



Penaeus monodon tropomyosin induces CD4 T-cell proliferation in shrimp-allergic patients

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ABSTRACT

Shellfish allergy affects approximately 2% of the population and can cause immediate hypersensitivity reactions such as urticaria, swelling, difficulty breathing, and, in some cases, anaphylaxis. Tropomyosin is the major shrimp allergen and binds IgE in two-thirds of patients. A total of 38 shrimp-allergic patients and 20 negative control subjects were recruited and evaluated on the basis of history, skin prick testing, specific immunoglobulin E (IgE) levels, and peripheral blood mononuclear cell proliferation in response to shrimp tropomyosin or shrimp tropomyosin-derived peptides. Of the classically allergic patients by history, 59% tested positive for serum shrimp IgE antibodies. Of patients with shrimp-specific IgE in sera, 70% also had significant IgE levels specific for shrimp tropomyosin. Peripheral blood mononuclear cells from classically shrimp-allergic patients proliferated in a dose-dependent manner in response to tropomyosin. In addition, a T-cell line derived from a shrimp-allergic patient proliferated specifically in response to tropomyosin-derived peptides. These studies suggest a strategy for immunotherapy using a tropomyosin-derived T-cell epitope vaccination.

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1. Introduction

Allergy to foods is an increasing problem in the American population; shellfish allergy affects approximately 2% of the adult population in the United States, and may affect up to 3.1% of adults 41 to 60 years of age [1]. Reactions include classical immediate hypersensitivity, such as urticaria, swelling, difficulty breathing, and, in some cases, anaphylaxis [1].

Tropomyosin, a 34-kDa muscle protein, is a major shrimp allergen and binds immunoglobulin E (IgE) in at least two-thirds of allergic patients [2]. However; other shrimp antigens, such as shrimp tRNA, Pen m2, and sarcoplasmic calcium-binding protein, have also been reported [3–5]. Tropomyosin is a pan-allergen, with amino acid sequence and allergenic properties similar to those in shellfish such as lobster and crab, as well as other arthropods, such as cockroach and mites [6–8]. Cross-reactivity to arthropod tropomyosin and shellfish occurs frequently in shrimp-allergic patients; tropomyosin is a major allergen from many invertebrate species [6,9,10].

Immunotherapy is a treatment in which the allergen is administered in increasing doses, either sublingually or subcutaneously, in an effort to tolerize T cells [11–14]. Immunotherapy has been a

successful method for desensitizing patients with allergies, including sensitivities to cat, pollen, and bee venom [15–19]. Sublingual immunotherapy is generally considered safer than injections; however, in the case of severe food allergies, systemic reactions may still occur. Immunotherapy with short peptides, or T-cell epitopes, has also been proposed as a safer method of desensitization, as peptides are too short to cross-link IgE on mast cells and can cause life-threatening anaphylaxis.

T-cell epitope immunotherapy has been reported to desensitize patient's allergic reaction to the bee venom phospholipase A2 protein, Cry j 1, a Japanese Cedar pollinosis antigen, and Fel d 1, the protein involved in cat allergies [20–23]. The mechanism, as in traditional therapy, is thought to involve induction of IL-10-secreting Treg cells that downregulate the activity of Th2 T lymphocytes [24–29]. In addition, IL-10 is known to provoke B cell switching to IgG4, which may act as a blocking antibody to IgE [13], [11] [17]. IL-10 can also decrease eosinophil and neutrophil recruitment.

In this study, we describe the clinical characteristics and result of skin testing and IgE responses to different shellfish and seafood antigens in a cohort of shrimp-allergic patients. We also demonstrate that *in vitro* stimulation with native tropomyosin protein and tropomyosin-derived peptides produce dose-dependent T-cell proliferative responses in shrimp-allergic patients. Our results are important for future identification of candidate T-cell epitopes that can be used for immunotherapy of patients with shrimp allergy.

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2. Subjects and methods

2.1. Study population

Patients and controls were recruited based on history of allergy to shrimp that included the time between exposure and reaction. Reactions included urticaria, erythema, swelling, vomiting, diarrhea, rhinitis, coughing, dyspnea, and loss of consciousness. After written consent, individuals were skin prick tested with commercially available allergen extracts of shrimp (family Penaeidae), crab (*Callinectes* spp.), lobster (*Homarus americanus*), mollusks (*Mercuraria* spp., *Ostrea/Crassostrea*, *Placopecten magellanicus*), and fish species (*Salmo salar*, *Salmo salar*, *Oncorhynchus mykiss*, *Thunnus* sp.), as well as saline solution as a negative control and histamine as a positive control (Greer, Lenoir, NC). Allergen solutions were applied on the volar skin of the arm, and wheal and flare reactions were read after 15 to 20 minutes. Mean wheal and flare diameters were calculated from the largest and perpendicular diameters. The Institutional Review Board at the University of Utah approved this protocol.

2.2. Total and specific IgE levels

Sera obtained from patients and controls were tested for total and specific IgE levels to shellfish-derived extracts using ImmunoCap assays according to the manufacturer's recommendations (Phadia, Uppsala, Sweden). Tropomyosin-specific immunocaps were made using purified biotinylated *Penaeus monodon* tropomyosin conjugated to streptavidin immunocaps at 0.25 mg/ml according to the manufacturer's recommendations.

2.3. T-cell proliferation assays

T-cell proliferation assays were performed in peripheral blood mononuclear cells (PBMC) from patients and controls added to 96-well, round-bottomed plates in culture media containing different concentrations of purified, native tropomyosin and tropomyosin-derived peptides as indicated. Cultures were incubated for 6 days and were pulsed overnight with tritiated thymidine. Radioactive label incorporation during T-cell proliferation was measured by liquid scintillation spectroscopy. T-cell proliferation was also measured using flow-cytometric analysis of cells stained with carboxy-fluorescein succinimidyl ester (CFSE) [30]. PBMC were stained with 0.5 μ mol/l CFSE and cultured for 7 days in the presence or absence of tropomyosin (1 μ g/ml). The dose of tropomyosin was determined by previous dose–response curves ranging from 0 to 10 μ g/ml. At the end of the 7-day culture, cells were stained with fluorescent-labeled monoclonal antibodies against CD4⁺, CD45RA⁺, and CD45RO⁺, and cells were analyzed by flow cytometry using FlowJo software (Tree Star, Ashland, OR).

The distributions of proliferation results were tested by the D'Agostino and Pearson normality test. According to the results of normality test, variables were analyzed by the paired *t* test or Mann–Whitney test. Levels of significance are reported as two-tailed *p* values. A *p* value <0.05 was considered significant. These data analyses were performed with the aid of Prim 5 software (GraphPad, La Jolla, CA).

2.4. Antigens and peptides

Tropomyosin was purified from frozen Black Tiger (*Penaeus monodon*) shrimp (Aquarius Fish Market, Salt Lake City, UT). Black Tiger shrimp is one of the major cultivated shrimp species worldwide. Briefly, 500 g of frozen Black Tiger shrimp (Aquarius Fish Co., Salt Lake City, UT) was blended in a food processor and extracted in 20 mmol/l KCl, 1 mmol/l KHCO₃, and 0.1 mmol/l dithiothreitol for 10 minutes, for a total of five times. Extract was discarded and the shrimp homogenate was filtered through cheese cloth and extracted three times in 1 L of 95% ethanol for 10 minutes and ex-

tracted three times in 1 L of diethyl ether for 10 minutes. The extract was discarded, and the shrimp homogenate was dried 2 hours or overnight in the hood and extracted overnight in high-salt buffer. Proteins were ammonium sulfate precipitated from shrimp extract and solubilized, and tropomyosin was precipitated by reducing the pH. The precipitate was solubilized and dialyzed into phosphate-buffered saline (PBS) [31]. Purity was validated by 12% Bis-Tris sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1) (Life Technologies, Grand Island, NY). The most abundant purified protein was identified as shrimp tropomyosin by mass spectrometry; peptides were analyzed using a nano-LC/MS/MS system comprising a nano-LC pump (2D-ultra, Eksigent) and an LTQ-FT mass spectrometer (ThermoElectron Corporation, San Jose, CA) and a MASCOT full database search (Matrix Science, Boston, MA).

Synthetic peptides were obtained from Mimotopes Ltd. (Melbourne, Australia) in sets consisting of 15-mers overlapping by 12 amino acids spanning the *P. monodon* tropomyosin molecule. Based on the scale of synthesis (~4 mg/peptide), each peptide was solubilized in 30 μ l of dimethyl sulfoxide and brought up to 10 mmol/l in PBS.

3. Results

Clinical history, skin prick testing, shrimp-specific IgE levels, and T-cell proliferation in response to shrimp tropomyosin were characterized in 58 subjects, including 38 shrimp-allergic patients and 20 negative control subjects with no history of shrimp allergy. History included questions regarding immediate type I reactions such as hives, swelling, runny nose, and tightness of the chest, as well as abdominal symptoms such as diarrhea and vomiting. Reactivity to allergens measured by skin prick testing included shrimp, as well as other crustaceans, mollusks, and fish (Table 1). Classical immediate type I allergic reactions were reported by 29 of 38 patients (76%), whereas abdominal symptoms, including vomiting and diarrhea, were reported in nine (24%) of the allergic subjects. Two of 20 negative control subjects (10%) were positive by skin prick testing.

The distribution of total and specific IgE levels in the cohort of 29 patients with classical immediate type I allergic manifestations and 20 negative controls are shown in Fig. 2 and Table 1. Total IgE levels were significantly increased in the 29 patients (median, 109 IU/ml; range, 8.16–1,074) compared with the 20 controls (median, 30.95 IU/ml; range, 3.16–153; *p* = 0.0024) (Fig. 2A). Eleven of 29 patients (38%) had total IgE levels above the normal reference range (0–180 IU/ml), whereas none of the controls had elevated total IgE levels. IgE levels to shrimp extracts were also significantly increased in the 29 patients (median, 0.84 kU/l; range, 0.35–50.80) compared with

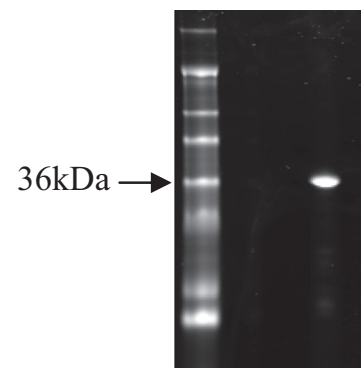


Fig. 1. SDS PAGE of purified *P. monodon* tropomyosin. Lane 1: Marker (SeeBlue Plus2, Life Technologies, Grand Island, NY). Lane 2: 1 μ g purified protein. The arrow points to the 36kDa marker. The predicted band size of shrimp tropomyosin is 34 kDa.

Table 1

Total and specific IgE and reactivity to fish and seafood allergens in study patients

Patient	Skin prick	Total IgE	Shrimp	Tropo	Lobster	Crab	Cod	Tuna	Salmon	Mackerel	Trout	Clam	Oyster	Scallop
2	+	944	8.15	2.69	7.93	7.12	<0.35	<0.35	<0.35	<0.35	<0.35	1.77	2.83	2.57
19	+	68.0	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
20	+	236	28.1	12.1	24.6	20.8	<0.35	0.76	<0.35	<0.35	<0.35	4.03	2.40	5.36
23	+	109	4.33	1.07	1.79	1.79	<0.35	<0.35	<0.35	<0.35	<0.35	0.48	0.45	0.37
24	+	417	1.94	<0.35	0.48	0.5	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
25	+	40.8	0.84	0.43	0.98	0.95	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
26	+	86.8	<0.35		<0.35	2.55	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
27	+	22.3	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
29	+	122	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
31	+	278	40.5	13.5	26.5	22	<0.35	<0.35	<0.35	<0.35	<0.35	6.49	5.60	4.62
32	+	111	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
33	+	1074	30.6	12.0	24.6	27.0	1.42	3.57	2.74	2.21	0.98	8.02	2.94	5.71
35	+	40.3	0.47	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
37	+	172	11.4	0.47	<0.35	2.49	<0.35	<0.35	<0.35	1.64	<0.35	<0.35	<0.35	<0.35
39	+	8.16	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
40	+	12.8	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
42	+	85.2	1.31	0.40	<0.35	1.06	<0.35	0.38	<0.35	0.79	<0.35	<0.35	<0.35	<0.35
43	+	408	20.3	0.48	0.41	0.62	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
44	+	52.6	1.9		0.67	0.73	<0.35	<0.35	0.39	<0.35	<0.35	<0.35	<0.35	<0.35
45	+	12.5	1.32	0.48	<0.35	0.83	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
46	+	406	50.8	26.9	47.0	44.9	<0.35	0.86	0.36	<0.35	<0.35	11.3	5.55	4.77
47	+	213	0.65	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
4	–	21.8	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
8	–	68.9	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
10	–	872	1.49	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
15	–	79.0	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
30	–	216	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
38	–	95.3	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
41	–	227	1.65	0.55	1.27	1.27	<0.35	<0.35	0.45	0.47	0.42	<0.35	<0.35	0.39

Tropo, tropomyosin.

Bold type refers to elevated levels of specific IgE.

the 20 controls (median, 0.1 kU/l; range, 0.1–0.21; $p < 0.0001$) (Fig. 2B). Of 29 patients, 17 (59%) had shrimp-specific IgE levels above the normal reference range (> 35 kU/l), whereas none of the control had elevated shrimp-specific IgE levels.

Patients with increased IgE levels to shrimp extracts were tested for IgE levels to shrimp tropomyosin and extracts from other shellfish species. We observed that 12 of 17 patients (70%) with elevated IgE levels to shrimp extracts in serum also had elevated IgE levels in response to to shrimp tropomyosin. Elevated IgE levels in shrimp tropomyosin-reactive patients were also observed to extracts from crab (88%), lobster (65%), scallop (41%), clam (35%), oyster (35%), tuna (24%), salmon (24%), trout (12%), and cod (6%) (Table 1).

3.1. T-cell proliferation in response to native tropomyosin stimulation *in vitro*

T-cell proliferation in response to tropomyosin challenge was measured in PBMC from shrimp-allergic patients and controls *in vitro*. Remarkably, the proliferative response of T cells from shrimp-allergic patients to tropomyosin was significantly increased compared with the response of T cells from controls (Fig. 3A). Proliferation was also measured by flow cytometry by incorporation of CFSE, which is sequestered inside the cytoplasm of viable cells and diluted during cell proliferation. Using this method to analyze the proliferation of subsets of CD4⁺ T cells, including naive T cells (CD4⁺, CD45RA⁺), and memory T cells (CD4⁺, CD45RO⁺), we found a significant increase in the number of proliferating CD4⁺ T cells of the memory subset (CD4⁺, CD45RO⁺) after tropomyosin stimulation (Fig. 3B), whereas the increase in the number of proliferating CD4⁺ T cells of the naive subset (CD4⁺, CD45RA⁺) was minimal (data not shown).

3.2. Specific T-cell responses to tropomyosin-derived peptides

Overlapping 15-mer peptides derived from the *P. monodon* tropomyosin protein sequence were tested for their ability to stimulate T-cell proliferation in selected shrimp-allergic patients. Several

peptides from tropomyosin protein elicited strong dose dependent proliferative responses in shrimp allergic patients compared to HLA-DR matched controls, suggesting that multiple peptides from the tropomyosin protein can be recognized by T cells in a variety of HLA backgrounds (Fig. 4). Moreover, a tropomyosin-specific T-cell line established from one patient by sorting and expanding proliferating T cells exhibited dose dependent proliferation in response to tropomyosin, but not to a control peptide from hepatitis C virus (Fig. 5).

4. Discussion

A cohort of shrimp-allergic patients has been identified and characterized on the basis of history as well as skin prick testing, analysis of serum IgE levels, and lymphocyte proliferation in response to shrimp tropomyosin. Most shrimp-allergic subjects exhibited a classical immediate-type hypersensitivity characterized by positive skin prick tests, a history of swelling, urticaria, and respiratory difficulties after shrimp ingestion. Of patients with a history of immediate shrimp sensitivity, 76% had a positive skin prick test. The existence of a group of patients with a history indicative of shrimp allergy but with negative skin tests suggests that these patients have lost sensitivity, possibly as part of the natural history of this disease. Furthermore, 59% of subjects with immediate type reactions according to history were positive for shrimp specific IgE, indicating that approximately one-third of subjects have non-IgE-mediated allergy or that enough time had elapsed since shrimp ingestion that IgE antibodies had disappeared from the circulation. Of shrimp IgE-positive subjects, 70% were also positive for shrimp tropomyosin-specific IgE, suggesting that 30% of allergic patients may be allergic to other shrimp proteins, such as tRNA, Pen m2, and sarcoplasmic calcium-binding protein, similar to findings from other studies [3–5]. Allergic reactions to crustaceans and mollusks are often cross-reactive, which is explained by the highly conserved amino acid sequences of tropomyosins among these species [6–8]. Consistent with sequence conservation

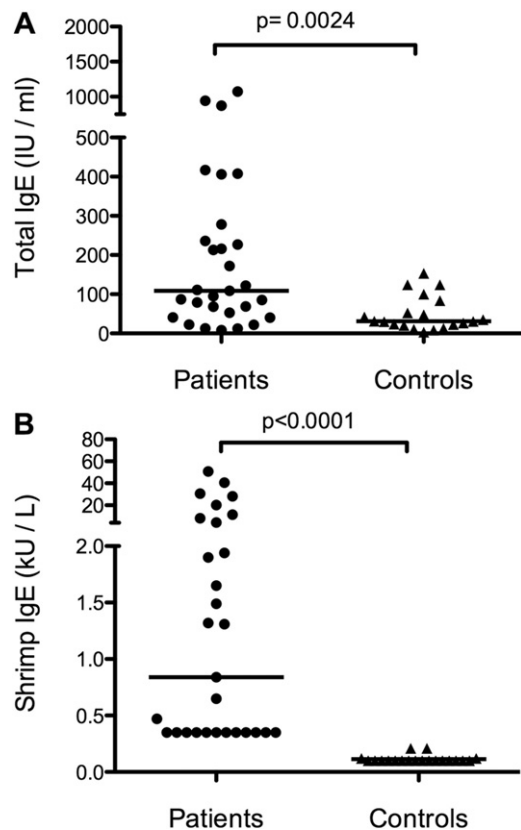


Fig. 2. Distribution of total IgE (A) and specific IgE levels to shrimp extracts (B) in the cohort of 29 patients with classical immediate type I allergic manifestations and 20 negative controls. Horizontal lines represent median values. Negative values differ slightly due to a slight change in the manufacturer's nonreactive baseline values.

of tropomyosin, we observed elevated IgE levels to extracts from crab, lobster, scallop, clam, oyster, tuna, salmon, trout, and cod that may be cross-reactive with tropomyosin.

Allergic responses are initially mediated through the presentation of allergens in the context of HLA class II molecules to naive CD4 T cells that mature into CD4 memory T cells. CD4 memory T cells stimulate B-cell differentiation into plasma cells that secrete allergen-specific IgE antibodies. Upon the re-exposure to the allergen, memory CD4 T cells will re-stimulate specific IgE antibody production. In this study we demonstrate that patients with clinical history of shrimp allergy and increased shrimp-specific IgE levels have dose-dependent proliferation of memory CD4⁺ T cells after *in vitro* stimulation with *P. monodon* tropomyosin. To our knowledge, this is the first study showing that tropomyosin, the most important shrimp-derived allergen, elicits memory T-cell proliferation in the context of HLA class II-restricted antigen presentation.

At present, the primary therapy for shellfish allergy is the avoidance of shellfish food. Although this is effective, it requires careful attention to the diet, and even then, allergenic food can be disguised in mixtures of other foods and can produce severe reactions. Desensitization shots can be dangerous because of the risk of anaphylaxis. Therefore, a vaccine using short peptides is likely to be much safer and to avoid the risk of anaphylaxis. Recent published reports on T-cell epitope peptide vaccines for the treatment of allergic diseases are most promising.

T-cell epitope immunotherapy has been reported to desensitize patient's allergic reaction to the bee venom phospholipase A2 protein, Cry j 1, a Japanese Cedar pollinosis antigen and Fel d 1, the protein involved in cat allergies [20–23]. The mechanism, as in traditional therapy, is thought to involve induction of IL-10-secreting Treg cells that downregulate the activity of Th2 T lymphocytes

[24–29]. In addition, IL-10 is known to provoke B-cell switching to IgG4, which may act as a blocking antibody to IgE [13], [11] [17]. IL-10 can also decrease eosinophil and neutrophil recruitment. In the case of cat allergy, treatment of 16 sensitive patients with T-cell peptides from the major cat allergen, Fel d 1, had profound effects; cutaneous late-phase reactions, stimulated by Fel d 1, diminished strikingly, and lymphocyte proliferation and PBMC production of IL-4, IL-13 and interferon- γ , stimulated by whole cat dander, decreased significantly. In addition, IL-10 production by PBMC increased significantly and the ability of the T cell peptide treated patients to tolerate exposure to cats significantly improved [23]. In patients with honey bee venom allergy, treatment with T-cell peptides from phospholipase A2 specifically reduced proliferation of PBMC to phospholipase A2 and production of TH1 and TH2 cyto-

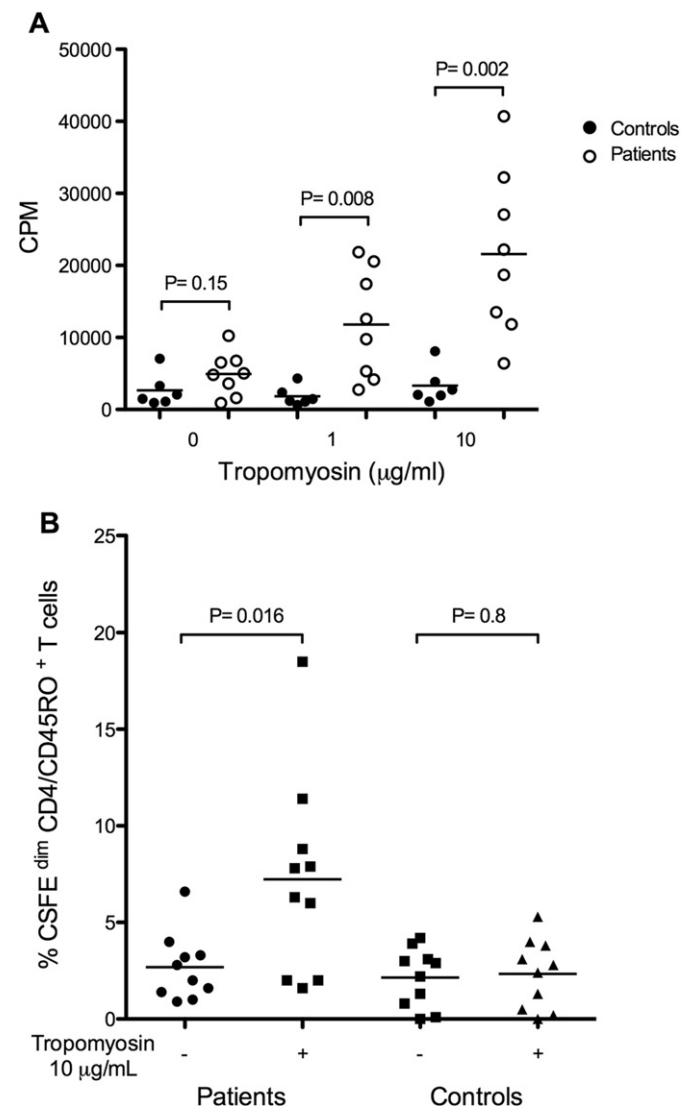


Fig. 3. (A) T-cell proliferation in response to native tropomyosin stimulation *in vitro*. *P. monodon* tropomyosin was added to peripheral blood mononuclear cells (PBMCs) from patients ($n = 8$) and controls ($n = 6$) at concentrations indicated on the x axis. Counts per minute (CPM) of incorporated tritiated thymidine from triplicate 6-day cultures are shown for each individual. Patients represented are 2, 20, 31, 33, 35, 37, 41, 46, and 6 negative controls [5,6,14,16,18,21]. (B) Percentage of proliferating memory T cells (carboxyfluorescein succinimidyl ester (CFSE)^{dim} CD4/CD45RO⁺) in patients ($n = 10$) and controls ($n = 10$) in the presence or absence of 10 μ g/ml of tropomyosin from *P. monodon*. Horizontal lines represent median values. Patients represented are 2, 20, 27, 31, 33, 35, 37, 43, 45, 46 and negative controls 5, 8, and 16 ARUP controls*) *Patients were positive for skin prick testing and history. Controls were individuals tested negative for skin prick, and normal IgE total and IgE shrimp.

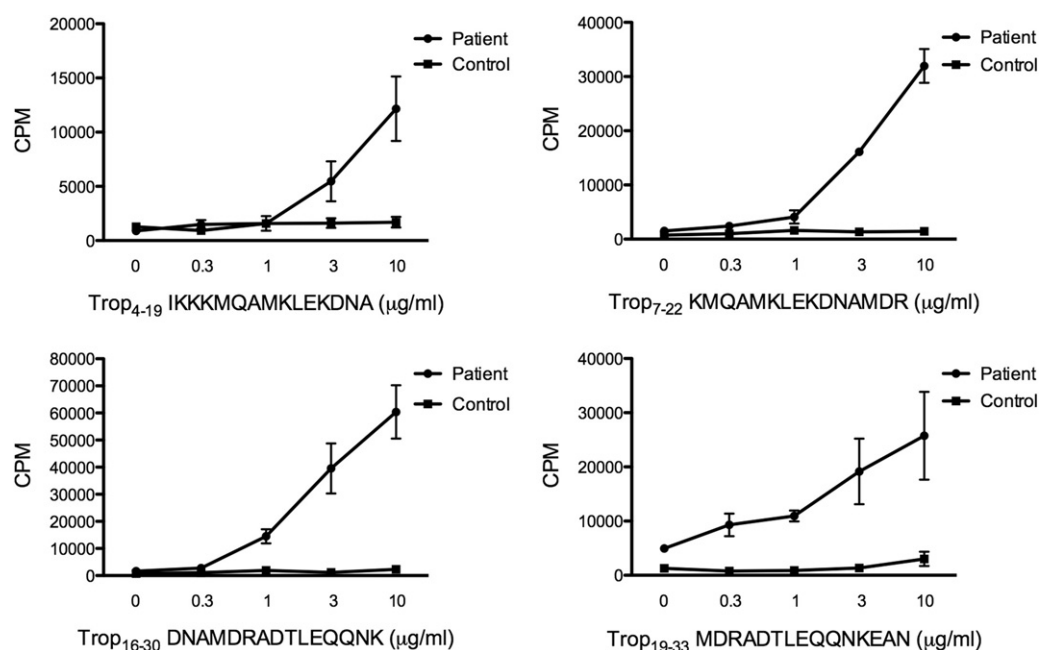


Fig. 4. Examples of T-cell proliferative responses to stimulation with tropomyosin-derived peptides. *P. monodon* tropomyosin-derived peptides were added to PBMCs from patients and controls at concentrations indicated on the x axis. Averages and standard error of CPM of incorporated tritiated thymidine from triplicate 6-day cultures are shown for each experiment.

kines [20]. After 2 months of peptide immunotherapy, three of the five patients tolerated sting challenge without anaphylaxis, although two patients developed erythema and urticaria with mild angioedema. Treatment of 12 additional bee venom sensitive patients with T-cell peptides from phospholipase A2 showed reductions in cutaneous late-phase reactions, PBMC proliferation, production of IL-13 and interferon- γ , and increases in IL-10 production [32]. These results encourage the belief that administration of peptides corresponding to T-cell epitopes may prove effective for allergen desensitization.

In this study, overlapping 15-mer peptides from the *P. monodon* tropomyosin protein sequence were used to stimulate T-cell proliferation in selected shrimp-allergic patients. The finding that multiple peptides from tropomyosin protein elicit strong dose-dependent proliferative responses in patients compared to HLA-DR matched controls, strongly suggests that combination of peptides

from tropomyosin protein can be used to modulate T-cell responses of shrimp allergic patients in a variety of HLA backgrounds. Further studies are necessary to precisely map and validate tropomyosin-derived T-cell epitopes and to determine the patterns of presentation by different HLA class II molecules.

Our results suggest an approach to exploit T-cell epitope vaccination as a desensitization therapy for allergy to shellfish. Knowledge of T-cell epitopes of shrimp tropomyosin will set the stage of future trials of T-cell epitope peptide therapy in allergy to shellfish. This is important because shrimp allergy can be disabling and potentially fatal. Severely affected individuals who might be afraid to visit a seafood restaurant because of cross-contamination would at least be able to accompany their friends and perhaps partake in small amounts of shrimp. At the very least, severe consequences such as anaphylactic shock could be eliminated with this approach.

Acknowledgments

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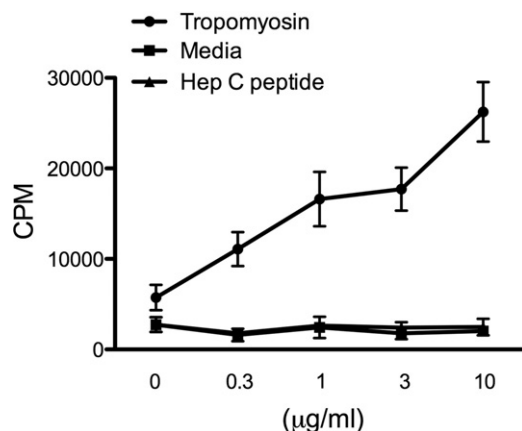


Fig. 5. Proliferative response of a tropomyosin-specific T-cell line. *P. monodon* tropomyosin and hepatitis C peptide (VIKGGRLHIFCHPKKKCD) were added to the T-cell line at concentrations indicated on the x axis. Averages and standard error of CPM of incorporated tritiated thymidine from triplicate 6-day cultures are shown for each experiment.

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