

Cross-reactivity between storage and dust mites and between mites and shrimp

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Abstract Many patients have sensitivities to multiple species of storage and house dust mites. It is not clear if this is because patients have multiple sensitivities to species-specific mite allergens or if these mites share many cross-reacting allergens. Our objective was to further define the cross-allergenicity between several species of storage and house dust mites using crossed-immunoelectrophoresis (CIE), crossed-radioimmuno-electrophoresis (CRIE), immunoblotting, and ELISA. CIE and CRIE reactions revealed that storage mites shared two cross-antigenic molecules and one of these bound IgE in a serum pool from mite allergic patients. Antibody in anti-sera built to each species of mite recognized many SDS–PAGE resolved proteins of other mite species and this suggested the potential for other cross-reactive allergens. Among patient sera, IgE bound to many different proteins but few had IgE that bound to a protein with common molecular weights across the mite species and this suggested mostly species-specific allergens. Antiserum built to each mite species precipitated one protein in shrimp extracts that bound anti-Der p 10 (tropomyosin) and IgE in the serum pool. Anti-Der p 10 showed strong binding to shrimp tropomyosin but very little to any of the mite proteins. ELISA showed the mite extracts contained very little tropomyosin. The storage and dust mites investigated contain mostly species-specific allergens and very small amounts of the pan-allergen tropomyosin compared to shrimp and snail.

Keywords Storage mite · House dust mite · Allergen · Tropomyosin

Introduction

Sensitivity to stored product mites is common in both rural (agricultural workers) and urban (non-agricultural) populations (Kondreddi et al. 2006; Luczynska et al. 1990; Musken et al. 2002; Puerta et al. 1993; Vidal et al. 2004; Yadav et al. 2006). Storage mites are prevalent in stored grains, hay and straw, poultry and livestock feed, and barn dust and have also been

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reported to occur in mattresses, bedding, carpets and furniture in homes where sometimes they are present in significant numbers (Boquete et al. 2006; Croce et al. 2000; de Oliveira et al. 2003; Valdivieso et al. 2006). Common species of these storage mites include *Acarus siro*, *Chortoglyphus arcuatus*, *Tyrophagus putrescentiae*, and *Lepidoglyphus destructor* among many other species. Some of these common storage mite species and their allergens along with house dust mites are periodically found in processed foods made from grains including baking mixes, cereals, oatmeal and flour and may induce anaphylactic reactions among persons after eating cooked or uncooked baking products (Sanchez-Borges et al. 2005; Wen et al. 2005).

Many studies demonstrate that individual patients have sensitivities to multiple species of storage and house dust mites. It is not clear if this is due to sensitization to many different species of mites or if this is because there are many cross-reactive allergens among the multiple species of storage and house dust mites and if there may be pan-allergens among these species.

Our understanding of the cross-allergenicity between different species of storage mites and between storage mite species and house dust mite species is limited. Generally, it is thought that cross-allergenicity among different species of storage mites and between various species of storage mites and house dust mites (*Dermatophagoides* spp. and *Euroglyphus maynei*) is very limited (Sidenius et al. 2001). However, most of the research along these lines has investigated cross-reacting epitopes (e.g., using RAST inhibition) and has not used techniques that allow the visualization of cross-reacting allergen molecules. Both species-specific and cross-reacting epitopes occur on the related house dust mite allergens Der p 1 and Der f 1 and on the allergens Der p 2 and Der f 2 (Heymann et al. 1986, 1989). Using antisera built in rabbits, it has been shown by crossed-immuno-electrophoresis (CIE) that many antigenic molecules in *Dermatophagoides farinae*, *D. pteronyssinus*, and *E. maynei* extracts cross-react (Arlan et al. 1987b, 1993) but this does not necessarily mean they are allergens for humans. However, some studies have shown some cross-inhibition of IgE binding between storage mites and house dust mite extracts (Chew et al. 1999; Harfast et al. 1992; Johansson et al. 1991, 1994; Luczynska et al. 1990; Puerta et al. 1993; van der Heide et al. 1998). Still others have found little cross-allergenicity between storage mites and between storage mite extracts and house dust mite extracts or allergens (Chew et al. 1999; Johansson et al. 1991; Puerta et al. 1993; van Hage-Hamsten et al. 1987; Ventas et al. 1992).

The purpose of this study was to further investigate the cross-reactive allergen molecules among the more common species of storage mites and house dust mites to which humans are exposed. We also investigated if any of the mite cross-reactivity was due to a pan allergen that also makes these mites cross-reactive with other invertebrates used as food such as shrimp and snails (escargot).

Materials and methods

Mite material and antisera

The storage mites *Chortoglyphus arcuatus* (CA), *Lepidoglyphus destructor* (LD), *Tyrophagus putrescentiae* (TP), and *Acarus siro* (AS) and the house dust mites *Dermatophagoides farinae* (DF), and *Euroglyphus maynei* (EM) were harvested from thriving laboratory cultures and soluble protein extracts were prepared as previously described

(Morgan and Arlian 2006). The protein contents of all extracts were determined by the method of Bradford (1976) using bovine serum albumin as standard.

Rabbit antisera to the house dust mites were prepared previously (Arlian et al. 1987a, 1993). Antisera to the storage mites were prepared by the Wright State University Laboratory Animal Resources facility using their standard polyclonal antibody production protocol.

Shrimp and snail material

Fresh snails (escargot) were purchased at a local grocery store and an extract was prepared essentially as described by DeWitt et al. (2004). Briefly, shells were removed and a weighed amount of material in an equal amount of water (W:V) was ground for 90 s in a blender. The resulting slurry was transferred to a 50 ml centrifuge tube, boiled for 10 min, allowed to cool and centrifuged for 15 min at 1,500g. The supernatant was collected, filtered through a 0.45 µm filter and frozen. Commercial shrimp immunotherapy extracts were purchased from two different vendors. One identified the source material as “*Penaeus* sp.” while the other supplier did not identify the species of shrimp used to prepare the extract. The protein contents of these extracts were also determined by the Bradford method (1976).

Tropomyosin

Tropomyosin from porcine muscle and rabbit antiserum to chicken gizzard tropomyosin were purchased from Sigma-Aldrich (St Louis, MO, USA). A mouse monoclonal antibody (1A6) to mite tropomyosin (Der p 10) was a gift from Martin Chapman of Indoor Biotechnologies (Charlottesville, VA, USA).

Human serum

The human serum used to probe gels and blots for IgE binding was provided by Kathleen Barnes of the Johns Hopkins University School of Medicine (Baltimore, MD, USA) and was collected under an approved protocol. The serum used in these experiments was collected from six individuals. All sera were commercially tested by ImmunoCAP and had scores of ≥ 4 to *D. farinae*, *D. pteronyssinus*, *Acarus* spp., *Lepidoglyphus* spp., and *Tyrophagus* spp. A pool was also prepared from these six sera and was used in some experiments as described below.

Crossed-immunoelectrophoresis (CIE), intermediate gel immunoelectrophoresis, and crossed-radioimmunoelectrophoresis (CRIE)

Homologous, heterologous, and intermediate gel crossed-immunoelectrophoresis were performed by standard methods as previously described and gels were stained with Coomassie blue (Arlian et al. 1987a, 2003; Axelsen 1983). Homologous CIE was used to visualize the antigenic molecules in the extracts of each storage mite species. With homologous CIE, the extract of one storage mite species was reacted with rabbit antiserum built to the same species. To visualize cross-reactivity between mite species, heterologous intermediate CIE was used to react the extract of one mite species with rabbit antiserum built to two different mite species. Heterologous CIE was also performed by reacting shrimp extract or porcine tropomyosin separated in the first dimension gel with rabbit antiserum to each individual house dust and storage mite species in the second dimension gel.

To visualize allergens, CRIE was also performed as previously described (Arlan et al. 2003). Briefly, unstained gels were incubated overnight in the human serum pool diluted 1:10 (V:V) in buffer followed by ^{125}I -anti-human IgE (0.36 $\mu\text{Ci/gel}$; Diamed Inc., Windham, ME, USA). Gels were washed, pressed and dried and subjected to autoradiography. The mouse monoclonal antibody to mite tropomyosin (Der p 10) was also used to probe selected CIE gels. These were developed as described for immunoblots following.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting

SDS–PAGE and immunoblotting were performed using 12% gels as previously described (Yadav et al. 2006). SDS–PAGE-separated proteins were transferred to membranes and blots were probed with rabbit antisera (1:500) followed by peroxidase-labeled goat anti-rabbit Ig (1:1000; Fisher Scientific, Pittsburgh, PA, USA) with detection using the mixed substrate of Young (1989). Selected blots were also probed with individual human sera (1:5) or with the human serum pool (1:10) followed by autoradiography as for CRIE.

To identify mite tropomyosin, selected blots and CIE gels were incubated in the mouse monoclonal antibody to mite tropomyosin (antibody 1A6 at 1:1000) followed by alkaline phosphatase labeled goat anti-mouse Ig (1:2000; Fisher Scientific). These were developed with AP blue membrane solution (Sigma-Aldrich).

Enzyme-linked immunosorbent assay (ELISA)

ELISA plates (Costar 3590, Fisher Scientific) were coated with 100 μl of the mouse monoclonal antibody to Der p 10 (1A6) diluted to 1.9 $\mu\text{g/ml}$ in 50 mM sodium carbonate/bicarbonate buffer, pH 9.6. Plates were sealed and placed at 4°C overnight. Plates were washed with PBST (Dulbecco's phosphate buffered saline with 0.05% Tween 20) and blocked for 1 h at room temperature with BPBST (1% bovine serum albumin in PBST). Antigen extracts were diluted in BPBST to 2,000, 200 and 20 μg protein/ml and loaded onto plates at 100 $\mu\text{l/well}$ for 2 h at room temperature. Plates were washed and incubated for another 2 h in rabbit antiserum to the various mites diluted 1:500 in BPBST. Plates were developed with peroxidase-labeled goat anti-rabbit Ig at 1:1000, and chromogenic substrate as previously described (Arlan et al. 1994).

Results

Homologous crossed-immunoelectrophoretic analysis of extracts of the storage mite species *C. arcuatus*, *L. destructor*, *T. putrescentiae*, and *A. siro* reacted with rabbit antiserum built to its own extract showed 14, 18, 12, and 12 antigen–antibody precipitates on Coomassie Blue stained gels, respectively. We then used intermediate gel CIE to directly investigate cross-reactivity among these four storage mite species. Intermediate gel CIE showed that at most only two molecules cross-reacted between extracts of two or three species of mites and in most cases only one molecule cross-reacted between them (Fig. 1). Incubation of these gels in the serum pool from the storage mite sensitive patients also showed very little IgE binding. The limited number of precipitates observed on these heterologous intermediate gels suggested that these mite species shared few cross-reacting immunogenic molecules and epitopes on these molecules. The few antigen–antibody precipitates and the similarity in electrophoretic mobility and the staining profiles of the

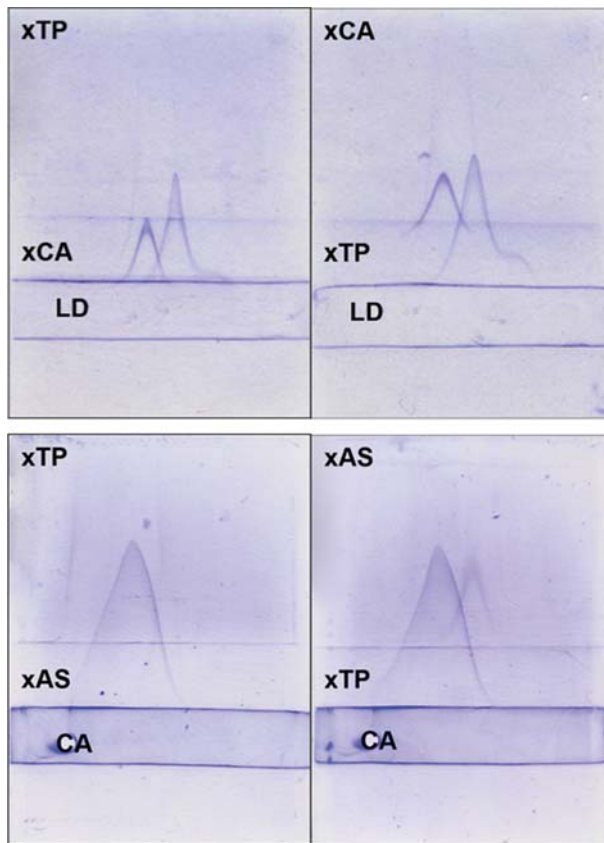


Fig. 1 Representative Coomassie blue stained heterologous intermediate gel CIE. CA, *C. arcuatus*; LD, *L. destructor*; TP, *T. putrescentiae*; AS, *A. siro*; x-, antiserum to the mite species indicated

cross-reacting molecules suggested the possibility that the cross-reactivity was due to the presence of a pan antigen/allergen shared by these mite species.

A known pan-allergen among many invertebrates is tropomyosin that has been shown to be a cross-reacting allergen of many arthropods (Jeong et al. 2006; Reese et al. 1999). Tropomyosin is a major allergen in shrimp (Pen a 1). Tropomyosin is a protein associated with the actin filament of muscle cells and other non-contractile cells and is involved in muscle contraction and other contractile activity of non-muscle cells of both vertebrates and invertebrates. When we reacted porcine (vertebrate) muscle tropomyosin with the various mite antisera, no antigen–antibody precipitates were observed. However, when shrimp extracts were reacted with antiserum to each of the house dust and storage mite species, a single electrophoretically identical protein was precipitated (Fig. 2a). CRIE of these gels showed these antigen–antibody precipitin peaks bound IgE when incubated in the serum pool from mite-allergic patients (Fig. 2b). Likewise, incubating one of these gels (shrimp vs. anti-*C. arcuatus*) in a monoclonal antibody directed against house dust mite tropomyosin (anti-Der p 10) showed anti-Der p 10 binding to the single precipitate and confirmed that this cross-reacting molecule was indeed tropomyosin (Fig. 2c). In addition, we ran another series of gels where the antigen/antibody ratios were altered. On these gels,

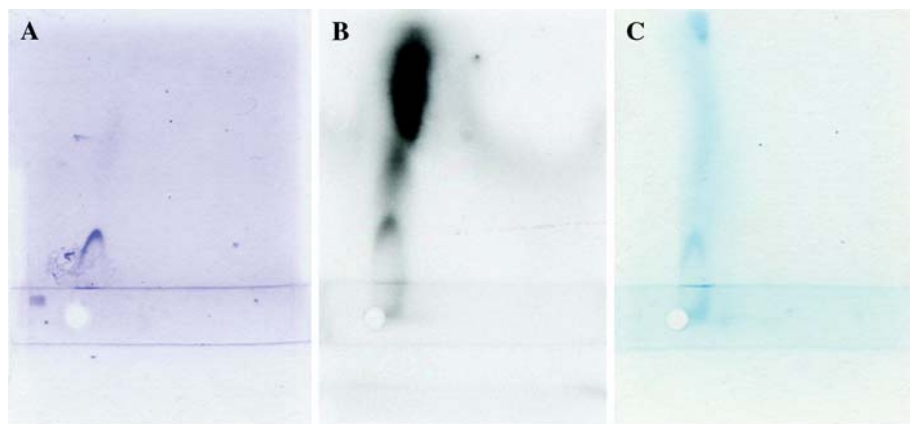


Fig. 2 Representative heterologous CIE reaction of shrimp extract with storage mite antiserum. In the experiment depicted here, shrimp extract was precipitated with anti-*C. arcuatus* antiserum. **a** Coomassie blue stained gel. **b** Gel probed with the human serum pool to reveal IgE binding. **c** Gel probed with anti-Der p 10

a second precipitate was observed when the shrimp extract was reacted with the antiserum to *A. siro* but this peak did not bind either human IgE or the anti-Der p 10 (data not shown).

The proteins in extracts of *C. arcuatus*, *L. destructor*, *T. putrescentiae*, *A. siro*, *D. farinae*, and *E. maynei* were also separated by SDS–PAGE. Western blotting using anti-*C. arcuatus*, anti-*L. destructor*, anti-*T. putrescentiae*, anti-*A. siro*, anti-*D. farinae*, and anti-*E. maynei* showed that the rabbit antiserum built to one species of storage mite recognized many proteins in the extracts of all the other species of mites while recognizing the most proteins in the homologous extract (Fig. 3). Anti-tropomyosin (from chicken gizzard muscle) showed little binding to any proteins in the mite extracts again suggesting there were few cross-reacting molecules or epitopes between the vertebrate tropomyosin and the tropomyosin in mites. Immunoblotting using serum from the six individual storage mite sensitive patients showed both differences in IgE binding by a given subject to proteins in extracts of the various storage and house dust mites as well as differences in the IgE binding from the various patients to proteins from a given mite species (Fig. 4; Table 1). In some cases, a serum contained IgE that recognized proteins of similar molecular weight in several different mite species. In other cases, IgE in the patient serum recognized a protein or a few proteins in one species but not proteins of similar molecular weight in other species. Likewise, a patient could recognize several or many proteins in one species but few or no proteins in another.

Immunoblotting was used to further investigate the cross-reactivity of these mites with shrimp and snails. The proteins in extracts of two commercial shrimp extracts and an extract that was prepared from fresh snails (escargot) were separated by SDS–PAGE. Western blotting with anti-*C. arcuatus*, anti-*L. destructor*, anti-*T. putrescentiae*, anti-*A. siro*, anti-*D. farinae*, and anti-*E. maynei* showed antibody binding to many proteins in the shrimp extracts and little binding to proteins in the snail extract (Fig. 3). Several of the mite anti-sera showed pronounced antibody binding to proteins of ~40 and 80 kDa in the shrimp extracts and in some of the mite extracts and to identical bands in the authentic tropomyosin from porcine muscle. Anti-chicken gizzard tropomyosin showed clear binding to two proteins of identical molecular weight in the two commercial shrimp extracts and

Fig. 3 SDS-PAGE immunoblots of mite, shrimp, and snail extracts (all loaded at 5 μ g/lane) and porcine tropomyosin (0.5 μ g/lane) probed with antiserum to mite extracts and to chicken gizzard tropomyosin (all 1:500). M, molecular weight markers; CA, *C. arcuatus*; LD, *L. destructor*; TP, *T. putrescentiae*; AS, *A. siro*; DF, *D. farinae*; EM, *E. maynei*; S, snail extract; X and Y, commercial shrimp extracts; T, tropomyosin

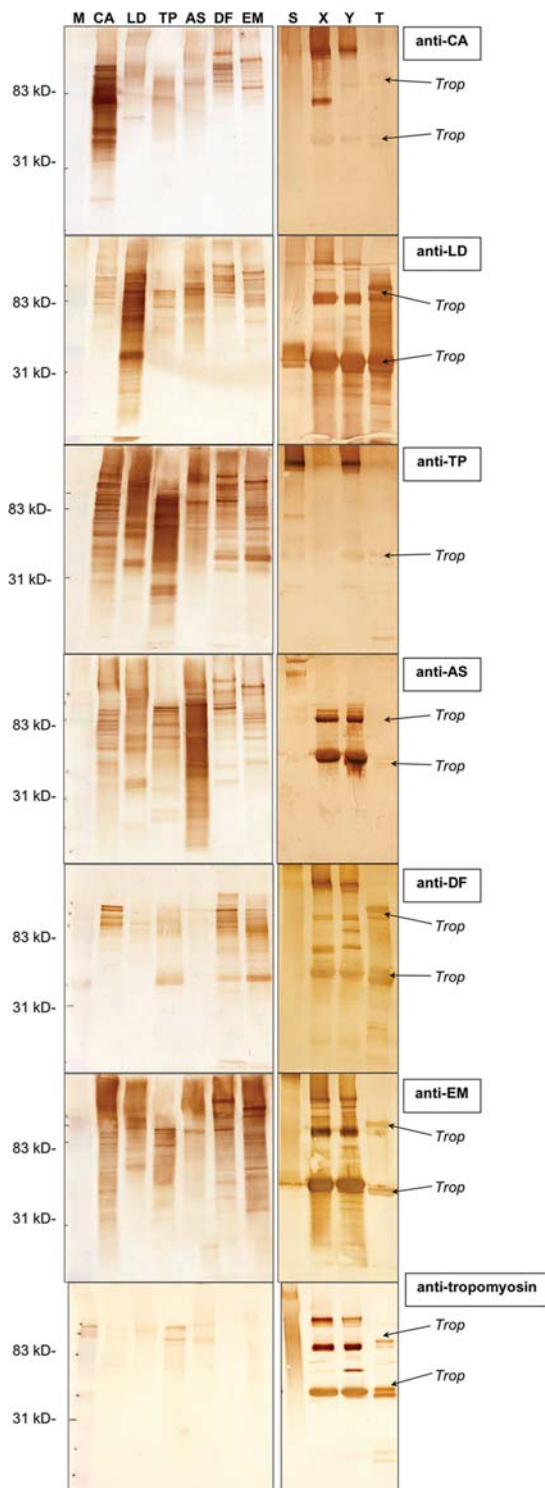


Fig. 4 Representative SDS–PAGE immunoblots of mite extracts (5 µg/lane) probed with human serum (1:5) from patients #2 (*top panel*) and #4 (*bottom panel*) to reveal IgE binding. M, molecular weight markers; CA, *C. arcuatus*; LD, *L. destructor*; TP, *T. putrescentiae*; AS, *A. siro*; DF, *D. farinae*; EM, *E. maynei*

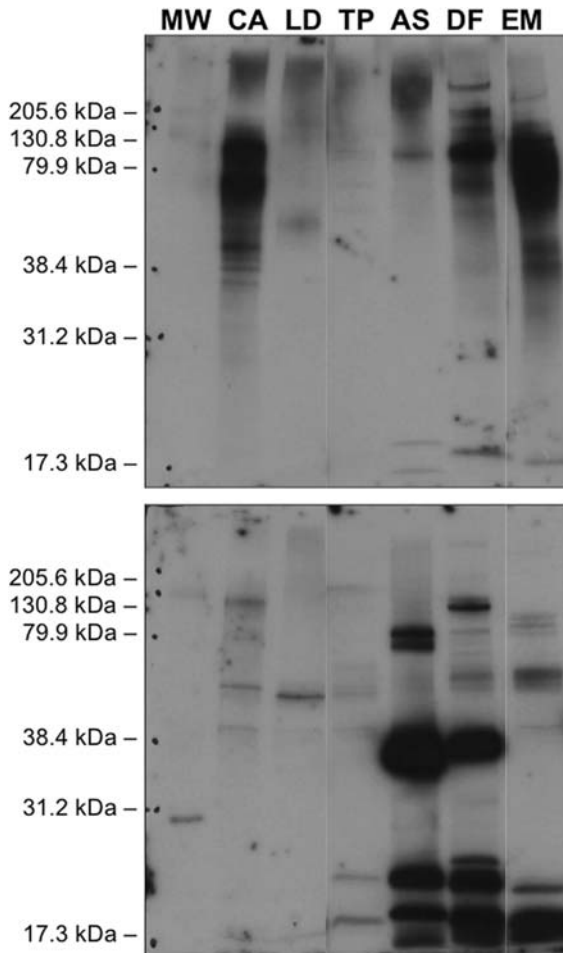
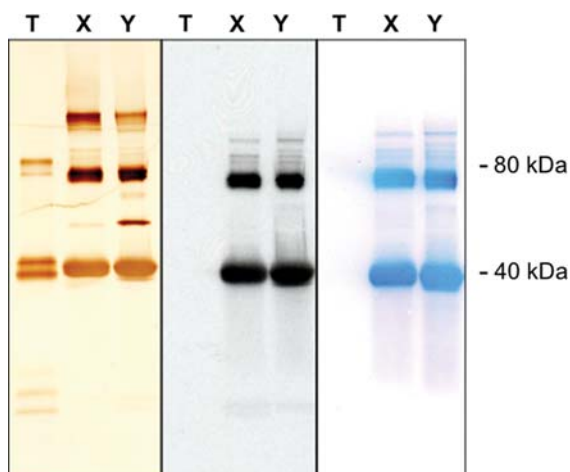


Table 1 Intensity of human IgE binding to SDS–PAGE immunoblots of various mite extracts probed with the serum of mite-sensitive patients

Mite extract	<i>C. arcuatus</i>	<i>L. destructor</i>	<i>T. putrescentiae</i>	<i>A. siro</i>	<i>D. farinae</i>	<i>E. maynei</i>
Human subject						
Patient 1	–	–	+	++	++	++
Patient 2	+++	+	+	+	++	+++
Patient 3	+	–	+	++	++	+
Patient 4	+	+	+	+++	+++	++
Patient 5	++++	+++	++	+	++	+
Patient 6	++++	+++	++	+++	+++	++

Scores were assigned to IgE-binding bands as they appeared on autoradiograms exposed for varying times from 1 (+++++) to 21 (+) days

Fig. 5 SDS-PAGE immunoblots of porcine tropomyosin (*T*) and shrimp extracts (*X* and *Y*) probed with antiserum to chicken gizzard tropomyosin (*left panel*), pooled human serum to reveal IgE binding (*center panel*), and anti-Der p 10 (*right panel*)



the pig tropomyosin. These proteins were ~ 40 and 80 kDa and likely corresponded to monomer and dimer forms of tropomyosin (Motoyama et al. 2006). Pooled serum from the mite-sensitive patients showed strong IgE binding to both of these proteins in the shrimp extracts but not to the pig tropomyosin (Fig. 5). The sensitivity of the individual patients whose serum was used to make the pool to shrimp was unknown but the binding to both shrimp and mites could be due to multiple sensitivities and not cross-reactivity.

We then developed an ELISA to compare the amounts of tropomyosin in mites with that in shrimp and snail. These ELISA reactions showed that all the mite and shrimp extracts contained tropomyosin that was captured by the monoclonal anti-Der p 10 and then recognized by the various mite antisera (Table 2). The amount of signal varied but the greatest amount was usually evident with the homologous reaction of an antigen (extract) and the antisera to this antigen (extract). The porcine tropomyosin was bound by the anti-Der p 10 but only the *A. siro* antiserum detected its presence in the ELISA format. The lowest mite extract doses tested ($2 \mu\text{g}/\text{well}$) were similar to the protein loads on the Western blots and did not contain enough tropomyosin to be detected by this method while the higher doses showed

Table 2 Absorbance values from the Der p 10 capture ELISA.

Antigen	Antiserum					
	<i>C. arcuatus</i>	<i>L. destructor</i>	<i>T. putrescentiae</i>	<i>A. siro</i>	<i>D. farinae</i>	<i>E. maynei</i>
<i>Tropomyosin</i> ^a	0.000	0.000	0.000	0.306	0.000	0.000
<i>C. arcuatus</i>	2.011	0.726	2.145	1.506	0.115	0.069
<i>L. destructor</i>	0.114	1.945	0.898	0.570	0.019	0.000
<i>T. putrescentiae</i> ^a	0.098	0.034	0.689	0.418	0.000	0.031
<i>A. siro</i>	0.422	0.829	1.271	3.000	0.105	0.193
Shrimp X	0.030	0.000	0.432	0.645	0.036	0.000
<i>D. farinae</i>	0.163	0.282	0.231	0.827	1.309	0.211
<i>E. maynei</i>	0.339	0.381	1.224	0.694	0.633	0.507

All wells were coated with a mouse monoclonal antibody to Der p 10. Various antigen extracts were then incubated in the wells followed by the various rabbit antisera as indicated. Plates were developed with peroxidase-labeled anti-rabbit antibody followed by a chromogenic substrate solution

^a These extracts were tested at $200 \mu\text{g}/\text{ml}$ while all others were at $2.0 \text{ mg}/\text{ml}$

stronger antibody binding which confirmed that tropomyosin is a pan-allergen present in all the mite and shrimp extracts but that its concentration in mite extracts is very low.

Discussion

Using intermediate gel CIE, we found that very few of the antigenic molecules (immunogenic) of one species of storage mite cross-reacted with those of another species or with those of the house dust mites, *D. farinae* and *E. maynei*. Homologous CIE reaction of an extract of each mite species with the rabbit anti-serum built to it gave many antigen–antibody precipitin complexes. In contrast, rabbit anti-serum built to each species of storage mite extract (*A. siro*, *C. arcuatus*, *L. destructor*, *T. putrescentiae*) did not precipitate many proteins from the extracts of other storage mite species in heterologous intermediate CIE reactions. With intermediate gel CIE, extract of one mite species was reacted with the antisera of two different mite species. Coomassie Blue staining of these gels showed that at most only two molecules reacted (precipitated) and were common between some of the extracts. In most cases only one molecule was common between most of them. The few antigen–antibody precipitates that did form with the heterologous intermediate CIE reactions had similar profiles (in terms of the peak position/molecular mobility, antigen–antibody complex height and staining characteristics) on the gel which suggested they were similar molecules. More importantly, these antigen–antibody complexes showed very little IgE binding after incubation in the sera of storage mite allergic (sensitive) patients. These CIE and CRIE results suggested that there were few cross-reactive molecules and allergenic epitopes on these immunogenic molecules and there is very little cross-allergenicity between molecules of each of the storage mites and between these four species of storage mites and the house dust mites, *D. farinae* and *E. maynei*. Therefore, it is likely that the serum used in this study came from subjects who had been exposed and actually sensitized to each individual mite species.

To further investigate the cross-antigenicity between various mite species, we separated the proteins in extracts of each storage and house dust mite, shrimp and snail by SDS–PAGE and then probed the separated proteins of each species using rabbit antisera for all the mite species and anti-tropomyosin by immunoblotting to detect antibody binding. The heterologous immunoblots showed that antibody in the anti-mite sera built to one species of mite bound to many proteins of the different mite species. The amount of binding varied between species but the immunoblots using anti-*T. putrescentiae*, anti-*A. siro* and anti-*E. maynei* contained antibodies that recognized the greatest number of proteins of different mite species. These immunoblot results seem puzzling because the heterologous intermediate gels showed so few antigen–antibody precipitates compared to the number of antibody binding proteins on blots prepared by SDS–PAGE. One explanation for this may be that the SDS–PAGE treatment changes the conformation of proteins and thus exposes internal epitopes that are not available for antibody to bind and precipitate in the CIE system where the extract contains intact molecules. The immunizing proteins in a mite extract may be hydrolyzed in vivo in the rabbit and thus the rabbit builds antibody to epitopes that are hidden in the internal structure of the natural protein. Using rabbit anti-mite sera, these results clearly show a higher degree of cross-reactivity (many molecules that are not allergens) between mite species than did the CIE reactions. Therefore, there is the potential for greater cross-allergenicity than we identified using intermediate gel CIEs probed with serum from storage mite sensitive patients. This possibility is supported by the immunoblots using the patients' sera. Patient sera that showed little binding to antigen–antibody

precipitates in the CRIE showed binding to more proteins using immunoblotting. However, the immunoblot results were very heterologous between mites and patients which suggests most allergens are species-specific. Among patients, IgE did not consistently bind to proteins of similar molecular weight across the various mite species.

As already mentioned, the profiles of the very few antigen–antibody precipitates for the various heterologous intermediate gel CIE reactions were similar and this suggested that these mites shared a pan immunogenic molecule. To determine if there might be a pan-allergen shared with other invertebrates, using heterologous CIE, we directly reacted a commercial shrimp extract with antiserum to each of the storage mites and to the house dust mites, *D. farinae* and *E. maynei*. In each of these reactions, a single antigen–antibody precipitate formed and all peaks had similar electrophoretic mobilities. This suggested that *C. arcuatus*, *L. destructor*, *T. putrescentiae*, *A. siro*, *D. farinae*, and *E. maynei* extracts shared a common immunogenic antigen (molecule) with the shrimp. The common antigen in these antigen–antibody precipitates bound monoclonal anti-Der p 10 (anti-*D. pteronyssinus* tropomyosin) and bound IgE from the serum of the mite allergic patients. Therefore, these data suggest that tropomyosin is a common allergen in the mites *A. siro*, *C. arcuatus*, *L. destructor*, *T. putrescentiae*, *D. farinae*, and *E. maynei* and in shrimp.

Shrimp tropomyosin (Pen a 1) and dust mite Der p 10 and Der f 10 share about 81% amino acid sequence homology (Ayuso et al. 2002). Shrimp allergen Pen a 1 has 8 IgE-binding epitopes and the amino acid sequence homology in the IgE-binding epitopes is very high (85–100%) for all eight epitopes (Ayuso et al. 2002). The epitopes differ in only 1–6 amino acid residues. A homolog of Der p 10 allergen is an immunodominant allergen of the sheep mite *Psoroptes ovis* (Huntley et al. 2004). Also, tropomyosin is a ubiquitous protein in the tissues of the parasitic red poultry mite *Dermanyssus gallinae* and has an 85% identity to Der p 10 (Nisbet et al. 2006). Therefore, our finding that tropomyosin is a common allergen in these storage mites and house dust mites is not surprising.

Serum from the patients that were ImmunoCAP-positive to storage mites showed strong IgE binding to three SDS–PAGE resolved bands in commercial shrimp extracts including two corresponding to tropomyosin (~ 40 and 80 kDa). It is not clear if the 80 kDa protein is an isoform (dimer) of tropomyosin. It was not known if the patients were sensitive to shrimp but clearly tropomyosin is present in shrimp and mites but its concentration in mites is so low that it is difficult to visualize on either CIE or immunoblots. The ELISA allowed larger amounts of mite protein to be tested thereby concentrating the tropomyosin and confirming its presence in all of the mite extracts. It also revealed that the amount of tropomyosin present in the mite extracts is very low. Practically speaking, shrimp extracts are prepared from organisms that are almost entirely muscle while mites have very little muscle and are predominantly exoskeleton and hemolymph.

In conclusion, this study showed that there is little cross-allergenicity between molecules of the different storage mite species *C. arcuatus*, *L. destructor*, *T. putrescentiae*, and *A. siro* and between molecules of these storage mites and the house dust mites *D. farinae* and *E. maynei*. These storage mites appear to be the source of mostly species-specific allergens and few of these seem to cross-react with shrimp and snail. However, immunoblotting using rabbit anti-mite serum showed that extracts of these mites contain other cross-reacting molecules/epitopes but it is not clear if any are allergenic until serum from a larger population of well defined patients can be evaluated. Tropomyosin (dust mite group 10 allergen) is present in small amounts in the mite extracts tested. Because there is little muscle in mites compared to shrimp, tropomyosin levels in mite extracts are quite low compared to those in shrimp extract. However, in specific populations, such as was reported for serum from patients in Zimbabwe, IgE recognition frequency to rDer p 10 can

be significant (55%) and this may reflect sensitization to the cross-reactive allergen tropomyosin (Westritschnig et al. 2003) even though low levels of this protein occur in mites relative to crustaceans. As has been previously suggested, because most allergenic molecules from stored product mites are species-specific, for diagnostic purposes, skin testing should be conducted with each mite species when storage mite sensitivity is suspected (Pittner et al. 2004; Yadav et al. 2006).

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References

- Arlan LG, Bernstein IL, Geis DP, Vyszynski-Moher DL, Gallagher JS, Martin B (1987a) Investigations of culture medium-free house dust mites III. Antigens and allergens of body and fecal extract of *Dermatophagoides farinae*. J Allergy Clin Immunol 79:457–466. doi:[10.1016/0091-6749\(87\)90363-0](https://doi.org/10.1016/0091-6749(87)90363-0)
- Arlan LG, Bernstein IL, Vyszynski-Moher DL, Gallagher JS (1987b) Investigations of culture medium-free house dust mites IV. Cross antigenicity between the house dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*. J Allergy Clin Immunol 79:467–476. doi:[10.1016/0091-6749\(87\)90364-2](https://doi.org/10.1016/0091-6749(87)90364-2)
- Arlan LG, Rapp CM, Fernandez-Caldas E (1993) Allergenicity of *Euroglyphus maynei* and its cross-reactivity with *Dermatophagoides* species. J Allergy Clin Immunol 91:1051–1058. doi:[10.1016/0091-6749\(93\)90219-6](https://doi.org/10.1016/0091-6749(93)90219-6)
- Arlan LG, Morgan MS, Vyszynski-Moher DL, Stemmer BL (1994) *Sarcoptes scabiei*: the circulating antibody response and induced immunity to scabies. Exp Parasitol 78:37–50. doi:[10.1006/expr.1994.1004](https://doi.org/10.1006/expr.1994.1004)
- Arlan LG, Morgan MS, Quirce S, Maranon F, Fernandez-Caldas E (2003) Characterization of allergens of *Anisakis simplex*. Allergy 58:1299–1303. doi:[10.1046/j.1398-9995.2003.00344.x](https://doi.org/10.1046/j.1398-9995.2003.00344.x)
- Axelsen NH (1983) Intermediate gel immunoelectrophoresis. Scand J Immunol 17(Suppl 10):141–149
- Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB (2002) Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Int Arch Allergy Immunol 129:38–48. doi:[10.1159/000065172](https://doi.org/10.1159/000065172)
- Boquete M, Iraola V, Fernandez-Caldas E, Arenas Villaroel L, Carballada FJ, Gonzalez de la Cuesta C, Lopez-Rico MR, Nunez Orjales R, Parra A, Soto-Mera MT, Varela S, Vidal C (2006) House dust mites species and allergen levels in Galicia, Spain: a cross-sectional, multicenter, comparative study. J Investig Allergol Clin Immunol 16:169–176
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. doi:[10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Chew FT, Yi FC, Chua KY, Fernandez-Caldas E, Arruda LK, Chapman MD, Lee BW (1999) Allergenic differences between the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. Clin Exp Allergy 29:982–988. doi:[10.1046/j.1365-2222.1999.00543.x](https://doi.org/10.1046/j.1365-2222.1999.00543.x)
- Croce M, Costa-Manso E, Baggio D, Croce J (2000) House dust mites in the city of Lima, Peru. J Investig Allergol Clin Immunol 10:286–288
- de Oliveira CH, Binotti RS, Muniz JRO, dos Santos JC, do Prado AP, de Pinho AJ (2003) Comparison of house dust mites found on different mattress surfaces. Ann Allergy Asthma Immunol 91:559–562
- DeWitt AM, Mattsson L, Lauer I, Reese G, Lidholm J (2004) Recombinant tropomyosin from *Penaeus aztecus* (Pen a 1) for measurement of specific immunoglobulin E antibodies relevant in food allergy to crustaceans and other invertebrates. Mol Nutr Food Res 48:370–379. doi:[10.1002/mnfr.200400015](https://doi.org/10.1002/mnfr.200400015)
- Harfast B, van Hage-Hamsten M, Ansotegui IJ, Johansson E, Jeddi-Tehrani M, Johansson SGO (1992) Monoclonal antibodies to *Lepidoglyphus destructor*: delineation of crossreactivity between storage mites and house dust mites. Clin Exp Allergy 11:1032–1037. doi:[10.1111/j.1365-2222.1992.tb03033.x](https://doi.org/10.1111/j.1365-2222.1992.tb03033.x)
- Heymann PW, Chapman MD, Platts-Mills TAE (1986) Antigen Der f I from the house dust mite *Dermatophagoides farinae*: structural comparison with Der p I from *D. pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. J Immunol 137:2841–2847

- Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE (1989) Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp.). *J Allergy Clin Immunol* 83:1055–1067. doi:[10.1016/0091-6749\(89\)90447-8](https://doi.org/10.1016/0091-6749(89)90447-8)
- Huntley JF, Machell J, Nisbet AJ, van den Broek A, Chua KY, Cheong N, Hales BJ, Thomas WR (2004) Identification of tropomyosin, paramyosin and apolipophorin/vitellogenin as three major allergens of the sheep scab mite, *Psoroptes ovis*. *Parasite Immunol* 26:335–342. doi:[10.1111/j.0141-9838.2004.00717.x](https://doi.org/10.1111/j.0141-9838.2004.00717.x)
- Jeong KY, Hong C-H, Yong T-S (2006) Allergenic tropomyosins and their cross-reactivities. *Protein Pept Lett* 13:835–845. doi:[10.2174/092986606777841244](https://doi.org/10.2174/092986606777841244)
- Johansson E, Borge A, Johansson SGO, van Hage-Hamsten M (1991) Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 21:511–518. doi:[10.1111/j.1365-2222.1991.tb01693.x](https://doi.org/10.1111/j.1365-2222.1991.tb01693.x)
- Johansson E, Johansson SGO, van Hage-Hamsten M (1994) Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their crossreactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 24:743–751. doi:[10.1111/j.1365-2222.1994.tb00985.x](https://doi.org/10.1111/j.1365-2222.1994.tb00985.x)
- Kondreddi PK, Elder BL, Morgan MS, Vyszynski-Moher DL, Arlian LG (2006) Importance of sensitization to *Tyrophagus putrescentiae* in the United States. *Ann Allergy Asthma Immunol* 96:124
- Luczynska CM, Griffin P, Davies RJ, Topping MD (1990) Prevalence of specific IgE to storage mites (*A. siro*, *L. destructor* and *T. longior*) in an urban population and cross reactivity with the house dust mite (*D. pteronyssinus*). *Clin Exp Allergy* 20:403–406. doi:[10.1111/j.1365-2222.1990.tb02801.x](https://doi.org/10.1111/j.1365-2222.1990.tb02801.x)
- Morgan MS, Arlian LG (2006) Enzymatic activity in extracts of allergy-causing astigmatid mites. *J Med Entomol* 43:1200–1207. doi:[10.1603/0022-2585\(2006\)43\[1200:EAIEOA\]2.0.CO;2](https://doi.org/10.1603/0022-2585(2006)43[1200:EAIEOA]2.0.CO;2)
- Motoyama K, Ishizaki S, Nagashima Y, Shiomi K (2006) Cephalopod tropomyosins: identification as major allergens and molecular cloning. *Food Chem Toxicol* 44:1997–2002. doi:[10.1016/j.fct.2006.06.018](https://doi.org/10.1016/j.fct.2006.06.018)
- Musken H, Fernandez-Caldas E, Maranon F, Franz J-T, Masuch G, Bergmann K-C (2002) In vivo and in vitro sensitization to domestic mites in German urban and rural allergic patients. *J Investig Allergol Clin Immunol* 12:177–181
- Nisbet AJ, Huntley JF, MacKellar A, Sparks N, McDevitt R (2006) A house dust mite allergen homologue from poultry red mite *Dermanyssus gallinae* (De Geer). *Parasite Immunol* 28:401–405. doi:[10.1111/j.1365-3024.2006.00862.x](https://doi.org/10.1111/j.1365-3024.2006.00862.x)
- Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, Kraft D, Valenta R (2004) Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 34:597–603. doi:[10.1111/j.1365-2222.2004.1930.x](https://doi.org/10.1111/j.1365-2222.2004.1930.x)
- Puerta L, Fernandez-Caldas E, Lockey RF, Caraballo LR (1993) Sensitization to *Chortoglyphus arcuatus* and *Aleuroglyphus ovatus* in *Dermatophagoides* spp. allergic individuals. *Clin Exp Allergy* 23:117–123. doi:[10.1111/j.1365-2222.1993.tb00306.x](https://doi.org/10.1111/j.1365-2222.1993.tb00306.x)
- Reese G, Ayuso R, Lehrer SB (1999) Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol* 119:247–258. doi:[10.1159/000024201](https://doi.org/10.1159/000024201)
- Sanchez-Borges M, Suarez-Chacon R, Capriles-Hulett A, Caballero-Fonseca F (2005) An update on oral anaphylaxis from mite ingestion. *Ann Allergy Asthma Immunol* 94:216–221
- Sidenius KE, Hallas TE, Poulsen LK, Mosbech H (2001) Allergen cross-reactivity between house-dust mites and other invertebrates. *Allergy* 56:723–733. doi:[10.1034/j.1398-9995.2001.056008723.x](https://doi.org/10.1034/j.1398-9995.2001.056008723.x)
- Valdivieso R, Iraola V, Estupinan M, Fernandez-Caldas E (2006) Sensitization and exposure to house dust and storage mites in high-altitude areas of Ecuador. *Ann Allergy Asthma Immunol* 97:532–538
- van der Heide S, Niemeijer NR, Hovenga H, de Monchy JGR, Dubois AEJ, Kauffman HF (1998) Prevalence of sensitization to the storage mites *Acarus siro*, *Tyrophagus putrescentiae* and *Lepidoglyphus destructor* in allergic patients with different degrees of sensitization to the house dust mite *Dermatophagoides pteronyssinus*. *Allergy* 53:426–430. doi:[10.1111/j.1398-9995.1998.tb03917.x](https://doi.org/10.1111/j.1398-9995.1998.tb03917.x)
- van Hage-Hamsten M, Johansson SGO, Zetterstrom O (1987) Predominance of mite allergy over allergy to pollens and animal danders in a farming population. *Clin Allergy* 17:417–423. doi:[10.1111/j.1365-2222.1987.tb02035.x](https://doi.org/10.1111/j.1365-2222.1987.tb02035.x)
- Ventas P, Carreira J, Polo F (1992) Purification and characterization of Lep d I, a major allergen from the mite *Lepidoglyphus destructor*. *Clin Exp Allergy* 22:454–460. doi:[10.1111/j.1365-2222.1992.tb00147.x](https://doi.org/10.1111/j.1365-2222.1992.tb00147.x)
- Vidal C, Boquete O, Gude F, Rey J, Meijide LM, Fernandez-Merino MC, Gonzalez-Quintela A (2004) High prevalence of storage mite sensitization in a general adult population. *Allergy* 59:401–405. doi:[10.1111/j.1398-9995.2003.00430.x](https://doi.org/10.1111/j.1398-9995.2003.00430.x)
- Wen D-C, Shyur S-D, Ho C-M, Chiang Y-C, Huang L, Lin M-T, Yang H-C, Liang P-H (2005) Systemic anaphylaxis after the ingestion of pancake contaminated with the storage mite *Blomia freemani*. *Ann Allergy Asthma Immunol* 95:612–614

- Westritschnig K, Sibanda E, Thomas W, Auer H, Aspöck H, Pittner G, Vrtala S, Spitzauer S, Kraft D, Valenta R (2003) Analysis of the sensitization profile toward allergens in central Africa. *Clin Exp Allergy* 33:22–27. doi:[10.1046/j.1365-2222.2003.01540.x](https://doi.org/10.1046/j.1365-2222.2003.01540.x)
- Yadav A, Elder BL, Morgan MS, Vyszynski-Moher DL, Arlian LG (2006) Prevalence of serum IgE to storage mites in a southwestern Ohio population. *Ann Allergy Asthma Immunol* 96:356–362
- Young PR (1989) Enhancement of immunoblot staining using a mixed chromogenic substrate. *J Immunol Methods* 121:295–296. doi:[10.1016/0022-1759\(89\)90174-9](https://doi.org/10.1016/0022-1759(89)90174-9)