

Immunoglobulin E antibody reactivity to the major shrimp allergen, tropomyosin, in unexposed Orthodox Jews

J. Fernandes*, A. Reshef†, L. Patton*, R. Ayuso*‡, G. Reese*§ and S. B. Lehrer*

*Tulane University Health Sciences Center, New Orleans, Louisiana, USA, and †Sheba Medical Center, Tel Hashomer, Israel

Summary

Background Assessment of allergic (IgE antibody-mediated) reactions to foods may become complicated by cross-reactivity that can occur among certain food families and between foods and seemingly unrelated allergens.

Objective The allergenic properties of tropomyosin (muscle-derived protein) have been recently demonstrated in invertebrates such as cockroaches, dust mites, and shrimp. In view of a possible cross-reactivity between food allergens and related allergens from animal sources, we designed a study to assess IgE antibody reactivity to the major shrimp allergen, Pen a 1, in an unexposed population of Orthodox Jews, who observe Kosher dietary laws that prohibit eating shellfish.

Methods Nine subjects, who reacted positively by skin tests to shrimp (*Penaeus setiferous*), were selected for the study. Subjects (two females, seven males) ranged in age from 14 to 32 years (mean 20.4). All subjects were strictly observant of Jewish tradition and had no prior exposure to seafood (regarded as a non-Kosher food). Serum was obtained from all the subjects and tested for IgE antibody reactivity to shrimp and dust mite.

Results All subjects reported symptoms of perennial allergic rhinitis, five had history of asthma, atopic dermatitis, and/or sinusitis. All had positive skin prick tests to shrimp and house dust mite (HDM) (*Dermatophagoides farinae*, *D. pteronyssinus*, or both); 2/7 subjects were positive to cockroach mix (*Blattella germanica* and *Periplaneta americana*). Sera of 4/9 subjects demonstrated specific IgE antibodies by RAST to shrimp (7.0–20.0%), 3/9 to Pen a 1 (6.3–24.1%), and 3/9 to shrimp or Pen a 1 by immunoblot. IgE binding to Pen a 1 was inhibited with either mite or cockroach extracts as demonstrated by RAST and/or immunoblot inhibition analysis.

Conclusions These studies indicate that IgE antibody reactivity to a major food allergen, shrimp, can occur in an unexposed population of individuals; some subjects allergic to HDM and/or cockroach show substantial IgE antibody reactivity to the major shrimp allergen Pen a 1 (tropomyosin). Based on inhibition with cockroach and/or dust mite extracts, this reactivity appears to be due to cross-reacting tropomyosins.

Keywords allergen cross-reactivity, cockroaches, dust mites, Orthodox Jews, shrimp reactivity, tropomyosin

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Introduction

Cross-reactivities occur among allergens within the same food group as well as between foods and non-food allergens. For example, some cross-reacting food allergens are phylogenetically closely related (those in legumes, fishes, crustaceans, fruits, or tree nuts), whereas other cross-reactivities have been described between foods and unrelated or distantly related

allergens especially pollens, such as the cross-reactivity between tomato and grass pollen [1–3].

The clinical relevance of food allergen cross-reactivity depends on the food in question. For example, the *in vitro* cross-reactivities detected among crustaceans are thought to be clinically relevant, e.g. shrimp-allergic subjects may have clinical symptoms after ingesting lobster and crab [4–6]. In contrast, cross-reactivities among legumes seem to be of less clinical importance, e.g. peanut-allergic subjects may show *in vitro* IgE antibody reactivity to beans or peas but usually have no clinical symptoms [7, 8].

Shellfish are a frequent cause of adverse allergic reactions among the food allergens of animal origin [9]. Most shellfish species known to elicit allergic food reactions belong to the class crustacea and include shrimp, crab, crawfish, and lobster. Shrimp has been thoroughly studied and its only

‡Present address: St. John's Episcopal Hospital, South Shore, 327 Beach 19 St., Far Rockaway, NY, 11691, USA

§Present address: Paul-Ehrlich-Institut, Paul-Ehrlich Str. 51–59, D-63225, Langen, Germany

Correspondence: Samuel B. Lehrer, PhD, Tulane, University Health Sciences Center, 1700 Perdido St. (SL-57), New Orleans, LA 70112, USA. E-mail: sblehrer@tulane.edu

major allergen has been identified as the muscle protein tropomyosin (Pen a 1) [10–12]. Tropomyosin has also been identified as an important allergen in invertebrates such as cockroach (Per a 7) [13], dust mites (Der p 10 and Der f 10) [14], chironomids [15] and silverfish (Lep s 1.1 and Lep s 1.2) [16], as well as other crustacea and molluscs and has been suggested as an important invertebrate cross-reacting allergen [2].

Determining the role of *in vivo* cross-reactivity in clinical allergic reactions is problematic since most patients are usually exposed to both foods and aeroallergens, and it is difficult to determine whether sensitization occurs through direct exposure or by exposure to cross-reacting allergens. We chose to study a unique population of Orthodox Jews because their adherence to strict kosher dietary laws prohibits them from consumption of shellfish. Based on these facts, the following study was designed to assess reactivity to shrimp tropomyosin and its possible cross-reactivity with dust mite and cockroach in the development of IgE antibody responses to shrimp (skin prick test (SPT), RAST and immunoblot) in a population of Orthodox Jews with no prior exposure to shellfish.

Material and methods

Skin prick tests (SPTs)

Approximately 900 patients reporting to an allergy clinic in Bnay-Braq, an ultraorthodox district near Tel-Aviv, Israel, were skin prick tested with the following allergens, obtained from Center Laboratories (NY, USA): trees (cypress, olive, eucalyptus, pecan, ailanthus – 1:20 w/v); grasses (five grass mix, three grass mix – 1:20 w/v); weeds (four weed mix – 1:20 w/v); *Parietaria officinalis* – 1:20 w/v; Mugwort – 1:20 w/v; mold mix (I and II), *Aspergillus fumigatus*; dust mite mix (*Dermatophagoides farinae*, *D. pteronyssinus*) 5000 AU each; feather mix (duck, goose, chicken) – 1:20 w/v; shellfish mix (oyster, crab, shrimp) – 1:20 w/v; and cockroach mix (*Blattella germanica* and *Periplaneta americana*) – 1:20 w/v. White shrimp (*Penaeus setiferus*) was prepared at Tulane Medical Center, New Orleans, LA, USA [17]. Allergen solutions were applied on the volar skin of the arm, lightly pricked by a lancet (prick lancetter, Hollister-Stier, Spokane, WA, USA), and weal (Oedema) and flare (erythema) reactions were read after 15–20 min. Positive reactions were traced by pen and transferred to a recording sheet by transparent adhesive tape. Mean weal and flare diameters were calculated from the largest and perpendicular diameters. A mean weal size of 3 mm or greater than the saline control or similar or larger than the positive control (histamine phosphate 2.75 mg/mL) was considered as positive.

Study Population

Nine patients who were positive by SPT to shrimp were selected for the study. All patients were Orthodox Jews who observed strict Kosher dietary laws that prohibit consumption of crustaceans and molluscs with confirmed histories of no exposure to these foods. All patients had positive skin tests to dust mite mix and two of the seven tested positive to cockroach. All patients (seven males, two females, mean age

20.4 years, range 14–32) had allergic rhinitis, two (B.S. and E.Y.) had asthma, and two (B.S. and S.N.) had atopic dermatitis as a young child or infant. No subjects reported food-related allergic symptoms. One subject (E.Y.) was receiving immunotherapy with dust mite (*D. farinae* and *D. pteronyssinus*) and grass allergens, at the time of the study. Informed consent for the study was obtained from the patients or their parents. This group of nine patients represents a small fraction of the randomly surveyed population.

Sera

Sera were collected from the nine unexposed Orthodox Jews who demonstrated positive SPTs to shrimp, dust mite, and/or cockroach as described above. A serum pool from four shrimp-allergic subjects was used as a positive control. Each serum came from subjects who were skin test and RAST positive (binding $\geq 3\%$) to shrimp and had histories of shrimp-induced food allergy.

Extracts

The preparation of shrimp and cockroach extracts has been previously described in detail [10, 18]. Briefly, raw white shrimp (*P. setiferus*) or brown shrimp (*P. aztecus*) was boiled for 15 min in ion-depleted water, peeled, and deveined. Cockroaches were purchased commercially and extracted as described previously. The boiled shrimp meat or cockroaches were homogenized in phosphate-buffered saline (PBS) pH 7.2. The homogenate was stirred overnight at 4 °C and then centrifuged (15 000 g). The supernatant was concentrated by Amicon-YM2 ultrafiltration (molecular weight cutoff <1 kDa; Danvers, MA, USA). The concentrate was re-centrifuged (15 000 g), dialysed (MWCO > 3500 Da), and stored in aliquots at –20 °C. Commercial mite extract (*D. pteronyssinus*) was obtained from Center Laboratories (Port Washington, NY, USA) and concentrated by dialysis and Amicon-YM2 ultrafiltration. Protein concentrations of the extracts were determined by the method of Bradford [17] using a commercial kit (Pierce Lab, Rockford, IL, USA) and BSA as a standard.

Isolation of the 36-kDa shrimp allergen by electroelution

Shrimp extract (locally purchased brown shrimp: *P. aztecus*) was prepared as described previously [5]. Pen a 1 was purified from shrimp extract by preparative SDS-PAGE (Model 491 PrepCell, BioRad, Hercules, CA, USA). Briefly, shrimp extract was separated on the 28 mm ID column using Laemmli extract discontinuous SDS-PAGE buffer system [19]. A 15-mm-high stacking gel (5% T, 1.5% C) poured on top of the 65-mm-high separation gel (11% T, 1.5% C) was used to separate shrimp proteins; the fractions containing Pen a 1 were collected and pooled.

RAST and RAST inhibition

Cellulose discs, activated with CNBr, were coupled according to the method of Ceska and Lundkvist, with Pen a 1 (0.1 µg/disc) [20]. Pen a 1-coated discs were incubated overnight with 100 µL undiluted serum in duplicate. For the RAST

inhibition assay, the discs were incubated with a diluted serum pool (1:5 or 1:10) in the presence of Pen a 1, shrimp, mite (*D. pteronyssinus*), German cockroach, American cockroach, or peanut extract as inhibitors. After washing with 0.9% NaCl solution, discs were incubated overnight with ^{125}I -labelled horse anti-human IgE (Sanofi Diagnostics Pasteur, Chasca, MN, USA) at 15 000 c.p.m./disc. After washing with saline solution, bound ^{125}I was determined in a γ -counter (Gamma 5500, Beckman Instruments, Irvine, CA, USA).

SDS-PAGE and IgE-immunoblot analysis

SDS-PAGE was performed with 12.5% T, 1.5% C separating gels and 5% T, 1.5% C stacking gels using the discontinuous buffer system according to Laemmli [19]. Extracts were mixed with sample buffer and boiled for 5 min. For purified Pen a 1, 1 $\mu\text{g}/\text{cm}$ and for all other extracts, 20 $\mu\text{g}/\text{cm}$ was loaded onto the gels and the electrophoresis was performed at 20 mA/gel ($V_{\text{max}} = 200 \text{ V}$) until the pyronine G tracking dye reached the bottom of the gel. For immunoblot analysis, electrophoretically separated proteins were transferred onto cyanogen bromide (CNBr)-activated nitrocellulose membranes [21] by semi-dry blotting at 0.8 mA/cm² – according to Kyhse-Andersen for 30 min [22]. The blots were blocked by Tris-buffered saline containing 0.05% Tween 20 (TBST) for 30 min.

The study subjects' and control sera were diluted (1:2) with TBST containing 1% BSA. For immunoblot inhibition analysis, serum pools, diluted (1:2), were mixed with Pen a 1 (50, 5 and 0.5 $\mu\text{g}/\text{mL}$), *D. pteronyssinus* (500, 50 and 5 $\mu\text{g}/\text{mL}$), or peanut extract (500 $\mu\text{g}/\text{mL}$). Each membrane was then incubated with test sera for 90 min and washed with TBST. To visualize the IgE-binding proteins, each membrane was incubated for 90 min with alkaline phosphatase-conjugated, monoclonal mouse anti-human IgE antibody (Southern Biotechnology Associates, Birmingham, AL, USA), diluted 1:1000 with TBST containing 1% bovine serum albumin (BSA) and washed three times with TBST. Finally, the IgE-binding proteins were detected using the substrate/chromogen mixture containing 450 μM 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and 400 μM nitro blue tetrazolium (NBT, Sigma Co., St Louis, MO, USA) dissolved in 100 mM Tris/HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl_2 [23]. The reaction was stopped with deionized water and the membranes were dried.

Results

SPTs

Table 1 shows the SPT results for the nine study patients. All nine patients tested positive to shrimp (*P. setiferus*); mean weal diameters ranged from 6 to 15 mm (mean 8.6 mm). All had positive tests to mite (*D. farinae*, *D. pteronyssinus*, or both) as well (range 6–15 mm, mean 10.1 mm). Of the patients tested to cockroach mix (*B. germanica* and *P. americana*), two were found positive (mean 5.5 mm). All six of the patients tested to shellfish mix (oyster, crab, and shrimp) were found positive (range 6–11 mm, mean 7.3 mm). Patients B.S. and S.N. were positive to shrimp, mite, and cockroach.

Table 1. Skin prick test results (Weal/flare mm) for study patients

Study subjects	<i>P. setiferus</i>	Mite mix	Cockroach mix	Shellfish mix
B.S.	15/35	10/30	5/10	NT*
S.N.	8/31	11/34	6/23	NT
A.S.	9/17	6/8	Neg	8/18
E.Y.	6/24	14/38	Neg	6/30
F.B.	8/23	10/26	Neg	11/17
G.H.	8/23	8/27	Neg	5/18
S.S.	8/24	15/42	Neg	NT
A.I.	7/30	9/24	Neg†	7/18
Y.A.	8/23	8/20	Neg	7/30

*NT – not tested.

†AI – converted to cockroach skin test positive (6/17) upon retesting.

RAST

Results of shrimp and Pen a 1 RASTs are presented in Table 2. RAST values were expressed as the mean percent bound of the total ^{125}I -labelled anti-IgE added. Positive RAST was defined as the percentage bound greater than 3%. Of the nine patients tested, four (44%) demonstrated positive IgE antibody responses to shrimp (RAST range 7.0–20.0%, mean 14.7%) and three (33%) were positive to Pen a 1 (RAST range 6.3–24.1%, mean 15.2%). Sera from subjects B.S., S.N., and E.Y. had significant IgE antibody reactivity to both shrimp and Pen a 1. For this reason, these three sera were pooled for the inhibition tests.

RAST inhibition

Figure 1 shows the RAST inhibition results of both the pool of three unexposed Orthodox Jews (patients B.S., S.N., and E.Y.) and the shrimp-positive control pool against Pen a 1. The results were expressed as the mean percent inhibition of the total specific binding. Inhibition values greater than the negative control inhibitor (peanut) were considered positive. Both the Orthodox Jew and shrimp sera pools' Pen a 1 RAST were inhibited by Pen a 1, shrimp, mite (*D. pteronyssinus*), German cockroach, or American cockroach extracts in a dose-dependent manner. The RAST with shrimp-allergic sera pool was inhibited to a greater degree, with Pen a 1 or shrimp,

Table 2. Individual IgE reactivities by RAST to shrimp and Pen a 1

Study subjects serum (undiluted)	% Binding	
	Shrimp (1 $\mu\text{g}/\text{disc}$)	Pen a 1 (0.1 $\mu\text{g}/\text{disc}$)
B.S.	20.0	24.1
S.N.	18.2	15.1
A.S.	13.7	1.3
E.Y.	7.0	6.3
F.B.	2.5	0
G.H.	0	0
S.S.	0	0
A.I.	0	0
Y.A.	0	0
Shrimp-allergic sera pool*	36.2	37.8

*Positive control.

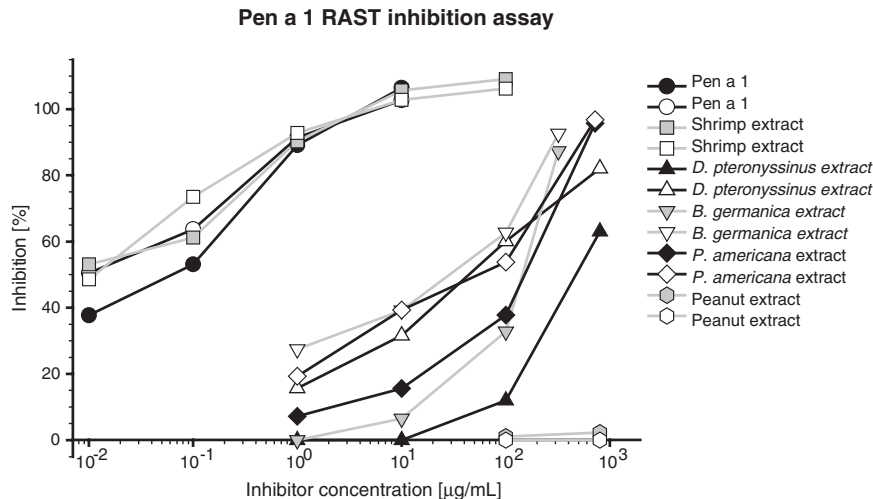


Fig. 1. RAST inhibition analysis: IgE antibody reactivities to purified Pen a 1 of serum pools of shrimp-allergic subjects (open symbols) and unexposed Orthodox Jews (filled symbols) were inhibited with Pen a 1, extract of *P. aztecus*, *D. pteronyssinus*, *B. germanica*, and *P. Americana*. Peanut extract served as negative control.

probably due to the lower mite and cockroach tropomyosin content. The peanut extract (negative control) showed essentially no inhibitor reactivity for either pool.

SDS-PAGE and IgE-immunoblot analysis

All nine patients were tested by immunoblot for IgE reactivity to shrimp and Pen a 1. Three (33%) of the nine patients showed reactivity to both shrimp and Pen a 1 (36-kDa protein band) (Fig. 2 a and b). Patients B.S. and S.N. showed strong reactivity to this band, whereas patient E.Y. demonstrated weaker reactivity. The shrimp-positive control sera pool demonstrated positive reactivity at the 36-kDa protein band as well as other protein bands with molecular weights ranging from 14 to 66 kDa.

Immunoblot inhibition analysis

Figure 3 shows the immunoblot inhibition of the Orthodox Jew pool to Pen a 1 by both the homologous inhibitor (Pen a 1) and heterologous inhibitors (mite *D. pteronyssinus* or peanut). As revealed by the reduction in IgE-binding intensity, Pen a 1 was inhibited by both the homologous inhibitor and mite in an almost identical dose-dependent manner. Peanut inhibitor, the negative control, showed no reduction in IgE-binding intensity.

Discussion

Tropomyosin has been identified as an important and/or major allergen in shrimp (Pen a 1), cockroach (Per a 7), and house dust mites (HDMs) (Der p 10 and Der f 10). This protein has also been identified as an allergen in a growing list of other foods and insects [2]. Tropomyosin belongs to a family of highly conserved proteins with multiple isoforms found in both muscle and non-muscle cells of vertebrates and invertebrates, with a high degree of amino acid sequence identity among different species [2]. Tropomyosin present in

the American cockroach or dust mites has a 75–80% identity with the tropomyosin from shrimp [2, 13].

Since cross-reactivity between different allergens occurs based on the shared similar or identical IgE-binding epitopes, these shared epitopes are probably the origin of the sensitization and development of allergic symptoms to allergens found in closely or distantly related species. Using synthetic overlapping peptides spanning the length of the major shrimp allergen, Pen a 1, eight IgE-binding epitopes were identified [24]. Four out of eight epitopes are identical to homologous regions in Der p 10 and Der f 10, and five out of eight are identical to homologous regions in Per a 7. In addition, even sequences in other arthropod epitopes that differ in one or more amino acids can still recognize and bind IgE antibodies from shrimp-allergic sera, although with somewhat lower intensity. Given the high degree of amino acid sequence identity between shrimp, mites, and cockroach tropomyosins and the evidence for cross-reactivity among the tropomyosins, it can be expected that shrimp-positive skin test and/or IgE antibody reactivity in subjects not exposed to shellfish might result from indirect sensitization by these cross-reacting allergens.

Of the nine patients in our study with positive SPTs to shrimp, three demonstrated IgE reactivity against the shrimp tropomyosin, Pen a 1, by RAST and immunoblot analysis. Because of the limited number of shrimp-reactive subjects studied, it is not possible to make any epidemiologic conclusions. Significant inhibition of IgE binding to Pen a 1 was demonstrated by RAST inhibition with dust mite, German cockroach, and American cockroach, as well as by immunoblot inhibition by dust mite. These results indicate that IgE sensitization to shrimp in this group of unexposed Orthodox Jews occurred as a result of indirect sensitization by cross-reacting tropomyosins between shrimp and either dust mite, cockroach, or both. The likelihood of inadvertent exposure to shrimp either through accidental ingestion of shrimp-containing foods or aerosolization of shrimp allergens is very unlikely, given the very strict adherence to Kosher dietary laws prohibiting the consumption of shellfish in this population.

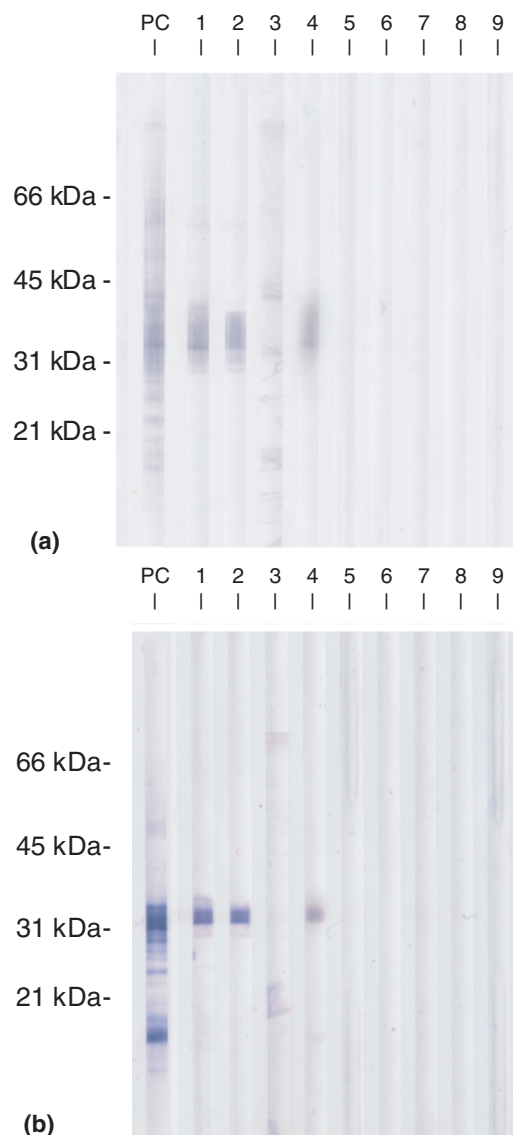


Fig. 2. Individual IgE antibody reactivity of Orthodox Jew patients 1–9 to Pen a 1 by immunoblotting. Shrimp-positive sera pool was used as a positive control (PC): (a) shrimp extract and (b) Pen a 1.

Indirect sensitization to cross-reacting allergens has been suggested by several reports in the literature. Sensitization by cross-reacting allergens was reported by van Ree et al. [25] who investigated the sera of 17 patients receiving immunotherapy for dust mite allergy for IgE antibodies against snail and shrimp. Three of the 17 patients, who had no prior reactivity, developed a positive RAST for shrimp and snail. Two of the three sera contained IgE antibodies against cross-reactive tropomyosin from mite, snail, and shrimp. These two patients had never eaten snail and both had a positive prick test for shrimp and snail and demonstrated clinical symptoms (oral allergy syndrome) after eating shrimp (but not snail). Dust mite immunotherapy has also been implicated in sensitization to limpet [26], squid [27], and snail [28, 29]. Alternatively, one study has suggested that sensitization to crustaceans may result in the development of allergy to mites and cockroach [30].

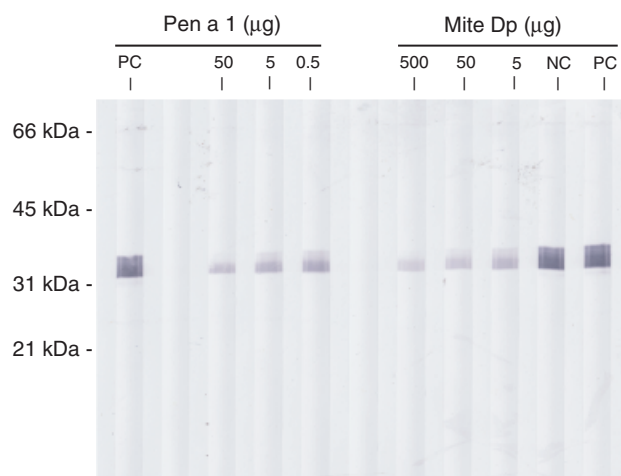


Fig. 3. Immunoblot inhibitions of the Orthodox Jew sera pool against purified Pen a 1. Inhibitions were performed with Pen a 1 (50, 5, 0.5 μ g) and Mite Dp (500, 50, 5 μ g). Shrimp-positive sera pool was used as a positive control (PC); peanut (500 μ g) was used as a negative control (NC).

Clinical reports from some regions of Europe (i.e. France, Italy), where snails are a popular food, have suggested an unusual frequency of patients with both food allergy to snails and allergic to the HDM [31, 32]. Some case reports of snail allergy in HDM-allergic patients have also demonstrated clinical allergic symptoms at the first ingestion of snails [32].

Our study demonstrates that IgE antibody reactivity to a major food allergen, shrimp, can occur in an unexposed population of atopic individuals (in the absence of dust mite immunotherapy with the exception of one patient). Based on inhibition with cockroach and/or dust mite extracts, this reactivity appears to be due to cross-reacting tropomyosins. This observation suggests that individuals may become inadvertently sensitized to certain foods without prior exposure. This finding has important implications for the interpretation of both skin test and IgE antibody reactivity.

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