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SUPPORTING INFORMATION

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Similar IgE binding patterns in Gulf of Mexico and Southeast Asian shrimp species in US shrimp allergic patients

To the Editor,

Shellfish allergy (SA) is a leading cause of food-induced anaphylaxis[1] and one of the most common causes of adult-onset food allergy worldwide, with 1%–3% of the United States (US) population affected.[2–4] Nearly half (45%) of US adults with SA report utilizing emergency services for SA symptoms over their lifetime,[2] remaining at-risk for lethal allergic reactions. Several allergenic proteins have been identified across shellfish species, including tropomyosin (TM), arginine kinase (AK), myosin light chain (MLC), sarcoplasmic calcium-binding protein (SCP), hemocyanin, troponin C, and triosephosphate isomerase.[5] (Table 1.) However, there are a large number of shrimp allergens that have been detected, but not yet characterized.[6] The allergens of major importance in SA are the muscle proteins TM and AK. TM, the major allergen with specific-IgE antibodies in ≤90% of SA patients, is associated with severe clinical reactivity. AK is a pan-allergen with cross-reactivity with crustaceans and cephalopods.[5]

Cross-reactivity has been observed clinically when SA patients ingest various invertebrate species with subsequent allergic reactions, but further study of shrimp sIgE binding between different shrimp species is needed.[7,8] This study examined the sIgE binding patterns to 2 shrimp species from the Gulf of Mexico and Southeast Asia in US SA patients.

Shellfish allergy patients with a history of shrimp-induced allergic reactions, allergic reaction with clinical oral food challenge and positive immediate skin prick testing (IHST) and/or shrimp sIgE ImmunoCAP™ levels were recruited from the Baylor College of Medicine (BCM) Allergy and Immunology Clinics. The study was approved by the BCM IRB and all participants provided written, informed consent. The patients underwent IHST to shrimp extract (mixture of *Penaeus borealis*, *Penaeus monodon*, *Metapenaeus barbata, and Metapenaeopsis joyner)*, *Dermatophagoides pteronyssinus (Der p1*, *Der p 10)*, *Dermatophagoides farinae (Der f1)*, cockroach, codfish, crab, lobster, and oyster using extracts from Greer™. The patients underwent prick and prick IHST to raw fresh shrimp (*Penaeus aztecus*), and cooked fresh shrimp (*Penaeus aztecus*). ImmunoCAP™ and ISAC™ customized testing by ThermoFisher™ assessed total IgE as well as sIgE levels for shrimp, recombinant *Penaeus aztecus* (TM), *Der p10*, *Der p1*, *Der p2*, recombinant *Penaeus monodon* AK, MLC, SCP, troponin C, crab, lobster, cockroach, clam, and oyster. Western blot (WB) analysis of sIgE binding profile to

Abbreviations: Der f1, *Dermatophagoides farinae*; Der p1, *Dermatophagoides pteronyssinus*; rPen a, recombinant *Penaeus aztecus*; rPen m, recombinant *Penaeus monodon*; SA, shrimp allergy; sIgE, specific IgE.

TABLE 1 Allergenic molecules present in crustacean species

Species frequently implicated		Allergen name (IUIS)	Biochemical name	Molecular weight	Heat stability	Physiological function	Route of exposure
Penaeus monodon (Black tiger prawn) – Pen m	1	Cha f 1, Cra c 1, Hom a 1, Lit v 1, Mel l 1, Met e 1, Pan b 1, Pen m 1, Por p 1	Tropomyosin	34–38 kDa	Highly heat-stable	Muscle contraction	Ingestion Inhalation
Litopenaeus vannamei (White leg shrimp) Lit v	2	Cra c 2, Lit v 2, Pen m 2	Arginine kinase	40–45 kDa	Stable	Energy metabolism in muscles	Ingestion Inhalation
Penaeus aztecus (Brown shrimp) Pen a	3	Hom a 3, Lit v 3, Pen m 3	Myosin light chain	17–20 kDa	Stable	Muscle contraction	Ingestion
Homarus americanus (American lobster) Hom a	4	Cra c 4, Lit v 4, Pen m 4, Pon l 4	Sarcoplasmic calcium-binding protein	20–25 kDa	Stable	Muscle contraction regulation	Ingestion
	5	Cra c 6, Hom a 6, Pen m 6	Troponin C	20–21 kDa	Unknown	Calcium dependent activation of muscle contraction	Ingestion
Crangon crangon (Sand shrimp) Cra c	6	Pen m 7	Hemocyanin	75 kDa	Stable	Copper-containing Oxygen transport protein, anti-microbial property	Ingestion Inhalation
Charybdis feriatus (Crucifix crab) Cha f	7	Arc s 8, Cra c 8	Triose-Phosphate isomerase	28 kDa	Labile	Glycolysis (energy metabolism)	Ingestion Inhalation
Portunus pelagicus (Blueswimmer crab) Por p	8	Pen m 13	Fatty acid-binding protein	20 kDa	Unknown	Transport protein for lipophilic molecules (fatty acids)	Ingestion

Note: Registered allergen names in accordance with WHO/IUIS Allergen Nomenclature.
Matricardi PM, et al., EAACI Molecular Allergology User's Guide. *Pediatr Allergy Immunol*, 2016. 27(Suppl.23): p. 1–236.

the major allergenic proteins TM, AK, MLC, and SCP in *Penaeus aztecus* (Brown shrimp) and *Penaeus monodon* (Black tiger shrimp) in raw and boiled extracts was performed.

PATIENT POPULATION

Fifteen SA patients between the ages of 2–17 years were enrolled. (Table 2) On oral food challenge, the most frequent symptoms were urticaria and angioedema. Forty percent had antihistamine treatment, one required steroids, and none required epinephrine or albuterol.

ANALYSIS OF ALLERGEN-sIgE

When examining sIgE binding patterns to the major allergens, WB revealed nearly identical sIgE binding profiles to tropomyosin, MLC, and SCP in both species in the heated shrimp extracts. (Figure 1) Eight subjects (53%) demonstrated sIgE binding to TM and six to SCP (40%). There was variability in the binding to other shrimp proteins, particularly in the unheated shrimp.[5] The proposed sIgE binding allergens have been identified by molecular weight,[5,6] and mass spectrometric in-gel analysis, (Table S1.) but need to be confirmed in future molecular studies.[9,10]

The presence of Southeast Asian Black tiger shrimp sIgE has been established and quantified through ImmunoCAP™ ISAC™ testing, with over 50% of patients having binding to TM, 33% to SCP, 27% to AK, and 13% each to MLC and troponin C.[6] Nevertheless, each individual patient's binding pattern in our study was similar between the two species, both unheated/raw (Figure 1A,B) and heated/boiled proteins (Figure 1C,D). Inhibitory immunoblot was performed which confirmed the identification of MLC and SCP. (Figure S1A,B) These similar sIgE binding patterns to the major allergens suggested conservation of the epitopes between different species of shrimp.

According to the Food and Agricultural Organization of the United Nations, *Penaeus aztecus* is native to the north-western Atlantic Ocean and Gulf of Mexico, while *Penaeus monodon* is native to the coasts of Southeast Asia, South Asia, East Africa, and Australia. Our study demonstrates shared epitopes among the major shrimp proteins for Gulf Brown shrimp and Southeast

TABLE 2 Demographics of US Shrimp Allergic (SA) population

	Total	
Gender		
Male	9 (60%)	
Female	6 (40%)	
Age (years)	9 (2–17)	
Race		
African American	5 (33.3%)	
Asian	1 (6.7%)	
Caucasian	3 (20%)	
Hispanic	6 (40%)	
Age of diagnosis (years)	4 (1–8)	
Skin test	Wheal (mm)	Flare (mm)
Shrimp extract	8 (0–42)	25 (0–56)
Raw shrimp	2 (0–8)	4 (0–25)
Cooked shrimp	1 (0–20)	1 (0–40)
Der p	5 (0–22)	15 (0–30)
Der f	9 (0–16)	20 (0–29)
Cockroach	4 (0–10)	10 (0–25)
Codfish	2 (0–30)	2.5 (0–48)
Crab	3 (0–20)	3 (0–25)
Lobster	2 (0–10)	4 (0–30)
Oyster	1 (0–7)	1 (0–15)
IgE (kU/L)		
Shrimp	3.35 (0–42.9)	
rPen a 1 (TM)	0.65 (0–41.1)	
Der p 10	0.68 (0–27.3)	
Der p 1	0 (0–100)	
Der p 2	0 (0–100)	
rPen m 2 (AK)	0 (0–0.81)	
rPen m 3 (MLC)	0 (0–4.01)	
rPen m 4 (SCP)	0 (0–15.1)	
rPen m 6 (TC)	0 (0–3.15)	
Total IgE	225 (2.71–5000)	
Crab	1.82 (0–33.5)	
Lobster	2.44 (0–50.2)	
Cockroach	0.59 (0–20.7)	
Clam	0.11 (0–8.95)	
Oyster	0 (0–8.25)	
Atopy history		
Asthma	9 (60%)	
AR	13 (86.7%)	
AD	8 (53.3%)	
FA other than shellfish	7 (46.7%)	
EGID	0 (0%)	
Onset of symptoms (minutes)	5 (5–30)	

TABLE 2 (Continues)

	Total
Symptoms	
Urticaria	4 (26.7%)
Angioedema	5 (33.3%)
Ocular	2 (12.5%)
Respiratory	0 (0%)
GI	0 (0%)
Treatment	
Antihistamine	6 (40%)
Epinephrine	0 (0%)
Steroids	1 (6.7%)
Albuterol	0 (0%)

Note: SA patients with a history of shrimp-induced allergic reactions, reaction on clinical oral food challenge and positive immediate skin prick testing (IHST) and/or shrimp sIgE ImmunoCAP™ and component ISAC™ levels were included. Data for age of onset, skin test, sIgE (kU/L) and onset of symptoms (minutes) presented as median (range).

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; EGID, eosinophilic gastrointestinal disease; FA, food allergy.

Asian Black tiger shrimp in US shrimp allergic subjects, implicating shared diagnostic and therapeutic potential for SA to varied shrimp species.

AUTHOR CONTRIBUTIONS

Sara Anvari, Contributed analysis tools, performed the analysis and wrote the paper. Shea Brunner, Contributed data, analysis tools and wrote the paper. Karen Tuano, Collected the data. Brenda Bin Su, Contributed analysis tools. Shaymaviswanathan Karnaneedi, Collected the data and performed analysis, and wrote the paper. Andreas L. Lopata, Conceived and designed analysis, performed the analysis and wrote the paper. Carla M. Davis, Conceived and designed analysis, collected data, and wrote the paper.

KEYWORDS

anaphylaxis, food allergy, shrimp allergy, shrimp oral immunotherapy, tropomyosin

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CONFLICT OF INTEREST

Drs. Anvari and Davis receive research contract funding from DBV Technologies, Regeneron and Aimmune Therapeutics. Dr. Davis is a

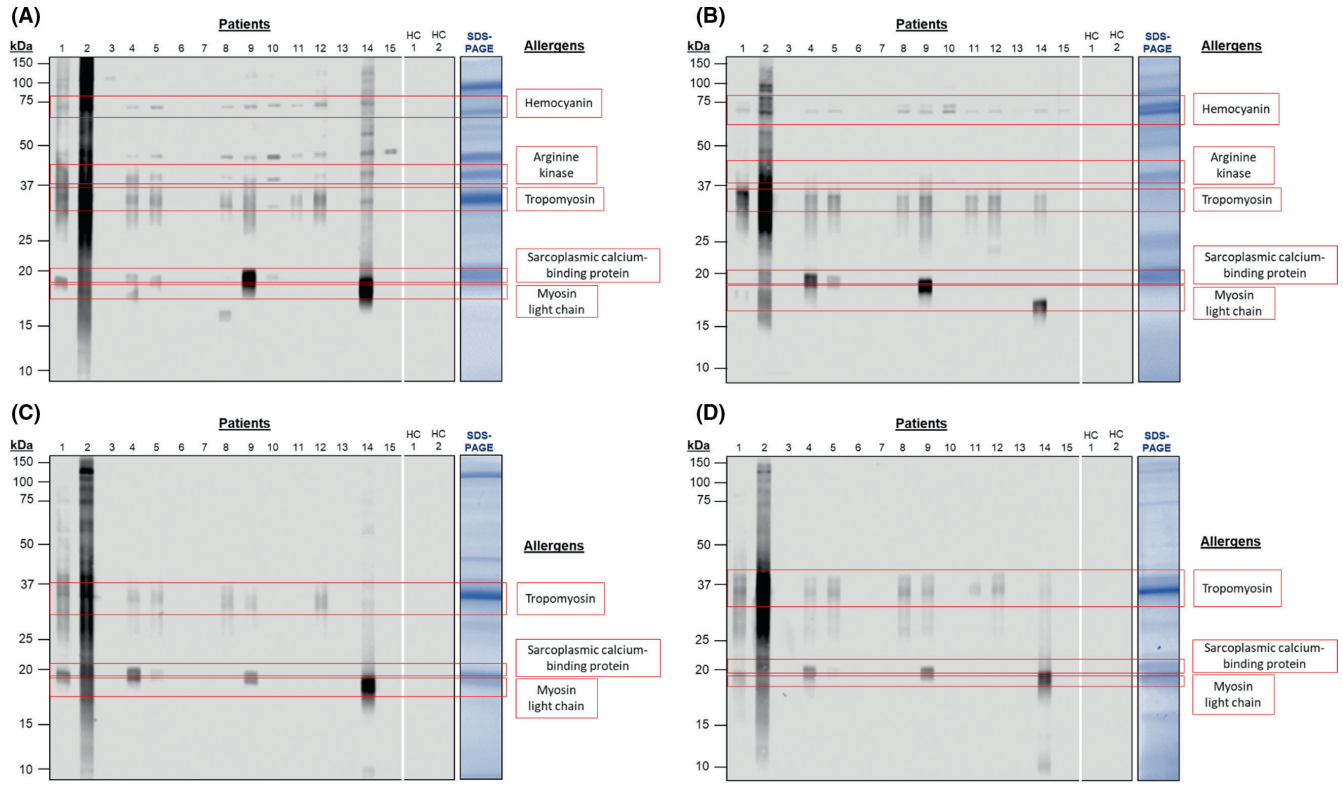

FIGURE 1 (A, B, C, D) Western blot analysis of the major and minor shrimp allergen proteins for different shrimp species in shrimp allergic and healthy controls (A) *Penaeus aztecus* (Brown shrimp), (B) *Penaeus monodon* (Black tiger shrimp), (C) heated extract of *Penaeus aztecus* (Brown shrimp), (D) heated extract of *Penaeus monodon* (Black tiger shrimp). The location of sIgE to proposed allergens are highlighted with red boxes. HC, healthy control

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SUPPORTING INFORMATION

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Anti-inflammatory effect of siRNAs targeted *IL-4* and *IL-13* in a mouse model of allergic rhinitis

To the Editor,

Allergic rhinitis (AR) is a chronic inflammatory disease of upper airways characterized by sneezing, itching, nasal congestion, and rhinorrhea. AR pathogenesis includes the elevation of allergen specific IgE in serum, inflammatory cell infiltration to nasal mucosa, and overexpression of Th2-cytokines (IL-4, IL-5, and IL-13). Current pharmacotherapy using intranasal corticosteroids, antihistamines, and anti-leukotriene medications results in symptom relief, but fail to regulate the underling pathogenesis. Anticytokine therapy mainly with monoclonal antibodies (Mab) is a promising approach for the treatment of asthma and other allergic conditions. Dupilumab targeting receptor subunit IL-4Rα (common for both IL-4 and IL-13) is now approved for severe asthma and atopic dermatitis treatment and was found efficient in uncontrolled persistent asthma and comorbid AR.[1] However, the high cost of this drug limits its broad implementation into clinical practice, and there is a need to develop effective and available therapies to suppress Th2 cytokines. RNA interference provides promising approach for anti-cytokine therapy of inflammation though sequence-specific silencing of disease-causing genes using small interfering RNAs (siRNAs).[2] In the current study, we aimed to evaluate the anti-inflammatory effect of nasal administration of siRNAs targeted *il-4* and *il-13* in mouse model of AR.

The mouse anti-IL-4 and IL-13 siRNAs[3,4] as well as branched (dendrimeric) cationic peptide LTP for nucleic acids delivery into mammalian cells, including lymphocytes (important source of IL-4 and IL-13)[5] were designed and tested previously. In the current study, we confirmed the ability of siRNA/LTP complexes to transfect lymphocytes. The anti-IL-4 and IL-13 siRNAs/LTP complexes (at mass ratio 1/12.5) substantially reduced the production of these cytokines in concanavalin A (ConA)-stimulated thymocytes compared with non-specific siRNA (Figure 1A, B).

To reveal the therapeutic potential of the siRNA-based complexes mice with OVA-induced AR were intranasally threated with the siRNAs/LTP complexes at doses of 5 and 15 μg/mouse. Corticosteroid budesonide which is widely used in the indications of AR and allergic asthma treatment served as a positive control. The group of intact mice treated with PBS only served as negative control. Intranasal instillation of the complexes containing siRNAs against IL-4 and IL-13 effectively suppressed the production of these Th2-cytokines by lymphocytes in the local lymph nodes (Figure 1C,D), while did not influence IFNγ level (Figure 1E). Moreover, we found decreased serum levels of OVA-specific IgE and IgG1, but not IgG2a (Figure 1F-H), that indicates the shift towards Th1 response after siRNA-mediated suppression of IL-4 and IL-13. It is important to note that non-specific siGFP did not exhibit effects on IL-4 and IL-13 expression neither in

Igor and Alexandra equivalent contribution.