

Greater epitope recognition of shrimp allergens by children than adults suggests that shrimp sensitization decreases with age

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Background: Shellfish allergy is a long-lasting disorder typically affecting adults. Despite its high prevalence, there is limited information about allergenic shrimp proteins and the epitopes implicated in such allergic reactions.

Objective: We sought to identify the IgE-binding epitopes of the 4 shrimp allergens and to characterize epitope recognition profiles of children and adults with shrimp allergy.

Methods: Fifty-three subjects, 34 children and 19 adults, were selected with immediate allergic reactions to shrimp, increased shrimp-specific serum IgE levels, and positive immunoblot binding to shrimp. Study subjects and 7 nonatopic control subjects were tested by means of peptide microarray for IgE binding with synthetic overlapping peptides spanning the sequences of *Litopenaeus vannamei* shrimp tropomyosin, arginine kinase (AK), myosin light chain (MLC), and sarcoplasmic calcium-binding protein (SCP). The Wilcoxon test was used to determine significant differences in z scores between patients and control subjects.

Results: The median shrimp IgE level was 4-fold higher in children than in adults (47 vs 12.5 kU_A/L). The frequency of allergen recognition was higher in children (tropomyosin, 81% [94% for children and 61% for adults]; MLC, 57% [70% for children and 31% for adults]; AK, 51% [67% for children and

21% for adults]; and SCP, 45% [59% for children and 21% for adults]), whereas control subjects showed negligible binding.

Seven IgE-binding regions were identified in tropomyosin by means of peptide microarray, confirming previously identified shrimp epitopes. In addition, 3 new epitopes were identified in tropomyosin (epitopes 1, 3, and 5b-c), 5 epitopes were identified in MLC, 3 epitopes were identified in SCP, and 7 epitopes were identified in AK. Interestingly, frequency of individual epitope recognition, as well as intensity of IgE binding, was significantly greater in children than in adults for all 4 proteins.

Conclusions: Children with shrimp allergy have greater shrimp-specific IgE antibody levels and show more intense binding to shrimp peptides and greater epitope diversity than adults. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Allergen, Crustacea, shellfish, shrimp, tropomyosin, arginine kinase, sarcoplasmic calcium-binding protein, myosin light chain, IgE epitope

Shellfish allergy, which includes crustaceans and mollusks, is among the most prevalent of the food allergies worldwide in both children and adults. It is also a long-lasting disorder, typically persisting into adulthood. However, only recently, efforts to characterize the proteins responsible for the induction of allergic food reactions to shrimp have been made. Shrimp is the crustacean most frequently implicated in allergic reactions among all shellfish. Shrimp allergens identified to date include tropomyosin,^{1,2} arginine kinase (AK),^{3,4} myosin light chain (MLC),⁵ and sarcoplasmic calcium-binding protein (SCP).⁶

In the last several years, the IgE-binding epitopes of different food allergens have been identified. Although food allergens contain both conformational and sequential epitopes, the importance of sequential epitopes in food allergy has been established. Sensitization to sequential epitopes has been associated with persistent milk and egg allergy. In addition, subjects with persistent milk or egg allergy are unlikely to tolerate baked foods, in which conformational epitopes are destroyed by intensive heating and only sequential IgE epitopes remain.⁷⁻⁹ Furthermore, for some foods, such as peanut, milk, and egg, a positive relationship has been found between recognition of certain sequential IgE-binding epitopes and the allergic reactivity of patients. Subjects with persistent allergy or histories of more severe reactions to peanut,¹⁰⁻¹³ milk,¹⁴⁻¹⁷ or egg^{18,19} were found to recognize larger numbers of IgE sequential epitopes. What is more interesting, sensitization to particular sequential IgE epitopes in older patients with persistent food allergy might differ from those recognized by

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Disclosure of potential conflict of interest: R. Ayuso receives research support from the Food Allergy Initiative. S. Sánchez-García has a short-term fellowship with the World Allergy Organization. J. Sastre receives lecture fees from Novartis, GlaxoSmithKline, Stallergenes, and UCB; has consultant arrangements with Phadia, Schering-Plough, and GlaxoSmithKline; and receives research support from Phadia, GlaxoSmithKline, and ALK-Abelló. H. A. Sampson has consultant arrangements with and is a 4% shareholder in Allertin Pharmaceuticals; receives research support from the Food Allergy Initiative and the National Institutes of Allergy and Infectious Diseases/National Institutes of Health; is a consultant/scientific advisor for the Food Allergy Initiative; has served as President of the American Academy of Allergy, Asthma & Immunology; and is 45% owner of Herbal Springs, LLC. The rest of the authors have declared that they have no conflict of interest.

Received for publication October 23, 2009; revised March 12, 2010; accepted for publication March 16, 2010.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2010.03.010

Abbreviations used

AK: Arginine kinase
 FDR: false discovery rate
 HSA: humans serum albumin
 MLC: myosin light chain
 PBS-T: PBS containing 0.05% Tween 20
 SCP: sarcoplasmic calcium-binding protein

children who are likely to outgrow their allergy.²⁰⁻²² Therefore IgE epitope mapping might become a useful tool to identify which subject are likely to outgrow their food allergies. Mapping IgE epitopes is also important for the development of mutated hypoallergenic proteins for immunotherapy of patients with shellfish allergy. In this article we describe the identification of IgE-binding epitopes of the 4 allergens identified to date in shrimp. As a second objective, we compared the IgE-binding profiles of adult and pediatric patients with shrimp allergy in an effort to characterize the epitope profiles associated with persistent shrimp allergy.

METHODS**Patient selection**

Sera were obtained from 53 patients with immediate allergic reactions after the ingestion of shrimp and increased serum shrimp-specific IgE levels. Sera from 7 nonatopic healthy volunteers with no history of food allergy were used as controls. All subjects' sera were tested by means of IgE immunoblotting to boiled and raw shrimp extract. Subjects with no recognition of shrimp proteins determined by means of immunoblot analysis were excluded from the study. Sera were collected from Spain (Hospital Infantil Universitario Niño Jesús, Madrid, Spain; Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria, Spain) and New York (Mount Sinai Medical Center, New York, NY). This study was approved by the Institutional Review Board of the Mount Sinai Medical Center.

Synthetic peptides and printing conditions

A library of overlapping peptides consisting of 15 amino acids with an offset of 3 corresponding to the primary sequence of the 4 allergens found in pacific white shrimp *Litopenaeus vannamei* Lit v 1 (tropomyosin), Lit v 2 (AK), Lit v 3 (MLC), and Lit v 4 (SCP) was commercially synthesized by using the PepStar technique (JPT Peptide Technologies, Berlin, Germany). Peptides were diluted (1:2) with Protein Printing Buffer (ArrayIt Corp, Sunnyvale, Calif) and printed in 2 sets of duplicates onto SuperEpoxy glass slides (ArrayIt Corp) by using the NanoPrint Microarrayer 60 (ArrayIt Corp). In addition, printed arrays included Protein Printing Buffer alone as negative control spots for background normalization, and fluorochrome-labeled BSA as a reference for grid alignment (positional grid controls). After printing, the slides were dried overnight at room temperature before use.

Immunolabeling

Immunolabeling was performed as previously, described with some modifications.²³ In brief, the slides were blocked with 400 μ L of 1% human serum albumin (HSA) in PBS containing 0.05% Tween 20 (PBS-T) for 60 minutes at room temperature, followed by incubation with 250 μ L of the patient's serum diluted 1:5 in PBS-T/HSA for 24 hours at 4°C. Slides were then washed with PBS-T and incubated for 24 hours at 4°C with a mix of 3 monoclonal biotinylated anti-human IgE antibodies, one from Invitrogen (Carlsbad, Calif) diluted 1:250, one from BD Biosciences Pharmingen (San Jose, Calif) diluted 1:250, and one as a gift from Phadia (Uppsala, Sweden) that was biotinylated in our laboratory and diluted 1:1,000 in PBS-T/HSA. Slides were then incubated for 3 hours at 31°C with Anti-Biotin-Dendrimer Oyster 550

(350; Genisphere, Hatfield, Pa) in Dendrimer Buffer (Genisphere) at 0.6 μ g/mL with the addition of 0.02 μ g/mL salmon sperm DNA (Invitrogen), followed by washing with PBS-T, 15 mmol/L Tris, 0.1 $\times$ PBS, and 0.05 $\times$ PBS. Slides were centrifuge dried and scanned with a ScanArray Gx (PerkinElmer, Waltham, Mass). Images were saved in TIF format.

Data analysis

The fluorescence signal of each spot was digitized with the program ScanArray Express (PerkinElmer), exported as comma-delimited text files, and transformed to z scores, as previously described.²³ An index z value of each peptide element was generated from the median of z scores of the 4 replicates. If an individual peptide sample had a z score exceeding 3 or 2, it was considered positive, indicating that the signal was above the background with a P value of less than .003 or .05, respectively.

Based on a previous study,¹² IgE-binding epitopes are defined as including 2 or more overlapping peptides, and therefore subsequent analysis was carried out, adjusting each z score to the median of itself and the 2 flanking peptides (weighted average z score), by using the following formula $z = 0.25 * z_{-1} + 0.5 * Z_0 + 0.25 * Z_{+1}$. An IgE-binding epitope for the population analyzed contained at least 2 contiguous peptides with a weighted average z score of greater than 3 for tropomyosin, MLC, and SCP or greater than 2 for AK. A lower z score was considered for AK for epitope identification because of the lower intensity of binding of the AK peptides. The Wilcoxon test and q values (calculated by the R project)²⁴ were used to determine statistical differences between weighted average z scores of atopic and negative control subjects and also between children and adults. A P value of less than .05 and a false discovery rate (FDR) of 0.05 were selected as significant thresholds. FDR and q values were used to adjust for the multiple comparisons in the peptide microarray study.

Inhibition assay with individual peptides

Inhibition experiments were done with selected peptides as inhibitors to demonstrate that the fluorescent signal of the peptide spots was specific (IgE mediated). One or 2 peptides were selected per protein, which were consistently recognized by the subject's serum with marked binding intensity, and included within an IgE-binding epitope. A pool was prepared from 5 subjects (3 children and 2 adults), each with shrimp-specific IgE levels of greater than 100 kU_A/L, who had shown recognition of those particular peptides. A serum/peptide inhibition mixture was prepared by mixing 25 μ L of a positive serum pool diluted 1:10 in PBS-T/HSA with 2.5 μ L of each peptide (1 mmol/L stock) at a final concentration of 16 mmol/L. The mix was incubated on a shaking platform for 2 hours at room temperature. Serum without peptide was processed in parallel as a control. After incubation, the serum mixture was applied onto the slides, and the immunolabeling was performed as described previously.

RESULTS**Patient selection**

A total of 53 subjects were recruited: 34 children (age, 3-18 years; mean age, 9 years) and 19 (36%) adults (age, 19-70 years; mean age, 31.4 years). Shrimp-specific IgE levels ranged from 1 to 100 kU_A/L (UniCAP; Phadia, Uppsala, Sweden), with a median shrimp-specific IgE level of 47 kU_A/L for children and 12.5 kU_A/L for adults. All subjects had a positive immunoblot result to raw or boiled shrimp extract (data not shown). Among 34 children, 64% had IgE levels of greater than 20 kU_A/L, 41% had IgE levels of greater than 50 kU_A/L, and 29% had IgE levels of greater than 100 kU_A/L, whereas among the 19 adults, 31% had IgE levels of greater than 20 kU_A/L, 26% had IgE levels of greater than 50 kU_A/L, and 15% had IgE levels of greater than 100 kU_A/L. The clinical characteristics of subjects included in this study are shown in Table E1 (available in this article's Online Repository at www.jacionline.org).

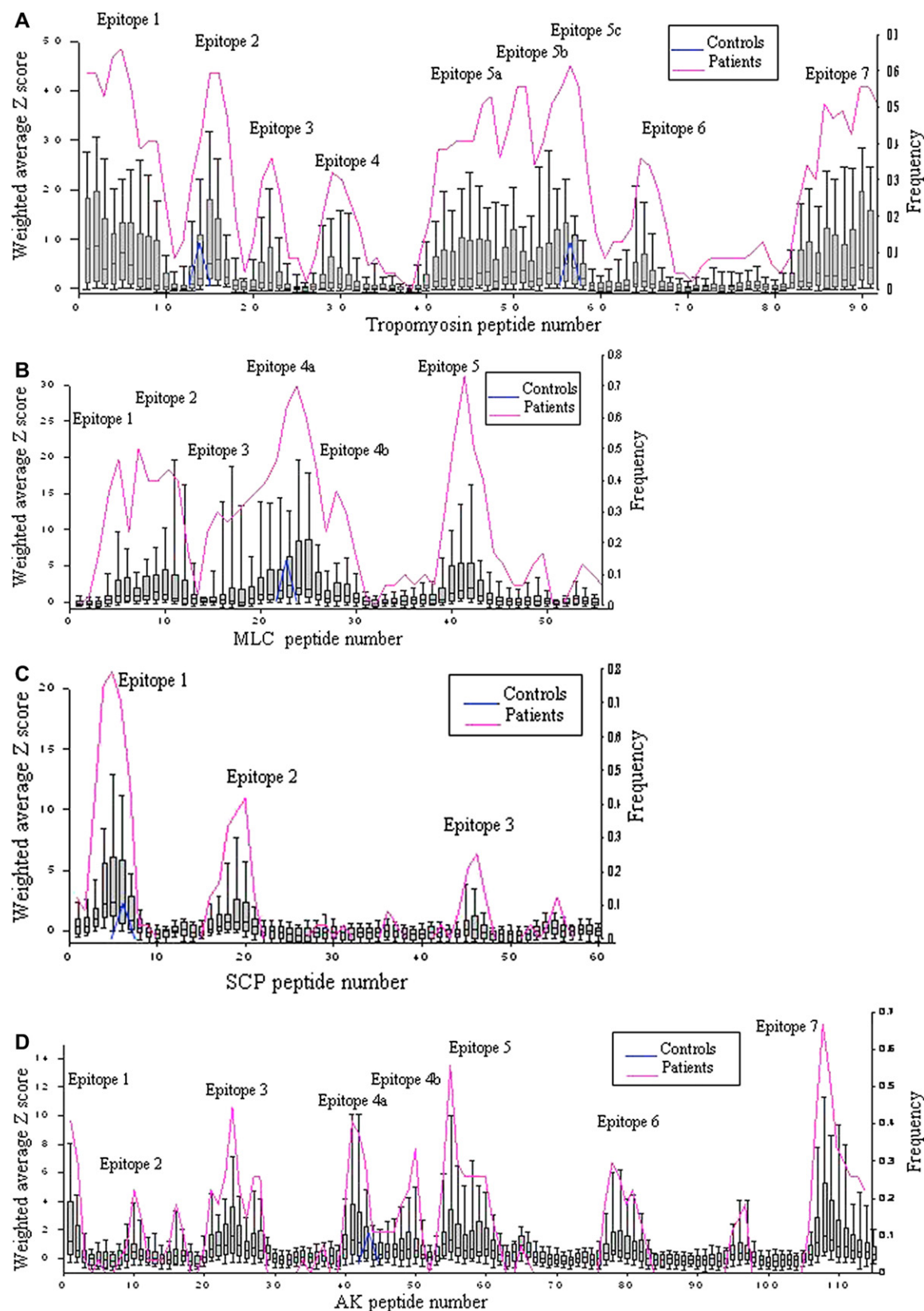


FIG 1. IgE epitope identification in tropomyosin, MLC, SCP, and AK. Peptides are represented on the x-axis. The fluorescent intensity of each peptide (weighted average z scores) is represented on the left y-axis. Boxes represent the median and 25% to 75% quartiles, and whiskers represent the 10% to 90% distribution. Right y-axis, Frequency of recognition of each peptide. **A**, Tropomyosin. **B**, MLC. **C**, SCP. **D**, AK.

TABLE I. IgE epitopes identified in tropomyosin, MLC, SCP, and AK with their frequency of recognition

Tropomyosin									
	Epitope 1	Epitope 2	Epitope 3	Epitope 4	Epitope 5a	Epitope 5b	Epitope 5c	Epitope 6	Epitope 7
Frequency	65%	60%	35%	30%	50%	55%	60%	35%	50%
AA	1-36	37-63	61-81	82-105	115-150	142-162	157-183	190-210	246-284
MLC									
	Epitope 1	Epitope 2	Epitope 3	Epitope 4a	Epitope 4b	Epitope 5			
Frequency	45%	50%	30%	70%	35%	75%			
AA	13-30	22-48	49-66	58-90	79-99	118-141			
SCP									
	Epitope 1				Epitope 2		Epitope 3		
Frequency	80%				40%		25%		
AA	10-36				49-72		130-147		
AK									
	Epitope 1	Epitope 2	Epitope 3	Epitope 4a	Epitope 4b	Epitope 5	Epitope 6	Epitope 7	
Frequency	45%	25%	50%	40%	30%	60%	30%	70%	
AA	1-18	25-42	64-96	121-141	142-159	160-192	232-255	319-342	

AA, Amino acid number.

Epitope identification of shrimp allergens: Tropomyosin (Lit v 1), AK (Lit v 2), MLC (Lit v 3), and SCP (Lit v 4)

The frequency of IgE binding to the different proteins, as determined by means of peptide microarray, was as follows: tropomyosin, 43 (81%) of 53; MLC, 30 (57%) of 53; AK, 27 (51%) of 53; and SCP, 24 (45%) of 53. The intensity of IgE binding as represented by the weighted average *z* score was higher for tropomyosin epitopes and MLC (Fig 1, A and B) and lower for SCP and AK (Fig 1C, and D).

An IgE-binding epitope was defined as containing at least 2 contiguous peptides. In some cases more than 4 or 5 contiguous peptides were bound by patients' IgE. Because they are likely to contain more than 1 epitope, those peptides are said to form an IgE-binding region; for example, tropomyosin epitopes 5a, 5b, and 5c form an IgE-binding region. The frequency of recognition of each peptide represents the number of patients with an average weighted *z* score of greater than 3 for that particular peptide divided by the total number of patients who recognize that allergen (Fig 1). Peptides are considered to represent an epitope when the average weighted *z* score is greater than 3 ($P < .003$) or 2 for AK ($P < .05$) and are recognized by at least 20% of the subjects who recognize that particular protein.

The epitopes identified in all 4 proteins and their frequencies of recognition (among the subjects who recognize that particular allergen) are represented in Table I. Regarding tropomyosin, 7 epitopes were identified: 4 of them confirm previously identified shrimp epitopes, and 3 are newly identified. New tropomyosin epitopes included epitopes 1, 3, 5b, and 5c (Fig 1, A). MLC has 5 epitopes (Fig 1, B), SCP has 3 epitopes (Fig 1, C), and AK has 7 epitopes (Fig 1, D). Peptides with statistically significant differences (*q* value $< .03$) between patients and control subjects, as determined by using the Wilcoxon test and FDR adjustment, are shown in Table E2 (available in this article's Online Repository at www.jacionline.org).

Five of 19 adults and 2 of 34 children showed no significant binding to any shrimp peptides. These subjects had shrimp-

specific serum IgE levels of 1 to 5 kU_A/L. One child and 1 adult recognized a single 14-kd protein by means of immunoblotting. The second child recognized tropomyosin with low intensity. Two of the remaining adults recognized multiple bands by means of immunoblotting (20-40-80 kd) but not tropomyosin, MLC, or SCP, and we did not see this pattern of recognition in children. The other 2 adults recognized a single undetermined protein of 80 to 100 kd, and one of them reported an anaphylactic reaction to shrimp (data not shown). Some children showed binding to proteins in this range but not in an isolated form. Among patients with anaphylaxis, patient 36 recognized tropomyosin and SCP (and AK with low intensity), patient 42 recognized tropomyosin and MLC, and patient 16 recognized none of the proteins tested by means of microarray (a nonidentified IgE-binding protein of 80-100 kd was recognized by means of immunoblotting).

Children with shrimp allergy show more intense and frequent allergenic epitope recognition than adults

Important differences were seen in the patterns of allergen and IgE epitope recognition by children and adults. The frequency of recognition of each protein in children was 94% for tropomyosin, 70% for MLC, 67% for AK, and 59% for SCP, whereas in adults tropomyosin was detected in 61%, MLC in 31%, AK in 21%, and SCP in 21%, making tropomyosin the most frequently recognized allergen in both patient groups. Compared with adults, children with shrimp allergy on average recognized more shrimp proteins and individual peptides than adults. In addition, the intensity of IgE binding was also greater in children (Fig 2). Peptides with statistically significant differences between children and adults, as determined by using the Wilcoxon test, are shown in Table E2 (available in this article's Online Repository).

Inhibition assay

Inhibition experiments were done with selected peptides as inhibitors to demonstrate that the fluorescent signal from the

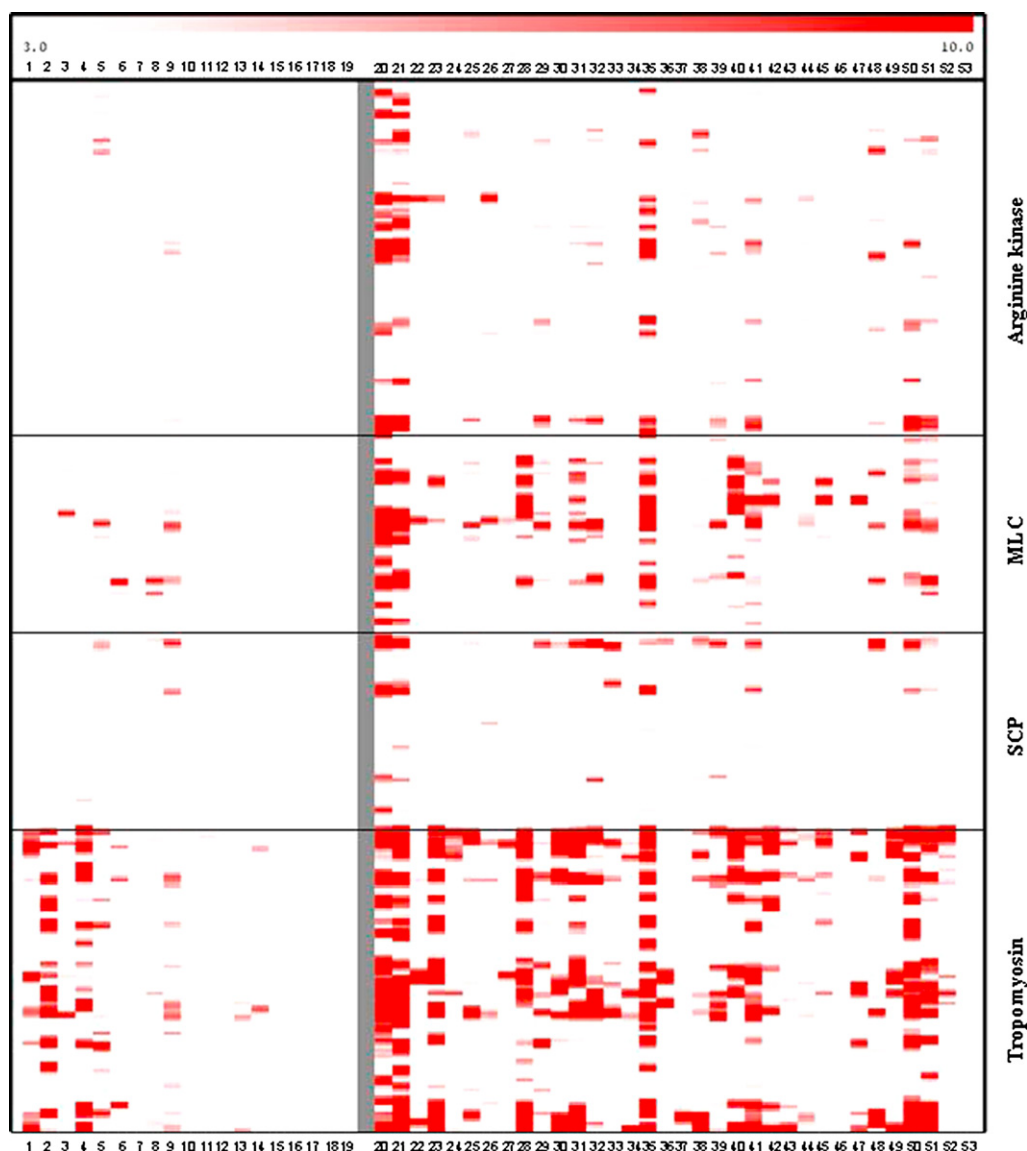


FIG 2. Comparison of shrimp epitope recognition by children and adults. All peptides from the 4 allergenic proteins are represented in rows. The recognition of peptides by each of the 53 subjects (1-19, adults; 20-53, children) are shown in the columns. The intensity of IgE binding is represented by weighted average z scores in a grading scale ranging from less than 3 (white) to 10 or greater (red).

peptide spots was specific (IgE mediated). For each protein, 1 or 2 peptides were selected that were bound by the subject's IgE antibodies and were included within an IgE-binding epitope. A serum pool was preincubated with individual peptides for 1 hour, followed by incubation of the serum/peptide mixture with the printed microarray slide. As expected, peptides inhibited IgE binding to the identical sequence on the printed slide, as well as peptides that were immediately adjacent. This is expected because each peptide overlaps with adjacent peptides by 12 of 15 amino acids. Interestingly, in the case of tropomyosin, peptide 15 (epitope 2: VHNLQKRMQQLNDL) inhibited not only the same sequence included in peptides 13 to 15 (epitope 2: VHNLQKRMQQLNDLDQVQES) but also peptides 2 to 3 (epitope 1: IKKKMQAMKLEKDNAMDR), peptides 29 to 31 (epitope 4: VAALNRRIQLEEDLERSEER), and peptides 84 to 85 (epitope 7: GKLQKEVDRLDEDELVNEK). Sequence

analysis of all these areas showed a common motif, "LEX₁X₂L" or "LEX₁X₂N," where X₁ can be D, E, N, or K and X₂ can be D or E (Fig 3).

DISCUSSION

Shellfish allergy is a long-lasting disorder typically persisting throughout life, with shrimp causing the majority of the allergic reactions.²⁵ Shellfish are responsible for the majority of emergency department visits in adults for food allergy and a significant number in children 6 years of age and older.²⁶⁻²⁸ Depending on local habits, shellfish might also be a significant cause of allergic reactions in 1- to 5 year-old children.²⁹

Despite its high prevalence, there is limited information about allergenic shrimp proteins, their IgE-binding epitopes, or whether there is a different allergen recognition pattern for children

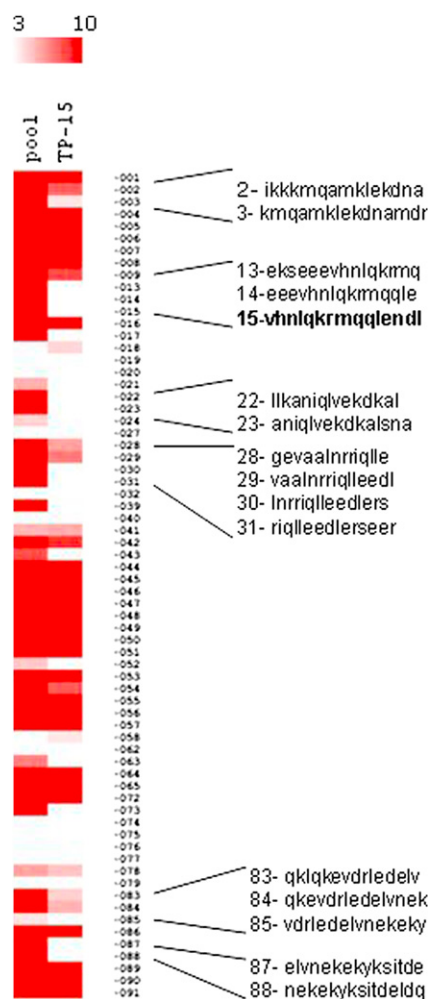


FIG 3. Inhibition of IgE binding to tropomyosin peptides. Tropomyosin peptides (1-91) are shown in rows. *Left column*, Peptides recognized by a pool of 5 subjects. *Right column*, Reactivity to the same peptides after pre-inhibition with tropomyosin peptide 15 (TP-15). The intensity of recognition is graded from white (z score < 3) to red (z score ≥ 10). In most peptides showing inhibition of IgE binding, the sequence LEX₁X₂L or LEX₁X₂N is seen.

compared with adults. In addition to the previously reported shrimp allergens tropomyosin and AK, our group has recently identified 2 new shrimp allergens that appear to be particularly important in the pediatric population.^{5,6} In this study we analyzed the frequency of recognition of the different shrimp allergens identified to date and mapped their sequential IgE-binding epitopes, comparing a pediatric and adult population to characterize epitope profiles that might be associated with persistent shrimp allergy.

Although most of the data regarding IgE epitope mapping of food proteins have been generated with the SPOTs membranes system (Genosys Biotechnologies, Woodlands, Tex),^{15,16,22,30-32} peptide microarray-based immunoassays for epitope mapping are proving more economical.^{11,23,33} In the case of shrimp tropomyosin, the IgE epitopes identified by means of microarray analysis confirm the results obtained previously with the SPOTs membrane system.³⁴ Furthermore, because a larger number of subjects can be tested, we have been able to identify a number of additional major IgE epitopes that had been missed with the

SPOTs system. All these data taken together confirm the utility of microarray analyses for IgE epitope mapping of food allergens for larger populations.

Children and adults with shrimp allergy show significant differences in allergen recognition in terms of frequency, diversity, and intensity (higher weighted average z scores) of epitope binding. Frequency of allergen recognition in children was about twice that of adults for most of the proteins (tropomyosin, 94% vs 61%; MLC, 70% vs 31%; AK, 67% vs 21%; and SCP, 59% vs 21%, respectively). Very intense recognition of tropomyosin and MLC peptides and less intense binding for SCP and AK suggest more sequential epitopes in tropomyosin and MLC and possibly more conformational epitopes in SCP and AK, given the intense binding determined by means of immunoblot analysis of recombinant SCP.⁶ Although tropomyosin peptides are frequently recognized by both children and adults, the other 3 proteins are recognized predominantly by children. This suggests tropomyosin as the allergen most associated with persistence of shrimp allergy into adulthood. However, because arthropod tropomyosins are highly cross-reactive³⁵⁻³⁷ and cross-reacting tropomyosins can also be recognized by IgE to cockroach and dust mite from subjects who tolerate shrimp,³⁸ tropomyosin might have limited specificity for the diagnosis of shrimp allergy. In addition, the potential confounding effect of other cross-reactive arthropod allergens, such as AK and MLC, also requires further evaluation; for example, shrimp AK has 82% sequence identity with the cockroach *Periplaneta americana* (Per a 9; accession no. EU429466)³⁹ and 78.7% identity with AK from the dust mite *Dermatophagoides pteronyssinus* (Der p 20; accession no. ACD50950). Also, shrimp MLC has significant sequence identity (52%) with cockroach MLC (Bla g 8; accession no. ABD47458).⁵ This emphasizes the need for component-based diagnosis with recombinant shrimp allergens and subjects with well-characterized shrimp allergy and subjects with well-characterized shrimp tolerance but arthropod allergy.

Interestingly, our study shows statistically significant differences in terms of allergenic epitope recognition between children and adults. Although children show significant epitope diversity in all 4 allergens, adults recognize tropomyosin epitopes preferentially. Similar tropomyosin epitopes are recognized by children and adults, although in general with lower intensity in adults, suggesting that such epitopes might be responsible for the persistence of shrimp allergy. Regarding the other allergens, although most SCP epitopes are not recognized by adults, some epitopes in AK and 1 in MLC show persistent IgE binding in older patients (no statistically significant differences compared with children), suggesting a possible role for those epitopes in persistent shrimp allergy as well. Given the fact that most of our subjects did not undergo a physician-supervised food challenge, we are not able to confirm which epitopes are truly relevant for persistent shrimp allergy because of potential cross-reactivity among tropomyosins and other allergens.

Our work is the first to show that sensitization to shrimp proteins is greater in children and that it appears to decrease with age. Because we have not performed a natural history study (ie, following subjects prospectively since childhood) and we do not know at what time the subjects were sensitized, we cannot be certain whether these differences in allergen and epitope recognition are due to distinct routes of sensitization in children and adults or stronger IgE responses when sensitized in childhood or whether shrimp IgE levels really decrease over time.

One could also argue that differences in allergen and epitope recognition in children and adults might be secondary to patient selection bias, resulting in higher shrimp-specific IgE levels in children than in adults. However, a comparison of peptide recognition by children and adults with similar shrimp-specific IgE levels (Fig 2: adults, 1-3; children, 40-42; all with shrimp-specific IgE levels of 100 kU_A/L) showed greater binding in children.

This has potential implications in the clinical setting. Because shellfish allergy has traditionally been seen as a persistent allergy, once patients are given a diagnosis of shellfish allergy, they typically avoid all shellfish for life. Although we have no doubt that some adults will continue to react to shrimp over time, it is possible that clinical reactivity might decrease as well, given that epitope recognition appears to be less in older subjects. This is supported by studies done with subjects with milk and peanut allergy, in which both persistent and severe food allergies have been found to be associated with increased epitope recognition.^{10,19} Subjects given diagnoses of shellfish allergy in the past who have decreased allergenic epitope recognition might benefit from an oral shrimp challenge to determine whether they have become tolerant over time. In fact, Daul et al⁴⁰ have reported that 30% of their subjects with shrimp allergy had a positive challenge result to shrimp. Also, a more recent report found that up to 50% of subjects with histories of shrimp allergy, positive skin prick test responses, or shrimp-specific serum IgE tolerated an oral challenge to shrimp.⁴¹ In this study most of the subjects passing the oral challenge were adults, whereas children reacted more frequently during the double-blind, placebo-controlled food challenge. In this sense IgE epitope mapping might provide a useful tool to help predict which subjects are likely to outgrow their shrimp allergy.

In conclusion, our work is the first to show that sensitization to shrimp allergens is greater in children and that it appears to decrease with age. Because our selection criteria were the same for children and adults, our results reflect shrimp allergen recognition and epitope diversity as seen in regular clinical practice.

We thank Chuck, Nancy, Sam and Alison Clarvit and the Food Allergy Initiative for supporting this project.

Clinical implications: In contrast to previous beliefs, sensitization to shrimp proteins appears to decrease with age. IgE epitope recognition might provide a useful tool to help predict which subjects are likely to outgrow their shrimp allergy.

REFERENCES

- Daul CB, Slattery M, Reese G, Lehrer SB. Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *Int Arch Allergy Immunol* 1994;105:49-55.
- Leung PS, Chu KH, Chow WK, Ansari A, Bandea CI, Kwan HS, et al. Cloning, expression, and primary structure of *Metapenaeus ensis* tropomyosin, the major heat-stable shrimp allergen. *J Allergy Clin Immunol* 1994;94:882-90.
- Garcia-Orozco KD, Aispuro-Hernandez E, Yepiz-Plascencia G, Calderon-de-la-Barca AM, Sotelo-Mundo RR. Molecular characterization of arginine kinase, an allergen from the shrimp *Litopenaeus vannamei*. *Int Arch Allergy Immunol* 2007;144:23-8.
- Yu CJ, Lin YF, Chiang BL, Chow LP. Proteomics and immunological analysis of a novel shrimp allergen, pen m 2. *J Immunol* 2003;170:445-53.
- Ayuso R, Grishina G, Bardina L, Carrillo T, Blanco C, Ibanez MD, et al. Myosin light chain is a novel shrimp allergen, Lit v 3. *J Allergy Clin Immunol* 2008;122:795-802.
- Ayuso R, Grishina G, Ibanez MD, Blanco C, Carrillo T, Bencharitwong R, Sanchez S, Nowak-Węgrzyn A, Sampson HA, et al. Sarcoplasmic calcium-binding protein is an EF-hand-type protein identified as a new shrimp allergen. *J Allergy Clin Immunol* 2009;124:114-20.
- Lemon-Mule H, Sampson HA, Sicherer SH, Shreffler WG, Noone S, Nowak-Węgrzyn A. Immunologic changes in children with egg allergy ingesting extensively heated egg. *J Allergy Clin Immunol* 2008;122:977-83, e1.
- Nowak-Węgrzyn A, Bloom KA, Sicherer SH, Shreffler WG, Noone S, Wanich N, et al. Tolerance to extensively heated milk in children with cow's milk allergy. *J Allergy Clin Immunol* 2008;122:342-7, e1-2.
- Vila L, Beyer K, Jarvinen KM, Chatchatee P, Bardina L, Sampson HA. Role of conformational and linear epitopes in the achievement of tolerance in cow's milk allergy. *Clin Exp Allergy* 2001;31:1599-606.
- Flinterman AE, Knol EF, Lencer DA, Bardina L, den Hartog Jager CF, Lin J, et al. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. *J Allergy Clin Immunol* 2008;121:737-43, e10.
- Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol* 2004;113:776-82.
- Shreffler WG, Lencer DA, Bardina L, Sampson HA. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. *J Allergy Clin Immunol* 2005;116:893-9.
- Beyer K, Ellman-Grunther L, Jarvinen KM, Wood RA, Hourihane J, Sampson HA. Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. *J Allergy Clin Immunol* 2003;112:202-7.
- Beyer K, Jarvinen KM, Bardina L, Mishoe M, Turjanmaa K, Niggemann B, et al. IgE-binding peptides coupled to a commercial matrix as a diagnostic instrument for persistent cow's milk allergy. *J Allergy Clin Immunol* 2005;116:704-5.
- Chatchatee P, Jarvinen KM, Bardina L, Vila L, Beyer K, Sampson HA. Identification of IgE and IgG binding epitopes on beta- and kappa-casein in cow's milk allergic patients. *Clin Exp Allergy* 2001;31:1256-62.
- Jarvinen KM, Chatchatee P, Bardina L, Beyer K, Sampson HA. IgE and IgG binding epitopes on alpha-lactalbumin and beta-lactoglobulin in cow's milk allergy. *Int Arch Allergy Immunol* 2001;126:111-8.
- Matsumoto N, Okochi M, Matsushima M, Kato R, Takase T, Yoshida Y, et al. Peptide array-based analysis of the specific IgE and IgG4 in cow's milk allergens and its use in allergy evaluation. *Peptides* 2009;30:1840-7.
- Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. *J Immunol* 1997;159:2026-32.
- Jarvinen KM, Beyer K, Vila L, Bardina L, Mishoe M, Sampson HA. Specificity of IgE antibodies to sequential epitopes of hen's egg ovomucoid as a marker for persistence of egg allergy. *Allergy* 2007;62:758-65.
- Cerecedo I, Zamora J, Shreffler WG, Lin J, Bardina L, Dieguez MC, et al. Mapping of the IgE and IgG4 sequential epitopes of milk allergens with a peptide microarray-based immunoassay. *J Allergy Clin Immunol* 2008;122:589-94.
- Jarvinen KM, Beyer K, Vila L, Chatchatee P, Busse PJ, Sampson HA. B-cell epitopes as a screening instrument for persistent cow's milk allergy. *J Allergy Clin Immunol* 2002;110:293-7.
- Chatchatee P, Jarvinen KM, Bardina L, Beyer K, Sampson HA. Identification of IgE- and IgG-binding epitopes on alpha(s1)-casein: Differences in patients with persistent and transient cow's milk allergy. *J Allergy Clin Immunol* 2001;107:379-83.
- Lin J, Bardina L, Shreffler WG, Andreae DA, Ge Y, Wang J, et al. Development of a novel peptide microarray for large-scale epitope mapping of food allergens. *J Allergy Clin Immunol* 2009;124:315-22.
- Storey JD, Taylor JE, Siegmund D. Strong control, conservative point estimation, and simultaneous conservative consistency of false discovery rates: a unified approach. *J R Stat Soc B* 2004;66:187-205.
- Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of seafood allergy in the United States determined by a random telephone survey. *J Allergy Clin Immunol* 2004;114:159-65.
- Clark S, Bock SA, Gaeta TJ, Brenner BE, Cydulka RK, Camargo CA. Multicenter study of emergency department visits for food allergies. *J Allergy Clin Immunol* 2004;113:347-52.
- Ross MP, Ferguson M, Street D, Klontz K, Schroeder T, Luccioli S. Analysis of food-allergic and anaphylactic events in the national electronic injury surveillance system. *J Allergy Clin Immunol* 2008;121:166-71.
- Piromrat K, Chinratanapisit S, Trathong S. Anaphylaxis in an emergency department: a 2-year study in a tertiary-care hospital. *Asian Pac J Allergy Immunol* 2008;26:121-8.
- Liu TH, Lin YR, Yang KC, Chou CC, Chang YJ, Wu HP. First attack of acute urticaria in pediatric emergency department. *Pediatr Neonatol* 2008;49:58-64.
- Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur J Biochem* 1997;245:334-9.
- Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, et al. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 1997;342:244-53.

32. Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999;103:535-42.
33. Lin J, Sampson HA. The role of immunoglobulin E-binding epitopes in the characterization of food allergy. *Curr Opin Allergy Clin Immunol* 2009;9:357-63.
34. Ayuso R, Lehrer SB, Reese G. Identification of continuous, allergenic regions of the major shrimp allergen pen a 1 (tropomyosin). *Int Arch Allergy Immunol* 2002;127:27-37.
35. Martinez A, Martinez J, Palacios R, Panzani R. Importance of tropomyosin in the allergy to household arthropods. Cross-reactivity with other invertebrate extracts. *Allergol Immunopathol (Madr)* 1997;25:118-26.
36. Asturias JA, Gomez-Bayon N, Arilla MC, Martinez A, Palacios R, Sanchez-Gascon F, et al. Molecular characterization of American cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. *J Immunol* 1999;162:4342-8.
37. Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *Int Arch Allergy Immunol* 2002;129:38-48.
38. Ayuso R, Reese G, Arruda LK, Chapman M, Lehrer SB. Identification of the IgE-binding epitopes in allergenic invertebrate tropomyosins per a 7, pen a 1 and Der p 10 with cockroach and shrimp-allergic sera. *J Allergy Clin Immunol* 2002;109(suppl):S94.
39. Chen JJ, Xia LX, Liu ZG, Liu W, Ji KM. Cloning, expression and purification of allergen arginine kinase from *Periplaneta americana* and its allergic activity. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 2008;26:356-60.
40. Daul CB, Morgan JE, Hughes J, Lehrer SB. Provocation-challenge studies in shrimp-sensitive individuals. *J Allergy Clin Immunol* 1988;81:1180-6.
41. Sanchez-Garcia S, Gamez C, Lopez E, Ibañez MD, Escudero C, Madero MF, et al. Allergy to shrimp: a double blind, placebo-controlled, food challenge study and allergens implicated in Spain. *J Allergy Clin Immunol* 2009;123(suppl):S23.

TABLE E1. Clinical characteristics and shrimp-specific IgE levels of all subjects included in this study

Subject no.	Center	Sex	Age (y)	Diagnosis	Reaction to shrimp	Shrimp-specific IgE (kU _A /L)
1	CI	M	34	AR	AE, U	>100
2	CI	F	38	A, AR	AE, U, C Urt	>100
3	CI	F	27	A, AR	OAS	93
4	CI	M	33	A, AR	OAS, AE, A	65
5	CI	F	22	AR	AE, RC	47.83
6	MS	M	30	AR	AE, U	15
7	MS	M	40	A	AE, U	>100
8	MS	F	34	A, AR	A	21
9	MS	F	19	A	RC, OAS	5.3
10	CI	M	26	AR	OAS	2.18
11	CI	F	19	AR	AE, U	11
12	MS	F	70	A	A, GI	0.9
13	CI	M	19		AE, RC	10
14	CI	F	40	A, AR	AE, A	14
15	CI	M	19	A, AR	AE	1.04
16	CI	F	34		ANA	1.07
17	CI	F	46		AE, U, A	0.64
18	CI	M	19	AR	U	14.1
19	MS	M	29	AR	AE, GI	1
20	M	M	17	A, AR, FA, AD	inh-AE, A, C	52.1
21	M	M	9.6	A, AR, FA	U	49.5
22	M	M	9.6	A, AR, FA, AD	U	11.3
23	M	M	13	A, AR, FA	inh-A, AE	82.4
24	MS	M	4	AR	V, dizzy	12
25	M	M	12	AR	AE, RC	47.4
26	M	F	11	FA	U	5.6
27	M	M	7	FA	U	14
28	M	M	5	A, FA	inh-AE	>100
29	MS	M	6	AD, FA, A, AR	AE, U, GI, C	1.9
30	MS	F	3	AD, FA	U	100
31	MS	M	8	AD, A, AR	GI, RC, U	100
32	MS	F	8	AR	AE, U, A	30
33	MS	M	18	AR	U, AE, A, GI	8
34	MS	M	3	AR, FA, AD	U, V	23
35	M	F	12	A, AR, FA, AD	inh-A	74.2
36	CI	M	16	A, AR, FA	ANA	>100
37	CI	M	15	AR	AE	55.4
38	MS	M	6	A, AR, AD, FA	AE, U	100
39	M	M	12	A, AR, FA	AE, U	11.7
40	M	F	6	A, AD	U	>100
41	M	F	9	AR	U	96.8
42	M	M	9	A	ANA	>100
43	M	M	8	A, FA	AE, U, A	7.8
44	M	M	15	A, FA	ANA	13
45	M	F	9	AR, AD	AE	53
46	M	M	12	AR, AD	U	1.3
47	M	M	4	AR	U	42.7
48	MS	M	8	AD, FA, A, AR	AE, U	6
49	MS	M	5	AD, FA	AE, C	100
50	MS	M	9	AD, FA, A, AR	AE, V	100
51	MS	F	5	AD, FA, A, AR	U	44
52	MS	M	3	AD, AR	U	80
53	CI	M	11	A, AR	U, GI	4.99

Subjects 1 to 19 are adults, and subjects 20 to 53 are children.

A, Asthma; AD, atopic dermatitis; AE, angioedema; ANA, anaphylaxis; AR, allergic rhinitis; C, cough; CI, Canary Islands; C Urt, contact urticaria; FA, food allergy; GI, gastrointestinal symptoms; inh-A, asthma by inhalation; inh-AE, angioedema by inhalation; M, Madrid; MS, Mt Sinai, NY; OAS, oral symptoms; RC, rhinoconjunctivitis; U, urticaria; V, vomiting.

TABLE E2. Amino acid sequences of tropomyosin, MLC, SCP, and AK peptides and identified epitopes

Tp-001#	mdaikkkmqamklek	MLC-001	rksgsrsssskrsk	SCP-001	mayswdnrvkyvrvy	AK-001#	madaaviekleagfk
Tp-002#	ikkkmqamklekdna	MLC-002	ksgsrsssskrsksg	SCP-002	swdnrvkyvrvymyd	AK-002#	aaviekleagfkkle
Tp-003#	kmqamklekdnamdr	MLC-003	srsssksrsksgggs	SCP-003	nrkyvrvymydidn	AK-003#	iekleagfkkleaat
Tp-004#	amklekdnamdradt	MLC-004	sskrsksgggsnvf	SCP-004#	kyvrvymydidnngf	AK-004#	leagfkkleaatdck
Tp-005#	lekdnamdradtleq	MLC-005#	rsksgsggsnvdfmf	SCP-005#	vrymydidnngfldk	AK-005	gfkkleaatdcksl
Tp-006#	dnamdradtleqqnk	MLC-006#	ksgggsnvdfmftqr	SCP-006#	mydidnngfldkndf	AK-006	kleaatdckslkky
Tp-007#	mradtleqqnkean	MLC-007#	ggsnvdfmftqrqva	SCP-007#	idnngfldkndfecl	AK-007	aatdckslkkytlk
Tp-008#	adtletqqnkeannra	MLC-008#	nvdfmftqrqvaefk	SCP-008#	ngfldkndfeclavr	AK-008#	dckslkkytlkevfv
Tp-009	leqqnkeannraeks	MLC-009#	dmftqrqvaefkegf	SCP-009	ldkndfeclavrtli	AK-009#	slkkytlkevfdkl
Tp-010	qnkeannraekseee	MLC-010#	trqvaefkegfqlm	SCP-010	ndfeclavrtltieg	AK-010#	kkyltkevfdklkdk
Tp-011	eannraekseeevhn	MLC-011#	qvaefkegfqlmrd	SCP-011	eclavrtltiegrge	AK-011	ltkevfdklkdkkts
Tp-012	nraekseeevhnllqk	MLC-012#	efkegfqlmrdkdqg	SCP-012	avrntltiegrgefsa	AK-012	evfdklkdkktslga
Tp-013#	ekseeevhnllqkrmq	MLC-013#	egfqlmrdkdqgvig	SCP-013#	ntltiegrgefsaday	AK-013	dklkdktstlsgatll
Tp-014#	eeevhnllqkrmqgle	MLC-014#	qlmrdkdqgvigktd	SCP-014#	iegrgefsadayann	AK-014	kdkktslsgatlldvi
Tp-015#	vhnllqkrmqqlendl	MLC-015	drldkdqgvigktdlrg	SCP-015#	rgefsadayannqki	AK-015#	ktlsgatlldviqsg
Tp-016#	lqkrmqqlendlldqv	MLC-016	kdqgvigktdlrgtfd	SCP-016#	fsadayannqkimrn	AK-016#	lgatlldviqsgven
Tp-017#	rmqqlendlldqvqes	MLC-017#	vigktdlrgtfdieg	SCP-017#	dayannqkimrnlnw	AK-017#	tlldviqsgvenlds
Tp-018#	qlendlldqvqesllk	MLC-018#	ktldlrgtfdiegria	SCP-018	annqkimrnlnwneia	AK-018	dviqsgvenldsgvg
Tp-019#	ndldqvqesllkani	MLC-019#	lrgtfdiegriatdq	SCP-019	qkimrnlnwneiaela	AK-019	qsgvenldsgvgiya
Tp-020#	dqvqesllkaniqlv	MLC-020#	tfdegriatdqeld	SCP-020#	mrlnwneiaeladfn	AK-020	venldsgvgiyapda
Tp-021#	qesllkaniqlvekd	MLC-021#	eigriatdqeldeml	SCP-021	lwneiaeladfnkdq	AK-021	ldsgvgiyapdaeeay
Tp-022#	llkaniqlvekdka	MLC-022#	riatdqeldemlada	SCP-022	eiaeladfnkdgevt	AK-022#	gvgiyapdaeeaytlf
Tp-023#	aniqlvekdkaalsna	MLC-023#	tdqeldemladapap	SCP-023	eladfnkdgevtvde	AK-023#	iyapdaeeaytlfapf
Tp-024	qlvekdkaalsnaege	MLC-024#	eldemladapapinf	SCP-024	dfnkdgevtvdefkq	AK-024#	pdaeeaytlfapldp
Tp-025	ekdkalsnaegevaa	MLC-025#	emladapapinfml	SCP-025	kdgevtvdefkqvavq	AK-025#	eeaytlfapldpiie
Tp-026	kalsnaegevaaln	MLC-026#	adapapinfmllnm	SCP-026	evtvdefkqvavqkhc	AK-026	tlfapldpiiedydh
Tp-027	snaegevaalnrriq	MLC-027#	papinfmllnmfae	SCP-027	vdefkqvavqkhcqgk	AK-027#	apldpiiedyhyvgf
Tp-028#	egevaalnrriqle	MLC-028#	infmllnmfaerqt	SCP-028	fkqvavqkhcqgkkyg	AK-028#	fdpiiedyhyvgfkqt
Tp-029#	vaalnrriqleedl	MLC-029#	tmlnmfaerqtges	SCP-029	avqkhcqgkkygdfp	AK-0293	iiedyhyvgfkqtdkh
Tp-030#	lnrriqleedlers	MLC-030	lnmfaerqtgesddd	SCP-030	khcqgkkygdfpgaf	AK-030	dyhyvgfkqtdkhpkn
Tp-031#	riqleedlerseer	MLC-031	faerqtgesdddvdv	SCP-031	qgkkygdfpgafkvf	AK-031	vgfkqtdkhpknkdfg
Tp-032#	lleedlerseerlnt	MLC-032	rqtgsedddvdvaka	SCP-032	kygdfpgafkvfian	AK-032	kqtdkhpknkdfgdnv
Tp-033#	edlerseerlntatt	MLC-033	gesdddvdvakafala	SCP-033	dfpgafkvfianqfk	AK-033#	dkhpknkdfgdnvsvf
Tp-034	erseerlntattkla	MLC-034	dddvdvakafalafad	SCP-034	gafkvfianqfkaid	AK-034#	pnkdfgdnvsvfnvd
Tp-035	eerlntattklaeas	MLC-035	dvvakafalafadeeg	SCP-035	kvfianqfkaidvng	AK-035	dfgdnvsvfnvdpeg
Tp-036	lntattklaeasqaa	MLC-036	akafalafadeegnid	SCP-036	ianqfkaidvngdgk	AK-036	dnvsvfnvdpegkfv
Tp-037	attklaeasqaades	MLC-037	flafadeegnidcfrh	SCP-037	qfkaidvngdgkvgl	AK-037	svfnvdpegkfvist
Tp-038	klaeasqaadeserm	MLC-038	fadeegnidcfrfhl	SCP-038	aidvngdgkvgldey	AK-038	nvdpegkfvistvr
Tp-039#	easqaadesermkrv	MLC-039	eegnidcfrfhlalm	SCP-039	vngdgkvgldeyrlid	AK-039	pegkfvistvrrogr
Tp-040#	qaadesermkrvlen	MLC-040#	nidcfrfhlalmwtg	SCP-040	dgkvgldeyrlidcit	AK-040	kfvistvrrogrsmq
Tp-041#	desermkrvlenrsl	MLC-041#	cdfrfhlalmwtgdkf	SCP-041	vgldeyrlidcitrsa	AK-041	istvrrogrsmgqyp
Tp-042#	ermkrvlenrslsde	MLC-042#	frhlalmwtgdkfssq	SCP-042	deyrlidcitrsafae	AK-042	rvrogrsmgqypfnp
Tp-043#	rkvlenrslsdeerm	MLC-043#	almtwgdkfssqead	SCP-043	rlidcitrsafaeveke	AK-043	cgrsmgqypfnpcit
Tp-044#	lenrslsdeermldal	MLC-044	twgdkfssqeadald	SCP-044	citrsafaevekeidd	AK-044	smgqypfnpcitseq
Tp-045#	rslsdeermldaleng	MLC-045	dkfssqeadaldalqm	SCP-045	rsafaevekeiddayn	AK-045	gypfnpcitseqyke
Tp-046#	sdeermldalengkle	MLC-046	ssqeadaldalqmdid	SCP-046	faevkeiddaynklt	AK-046#	fnpcitseqykemea
Tp-047#	ermldalengklearf	MLC-047	eaddaldalqmdiddg	SCP-047	vkeiddaynkltted	AK-047#	clitseqykemeakvs
Tp-048#	dalenqklearfiae	MLC-048	daldalqmdiddgkid	SCP-048	iddaynkltteddrk	AK-048#	esqykemeakvsstl
Tp-049#	enqklearfiaeaead	MLC-049	dqmdiddgkidvqg	SCP-049	aynkltteddrkagg	AK-049#	ykemeakvsstlssl
Tp-050#	lkearfiaeaeadrky	MLC-050	diddgkidvqgviq	SCP-050	kltteddrkagglit	AK-050	meakvsstlssllege
Tp-051#	arfiaeaeadrkydev	MLC-051	dgkidvqgviqmlt	SCP-051	teddrkagglitlery	AK-051	kvsstlssllegelkg
Tp-052#	laeeadrkydevark	MLC-052	kidvqgviqmltagg	SCP-052	drkagglitleryqdl	AK-052	stlssllegelkgtyy
Tp-053#	eadrkydevarklam	MLC-053#	vqgviqmltagggdd	SCP-053	aggltleryqdliaq	AK-053	sslegelkgtyyplt
Tp-054#	rkydevarklamvea	MLC-054#	viqmltagggddaaa	SCP-054	ltleryqdliaqfis	AK-054#	egelkgtyypltgms
Tp-055#	devarklamveadle	MLC-055	mltagggddaaaeeaa	SCP-055	eryqdliaqfisnpsd	AK-055#	lkgtyypltgmskev
Tp-056#	arklamveadlerae			SCP-056	qdliaqfisnpsdesc	AK-056#	tyypltgmskevqqk
Tp-057#	lamveadleraeera			SCP-057	yaqfisnpsdescasc	AK-057#	pltgmskevqqklid

(Continued)

TABLE E2. (Continued)

Tp-058	veadleraeeraetg	SCP-058	fisnpdescsacylf	AK-058#	gmskevqqkliddhf
Tp-059	dlraeeraetgesk	SCP-059	npdescsacylfgpl	AK-059#	kevqqkliddhffk
Tp-060	raeeraetgeskive	SCP-060	escsacylfgplkvv	AK-060#	qqkliddhffkegd
Tp-061	eraetgeskivelee			AK-061#	liddhffkkgdrfl
Tp-062	etgeskiveleelr			AK-062#	dhffkkgdrflqaa
Tp-063#	eskiveleelrvvg			AK-063	lfkkgdrflqaanac
Tp-064#	iveleelrvvgnnl			AK-064	egdrflqaanacryw
Tp-065#	leelrvvgnnksl			AK-065#	rflqaanacrywpag
Tp-066#	elrvvgnnkslevs			AK-066#	qaanacrywpagrgi
Tp-067#	vgnnkslevseek			AK-067	nacrywpagrgiyhn
Tp-068#	nnkslevseekanq			AK-068	rywpagrgiyhndnk
Tp-069	kslevseekanqree			AK-069	pagrgiyhndnktfl
Tp-070#	evseekanqreeayk			AK-070	rgiyhndnktflvvw
Tp-071#	eeekanqreeaykeqi			AK-071	yhndnktflvvwnee
Tp-072#	anqreeaykeqikt			AK-072	dnktflvvwneedhl
Tp-073	reeaykeqiktlnk			AK-073#	tlflvvwneedhlri
Tp-074#	aykeqiktlnklka			AK-074#	vvwneedhlriismq
Tp-075#	eqiktlnklkaaea			AK-075	needhlriismqmgg
Tp-076#	ktlnklkaaeaeae			AK-076	dhlriismqmggdglg
Tp-077#	tnklkaaeaeaeae			AK-077	riismqmggdglqv
Tp-078#	lkaaeaeaeaeae			AK-078	smqmggdglqvfrl
Tp-079#	aearaeaeaeae			AK-079#	mggdglqvfrltsa
Tp-080	raeaeaeaeae			AK-080#	dlqvfrltsavne
Tp-081	faeaeaeaeae			AK-081#	qvfrltsavniek
Tp-082	rsvqklqkevdrled			AK-082	rrltsavniekrip
Tp-083#	qklqkevdrledelv			AK-083	tsavniekripfsh
Tp-084#	qkevdrledelvnk			AK-084	vniekripfshhdr
Tp-085#	vdrledelvnkeky			AK-085	iekripfshhdrfg
Tp-086#	ledelvnkekyksi			AK-086	ripfshhdrfgfltf
Tp-087#	elvnkekyksitde			AK-087	fshhdrfgfltfcpt
Tp-088#	nekekyksitdeldq			AK-088	hdrfgfltfcptnl
Tp-089#	ekyksitdeldqtf			AK-089	lgtfcpnlgtttv
Tp-090#	ksitdeldqtfels			AK-090	ltfcpnlgtttrv
Tp-091#	tdeldqtfelselgy			AK-091	cptnlgtttrvsvhi
				AK-092	nlgtttrvsvhiklp
				AK-093	tttrvsvhiklpkla
				AK-094	rasvhiklpklaanr
				AK-095#	vhiklpklaanrekl
				AK-096#	klpklaanrekleev
				AK-097#	klaanrekleevagk
				AK-098	anrekleevagkynl
				AK-099	ekleevagkynlqvr
				AK-100	eevagkynlqvrgtr
				AK-101	agkynlqvrgtrgeh
				AK-102	ynlqvrgtrgehea
				AK-103	qvrgtrgeheaegg
				AK-104	gtrgeheaeggidy
				AK-105	geheaeggidydis
				AK-106	teaeggidydisnkr
				AK-107#	eggidydisnkrmg
				AK-108#	idydisnkrmgltf
				AK-109#	isnkrmgltfqav
				AK-110#	krmgltfqavkem
				AK-111#	mgltfqavkemqdg
				AK-112#	tefqavkemqdgile
				AK-113#	qavkemqdgilelik
				AK-114#	kemqdgilelikiek
				AK-115	qdgilelikiekem

Identified epitopes in all 4 proteins are shown with a yellow background. In tropomyosin epitopes previously identified are shown in a box. After FDR adjustment, peptides in all 4 proteins with statistically significant differences between patients and control subjects are marked with the symbol “#” next to the peptide number. Peptides with statistically significant differences between children and adults are shown in boldface.

Tp, Tropomyosin.