

Review

Seafood Allergy and Allergens: A Review

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Abstract: Seafoods are composed of diverse sea organisms and humans are allergic to many of them. Tropomyosin is a major allergen in many shellfish, especially crustacea and mollusks. Interestingly, tropomyosin has also been identified as an important allergen in other invertebrates including dust mites and cockroaches, and it has been proposed by some to be an invertebrate pan allergen. Different regions of shrimp tropomyosin bind IgE; 5 major IgE-binding regions have been identified in shrimp tropomyosin containing 8 epitopes. Mutations of these shrimp allergenic epitopes can reduce seafood allergenicity; methods utilizing such mutations will provide safer vaccines for more effective treatment of seafood-allergic patients, and in the future less-allergenic seafood products for consumption.

Key words: seafood, shrimp, allergy, tropomyosin, epitopes, IgE antibody reactivity.

INTRODUCTION

Since ancient times the sea has been a major source of food for human populations. In 1994 world commercial fishery landings exceeded 100 million metric tons. In addition to commercial fishing, aquaculture supplies an increasing amount of seafood such as catfish, shrimp, and salmon. Associated with the increased harvesting and farming of seafood is the general increase in consumption of seafood in the United States, particularly during the last decade (Table 1). As of 1997 the per capita consumption of sea-

food was 14.6 pounds, and consumer expenditures on seafood products exceeded 38 billion dollars, most of which was spent in food service establishments.

Edible seafood, although grouped together, comprise 3 major divisions of sea organisms, the Chordata, the Mollusca, and the Arthropoda, each of which belongs to a different phylum. The major component of the Chordata is the class Osteichthyes or ray-fin fishes. The Mollusca include snails and abalone (gastropods), mussels, oysters, scallops, clams (bivalves), and squid and octopus (cephalopods). The Arthropoda phylum contains the major class crustacea, which includes shrimp, crabs, lobster, and crawfish. The seafood species most frequently consumed in the United States are shown in Table 2.

With the advent of new technological methods, including improved transportation and preservation, fresh seafood is now more readily available in areas that are far removed from where it is grown and harvested. In addition,

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Table 1. U.S. Seafood Consumption 1970–1997 (in pounds per capita)^a

Seafood	1970	1980	1990	1991	1992	1993	1994	1995	1996	1997
Fresh/frozen	6.9	7.9	9.6	9.7	9.9	10.2	10.4	10	10	9.9
Canned	4.5	4.3	5.1	4.9	4.6	4.5	4.5	4.7	4.5	4.4
Cured	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total	11.8	12.5	15.0	14.9	14.8	15.0	15.2	15	14.8	14.6

^aFrom National Marine Fishery Service, Commerce Department, Washington, D.C.

Table 2. Top 10 U.S. Seafoods 1990–1997 (in pounds per capita)^a

Seafood	1997	1996	1995	1994	1993	1992	1991	1990
Total	14.6	14.8	15.0	15.2	16.0	14.8	14.9	15.0
1. Canned tuna	3.100	3.200	3.400	3.300	3.500	3.500	3.600	3.200
2. Shrimp	2.700	2.500	2.500	2.600	2.500	2.500	2.400	2.200
3. Alaska pollock	1.640	1.620	1.520	1.520	1.200	1.230	0.990	1.270
4. Salmon	1.299	1.440	1.190	1.113	0.995	0.871	0.970	0.730
5. Cod	1.057	0.918	0.983	0.928	1.030	1.076	1.120	1.380
6. Catfish	1.016	0.899	0.864	0.855	0.988	0.907	0.770	0.700
7. Clams	0.461	0.518	0.567	0.544	0.589	0.616	0.580	0.610
8. Crabs	0.424	0.333	0.319	0.312	0.375	0.333	0.320	0.290
9. Flatfish	0.330	0.384	0.302	0.361	0.623	0.507	0.380	0.570
10. Halibut	0.286	0.203	0.188	0.215	0.243	0.247	0.208	0.248

^aFrom the National Fisheries Institute, Arlington, Va.

seafood meat such as surimi (protein derived from Alaska pollock) has been used as the basis of producing a variety of food products, such as imitation crabmeat, shrimp, sausages, sauces, and flavors. Thus consumption and subsequent exposure to some seafood proteins may not always be obvious to consumers allergic to such foods. Allergic reactions can and do develop to seafood products (Taylor and Bush, 1988). Studies in our laboratory evaluating seafood-induced allergies in 30 shrimp-sensitive and 37 fish-allergic individuals found that the most frequently reported sign was generalized itching (ranging from 69% to 90%). Development of hives occurred in 69%, while more than half of both populations (51% and 52%, respectively) reported swelling of the lips and tongue. Some 50% of both groups reported pulmonary symptoms, gastrointestinal symptoms, and anaphylactic shock (Table 3) (O’Neil et al., 1993; Daul et al., 1988, 1993a, 1993b). Thus the food-induced allergic reactions that develop to seafood products are typical of those types of reactions that are reported to a variety of other products that cause food allergy (Sampson and Metcalfe, 1991; Lehrer and Reese, 1998).

In addition, occupational reactions have been reported to occur in a variety of seafood workers (Table 4); these

Table 3. Clinical Symptoms and Signs Observed in Double-Blind Placebo-Controlled Food Challenges with Fish and Shrimp in Allergic Subjects

Symptoms/Signs	Fish allergy, % (<i>n</i> = 37)	Shrimp allergy, % (<i>n</i> = 30)
Generalized itching	69	90
Urticaria	69	61
Swelling of lips and tongue	51	52
Pulmonary	21	42
Gastrointestinal	24	35
Shock	8	13

include fishermen, fish and prawn workers, seafood processing workers, cannery workers, restaurant cooks, and a number of other workers associated with other aspects of the seafood industry and seafood products (Cartier et al., 1984, 1986; Desjardins et al., 1995; Lemiere et al., 1996; O’Neil and Lehrer, 1996). One of the best-investigated seafood industries for occupational allergic reactions is the snow crab industry. Studies performed by Cartier and Malo (Cartier et al., 1984) demonstrated that workers in this

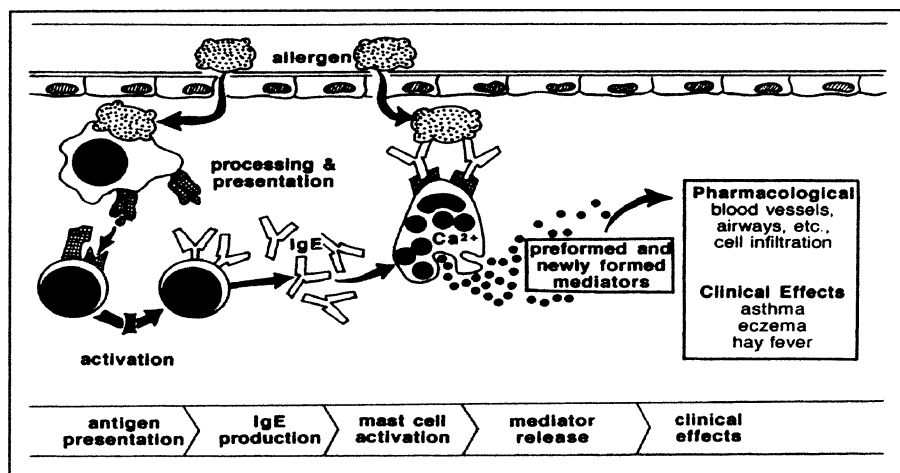


Figure 1. Induction and provocation of an allergic reaction.

industry were exposed to occupational allergens through direct contact with seafood products as well as inhalation of bits of seafood or water droplets generated during seafood processing. In their initial report, of the 303 crab workers investigated, over one third reported asthma, 18% reported rhinitis or conjunctivitis, and almost 24% reported some sort of skin rash (Cartier et al., 1984).

In both consumers and seafood workers, these reactions are due to the development of IgE antibodies (Figure 1). IgE is the class of serum immunoglobulins that are responsible for type I immediate hypersensitivity reactions. When a protein or immunologically active protein fragment crosses the mucosal membrane surfaces, and is processed by macrophages and presented to T and B lymphocytes, stimulation of the production of protein-specific IgE antibodies can result. These antibodies have the ability to bind to the surfaces of mast cells and basophils; the subsequent interaction or exposure to allergen can cross-link two or more cell-membrane-bound IgE antibodies, leading to release of preformed mediators as well as synthesis and release of newly formed mediators. These mediators are potent, pharmacologically active molecules that cause significant change in cell migration, mobility, and dilation of blood vessels as well as smooth muscle contraction. All of these events result in the symptoms observed during an allergic reaction (Sampson and Metcalfe, 1991; Lehrer et al., 1999).

SEAFOOD ALLERGENS

During the last 20 years, a number of major allergens have been identified in allergenic seafood (Table 5). The best-studied finned fish species is codfish *Gadus callarias* whose major allergen is Gad c 1. This parvalbumin protein was

one of the first allergens to be characterized and sequenced, over 20 years ago by Elsayed and associates (Elsayed et al., 1972; Elsayed and Benuich, 1975). Additional major allergenic seafoods such as other species of fish and crustacea (particularly shrimp) have been investigated in recent times. Three different research teams have identified the major shrimp allergen as the muscle protein tropomyosin (Daul et al., 1993a, 1993b, 1994; Shanti et al., 1993; Leung et al., 1994). Subsequent studies have indicated that tropomyosin is also the major allergen present in other crustaceans such as lobster (Leung et al., 1998a; Mykles et al., 1998) and crab (Leung et al., 1998b), and also in insects such as the cockroach (Santos et al., 1999; Asturias et al., 1999), and arachnids such as house dust mites (Aki et al., 1995). Allergenic tropomyosins have also been identified in mollusks (Leung et al., 1996) such as squid (Miyazawa et al., 1996), oyster (Ishikawa et al., 1998a), snail (Ishikawa et al., 1998b), and mussels, clams, and scallops (Chu et al., 2000).

Our studies of shrimp allergens began many years ago with investigations by Daul and coworkers, who demonstrated that the major shrimp allergen was a 36-kDa protein to which more than 80% of shrimp-allergic subjects react. Immunoblotting analysis of two different species of shrimp, *Penaeus aztecus* and *Penaeus setiferus* (newly classified as *Farfantepenaeus aztecus* and *Litopenaeus setiferus*, respectively), in cooked or uncooked form, confirmed that the major allergen was the 36-kDa protein. This protein was present not only in extracts of shrimp meat but also in the liquid in which shrimp had been boiled. The homology of known amino acid sequences as well as the deduced amino acid sequence from complementary DNA indicated this molecule was indeed the shrimp muscle protein tropomyosin (Pen a 1). Tropomyosin (Pen a 1, Pen i 1, Met e 1) is

Table 4. Seafood Agents that Induce Occupational Reactions in Seafood Workers

Agent	Exposure	Disease	Reference(s)
Prawns	Processing	Asthma, dermatitis	Gaddie et al., 1980; McSharry et al., 1987
Crab	Processing	Asthma	CDC, 1982
King Crab	Processing	Asthma	Orford et al., 1985
Snow Crab	Processing	Asthma	Cartier et al., 1986 & 1984
Oyster	Culturing	Asthma	Nakashima, 1969; Wada et al. 1967
Shrimpmeal	Aquaculture	Asthma	Carino et al., 1985
Fishmeal	Factory workers	Asthma	Droszcz et al., 1981
Mother of Pearl	Button factory workers	Asthma	Tas, 1972
Seasquirt	Oyster shuckers	Asthma	Jyo et al., 1974/1975
Seashells	Shell grinders	Asthma	Kim et al., 1982
Fish, mustard	Fish factory workers	Dermatitis, contact urticaria	Kavli et al., 1987
Shrimp	Fishmarket workers	Allergic contact urticaria	Fisher, 1988
Shrimp, fish	Caterers	Contact urticaria, dermatitis	Cronin, 1987; Maibach, 1986
Oysters	Oyster shuckers	Dermatitis	Yamura et al., 1966
Mussels	Mussel processors	Dermatitis	Zhoutyi et al., 1973
Fish and shellfish	Food handlers	Contact dermatitis	Hjorth et al.,1976
Fish, rubber boots	Fishermen	Skin diseases	Rasheia-Kotel’ba, Chojecka et al., 1979; Sabatini, 1969
Fish	Fish workers	Contact urticaria	Beck et al., 1983
Fish, lobster	Cooks	Contact urticaria, asthma	Goransson, 1981; Patel et al., 1992
Bryozoa	Trawlermen	Dermatitis, eczema	Newhouse, 1966
Fishnets	Fishnet repairers	Dermatitis	Rasheia-Kotel’ba, Khoetska et al., 1979
Shrimp, scallop	Restaurant workers	Asthma	Goetz et al., 2000
Clam, scallop	Food company workers	Asthma	Desjardins et al., 1995
Cuttlefish		Contact dermatitis	Burches et al., 1992
Lobster, shrimp	Processors	Asthma	Lemiere et al., 1996
Fish	Fish processing	Asthma	Rodriguez et al., 1997
Gammarus shrimp	Fish factory worker	Asthma	Baur et al., 2000

the only major shrimp allergen of several species investigated, *Penaeus aztecus*, *Penaeus indicus* (now called *Fenneropenaeus indicus*), and *Metapenaeus ensis*, respectively (Daul et al., 1993b; Shanti et al., 1993; Leung et al., 1994), and accounts for more than 84% of the total IgE antibody reactive to shrimp from shrimp-allergic subjects (Morgan et al., 1990).

TROPOMYOSIN STRUCTURE IN RELATION TO IgE ANTIBODY REACTIVITY

Tropomyosin is a major muscle protein present in all living animals. These molecules appear to be highly conserved based on the substantial amino acid sequence identity of tropomyosins from unrelated species. For example, shrimp tropomyosin amino acid sequence shares nearly 60% sequence identity with tropomyosins of vertebrates, mole-

cules that are not allergenic. Tropomyosin has a rather unique structure in that it is composed of two polypeptide chains, each in α -helix formation coiled around one another in the coiled-coil formation (Smillie, 1979).

Although the structure of tropomyosin is well known, little information about its B cell epitopes (IgE-binding epitopes) and no information about the T cell epitopes are available. Shanti et al. (1993) first identified two IgE-binding regions of Pen a 1 by analyzing IgE antibody reactivities of shrimp-allergic patients and control subjects to nine tryptic fragments of Pen i 1. Two peptides, nine (Pen i 1 [50–66]) and six (Pen i 1 [153–161]) amino acid residues long, were identified as important IgE-binding epitopes, since they inhibited more than 50% of specific IgE reactivity to Pen i 1. The region Pen i 1 (153–161) partially overlaps with one of the three IgE-binding regions of Pen a 1 (136–148, 157–179, and 262–282), previously identified using a recombinant peptide library of Pen a 1 (Reese et al.,

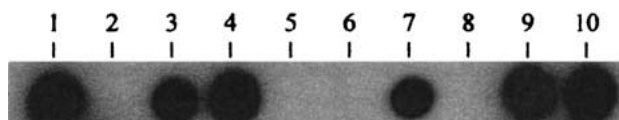


Figure 2. Amino acid substitutions in epitope 5b. Pen a 1 epitope 5b: KYKSTIDE (266–273) (spots 1 and 10); spot 2 (F substituted for S at position 269), spot 3 (C substituted for T at position 271), spot 4 (D substituted for E at position 273), spot 5 (F substituted for S, and C substituted for T), spot 6 (F substituted for S, and D substituted for E), spot 7 (C substituted for T, and D substituted for E), spot 8 (F substituted for S, C substituted for T, and D substituted for E), and spot 9 (S substituted for T at position 271).

1997). In this study Reese et al. screened a unidirectional expression cDNA library from *P. aztecus* tail muscle with a Pen a 1-specific monoclonal antibody, identifying four positive clones. All four recombinant proteins were recognized in immunoblot analysis by serum IgE from shrimp-allergic individuals. One of the clones (P7) was purified and used to construct a peptide library (Novatope epitope mapping system). The library was screened with individual serum from shrimp-allergic subjects, identifying four IgE-reactive peptides (regions E2 [167–179], E3 [136–148], E4 [262–282], and E6 [157–169]) that were between 13 and 21 amino acids long (Reese et al., 1997). All the peptides were located in the second half of the molecule including the Carboxyl terminus.

In order to identify areas of the tropomyosin molecule that contain important IgE-binding regions, the following strategy was used by Ayuso et al. (1999, 2002). Thirty-six overlapping peptides (15 amino acids long, offset 6 amino acids) were synthesized that spanned the entire sequence of Pen a 1 shrimp tropomyosin allergen. Testing the sera from 18 shrimp-allergic subjects, reactive to Pen a 1, these synthetic peptides were screened for IgE antibody reactivity. The prevalence and intensity of IgE antibody binding identified five major IgE-reactive, IgE-binding regions in shrimp tropomyosin (Pen a 1; Ayuso et al., 1999, Ayuso et al., 2002). Region 1 was composed of peptide 8; region 2, peptides 15 and 16; region 3, peptide 23; region 4, peptide 32; and region 5, peptides 42 to 46. The size of several IgE-binding regions and composition of two or more overlapping peptides suggest that they may contain more than one allergenic epitope. Further efforts were directed at identification of the IgE-binding epitopes within each region. The IgE-binding epitope was defined as the smallest sequence of amino acids that yields maximal IgE binding. In order to achieve this, region-reactive sera from shrimp-allergic individuals was used. The

same system of overlapping synthetic peptides was employed using shorter peptide lengths with varying sizes in order to identify the minimal peptide that would bind IgE. All peptides were synthesized and tested as described previously.

The IgE-binding epitopes were identified using sera from three to eight shrimp-allergic individuals who recognized that particular region. In this way we were able to identify eight IgE-binding epitopes contained within the five IgE-binding regions of Pen a 1: epitope 1 in region 1, epitope 2 in region 2, epitopes 3a and 3b in region 3, epitope 4 in region 4, and epitopes 5a, 5b, and 5c in region 5 (Reese et al., 1999a, 1999b). In some cases the same epitope was recognized with maximal intensity by all subjects showing IgE reactivity to a certain region. In other cases a common sequence was identified (common core), recognized by all subjects tested to that particular region but the length of the whole epitope recognized varied with the individual. Interestingly, the minimum IgE-binding peptide sites varied in length from 8 to 15 amino acids long, depending on the region and the subject studied. In general, for each subject, the epitope with maximal IgE-binding capacity was preceded and followed by shorter or longer peptides with weaker reactivity.

AMINO ACIDS ESSENTIAL TO IgE BINDING

Eight IgE-binding epitopes were defined in five major IgE-binding regions of shrimp tropomyosin; further studies determined which amino acids were essential for IgE antibody binding. Study of shrimp allergen epitopes has an advantage in that natural counterparts (nonallergenic tropomyosins) that exist in other foods to which we are exposed can provide templates for amino acid substitution. To study the effects of amino acid substitutions on the IgE-binding capacity of an allergenic epitope, the amino acid sequences within the individually identified Pen a 1 IgE-binding epitopes were compared with corresponding sequences of allergenic invertebrate and nonallergenic vertebrate tropomyosins. IgE antibody reactivities of shrimp-allergic subjects to the identified Pen a 1 epitopes were compared with reactivities to mutated peptides using amino acids from homologous vertebrate and invertebrate sequences.

Allergenic tropomyosins include those of crustaceans (Hom a 1 of American lobster *Homarus americanus*), insects (Per a 7 of the American cockroach *Periplaneta americana*), and arachnids (Der p 10 of the house dust mite *Dermatophagoides pteronyssinus*). Nonallergenic vertebrate tropo-

Table 5. Major Allergens Identified in Allergenic Seafood^a

Origin	Name	MW, kDa	Sequence data	Reference
<i>Gadus callarias</i> (cod)	Gad c 1; allergen M	12.3	C	Elsayed and Bennich, 1975
<i>Salmo salar</i> (Atlantic salmon)	Sal s 1; parvalbumin	11.9 ^b	C	Lindstrom et al., 1996, Van Do et al., 1999
<i>Metapenaeus ensis</i> (shrimp)	Met e 1; tropomyosin	32.8	C	Leung et al., 1994
<i>Homarus americanus</i> (American lobster)	Hom a 1, tropomyosin	32.8	C	Mykles et al., 1998
<i>Panulirus stimpsoni</i> (spiny lobster)	Pan s 1, tropomyosin	32.8	C	Leung et al., 1998a
<i>Charybdis feriatus</i> (crab)	Cha f 1, tropomyosin	32.8	C	Leung et al., 1998b
<i>Penaeus aztecus</i> (shrimp) (<i>Farfante penaeus aztecus</i>)	Pen a 1; tropomyosin	36	P	Daul et al., 1994
<i>Penaeus indicus</i> (shrimp) (<i>Fenneropenaeus indicus</i>)	Pen i 1; tropomyosin	34	C	Shanti et al., 1993
<i>Todarodes pacificus</i> (squid)	Tod p 1; tropomyosin	38	P	Miyazawa et al., 1996
<i>Haliotis midae</i> (abalone)	Hal m 1	49	—	Lopata et al., 1997

^aFrom the WHO/IUIS standardization committee (<ftp://biobase.dk/pub/who-iuis/allergen>).

^bMolecular weight MW of Sal s 1 calculated based on its GenBank sequence.

myosins include salmon fast muscle tropomyosin (TM), chicken α -TM, chicken β -TM, mammalian α -TM, and mammalian β -TM from skeletal muscle. All possible substitution combinations (up to five per peptide) resulting from the differences between Pen a 1 epitopes and the homologous vertebrate and invertebrate sequences were tested using overlapping peptides (Genosys). Since the minimal size of the IgE-binding epitopes varied for each subject, the amino acid substitutions for those epitopes were analyzed individually.

The results of amino acid substitutions demonstrated several important patterns. First, single amino acid substitutions may abolish, decrease, increase, or not modify IgE binding to the modified peptide. Most of the critical amino acid substitutions that led to a significant decrease or total loss of IgE binding to the identified epitopes were in the center of the epitope rather than in peripheral parts of the peptide. In addition, IgE reactivity to the modified peptides decreased with the number of substitutions included, demonstrating that, in general, peptides with two or more amino acid substitutions show little or no IgE recognition from shrimp-allergic sera. A majority of the critical amino acid substitutions that abolished IgE binding to the epitope were nonconservative substitutions (substitution of 1 amino acid for another belonging to a different amino acid group) rather than conservative ones (within the same amino acid group). For example, in region 5, of the amino acid substitutions that reduced or abolished IgE binding to an epitope, 64% were nonconservative and 36% were

conservative. Similarly, for every amino acid substitution that had no effect or increased IgE binding to that epitope, 69% were conservative and 31% were nonconservative.

Interestingly, increased IgE binding of some modified peptides with substitutions at certain positions of a particular epitope was observed. Certain individual amino acid substitutions appeared to be more significant than others in decreasing or abolishing the IgE binding to a particular epitope in most subjects: in epitope 1, there are six individual substitutions, each of which abolishes IgE binding to the modified Pen a 1 peptide; in epitope 2, one substitution; in epitopes 3a and b, three substitutions; in epitope 3a, two substitutions; in epitope 5b, one substitution, and in epitope 5c, five substitutions. For example, substitution of arginine (R) for aspartic acid (D) in position 255 of epitope 5a (251–259), or serine (S) for phenylalanine (F) in position 269 of epitope 5b (266–273), abolished IgE binding to the mutated peptide in all three or four subjects tested, respectively. An example is shown in Figure 2. These studies show that single amino acid substitutions can significantly decrease or abolish IgE antibody binding to the mutated peptides.

IMPLICATIONS OF SHRIMP EPILOPE SUBSTITUTIONS

Immunotherapy of IgE-mediated diseases has been used since the beginning of this century (Noon and Cantab,

1911; Freeman, 1911), and is currently indicated for patients with allergic rhinitis and some with asthma (Durham et al., 1999; Walker et al., 2001). However, injection immunotherapy to desensitize food-allergic subjects has not been recommended, due to the anaphylactic potential associated with the high IgE-binding capacity of purified food allergens. Thus avoidance of the offending foods is the only therapy recommended for food-allergic subjects. Unfortunately, some food allergens such as peanuts, soy, and egg are used as supplements in many different foods, which makes accidental consumption almost inevitable. Therefore, novel strategies are being developed to reduce the risk of allergic reactions, such as immunologic manipulation of the food-allergic subject (mucosal vaccines, peptide immunotherapy, oral immunotherapy, immunotherapy with recombinant allergens), or food alteration through genetic engineering for the production of hypoallergenic foods that sensitized subjects could tolerate.

The identification of the major IgE-binding epitopes of Pen a 1 (shrimp tropomyosin), and the amino acids within these epitopes that are critical for IgE binding, are important for different reasons. First, they provide the information to alter tropomyosin cDNA nucleotide sequence for the production of a hypoallergenic tropomyosin variant. Because this method is very specific, one can render allergenic proteins nonallergenic without substantially altering the protein's function, which is essential in the case of functional proteins such as tropomyosin. Modified tropomyosin would be expressed from one shrimp generation to the next, creating a new hypoallergenic variant.

Second, the production of recombinant tropomyosin becomes possible, which provides a promising therapeutic option for food-allergic individuals (Marshall and Davis, 1997). To be effective in clinical practice, therapeutic recombinant allergens need to have reduced IgE-binding capacity (and therefore low risk of IgE-mediated adverse reactions), while retaining their T cell epitopes intact to allow for the induction of tolerance (Ferreira et al., 1998). Such recombinant allergens with low IgE-binding capacity could allow higher doses of allergen per injection, decreasing the amount of interleukin 4 produced by CD4 T cells, and shifting the Th2 allergic response to a Th1 inflammatory response (Secrist et al., 1995). In our study we have identified at least one amino acid substitution in each one of the major Pen a 1 epitopes that abolish IgE-binding of different tropomyosin epitopes. Although tropomyosin T cell epitopes have not been fully identified, these findings still represent a

promising therapeutic tool for the treatment of shrimp-allergic subjects.

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