Allergy, Asthma &
Immunology Research

AAIR

Original Article

Check for updates

Defining Biomarkers to Predict Natural Resolution in Shrimp Allergy

Surapon Piboonpocanun (ID),[1] Suttipong Ittiporn (ID),[2] Pisit Ubonsri (ID),[1]
Anchalee Wangtan (ID),[2] Punchama Pacharn (ID),[2] Nualanong Visitsunthorn (ID),[2]
Orathai Jirapongsananuruk (ID) [2*]

[1]Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakhonpathom, Thailand
[2]Division of Allergy and Immunology, Department of Pediatrics, Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok, Thailand

OPEN ACCESS

Correspondence to
Orathai Jirapongsananuruk, MD
Division of Allergy and Immunology,
Department of Pediatrics, Faculty of Medicine
Siriraj Hospital, Mahidol University, 2 Wanglang
Road, Bangkoknoi, Bangkok 10700, Thailand.
Tel: +66-11-662-419-7000 (ext. 95670)
Fax: +66-11-662-418-4223
Email: orathai.pib@mahidol.ac.th
        jirapongo@yahoo.com

ORCID iDs
Surapon Piboonpocanun (ID)
https://orcid.org/0000-0003-4374-8003
Suttipong Ittiporn (ID)
https://orcid.org/0000-0003-4504-9644
Pisit Ubonsri (ID)
https://orcid.org/0000-0003-0614-4462
Anchalee Wangtan (ID)
https://orcid.org/0000-0002-6487-2364
Punchama Pacharn (ID)
https://orcid.org/0000-0002-2507-2057

ABSTRACT

Purpose: Tolerance to shrimp has been reported in some patients with a history of shrimp allergy. The predictors of the natural resolution of shrimp allergy have not been widely explored. This study aimed to investigate the role of specific IgE (sIgE) and specific IgG4 (sIgG4) to shrimp extracts and the cross-reactive shrimp allergens tropomyosin (TM), arginine kinase (AK) and myosin light-chain (MLC), as markers of persistent or resolved shrimp allergy (PSA or RSA).
Methods: Seventeen patients with a 10-year history of allergy to *Penaeus monodon* (*Pm*) and/or *Macrobachium rosenbergii* (*Mr*) were recruited. Oral shrimp challenges identified 10 patients with PSA and 7 patients with RSA. Sera from these patients were evaluated for sIgE and sIgG4 to *Mr* and *Pm* extracts as well as to TM, AK and MLC.
Results: The levels of sIgE to *Mr* and *Pm* extracts were lower in the RSA than in the PSA groups ($P = 0.05$ and $P = 0.008$, respectively), but sIgG4 or sIgG4:sIgE ratio did not show statistical significance. The sIgE to AK and MLC, but not TM, were lower in the RSA group than in the PSA group ($P = 0.009$ and $P = 0.0008$, respectively). There was no difference in sIgG4 to TM, AK and MLC between both groups. The ratio of sIgG4:sIgE to MLC, but not TM or AK, was higher in the RSA than in the PSA group ($P = 0.02$). A higher diversity of sIgE to shrimp components was found in the PSA group than in the RSA group ($P = 0.006$).
Conclusions: Specific bioassays can be used to identify patients with RSA. Oral shrimp challenges in these patients may provide a higher rate of passing the challenges and finally reintroducing shrimp in their diet.

Keywords: Food hypersensitivity; crustacea; shrimp; immune tolerance; biomarkers; immunoglobulin E; tropomyosin; arginine kinase; myosin light-chains

INTRODUCTION

Shrimp is one of the most common causes of allergic reactions in children and adults.[1-3] It is also the leading cause of food-induced anaphylaxis, especially in Asian countries.[4,5] At least 5 shrimp allergens have been identified in many different populations worldwide. Shrimp allergens can be grouped as cross-reactive or pan-allergens and non-cross-reactive allergens. The major cross-reactive shrimp allergen is tropomyosin (TM), a 38 kDa protein found in

Nualanong Visitsunthorn
https://orcid.org/0000-0002-4342-5100
Orathai Jirapongsananuruk
https://orcid.org/0000-0002-1603-1106

muscle and non-muscle cells.[2,6] The second cross-reactive allergen is arginine kinase (AK), a 40 kDa phosphotransferase that plays a critical role in energy metabolism.[7] Other important cross-reactive allergens include myosin light-chain (MLC) and sarcoplasmic calcium-binding protein (SCBP), 20 kDa muscle proteins with calcium-binding function.[6] In addition, shrimp hemocyanin was first identified as a non-cross-reactive allergen.[8] The hemocyanin is a hetero-hexamer of 77 kDa monomers and functions as an oxygen carrier.[2]

Although the natural resolution of food allergy has been observed in children suffering from egg, milk and soy, it was thought that only a minority of children with shrimp allergy outgrow their food allergies. Moreover, adult-onset shrimp allergy is also considered a lifelong condition.[9] Patients with shrimp allergy are generally instructed to strictly restrict the ingestion of shrimp due to the risk of severe allergic reactions. Patients who had food allergies and their families reported anxiety, depression and impaired quality of life. Therefore, identifying patients who outgrow food allergy may improve the quality of life in patients and their families.[10] Recently, our group has examined the natural resolution of shrimp allergy among non-anaphylactic shrimp-allergic patients 10 years after the first diagnosis and reported that 46% of the patients had resolved shrimp allergy (RSA), while 54% had persistent shrimp allergy (PSA).[11] Interestingly, patients with RSA had significantly smaller shrimp skin test responses than did patients with PSA at both diagnosis and follow-up, suggesting an important role of specific IgE (sIgE) as a potential biomarker for RSA.[11]

Both sIgE and specific IgG4 (sIgG4) may play a role in the resolution of food allergy. Many studies reported that patients with resolved food allergies had low titers of serum sIgE to the corresponding food allergens.[12,13] However, the role of sIgG4 in predicting resolved food allergies was controversial.[12-14] Nevertheless, the reactivity of sIgE and sIgG4 to shrimp cross-reactive allergens has not been explored in PSA and RSA patients. Therefore, we aimed to examine the interaction of sIgE and sIgG4 to known shrimp allergens in PSA and RSA patients. We also investigated the potential biomarker identifying the status of shrimp allergy in these patients.

MATERIALS AND METHODS

Subjects
This study was approved by the Institutional Review Board (COA No. 017/2559, EC1). It is a prospective study that followed patients diagnosed with shrimp allergy to *Penaeus monodon* (*Pm*), *Macrobrachium rosenbergii* (*Mr*), or both from our previous study conducted during the period 2005-2006.[15] The current oral food challenges (OFCs) to shrimp were conducted during follow-up in the period of 2015–2016. The sera of patients were collected on the same day of current challenges for further analysis. Due to the ethical concern, patients who had a previous history of anaphylaxis or had a recent allergic reaction to shrimp in the past 6 months were not included. We excluded patients with underlying diseases such as cardiovascular, hepatobiliary and renal diseases, severe systemic infection, and pregnancy. Patients with allergic diseases, such as allergic rhinitis, urticaria, atopic dermatitis, and asthma, were stable for more than 7 days before OFC. The peak expiratory flow rates were more than 70% of predicted value on the day of OFC. Of the 46 patients who met the inclusion criteria, 9 refused to participate and 20 could not be contacted. The remaining 17 patients were recruited. Written informed consent was obtained from all participants.

OFC

The methods and results of OFCs with shrimp were described in our previous report.[11] In brief, a 3-step challenge protocol was completed with multiple doses in 2 of the steps. Each step was performed 15 minutes apart. The first step consisted of raw lyophilized shrimp in a capsule with a cumulative provocation dose (PD) of 7.5 g. The second step was to identify oral-mucosal reactions by swabbing cooked shrimp onto the inner lips, placing it into the mouth without chewing for 5 minutes, and then removed. The third step was open feeding of cooked shrimp with a cumulative PD of 15 g. A positive reaction to 1 or more steps of the OFC was considered a positive challenge. Patients who previously had a positive reaction to both shrimp species were challenged 2–4 weeks later with other shrimp species. Anaphylaxis was diagnosed according to the standard criteria.[16] Patients with a negative shrimp challenge were designated RSA and a positive challenge was assigned to PSA. Patients who were previously diagnosed as having allergies to both shrimp species would need to have negative OFC to both shrimp species in order to be categorized as RSA. The sera of these patients were collected on the same day of OFC while obtaining the intravenous access and were stored at −20°C until further analysis. Sera from healthy subjects who had no allergic diseases were used as controls.

Expression and characterization of recombinant TM, AK and MLC

Recombinant TM was expressed from transformed *Escherichia coli* cells containing plasmid pET-28b ligated with TM cDNA (Mac r1.0101, nucleotide accession# GU369816) as previously reported.[17] The TM was purified from lysis of transformed *E. coli* cells before IgE reactivity was confirmed with the sera of shrimp-allergic patients.

Recombinant AK and MLC were also expressed from plasmid pET-28b-AK or pET-28b-MLC transformed *E. coli* cells. The cDNA of AK (nucleotide accession# DQ975203) and MLC (nucleotide accession# EU449515) of pacific white leg shrimp *Litopenaeus vannamei* (*Lv*) were synthesized (GenScript, Piscataway, NJ, USA). Both recombinant AK and MLC were also purified from cell lysis of transformed *E. coli* cells before IgE reactivity was confirmed by immunoblot using the sera of shrimp-allergic patients.

Indirect binding of serum IgE and IgG4 to shrimp extract and cross-reactive allergens

Shrimp extract of *Pm* and *Mr* was prepared as previous described.[8] To measure serum IgE and IgG4 to allergen in the *Pm* and *Mr* extract by indirect binding ELISA, shrimp extract was diluted in phosphate-buffered saline (PBS) and coated individually at 500 ng per well on 96-well Maxisorb plates (Nunc™, Thermo Fisher Scientific, Waltham, MA, USA) and incubated at 4°C overnight. Moreover, to measure serum IgE and IgG4 binding to recombinant TM, AK and MLC, each recombinant allergen was also diluted in PBS and coated individually at 500 ng per well on 96-well Maxisorb plates (Nunc™, Thermo Fisher Scientific, Waltham, MA, USA) and incubated at 4°C overnight. The coated 96-well plate was washed with PBS-A (PBS+3% non-fat dried milk). Sera of patients and controls were diluted at 8:1 in PBS-A. Diluted sera were incubated with coated allergens in PBS-A for 2 hours at room temperature. The plate was washed before either diluted HRP-labelled goat IgG anti-human IgE or diluted HRP-labelled rabbit IgG anti-human IgG4 antibodies was added into the designated wells. Substrate (3,3′,5,5′-tetramethylbenzidine or TMB, Thermo Fisher Scientific) was added to each well before absorbance at $OD_{650\,nm}$ was measured.

Statistical analysis

Biomarkers were compared between PSA and RSA groups using the Mann-Whitney U test since the data were not normally distributed. GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA) was used for data analysis. A P value ≤ 0.05 was considered statistically significant.

RESULTS

Seventeen patients who were previously diagnosed as having Mr allergy, Pm allergy, or both were recruited. Three of these patients had an isolated allergy to Mr (Mr1–3), another 3 had an isolated allergy to Pm (Pm1–3) and 11 had an allergy to both shrimp species (B1–11). The patients' sex, age, as well as results and symptoms upon OFC, are shown in **Table**.

Upon re-evaluation, 10 patients were categorized as having PSA and 7 as RSA. Patients were further grouped based on their previous reactions to shrimp species. Of 10 PSA patients, 2 were previously allergic to Pm (Pm1–Pm2), while 8 were allergic to both Mr and Pm (B1–B8). Two patients who were previously allergic to both shrimp species (B7–8) refused to receive the second challenge after having an allergic reaction to the first challenge. Four patients (B1, B2, B4 and B5) were positive to both Mr and Pm, while 3 (B3, B6 and B7) were positive to Mr, and B8 was positive to Pm (**Table**).

Patients with dual shrimp allergy were categorized as having RSA only when they had a negative challenge to both shrimp species. Among 7 RSA patients, 3 were previously allergic to Mr (Mr1–Mr3), 1 to Pm (Pm3), and another 3 to both Mr and Pm (B9–B11). Upon re-evaluation, 1 patient (Pm3) was negative to Pm, 3 (Mr1–M3) were negative to Mr, and 3 (B9–B11) were negative to both Pm and Mr (**Table**). A total of 26 challenges were done; 6/13 (46%) challenges to Pm, and 6/13 (46%) challenges to Mr were negative.

Table. Demographic data from patients with persistent or resolved shrimp allergy

Shrimp allergy status	Sex	Current age (yr)	Results of Mr OFC	Symptoms upon Mr OFC	Results of Pm OFC	Symptoms upon Pm OFC
Persistent shrimp allergy						
Pm1	Male	26	NP	NP	Positive	LI
Pm2	Female	18	NP	NP	Positive	TI
B1	Male	23	Positive	LI, TI	Positive	TI
B2	Male	18	Positive	U. N/V, AP, A	Positive	U
B3	Female	18	Positive	LI, TI	Negative	No
B4	Female	21	Positive	TI, U, LSI	Positive	AG, R, A
B5	Male	18	Positive	F	Positive	TI, LI
B6	Male	24	Positive	R	Negative	No
B7	Male	18	Positive	TI, LI, U, N/V, AP, A	NP	NP
B8	Male	20	NP	NP	Positive	AP
Resolved shrimp allergy						
Pm3	Male	19	NP	NP	Negative	No
Mr1	Male	19	Negative	No	NP	NP
Mr2	Male	19	Negative	No	NP	NP
Mr3	Female	25	Negative	No	NP	NP
B9	Male	23	Negative	No	Negative	No
B10	Female	24	Negative	No	Negative	No
B11	Female	24	Negative	No	Negative	No

A, anaphylaxis; AG, angioedema; AP, abdominal pain; B, both Mr and Pm shrimp allergy; F, flushing; LI, lip itching; LSI, lip swelling and itching; Mr, *Macrobrachium rosenbergii*; NP, not performed; N/V, nausea and/or vomiting; OFC, oral food challenge; Pm, *Penaeus monodon*; R, rhinitis; TI, throat itching; U, urticaria.
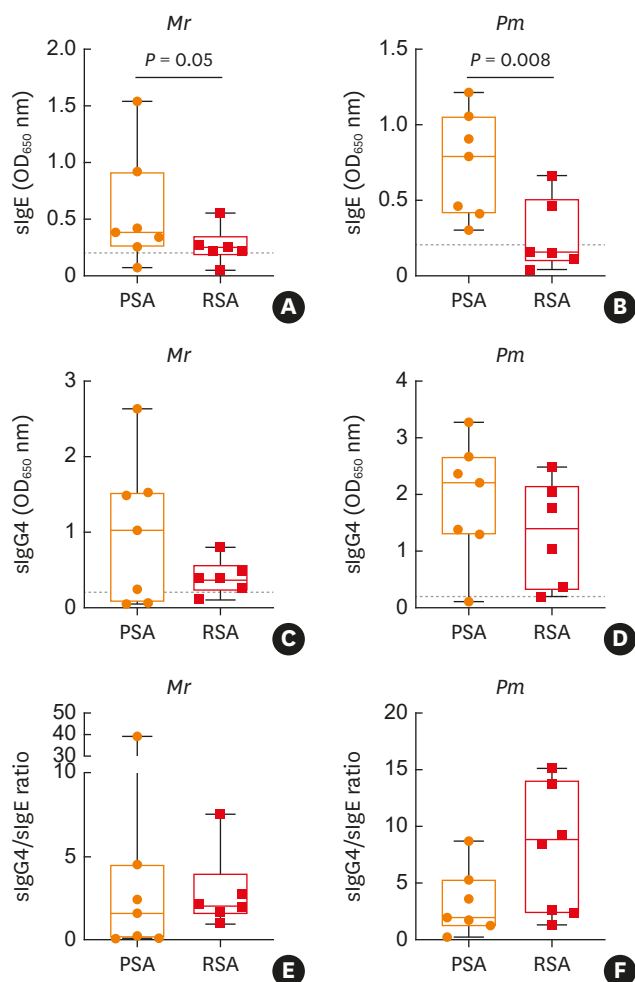
Fig. 1. The levels of sIgE, sIgG4 and the ratios of sIgG4:sIgE to allergens in *Mr* and *Pm* shrimp extracts in PSA and RSA groups.

The dotted lines are presented as $OD_{650\,nm}$ values of bound antibodies from non-allergic donors. Each plotted symbol represents duplicate data from each patient.

sIgE, specific IgE; sIgG4, specific IgG; *Mr*, *Macrobrachium rosenbergii*; *Pm*, *Penaeus monodon*; PSA, persistent shrimp allergy; RSA, resolved shrimp allergy.

Binding of sIgE and sIgG4 to allergens of shrimp *Mr* and *Pm* extracts as well as sIgG4:sIgE ratios in PSA and RSA groups

After determining the levels of sIgE and sIgG4 binding to allergens in *Mr* or *Pm* extract, patients were categorized as PSA or RSA groups according to the result of the OFC to each shrimp species (**Fig. 1**). For *Mr* shrimp allergy, patients Mr1–M3 and B9–B11 were categorized as RSA, while patients B1–B7 were categorized as PSA. For *Pm* shrimp allergy, patients Pm3, B3, B6 and B9–B11 were categorized as RSA, and patients Pm1–Pm2, B1, B2, B4, B5 and B8 were categorized as PSA.

When the levels of sIgE binding to allergens in *Mr* and *Pm* extracts were determined, RSA patients had significantly lower sIgE to allergens in *Mr* (**Fig. 1A**) and *Pm* (**Fig. 1B**) extract than PSA patients ($P = 0.05$ and $P = 0.008$, respectively).

In contrast, the levels of sIgG4 to allergens in *Mr* (**Fig. 1C**) and *Pm* (**Fig. 1D**) extracts of RSA patients were not significantly different from those of PSA patients ($P = 0.68$ and $P = 0.45$,

respectively). The ratios of sIgG4:sIgE to allergens in *Mr* (**Fig. 1E**) and *Pm* (**Fig. 1F**) extracts in RSA patients were not significantly different from those of PSA patients (*P* = 0.94 and *P* = 0.24, respectively).

Binding of sIgE and sIgG4 to 3 shrimp cross-reactive allergens as well as sIgG4:sIgE ratios in PSA and RSA groups

To examine the role of allergen components in the natural resolution of shrimp allergy, the levels of sIgE and sIgG4 to the cross-reactive shrimp allergens (TM, AK and MLC) were determined in PSA and RSA groups. The sIgE (**Fig. 2A**), sIgG4 (**Fig. 2B**) and sIgG4:sIgE ratio (**Fig. 2C**) to TM in RSA and PSA groups were not significantly different (*P* = 0.08, *P* = 0.49 and *P* = 0.81, respectively). The level of sIgE (**Fig. 2D**), but not sIgG4 (**Fig. 2E**) or sIgG4:sIgE ratio (**Fig. 2F**), to AK was significantly lower in the RSA group than in the PSA group (*P* = 0.009, *P* = 0.55 and *P* = 0.13, respectively). The sIgE (**Fig. 2G**) to MLC was significantly lower in the RSA group than in the PSA group (*P* = 0.0008). However, the sIgG4 (**Fig. 2H**) to MLC was not significantly different between the RSA and PSA groups (*P* = 0.81). In contrast, the sIgG4:sIgE ratio to MLC was significantly higher in the RSA group than in the PSA group (*P* = 0.02, **Fig. 2I**).
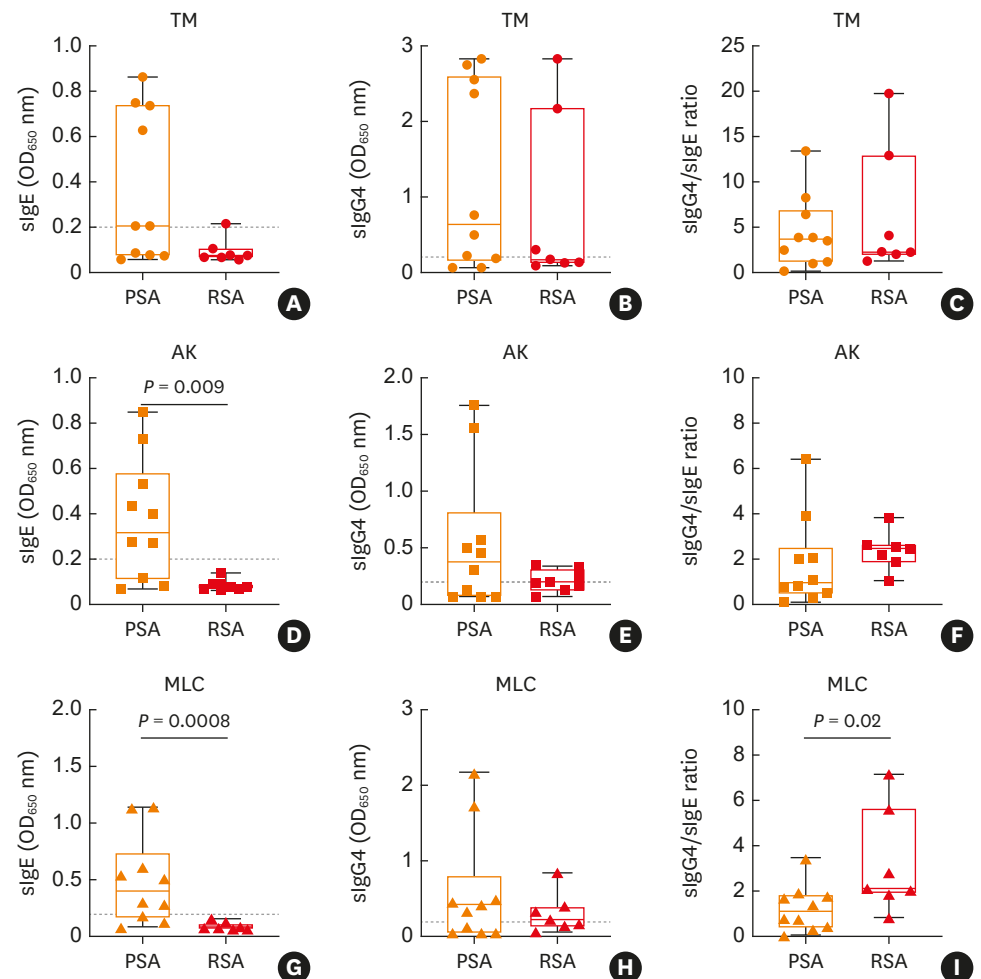

Fig. 2. The levels of sIgE, sIgG4 and the ratios of sIgG4:sIgE to shrimp cross-reactive allergens in PSA and RSA groups. Each plotted symbol represents duplicate data of one patient.
sIgE, specific IgE; sIgG4, specific IgG4; PSA, persistent shrimp allergy; RSA, resolved shrimp allergy; TM, tropomyosin; AK, arginine kinase; MLC, myosin light-chain.
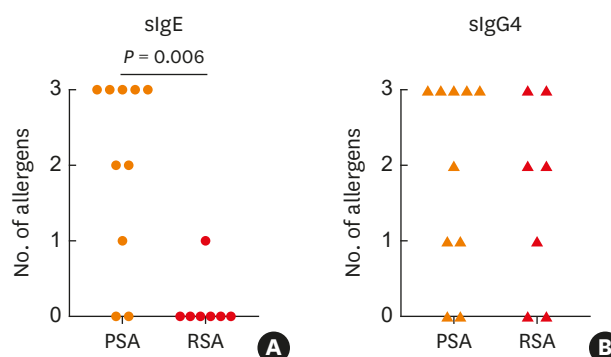
Fig. 3. The number of allergens bound to each patient's sIgE and sIgG4 in PSA and RSA groups. Each symbol represents one patient.
PSA, persistent shrimp allergy; RSA, resolved shrimp allergy; sIgE, specific IgE; sIgG4, specific IgG4.

The number of allergen components bound to patients' sIgE was higher in the PSA group than RSA group ($P = 0.006$, **Fig. 3A**). In contrast, the number of allergen components bound to the patients' sIgG4 was not significantly different in either group ($P = 0.56$, **Fig. 3B**).

DISCUSSION

Development of oral tolerance involves suppression of Th2 cells, generation of Treg cells, suppression of effector T cells, decreased production of IgE, increased production of IgG4 by B cells, induction of IL-10–producing DCs and suppression of basophil, eosinophil and mast cell activation.[18] Growing evidence suggests that the balance between antigen-sIgE and sIgG4 is important for acquiring food tolerance.[19] In oral immunotherapy (OIT), sIgG4 increases, while sIgE initially increases, but then declines after several months of therapy.[20-22] Since sIgG4 may increase with allergen exposure, many studies have explored the sIgG4:IgE ratio as a marker for food tolerance. In the LEAP study, the early introduction of peanuts decreased the development of peanut allergy in high-risk children and was associated with increased peanut-sIgG4 and peanut-sIgG4:IgE ratio.[23] A proposed mechanism of desensitization through sIgG4 involves the competitive binding of sIgE to the same allergen and blocking allergen-triggered activation of effector cells.[14,24,25] Thus, determining differences in sIgE and sIgG4 to shrimp allergens may help us to identify markers of RSA.

Although it was proposed that patients with resolved food allergy would have lower food-specific IgE and higher food-specific IgG4 or IgG4:IgE ratio than patients with persistent food allergy, the data from recent studies were conflicting. In a cohort study by Wood *et al.*,[12] patients with resolved milk allergy had lower levels of milk sIgE compared to those with persistent milk allergy. However, the milk to sIgG4 or milk to IgG4:IgE ratio were not predictive of resolution. Another cohort study by Sicherer *et al.*[13] demonstrated that both sIgE and sIgG4 to egg were lower in patients with resolved egg allergy compared to those with persistent egg allergy. Santos *et al.*[14] studied peanut-allergic (PA), peanut-sensitized but nonallergic (PS) and non-peanut-sensitized nonallergic (NA) children, and discovered that PS children had higher levels of peanut-sIgG4 as well as peanut-specific IgG4:IgE ratios compared to PA children. Our study supported the previous study[12] that RSA patients had significantly lower sIgE to shrimp extracts compared to PSA patients. However, sIgG4 or the sIgG4:sIgE ratio to shrimp extracts did not predict RSA or PSA.

Patients with food allergy may be sensitized to multiple allergens in a single food source. Therefore, evaluating the levels of sIgE, sIgG4 and the ratio of sIgG4:sIgE to allergen components may allow more detailed profiling of the allergen-sIgE and allergen-sIgG4 repertoires. In children with cow's milk protein allergy (CMPA), the β-lactoglobulin (BLG)-sIgG4 level and BLG-sIgG4:CM-sIgE ratio, but not casein-sIgG4 level or casein-sIgG4:CM-sIgE ratio, were higher in CMPA patients at the tolerance phase compared to the symptomatic phase.[26] In a study of egg-allergic children, baked-egg tolerant patients had significantly lower levels of egg-, ovalbumin- and ovomucoid-sIgE compared to baked-egg-reactive patients.[27] Another study showed that a high IgG4:IgE ratio to ovomucoid and ovalbumin was the marker of tolerance to the baked egg in egg-allergic children.[28] In this study, we further investigated the levels of sIgE and sIgG4 against 3 cross-reactive allergens and revealed that the levels of sIgE to AK and MLC, but not to TM, were significantly lower in the RSA group. The ratio of sIgG4:sIgE to MLC, but not to TM or AK, was also significantly higher in the RSA group.

Ayuso et al.[29] compared sIgE to shrimp TM, AK, MLC and SCBP between children and adults who had a history of IgE-mediated shrimp allergy. Compared to adults with shrimp allergy, children with shrimp allergy had greater frequency of sIgE and individual epitope recognition as well as increased intensity of IgE binding to all 4 allergen components. The authors observed that sIgE to TM, but not to the other 3 components, was frequently found in both children and adults, and postulated that TM might be associated with PSA. Our study could not confirm this hypothesis since 6/10 PSA patients had an undetectable level of sIgE to TM. In contrast, most PSA patients had sIgE to AK and MLC, suggesting that these components were important predictors of PSA. Of interest, the number of allergen components bound to patients' sIgE was higher in the PSA group. This finding was supported by the HealthNuts study which showed that the diversity of sIgE to multiple egg allergens (Gal d 1, 2, 3 and 5) predicted persistent egg allergy at 4 years of age.[30]

The strength of our study was that we followed patients who had confirmed shrimp allergy by OFC and identified the PSA or RSA patients by OFC after 10 years. Our long-term follow-up study facilitated the recruitment of real shrimp-allergic patients and finally confirmed that 46% of them could outgrow shrimp allergy. Our study was unlike a cross-sectional one that could only identify shrimp-allergic or non-allergic patients. However, the limitations of our study were the small number of PSA and RSA patients. The serum samples of patients at the diagnosis in 2005–2006 were not obtained for sIgE and sIgG4 to shrimp extracts and shrimp allergen components; therefore, they could not be compared to the current biomarker data. Also, a single serum dilution (1:10) was used because the sIgE levels varied among patients. The 1:10 dilution was predetermined based on immunoblot and ELISA at various dilutions.

In conclusion, patients with RSA had significantly lower sIgE to shrimp extracts, AK and MLC as well as significantly higher sIgG4:sIgE ratios to MLC compared to PSA patients. PSA patients had more diverse sIgE binding to shrimp allergen components than RSA patients. These specific bioassays can be used to identify patients with RSA. Performing oral shrimp challenge in these patients may provide a higher rate of passing the challenge and finally reintroducing shrimp in their diet.

ACKNOWLEDGMENTS

REFERENCES

1. Wai CYY, Leung NYH, Chu KH, Leung PSC, Leung ASY, Wong GWK, et al. Overcoming shellfish allergy: how far have we come? Int J Mol Sci 2020;21:2234.
   PUBMED | **CROSSREF**

2. Lopata AL, Kleine-Tebbe J, Kamath SD. Allergens and molecular diagnostics of shellfish allergy: Part 22 of the Series Molecular Allergology. Allergo J Int 2016;25:210-8.
   PUBMED | **CROSSREF**

3. Santadusit S, Atthapaisalsarudee S, Vichyanond P. Prevalence of adverse food reactions and food allergy among Thai children. J Med Assoc Thai 2005;88 Suppl 8:S27-32.
   PUBMED

4. Jirapongsananuruk O, Bunsawansong W, Piyaphanee N, Visitsunthorn N, Thongngarm T, Vichyanond P. Features of patients with anaphylaxis admitted to a university hospital. Ann Allergy Asthma Immunol 2007;98:157-62.
   PUBMED | **CROSSREF**

5. Ganapathy S, Lwin Z, Ting DH, Goh LS, Chong SL. Anaphylaxis in children: experience of 485 episodes in 1,272,482 patient attendances at a tertiary paediatric emergency department from 2007 to 2014. Ann Acad Med Singapore 2016;45:542-8.
   PUBMED

6. Faber MA, Pascal M, El Kharbouchi O, Sabato V, Hagendorens MM, Decuyper II, et al. Shellfish allergens: tropomyosin and beyond. Allergy 2017;72:842-8.
   PUBMED | **CROSSREF**

7. Yao CL, Ji PF, Kong P, Wang ZY, Xiang JH. Arginine kinase from *Litopenaeus vannamei*: cloning, expression and catalytic properties. Fish Shellfish Immunol 2009;26:553-8.
   PUBMED | **CROSSREF**

8. Piboonpocanun S, Jirapongsananuruk O, Tipayanon T, Boonchoo S, Goodman RE. Identification of hemocyanin as a novel non-cross-reactive allergen from the giant freshwater shrimp *Macrobrachium rosenbergii*. Mol Nutr Food Res 2011;55:1492-8.
   PUBMED | **CROSSREF**

9. Savage J, Johns CB. Food allergy: epidemiology and natural history. Immunol Allergy Clin North Am 2015;35:45-59.
   PUBMED | **CROSSREF**

10. Feng C, Kim JH. Beyond avoidance: the psychosocial impact of food allergies. Clin Rev Allergy Immunol 2019;57:74-82.
    PUBMED | **CROSSREF**

11. Ittiporn S, Piboonpocanun S, Pacharn P, Visitsunthorn N, Thongngarm T, Jirapongsananuruk O. Natural resolution of non-anaphylactic shrimp allergy in patients diagnosed 10 years earlier by oral food challenge. Asian Pac J Allergy Immunol 2021;39:249-57.
    PUBMED | **CROSSREF**

12. Wood RA, Sicherer SH, Vickery BP, Jones SM, Liu AH, Fleischer DM, et al. The natural history of milk allergy in an observational cohort. J Allergy Clin Immunol 2013;131:805-12.
    PUBMED | **CROSSREF**

13. Sicherer SH, Wood RA, Vickery BP, Jones SM, Liu AH, Fleischer DM, et al. The natural history of egg allergy in an observational cohort. J Allergy Clin Immunol 2014;133:492-9.
    PUBMED | **CROSSREF**

14. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. J Allergy Clin Immunol 2015;135:1249-56.
    PUBMED | **CROSSREF**

15. Jirapongsananuruk O, Sripramong C, Pacharn P, Udompunturak S, Chinratanapisit S, Piboonpocanun S, et al. Specific allergy to *Penaeus monodon* (seawater shrimp) or *Macrobrachium rosenbergii* (freshwater shrimp) in shrimp-allergic children. Clin Exp Allergy 2008;38:1038-47.
   PUBMED | CROSSREF

16. Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol 2006;117:391-7.
   PUBMED | CROSSREF

17. Kumjim S, Jirapongsananuruk O, Piboonpocanun S. Cloning and characterization of recombinant tropomyosin of giant freshwater shrimp *M. rosenbergii* to determine major allergens causing allergic reactions among shrimp-allergic children. Asian Pac J Allergy Immunol 2016;34:229-35.
   PUBMED | CROSSREF

18. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. J Allergy Clin Immunol 2018;141:11-9.
   PUBMED | CROSSREF

19. Schmiechen ZC, Weissler KA, Frischmeyer-Guerrerio PA. Recent developments in understanding the mechanisms of food allergy. Curr Opin Pediatr 2019;31:807-14.
   PUBMED | CROSSREF

20. Smeekens JM, Kulis MD. Evolution of immune responses in food immunotherapy. Immunol Allergy Clin North Am 2020;40:87-95.
   PUBMED | CROSSREF

21. Hardy LC, Smeekens JM, Kulis MD. Biomarkers in food allergy immunotherapy. Curr Allergy Asthma Rep 2019;19:61.
   PUBMED | CROSSREF

22. Vickery BP, Lin J, Kulis M, Fu Z, Steele PH, Jones SM, et al. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. J Allergy Clin Immunol 2013;131:128-134.e1-3.
   PUBMED | CROSSREF

23. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med 2015;372:803-13.
   PUBMED | CROSSREF

24. Scott-Taylor TH, Axinia SC, Amin S, Pettengell R. Immunoglobulin G; structure and functional implications of different subclass modifications in initiation and resolution of allergy. Immun Inflamm Dis 2018;6:13-33.
   PUBMED | CROSSREF

25. Tordesillas L, Berin MC. Mechanisms of oral tolerance. Clin Rev Allergy Immunol 2018;55:107-17.
   PUBMED | CROSSREF

26. Sommanus S, Kerddonfak S, Kamchaisatian W, Vilaiyuk S, Sasisakulporn C, Teawsomboonkit W, et al. Cow's milk protein allergy: immunological response in children with cow's milk protein tolerance. Asian Pac J Allergy Immunol 2014;32:171-7.
   PUBMED | CROSSREF

27. Berin MC, Grishin A, Masilamani M, Leung DYM, Sicherer SH, Jones SM, et al. Egg-specific IgE and basophil activation but not egg-specific T-cell counts correlate with phenotypes of clinical egg allergy. J Allergy Clin Immunol 2018;142:149-158.e8.
   PUBMED | CROSSREF

28. Caubet JC, Bencharitiwong R, Moshier E, Godbold JH, Sampson HA, Nowak-Węgrzyn A. Significance of ovomucoid- and ovalbumin-specific IgE/IgG$_4$ ratios in egg allergy. J Allergy Clin Immunol 2012;129:739-47.
   PUBMED | CROSSREF

29. Ayuso R, Sánchez-Garcia S, Lin J, Fu Z, Ibáñez MD, Carrillo T, et al. Greater epitope recognition of shrimp allergens by children than by adults suggests that shrimp sensitization decreases with age. J Allergy Clin Immunol 2010;125:1286-1293.e3.
   PUBMED | CROSSREF

30. Dang TD, Peters RL, Koplin JJ, Dharmage SC, Gurrin LC, Ponsonby AL, et al. Egg allergen specific IgE diversity predicts resolution of egg allergy in the population cohort HealthNuts. Allergy 2019;74:318-26.
   PUBMED | CROSSREF