

B- and T-cell epitopes of tropomyosin, the major shrimp allergen

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Seafood allergy is often encountered on ingestion of crustaceans such as shrimp, lobster, crab, and crayfish (1). On eating cooked shrimp, sensitive individuals experience a wide spectrum of reactions ranging from abdominal discomfort to anaphylaxis. The presence of cross-reacting heat-stable allergens in crustacean food was first recognized by Hoffman et al. (2) and Lehrer et al. (3). Subsequently, the major allergen was isolated and characterized from the shrimp species *Penaeus indicus* (Pen i 1) (4) and *P. aztecus* (Pen a 1) (5). Pen i 1 (originally designated Sa-II) and Pen a 1, with mol. mass of 34 and 36 kDa, respectively, contain 301 and 312 amino-acid residues with a predominance of glutamate/glutamine and aspartate/asparagine.

Tropomyosin, the major shrimp allergen

A comparison of amino-acid sequences of different peptides generated by proteolysis of Pen i 1 revealed 86% homology with tropomyosin from *Drosophila melanogaster*. Subsequent studies established that Pen i 1 (6) and Pen a 1 (7) are indeed tropomyosin. Amino-acid sequence analysis of Pen i 1 revealed the presence of repeating heptads of the form $(a-b-c-d-e-f-g)_n$, where *a* and *d* are nonpolar residues, which is a unique feature of tropomyosins. In isoelectric focusing, it behaves like an isoallergen (pI 4.8-5.4) and in SDS-PAGE, in the presence of 6 M urea, exhibits a unique migration pattern with an apparent mol.

mass of 50 kDa, a feature characteristic of the muscle protein, tropomyosin.

Leung et al. (8) determined the primary structure of the major allergen from another species of shrimp, *Metapenaeus ensis*, by applying recombinant DNA technology and amino-acid sequence determination of an IgE-reactive cDNA clone, Met e I. The nucleotide sequence of this cDNA clone revealed an open reading frame of 281 amino-acid residues, coding for a protein of 34 kDa. Screening a unidirectional expression cDNA library from shrimp tail muscle with a Pen i 1-specific monoclonal antibody, Reese et al. (9) identified four positive clones. Based on DNA sequence analysis, the authors confirmed that three of the recombinant proteins expressed in *E. coli* corresponded to shrimp tropomyosin. A peptide library of Pen a 1 was constructed, revealing four IgE-reactive recombinant peptides which exhibited varying degrees of sequence homology with tropomyosins from cockroach, fruitfly, house-dust mite, and other animal species. The availability of recombinant shrimp allergen may allow new therapeutic approaches to patients sensitive to certain seafood.

It is increasingly recognized that tropomyosin is the major allergen from several other sources including other invertebrates such as shellfish (10), mollusk (11), squid (12), and cockroach (13). In the course of a study of house-dust-mite (*Dermatophagoides pteronyssinus*) allergens, Wittenman et al. (14) obtained a monoclonal antibody (mAb) that

reacted not only to mite extracts but also to extracts of insects and other invertebrates such as shrimp. These authors have shown tropomyosin to be involved in cross-reactivity between mite, shrimp, and insects. Similarly, native and recombinant tropomyosin from *D. farinae* also exhibited a high degree of cross-reactivity across the species (15). Recently, an allergenic recombinant tropomyosin (Der p 10) from *D. pteronyssinus* was shown to share more than 65% identical residues with other invertebrate tropomyosins (16). This mite allergen reacted with shrimp antitropomyosin antibodies and had IgE reactivity with a frequency of 5.6% in sera from patients allergic to mite.

B-cell epitopes of tropomyosin

Cross-reactivity studies using polyclonal sera of shrimp-sensitive patients and Pen i 1-specific mouse anti-idiotypic antibodies have attributed the allergenic cross-reactivity observed among related crustaceans to the presence of highly conserved IgE-binding epitopes on tropomyosin (6). The sequences of two IgE-binding B-cell epitopes identified from tryptic digests of shrimp tropomyosin are shown in Table 1. A comparison of these sequences with tropomyosin from fruit fly, fish, chicken, rat, and man shows a higher degree of identity in peptide 1 (MQQLENDLDQVQESLLK) between different species of shrimp and *Drosophila* tropomyosin than other species. Peptide 2 (FLAEEADRK), on the other hand, is strikingly similar among the animal species, except in the first two amino-acid residues. Sequence analysis of the whole protein also revealed other variable regions, which may include other unidentified B-cell epitopes (8). These additional IgE-binding epitopes could have been lost during tryptic digestion (8). IgE and mAb reactivities of Pen a 1 to peptide fragments generated by CNBr cleavage or digestion with endopeptidases suggest that IgE-binding epitopes are restricted to certain regions of the Pen a 1 molecule (17).

T-cell epitopes of tropomyosin

From the deduced primary amino-acid sequence reported for *M. ensis*, we have identified candidate T-cell epitopes on tropomyosin, and examined the ability of their synthetic peptides to modulate allergic responses *in vivo*. The primary amino-acid sequence was scanned for regions with amphipathic helices, and six peptides corresponding to putative T-cell epitope regions were identified on the basis of a computer algorithm developed by Berzofsky (18, 19) and synthesized with a solid-phase synthesizer (Table 2). None of the synthetic peptides

Table 1. Comparison of amino-acid sequence of putative IgE-binding epitopes of shrimp tropomyosin with corresponding sequences from other organisms

Source	Peptide 1 (aa 47–63)	Peptide 2 (aa 150–158)
Shrimp		
<i>P. indicus</i>	MQQLENDLDQVQESLLK	FLAEEADRK
<i>M. ensis</i>	MQQLENDLDQVQESLLK	FLVEEARDK
<i>Drosophila</i>	IQTVENELDQTQEALTL	FLAEEADKK
Rat	LKGTEDELDKYSKALKD	HIAEADARK
Fish (zebra fish)	LKATEDELDKYSEALKD	HIAEEADRK
Chick	LKGTEDEVEKYSKSVKD	HIAEEADRK
Rabbit	LRASEDERDRVLKELHK	HIAEESDRK
Human	LKGTEDELDKYSKALKD	HIAEEADRK

bound to IgE antibodies from a shrimp-sensitive patient's serum or to tropomyosin-specific mouse IgE antibodies. Dot-blot analysis and competitive ELISA-inhibition assay also confirmed the lack of reactivity of various peptides to tropomyosin-specific IgE antibodies (data not shown). Interestingly, one of the predicted T-cell epitopes spanning residues 151–171 (peptide 4), although overlapping with the previously identified B-cell epitope (150–158), did not bind to IgE. This confirms our previous observation that replacement of phenylalanine at 150 in tropomyosins from various mammalian species makes them lose their IgE-binding ability.

Having established the IgG- and IgE-binding properties of the synthetic peptides, we tested them for the ability to induce *in vitro* proliferation of splenocytes isolated from mice immunized with native tropomyosin in a recall assay (data not shown). Peptide 6 was able to induce proliferation comparable to the native allergen, indicating that this peptide spanning the region 261–281 of shrimp tropomyosin is a putative T-cell epitope.

The potential of peptide 6 to downregulate an established allergen-specific antibody response *in vivo*

Table 2. Sequence of synthetic peptides encompassing predicted T-cell epitopes of shrimp tropomyosin

Peptide	Sequence	Residue numbers
1	N K E A N N R A E K S E E E V H N L Q K R M Q Q L E N T L D Q V Q E S L L K A N N	26–66
2	N R R I Q L L E E D L E R S E E R L N T A T T K L	86–110
3	T A T T K L A E A S Q A A D E S E R M R K V L E N R S L	105–132
4	L A E E A D R K Y D E V A R K L A M V E A	151–171
5	A E F A E R S V Q K L Q K E V D R L E D E L E N E K	236–261
6	K E K Y K S I T D E L D Q T F S E L S G Y	261–281

was investigated. BALB/c mice were administered alum-adsorbed tropomyosin intraperitoneally on days 0 and 14. On day 28, mice were administered peptide 6 (50 µg) along with complete Freund's adjuvant by the subcutaneous route. This was followed by three injections in incomplete Freund's adjuvant at the same dose. The animals were bled at 10-day intervals, and downregulation of the response was studied. As can be seen in Fig. 1, after administration of the peptide, the animals were able to maintain the reduced titers of both tropomyosin-specific IgG and IgE beyond 30 days. Clinical manifestations of an allergic response occur after an increase in serum titers of allergen-specific IgE beyond a threshold level; therefore, the reduction in the titers observed in the peptide-treated mice is significant. It appears that the reduction of the ongoing tropomyosin-specific immune response may possibly be due not to tolerization but to an alteration in the cytokine profiles or the activation of allergen-specific T-suppressor cells (20). To our knowledge, we have demonstrated for the first time that a synthetic peptide of the major crustacean allergen, tropomyosin, encompassing the T-cell epitope, is capable of downregulating tropomyosin-specific IgG and IgE responses.

Summary

The major crustacean allergen characterized from different species of shrimp is the muscle protein tropomyosin. Two shared epitopes corresponding to 47–63 and 150–158 of the deduced amino-acid sequence of the brown shrimp, *M. ensis*, were

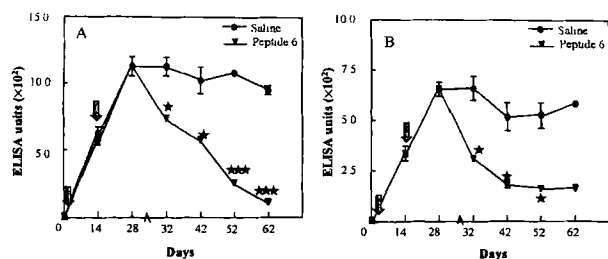


Fig. 1. Suppression of ongoing shrimp tropomyosin-specific antibody response in mice after administration of synthetic T-cell peptide (peptide 6). Data shown represent ELISA units $\pm$ SEM of allergen-specific serum IgG (A) and IgE (B) levels determined by AB-microELISA at serum dilution of 1:8000 (chosen as representative serum dilution showing half maximal value in linear range of serum dilution curve). Saline (with adjuvant)-treated mice were used as controls. Serum dilution curves for saline-treated control mice with or without adjuvant were essentially superimposable. * $P < 0.05$; *** $P < 0.001$. Where error bars are not shown, error bars were too close to be diagrammed. For analytic experimental details, see Shanti et al. (6).

identified as IgE-binding B-cell epitopes. A 21-mer peptide spanning the amino-acid residues 261–281 was identified as a putative T-cell epitope capable of reducing ongoing tropomyosin-specific IgG and IgE responses in a mouse model. These observations suggest that peptide immunotherapy may also be effective in the treatment of food hypersensitivity.

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