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Possible induction of food allergy during mite immunotherapy

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Sera of 17 patients receiving immunotherapy for house-dust mite allergy were tested for IgE antibodies against snail and shrimp. Serum samples were taken at the start of immunotherapy and 14–20 months later. While the average IgE response to mite, Der p 1, and Der p 2 did not alter significantly, the average response to snail showed a significant increase. This included two conversions from negative to strongly positive. These novel IgE antibodies against snail were shown to be cross-reactive with mite. Three patients had a positive RAST for shrimp. For one of them, a strong increase of IgE against shrimp (and snail) was observed. In 2/3 snail/shrimp-positive sera, IgE antibodies against the cross-reactive allergen tropomyosin from mite, snail, and shrimp were demonstrated. A clear IgE response to snail (>10% binding in a snail RAST) was confirmed by a positive skin prick test (SPT) for 6/10 patients. The two patients with antitropomyosin IgE also had a positive SPT for shrimp, and demonstrated the oral allergy syndrome (OAS) after eating shrimp. The observations in this study indicate that house-dust mite immunotherapy is accompanied by the induction of IgE against foods, including tropomyosin-reactive IgE. Food allergy (OAS) was observed in patients that had IgE antibodies against this cross-reactive allergen. In conclusion, induction of IgE during mite immunotherapy might occasionally cause allergy to foods of invertebrate animal origin.

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Food-allergic symptoms after consumption of invertebrate animals (e.g., seafood) are often seen in house-dust-mite-allergic patients (1–11). Cross-reactivity of IgE between house-dust mite and such foods was demonstrated, for example, for shrimp (12) and snail (2, 3, 7, 8, 10, 11). The major shrimp allergen Pen a 1 (tropomyosin, a homodimer with two chains of approximately 35 kDa each) was identified as one of the allergens involved in cross-reactivity among house-dust mite, insects, and food allergens (12).

Careful study of the case history of patients can give information about the route of sensitization. In several studies, patients with a long history of inhalant allergy to house-dust mite were reported as subsequently developing food allergy to invertebrates. Banzet et al. (3) reported the case histories of 12 patients with well-documented respiratory allergy to house-dust mite who developed clinical manifestations after consumption of snails. In a study by Carrillo et al. (6), six mite-allergic patients were shown to have acquired allergy to limpet. A striking similarity between both studies was the high number of patients receiving mite immunotherapy: 7/12 and 5/6, respectively. In most of these patients, food allergy occurred after a prolonged period of immunotherapy. Although this is suggestive, the authors of these studies did not hypothesize a causal link between mite immunotherapy and the induction of food allergy.

The aim of this study was to investigate whether mite immunotherapy is accompanied by the induction of IgE antibodies to foods such as snail and shrimp, and, if so, whether this was accompanied by food-allergic symptoms. In addition, the possible involvement of the cross-reactive allergen tropomyosin was investigated. The study was performed in a group of 17 patients with house-dust mite allergy who were receiving immunotherapy.

Material and methods

Patients and sera

All patients ($n=17$) were from the area around Ancona (Italy) and had house-dust-mite-related

rhinitis and asthma, confirmed by a strongly positive skin test. Patients were not selected on food-allergic symptoms. All received mite immunotherapy with a mixture of *Dermatophagoides pteronyssinus* and *D. farinae* (Abelló, Madrid, Spain). Serum samples were taken on day 0 and 14–20 months after the start of therapy.

Sera of patients ($n=23$) from the area around Bordighera (Italy) with allergy to *Parietaria* pollen but without mite allergy served as control sera. These patients were receiving immunotherapy with either a *Parietaria* allergoid preparation (Purethal *Parietaria*, HAL Allergen Laboratories, Haarlem, The Netherlands) or placebo. Serum samples were taken at the start of therapy (October–January) and approx. 18 months later.

Allergen extracts

Dried *D. pteronyssinus* house-dust mites and spent-medium of mite cultures were obtained from the Commonwealth Serum Laboratories (Melbourne, Australia). Shrimp (*Crangon crangon*) were obtained from the local fish store (Amsterdam, The Netherlands). Extracts of both mites and shrimp were made as described elsewhere (12). Freeze-dried extract of snail (*Helix aperta*) was obtained from Lofarma Allergeni (Milan, Italy) (2).

Purified allergens

Der p 1 was affinity-purified from house-dust mite extract with Sepharose-coupled Der p 1-specific monoclonal antibodies (13). Der p 2 was obtained as a recombinant fusion protein with a peptide containing six histidines (paper in preparation), allowing purification by Ni^{2+}-chelate affinity chromatography (14).

RAST (inhibition)

The radioallergosorbent test (RAST) was performed as described elsewhere (15). The following amounts of allergen extracts were coupled to 100 mg CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden): 1.2 mg (protein) of house-dust mite extract, 5 mg (dry weight) of shrimp extract, and 4 mg (dry weight) of snail extract. Per test, 1.5 mg of Sepharose was used. Of Der p 1 and Der p 2, 200 µg and 120 µg were coupled, respectively. In this case, 0.5 mg of Sepharose was used per test. Results were expressed as percentages of radiolabeled sheep-antihuman IgE bound to the Sepharose. Comparison of IgE responses before and in the course of immunotherapy was done with the paired Student's *t*-test.

For RAST inhibition, serum was preincubated for 2 h with allergen extract before addition of Sepharose-coupled allergen. Inhibition was performed at nonsaturating serum concentrations. Results were expressed as percentages of the non-inhibited value (PBS/0.3% [w/v] BSA/0.1% [v/v] Tween-20 added instead of inhibitor).

Tropomyosin RAST

Measurement of IgE antibodies against tropomyosin was performed with Sepharose-coupled monoclonal antibodies (1A6) reactive with invertebrate tropomyosins (12) after incubation with *D. pteronyssinus* extract, *H. aperta* extract, or shrimp extract. Before addition of serum, unbound material was washed out. In this way, Sepharose with either monoclonal antibody-bound mite tropomyosin, snail tropomyosin, or shrimp tropomyosin was obtained, and was further used in a normal RAST.

Sepharose-coupled monoclonal antibody 1A6 was also used for comparison of tropomyosin content of whole-body and spent-medium mite extracts. In this case, serial dilutions of allergen extract were allowed to bind to the Sepharose. Detection of tropomyosin was performed with a human serum with a high level of IgE antitropomyosin (12).

Immunoblotting

After separation of allergen extracts by SDS–PAGE, electrophoretic transfer to nitrocellulose (1 mA/cm^2 for 1 h) was performed semidry on a Novablot electrophoretic transfer apparatus (Pharmacia). After blocking with PBS/1.8% (w/v) BSA/0.1% (v/v) Tween-20, the blots were cut into strips of 4–6 mm × 12.5 cm before immunoprobing. Strips were incubated with 150 µl serum. Detection of bound IgE was performed with radiolabeled polyclonal sheep antibodies against human IgE. For identification of the tropomyosin band, mAb 1A6 was used in conjunction with polyclonal goat antibodies against mouse IgG (12).

Skin prick test

Skin prick tests (SPT) for house-dust mite (*D. pteronyssinus* and *D. farinae*) and for shrimp were performed with commercial extracts (Abelló, Madrid, Spain, and Dome/Hollister-Stier, Bayer Milano, Milan, Italy, respectively) by the method of Dreborg (16). For snail, SPT was performed with a 5% snail extract (Lofarma Allergeni). A wheal of 7 mm^2 or more was considered to be positive.

Results

Sera, obtained at the start and 14–20 months after the start of immunotherapy, were tested in RAST for *D. pteronyssinus* (Der p 1, Der p 2) snail, and shrimp (Fig. 1). At the start of therapy, all patients had clearly positive RAST for mite (>5% binding), and 13/17 (>75%) patients for snail. In the control group of *Parietaria* patients, clearly positive RAST for snail was found in only 3/23 (<15%) patients (16.2%, 7.1%, and 5.8% binding, respectively). In the mite patients, clearly positive RAST for shrimp was found in 3/17 (<20%) patients. IgE against shrimp was below blank (<2% binding) for 10 patients.

Although IgE against *D. pteronyssinus* clearly increased in one case (AN9: from 13.1% to 30.1% binding), the average percent binding did not alter (20.3 ± 2.7 (SE) to 22.6 ± 3.0; $P > 0.2$). Average IgE titers against the major mite allergens, Der p 1 and Der p 2, did not show significant changes either. In contrast, IgE against snail clearly increased. The average percent binding changed from 7.4 ± 2.0 to 13.9 ± 3.5 ($P < 0.01$). The most extreme increases were observed for AN9 and AN17: from 3.2% to 49.4% and from 2.8% to 33.9%, respectively. In the *Parietaria* control group, the average percent binding did not change significantly (1.7 ± 0.3 to 1.6 ± 0.2). As was observed for IgE to *D. pteronyssinus* and snail, AN9 showed a strong increase of IgE to shrimp: from 9.0% to 44.9%.

For AN8, AN9, AN11, and AN17, the RAST for snail was inhibited by mite extract (shown for AN9 and AN17 in Fig. 2). At 150 µg of mite protein per test, binding of IgE to snail was inhibited for >70%. This illustrates that the (new) IgE response to snail is (largely) cross-reactive with house-dust mite.

For identification of the allergen(s) involved in this cross-reactivity between house-dust mite and snail, serum samples of five patients were analyzed by SDS–PAGE/immunoblot (Fig. 3). Four of these (AN1, AN9, AN16, and AN17) had demonstrated a clear increase for IgE against snail. This was confirmed on immunoblot. For AN9, strong binding was induced to an allergen of approximately 35 kDa, suggesting that this might be the cross-reactive allergen tropomyosin. AN11 recognized this allergen already at the start of immunotherapy.

Sepharose-coupled mAb 1A6 was allowed to bind tropomyosin from either mite, snail, or shrimp extract, and was subsequently used in RAST for tropomyosin (Fig. 4). IgE from serum AN9 and AN11 recognized tropomyosin from all three allergen sources, comfirming the identification of the band of approximately 35 kDa as tropomyosin.

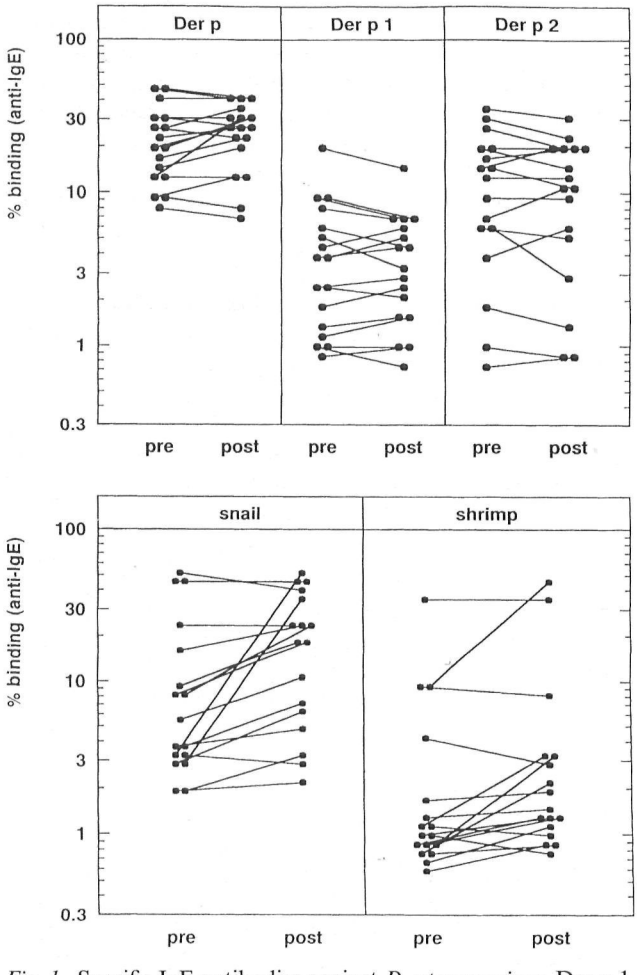

Fig. 1. Specific IgE antibodies against *D. pteronyssinus*, Der p 1 and 2, and snail and shrimp were measured at day 0 and 14–20 months after start of immunotherapy. RAST results are expressed as percent binding. For patient AN11, second values were available only for Der p 1 and 2 and snail.

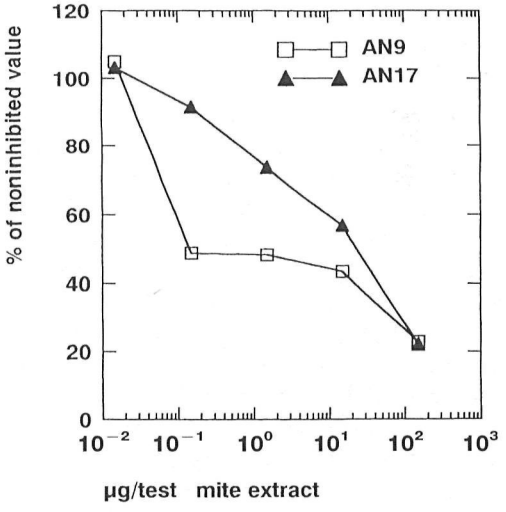

Fig. 2. Binding of IgE (patients AN9 and AN17) to snail was inhibited with *D. pteronyssinus* extract. Results are expressed as percentages of noninhibited value.

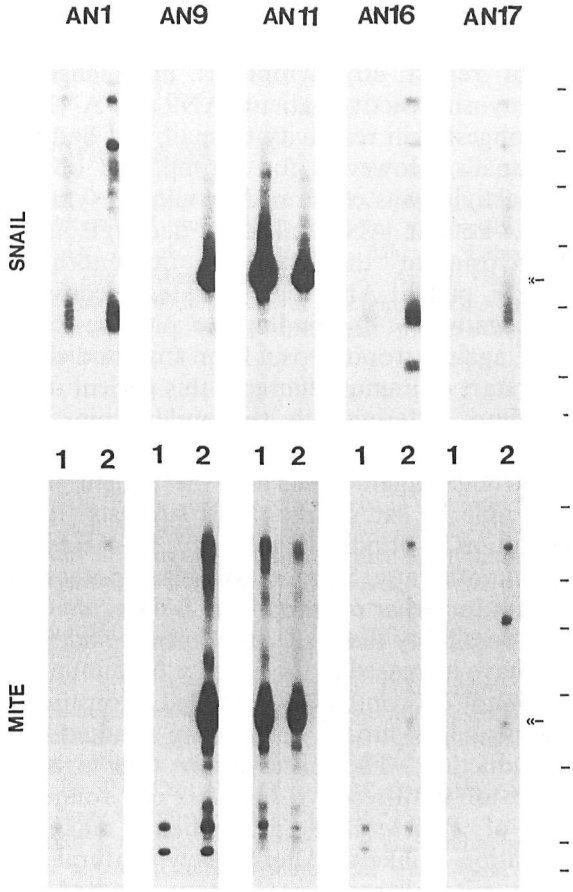

Fig. 3. IgE-immunoblot analysis was performed with five sera, after separation by SDS–PAGE of snail extract and *D. pteronyssinus* extract. 1) day 0 sample; 2) 14–20-month sample. Arrow indicates tropomyosin band. On right side, position of molecular mass is indicated, from top to bottom: 200.0, 97.4, 68.0, 43.0, 29.0, 18.4, and 14.3 kDa.

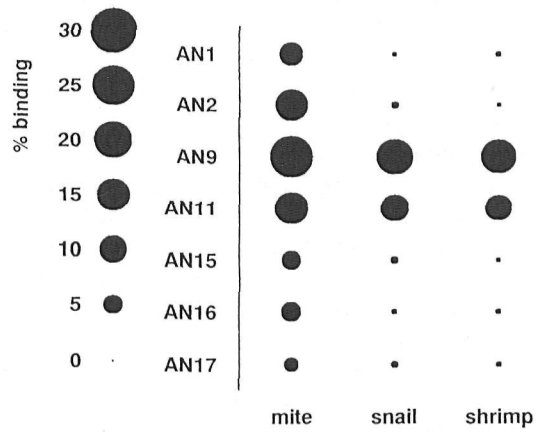

Fig. 4. Binding of IgE to tropomyosin from mite, snail, and shrimp. Sera not shown had percent binding below 5% for all three tropomyosins.

Recognition of mite tropomyosin was not always accompanied by recognition of tropomyosin from snail and shrimp (e.g., AN2). For AN9, tropomyosin-specific IgE appeared in the course of immunotherapy (Table 1).

For mite immunotherapy, both total culture extracts (bodies plus spent-medium) and whole-body extracts are used. Tropomyosin is a muscle protein and is therefore not secreted by the house-dust mite, like, for example, the major allergen Der p 1, which is also found in fecal particles. A 2% whole-body extract (685 kU Der p 1/ml) and a 2% spent-medium extract (417 kU Der p 1/ml) were compared for tropomyosin content. Spent-medium extract contained about 1000 times less tropomyosin relative to Der p 1 (Fig. 5). When expressed relative to Der p 2, the difference was still a factor of 100 (not shown).

All patients were skin prick tested with snail and shrimp extract, with the exception of AN2 and AN16. Of patients with a clearly positive RAST for snail (>10% binding), 6/10 demonstrated concordant SPT (Table 2). All three patients with positive RAST for shrimp showed concordant SPT. The strongest skin reactivity for both foods was observed in those patients who had a clear response to tropomyosin (AN9 and AN11). AN9 and AN11 were also the only patients who had clinical symptoms (oral allergy syndrome) after eating shrimp. No patient reported symptoms related to snail.

Table 1. Measurement of IgE antibodies against tropomyosin from mite, snail, and shrimp in serum of patient AN9. Results are expressed in percent binding of radiolabeled anti-IgE

	IgE antitropomyosin	
Patient AN9	day 0	day 450
Mite	1.9	26.6
Snail	0.4	19.3
Shrimp	0.5	17.9

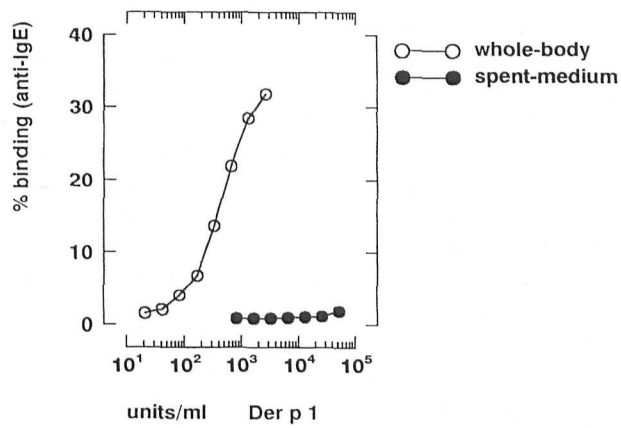

Fig. 5. Binding of tropomyosin-specific IgE to 1A6-bound allergen from whole-body *D. pteronyssinus* extract and spent-medium extract. Results are expressed in percent binding. Relative to Der p 1 content, spent-medium extract contains about 1000 times less tropomyosin.

Table 2. Skin prick test results (in mm^2) for snail and shrimp, and symptoms related to these foods, are summarized. No symptoms were reported for snail. However, six patients had never eaten snails (marked with ?). Two patients with strongest skin reactivity to shrimp demonstrated OAS after eating shrimp. No data are available (NA) for patients AN2 and AN16, who discontinued immunotherapy

	Snail			Shrimp		
Patient no.	SPT	Symptoms	RAST	SPT	Symptoms	RAST
AN1	–	–	17.2	–	–	1.3
AN2	NA	NA	40.9	NA	NA	3.1
AN3	–	–	4.8	–	–	2.1
AN4	–	–	2.3	–	–	0.9
AN5	–	–	3.4	–	–	1.2
AN6	–	–	22.1	–	–	1.8
AN7	7	–	38.8	7	–	7.9
AN8	–	–	18.5	–	–	0.9
AN9	28	?	49.4	63	OAS	44.9
AN10	–	?	3.0	–	–	2.9
AN11	28	?	43.5	60	OAS	33.9
AN12	7	?	11.3	–	–	0.8
AN13	–	–	6.0	–	–	1.1
AN14	–	?	7.4	–	–	1.5
AN15	8	?	24.7	–	–	1.0
AN16	NA	NA	22.7	NA	NA	1.3
AN17	19	–	33.9	–	–	3.4

However, 6/15 patients who underwent skin prick test had never eaten snail, including AN9 and AN11.

Discussion

Earlier studies (1–4, 6–8, 10, 11) reported that IgE to snail is prevalent in house-dust-mite-allergic patients. In several of these reports, cross-reactivity of IgE between house-dust mite and snail was demonstrated (2, 3, 7, 8, 10, 11). In this study, 13/17 patients had clearly positive RAST to snail. In a group of *Parietaria*-allergic patients, IgE reactivity to snail was almost absent (3/23). These observations confirm the close link between mite allergy and food allergy to invertebrates such as the snail. It was further demonstrated that immunotherapy for house-dust mite allergy is accompanied by an increase of (cross-reactive) IgE antibody titers against foods of invertebrate animal origin, such as snail and shrimp. Tropomyosin was identified as one of the (cross-reactive) allergens involved (AN9 and AN11) for both snail and shrimp. Tropomyosin was not the only allergen to which increased reactivity was observed. Snail immunoblots revealed increased IgE reactivity with two bands between 20 and 30 kDa, and with multiple bands above 45 kDa (AN16 and AN17).

Skin prick tests for snail and shrimp showed that these food allergens were biologically active in most patients with positive RAST: 6/10 and 3/3, respectively. For snail, data on clinical symptoms were incomplete, since most of the patients with positive SPT had never eaten snails. Two patients (AN7 and AN17) with positive RAST and SPT for snail did not report any symptoms upon ingestion. Tropomyosin-reactive patients AN9 and AN11 had the strongest skin reactivity to snail, but had never eaten snails. However, for shrimp, the observed skin reactivity was confirmed by clinical symptoms (OAS). Patient AN11 already had IgE against tropomyosin at the start of immunotherapy. However, patient AN9 demonstrated a conversion from negative (<1% binding) to positive (>15%) for IgE against tropomyosin from snail and shrimp. At the start of immunotherapy, this patient used to eat shrimp, suffering only very mild itching in the mouth. During immunotherapy, symptoms (OAS) were strongly aggravated, and the patient was no longer able to eat shrimp. This suggests that the aggravation of food allergy might be a side-effect of immunotherapy. Support for this hypothesis can be found in earlier case reports (3, 6).

The possibility that IgE titers to snail and shrimp might have increased in the absence of immunotherapy cannot be completely excluded. Consumption of snail and/or shrimp could have resulted in the IgE induction. The observation that a similar increase of snail-reactive IgE was not found in a group of *Parietaria*-allergic patients makes this explanation unlikely. Alternatively, natural exposure to mite allergens could have resulted in an increase of IgE to mite allergens, including allergens cross-reactive with snail. However, IgE titers to total mite, Der p 1, and Der p 2 did not increase. It is unlikely that natural exposure to mite would suddenly induce IgE to cross-reactive allergens, but not to mite-specific allergens. A possible explanation of the observed induction of IgE is the immunotherapy treatment. During immunotherapy, allergens enter the body through another route, and at higher concentrations. This might result in (increased) IgE responses to proteins that are not very potent allergens upon natural exposure.

The induction of IgE with new specificities in the course of immunotherapy has been reported earlier (17, 18). Clinical implications were, however, not indicated. This study demonstrates that new IgE responses (e.g., to tropomyosin) may have clinical implications. In the case of mite immunotherapy, the presence of relatively high levels of tropomyosin might be a risk factor for induction of food allergy. Tropomyosin levels are much higher in whole-body house-dust mite extracts than in spent-medium extracts. A trend toward application of whole-body instead of spent-medium extracts (to avoid contamination with growth medium-derived allergens) might, therefore, be reconsidered. Possibly, immunotherapy with purified (recombinant) major allergens would be a better alternative. In the mean-

time, physicians should be alert to the development of food allergy in patients receiving mite immunotherapy.

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