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Shellfish allergy: "what is on the menu?"

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Abstract

IgE-mediated shellfish allergy constitutes an important cause of food-related adverse reactions. Shellfish are classified into molluscs and crustaceans, the latter belonging to the class of arthropoda. Among crustaceans, shrimps are the most predominant cause of allergic reactions and thus more extensively studied. Several classes of major and minor allergens have been identified and cloned. Amongst them invertebrate tropomyosin, arginine kinase, myosin light chain, sarcoplasmic calcium-binding protein and hemocyanin are the most relevant. Shellfish can trigger non-immune and immune adverse food reactions that are sometimes difficult to discriminate. The clinical presentation of shellfish allergy varies from mild allergic reactions to severe life-threatening anaphylaxis and can also present as a food-dependent exercise-induced anaphylaxis. In clinical practice, physicians generally rely on quantification of specific IgE antibodies and skin testing, however correct diagnosis of shellfish allergy is not always straightforward mainly because of uncertainties associated with the correct identification of the offending species by the patient and the low specificity of traditional diagnostics given cross-reactivity issues. In cases where traditional tests yield equivocal or negative results additional diagnostics like basophil activation tests and food challenges might be required. Hitherto, avoidance of the culprit and clinically significant cross-reacting species remains the cornerstone of correct treatment.

Key words: allergy; cross-reactivity; crustaceans; epidemiology; IgE; molluscs; shrimp

Word count: 4108

Abbreviations:

CCD; Crossreactive Carbohydrate Determinant

DBPCFC; Double-Blind Placebo-Controlled Food Challenge

FcεRI; high-affinity IgE receptor

FDEIA; Food Dependent Exercise Induced Anaphylaxis

GDPH; Glyceraldehyde Phosphate DeHydrogenase

MLC; myosin light chain

OAS; Oral Allergy Syndrome

SCP; Sarcoplasmic Calcium-binding Protein

SERCA; Smooth Endoplasmic Reticulum Ca^{++} ATPase

sIgE; specific Immunoglobulin E

SPT; Skin Prick Test

TpC; Troponin-C

Introduction

Despite the name, shellfish are not fish but simply water dwelling animals. “Shellfish” is a fisheries and culinary term for exoskeleton-bearing aquatic invertebrates, comprising various species of crustaceans, molluscs and echinoderms (Figure 1). Although predominantly harvested from saltwater, some species are found in freshwater and on land, such as the blue land crab (*Cardisoma guanhumi*). As addressed by Lee *et al* (1), within the phylum crustaceans “shrimps” and “prawns” are loosely used terms and are often utilized interchangeably. A “shrimp” in the USA is known as a “prawn” in Australia and the United Kingdom. This generic use might be confusing because it may refer to species belonging to separate families. Within the class *Decapoda* (that also includes crabs, crayfish and lobster), “shrimps” and “prawns” usually refer to the group *Penaeidae*. However, another taxonomical separate *Decapoda* family exists, the *Caridae*, which is also referred to as “shrimp”. Well-known edible representatives of the *Caridae* are the giant fresh water shrimp (*Macrobrachium rosenbergii*) and the northern shrimp (*Pandalus borealis*).

Adverse reactions to crustacean and molluscan shellfish can be defined as any aberrant reaction after its consumption, and can be of toxic/infectious or non-toxic origin (Figure 2). Toxic-reactions can occur in anyone, provided the ingested dose of natural or contaminating toxin is sufficient (e.g. phycotoxins such as saxitoxins, ciguatoxins, brevetoxins, gambierol, palytoxins). Infectious reactions can be caused by parasites (e.g. the nematode *Anisakis simplex*), bacteria (e.g. *Vibrio*, *Klebsiella*, *Pseudomonas*) and/or viruses, particularly Hepatitis A and norovirus. Non-toxic/infectious reactions depend on the individual susceptibilities and may originate from both a non-immunological or immunological (type I hypersensitivity or IgE mediated allergy) mechanism, resulting from exposure to the shellfish itself or to various non-seafood components in the product. Well-known examples of non-immunological reactions that can mimic shellfish allergy are histamine poisoning and adverse reactions to various additives such as preservatives and spices. Because of the similarity between immunological and non-immunological reactions, knowledge of the underlying pathomechanism and easy access to reliable *in vitro* and *in vivo* diagnostic tools is of paramount importance for correct diagnosis and management.

Epidemiology

In the absence of well-controlled epidemiologic studies using controlled food challenges for diagnosis, data on the exact prevalence of shellfish allergy remain exceedingly scarce. Nevertheless, it seems that the prevalence of shellfish sensitization and allergy is increasing and might exhibit geographical and age-dependent differences, mainly as a result of different nutritional habits, exposure to cross-reactive allergens and last but not least the applied screening technique. For instance, a telephone survey conducted in the USA in 2002 revealed that 2% of the 14.948 participants had experienced an allergy to seafood and that seafood allergy was almost 5 times more prevalent in adults than in children (2). The rate of adverse reactions to crustaceans and molluscs was respectively 38% and 49%, and only 14% of patients with crustacean allergy reported also allergic symptoms upon exposure to molluscs, suggesting no uniform clinical cross-reactivity between crustaceans and molluscs. Note, however, that telephone surveys are unable to discriminate between genuine allergies and non-immunological reactions. In a recent European survey conducted between 2005 and 2009, the estimated weighted prevalence of IgE sensitization to shrimp in adults varied between 2.76% in Reykjavik (Iceland) and 6.89% in Zurich (Switzerland) (3). It cannot be excluded that these sensitization rates (partially) result from sensitization to alternative causes as the highest consumption of seafood in Europe is reported in Iceland (91 kg live weight per capita per year) (4). In a systematic review of western/northern European studies, the overall pooled estimates for all age groups of self-reported lifetime prevalence of allergy to shellfish was 1.3% (95% confidence interval: (0.9-1.7%) and the prevalence of food-challenge-defined allergy to shellfish was 0.1% (0.06-0.3%) (5). In various Asian countries, shellfish is ranked amongst the top most common causes of food allergy (1) with self-reported rates of shellfish allergy readily attaining 5% of adolescents in the Philippines and Singapore. In another study conducted between 2003 and 2006, showed a positive skin response to shellfish in 39% of Singaporean children with food allergy (6).

Pathomechanisms and routes of sensitization

Shellfish allergy mainly presents as genuine IgE-mediated food allergy that originates from a primary sensitisation through exposure via the gut where various immunologic and non-immunologic mechanisms prevent the food allergen to enter the body and/or to elicit an immune reaction. However, some food allergens “escape/evade” from these surveillance mechanisms and encounter specialized antigen-presenting cell populations in the gut that govern cell priming and polarization (generally to Th2). In IgE-mediated allergy, this process finally triggers immunoglobulin isotype switching resulting in the synthesis and secretion of allergen-specific IgE (sIgE) antibodies by plasma cells. Tissue-resident effector cells, mainly mast cells, are loaded with this sIgE that preferentially binds to the high-affinity IgE receptor (FcεRI). Upon re-exposure to the allergen, the cross-linking of neighbouring sIgE antibodies bound to the high-affinity IgE receptor (FcεRI) causes effector cell activation and release of a myriad of mediators responsible for the allergic reaction. Sensitization to shellfish allergens can also occur via the cutaneous (7, 8) and respiratory route (8-10). Furthermore, IgE-mediated shellfish allergy can also be acquired indirectly and result from cross-reactivity towards structural homologous epitopes present in non-related invertebrates such as dust and storage mites (11-15) and cockroaches (16-18). Whether house dust mite immunotherapy can induce shellfish allergy remains controversial with pros (19-21) an cons (22), and might be related to differences in particular components (e.g. tropomyosin) present in the immunotherapy preparations. Finally, it is of note that "genuine" shellfish allergy can result from an IgE-mediated allergy to (hidden) contaminants (e.g. *Anisakis simplex* parasiting shellfish).

Clinical manifestations

As reviewed by Lopata *et al* (23) and Taylor SL (24), IgE-mediated shellfish allergy presents as a variety of symptoms related to the site and extent of mast cell and/or basophil degranulation that may occur within minutes after ingestion of the allergen. However, late-phase reactions have also been reported up to 6-8 hours after ingestion, particularly to snow crab, cuttlefish, limpet and abalone. In oral allergy syndrome (OAS), often experienced by crustacean allergic patients, the reaction is limited to the oropharynx (itching or tingling of the lips, tongue, palate, and throat, swelling of lips and/or tongue), or throat with sensation of tightness and/or hoarseness, dysphonia, and/or dry cough. Gastrointestinal symptoms may include abdominal pain, nausea, vomiting, diarrhoea, and colic. In other cases more systemic reactions may involve skin and mucosa (pruritus, erythema, acute urticaria and/or angioedema), nose and eyes (rhinorrhoea, sneezing, nasal itching and/or congestion, tearing, burning or itching eyes, redness of eyes), lungs (cough, dyspnoea, wheezes, tightness). In severe cases, patients may present with cardiovascular symptoms including hypotension, shock and cardiac dysrhythmias. Fatalities have been reported, even by exposure to trace amounts hidden in a kiss. An interesting observation regarding the clinical manifestations of shrimp allergy is the intra-individual variation of clinical severity in which reactions vary from mild to life-threatening. Possible explanations for these variations are: differences in allergenicity of the species consumed, effect of cooking and boiling (see below), the presence of aggravating co-factors (intake of non-steroidal anti-inflammatory drugs, alcohol, menstruation, proton pump inhibitors) and variation in the part of the shrimp eaten (25). A particular clinical presentation of shellfish allergy is the food-dependent exercise-induced anaphylaxis (FDEIA). In this condition, anaphylaxis occurs within 2-6 hours after consumption of food, but in the absence of exercise ingestion of the food is tolerated (26-28). Another important element appears to be the ingested dose needed to cause anaphylactic reactions. Bernstein *et al* (29) reported that patients in a double-blind placebo-controlled challenge (DBPCFC) might react to 14 g of freeze dried cooked shrimp. Daul *et al* (30) reported positive challenges at a cumulative dose of 16 g of whole cooked shrimp. In another study some patients already reacted at doses of about 1-3 g lyophilized cooked shrimp, whereas the eliciting dose for raw lyophilized shrimp generally exceeded 15 g (31). Using DBPCFC, the accumulated amount of dried snail causing a 20% drop in FEV1 was as little as 120 mg (21). Allergic symptoms not only result from ingestion of shellfish but can also be triggered by inhaling (cooking) vapours that contain airborne allergen and handling food in the domestic and occupational environments (7, 10, 32). In these types of exposure anaphylaxis is rare and symptoms mainly manifest as rhinoconjunctivitis, bronchospasms and/or dermatitis. The prevalence of occupational asthma in shellfish-processing personnel is estimated between 2% and 36%, and occupational protein dermatitis between 3-11% (8, 9, 23, 32). Occupational sensitization might also occur in restaurant workers (33).

Allergens

Table 1 summarises the most relevant shellfish allergens that have currently been characterized. Tropomyosin is considered to be the major allergen in shellfish allergy. Actually, already in the early eighties Hoffman *et al* (34) identified a heat-stable IgE-binding allergen in shrimps, that was later identified as tropomyosin in brown shrimps (*Penaeus aztecus*) reacting with 28/34 (82%) of shrimp-sensitive individuals (35). Moreover tropomyosin has been identified as a pan-allergen of many invertebrate species including various crustaceans (shrimp, lobster, crab), molluscs (mussels, oysters, scallops, octopus, squids, snails, abalones, whelk, clams, razor shell), cockroaches and mites (11, 17, 18, 36-43). Tropomyosins are present in both muscle and non-muscle cells. In striated muscle tropomyosins mediate the interaction of troponin-actin complex to regulate muscle contraction. Note that, tropomyosins from crustaceans share a high homology (up to 98%), whereas the amino acid sequence identity between crustacean and mollusc tropomyosin is about 65%. Vertebrate tropomyosins, that share about 55% of sequence homology with invertebrate tropomyosin, seem to be non-allergenic (40). At present, different IgE-binding B cell and putative T cell epitopes have been described in tropomyosin (44-49) and it has been suggested that children recognize a greater epitope repertoire than adults suggesting shrimp sensitization to decrease over age (47). In this study IgE-binding epitopes were also identified in arginine kinase, myosin light chain and sarcoplasmic calcium-binding protein.

Recently a recombinant Pen a 1, the tropomyosin from *Penaeus aztecus* has become available for molecular diagnostic testing.

In addition to tropomyosin, several other allergenic components have been identified in shellfish. In 2003 Yu *et al* identified a novel allergen in *Penaeus monodon* (black tiger shrimp) (50). The allergen was designated as Pen m 2 and was demonstrated to have arginine kinase activity. Arginine kinase is highly abundant in invertebrate muscle and hitherto, arginine kinases have also been described in various other shellfish species as well as other invertebrates such as mites, cockroaches, crickets, silk worms and the Indian meal moth (13, 51-61). Unlike tropomyosins, arginine kinases display unstable physicochemical properties and do not resist thermal and acid-base treatment (62, 63).

A third allergen in shellfish is myosin light chain (MLC) that was first described by Ayuso *et al* (64) in the Pacific white shrimp *Litopenaeus vannamei* and designated as Lit v 3 and subsequently also in various taxonomically distant and related invertebrates such as other shrimp species (47, 65, 66) and cockroaches. Like tropomyosin and arginine kinase, MLC display a significant amino acid sequence homology with homologues such as Bla g 8 from the German cockroach (*Blatella germanica*) (64). MLC molecules are highly resistant to heat, acid–alkali, and digestion, and retain weakly IgE-binding activity when their secondary structure is altered (67).

A forth shellfish allergen is the heat resistant sarcoplasmic calcium-binding protein (SCP), an EF-hand-type protein. It constitutes an allergen in various crustaceans such as shrimp and crayfish (13, 47, 60, 65, 66, 68-70). Like other allergenic components, SCPs from taxonomically related species demonstrate a significant amino acid sequence homology that varies between 80-98% for crustaceans, whereas homology between crustacean and molluscan SCPs is only 15-21% (71). Various epitopes have been identified for shrimp MLC, AK and SCP (47, 49).

Hemocyanin, isolated from hemolymph is another heat stable shrimp allergen (58, 60, 72, 73) and cross-reacts with its homologue in house dust mite (60) and probably also snail (74). In one of these studies, the authors elegantly demonstrated that sensitization to hemocyanin can lead to a selective allergy to the giant fresh water shrimp (*Macrobrachium rosenbergii*) in patients tolerating a DBPCFC with black tiger shrimp (*Penaeus monodon*) (72). The explanation for these selective allergies should probably be sought in the low amino acid sequence homology of only 19-27% between both shrimp species that respectively belong to the *Caridae* and *Penaidae*.

Troponin-C (TpC) was first described as an allergen in North Sea shrimp (*Crangon crangon*, Cra c 6) (66), recognized by 9/31 (29%) of the patients and later in the northern shrimp (*Pandalus borealis*), recognized by 33% of the patients (75). In a recent study by Pascal *et al* (15) sensitization to TpC was found in 17.2% of 58 shrimp allergic patients. TpC has also been identified in cockroach (76) and storage mites (*Tyrophagus putrescentiae*) (77). Other potential allergens identified in shellfish are paramyosins (identified in many mollusc species (78) and *Anisakis simplex* (79)), triose phosphate isomerases such as Cra a 8 (65, 66), myosin heavy chain (73), α- and β-actin (13, 43, 59), smooth endoplasmic reticulum Ca^{++} ATPase (SERCA) (59), glyceraldehyde phosphate dehydrogenase (GDPH), titin (65), ubiquitin (13).

As indicated in the table 1, several allergenic components such as tropomyosin, MLC and SCP are resistant to heat. Moreover, boiling might even enhance their allergenicity (42, 80), e.g. by creating neo-epitopes during Maillard reactions (81). In the study by Jirapongsananuruk *et al* (31), the proportion of positive challenges to raw shrimp was lower than to cooked shrimp. For a review on the effect of various physical and chemical treatments on the allergenicity of various shellfish tropomyosins the reader is referred elsewhere (82).

Cross-reactivity

Cross-reactivity between shellfish species is a common phenomenon and involves several components that are currently extending beyond the major allergen tropomyosin. As a matter of fact, cross-reactivity between shellfish species might involve almost all allergenic components that have been identified so far. However, this paragraph focuses on tropomyosin as most of our knowledge gathered today relates to this component. As mentioned above, amino acid homology between the tropomyosins of phylogenetically related crustaceans attains up to 98% (83, 84). For molluscan shellfish, a comparable intra-class amino acid similarity exists for tropomyosins of gastropods (85-91%) (85, 86), cephalopods (91-100%) (87) and bivalves (70-100%). The homology between the different molluscan classes varies from 68-100%. In contrast, amino acid homology between crustacean and molluscan tropomyosin is lower, 56-68% (87). For example, sequence homology between Tod p 1 from the cephalopod *Todarodes pacificus* and Pen o 1 from *Penaeus orientalis* (Chinese white shrimp) is 62% (36). There is 81% of amino acid homology of tropomyosin between shrimp and mites, and 82% between shrimp and cockroach. Sequence homology between tropomyosin from shrimp and the nematode *Anisakis simplex* (Ani s 3) is 75% (88) and between shrimp and fish (e.g. tilapia (Ore m 4, *Oreochromis mossambicus*) is about 55% (89). Obviously, this high molecular homology of shellfish tropomyosins translates to significant degrees of IgE cross-reactivity between various shellfish species (36, 37, 83, 84, 86, 90, 91) or between shellfish and other invertebrates (11, 13, 17, 18, 40, 46, 88, 92-95). For example, Leung *et al* demonstrated that sera from shrimp allergic patients contain IgE antibodies against molluscs, grasshopper, cockroach and fruit fly but not to chicken or mouse (90). Furthermore, from these papers it appears that particularly house dust mite and cockroach tropomyosins have been demonstrated to account for the presence of sIgE to shrimp, even in unexposed subjects such as Orthodox Jews who abstain from shellfish according to kosher law (96). The opposite, with the occurrence of mite and cockroach allergy as a result of primary sensitization to crustaceans has also been suggested (46). Besides that, species-specific allergies have been demonstrated (2, 31, 97), as demonstrated by Jirapongsananuruk *et al* (31), in which the idea of shellfish allergy as a pan-allergy was rejected by demonstrating specific allergies to *Penaeus monodon* and *Macrobrachium rosenbergii*.

With this in mind, the recommendation that all patients with shellfish allergy should strictly avoid all crustaceans, molluscs and edible insects may not be absolutely accurate. Larger studies with challenge-documented patients focusing on the understanding of clinical cross-reactivity are absolutely mandatory to better understand and properly manage shellfish allergy. It is also a general misbelieve that shellfish allergens cross-react with fish allergens. As a matter of facts, the major fish allergen is parvalbumin that cross-reacts with other parvalbumins (e.g. in frog) (98). Tropomyosin is not a major allergen in fish. Actually the particular finding of a high prevalence of sIgE antibodies to tropomyosin found in fish allergic patients published by Liu *et al* (89) probably results from the fact that the majority of patients had inflammatory bowel disease, and these patients probably synthesise antibodies against human tropomyosin isoform 5 that has a C-terminal peptide virtually identical to Ore m 4, the tropomyosin of tilapia.

Diagnosis

Diagnosis of shellfish allergy starts with a thorough clinical history complemented with quantification of sIgE antibodies, skin testing, basophil activation assays and when needed controlled oral challenges. However, correct diagnosis is not always straightforward. Some difficulties and uncertainties are associated with the identification of the ingested species that is compounded by the misuse of popular names. For example, we observed a series of patients with an allergy that was initially erroneously attributed to scampi, or Norwegian lobster (*Nephrops norvegicus*). However, a careful reading of the package leaflets revealed that the reactions must have resulted from a giant freshwater shrimp (*Macrobrachium rosenbergii*), frequently used as a surrogate for scampi (99). Moreover, by closely inspecting the leaflets of different brands, "scampi" appears to be a very popular and fancy name used for various shrimp species. Clinical history can also be difficult to address due to the presence of co-factors, the similarity between non-immunological and immunological reactions, the fact that patients are sensitized to allergens present in particular body parts (25) or the presence of a hidden (non-seafood) allergen.

For many physicians detection of sIgE constitutes the first measure to confirm their clinical suspicion of a shellfish allergy. To detect sIgE in serum several assays are currently available: shrimp mix (*Pandalus borealis*, *Penaeus monodon*, *Metapenaeopsis barbata*, *Metapenaeus joyneri*), lobster (*Homarus gammarus*), langust (spiny lobster, *Palinurus spp*), crab (*Chionocetes spp*), clam (*Ruditapes spp*), crayfish (*Astacus astacus*), oyster (*Ostrea edulis*), Pacific squid (*Todarodes pacificus*), squid (*Logilo spp*), scallop (*Pecten spp*), snail (*Helix aspersa*), and finally rPen a 1 (a recombinant tropomyosin from *Penaeus aztecus* that is free of CCD). However, the predictive value of sIgE quantification to identify true allergic individuals is not absolute. The presence of sIgE antibodies is not always associated with clinical reactions; in contrast patients with a compelling history might not have detectable sIgE antibodies (99-101), therefore many studies have been evaluating the added value of component resolved diagnosis (CRD). In this context sIgE to tropomyosin from *Penaeus aztecus/P. setiferus* has been shown to be more specific than sIgE to the shrimp mix with respectively 2 and 7 clinically irrelevant results out of 28 individuals tolerant to Pacific white shrimp (*L. vannamei*) (100). Similar results have been demonstrated by Gamez *et al* (102), who found shrimp sIgE and rPen a 1 to be positive in respectively 61% and 33% of shrimp tolerant individuals. Whether higher values of sIgE to shrimp and rPen a 1 are suggestive for mollusc allergy in crustacean allergic patients, needs to be confirmed in larger series (97). Unfortunately, tropomyosin, although a major allergen in various shellfish species, does not cover the entire IgE anti-shrimp response and additional components such as arginine kinase, MLC, SCP, TpC and triose phosphate isomerase might be necessary to establish correct diagnosis in particular cases and to study cross-reactivity with other invertebrates (15, 39, 60, 66, 72, 103). In our own series, sIgE reactivity to tropomyosin (rPen a 1) was demonstrable in only 5 out of 20 patients allergic to *Macrobrachium rosenbergii*. In these cases adding a hemocyanin sIgE assay to the diagnostic instrumentation could have optimized serological diagnosis (56). Bauermeister *et al* demonstrated that using a panel of 6 recombinant proteins (tropomyosin, arginine kinase, MLC, SCP, TpC and triose phosphate isomerase) from the North Sea shrimp *Crangon crangon* identified 90% of shrimp allergic patients (66). Similar observations were made by Pascal *et al* (15) who demonstrated tropomyosin to be the dominant allergenic component recognized in 83% of shrimp allergic patients and 44% of shrimp tolerant individuals. Of the other 9 components tested, arginine kinase had the highest sensitivity (48%), followed by MLC (38%) and SCP (34%). However, even with the complete panel the false negativity rate was still 12%.

Furthermore, whether epitope recognition constitutes a reliable technique to document shellfish allergy remains to be further confirmed, although several informative IgE binding epitopes have been identified (15, 49).

Skin prick tests (SPT) permit to rapidly investigate patients with a clinical suspicion of shellfish allergy. However, like sIgE in serum, SPT do not demonstrate an absolute predictive value for allergy and performances might significantly differ according to the used extract. For example, Carnes *et al* (80), demonstrated that boiled crustacean extracts might identify (slightly) more patients than raw extracts. More recently, Jirapongsanaruk *et al* (31) showed that prick-prick tests with fresh shrimp or home-made shrimp extract might have a somewhat higher sensitivity than a commercial SPT extract (>97% vs. 88%), provided an extract-specific decision threshold is applied. However, like for quantification of sIgE, specificity of SPT was disappointingly low (0%-37.5%).

Upon encounter of allergens that bridge membrane-bound sIgE molecules mast cells and basophils not only secrete quantifiable mediators but also up-regulate the expression of various membrane proteins that can easily be quantified using flow cytometry (104). For instance, we have shown the technique to be particular useful to diagnose allergy from *Macrobrachium rosenbergii*, a condition in which quantification of sIgE and skin tests frequently yielded a false negative result (99).

Although DBPCFC is considered to be the gold standard in food allergy diagnosis, it has not entered mainstream application mainly because of ethical, practical and economic reasons. Actually, DBPCFC remains a dangerous endeavour and is costly. As already addressed in the section on clinical manifestations, the minimal dose eliciting symptoms might vary considerably according to the source and thermal processing of the food. For the time being there is no standardized protocol for DBPCFC with crustaceans.

Therapy

For the moment, in the absence of a cure, strict allergen avoidance of the culprit and clinically relevant cross-allergens remains the corner stone of shellfish therapy. However, correct therapeutic management of shellfish allergy might not always be straightforward for several reasons. First, correct identification of the culprit species is not always evident and problems are compounded by unspecific terminology. Second, because of the low specificity of traditional diagnostics overdiagnosis is not exceptional, mainly in atopic patients suffering from house dust mite and cockroach allergy. Third, patients may develop symptoms upon exposure to airborne allergen (e.g. cooking vapours). Forth, exposure to hidden allergen cannot always be ruled out. Fifth, in some cases a cofactor may be needed for the clinical expression of sensitization. Whether the development of hypoallergenic mutant components (105) or T cell epitopes (45) will enable the development of immunotherapeutics to treat shellfish allergy remains to be established.

Table 1.

Allergenic components in shellfish (adapted from (65) and (82))

Component	MW Weight (kDa)	Allergen nature and function	Heat resistance	Examples (not exhaustive)	Route of exposure
Tropomyosin	34-39	Muscle contraction	Yes	Pen a 1, Lit v 1, Tod p 1, Der p 10, Der f 10, Bla g 7, Per a 7, Hom a 1, Cra c 1, Cha f 1, Mac ro 1,	Ingestion Inhalation
Arginine kinase	40-45	Energy for muscle contraction	No	Pen m 2, Lit v 2, Cra c 2, Art fr 2, Der p 20, Bla g 9, Per a 9	Ingestion Inhalation
Myosin light chain	17-20	Muscle contraction	Yes	Lit v 3, Cra c 5	Ingestion
Sarcoplasmic calcium-binding protein	20-25	Regulation muscle contraction	Yes	Lit v 4, Pen m 4, Cra c 4	Ingestion
Hemocyanin	± 75	Copper containing protein – oxygen carrier	Yes	Bla g 3, Per a 3	
Troponin-C	17.7	Muscle contraction / relaxation	Yes	Cra c 6, Bla g 6	
Triose phosphate isomerase	26-29	Glycolysis	No	Cra c 8	Ingestion Inhalation
Paramyosin	100	Muscle contraction			
Titin	70	Passive muscle elasticity	Yes		Ingestion
Fructose 1,6 biphosphate aldolase	39-43	Glycolysis	No		Ingestion Inhalation
Myosin heavy chain	225	Muscle contraction			
α-actin	31-42	Muscle contraction			
SERCA	113	Enzyme			
GADPH	37	Enzyme			
Ubiquitin	8.5	Enzyme	No		

Pen a: *Penaeus aztecus* (brown shrimp), Lit v: *Litopenaeus vannamei* (Pacific white shrimp), Pen m: *Penaeus monodon* (black tiger shrimp), Art fr: *Artemia franciscana* (San Francisco brine shrimp, Tod d 1: *Todarodes pacificus* (Japanse common squid), Der p: *Dermatophagoides pteronyssinus* (house dust mite), Der f: *D. farinae* (flour mite), Bla g: *Blatella germanica* (German cockroach), Per a: *Periplaneta americana* (American cockroach), Hom a: *Homarus americanus* (American lobster), Cra c: Crangon crangon (North Sea shrimp), Cha f: *Charybdis feriatus* (crucifix crab), Mac ro: *Macrobrachium rosenbergii* (giant fresh water shrimp)
For allergen components see www.allergome.org and www.allergen.org

Figure legends:

Figure 1: Classification of edible crustacean and molluscan shellfish species

Figure 2: Shellfish adverse food events

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We attest to the fact that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission. We have no conflict of interest to declare.

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