

Cross-reactivity between terrestrial snails (*Helix* species) and house-dust mite (*Dermatophagoides pteronyssinus*).

II. *In vitro* study

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Epidemiologic and *in vitro* data have shown that the association of house-dust mite (HDM) allergy and snail allergy in the same patients was due to cross-reactivity between HDM and snail allergenic components. However, the cross-reacting allergen(s) have not yet been identified. *In vitro* reactivity of seven patients' sera to the various extracts and hemolymph of four different *Helix* snail species was analyzed by IgE detection on immunodots and Western blots. Cross-reactivity between snails and *Dermatophagoides pteronyssinus* was assessed by immunodot and ELISA inhibition in two patients. Heterologous inhibition of the snail immunodot and ELISA was observed in one serum. Western blotting showed a specific binding on all four snail species extracts; molecular weights of snail allergens ranged from <21 to 200 kDa. Marked individual differences were observed in the seven sera under study; most sera demonstrated IgE recognition of multiple bands, illustrating that no single allergen is responsible for cross-reactivity between snail and mite. These results confirm that cross-reactivity exists between snails of the *Helix* genus and HDM. This cross-reactivity, involving more than a single allergen, may be of clinical significance in atopic patients allergic to *D. pteronyssinus*. The identity of the cross-reacting allergens remains to be determined. Potential candidates include the thermostable minor allergens of *D. pteronyssinus*, tropomyosin and hemocyanin.

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Several clinical reports, especially from European countries where snails are commonly eaten, have suggested that true cross-reactivity may exist between snail and house-dust-mite (HDM) allergens, since patients with HDM allergy presented similar symptoms after sea- or land-snail consumption (1–8). True cross-reaction had never been demonstrated until Amoroso et al. reported heterologous inhibition of snail RAST by *Dermatophagoides farinae* (D. f.). The study was performed on the serum of allergic patients in Sicily, Italy, who had asthmatic attacks after special holiday meals which included the snail *Euparipha pisana* (9). In the Canary Islands, Carillo et al. described two cases of allergy to the limpet, a sea mollusk of the Gastropoda class, in patients sensitized to *D. pter-*

onyssinus (D. pt), and showed unexplained cross-inhibition, by D. pt extracts, of the RAST with limpet extracts (10). Similar results were obtained in squid hypersensitivity (11). Tropomyosin, a highly conserved muscle protein, identified as the major allergen in HDM and shrimp (12), was proposed as the best candidate for the cross-reactive allergen in HDM and snails (6, 13). However, a detailed report on 28 patients allergic to both HDM and snails clearly showed that the problem was not so simple: more than a single allergen was shared by HDM and land or sea snails, and IgE binding to tropomyosin was observed in only two patients under study (14). Another potential allergen candidate was the parasitic mite *Riccardoella limacum*, which frequently infests

land snails (15). However, this possibility was ruled out by

- 1) the observation of cross-reactivity between HDM and sea snails which are not infested by mites
- 2) the direct demonstration of no *in vivo* skin reactions to *Riccardoella* extracts in patients with HDM and snail allergy (16).

The aims of this study were thus

- 1) to assess *in vitro* reactivity of the sera from HDM-allergic patients with clinical symptoms after snail ingestion to snail extracts and snail hemolymph by IgE detection on immunodots and Western blots
- 2) to demonstrate cross-reactivity between snails and D. pt by immunodot and ELISA inhibition.

Material and methods

Snail aqueous extracts

Extracts were prepared from four *Helix* species bred in the laboratory of the Research Group on Snail Biology, University of Franche-Comté, Besançon: *H. aspersa aspersa* (small gray snail), *H. aspersa maxima* (large gray snail), *H. lucorum* (Turkish snail), and *H. pomatia* (Burgundy snail); class Gastropoda, family Helicidae. A native crude extract (NE) was prepared from each species. The whole body of the snail was separated from the spiral shell. The two parts of the whole body, the foot and the visceral mass, were crushed in phosphate-buffered saline (PBS), and the mixtures were centrifuged at 8000 g for 15 min. The raw supernatant (R extract) was divided into two volumes. One volume was heated in a water-bath for 15 min at 100°C (H extract).

Hemolymph was obtained through a direct puncture of the snail heart, and mucus by gentle pressure on the snail. Hemolymph and mucus were also divided into two volumes. One volume was heated in a water-bath for 15 min at 100°C.

Dermatophagoides (*D. pt*) extracts

D. pt extracts were purchased from Stallergènes, Fresnes, France.

Patients

The seven patients under study had developed clinical manifestations after eating snails and were known to be allergic to D. pt and/or D. f. All seven patients had had asthmatic episodes after consumption of snails. Patient F, especially, had very severe asthmatic attacks both after eating snails and after

inhalation during snail cooking. Two patients had anaphylactic shock, and three presented face edema together with other allergic symptoms. In patient A, a semidelayed local reaction, with erythema, swelling, and pain at the site of previous HDM-specific immunotherapy, was the only clinical manifestation. This recurred at each snail consumption. All of them, except patient F, who refused any *in vivo* test with snail extracts because of the severity of his symptoms, were positive to prick tests with commercial D. pt extract as well as to those performed with a Stallerpoint® lancet (Stallergènes, Fresnes, France) by the prick + prick technique (17) with commercially preserved *H. pomatia* snails (Romanzini, La Rivière-Druegon, France).

Human sera

Serum samples were obtained from patients A–G (Table 1). On four of them (patients A–D), both *in vivo* prick tests and *in vitro* studies were performed with the same snail extracts and hemolymph from four *Helix* species and *R. limacum* extracts. The results of the *in vivo* study have been reported separately (16). Serum from four non-allergic laboratory staff members, with no history of clinical manifestations at snail ingestion, and with negative skin prick tests to pneumallergens, were also collected and used for *in vitro* tests. All the sera were kept frozen at –80°C.

Specific IgE immunodots

Snails (*H. aspersa aspersa*) were pulverized and stirred for 2 h in NH_4HCO_3 , pH=8, EDTA 1 mmol/l at 4°C. The mixture was centrifuged for 30 min at 10 000 g at 4°C. The supernatant was put through Whatman filters nos. 4 and 40. Ammonium sulfate was then added in the proportion of 29.5 g/100 ml of liquid, and the solution was stored at 4°C overnight. After centrifugation (10 000 g for 30 min at 4°C), the precipitate was resuspended in water, and the solution was dialyzed overnight at 4°C across a 3.5-kDa cutoff membrane. After dialysis, the pH was adjusted to 7.0, and the solution was centrifuged. The supernatant was then lyophilized, aliquoted, and stored at –20°C. The protein concentration of snail extract, 4.7 mg/ml, was measured by the BCA protein assay reagent (Pierce, Rockford, Chemical Co, IL, USA), according to the manufacturer's instructions.

Specific IgE antibodies to snails and D. pt were determined with immunodots. A volume of 10 µg of protein snail whole-body extract and 10 of D. pt extract were applied to nitrocellulose sheets (0.22 µm) (Schleicher and Schüell, Dassel, Germany). After drying, the membranes were

Table 1. Molecular weights of allergenic fractions* of different snail extracts and *D. pteronyssinus* extract

D. pt	Hpo	Hpo		Hpo		Hpo	Hlu		Ham		Haa
	He R	F/H	F/R	VM/H	VM/R	Mucus	F/R	VM/R	VM/H	VM/R	F/R
	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200		> 200	> 200
	200	208	208	208	208						
	150	170	177	177	177		177	162			
100	123	117			100-83		135-95				107-79
91	91	95		91					91-79	91-79	
83	71	79		76	77-69		77				
68		69	67				69	67-60			69
63				63							
60											
52	54			54	54			54	54	54	
		55-47	55-47	53-49	53-49		55-47	53-49			
46.7											
40		40	40	40							
33-29		33	33	33			33-32				33
27							29-25	29-26			30-28
							24-23				
< 21		< 21	< 21	< 21	< 21			< 21	< 21	< 21	

Hpo: *Helix pomatia*; Hlu: *Helix lucorum*; Ham: *Helix aspersa maxima*; Haa: *Helix aspersa aspersa*; D. pt: *Dermatophagoides pteronyssinus*; He: hemolymph; F: foot; VM: visceral mass; R: raw; H: heated.

* Immunoblot obtained with serum from patient D.

incubated with blocking buffer PBS/BSA 3% for 60 min at 37°C. Patient sera diluted 1 : 10 in blocking buffer were added, and the membranes were incubated overnight at room temperature with continuous shaking. Strips were then washed five times for 5 min with washing buffer (PBS/Tween 0.05%). The bound IgE antibodies were detected by ¹²⁵I-labeled antihuman IgE (Pharmacia). Then, autoradiography was performed at -80°C for 3-7 days with Kodak X-omat films (Kodak, Heidelberg, Germany). Control strips were processed with pooled sera from four nonallergic subjects.

Inhibition of immunodots

For immunodot inhibition, aliquots of patient sera D and E (20 µl) preincubated with 180 µl of different quantities of snail whole body or D. pt extracts ranging from 0 to 240 µg, for 4 h at room temperature with shaking, were analyzed by immunodot assay, as described above. Sheets were then washed with PBS/Tween 0.05% buffer, and bound IgE antibodies were detected by ¹²⁵I-labeled rabbit antihuman IgE (Pharmacia). Control inhibition was performed with *Dactylis glomerata* extract (Allerbio, Varennes-en-Argonne, France).

Specific IgE ELISA

One microtiter plate was coated with different dilutions of *H. pomatia* whole-body extracts (protein concentration: 0.01 mg/ml) and a second

microtiter plate with different dilutions of D. pt extract. Both were left overnight at 4°C. After the plates were rinsed with PBS/Tween 0.05%, they were left with PBS/BSA 3% for 2 h at 37°C. After washing, 100 µl of patient D serum diluted 1/2 was distributed in several wells, and then incubated overnight at room temperature. After washing with PBS/Tween 0.05%, peroxidase-labeled antihuman IgE (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA), diluted at 1/100 in PBS/BSA 3%, was added; 200 µl of peroxidase substrate (4-amidoantipyrine) was then distributed in the wells, and the optical densities (OD) were read 30-60 min later at 450 nm on a spectrophotometer (Ortho-Diagnostic System, Raritan, NJ, USA).

Inhibition of ELISA

Microtiter plates were coated with either D. pt or snail extract and left overnight at 4°C. The empty protein-binding sites on the plates were blocked for 2 h at 37°C with PBS/BSA 3%. The potentially cross-reacting allergen was preincubated with patient D serum for 2 h. The allergen was diluted in PBS/BSA 3% in order to obtain different inhibition concentrations. The preincubated serum was then incubated in the coated wells of the microtiter plates overnight at room temperature and shaken constantly. After washing, incubation with peroxidase-labeled antihuman IgE diluted at 1/100 (v/v) in PBS/BSA 3%, and subsequent incubation with

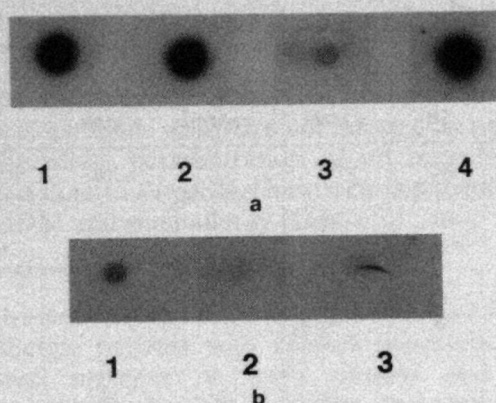


Fig. 1. Immunodot inhibition (patient serum D). a) Volume of 10 μ g of *D. pteronyssinus* (D. pt) proteins was blotted onto nitrocellulose. Inhibiting extracts – 1: none; 2: 240 μ g of snail proteins; 3: 210 μ g of D. pt proteins; 4: 50 μ g of *D. glomerata* proteins. b) Volume of 10 μ g of snail proteins was blotted onto nitrocellulose. Inhibiting extracts – 1: none; 2: 210 μ g of snail proteins; 3: 240 μ g of D. pt proteins.

the substrate, the reaction was read at 450 nm. Control inhibition was performed with *D. glomerata* extract (Allerbio, Varennes-en-Argonne, France). Percent inhibition was calculated by the following formula:

$$\frac{100 - (\text{OD serum with preincubation})}{\text{OD serum without preincubation}} \times 100$$

Sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE)

Proteins from the different snail body parts were analyzed and compared to D. pt profile protein with 8–25% polyacrylamide gels and the Phastsystem apparatus (Pharmacia, Uppsala, Sweden). Quantities ranging from 2 to 10 μ g of snail or D. pt extract (Alyostal, Stallergènes, Fresnes, France) in buffer containing 1% β -mercaptoethanol were put on the minigel. Migration and silver staining were performed according to the manufacturer's instructions (Pharmacia).

Specific IgE immunoblots

Each snail aqueous extract (21 μ l of a solution containing a protein concentration of 1.5 mg/ml) was fractionated by 12% polyacrylamide-gel electrophoresis (Mini-Protean II system, BioRad Laboratories, Richmond, CA, USA). The separated antigens were transferred overnight at 4°C from the separation gel to a nitrocellulose membrane (Schleicher and Schüell, Dassel, Germany; 0.22 μ m) by electrophoretic transfer. The empty protein-binding sites on the nitrocellulose mem-

brane were blocked in buffer containing 3% bovine serum albumin (BSA) for 60 min at 37°C. The nitrocellulose membranes with blotted proteins were incubated overnight with human sera diluted 1/2 (v/v) in PBS/BSA 3%. After washing with PBS/Tween 0.05%, the strips were incubated with ^{125} I-labeled antihuman IgE (Pharmacia, Uppsala, Sweden), 0.5 μ g/5 ml, 6 μ Ci, diluted 1/2 in PBS/BSA 3% for 3 h. The strips were washed, dried at 37°C, and loaded into Kodak X-omat cassettes with regular intensifying screens (Kodak, Heidelberg, Germany). Cassettes were stored at –80°C for 4–20 days. Films were developed, and those with optimum exposure quality were analyzed visually. Control strips were processed with pooled sera from four nonallergic subjects.

Results

Immunodots and immunodot inhibition

Immunodots showed the presence of IgE specific to snail in all seven sera from the patients under study and none in control subject sera. Homologous inhibition of D. pt and snail immunodots was obtained with patient sera D (Fig. 1a and b) and E (data not shown). Heterologous inhibition of the “snail” immunodot after incubation with D. pt extract was significant only with patient D serum (Fig. 1a and b), and not with patient E serum. However, significant heterologous inhibition of the D. pt immunodot after incubation with snail extracts was never obtained with either serum, nor was it obtained with four other sera from patients with reactivity to both snail and D. pt, which are not included in this study (data not shown).

ELISA inhibition

With patient D serum, the inhibition percentage in homologous inhibition was 82% with the D. pt extract (OD: 0.0795 vs 0.4315), but only 29% (OD: 0.1885 vs 0.2660) with the snail extracts. The heterologous inhibition was 59% when sera were preincubated with D. pt extract (OD: 0.1100 vs 0.2660) and 22% when preincubated with snail extracts (OD: 0.3455 vs 0.4315).

Electrophoretic profile of snail proteins

The electrophoretic profiles of snail proteins from the different snail extracts are shown in Fig. 2. Most of the proteins were thermostable and were present in the different species of snails and the different parts of the snails under study.

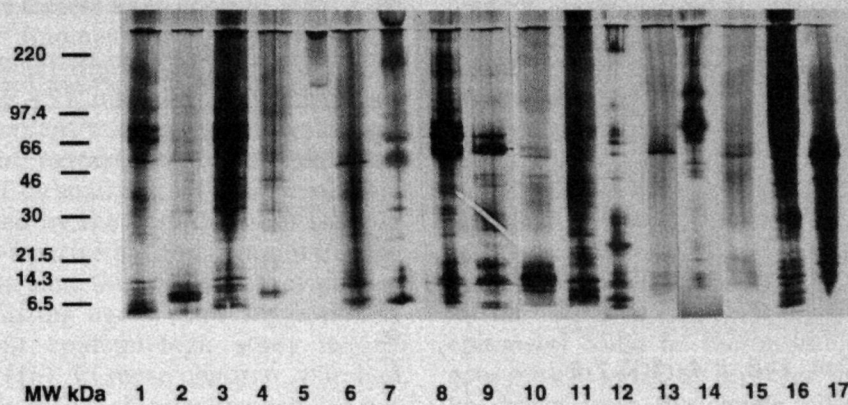


Fig. 2. SDS-PAGE analysis of different snail extracts. Lane 1) *Helix aspersa maxima*, visceral mass heated (H); lane 2) *H. aspersa maxima*, foot (H); lane 3) *H. aspersa maxima*, visceral mass, raw (R); lane 4) *H. aspersa maxima*, foot (R); lane 5) *H. pomatia*, hemolymph; lane 6) *H. lucorum*, foot (H); lane 7) *H. lucorum*, visceral mass (H); lane 8) *H. lucorum*, foot (R); lane 9) *H. lucorum*, visceral mass (R); lane 10) *H. pomatia*, foot (H); lane 11) *H. pomatia*, visceral mass (H); lane 12) *H. pomatia*, mucus; lane 13) *H. aspersa aspersa*, foot (H); lane 14) *H. aspersa aspersa*, visceral mass (H); lane 15) *H. aspersa aspersa*, foot (R); lane 16) *H. aspersa aspersa*, visceral mass (R); lane 17) *D. pteronyssinus*.

Immunoblots

Western blotting with *D. pt* extracts confirmed the presence of specific IgE against different mite fractions in all patients' sera (A–G, Figs. 3 and 4; Tables 1 and 2), and in none from the control subjects. Extracts from the four *Helix* species were tested by Western blotting with the serum of patient D, who exhibited very positive skin prick tests to all species of snails (Table 1 and Fig. 3). With this serum, IgE binding occurred on all four species extracts with intense binding to *H. pomatia* and *H. lucorum* allergens. Molecular weights (MW) and location of snail allergens ranging from <21 to 200 kDa are summarized in Table 1. For *H. pomatia* species, preheated and raw allergen extracts did not exhibit very different patterns (Fig. 3). In visceral mass extract, an allergen of 54 kDa was revealed that was not present in *H. pomatia* foot extract (R or H). Conversely, a protein of 67–69 kDa was found only in foot extract (R and H). A strong IgE response directed against an allergen of 49–53 kDa was found in both visceral mass and foot extracts (R and H). Other allergens with high MW (>200–170 kDa) were found in both visceral mass and foot extracts. In the other species (*H. lucorum*, *H. aspersa aspersa*, and *H. aspersa maxima*), allergens of 54, 49–53, and 67 kDa were also found, but three other allergenic components with MW of 35–32, 29–25, and 24–23 were detected only in *H. lucorum* and *H. aspersa aspersa* extracts.

The other sera were tested on *H. pomatia* and *H. lucorum* extracts, since these species caused the highest skin reactivity (16). In 5/6 sera under study (B, C, E, F, and G), specific IgE were bound to either *H. pomatia* or *H. lucorum* proteins (Fig. 4

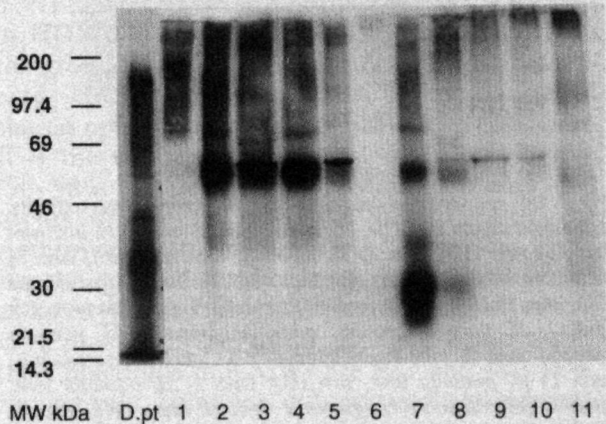


Fig. 3. IgE reactivity against different snail and *D. pteronyssinus* extracts (patient serum D). D. pt: *D. pteronyssinus*; lane 1) *H. pomatia*, hemolymph; lane 2) *H. pomatia*, foot, heated (H); lane 3) *H. pomatia*, visceral mass (H); lane 4) *H. pomatia*, foot, raw (R); lane 5) *H. pomatia*, visceral mass (R); lane 6) *H. pomatia*, mucus; lane 7) *H. lucorum*, foot (R); lane 8) *H. lucorum*, visceral mass (H); lane 9) *H. aspersa maxima*, visceral mass (H); lane 10) *H. aspersa maxima*, visceral mass (R); lane 11) *H. aspersa aspersa*, foot (R).

and Table 2). Patient A serum response was negative to both extracts, but positive to *H. aspersa aspersa* proteins (data not shown). Sera E, F, and G reacted to both *H. pomatia* and *H. lucorum* allergens, serum B reacted to *H. lucorum* extracts, and serum C reacted mainly to *H. pomatia* allergens. For all these sera, allergens of MW similar to those found for serum D were observed. In both *H. pomatia* and *H. lucorum* extracts, allergens of high MW (170–200 kDa) and proteins located in the range of 49–54 kDa were detected, and in

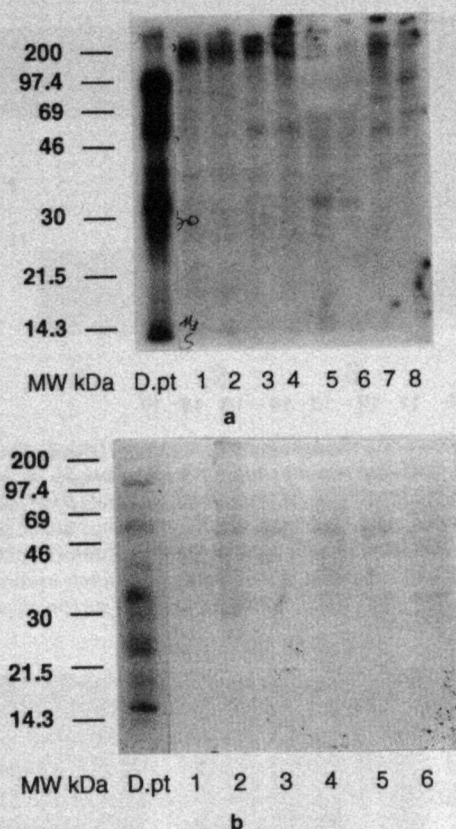


Fig. 4. IgE reactivity against snail and *D. pteronyssinus* extracts. a) Patient serum E. D. pt: *D. pteronyssinus*; lane 1) *H. lucorum* foot, heated (H); lane 2) *H. lucorum* foot, raw (R); lane 3) *H. lucorum* visceral mass (H); lane 4) *H. lucorum* visceral mass (R); lane 5) *H. pomatia* foot (H); lane 6) *H. pomatia* foot (R); lane 7) *H. pomatia* visceral mass (H); lane 8) *H. pomatia* visceral mass (R). b) Patient serum F. D. pt: *D. pteronyssinus*; lane 1) *H. pomatia* foot, raw (R); lane 2) *H. pomatia* foot, heated (H); lane 3) *H. pomatia* visceral mass (R); lane 4) *H. pomatia* visceral mass (H); lane 5) *H. lucorum* visceral mass (R); lane 6) *H. lucorum* visceral mass (H).

H. lucorum extract, allergens averaging 33–34 kDa were also found. It should be noted that in the seven sera under study marked individual differences were observed in number of allergenic fractions as well as MW.

Discussion

The results obtained in this study strongly confirm that cross-reactivity exists between snails of the *Helix* genus and HDM. This cross-reactivity may be of clinical significance in atopic patients allergic to *D. pteronyssinus*. We tested cross-inhibition between snails and *D. pteronyssinus* in two sera, using two different techniques. Using the two techniques, immunodot inhibition and ELISA inhibition, we were able to demonstrate cross-inhibition of IgE binding after preincubation of one serum with

Table 2. Molecular weights of allergenic fractions of *D. pteronyssinus*, *H. pomatia*, and *H. lucorum* extracts

Immunoblot obtained with serum from patient B				
D. pt	Hlu VM/H			
	> 200			
150-130				
60-51	54-43			
42-40				
37-33	33			
31-29				
26				
21				
16	16			
15				
14				

Immunoblot obtained with serum from patient C				
D. pt	Hpo		Hlu	
	F/R	VM/R	F/R	VM/H
> 200	> 200	> 200	> 200	> 200
140-125	140			
120-110	100	100		
92-72	86-66	83-71		
72-63				
	54-49	56-48		
43-40	43	43		
36-33	32	32		
31-30		29		
< 21	< 21	< 21		< 21

Immunoblot obtained with serum from patient F					
D. pt	Hpo			Hlu	
	F/H	VM/R	VM/H	VM/R	VM/H
150-130					
60-51					
	54-50	54-50	54-50	54-50	54-50
42-40	42-39				
37-33	34-33		34-33	34-33	34-33
31-30					31
27					
26					
< 21					

Immunoblot obtained with serum from patient G						
D. pt	Hpo		Hlu			
	F/R	F/H	F/R	F/H	VM/R	VM/H
> 200	> 200	> 200	> 200	> 200	> 200	> 200
112	128	128	160-95	160-95		
91			66-61	66-61		
16-14						

D. pt: *Dermatophagoides pteronyssinus*; Hpo: *Helix pomatia*; Hlu: *Helix lucorum*; F: foot; VM: visceral mass; R: raw; H: heated.

D. pteronyssinus extracts only, and not with snail extracts. Similar findings have been reported recently by De Maat-Bleeker et al. (6) and van Ree et al. with RAST inhibition (14): the snail RAST for their patient was significantly inhibited by *D. pteronyssinus* extract, whereas no inhibition of the mite RAST by snail extract was found. This could be explained by the very different relative amounts of cross-reactive antigens present in mite and snail extracts, and this suggests that mite is usually the sensitizing agent. This suggestion is confirmed by our epidemiologic study in 169 allergic children (16). In these children, who had never eaten snails before skin testing and did not complain of allergic reactions to shellfish, snail sensitization, occurring in 30% of the children allergic to HDM, was probably due to primary sensitization by HDM.

The profiles of IgE binding to snail protein fractions obtained from all seven sera under study also confirm that

- 1) the shared allergens are not species-specific, but the relative amount of a given allergen contained in a given extract may be responsible for the apparently varying responses observed with a given serum
- 2) there is more than a single allergen involved in the cross-reactivity between HDM and snails.

This could explain the relatively high frequency of latent snail allergy, disclosed by prick testing in unselected populations of HDM-allergic patients (4, 8, 9, 16). The prevalence of clinical manifestations is obviously related to the alimentary habits of the geographic area, a fact which explains why most of the reported cases came from Italy and eastern France. However, the cross-reacting allergen(s) remains to be identified and, as already mentioned, tropomyosin is no longer the only candidate (14).

In fact, tropomyosin, the major shrimp allergen (12), has recently been proposed as the cross-reactive allergen in shrimp, mites, and insects (13). Its MW (36 kDa) and the observation of associated clinical manifestations after the ingestion of crustaceans in two of our patients, whose sera reacted to snail protein fractions of average MW 33–34 kDa, suggest that tropomyosin may be central to the complex mite-mollusk-crustacean cross-reactivity observed in these particular patients. As suggested previously by Mistrello et al., hemocyanin would also be a good candidate (5).

Our immunoblot findings give some indications about potential antigens which may be shared by *Helix* and *Dermatophagoides* species. Many different allergenic protein fractions were recognized by the patients' sera. We were able to confirm IgE

binding to snail extracts with all the sera under study; however, there was a large variety of individual IgE-dependent immune responses. Cross-reacting allergens were thermostable and present in all parts of the snails, including hemolymph, and in all the *Helix* species tested, although *H. pomatia* and *H. lucorum* seemed to give the highest *in vivo* (16) and *in vitro* reactivity. The prevalence of associated sensitization and comparison of the MW of the allergenic fractions of *D. pt* and snail extracts in the same patient suggest that the shared epitope(s) could be thermostable "minor" allergens present in HDM; however, no conclusions can be drawn from the simple observation of IgE-binding fractions of similar MW on mite- and snail-extract immunoblots. Thermolabile allergens, such as Der p I, can certainly be excluded, but, in view of the IgE-binding frequency in populations of atopic patients and their MW (18–20), there are many potential candidates, such as Der p IV (56–60 Da), Der p V (15 kDa), and Der p VI and Der p VII (22–28 kDa). Additional allergens, present in HDM but less extensively characterized than the groups I–VII allergens, must also be considered. Hemocyanin could also be the source of shared epitopes in mites, snails, and crustaceans. It is the major component of snail hemolymph (90–98% of hemolymph-protein content) and occurs in two phyla of the invertebrates, the Arthropoda (including both Crustacea and Arachnida) and the Mollusca, in which it serves as an oxygen-carrier (21). Hemocyanin is present in *H. pomatia* in two forms, α - and β -hemocyanin; their functional unit MWs are 52.7 and 48.7 kDa, respectively. However, different molecular forms, due to the quaternary structure of the molecule, leading to different sedimentation rates ranging from 11 to 100 s, have been described (21). IgE binding to a 49–53-kDa protein fraction of the snail extracts was observed in 6/7 sera under study. Three patients who exhibited this type of reactivity *in vitro*, and who participated in the *in vivo* study, had positive prick tests with hemolymph (A, B, and C) (16). An antiserum raised against β -fructosidase isolated from the cell walls of suspension-cultured carrot cells has been shown to cross-react with hemocyanin of *H. pomatia* as well as with various seed plants, mollusks, and insects, but not with vertebrates, fungi, or algae (22). The epitope identified as a small, complex xylose-containing, asparagin-linked glycan is shared by many species commonly involved in allergic reactions (such as *D. glomerata*, *Allium sativum*, *Apis mellifera*, and *Mytilus* species) (22). These cross-reactions have been demonstrated for IgG, but they could also involve IgE; this hypothesis has yet to be tested.

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