

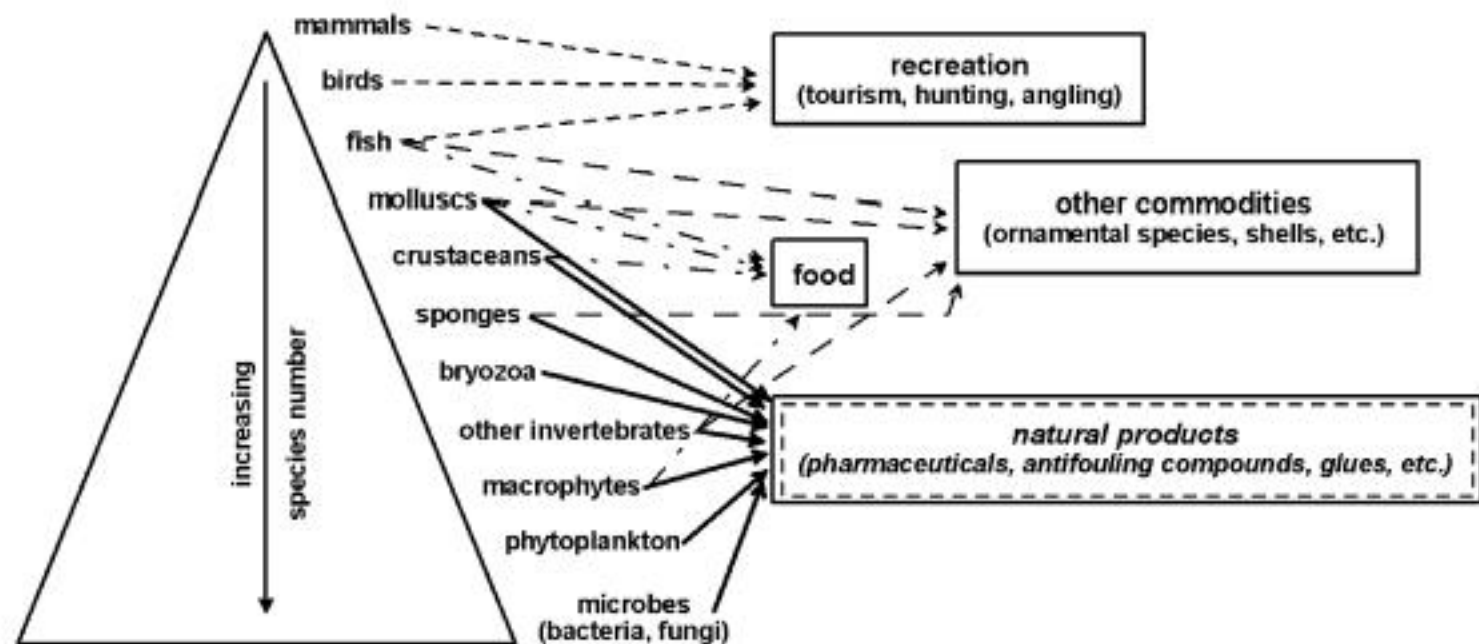


Fig.1 Diagram illustrating the services rendered by various classes of marine organisms to man

Seafood Allergy: Lessons from Clinical Symptoms, Immunological Mechanisms and Molecular Biology

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Abstract Food allergy consists of a wide range of disorders that result from adverse immune responses to dietary antigens. Manifestations of allergic response includes acute, potentially fatal anaphylactic reactions and a variety of chronic diseases that mainly affect the gastrointestinal tract, skin, and respiratory tract. Tools for clinical diagnosis and management, which have not changed much in the past two decades, include the clinical history, tests for specific IgE antibody to suspected foods, elimination diets, oral food challenges, and provision of medications such as epinephrine for emergency treatment. On the other hand, recent immunological and molecular biological research have

enhanced our understanding of the mechanisms of these disorders and revealed the identities of many food allergens. Here, we will discuss seafood allergies with respect to the clinical manifestations, diagnosis, immunological mechanisms, and molecular biology of seafood allergens. Furthermore, potential applications and future directions in the clinical management of seafood allergies are discussed.

Keywords Hypersensitivity · IgE · Tropomyosin · Epitopes · Allergen · Seafood

1

Food Allergy and Society

Allergies are the sixth leading cause of chronic diseases with approximately 9–16% of people suffering from allergies in the United States [1]. Treatment of allergies has a great economic impact in the United States, amounting to \$18 billion annually in health care costs [2]. Some of the different types of allergies are allergic drug reactions, which make up about 5–10% of all negative drug reactions [2], and food allergies, which occur in children less than 6 years old and approximately 1–2% of adults [3, 4]. Hughes and Mills [6] stated that around 33% of anaphylactic reactions are caused by food. About 4–8% of children suffer from some forms of food allergy [7, 8]. The death count from food allergies adds up to about 100 Americans per year, with most of them being children [5]. Gastrointestinal manifestations of food allergies include nausea, emesis, diarrhea, and abdominal cramping. Dermatological reactions (flushing, urticaria, angioedema and atopic dermatitis) [9, 10], respiratory reactions (wheezing and rhinitis) and ocular reactions (conjunctivitis) [11, 12] are frequent in patients with food allergies.

Food is an intrinsic part of human civilization. In recent years, seafood has been considered a healthier component of the human diet due to an increasing concern about dietary fat and cholesterol. In 2001, seafood consumption reached 14.8 lb per capita in the United States. Shrimp and tuna with an average per capita consumption of 3.4 and 2.9 lb, respectively, were the first and second most popular seafood items. Physiologically, food is being catabolized to provide energy and nutrients. Although the intake and breakdown of food are taken very much for granted in human life, food is frequently implicated in a variety of maladies, including food allergies. Currently, the only therapy for food allergy is strict avoidance of the food that causes allergy. Chemicals including cromoglycate histamine receptor antagonists and ketotifen are not recommended as prophylactic treatment of food allergy because of the conflict of their efficacy [13, 14]. Occupational hypersensitivity affects a wide spectrum of people ranging from fishermen, processing workers, fish meal factory workers, oyster shuckers, caterers, and cooks [15–18]. The growing demand for seafood and the increasing risk of getting seafood allergy make it important to elucidate the clinical symptoms, diagnosis, and immunological

mechanisms of seafood allergy and to identify the seafood allergen(s) at the molecular level.

2

Clinical Symptoms of Food Allergies

2.1

Gastrointestinal Manifestations of Food Allergy

Since the gastrointestinal tract is the first site to come into contact with food allergens, gastrointestinal symptoms are frequent in patients with food allergy. IgE-mediated reactions that cause nausea, vomiting, abdominal pain, and diarrhea may occur within minutes of ingestion. Repeated ingestion of allergenic foods may lead to poor appetite, intermittent abdominal pain, and malabsorption. The direct contact of allergenic food with the oral mucosa that causes itching and swelling of the lips, tongue, palate, and throat is called the oral allergy syndrome. Aside from IgE-mediated reactions, food allergies may cause other forms of gastrointestinal pathologies, including eosinophilic oesophagitis or gastroenteritis. Symptoms include gastroesophageal reflux, vomiting, abdominal pain, failure to thrive, low serum albumin, blood vomiting, and intestinal obstruction [19]. Elimination of the offending foods will lead to resolution of symptoms in 3–8 weeks.

2.2

Dermatological Manifestations of Food Allergy

IgE-mediated reactions to food often present as urticaria (hives) and angioedema (swelling). The onset of symptoms is rapid, sometimes within minutes of ingestion, and usually lasts for less than 24 h. However, patients are usually able to identify and avoid offending foods that cause the symptoms. Direct skin contact can also elicit a reaction, and this is sometimes seen in fishmongers and kitchen workers. It is now clear that food allergy is an important factor in the development of eczema in young children. In a recent study, 37% of children with moderate to severe eczema were found to be allergic to food by serum IgE tests and food challenge [20]. Although one-third of children with eczema and food allergies “outgrow” their allergies within 1–3 years [21], shellfish allergies tend to be an exception. It is therefore pertinent that all children with moderate to severe eczema should be tested for food allergies and, if present, dietary advice should be given. A 2–3 week elimination diet followed by gradual reintroduction of suspected foods one by one while keeping a symptom diary is often sufficient for diagnosis.

2.3

Respiratory Tract Manifestations of Food Allergy

Respiratory symptoms are common in patients who develop food-induced anaphylaxis, and are invariably present in fatal or near-fatal cases. Patients with a history of asthma or a history of previous severe food allergic reactions are especially susceptible to severe anaphylaxis. Food allergy is rarely a factor in allergic rhinitis; a survey of 323 patients with chronic rhinitis revealed that only two patients had nasal symptoms during blinded food challenges [22]. A study of 88 children with eczema and asthma revealed that 15% wheezed during food challenge, and 8% demonstrated a greater than 20% drop in lung function [23]. However, food-induced respiratory symptoms appear to be rare in patients without eczema.

2.4

Systemic Manifestations of Food Allergy

Systemic anaphylaxis is the most dangerous of all allergic reactions, and food allergy is the most common cause of anaphylaxis [24]. Any food protein can theoretically cause anaphylaxis but shellfish is especially likely. In our series of 84 shellfish allergic patients in Hong Kong, one-third gave a history of anaphylaxis. Reactions invariably occur within 60 min and may include urticaria, angioedema, respiratory difficulties, low blood pressure, throat swelling, abdominal pain, vomiting, and diarrhea. Severe reactions will lead to asphyxia, vascular collapse, irregular heartbeat, or myocardial infarction. The quantity of food required to induce a reaction is dependent on patient sensitivity, potency of the food allergen, and other unknown factors. Shellfish allergens may invoke a fatal reaction in microgram amounts. The sensitivity of individual patients may change with time. Some patients may have tolerated a certain amount of a particular food without significant ill effects in the past, but nevertheless developed a severe reaction when they ingest a similar amount a second time. One interesting form of anaphylaxis is the exercise-induced, food-specific anaphylaxis. These patients develop anaphylaxis if they exercise within 2–4 h of ingesting a food that they are allergic to. However, ingesting the same food without exercise will not elicit a reaction. Shellfish has been found to cause 8% of exercise-induced anaphylaxis [25].

3

Diagnosis of Food Allergies

The history is the most important tool in the diagnosis of food allergy. However, it is frequently inaccurate and only about 40% of such histories can be verified by food challenges [26]. Important points to note in the history in-

clude the nature of the reaction, the foods ingested at the time, the timing of the reaction relative to food ingestion, repeatability and frequency of the reaction, and other exacerbating factors such as exercise. Knowledge of food allergy patterns found in a community is also helpful in discerning the likely culprit. One must also bear in mind the “hidden allergens”, especially in processed food. For example, shellfish is often used in Chinese dumplings and shells are sometimes added to soup for flavor. Cross-contamination of cooking utensils can also cause problems. A food diary is often a helpful tool in revealing these hidden allergens. If an IgE-mediated food allergy is suspected, skin prick tests (SPT) are useful in screening for likely culprits. One must bear in mind that SPT have a low positive predictive value (hence high false-positive rate) but a high negative predictive value. Treatment based solely on positive SPT results would subject patients to unnecessary food avoidance. For patients who are on antihistamines or have significant skin pathologies that preclude the use of skin tests, measurement of serum food-specific IgE by the radioallergosorbent test (RAST) or the Pharmacia CAP test is warranted. When a list of possible candidate food allergens can be established, a diagnostic elimination diet can support the diagnosis. If the offending allergens are eliminated, there should be significant improvement in symptoms. After two weeks of allergen elimination, a food challenge should be carried out. In cases where there is a risk of severe anaphylaxis, or if the history is strongly supported by SPT results, then food challenge can be omitted. Food challenges can be done open, single-blind or double-blind. Double-blind, placebo-controlled food challenge is the gold standard for the diagnosis of food allergies and should be used when patient perception might significantly influence symptom assessment.

4

Immunological Mechanisms

Ingested food is first processed through the gastrointestinal tract, which forms an extensive barrier to the outside environment. Although the mucosal immune system associated with the intestinal tract (gut-associated lymphoid tissue) is proficient in inhibiting immune responses to non-dangerous antigens and mounting a rapid response to pathogens, about 2% of ingested food is absorbed and transported throughout the body in an immunologically active form [4, 27]. Food antigens, are typically poor immunogens and induce a state of unresponsiveness (oral tolerance). Thus intact food antigens do not typically cause clinical symptoms because most individuals acquire tolerance. For example, unresponsiveness of T cells to ingested food proteins is believed to be due to T cell anergy. The regulation of T-cell responses in the intestinal mucosa by intestinal epithelial cells as antigen-presenting cells, the secretion of IL-10 and IL-4 by dendritic cells residing with the non-inflammatory en-

vironment of the Peyer's patches, and the production of transforming growth factor beta by T regulatory cells in the mucosal lymphoid tissue in response to low dose antigen are believed to contribute to oral tolerance [28–30].

Despite the phenomenon of oral tolerance, one may be sensitized to food allergens in the gastrointestinal tract after ingestion of food, which is classified as class 1 food allergy or after inhalation of an airborne allergen that crossreacts with a specific food, which is considered a class 2 food allergy [31]. IgE-mediated reactions develop when food-specific IgE antibodies residing on mast cells and basophils recognize and bind to circulating ingested food allergens and thus activate the cells to release a number of potent mediators and cytokines.

A typical sequence of events in the development of a hypersensitive food reaction consists of exposure to the antigen, activation of Th2 cells specific for the antigen, production of IgE antibodies specific for the antigen, binding of IgE to Fc receptors of mast cells, releasing of mediators upon exposure to the antigen, and subsequent pathology reaction. The development of an IgE-mediated response to an allergen is the result of a series of molecular and cellular interactions, involving antigen-presenting cells (APCs), T cells, and B cells (Fig. 1). APCs present small peptides in the context of MHC class II molecules to T cells. The binding of allergen/MHC class II complex to the complementary T cell receptor signals the T cells to proliferate and generate signals to promote an IgE response [32, 33]. Recent studies have substantiated the understanding of the roles of T cells in allergic inflammatory responses. A combination of interleukin 4 (IL-4), IL-5, IL-10, IL-13 produced by a subset of CD4+ T cells (termed Th2 cells) are found in increased abundance in allergic individuals [35]. CD4+ T cells play a role in the enhancement, recruitment, growth, and differentiation of other cell types in allergic inflammatory responses through cytokine secretion [34]. CD4+ T cells secrete IL-4 and IL-13, which enhance the induction of IgE synthesis in B cells, mast cell growth, and recruitment of lymphocytes, mast cells, and basophils to sites of inflammation [35]. In addition, CD4+ T cells produce IL-5, which enhances the growth and differentiation of eosinophils and B cells, and IL-10, which enhances the growth differentiation of mast cells and inhibits the production of IFN- γ . This may partly explain why non-allergic individuals are asymptomatic because they develop a Th1 response, which inhibits IgE synthesis and mast cell and eosinophil differentiation. A recent study by Watanabe et al. [36] suggested that food antigen might negatively select Th cells for IgE response to the food antigen by preferential deletion of Th1 cells in the liver. A novel mechanism of peripheral T cell response in atopic disease was described by Akdis et al. [37]. The Th1 compartment of activated memory/effector T cells selectively undergoes activation-induced cell death and thus skews the immune response towards surviving Th2 cells. The apoptosis of circulating memory/effector T cells was confined to atopic individuals whereas healthy controls showed no evidence for enhanced T cell apoptosis *in vivo*. It is interesting to note that IL-25, a recently reported cytokine, has

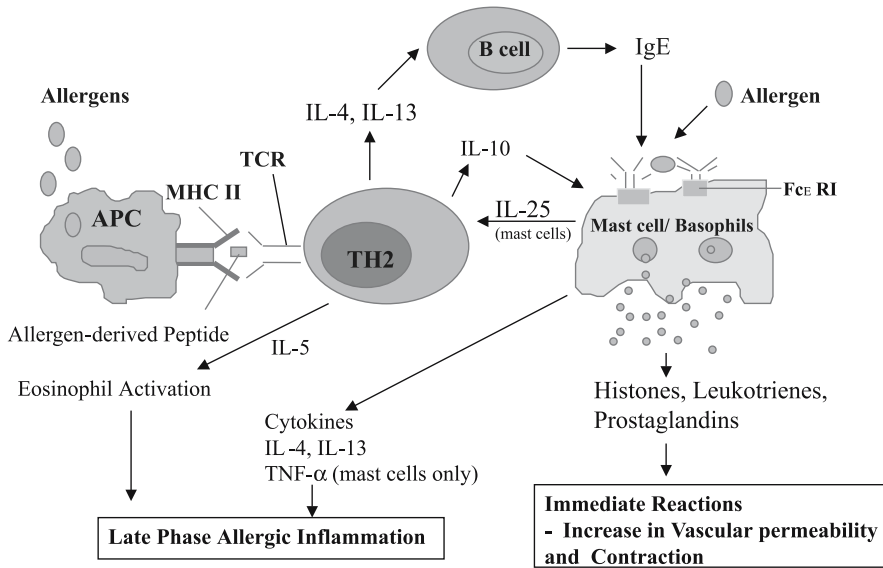


Fig. 1 Seafood allergens are first processed and presented by the antigen-presenting cells (APC) through the major histocompatibility complex class II (MHC II). CD4⁺ helper T cells (Th2) then recognize the allergens via the interaction between the T cell receptor (TCR) and MHC II, while IL-4 and IL-13 are secreted to promote the formation of plasma cell from B cell. Specific IgE are released by plasma cells and bind to the IgE receptors (FcεRI) on the surface of mast cells or basophils. Upon subsequent exposure to the same allergen, the cross-linking of the IgE receptors induce the degranulation of mast cells or basophils. A potent Th2 response inducer IL-25, is also secreted by mast cells. IL-10 is secreted by Th cells as positive feedback to mast cells. Chemical mediators released by the degranulation include histamine, prostaglandins, proteoglycans, leucotrienes, platelet-activating factors and cytokines, which are responsible for the hypersensitivity reaction. The secretion of IL-4 and IL-13 by mast cells as well as IL-5 secreted by Th2 cells lead to late phase allergic reactions

been suggested to play a significant role in allergic diseases via the production of IL-4, IL-5 and IL-13, and eotaxin [38], perhaps by mast cells [39]. IL-25 might play a significant role in the development of food hypersensitivity reactions. Although it is generally agreed that allergy is caused by the development of allergen-specific Th2 cells in allergic individuals, this is a much simplified picture. Furthermore, the recent discovery of a different subset of CD4⁺ T regulatory (Tr) cells and the manifestation of cytokine- (IL-10 and TGF-β) dependent suppression of inflammatory response by CD4⁺ CD25⁻ (Tr) T cells and cytokine-independent suppression by CD4⁺ CD25⁻ T cells substantiate the hypothesis that Tr cells have a critical role in maintaining immune homeostasis in vivo [40, 41]. The exact mechanism of the Th1/Th2 paradigm is not fully understood and is likely to be influenced by a number of genetic and environmental factors [42].

The mast cell IgE and IgE receptor network plays an important role in regulating an allergic reaction [43, 44]. IgE antibodies are bound to a high affinity Fc receptor specific for ϵ heavy chains (Fc ϵ RI) that are expressed on mast cells, basophils, and eosinophils. Each Fc ϵ RI molecule is composed of one α chain that mediates IgE binding, and a β and two γ chains that are responsible for signaling. The amino terminal extracellular portion of the Fc ϵ RI α chain consists of two Ig-like domains and constitutes the IgE binding site. The β chain of the Fc ϵ RI chains consists of a single immunoreceptor tyrosine-based activation motif (ITAM) in the carboxyl terminus and the two identical γ chains, each containing one ITAM [45, 46].

In an individual who is allergic to a particular antigen, a large proportion of the IgE bound to mast cells is specific for that antigen. In contrast, in non-atopic individuals, the mast cell's associated IgE are highly heterologous in antigen specificity. Thus, exposure to the allergen will cross-link sufficient IgE molecules in an allergic individual but will be less likely to produce the same effect in non-atopic individuals. Upon IgE cross-linking of the Fc ϵ RI, the Lyn tyrosine kinase phosphorylates the ITAM of the β and γ chains of the Fc ϵ RI. Syk tyrosine kinase is then recruited to the ITAMs of the γ chain, and activated, thus phosphorylating other proteins in the signaling cascade [47]. Several key signaling players are involved in the IgE-mediated immune response: (a) Phosphorylation of the γ isoform of phosphatidylinositol-specific phospholipase (PLC γ), which catalyses the breakdown of phosphatidylinositol biphosphate to inositol triphosphate (IP3) and diacylglycerol (DAG); (b) activation of cytosolic phospholipase A₂ (PLA₂), which subsequently hydrolyzes membrane phospholipase eventually leading to the release of mediators including arachidonic leukotrienes especially LTC₄ and prostaglandin D₂; and (c) nuclear translocation of transcription factors including nuclear activator of activated T cells (NFAT) and NF- κ B, which are important in the stimulation of transcription of cytokines [48, 49]. Mast cell activation is also negatively regulated by cyclic adenosine monophosphate (cAMP). Activated cAMP activates protein kinase A, which in turn inhibits mast cell degranulation. In addition, the Fc ϵ RI activation pathway is regulated by the inhibitory Fc receptor (Fc γ RIIb), which contains a immunoreceptor tyrosine-based inhibitory motif (ITIM). Phosphorylation of ITIM leads to the recruitment of the tyrosine phosphatase Sh2 domain-containing inositol 5-phosphatase (SHIP) and inhibition of IgE-mediated Fc ϵ RI signaling [50, 51].

The effector functions of mast cells are mediated by soluble molecules released upon activation. The key mediators include: (a) histamines; (b) granule proteins such as tryptase, chymase, carboxypeptidase A, cathepsin G, and proteoglycans such as heparin [52]; (c) lipid mediators such as prostaglandin D₂ and leukotrienes that have a variety of effects on blood vessels, bronchial smooth muscle, and leukocytes; and (d) cytokines including TNF, IL-1, IL-4, IL-5, IL-6, IL-13, MIP-1 α , MIP-1 β [53, 54], and a potent Th2 response inducer IL-25 [39]. In addition to mast cells, Fc ϵ RI are also expressed on basophils

Table 1 Mediators produced by mast cells, basophils and eosinophils and their physiological functions

Cell type	Mediators	Physiological functions
Mast cells and basophils		
Preformed cytoplasmic granules	Histamines	Increase vascular permeability, stimulate smooth muscle contraction
	Neutral proteases (tryptase, chymase), acid hydrolyases, cathepsin G, carboxypeptidase A	Tissue damage/remodelling
Lipid mediators	Prostaglandin D2	Vasodilation, bronchoconstriction, neutrophil chemotaxis
	Leukotriene C4, D4, E4	Prolong bronchoconstriction, mucus secretion, increase vascular permeability
	Platelet activating factor	Bronchoconstriction, increase vascular permeability, chemotaxis of leukocytes
Cytokines	IL-3	Promotes mast cell proliferation
	TNF- α , MIP-1 α ,	Inflammation
	IL-4, IL-13	Th2 differentiation
	IL-5	Promotes eosinophil production and activation
Eosinophils		
Preformed cytoplasmic granules	Peroxidase, lysosomal hydrolases	Tissue damage/remodelling
Lipid mediators	Leukotriene C4, D4, E4	Prolong bronchoconstriction, mucus secretion, increase vascular permeability
	Lipoxins	Inflammation
Cytokines	IL-3, IL-5, GM-CSF	Promotes eosinophil production and activation
	IL-8, IL-10, MIP-1 α	Leukocytes chemotaxis

and eosinophils. The ability of eosinophils to release granules is enhanced by IL-5 [55]. Activated eosinophils produce and release lipid mediators such as PAF, prostaglandins, and leukotrienes [56–59] (Table 1) in a similar fashion to mast cells and basophils.

5 Molecular Biology of Seafood Allergens

5.1 History

Prausnitz and Küstner [60] performed a classical experiment by injecting serum from a fish-allergic patient into the skin of a non-atopic individual. The injection site was then challenged with fish extract and the allergic responses observed. This study represents an early study on seafood allergy. The fish allergen, known as Allergen M (later designated as Gad m 1 or Gad c 1), isolated from the Atlantic cod (*Gadus morhua* = *G. callarias*) in 1969 is among the first food allergens identified [61]. Crustaceans such as shrimp, lobster, and crab have long been known to be the most common allergic food items among seafood [62, 63]. Some allergic patients not only developed allergic reactions to ingested shrimp, but also developed urticaria after handling shrimp directly, or wrapping containing shrimp or other crustaceans such as lobster or crab [64]. Shrimp water or vapor from cooked shrimp was shown to be a source of allergen in susceptible individuals [65]. Such contacts present a threat of occupational allergy among seafood-processing workers [18]. Some patients even developed adverse reactions towards crustaceans in the absence of previous contact [11]. Although isolation of shrimp allergens by biochemical methods was reported in the 1980s [64, 66], the identity of shellfish allergens remained elusive till the 1990s, despite the fact that a number of allergens had been identified from common food items such as lactoglobulin, lactalbumin, and casein from cow's milk, and ovalbumin and ovomucoid from hen's egg in 1970–1980.

5.2 Studies of Fish Allergens

The allergen in fish was first identified in the Atlantic cod [61, 67–70]. The allergen (Gad c 1) consists of 113 amino acids and a glucose molecule, with a molecular weight of about 12 kDa. The allergen belongs to a family of parvalbumins, a group of calcium-binding proteins. Parvalbumins are present in high amounts in white muscles of lower invertebrates [71] and in lower amounts in fast twitch muscles of higher vertebrates [72]. They function in calcium buffering and are involved in the relaxation process of muscles [73].

Analysis of amino acid sequences indicates that the parvalbumin protein family could be subdivided into an α group and a β group [74]. Lindstrom et al. [75] purified parvalbumin in the Atlantic salmon (*Salmo salar*) and confirmed its allergenicity. The allergen was designated as Sal s 1. A probe based on the amino acid sequences of Sal s 1 was used to screen a salmon muscle cDNA library to identify two distinct cDNA clones representing two different parvalbumin genes. The deduced amino acid sequences of both clones belong to the β -lineage of parvalbumins. This study represents the first molecular cloning study of a fish allergen. Van Do et al. [76] further confirmed this finding.

Das Dore et al. [77, 78] determined the DNA sequence of parvalbumin allergen (Gad m 1) in the Atlantic cod. This recombinant allergen of a molecular weight of 11.5 kDa had 62.3% identity with Gad c 1 and surprisingly, 75% with Sal s 1. The parvalbumin gene of the Atlantic cod was also cloned by Van Do et al. [79]. Using RT-PCR, two distinct cDNA clones, named T1 and T2, were identified in the white muscle. Both belong to parvalbumin β group with high sequence similarity to other members of the family Gadidae. When we compare the amino acid sequences among Gad c 1 [70], Gad m 1 [77, 78], T1, and T2 [79], the identity of Gad m 1 and T2 is 94.3%, suggesting these two allergens represent very similar, if not identical isoforms. Their identities to the other cod allergens are less than 72%, suggesting that the allergens represent distinct isoforms of parvalbumin. Parvalbumin has also been characterized as the allergen in horse mackerel [80] and carp [81, 82]. A list of known fish allergens is shown in Table 2. It should be noted that while parvalbumin represents the major cross-reactive fish allergen, other fish allergens have been reported in a number of fish species [83–86].

The cod allergen is the most intensively investigated allergen and serves as a model for epitope mapping of fish allergen [87]. As parvalbumin, the structure of Gad c 1 consists of three domains, AB, CD, and EF. A series of studies using tryptic and synthetic peptides as inhibitors of RAST showed that Gad c 1 contains at least five IgE-binding sites distributed along its polypeptide chain at residues 13–32, 33–44, 49–64, 65–74, and 88–96 [88–96]. Peptide 13–32 is found in the AB domain. Peptide 33–44 and 65–74 are found on the axis joining AB and CD domains and the axis joining the CD and EF domains, respectively. A high amino acid homology in these two regions suggests the presence of repeated allergenic determinants in Gad c 1. Peptide 49–64 of the Ca^{2+} -coordinating CD domain consists of two repetitive sequences (D-E-D-K) and (D-E-L-K) that are important for antibody binding. This peptide is also cross-reactive with birch pollen allergen [97]. Peptide 88–96 is found in the Ca^{2+} -coordinating domain EF. Interestingly Ca^{2+} depletion reduces specific IgE binding to Gad c 1 [98]. This observation has also been confirmed in the study on carp parvalbumins (Cyp c 1.01 and Cyp c 1.02) [82]. The reduction in IgE binding is possibly due to unfolding of the protein or a change in conformational epitopes. Other Ca^{2+} -binding proteins

have been identified as allergens in plants and autoallergens in man [99]. Protein-bound Ca^{2+} is required for IgE binding to Ca^{2+} -binding plant allergens [100, 101].

5.3

Studies on Crustacean Allergens

The molecular identity of the shrimp allergen was elucidated in the early 1990s by a number of research groups. Shanti et al. [102] reported that there is high (86%) amino acid sequence homology between allergen Sa-II (also referred as Pen i 1) from the shrimp *Penaeus indicus* [66] and the muscle protein tropomyosin of the fruit fly *Drosophila melanogaster*. Tropomyosins are a family of actin filament-binding proteins with distinct isoforms found in muscles, brain, and various non-muscle cells. In association with the troponin complex, tropomyosins function in the regulation of calcium-sensitive interaction of actin and myosin. Leung et al. [103] first reported the cloning of the major shrimp allergen from *Metapenaeus ensis*, called Met e 1, by screening a cDNA library of shrimp muscle with sera from patients with shellfish allergy. The cDNA of Met e 1 exhibited an open reading frame of 281 amino acids, coding for a 34-kDa protein. This molecule had high homology to *Drosophila* tropomyosin. Another shrimp allergen, Pen a 1, was also identified in the same year from the shrimp *Penaeus aztecus* [104]. The identity of Pen a 1 as tropomyosin has subsequently been confirmed by molecular cloning and nucleotide sequence analysis [105]. Comparison of the amino acid sequences of antigens Sa-II, Pen a 1 and Met e 1 showed that they are similar or identical allergens.

The identity of tropomyosin as an allergen has subsequently been confirmed in other crustaceans. Leung et al. [106, 107] identified the allergen Pan s 1 from the spiny lobster *Panulirus stimpsoni*, Hom a 1 from the American lobster *Homarus americanus*, and Cha f 1 from the crab *Charybdis feriatus*. All three have similar molecular weight (34 kDa) and their deduced amino acid sequences are highly homologous to tropomyosin. While Cha f 1 appears to be slow muscle tropomyosin, the other crustacean allergens identified (Met e 1, Pen a 1, Hom a 1, and Pan s 1) are possibly fast muscle tropomyosins.

While tropomyosin is the major allergen among crustaceans, there have been reports on the presence of multiple allergens [108, 109]. Yu et al. [110] recently identified a novel allergen designated as Pen m 2 from the shrimp *Penaeus monodon* by two-dimensional immunoblotting using sera from patients with shrimp allergy. The allergen was cloned and the cDNA contained 1071 bp of open reading frame encoding a 356 amino acid protein with a theoretical molecular weight of 39.9 kDa. The sequence of this protein showed similarity (60%) to arginine kinase of crustaceans (*Penaeus japonicus*). Pen m 2 exhibited arginine kinase activity and reacted with IgE

Table 2 Summary of seafood allergens identified

Source of allergen	Nomen- clature	MW (kDa)	Identity	cDNA cloned	Reference
Fish					
<i>Gadus morhua</i> = (<i>Gadus callarias</i>) (Atlantic cod)	Gad m 1	12	Parvalbumin	–	61, 67
<i>Salmo salar</i> (salmon)	Sal s 1	12	Parvalbumin	+	75
Horse mackerel	Nil	12	parvalbumin	–	80
<i>Cyprinus carpio</i> (common carp)	Cyp c 1.01	11.4	Parvalbumin	+	81, 82
	Cyp c 1.02	11.4	Parvalbumin	–	
Crustacea					
<i>Metapenaeus ensis</i> (Greasy back shrimp)	Met e 1	34	Tropomyosin	+	103
<i>Penaeus aztecus</i> (Northern brown shrimp)	Pen a 1	36	Tropomyosin	+	104
<i>Penaeus indicus</i> (Indian white shrimp)	Pen i 1	38	Tropomyosin	–	66, 102
<i>Penaeus monodon</i> (Giant tiger shrimp)	Pen m 2	39.9	Arginine kinase	+	110
<i>Homarus americanus</i> (American lobster)	Hom a 1	34	Tropomyosin	+	107
<i>Panulirus stimpsoni</i> (spiny lobster)	Pan s 1	34	Tropomyosin	+	107
<i>Charybdis feriatus</i> (crab)	Cha f 1	34	Tropomyosin	+	106
Mollusca					
<i>Haliotis midae</i> (abalone)	Hal m 1	49	Nil	–	119
<i>Haliotis diversicolor</i> (abalone)	Hal d 1	38	Tropomyosin	+	128
<i>Turbo cornutus</i> (turban shell)	Tur c 1	35	Tropomyosin	–	125
<i>Helix aspersa</i> (brown garden snail)	Hel as1	36	Tropomyosin	+	120
<i>Perna viridis</i> (mussel)	Per v 1	38	Tropomyosin	+	128
<i>Placopecten magellanicus</i> (sea scallop)	Nil	30	Tropomyosin	+	127
<i>Chlamys nobilis</i> (scallop)	Chl n 1	38	Tropomyosin	+	128
<i>Crassostrea gigas</i> (Pacific oyster)	Cra g 1.01	35	Tropomyosin	–	117, 123,
	Cra g 1.02	35	Tropomyosin	–	124, 126
	Cra g 1.03	31	Tropomyosin	+	
<i>Todarodes pacificus</i> (squid)	Tod p 1	38	Tropomyosin	–	129
<i>Octopus vulgaris</i> (octopus)	Oct v 1	31–34	Tropomyosin	–	130

from shrimp-allergic patients. Strong reactivity of purified arginine kinase from shrimp (*Metapenaeus ensis*), lobster (*Homarus gammarus*), crawfish (*Metanephrops thomsoni*), and crab (*Scylla serrata*) with anti-Pen m 2 anti-

body and sera from shrimp-sensitive patients indicates that arginine kinase as a common allergen among crustaceans, in addition to tropomyosin. Arginine kinase has also been designated as an allergen in the moth (*Plodia interpunctella*) [111]. A list of known crustacean allergens is included in Table 2.

5.4

Studies of Mollusk Allergens

The mollusks commonly consumed include members of class Gastropoda (e.g. limpet and abalone), class Bivalvia (e.g. scallop, clam, mussel, and oyster) and class Cephalopoda (cuttlefish, squid, and octopus). There have been a number of reports on allergies to mollusks including allergies to snail [112], abalone [113], limpet [114, 115], cuttlefish [116], and squid [117]. Leung et al. [118] showed that sera from patients with shrimp allergies reacted specifically to a 38-kDa protein in a wide variety of mollusks from the three classes. The IgE-binding reactivity of the sera to mollusk extract could be inhibited by preabsorption with recombinant shrimp tropomyosin Met e 1, suggesting that tropomyosin is a common allergen among crustaceans and mollusks. The identity of tropomyosin as the allergens in mollusks has subsequently been confirmed in the three groups of mollusks.

For gastropods, Lopata et al. [119] demonstrated the IgE reactivities of abalone (*Haliotis midae*) sensitive subjects to two major allergens with molecular weights of 38 kDa and 49 kDa. While the 38-kDa protein was believed to be tropomyosin, the identity of the 49-kDa allergen, named Hal m 1, remains to be identified. Ishikawa et al. [124, 125] purified a 35-kDa allergen (Tur c 1) in the turban shell *Turbo cornutus*. The amino acid composition and partial amino acid sequences of Tur c 1 imply that it is tropomyosin. Asturias et al. [120] isolated and cloned tropomyosin from *Helix aspersa* (brown garden snail) and found that the tropomyosin (Hel as 1) was a 36-kDa protein and shared 84% of its amino acid sequence identity with abalone (*Haliotis diversicolor*), 70% with mussel (*Mytilus edulis*), and 72% with scallop (*Chlamys nobilis*) tropomyosins. Western blot inhibition of different mollusk and crustacean extracts showed cross-reactivity between *Helix aspersa*, cuttlefish, octopus, sea snail, and shrimp. The IgE-binding capacity of the recombinant Hel as 1 was found to be weaker than its natural counterpart, possibly due to the absence of some epitopes or improper folding of the recombinant protein. These studies demonstrated that tropomyosin is indeed a major allergen in various gastropod mollusks.

Among bivalves, it is well known that oysters can cause hypersensitive reactions upon ingestion, as well as occupational reactions in sensitized workers [16, 121]. Oyster and crustacean allergens possibly share common antigen epitopes, i.e., the common primitive allergenic structure shared by the two taxa is conserved in evolution [122]. Ishikawa et al. [123] purified

two biochemically similar allergens (Cra g 1 and 2) from the Pacific oyster *Crassostrea gigas* by gel permeation chromatography, ion exchange FPLC, and reverse-phase HPLC. Their molecular weights and amino acid composition strongly suggest that they are isoforms of tropomyosins [123–125]. Leung and Chu [126] indicated that Cra g 1 and 2 represent the same or similar isoforms of oyster tropomyosins and they should be designated as Cra g 1.01 and Cra g 1.02. In the same study, tropomyosin (Cra g 1.03) was confirmed to be the oyster allergen using recombinant DNA technology. Patwary et al. [127] isolated and characterized cDNA clones that encode tropomyosin from adductor muscle of sea scallop (*Placopecten magellanicus*). The cDNAs encoded an open reading frame of 284 amino acids and this protein had a molecular weight of approximately 30 kDa. The amino acid sequence is about 70% identical to tropomyosins from other mollusks. Using reverse transcriptase-PCR, Chu et al. [128] amplified tropomyosin cDNA from abalone (*Haliotis diversicolor*), scallop (*Chlamys nobilis*), and mussel (*Perna viridis*). The cDNAs were cloned and expressed and the IgE reactivity of the recombinant proteins was demonstrated. A comparison of amino acid sequences of tropomyosins of the three mollusks to those of a wide variety of mollusks available from the GenBank database shows that they are highly conserved (identity > 68%).

Allergens have also been identified as tropomyosins in cephalopods. Miyazawa et al. [129] isolated a 38-kD heat-stable allergen (Tod p 1) from the squid *Todarodes pacificus* by column chromatography. The peptide sequences of Tod p 1 exhibited significantly high homology to the snail tropomyosin. Ishikawa et al. [130] identified a major allergen, Oct v 1, in muscle of octopus (*Octopus vulgaris*) by gel filtration and liquid chromatography. Its molecular weight, amino acid composition, and partial amino acid sequence indicate that this allergen is tropomyosin. Competitive ELISA inhibition experiments suggested cross-reactivity between Oct v 1 and Tur c 1 (turban shell *Turbo cornutus* allergen). A list of known mollusk allergens is shown in Table 2.

5.5

Cross-reactivity Among Different Seafood

Food allergens are able to sensitize and elicit IgE reactions after oral exposure. Related food items are believed to trigger the same responses, leading to cross-reactivity. For example, Ayuso et al. [131] demonstrated cross-reactivity between meats. IgE reactivities to beef and lamb were most frequent, followed by venison, pork, and chicken. The possible reason of cross-reactivity would be the presence of shared or common epitopes on antigens and the conformational similarity of epitopes to which the antibody would bind with similar affinity.

In fish, Hansen et al. [132] studied the reactions of clinically cod-sensitive patients to other fish species by skin prick test, specific IgE tests, histamine release test, and immunoblotting. IgE-binding ability to cod, mackerel, herring,

and plaice were analyzed using sera from eight cod-allergic patients. RAST inhibition assay revealed cross-reactivity between cod and other fish species, but not between cod and shrimp nor milk. Similar studies confirm that parvalbumin exhibits cross-reactivity among different fish species [82, 98]. For instance, the loss of most of the IgE reactivity to cod, tuna, and salmon total fish extract in sera from fish-allergic patients preincubated with recombinant carp parvalbumin (Cyp c 1.01) suggests common epitopes are present in fish [82]. These findings are consistent with numerous reports on in vivo cross-reactivity among fish species [133–139].

The cross-reactivity among crustaceans has long been documented [117, 122, 140, 141]. Daul et al. [142] demonstrated that monoclonal antibodies against shrimp Pen a 1 exhibited similar reactivity patterns to crayfish, crab, and lobster antigens. Leung et al. [106, 107, 118] demonstrated that sera from shellfish-allergic patients lost their IgE reactivity to crude crustacean extracts when they were preincubated in recombinant proteins Met e 1, Pan s 1 or Hom a 1. These studies represent the first evidence of cross-reactivity at the molecular level between different crustacean species that accounts for previous results on the allergic cross-reactivity between crustaceans [117, 122, 140, 141]. It suggests that crustacean tropomyosins share common allergic epitopes.

Cross-reactivity among mollusks has been reported. Lopata et al. [119] demonstrated cross-reactivity in a variety of mollusks including abalone (*Haliotis midae*), snail (*Helix aspersa*), white mussel (*Donax serra*), black mussel (*Mytilus galloprovincialis*), oyster (*Crassostrea gigas*), and squid (*Loligo vulgaris*) by binding with abalone-sensitive sera. Chu et al. [128] demonstrated that sera of shellfish-allergic subjects lost their IgE reactivities to mollusk extract after preincubation in recombinant tropomyosins of mollusks or crustaceans. The results confirmed that tropomyosin is the major cross-reactive mollusk allergen.

Cross-reactivity between crustaceans and mollusks has been widely reported [114, 115, 117, 122]. For example, Goetz & Whisman [17] reported a case of a seafood handler who was sensitive to both shrimp and scallop. Cross-reactivity between the crustaceans and mollusks at the molecular level was demonstrated by Leung et al. [118]. Immunoblotting of sera from subjects sensitive to shrimp showed positive results against muscle extracts from bivalves (mussel, fan shell, clam, oyster, and scallops), gastropods (abalone and whelk) and cephalopods (cuttlefish, squid, and octopus). Thus tropomyosin is the major cross-reactive allergen among crustaceans and mollusks. Leung et al. [118] also showed that sera from subjects with crustacean allergy demonstrated IgE reactivities against insects, suggesting that allergic cross-reactivity occurs among all arthropods (see next section). Thus tropomyosin serves as a good molecular model for investigating the relationship between protein structure and allergenicity. It should also be noted that the IgE against shellfish tropomyosins does not cross-react with vertebrate tropomyosins.

5.6

Tropomyosins as Allergens in other Invertebrate Groups

Leung et al. [118] showed that sera from subjects with crustacean allergy demonstrated IgE reactivities against insects, suggesting that allergic cross-reactivity occurs among arthropods. Crespo et al. [143] demonstrated cross-reactivity between shrimp (*Pandalus borealis*) and German cockroach (*Blattella germanica*). Immunoblotting using sera of patients with shrimp allergy showed the strongest IgE binding to both shrimp and cockroach extracts, at 30 and 34 kDa, respectively. Inhibition studies by immunoblotting showed that the IgE-binding capacity of German cockroach was totally abolished by the shrimp extract, while the binding capacity of shrimp was partially affected by German cockroach extract. Witteman et al. [141] also identified tropomyosin as a cross-reactive allergen among the arthropod groups Crustacea, Arachnida (such as mites), and Insecta.

The allergic nature of tropomyosin has been well documented in mites. For example, Asturias et al. [144] cloned and expressed the tropomyosin allergen (Der p 10) from *Dermatophagoides pteronyssinus*. Tropomyosin allergen from dust mite *Lepidoglyphus destructor* (Lep d 10) has also been identified [145]. The recombinant allergen Blo t 10 from the mite *Blomia tropicalis* shared 94–98% protein sequence homology with other mite allergens including Lep d 10, Der p 10, and Der f 10 (from *Dermatophagoides farinae*) [146]. Skin prick tests using patients sensitive to mite allergens showed exclusive reactivity to Blo t 10 and Der p 10, indicating the presence of shared epitopes in the two allergens. Positive skin reactivity of seafood allergy patients to Blo t 10 suggested cross-reactivity between Blo t 10 and seafood. It should be noted that other allergens have been reported in mites. For instance, group I allergens in mites, such as Pso o 1 from sheep scab mite *Psoroptes ovis* [147] are papain-superfamily of cysteine proteases.

Tropomyosin has also been identified as an insect allergen. For instance, Asturias et al. [148] and Santos et al. [149] cloned and expressed the American cockroach (*Periplaneta americana*) tropomyosin (Per a 7). Tropomyosin expressed in both studies showed high sequence homology to tropomyosin of other invertebrates. The DNA sequences of Per a 7 from both studies differ in several nucleotides, suggesting that they are tropomyosin isoforms. Thus the cockroach tropomyosin allergens identified by Asturias et al. [148] and Santos et al. [149] should be designated as Per a 7.01 and Per a 7.02, respectively. The tropomyosin of the silverfish (*Lepisma saccharina*) was also characterized to be allergic [150]. The allergen from this primitive insect can be recognized by sera from patients with household insect allergy.

Anaphylactic reactions can be caused by *Anisakis simplex*, a parasitic nematode of fish, after ingestion of parasitized fish [151, 152]. The nematode was shown to induce specific IgE-mediated reactions [153] and is an important etiologic factor in acute urticaria after ingestion of fish [154]. Leung

& Chu [126] suggested that tropomyosin is the allergen in the nematode, based on its high sequence homology with arthropod tropomyosins. Johansson et al. [155] reported cross-reactivity between this nematode and insects.

5.7

Epitopes of Tropomyosin Allergens

Tropomyosin is the cross-reactive allergen among crustaceans, mollusks, mites, and insects, yet there is no cross-reactivity between tropomyosins from these invertebrates and the vertebrates [118]. Likewise, extracts of different fish species did not significantly inhibit shrimp RAST [119], indicating the presence of unique IgE-binding epitopes in tropomyosins from the various groups of invertebrates. Moreover, the cross-reactivity between various tropomyosin allergens suggests the presence of common IgE-binding epitopes. To characterize IgE epitopes of Pen a 1, a peptide library was constructed to express 10–30 amino acid peptides of this allergen, which were screened with sera from allergic patients [105, 156, 157]. Reese et al. [105] identified four IgE-binding peptides, namely peptide E2 (residues 167–179), E3 (136–148), E4 (262–282) and E6 (157–169). Peptide E6 partially overlaps with an IgE-reactive peptide (residues 153–161) of Pen i 1 [98]. Reese et al. [157] showed that the center and C terminus of Pen a 1 contain most of the IgE-binding sites. A 21-mer peptide (residues 264–284) at the C terminus that overlaps most of peptide E4 was suggested as a putative T-cell epitope, which could reduce the IgE reactivity in a mouse model [158].

In the most recent study, Ayuso et al. [156] analyzed eight IgE-binding epitopes in five regions of Pen a 1 based on the reactivity of sera from shrimp-allergic subjects with synthetic peptides having different amino acid substitutions present in the lobster *Homarus americanus*, cockroach *Periplaneta americana*, and house dust mites *Dermatophagoides pteronyssinus* and *D. farinae*. The cross-reactive epitopes are: residues 43–55 (epitope 1), residues 87–101 (epitope 2), residues 137–141 (epitope 3a), residues 144–151 (epitope 3b), residues 187–197 (epitope 4), residues 249–259 (epitope 5a), residues 266–273 (epitope 5b), and residues 273–281 (epitope 5c). IgE reactivity of shrimp-allergic sera to peptides with homologous amino acid sequence of the other invertebrates is the basis of cross-reactivity between these species.

By comparing amino acid sequences of these epitopes with corresponding sequences in other tropomyosin allergens (Fig. 2), we could classify the eight epitopes into three different types depending on the degree of identity between different taxa. First, epitope 5a is almost identical among all species. This may represent the common epitope among crustaceans, insects, mites, and mollusks. In the second type, including epitopes 2, 3a, 3b, and 4, the amino acid residues are highly conserved in arthropods (crustaceans, insects, and mites), but are variable when compared with mollusks. These may repre-

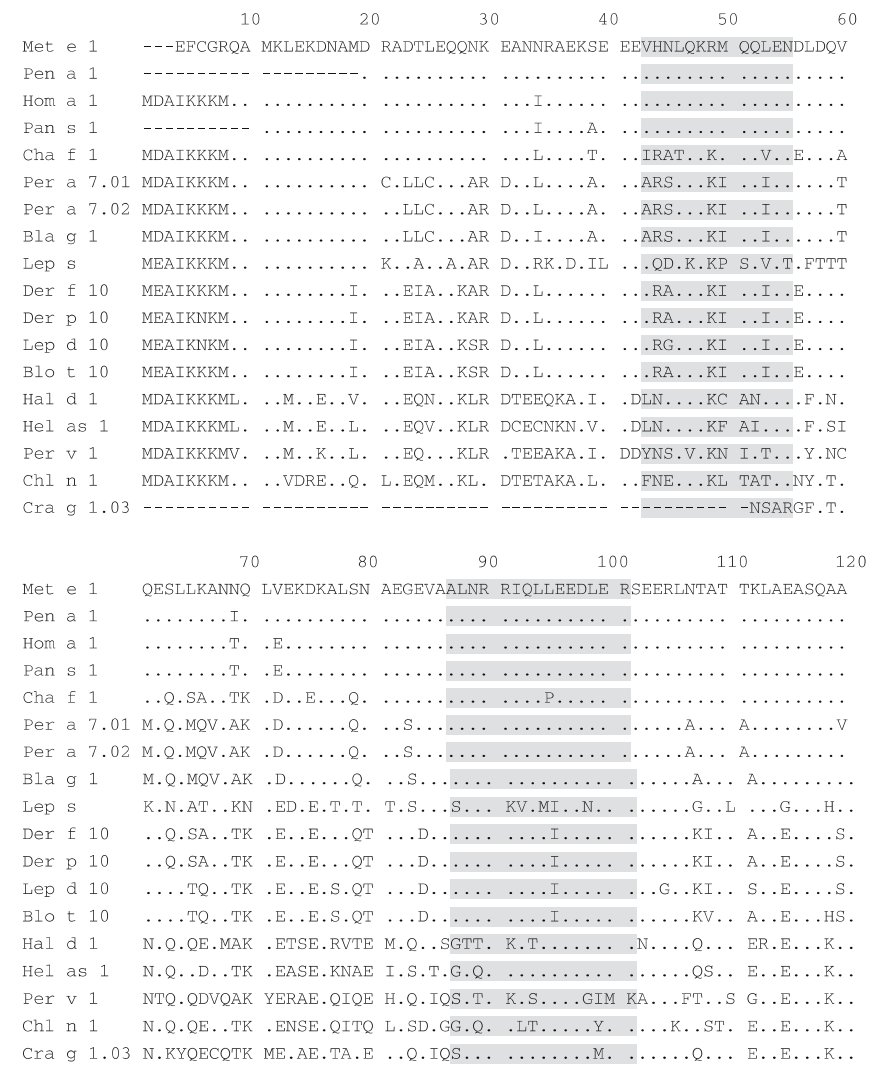


Fig. 2 Amino acid sequence comparison of tropomyosins identified as allergens in various taxonomic groups. Crustaceans: Met e 1 from *Metapenaeus ensis* (shrimp), Pen a 1 from *Penaeus aztecus* (shrimp), Hom a 1 from *Homarus americanus* (American lobster), Pan s 1 from *Panulirus stimpsoni* (spiny lobster), and Cha f 1 from *Charybdis feriatius* (crab). Insects: Per a 7.01 from *Periplaneta americana* (American cockroach), Per a 7.02 from *P. americana*, Bla g 1 from *Blattella germanica* (German cockroach), and Lep s from silverfish (*Lepisma saccharina*). Mites: Der f 10 from *Dermatophagoides farinae* (house dust mite), Der p 10 from *D. pteronyssinus* (house dust mite), Lep d 10 from *Lepidoglyphus destructor* (dust mite), and Blo t 10 from *Blomia tropicalis*. Mollusks: Hal d 1 from *Haliotis diversicolor* (abalone), Hel as 1 from *Helix aspersa* (brown garden snail), Per v 1 from *Perna viridis* (green mussel), Chl n 1 from *Chlamys nobilis* (scallop), and Cra g 1.03 from *Crassostrea gigas* (Pacific oyster). IgE binding epitopes of Pen a 1 [156] are shaded

	130	140	150	160	170	180
Met e 1	DESERMRKVL	ENRSLSDER	MDALENQLKE	ARFLAEEADR	KYDEVARKLA	MVEADLERAE
Pen a 1						
Hom a 1						
Pan s 1						
Cha f 1						
Per a 7.01	A..I..	.SKG.A....		..M....K		
Per a 7.02	A..I..	.SKG.A....		..M....K		
Bla g 1	A..I..	.SKG.A....		..M....K		
Lep s	..AS..C...	QQ....	..Q..T....	..M...D..G	.S...S..M.	Q..D...V..
Der f 10	M.	.H..IT....	..G.....	..MM..D...		
Der p 10	M.	.H..IT....	..EG.....	..MM..D...		
Lep d 10	M.	.H..IT....	..EG..S....	..MM..D...		
Blo t 10	M.	.H..IT....	..G..S....	..MM..D...		
Hal d 1	GAR..	.S...A..D..	I.Q..A....	KYI..D.E.	A....	IT.V.....
Hel as 1	G....	.S...A..D..	L.G..A....	KYI..D.E.	..F..A....	IT.V.....
Per v 1	N....	..LNSGND..	I.Q..K..T..	..KWI....K	..E.A....	IT.V.....
Chl n 1	N....	.G..N..Y...	I.E..K..ET	..KNV.TD..H	..F..A....	IT.V.....
Cra g 1.03	N....	..LNNAS...	T.V..K..T..	..KLI....K	A....	IT.V.....

	190	200	210	220	230	240
Met e 1	ERAETGESKI	VELEEEIRVV	GNNLKSLEVS	EEKANQREEA	YKEQIKTLTN	KLKAAEARAE
Pen a 1						
Hom a 1					..A.	
Pan s 1	F.....					
Cha f 1	S.....			T	A.	
Per a 7.01	S.....			L...E	..Q.....T	R..E.....
Per a 7.02	S.....			L...E	..Q.....T	R..E.....
Bla g 1				L...E	..Q.....NT	R..E.....
Lep s	D.VKS.D...	M.....K..	..S.....	V.E	..R.....V	..E.....
Der f 10				..Q.....	..EQ..RIM.A	..E.....
Der p 10				..Q.....	..EQ..RIM.T	..E.....
Lep d 10				..Q.....	..EQ..RIM.T	..E.....
Blo t 10	T.....			..Q.....	..EQ..RMM.G	..E.....
Hal d 1	A.L.AA.A..	L.....K..	..M....I.	..QE.S...DS	..E.T.RD..Q	R..D..N..T
Hel as 1	A.L.AA.A..	L.....K..	..M....I.	..QE.S...DS	..E.T.RD..Q	R..D..N..S
Per v 1	A.L.AA.A.V	ID...Q..T..	..A.I.T.Q.Q	NDQ.S...DS	..E.T.RD...	R..D..N..T
Chl n 1	T.L.AADA.V	L.....T..	..A.I.T.Q.Q	NDQ.S...DS	..E.T.RD..K	S..D..N..T
Cra g 1.03	A.L.AA.A.V	L.....K..	..M....I.	..QE.S...DS	..E.T.RD..Q	R..D..N..T

Fig.2 (continued)

sent epitopes that are common to arthropods, but distinct from mollusks. In the third type, including epitopes 1, 5b, and 5c, the amino acid residues are similar within each of the above four taxa but vary considerably between the taxa. These may represent epitopes that are specific only to crustaceans.

The presence of these different types of epitopes may account for the finding by Leung and Chu [128] that pre-absorption of sera from oyster allergy subjects with recombinant crustacean allergen was able to remove most but

	250	260	270	280
Met e 1	FAERSVQK LQ KEVDRL	DEL VNEKEKYKSI	TDELDQTFSE	LSGY
Pen a 1				..
Hom a 1				..
Pan s 1				..
Cha f 1				-----
Per a 7.01			.H.....	F. C.D. M. T. A..
Per a 7.02			.H.....	F. C.D. M. T. I.N
Bla g 1			.H.....	Y. C.D. M. T. I.N
Lep s	Y..KY.K.		GIN.DR.RAL	A..M....A.
Der f 10			.H.....	S.....A. T..
Der p 10		G.....	.H.....	S.....A. T..
Lep d 10			.H.....	S.....A. T..
Blo t 10			.H.....	S.....A. T..
Hal d 1	E...T.S.		LA.....	A. S.....A. A..
Hel as 1	E...T.S.		LA.....	R..AT S.....S.A. A..
Per v 1	E...T.S.		LT.....	A. S.....A.A. A..
Chl n 1	E...Q.V.		LA.....	R..A. S.D.....A. IA..
Cra g 1.03	E...T.S.		LA.....	R..A. S.....A. A..

not all of the IgE reactivity to either recombinant oyster allergen or extract. Generally speaking, there appears to be cross-reactive and distinct IgE epitopes in the tropomyosin allergens from different taxonomic groups.

Epitopes in mollusk tropomyosins have been reported. Ishikawa et al. [124, 125] proposed a 14-mer peptide (IQLLEEDMERSEER, residues 92–105) as the IgE-binding epitope in the oyster allergen Cra g 1.01 and 1.02. Yet the epitope in Tur c 1 (from the turban shell *Turbo cornutus*) is different and resides at the C-terminus (residues 245–284) of the tropomyosin molecule [125]. In the allergen Oct v 1 from the octopus *Octopus vulgaris*, two IgE-binding epitopes were identified at the central region (residues 77–112 and 148–160) and one at C-terminal region (residues 269–281) [124]. Residues 269–281 is nested within the epitope of Tur c 1 and its sequence is highly conserved among mollusks. This may represent the common epitope among mollusks. Peptide 77–112 of Oct v 1, which is the most reactive site for IgE binding, include the sequence of the epitope (residues 92–105) in Cra g 1. While residues 92–105 are rather conserved among different mollusks, the other regions in the residues 77–112 are highly variable. Interestingly, the amino acid residues 92–105 in the oyster epitope are almost identical to the corresponding residues in most arthropod tropomyosins, although the residues differ significantly between arthropods and some mollusks. It should also be noted that this oyster epitope overlaps with epitope 2 in Pen a 1. Clearly more intensive studies are necessary to clarify the common and distinct epitopes in tropomyosin allergens.

6

Applications and Future Directions

The molecular identification of seafood allergens, mapping of epitopes, expression of recombinant allergens, and the elucidation of the immunological mechanisms of allergies set the cornerstones and thus provide significant knowledge in the potential applications in the diagnosis and design of therapeutic modalities in seafood allergies. In addition, the recent development of gene transfer technology in lower invertebrates, including shrimp, may allow the future development of non-allergic transgenic shrimps for human consumption.

6.1

Diagnosis and Profiling

The enormous immune repertoire and specific recognition of antigens by antibodies are among the most fascinating phenomena of the human body. Interestingly, the specificity of antibody/antigen reactions has formed the molecular principle of immunodiagnosis. Early immunoassays capable of multiplex analysis include ELISA, fluorescent-based immunoassays, and radioimmunoassays performed in microtiter plates and arrays of peptides on pins, western blot analysis, and bacterial colonies/phage plaque-based assays [156, 160]. However, these methods are limited by requirement for relatively large quantities of clinical samples. In the late 1980s, several groups proposed the use of miniaturized as well as addressable immunoassays and photolithography-generated arrays [161, 162]. This idea was further advanced by robotic printing devices in the generation of DNA microarrays [163] and was subsequently applied in the fabrication of protein arrays on microscope glass slides [164, 165]. Recent advances in microarrays have focused on the capability of high throughput detection of antigens using submicroliter quantities of biological fluids [166].

In vitro diagnosis of allergy is based on the detection of allergen-specific IgE antibodies in the sera of atopic patients. Current available forms of diagnosis rely on allergen extracts prepared from allergen-containing biological materials, which are a variety of allergenic and non-allergenic components and are difficult to standardize [167–170]. With the molecular identification of allergens and availability of recombinant proteins, protein microarrays have been developed to profile allergen-specific antibodies from human sera [171–173]. Allergen protein array thus allows high throughput determination and monitoring of IgE reactivity profiles to a large number of allergens by using minute amounts of serum with sensitivity between 0.16–2 $\mu\text{g/L}$ of allergens [172]. Since small bioactive molecules such as synthetic peptides conjugated to macromolecules can be applied in microarray technologies, with the growing knowledge of IgE epitopes of allergens, peptide epitopes may also be used in allergen microarrays.

6.2

Immunotherapy

One of the most exciting recent developments in the treatment of food allergy is novel immunotherapeutic strategies designed to alter the immune system's response to food allergens. These strategies are now being examined in animal models as potential treatment modalities. They include plasmid DNA immunotherapy, immunostimulatory sequence-modulated immunotherapy, allergen-peptide immunotherapy, and IgE binding site modified protein immunotherapy. All of these methods strive to elicit a Th1-type response or tolerance from the immune system in response to a specific food allergen.

In the search for a novel immunotherapy with a low risk-to-benefit ratio, immunostimulatory CpG motif DNA sequences have recently been shown to provide an excellent tool for designing safer and more efficient forms of allergen immunotherapy. These DNA-based immunotherapeutics include allergen gene vaccines, immunization with allergen-DNA conjugates, and immunomodulation with immunostimulatory oligodeoxynucleotides. All three DNA-based immunotherapeutics have been shown to be very effective in animal models of allergic diseases and, at present, allergen-DNA conjugates are being tested for their safety and efficacy in allergic patients [174]. Plasmid DNA immunization entails the introduction of a plasmid DNA encoding a specific allergenic protein. The plasmid DNA is taken up by antigen-presenting cells and the expressed allergen is presumably presented on the surface of APC in the context of MHC. The presentation of endogenous proteins is believed to induce Th1 response with the upregulation of IFN- γ and suppression of IgE production. A recent study showed that genetic immunization with bovine β -lactoglobulin cDNA induces a preventive and persistent inhibition of specific anti-BLG IgE response in mice, but the window of response appears to be highly restricted [175]. Oral immunization with chitosan DNA particles has been shown to be effective in reducing allergen-induced anaphylaxis in an animal model of peanut allergy [176]. The effect of immunostimulatory sequence-modulated immunotherapy in food allergies has also been under active investigation [174, 177]. In general, primary gene and protein/immunostimulatory sequence oligodeoxynucleotide vaccination of Th2-sensitized mice significantly reduced the risk of death after anaphylactic challenge. In addition, gene and protein/ISS-ODN vaccination reduced post-challenge plasma histamine levels. Analysis of the immune profiles of mice receiving DNA-based vaccines showed that both gene and protein/ISS-ODN vaccination effectively prevented the development of Th2 biased immune profiles after sensitization. In contrast, vaccination with protein alone, the experimental equivalent of the traditional protein-based immunotherapy reagents used in clinical practice, provided no protection from anaphylaxis, and did not prevent the development of a Th2 biased immune profile after allergen sensitization [178–180]. These studies support the po-

tential application of DNA-based vaccination for the desensitization of food allergic individuals .

Specific immunotherapy (SIT), whereby an individual is repeatedly injected with increasing doses of allergen extract is perhaps the only effective therapeutic intervention of allergy. Over time, the individual becomes less reactive to the allergen, perhaps due to CD4+ T cell tolerance. However, traditional injection immunotherapy for food allergy is not recommended due to high rate of adverse systemic reactions, including anaphylaxis and death [181]. On the other hand, peptide immunotherapy reduces the allergen to its component CD4+ T cell epitopes. This method relies on HLA-binding peptides and thus it is important to verify the population-based immunodominant peptides for each ethnic group under consideration. Peptides are administered in order to induce antigen specific CD4+ T cell tolerance. Because the peptides cannot crosslink IgE, they are considered a safer therapy for SIT than intact allergens. This method is effective in animal models, and is thus potentially promising in human immunotherapy [182, 183]. Recombinant allergens can be genetically modified to reduce IgE binding, but keeping the T cell epitopes intact. This approach has been shown to be effective in inducing tolerance and offers a safe immunotherapeutic treatment of allergy [184–187].

It should be noted that the use of IgE binding site modified proteins or peptide epitopes to induce allergen non-responsiveness requires the identification of individual allergens to be included in a therapy regimen on a per patient basis. Moreover, knowledge on T cell epitopes in the allergen of interest is necessary so that the T cell epitopes can be retained in the construction of IgE binding modified proteins or the design of peptides. Since most individuals with allergies are responsive to many allergens, a cocktail approach may be sought in either peptide or modified IgE binding protein SIT. Although allergen-peptide immunotherapy and IgE binding site modified protein immunotherapy for food allergy have not reached the stage of clinical trials, their potential for food allergy therapy is evident.

There is currently no treatment available for seafood allergy. Therefore, patients must be taught to recognize and avoid seafood allergens. Those patients at risk of anaphylaxis must carry injectable epinephrine. The use of a humanized anti-IgE (Omalizumab) might reduce the risk of serious reactions. This product inhibits the binding of IgE to its receptors, and has been approved for the treatment of asthma. Data from peanut-allergic subjects indicates that it can significantly increase the amount of peanut allergen tolerated, thus protecting the patients from inadvertent exposure [188]. However, there is no data on seafood-allergic subjects and long-term efficacy is unknown. Another approach is the use of allergen immunotherapy to induce immunologic tolerance. This approach is being used successfully in the treatment of allergic rhinitis and asthma, but the risk is unacceptably high in subjects with food allergy [181]. Nevertheless, there have been

anecdotal reports of successful oral desensitization in food allergic subjects [189].

6.3

Non-allergenic Seafood

With the advent of molecular biological technology together with the increasing knowledge on the immunological mechanisms and epitopes of the seafood allergens, the generation of non-allergenic seafood may be at hand. Gene transfer technique is a powerful approach for changing the heritable traits of domesticated animals. This technique has been successfully applied in many fish species, with the production of stable lines suitable for commercial production [190]. Using DNA transformation technology, allergic structures in seafood can be modified by changing the amino acid sequences in epitopes to avoid allergy. Technological advances in genetic transformation in marine invertebrates including shrimp were unsatisfactory until recent years. In the case of transgenic shrimp, Preston et al. [191] first demonstrated microinjection as a reliable technique for delivering DNA to early embryos. Kau et al. [192] have recently developed an *in vivo* gene transfer technique for producing transgenic shrimp, in which gene constructs were injected into spermatophores and electroporation was used to assist the entry of constructs into oocytes. These studies provide significant groundwork for the future development of genetic transformation in marine organisms, including non-allergic transgenic seafood.

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