there are very few studies in which

the cross-reactivity among food allergens and aeroallergens, from an animal source, has been evaluated. Recently, several publications have described the occurrence of egg allergy combined with hypersensitivity to bird excrements or feathers in adults. This observation led to the term "bird-egg syndrome". It has been shown that egg-yolk proteins, particularly egg-yolk α -livetins (chicken serum albumin), are responsible for this cross-reactivity of specific IgE antibodies (28). Food allergy caused by snails seems to prevail in patients sensitized to *Dermatophagoides* mites (1). In the same way, Eriksson et al. reported that cross-reactivity exists between larvae of chironomids (nonbiting midges) and shrimp (6). Cross-reactivity between shrimp and cockroach extracts has been suggested by O'Neil et al. (19). Taking this as a basis, we have evaluated the extension of shrimp/cockroach cross-reactivity in a wide sample of patients.

Material and methods

Sera

Serum samples were collected from 89 patients, younger than 14 years of age, with serum specific IgE to Atlantic (northern) shrimp (*Pandalus borealis*) and/or German cockroach (*Blattella germanica*). These patients were evaluated in the Allergy Department of "La Paz" Children's Hospital of Madrid, Spain, for allergic rhinitis and/or asthma and/or food hypersensitivity, by means of clinical history, skin prick testing, *in vitro* specific IgE, and oral challenges. Each serum was tested for either antishrimp or antickroach-specific IgE, when it had not been carried out previously. Sera were stored at -70°C until use.

Sera from 20 of 89 patients selected by means of similar levels of specific IgE to both allergens and higher than 10 kU/ml were pooled and stored at -70°C , to be used in the inhibition-assay studies and immunoblotting.

Allergen extracts

Freeze-dried raw material from whole body of German cockroach (GC) (*B. germanica*) (Lot no. 89-031) was kindly provided by Biopol Laboratory, Inc (Spokane, WA, USA) through Abello Laboratories (Madrid, Spain). This material was resuspended with PBS (10% w/v). The mixture was stirred overnight at 4°C and was centrifuged at 12 000 g for 20 min at 4°C . The supernatant was subsequently dialyzed (Spectra/por, Spectrum Medical Industries, Inc, Los Angeles, CA, USA, molecular mass cutoff > 3500 Da) at 4°C against 2000 vol of 10 mM of glycine for 20 h, centrifuged at 30 000 g for 20 min at 4°C , and filtered through a 0.45- μm Millipore

filter (Millipore Corp., Bedford, MA, USA). The material was finally freeze-dried.

Atlantic (northern) shrimp (*P. borealis*), purchased from a local seafood store, was boiled for 15 min in ion-depleted water. The meat of the cooked shrimp was removed and homogenized in PBS, pH 7.2, for 10 min in a Waring blender (Waring Products Division, New Hartford, USA). After mixing overnight at 4°C with agitation, the extract was centrifuged at 12 000 g, and supernatants were dialyzed (Spectra/por, Spectrum Medical Industries, Inc., Los Angeles, CA, USA, molecular mass cutoff > 3500 Da) against 10 mM glycine and recentrifuged (23 000 g). The material was finally freeze-dried. Determination of protein concentration in both the boiled Atlantic shrimp (BAS) and GC extracts was performed according to the Lowry et al. method (15).

Allergen-specific IgE levels

BAS- and GC-specific IgE levels in all 89 individual sera and in the serum pool were determined by an enzymatic immunoassay using ImmunoCAP (cellulose sponge) as solid phase for allergens (CAP-*FEIA*), performed according to the manufacturer's instructions (Pharmacia Diagnostics, Uppsala, Sweden). BAS and GC extracts coupled to the ImmunoCAP (Pharmacia Diagnostics, Uppsala, Sweden) were used as solid phase in the CAP-*FEIA* assays. Results were expressed in kU/l. Significant reactivity was determined to be higher than or equal to 0.35 kU/l, according to the manufacturer's instructions. CAP-*FEIA* reactivity to BAS and GC was compared by linear regression of the logarithmically transformed values for each serum. Anti-*Lolium perenne*, anti-*Alternaria alternata*, and anti-ovalbumin IgE were determined by the same CAP-*FEIA* method in the pooled serum.

CAP-*FEIA* inhibition

The ImmunoCAP-inhibition procedure was a modification of the Gleich et al. RAST-inhibition method (7). Several concentrations (0.1, 1, 10, and 100 $\mu\text{g}/\text{ml}$, final concentrations) of competing fluid-phase antigens (BAS or GC), diluted with PBS, were preincubated with an equal volume of pooled serum overnight with intermittent shaking at room temperature. Positive controls were diluted and preincubated in PBS alone. After preincubation, the mixtures were centrifuged and 50- μl aliquots, in duplicate, were incubated at room temperature with the ImmunoCAP, with either the same or another extract, for measurement of BAS- and GC-specific IgE concentrations, as determined by the CAP-*FEIA* procedure. Percent inhibition of BAS- and GC-specific IgE for each soluble antigen plotted on semi-

log graph paper, with the y-axis as percentage inhibition and the x-axis as log concentration ($\mu\text{g/ml}$) of competing fluid-phase antigen. As suggested by Schröder & Yman (23), at least two points on either side of 50% inhibition level were used for calculation of the slope. Pearson's correlation coefficient of the line was determined from such points to be >0.90 . For each competing fluid-phase antigen evaluated, the graph of inhibition was determined by linear regression. Similarity in slopes was a reflection of antigenic similarity.

SDS-PAGE and immunoblotting

The electrophoresis procedure was a modification of the method of Sutton et al. (25). SDS-PAGE was performed with a 12% polyacrylamide separating gel and a stacking gel of 4%. Twenty microliters of a 1-mg/ml solution of each allergenic extract was applied to each well. Electrophoresis was performed for 1 h at 130 V per gel for the 8 by 7.5 cm gels (Mini-Protein II system, Bio-Rad Laboratories). To ensure proper protein separation and visualization, Coomassie brilliant blue (Sigma Chemical Co., St Louis, MO, USA) stains were done on gels. Proteins were electrophoretically transferred from the separating gel to a nitrocellulose membrane in a transfer buffer (Tris-glycine) with 20% methanol (26). This procedure was done in a minitransfer apparatus (Bio-Rad Laboratories).

After removal from the transblot apparatus, the nitrocellulose was placed in blocking solution (1% BSA/TBS Tween) for 1 h at room temperature. The nitrocellulose blot was then washed four times with TTBS (TBS with 0.1% Tween 20) and incubated with the pooled serum (1:10 vol/vol dilution) for 1 h at room temperature with rocking. The nitrocellulose blot was again washed with TTBS four times, and alkaline-phosphatase-conjugated goat antihuman IgE (1:200 vol/vol of TBS, Caltag Laboratory, San Francisco, CA, USA) was added and incubated at room temperature with rocking for 1 h. After an additional wash with TBS three times, the blot was developed with 250 μl of 30 mg/ml of NBT in 70% DMF and 250 μl of 15 mg/ml BCIP in DMF solutions in 25 ml of carbonate buffer (0.2 mol/l, pH 9.8) at room temperature for 15 min. The reaction was then stopped by decanting the developing solution and incubating the nitrocellulose blot for 10 min with distilled water. The blot was then air-dried.

Immunoblot inhibition

Aliquots (1.5 ml) of the serum pool were incubated overnight with BAS or GC extracts at a final concentration of 0.1 mg/ml protein (final serum dilution 1:10). Mixtures were then centrifuged and each

supernatant was incubated with BAS- or GC-blotted nitrocellulose membranes, followed by immunodetection as described above. Ovalbumin (Sigma, Chemical Co, St Louis, MO, USA), at a final concentration of 0.1 mg/l, was used as control extract to demonstrate specificity of inhibitory activity.

Results

Correlation between antishrimp and antickroach allergen IgE levels

Serum levels of both antishrimp and antickroach allergens IgE were found to be positive in 76 of the 89 (85.4%) patients' sera tested. A positive anti-GC IgE was very rare in the absence of detectable anti-BAS IgE (five of 89 patients tested). In these five sera, values of antickroach IgE were lower than or equal to 1.7 kU/l (range 0.71–1.7 kU/ml). Eight of 89 patients were found to have antishrimp IgE, but not antickroach IgE.

In both assays, values obtained with 70 sera from patients with BAS- and GC-specific IgE levels within quantitative range of the assay (0.35–100 kU/l) showed a log-normal distribution (Fig. 1). Linear regression analysis on this double-log plot revealed a correlation coefficient (r) of 0.73 (range 0.60–0.82, $P < 0.05$). The inclusion of negative data (allergen-specific IgE < 0.35 kU/l) and values over 100 kU/l in the analysis had little effect on the correlation coefficient ($r = 0.7$; 0.57–0.79, $P < 0.05$).

CAP-FEIA inhibition studies

Inhibition CAP-FEIA was performed to assess whether the correlation shown in Fig. 1 was due to immunologic cross-reactivity. In the serum pool with a pronounced IgE reaction to both BAS (38.4 kU/l) and GC (25.8 kU/l), dose-dependent inhibition was



Fig. 1. Values obtained in 70 sera from children with BAS- and GC-specific IgE levels within quantitative range of assay (0.35–100 kU/l) showed log-normal distribution.

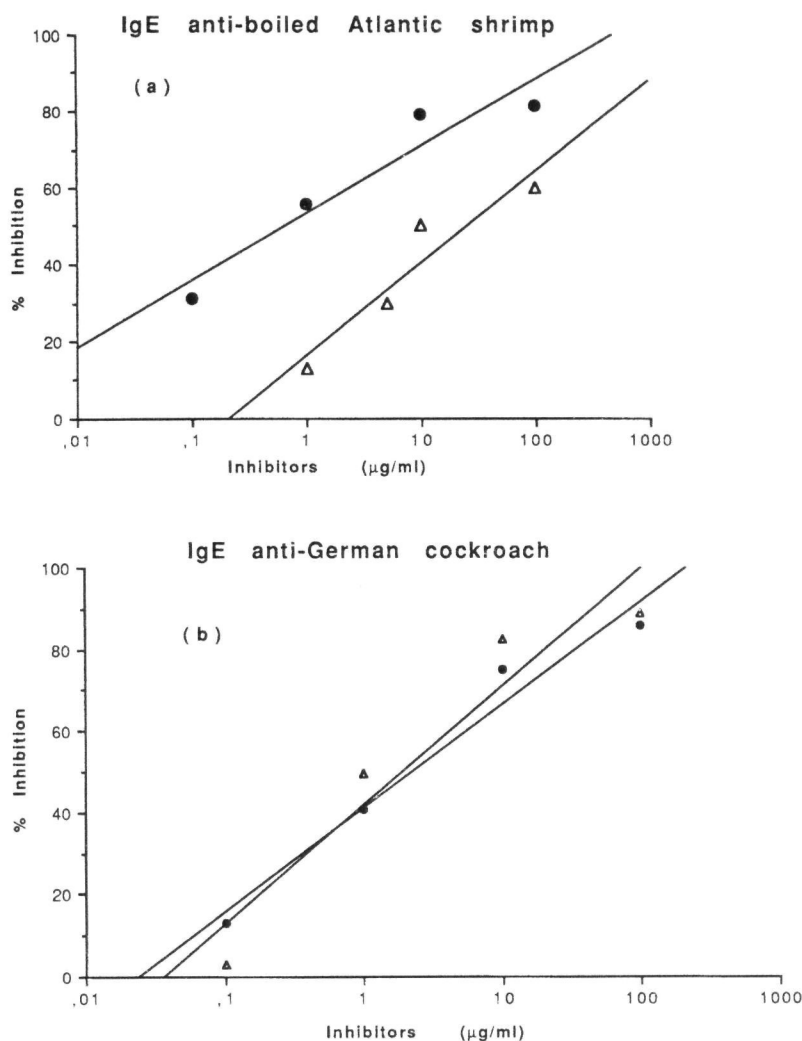


Fig. 2. Inhibition of (a) specific IgE anti-BAS (solid phase) and (b) specific IgE anti-GC (solid phase) with GC (fluid phase) in triangles and BAS (fluid phase) in circles. Difference between slopes was not significant. In (a), concentrations that inhibited 50% were 0.7 $\mu\text{g/ml}$ for homologous (BAS) and 22 $\mu\text{g/ml}$ for heterologous (GC) inhibitors; in (b), 1.9 $\mu\text{g/ml}$ for homologous (GC) and 2.1 $\mu\text{g/ml}$ for heterologous (BAS) inhibitors.

observed after preincubation with either of the two extracts. The extracts showed no quantitative differences in their capabilities to inhibit IgE reactions with the same extract. The GC extract had lower fluid-phase inhibitory capacity (60%), with the other extract coated to the solid phase, than BAS allergens (89%) with cockroach extract in the solid phase. The comparisons of inhibition curves using GC or BAS extracts coated to the solid phase and GC and BAS as inhibitors showed that the difference between the slopes did not differ significantly (t -test, $P < 0.05$) (Fig. 2). The allergen concentrations which yielded a 50% inhibition were 0.7 $\mu\text{g/ml}$ for homologous BAS and 1.9 $\mu\text{g/ml}$ for homologous GC; BAS vs GC 2.1 $\mu\text{g/ml}$ and GC vs BAS 22 $\mu\text{g/ml}$.

To rule out the possibility that the observed cross-inhibition was due to nonspecific binding of IgE, similar experiments with ovalbumin, *L. perenne*, and *A. alternata* allergens were performed using the serum pool with a known level of appropriate specific IgE (ovalbumin 42.3 kU/l, *L. perenne* 20.9 kU/l, and *A. alternata* 23.1 kU/l). None of these IgE reactions were affected by preincubation of the sera with either BAS or GC extracts at the same concentrations.

Immunoblot and immunoblot inhibition

BAS allergens. Approximately 12 of 29 protein bands obtained by SDS-PAGE showed IgE-binding capacity, ranging in molecular mass from 25 to 90 kDa (Fig. 3). The strongest allergenic activity was a double band between 36 and 43 kDa. Another double band around 27 kDa was not evident in the cockroach pattern.

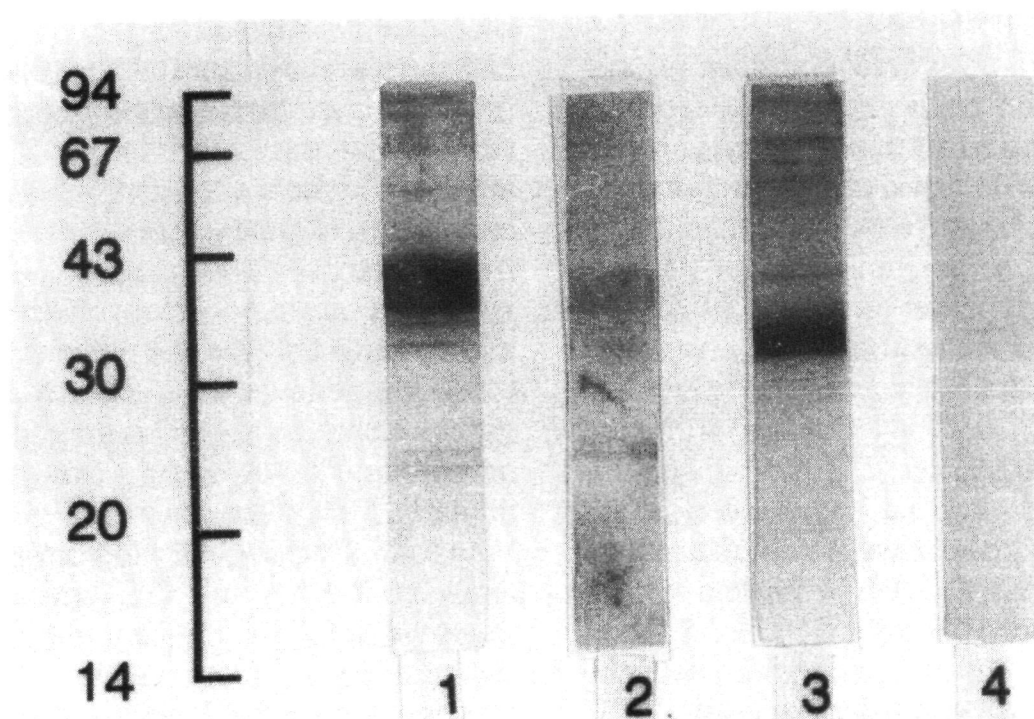


Fig. 3. SDS-PAGE immunoblot analysis of BAS (lane 1) and GC (lane 3) extracts. SDS-PAGE immunoblot inhibition analysis of BAS (solid phase), performed with GC extract (lane 2), and GC (solid phase), with BAS extract (lane 4).

GC allergens. The immunoprint pattern had fewer IgE-binding bands. The most important one was a double band between 30 and 36 kDa (Fig. 3).

Immunoblot inhibition. The homologous inhibition for both allergens was total. The inhibition of BAS with GC was partial, being stronger at high molecular mass bands. The blot inhibition of GC with soluble BAS was total (Fig. 3). Ovalbumin was used as the control allergen for specificity of inhibitory activity, and its band pattern was unaffected by either allergen. Ovalbumin was used as inhibitor blot extract at the same concentration without any effect on BAS or GC band patterns.

Discussion

Shrimp is among the foods most frequently associated with IgE-mediated type I hypersensitivity (30). Earlier, evidence of cross-reactivity between shrimp and cockroach extracts had been found by rocket electrophoresis and RAST inhibition (19). However, the extension of these findings was not further evaluated. The current study confirms that both Atlantic shrimp and German cockroach have significant, and similar, allergenic activity. In our selected individuals, positive specific IgE to both BAS and GC was found in 85.4% of the sera evaluated. BAS and GC CAP-FEIA results were found to be highly and significantly correlated ($r = 0.73$, $P < 0.05$). Therefore, CAP inhibition studies were performed with BAS and/or GC-coupled ImmunoCAP, with BAS and/or GC as inhibiting allergens. These inhibition experiments showed that BAS extracts inhibited CAP with GC, and vice versa. This indicates that both allergen sources contain structures sharing similar IgE-binding sites.

Allergenic characteristics of several shrimp species, as well as German cockroach, have been extensively studied. During an attempt to isolate shrimp allergens by Nagpal et al., evidence was obtained that shrimp ribonucleic acid (tRNA) was capable of eliciting a specific IgE response. This fact has not been mentioned by any other group (17). Subsequently, these investigators identified and characterized two heat-stable protein allergens, designated as Sa-I (8.2 kDa) and Sa-II (34 kDa), from boiled shrimp (*Penaeus indicus*) extracts. Solid-phase inhibition studies revealed that Sa-I and Sa-II share 54% of the allergenic epitopes, suggesting that Sa-I may be a fragment of Sa-II (18). A protein with a molecular mass of approximately 34 kDa isolated from prawn, lobster, and crab cross-reacts with the major shrimp allergen (11). Additionally, the Sa-II major allergen (Pen i 1) has been identified as the shrimp muscle tropomyosin with an 86% homology with tropomyosin from *Drosophila melanogaster* (24).

A major heat-stable shrimp allergen, Met e 1 from *Metapenaeus ensis*, has also been cloned and expressed. It is a 34-kDa protein highly homologous with multiple isoforms of tropomyosin (13). Recently, Daul et al. demonstrated that Pen a 1, the major shrimp allergen from *Penaeus aztecus*, has significant homology (60–87%) with the muscle protein tropomyosin from various species. The greatest homology (87%) was noted with tropomyosin of the fruit fly (*D. melanogaster*) (5). Lin et al. identified six components, ranging in molecular mass from 86 to 39 kDa, as shrimp allergens (*Parapenaeus fissurus*). The component of about 39 kDa (with isoelectric point values from 5.1 to 5.6) showed the highest frequency of IgE-binding and was considered to be one of its major allergens, being designated as Par f 1 (14). The shrimp species *P. borealis* is commonly eaten in our area; however, no data about allergens present in this species have previously been reported. Immunoblot analyses in the present study showed at least 12 protein bands (25–90 kDa) that bound IgE in BAS. In particular, this extract showed a dense IgE-binding component between 36 and 43 kDa.

Regarding German cockroach, we found the strongest IgE-binding area at 30–35 kDa, but other bands at > 50 kDa also exhibited significant activity. Several allergens of German cockroach have been isolated and characterized. Schou et al. reported the purification of an acidic allergen from *B. germanica* extracts that has been designated Bla g 1 (22). This is a protein around 25 kDa, pI 3.5, which dissociates into 6-kDa peptides, suggesting a multimeric structure. A second *B. germanica* allergen, designated Bla g 2, has been isolated with monoclonal antibodies (20). Bla g 2 is a 36-kDa heat-sensitive protein, pI 5.2, which has also been identified by Helm et al. (8), who provided evidence of the presence of shared allergenic activity in Asian (*Blattella asahinai*), German (*B. germanica*), American (*Periplaneta americana*), and Oriental (*B. orientalis*) cockroach species (9). In addition, different binding profiles have been noted with the serum IgE from different patients with GC allergy. In this study, we used a serum pool from children sensitized to GC and BAS to analyze the presence of common structural components in these extracts. Presumably, a different pattern of IgE binding to GC proteins could be obtained by using sera from adult patients, particularly if they are sensitized solely to GC.

In our study, CAP and immunoblot inhibition analysis of BAS and GC extracts strongly suggest that several IgE-binding components could be shared by these extracts. In addition, the specificity of these results is supported by proper controls for CAP and immunoblot inhibitions. IgE-binding to ovalbumin was unaffected by preincubation of the serum pool with BAS and GC, nor did preincubation of the

serum pool with ovalbumin (negative noninhibitory control extract) affect the IgE-binding pattern to GC and BAS. Baldo & Panzani reported the detection of IgE antibodies to a wide range of insect species, including GC, in subjects with suspected inhalant allergies to insects. These authors pointed out that "panallergy" to insects may occur in subjects who have been sensitized to one or a few insects, and that allergenic similarities may extend to at least some other noninsect members of the phylum Arthropoda (2). Recently, Witteman et al. identified tropomyosin as a cross-reactive allergen among members of the classes Crustacea (*Crangon crangon*), Arachnida (*D. pteronyssinus*), and Insecta (chironomid, mosquito, and *B. germanica*) (31). However, there are no previous studies of the allergen content of *P. borealis* or of its cross-reactivity with other arthropods. It is possible that, although tropomyosin may be an important "panallergen", other common structural components could also be present in different arthropods.

It could be important to determine the clinical significance of cross-allergy to both allergens, in which exposure occurs through different routes. In this way, our ongoing clinical research is focusing on the clinical profile (respiratory symptoms after cockroach exposure and/or clinical manifestations following shrimp ingestion) of patients sensitized to both shrimp (*P. borealis*) and German cockroach.

In summary, this study demonstrated an extensive cross-allergenicity between boiled Atlantic shrimp, an important food allergen, and German cockroach, which has an increasing role as an indoor aeroallergen in allergic asthma. These results confirm other reports of cross-reactivity among members of the phylum Arthropoda, exposure occurring by different routes. Additionally, allergens in the widely consumed shrimp species (*P. borealis*) were identified through immunoblotting with a serum pool from patients sensitive to shrimp. Further studies will be needed to elucidate the role of exposure to common allergenic components, coming from different sources and routes, in eliciting clinical manifestations.

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