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Identification of a Cross-Reactive Allergen (Presumably Tropomyosin) in Shrimp, Mite and Insects

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Key Words

Insects, Shrimp

Dermatophagoides pteronyssinus

Cross-reactivity

House dust mite

Crustacea

Tropomyosin

Monoclonal antibody

Abstract

A monoclonal antibody to *Dermatophagoides pteronyssinus* is described that cross-reacts with an IgE-binding antigen present in insects, *Crustacea* (e.g. shrimp) and other invertebrates. By means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, gel filtration and immunofluorescence it was shown that this monoclonal antibody presumably recognizes tropomyosin. Tropomyosin was shown to be involved in cross-reactivity between mite, shrimp and insects in shrimp-allergic patients.

Introduction

In the course of our study on allergens of the house dust mite we obtained a monoclonal antibody (mAb) that reacted not only with mite extracts, but also with extracts of insects and other invertebrates (e.g. shrimp). This observation indicated the presence of a cross-reactive antigen in mite, shrimp and insects. In the literature it has been suggested that there are cross-reacting IgE antibodies against mite, insects and *Crustacea* [1–6]. Invertebrates are known to be strong sensitizers for IgE-mediated immune responses. The most important species in many parts of the world are mites. However, insects have also been found to contribute to inhalant allergies, e.g. cockroaches [7–9], moth [10], non-biting midges [5] and caddis fly [11].

Several patient studies have shown that high reactivity against insects is frequently accompanied by high reactivity against mites [1, 2].

Crustacea are a common cause of ingestant allergic disease [12]. The characterization of shrimp allergens is discussed in a number of studies [13–15]. Very recently, *Pen a* 1

or Sa-II, a major shrimp allergen, has been identified as tropomyosin [16, 17].

There have been some reports in the literature about asthma attacks after eating snails, *Crustacea* or other nonfish seafoods in patients with allergy to mites and/or insects [1, 4]. Cross-reactivity between *Arthropoda* and *Crustacea* has been observed; for instance, Eriksson et al. [5] showed cross-reactivity between chironomids and shrimp in 1988. Cross-reactivity between snails and *Dermatophagoides pteronyssinus* was described by Ardito et al. [6] in 1990.

In this study we investigate cross-reactivity between *D. pteronyssinus*, *Crustacea* and insects. Tropomyosin was found to be a cross-reactive allergen. A cross-reactive mAb against tropomyosin is described.

Materials and Methods

Patients

Two groups of sera were obtained from our diagnostic department. Group A (n=10) was selected on the basis of IgE antibodies against mite and did not have IgE antibodies against shrimp. Group B (n=3)

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selected on the basis of allergy symptoms after eating shrimp and IgE antibodies against shrimp and mite. IgE antibodies were determined as described in the section 'RAST'.

Allergens

Dried *D. pteronyssinus* mites were obtained from the Commonwealth Serum Laboratories (CSL Melbourne, Australia). Mites were reared for 12 h in phosphate-buffered saline (PBS) (10 mM phosphate, 140 mM saline), containing 0.1% Tween 20, at pH 7.4 at 4°C (2% mite spent medium from the same source was extracted in the same way).

The shrimps, oysters, crabs and mussels were obtained locally (Amsterdam). They were cleaned, crushed and extracted at room temperature (10% w/v) in water, maintained at pH 8, for 4 h, dialyzed against distilled water and lyophilized. The shrimps (*Crangon crangon*) were extracted at 100°C (10% w/v) for 15 min, defatted by Freon extraction and dialyzed against distilled water.

Chironomids (aquarium fish food) were obtained locally (Amsterdam). Mosquito extract was obtained from Diephuis Laboratoria (Diephuis, The Netherlands). Cockroaches (*Blattella germanica*) were obtained from a household in Amsterdam. The insects were extracted at room temperature (10% w/v) in PBS, containing 0.1% Tween 20 for 4 h at pH 7.4.

Gel Filtration

Gel filtration was performed with 2 ml 2% (w/v) *D. pteronyssinus* extract on a 200-ml column of ACA-54 (Sepracor, France). Fractions were monitored for *Der p* I and *Der p* II by radioimmunoassay (RIA) [9] and for amylase using the iodine starch reaction.

Monoclonal Antibody

ALB/c mice were immunized subcutaneously with 0.05 mg of 5–80 kD molecular weight fraction of *D. pteronyssinus* extract in incomplete Freund's adjuvant (Difco Laboratories, Detroit, Mich.), and boosted after 6 weeks with the same dose in incomplete Freund's adjuvant (Difco). Three days before removal of the spleen, a booster injection was administered intravenously with the same dose. Cell fusion was performed as described by Astaldi et al. [20]. Antibody-producing (IgG1 $\times$ light chain) hybridomas were selected by limiting dilution [21]. Monoclonal 1A6 antibodies were selected from culture supernatant by affinity chromatography on protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). Anti-1A6 was selected based on radioallergosorbent test (RAST) and indirect RAST with *D. pteronyssinus* extract or insect extract coupled to Sepharose (see RAST). The antibody was coupled to CNBr-activated Sepharose (4 mg antibody/100 mg Sepharose).

RAST

RAST was performed as described previously [22]. For the *D. pteronyssinus* RAST, mite-body extract, 3.6 mg protein, was coupled to 1 ml packed gel (equivalent to 1 ml packed gel) of CNBr-activated Sepharose (Pharmacia). For the oyster, crab, mussel and shrimp, 15 mg of the mite extract was coupled to 300 mg of CNBr-activated Sepharose. For the insects, 6 mg protein was coupled to 300 mg of CNBr-activated Sepharose. Tests were performed by incubating 50 μ l serum or 1A6 supernatant (30 μ g IgG/ml) with 0.5–1.5 mg allergen-coupled Sepharose. Immunodetection of IgE was performed with 1 ng labeled sheep anti-human IgE (MH25-01; CLB, Amsterdam, The Netherlands) in 200 μ l PBS, containing 0.1% Tween 20 and 0.3% bovine serum albumin (BSA). Mouse IgG was detected with 1 ng ^{125} I-

labeled rat anti-mouse IgG (CLB). The results were expressed as percentage of total counts added.

For the indirect RAST the Sepharose-coupled 1A6 monoclonal was incubated for 24 h with *D. pteronyssinus* extract or shrimp extract. After removal of the nonabsorbed components by washing, serum of a shrimp-allergic patient was added, and binding of IgE was detected with radiolabeled anti-IgE.

RAST inhibition assays were performed as follows: 50 μ l allergen solution was incubated with 50 μ l serum for 2 h at room temperature; this mixture was then incubated overnight with the Sepharose-coupled allergen extract and followed by the normal RAST procedure. The amount of allergosorbent was adjusted to give 10–20% binding of radioactivity in absence of inhibitor.

Solid-Phase Absorption and Depletion of Extracts for Tropomyosin

Tropomyosin-depleted shrimp extract was prepared by taking the supernatant after incubation of 0.1 ml shrimp extract (0.85 mg/ml) with 100 mg 1A6-Sepharose for 12 h at 4°C. As a control experiment the same amount of shrimp extract was incubated with 100 mg glycine-Sepharose. The result of the depletion was measured in the indirect RAST, with 1A6 coupled to Sepharose, 1A6-depleted shrimp extract or glycine-depleted shrimp extract and serum of a shrimp-allergic patient (see RAST). The amount of tropomyosin in the depleted extract was reduced by a factor of 1,000 compared with the glycine-depleted extract.

Sodium Dodecyl Sulfate-Polyacrylamide Gel (SDS-PAGE)

SDS-PAGE was performed according to the procedure of Laemmli [23]. Allergen extracts were applied to a polyacrylamide gradient gel (10–20%) under reducing conditions. Subsequently gel proteins were electrophoretically transferred to a nitrocellulose sheet by the Western blotting technique according to Burnette [24]. The blots were incubated for 2 h in 100 ml of PBS, containing 10 mM EDTA, 0.1% (w/v) Tween 20, 0.45% (w/v) BSA and 0.05% (w/v) NaN_3 at room temperature. Blots were incubated in a blotting incubation apparatus as developed by Westgeest et al. [25]. After incubation with mAb 1A6, a second antibody (1 ng ^{125} I-goat anti-mouse IgG) was used. Both incubations were performed overnight in 4 ml of PBS, containing 10 mM EDTA, 0.1% Tween 20, 0.3% BSA and 0.05% NaN_3 . Between and after the incubations the blots were washed 5 times with PBS, containing 0.1% Tween 20. Autoradiography was performed by exposing immunoblots to X-ray films at -70°C for 48 h with an intensifying screen.

Agar Electrophoresis

Agar electrophoresis was performed according to Johansson [26]. 3 μ l of 2% (w/v) *D. pteronyssinus* extract (1.2 mg/ml protein, determined with Pierce BCA Protein Assay Reagent according to the manufacturer's instructions) was applied to agar gel. After electrophoresis, the proteins were transferred to a nitrocellulose sheet. After incubation with mAb 1A6 a second antibody (1 ng ^{125} I-goat anti-mouse IgG) was used. Autoradiography was performed by exposing immunoblots to X-ray films at -70°C for 48 h with an intensifying screen.

Immunohistochemical Methods

Shrimps were snap-frozen in liquid nitrogen. 4- μ m cryostat sections were cut, air-dried and fixed in acetone for 10 min. Fixation and all subsequent washes and incubations were performed at room temperature. After fixation, sections were washed for 5 min in three changes of PBS, incubated for 30 min with protein A-purified mAb

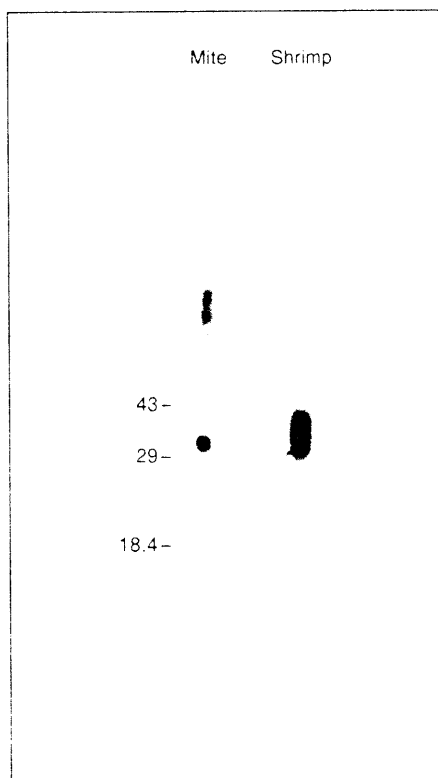


Fig. 1. Autoradiography of SDS-PAGE on shrimp and mite extract after incubation with mAb 1A6.

1A6, 0.1 mg/ml, washed again in three changes of PBS and incubated for 30 min with FITC-labeled goat anti-mouse (5 mg/ml, 1:100; CLB). After these incubations the sections were again washed in three changes of PBS. As a negative control we used another mAb anti-mite (anti-Der p II; CLB).

Isoelectric Focusing

Isoelectric focusing was performed with a Pharmacia Kit according to the manufacturer's directions. 1 µg of affinity purified tropomyosin was applied to the Ampholine PAG plate (pI 4.0–6.5). Detection was performed by immunoblot with mAb 1A6 as described above.

Results

Specificity of mAb 1A6

The antibody reacted with a mite component of 34–36 kD in SDS-PAGE immunoblot under reducing conditions (fig. 1). It reacted with a component with an apparent MW of >80 kD in gel filtration and an electrophoretic mobility close to human serum albumin in agar electrophoresis. A pI of 5.0 was measured. The 1A6 antibody reacted not

only with mite extract, but also with extracts of various insects (chironomid, mosquito and cockroach) and *Crustacea*, e.g. shrimp (fig. 2). By immunofluorescence microscopy on shrimp, muscle staining was observed (fig. 3). On the basis of these results we assume that the mAb 1A6 recognizes tropomyosin.

Identification of IgE Antibodies to Tropomyosin

To investigate the role of tropomyosin as a cross-reactive allergen, we performed an indirect RAST. In the indirect RAST, Sepharose-coupled 1A6 monoclonal was incubated with *D. pteronyssinus* extract. None of the ten sera of group A (mite-positive, shrimp-negative sera) reacted in the indirect RAST, but all three sera of group B (mite- and shrimp-positive) were strongly positive (fig. 4). This suggests that only the mite-positive sera that also react with shrimp, have IgE antibodies against tropomyosin. The control experiment with an irrelevant Sepharose-coupled mAb was negative.

To determine whether the observed co-occurrence of IgE antibodies against shrimp and mite is based on cross-reactivity of these antibodies, the sera of group B were tested in a RAST inhibition assay. In the case of serum I and II, the RAST for mite was completely inhibited by shrimp extract and the RAST for shrimp partly by mite extract (fig. 5 shows the result with serum II). In case of serum III, the RAST for mite was only partially inhibited by shrimp.

To investigate if tropomyosin is responsible for this cross-reactivity, we performed a mite RAST inhibition with supernatants of 1A6 solid phase absorbed extracts of shrimp. The amount of tropomyosin in the depleted extract was reduced by a factor of 1,000 as described above. Tropomyosin-depleted shrimp extract was a 100-fold less potent inhibitor of the mite RAST compared to the control (glycine-depleted shrimp extract) (fig. 6).

Discussion

Tropomyosin has recently been identified as major shrimp allergen *Pen a* I or Sa-II [16, 17, 27, 28]. It is a highly conserved protein and 1 out of 3 mAbs to shrimp tropomyosin was found to cross-react with insect tropomyosin [29]. We assume that our mAb 1A6 reacts with tropomyosin, because it reacts with a major shrimp allergen with the physicochemical properties described for tropomyosin: a homodimer of a 36-kD filamentous monomer with a pI of 4.8–5.4. The high apparent MW on gel filtration indicates an anisomorphic structure. Furthermore, by immunofluorescence microscopy on shrimp, muscle staining was observed.

Fig. 2. Reactivity of mAb 1A6 to mite, various insects and *Crustacea*. The mAb 1A6 was incubated with the allergen Sepharoses. Mouse IgG was detected with ^{125} I-labeled rat anti-mouse IgG.

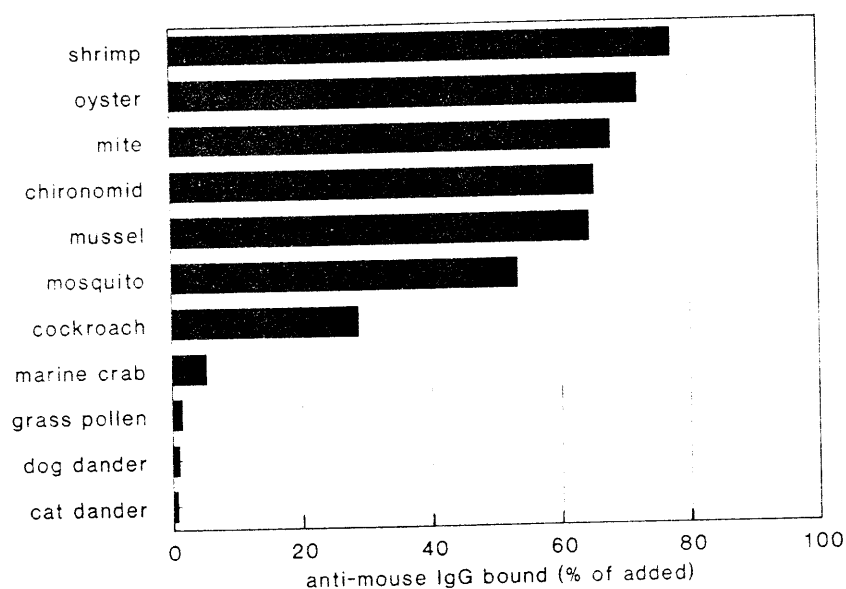


Fig. 3. Shrimp incubated with mAb 1A6. A clear muscle staining was observed.

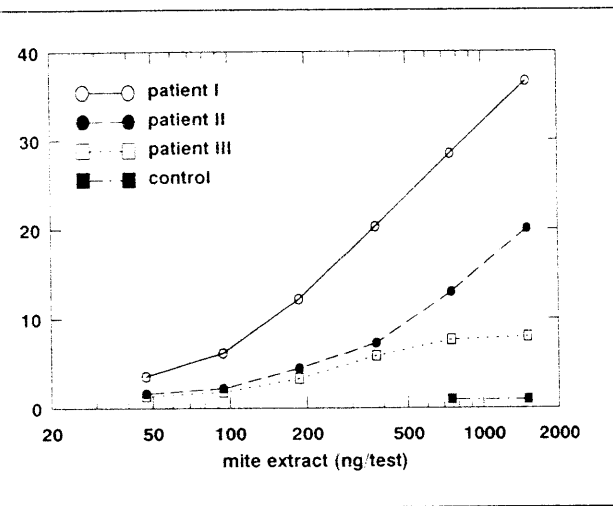
The finding, in several studies, that many mite-positive sera reacted with insects [1] or *Crustacea* [6], suggested that the sera contained cross-reacting antibodies. In this study we identified tropomyosin as a cross-reactive allergen. In our laboratory we have indications for additional mite allergens that cross-react with insects.

An interesting question is which allergen induces the immune response. In case of sera I and II it seems to be shrimp

tropomyosin because the IgE binding to mite can be completely blocked by shrimp. However, in serum III we found only a partial inhibition of the mite RAST by shrimp. The initial sensitization route may be different for different patients.

The clinical relevance of tropomyosin as a cross-reactive allergen is still unclear. In order to quantify the cross-reactivity between mite and shrimp, we tested 139 mite-positive

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g. 4. Results of the indirect RAST: mAb 1A6 coupled to Sepharose catches the IgE binding mite component in three sera of shrimp-ic patients and binding of IgE was detected with radiolabeled gE. The control shows the result with an irrelevant mAb.

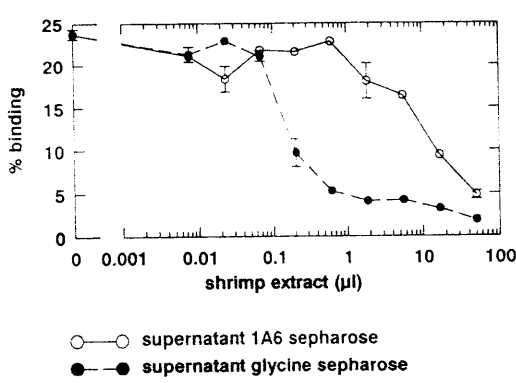


Fig. 6. Mite RAST inhibition of serum II with mAb 1A6 depleted and undepleted shrimp extract.

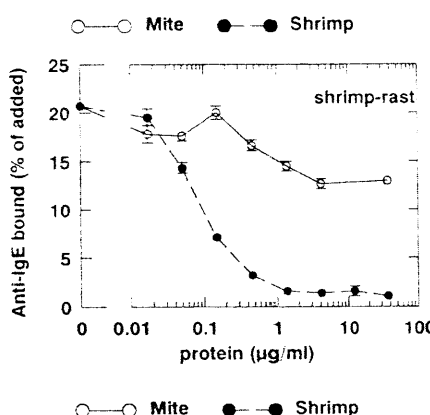
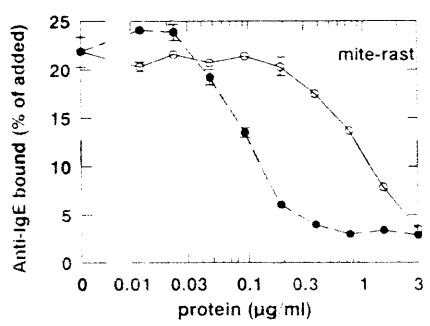


fig. 5. RAST inhibition data of serum II. The mite RAST is completely inhibited by shrimp and the shrimp RAST partly by mite.

sera for the presence of IgE antibodies to shrimp. From these sera, 15 (11%) were positive in the shrimp RAST (% binding more than 5% of total radioactivity). We do not know whether these sera were from people who had complaints of shrimp allergy. Skin tests and histamine release tests need to be done to clarify the clinical relevance.

In the literature there are indications for clinical relevance of the cross-reactivity between mite and shrimp. Banzet et al. [3] observed serious clinical manifestations after eating snails in a few patients who had received immunotherapy against mite. These patients developed also an erythematous and indurated reaction of the skin and subcutaneous tissue of the region of the arm in which the mite desensitization injections had been administered during previous years.

We found that mite body extract contains more tropomyosin than spent medium extract. Therefore, in case of mite immunotherapy in a patient with concomitant allergy to *Crustacea* the use of an extract which contains relatively more mite excreta than mite body components might be preferable.

The clinical implications of the cross-reactivity between shrimp and mites require further study.

Acknowledgments

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