

Hypersensitivity Reactions to Crustacea and Mollusks

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Introduction

Shellfish are a frequent cause of adverse food reactions in hypersensitive individuals (1,2). The exact prevalence of sensitivity is unknown; however, we have estimated that more than 250,000 individuals, in the United States alone, have developed or are at risk of developing immunologically mediated adverse reactions to shellfish (3).

Exposure is the principal environmental factor related to the development of any food hypersensitivity (4). Therefore, a higher prevalence of reactivity to shellfish, in general, and to specific ones, in particular, would be expected in populations where these foods are consumed habitually. There is no published information on differences in the prevalence of reactivity to seafood by geographic area; however, individuals living near coastal areas probably have the highest prevalence of shellfish hypersensitivity. It is our clinical impression, for example, that adverse reactions to shellfish, particularly shrimp, are common among patients in the Gulf South area of the United States.

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Invertebrate species that are known to elicit adverse food reactions belong to two phyla—Arthropoda and Mollusca; however, in some cultures, members of Coelenterata (jellyfish and sea anemones) and Echinodermata (sea urchins and sea cucumbers), are eaten. The latter two groups of invertebrates have the potential to induce adverse responses in exposed individuals; however, food hypersensitivities have not been reported. The phylum Arthropoda surpasses all other animal groups with regard to number of species and number of individuals; however, except in Western societies, only crustaceans are eaten. The most popular of these belong to the order Decapoda and include freshwater and marine shrimp, crabs, crawfish, and lobsters. Among the invertebrate phyla, mollusks are second only to arthropods in number of species. Over 80,000 living species have been described; however, in North America, only pelecypods (oysters, clams, scallops, and mussels) have gained widespread culinary acceptance. In other areas of the world, many types of mollusks are also eaten, including snails and limpets (class Gastropoda), and squid, octopods, and cuttlefish (class Cephalopoda). Together, decapods and pelecypods constitute the artificial grouping known as “shellfish.” More detailed taxonomic relationships among these groups of invertebrates are discussed in the first article of this volume.

Clinical Features **Associated with Hypersensitivity Reactions**

Symptoms Following Ingestion of Shellfish or Inhalation of Shellfish Cooking Vapors

The appearance of adverse symptoms resulting from ingestion of shellfish or inhalation of cooking vapors of crustaceans or mollusks is almost always immediate in onset, that is, occurring within 2 h of exposure (5–12). Dual and isolated late phase asthmatic reactions have, however, also been reported in workers exposed to steam generated during the boiling of snow crabs (13). Case reports of adverse reactions occurring 3–6 h after ingestion of cuttlefish (14) or limpets (6) have also been published. In some instances of hypersensitivity, strenuous exercise (15) or concomitant exposure to light (16) are necessary cofactors for the development of symptoms.

Table 1
Symptoms Associated with Shrimp Ingestion in Shrimp-Sensitive Subjects

Symptom	Atopic <i>n</i> = 16 (%)	Nonatopic <i>n</i> = 17 (%)	Total <i>n</i> = 33 (%)
Urticaria/angioedema	14 (88)	14 (83)	28 (85)
Gastrointestinal	7 (44)	6 (35)	14 (40)
Pulmonary	5 (31)	4 (24)	9 (27)
Systemic anaphylaxis	7 (44)	0 (0)	7 (21)
Generalized pruritus	0 (0)	2 (12)	2 (6)
Other symptoms	1 (6)	1 (6)	2 (6)

Objective Symptoms

Immediate symptoms experienced by shellfish hypersensitive individuals are consistent with the anaphylactic spectrum; symptoms include urticaria and angioedema, laryngeal edema, asthma and rhinitis, vomiting and diarrhea, and, in the most severe cases, frank shock. With the exception of shock, the frequency with which individuals with a history of shrimp intolerance report specific adverse symptoms is approximately equal in atopic and nonatopics (Table 1). The majority of individuals with shrimp hypersensitivity that can be documented by double-blind placebo-controlled food challenge (DBPCFC) are, however, atopic (10).

Subjective Symptoms

Among 30 shrimp-sensitive patients who underwent DBPCFC, oropharyngeal pruritus, the least severe symptom and the most difficult to document objectively, was the most commonly reported complaint. Twenty-one (70%) individuals challenged with shrimp extract reported pruritus, either alone (*n* = 18) or in conjunction with other symptoms (*n* = 3) (10). Typically, pruritus began within 5 min of ingestion of shrimp extract and it resolved spontaneously within 15–20 min. In some patients, pruritus may be a forerunner of a more severe reaction. Thirty-three percent (6/18) of our shrimp challenge subjects who reported pruritus or subjective “pharyngeal swelling” went on to develop objective symptoms at a higher test dose of shrimp extract (10).

The significance of subjective symptoms like oropharyngeal pruritus should not be minimized. In 14 fatal cases of food-induced

anaphylaxis, symptoms of some patients were originally considered to be minimal by the patients or by the care giver (4,17). In seven such cases (4), patients initially felt only some tingling in the mouth and throat discomfort. A progression to more severe symptoms including abdominal cramps and vomiting, respiratory compromise, and hypotension took place over the next 60–90 min. Such severe life threatening reactions are most often associated with peanuts, nuts, fish, and shellfish (4) and appear to be more common in atopic individuals. In shrimp hypersensitive individuals, as little as 1–2 g of the offending food may trigger a life-threatening reaction (18).

Symptoms

Following Cutaneous Exposure to Shellfish

Contact dermatitis, urticaria, or pruritus is often reported by individuals who handle shrimp, crawfish, or crab (19–21). Although the majority of cutaneous reactions have been reported in subjects who are exposed occupationally; cutaneous reactions can also occur in hypersensitive subjects in nonoccupational settings. For example, a significant proportion of our shrimp hypersensitive patients have reported dermatitis. The exact prevalence of cutaneous reactivity in either occupational or domestic settings has not been established.

Urticaria resulting from cutaneous exposure could result from abrasion of the skin from handling the hard chitinous shells of crustacea; this, in effect, is a prick skin test “au naturale.” In some individuals, breaks in the natural skin barrier could be the exposure route for shellfish allergens. Care should be taken, however, to distinguish between true hypersensitivity reactions of the skin and simple physical trauma.

Pathogenesis

Immune Mechanisms

IgE-Mediated Hypersensitivity

The nature of adverse reactions resulting from ingestion or inhalation of shellfish cooking vapors and their temporal pattern of onset are suggestive of an IgE-mediated pathogenesis. In patients with a history of immediate adverse reactions following ingestion of shrimp (5,9,10), limpet (7), grand keyhole limpet (6), squid (8), or snail (12) specific IgE antibodies were demonstrated by prick skin

and radioallergosorbent testing (RAST). Basophils from limpet-sensitive patients also demonstrated dose-dependent histamine release following in vitro challenge with limpet extract (7).

The clinical relevance of shellfish-specific IgE antibodies can be seen in our studies of shrimp hypersensitivity. Eight of nine (89%) individuals with a positive shrimp challenge had a positive prick skin test to shrimp extract (10). The single patient with a negative skin test experienced only mild urticaria during open challenge. All patients reporting oropharyngeal pruritus also had a positive skin test to shrimp. A positive prick skin test coupled with a RAST value of >11% label bound had an 87% probability of predicting a positive shrimp challenge. It should be noted that our study population was chosen based on their history of immediate reactivity to shrimp; therefore, it may not be possible to extend these findings to all cases of shrimp hypersensitivity.

Non-IgE-Mediated Hypersensitivity

Circulating shrimp-specific IgG, IgM, and IgA antibodies have also been detected in shrimp hypersensitive subjects and in controls (10,22,23). Challenge positive individuals were shown to have significantly higher levels of shrimp-specific IgG and IgG₂ antibodies than patients whose sensitivity could not be confirmed by challenge (22,23). Shrimp specific salivary IgG and IgA levels were also quantitated in these subjects (24). Lower IgA:albumin ratios were found in those subjects with a negative shrimp challenge, suggesting that secretory IgA does not act as a barrier to the ingress of shrimp allergens. Clearly, the potential role of all specific isotypes and IgG subclasses in providing protection from or eliciting adverse reactions to shellfish in hypersensitive individuals needs additional study.

Nonimmune Mechanisms

Most patients with a history of adverse reactions to shellfish purport to have food "allergies;" however, not all of these reactions are actually caused by immune mechanisms. Since patients not suffering from a true hypersensitivity often need no treatment and can usually continue to eat shellfish with impunity, it is important to make this distinction. Examples of nonimmune mediated adverse reactions range from simple gastroenteritis to anaphylactoid reactions resulting from the ingestion of seafood contaminated with algal

or other naturally occurring toxins (25). The presence of food additives, including sodium metabisulfite, are of questionable significance in seafood-related reactions.

There is a commonly held belief in the medical community that there is an association between adverse reactions to radiocontrast media and shrimp hypersensitivity. There is, however, no objective information to substantiate this. In fact, in a study of 23 patients with recent histories of adverse reactions to radiocontrast media, no association with IgE-mediated reactions to seafood was demonstrated (26). Our own studies substantiated these findings.

Diagnosis

History

As with the assessment of any patient with suspected allergy, a detailed medical history is critical. Information should also be obtained concerning the putative foods provoking reactions; the nature of the symptoms—including the temporal relationship between the appearance of symptoms and ingestion of the food, and their reproducibility; and whether exercise exacerbates the reaction. Since challenge-documented shellfish hypersensitivity appears to be more common in atopic subjects (10), the atopic status of a patient should be determined by history and by prick skin testing with a panel of common inhalant allergens.

Skin Testing with Shellfish Extracts

Prick skin testing is the method of choice for demonstrating tissue-bound IgE (27,28). However, results from prick skin testing with many food extracts do not correlate as well with symptoms of food allergy as do results with inhalant allergens (4,29). Reasons for this probably include lack of appropriate extracts and failure to test patients who are truly hypersensitive. Shellfish, however, are among those foods in which skin test results are believed to correlate reliably with hypersensitivity reactions (10,30).

Prevalence of Skin Reactivity in Shellfish-Sensitive Individuals

Our studies of shrimp allergic patients (5,9,10,22,23) suggest that prick skin testing with shellfish extracts is an important part of the evaluation of shellfish-intolerant patients. Virtually all skin test

Table 2
Prevalence of Positive Skin test to Crustacea Extracts*

Extract	Crustacea-sensitive			Control subjects		
	Atopic <i>n</i> = 16 (%)	Nonatopic <i>n</i> = 17 (%)	Total (%)	Atopic <i>n</i> = 13 (%)	Nonatopic <i>n</i> = 16 (%)	Total (%)
Shrimp	15 (94)	13 (76)	85	2 (15)	0 (0)	7.5
Crab	13 (81)	11 (65)	73	2 (15)	0 (0)	7.5
Crayfish	13 (81)	10 (59)	70	1 (7.5)	0 (0)	3.8
Lobster	15 (94)	12 (71)	82	2 (15)	0 (0)	7.5

*Immediate wheal and flare at 10 mg/mL of crustacea extract.

reactions are immediate (within 15 min); however, rare isolated delayed reactions have also been reported (9). In one series of patients, 94% of atopic individuals who reported shrimp intolerance were skin test positive; this figure contrasts sharply with those for atopic (15%) or nonatopic shrimp-tolerant controls (0%) (Table 2). Similar results were obtained for skin test reactions to other crustacean allergens. All atopic and most (82%) nonatopic sensitive subjects had at least one positive prick skin test to a crustacea allergen (9).

Morgan and associates also showed that, in 36 subjects with a history of shrimp intolerance, skin reactivity was related to clinical history (31). Subjects with a history of pulmonary reactions following exposure to shrimp had a higher prevalence of skin test positivity to shrimp extract (85.7%) than did subjects without them (54.5%; $p = 0.05$).

In Vitro Quantitation of Shellfish-Specific Antibodies

Circulating shellfish-specific IgE antibodies have been quantified using the RAST (5,6,10,22,32) and other solid phase in vitro assays (7,8). Other isotype and IgG subclass specific antibodies have been measured using enzyme linked immunosorbent assays (ELISA) (9,10,22,23). Shellfish-specific antibody levels can be used to help determine immunopathogenetic mechanisms, predict which patients have the greatest likelihood of responding to challenge (10), monitor time and diet related changes in sensitivity (22), evaluate the presence of shared allergenic determinants among different species (6-8,32,33), and screen patients' sera for more sophisticated tests (34,35).

The importance of the direct RAST in the evaluation of shrimp hypersensitive patients has been demonstrated repeatedly. Elevated shrimp RASTs have been detected in up to 75% of atopic patients who report a history of shrimp intolerance (5). Although the percentage of individuals with elevated RAST values varied slightly with the population studied (9) it is noteworthy that no false positive reactions were reported. In individuals with DBPCFC-documented shrimp-hypersensitivity, RAST reactivity correlated with symptom score ($p < 0.05$), shrimp prick skin test titration end points ($p < 0.001$), and threshold dose for the development of objective symptoms ($p < 0.001$).

Quantitation of circulating shrimp-specific antibodies, including IgE, over a 24-mo period in patients with DBPCFC-documented shrimp hypersensitivity who eschewed shrimp has demonstrated the persistent nature of the immune response to shrimp in the absence of antigen exposure (22). The exact duration of these antibodies and whether they constitute the basis of a permanent shrimp hypersensitivity remains to be determined.

Double-Blind Placebo-Controlled Food Challenge

The "gold standard" for diagnosis of food allergy and for identification of the offending agent is the DBPCFC under controlled conditions (36). Provocative challenges are highly sensitive and specific; however, without concomitant laboratory tests on patient's sera or other biologic samples, they cannot provide information on the pathogenesis of reactions. Moreover, challenge is costly, time consuming, and carries a risk for the patient.

There are a variety of protocols available for DBPCFCs (36); however, we have developed one specifically to test shrimp hypersensitive individuals (10). This method can easily be adapted for use with other foods. Challenge consists of two phases. The first is double-blind-placebo-controlled. For that phase, increasing doses of shrimp extract (8 mg, 32 mg, 128 mg [equivalent to 16 shrimp]) or saline (placebo) are masked with vanilla ice cream mixed with grape flavoring (Syrpalta; Emerson Laboratories, Texarkana, TX). For each challenge, a test or placebo mixture is given at hourly intervals until objective symptoms occur or until the maximum dose is administered. If the subject tolerates the highest concentration of shrimp extract, open challenge with 16 whole shrimp, approx 64 g, is per-

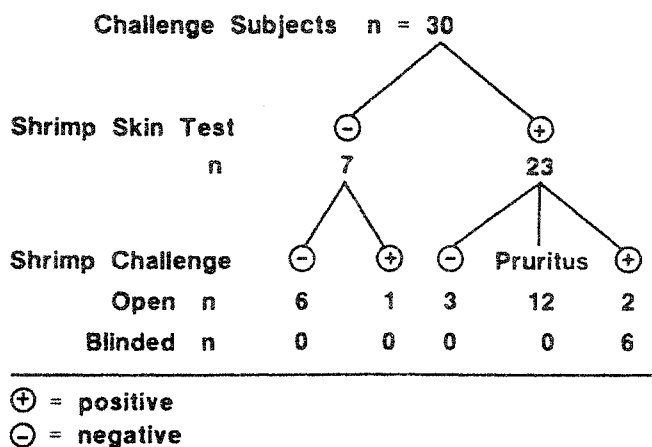


Fig. 1. Relationship of shrimp prick skin test reactivity to shrimp challenge response. From ref. 10. Reprinted with permission.

formed. Challenge is considered to be positive if objective symptoms occur during either phase.

Our challenge procedure was used to test 30 individuals with histories of immediate adverse reactions following ingestion of shrimp. Historical shrimp-induced symptoms in these subjects were: pruritus (90%), urticaria (61%), angioedema (52%), pulmonary difficulties (52%), gastrointestinal symptoms (35%), and shock (10%). Prior to challenge, end point prick skin test titration was performed and sera were obtained for immunologic assays. Only 9/30 (30%) subjects had a positive challenge (Fig. 1); all positive reactions occurred within 1 h of challenge. With the exception of urticaria, challenge-induced symptoms in the 9 reactors were similar to those reported historically (Table 3).

It is noteworthy that pruritus occurred in all 9 reactors and in 12 of the skin-test positive nonreactors. In this subgroup of 12, 7 (58%) first experienced pruritus during the blinded phase of the challenge. It is probable that masking the challenge substance in a vehicle that allows contact of this substance with the bucal mucosa, rather than via capsules or a feeding tube, maximizes the possibility of triggering oropharyngeal pruritus. Use of such a vehicle, however, more closely approximates the natural route of exposure.

Our finding that only 30% of individuals with a convincing history of shrimp hypersensitivity had a positive DBPCFC is consistent

Table 3
Comparison of Historically Reported Symptoms
with Challenge-Induced Symptoms in Positive Challenge Responses

Symptoms (<i>n</i>)	Produced during challenge	Reported history (<i>n</i>)	Reproduction of symptom (%)
Pruritus	9	9	100
Urticaria	1	6	17
Angioedema	3	7	43
Pulmonary	4	6	67
GI	4	6	67
Shock	0	0	0

with those from other studies of a variety of foods (37–40). One reason for this difference could be that the reported reactions were not actually to shrimp, but to another food or food product habitually ingested with shrimp, for example seasoning spices. Loss of food sensitivity is another potential reason for these differences (41) although this appears unlikely in the case of shellfish allergy (5,22). Lastly, there is the difference between perceived and documented sensitivity; which in turn, is partially dependent on confusion over what constitutes a true allergic reaction. All of these reasons underscore the necessity to document objectively, by provocative challenge, shellfish (or other food) hypersensitivity.

Treatment

Avoidance is the only currently accepted “treatment” of food allergies (42). With a discrete food entity, like shellfish, avoidance is usually easy; however, on occasion, these foods may be “hidden” in sauce, dressing, or prepared foods. For example, surimi, a seafood product, usually prepared from pollock, which is used in the manufacture of food products resembling crab-meat, beef, or pork and subsequently in artificial shellfish preparations, hot dogs, ham, or pizza toppings (43), may contain up to 10% crab meat or include crab extract. Therefore, shellfish hypersensitive subjects should be instructed always to read labels and, if in doubt, to inquire as to the ingredients used in a particular dish.

Antihistamines may ameliorate symptoms following accidental ingestion (44,45); however, they are not effective for prevention. Since

sodium cromolyn was shown to be ineffective in controlling adverse symptoms in DBPCFC, it can be neither recommended nor condoned as a prophylactic agent (46). Moreover, use of any prophylactic medication may give patients the mistaken belief that they are free to eat shellfish with impunity.

Individuals with a history of facial angioedema, laryngeal edema, pulmonary symptoms, or frank shock should be taught to self-administer epinephrine and to have it available (17). Identification bracelets (Medic-Alert) can also be purchased.

Other treatments commonly used for allergic diseases, notably immunotherapy, have not been widely used with food allergens. Recently, the potential utility of rush injection immunotherapy was demonstrated in peanut-sensitive patients (47); however, at this time this type of food immunotherapy should not be undertaken in the practice setting (48). Controlled clinical trials must be conducted before immunotherapy can be recommended as a general treatment for any food hypersensitivity.

Shellfish Allergens

Preparation of Shellfish Extracts

Commercial extracts are available for a variety of shellfish; however, there are several caveats to using them: Individual extracts may be prepared from multiple species or genera (49), extracts from unusual species may not be available, and their potency has not been adequately evaluated. Therefore, for use in *in vivo* and *in vitro* assays, we have prepared extracts of raw and cooked shellfish and of the water used to cook shellfish (5,32,33,49-51).

To prepare cooked-meat extracts, shellfish were cooked in ion-depleted water for 15 min. Meat of cooked or raw shellfish was mixed with phosphate buffered saline (PBS) for 3 min in a Waring blender. After an overnight extraction at 4°C, extracts were centrifuged (27,000g); and the supernatant was concentrated on an Amicon (Danvers, MA) YM 2 filter (mol wt exclusion >2000 Da) and dialyzed (mol wt exclusion 3500 Da) against PBS. Protein levels were quantified; and extracts were aliquoted and frozen at -20°C. Cooking water was concentrated and dialyzed as described for preparation of meat extracts. For shrimp, meat and water extracts have very similar protein content (Fig. 2). Prior to use in *in vivo* studies, all extracts were

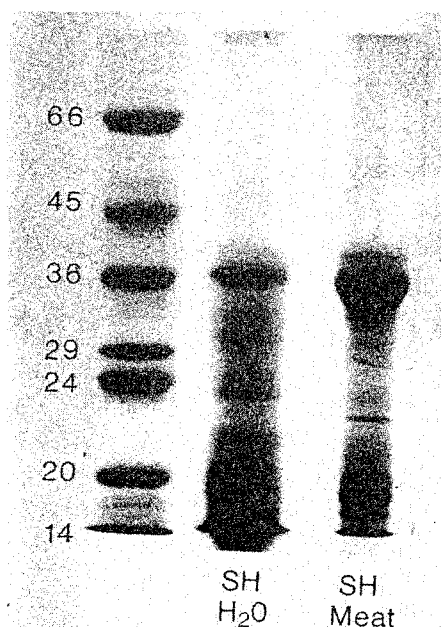


Fig. 2. SDS-PAGE separation of proteins in meat and boiling water extracts of brown and white shrimp.

sterile filtered ($0.45\ \mu$) and tested for the presence of hepatitis B surface antigen. Additionally, skin test reagents were mixed with sterile glycerol (1:1). Similar methods have been reported for preparation of skin test reagents for other species of shellfish (6–8).

Identification and Isolation of Major Shellfish Allergens

Shrimp Allergens

Purified, characterized shellfish allergens are necessary for the development of improved diagnostic and therapeutic agents. Shrimp extracts have been used as a model for the isolation and characterization of shellfish allergens (35,52–55). Hoffman and coworkers (52) were the first to identify and partially characterize shrimp allergens. Eleven of 11 serum samples (100%) from shrimp hypersensitive individuals had circulating IgE antibodies directed against a heat stable allergen, Antigen II, isolated from shrimp. That 38 kDa allergen had an isoelectric point of 4.5 and contained approx 4% carbohy-

drate. Further, Antigen II inhibited a cooked shrimp RAST by 92%. A second, less important allergen, Antigen I, was isolated from raw shrimp. Antigen I, a dimer of two polypeptide chains, had a mol wt of 21 kDa.

An antigen (Sa-II) similar to or identical to Antigen II was also isolated from shrimp (*Penaeus indicus*) by Nagpal and associates (53); however, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Sa-II has a slightly lower mol wt—34 kDa. These investigators also isolated a second heat stable protein antigen (Sa-I) (53) and what appears to be a tRNA allergen (Sa-III) (54). Although initially intriguing, the existence of a shrimp tRNA allergen has not been confirmed by other investigators. Both investigative groups used cumbersome isolation techniques and evaluated these allergens with only a limited number of patient's sera.

Confirmation of the importance of these allergens, as well as the evaluation of other shrimp antigens for allergenic properties, remained to be performed. Therefore, to assess more fully major allergens present in shrimp, proteins in cooked and raw meat from white (*P. setiferus*) and brown (*P. aztecus*) shrimp, as well as their cooking waters, were separated using SDS-PAGE (35,56). Coomassie blue staining of the different extracts demonstrated qualitatively similar patterns of multiple protein bands (Fig. 2). When these bands were probed with sera from two groups of RAST positive shrimp hypersensitive patients and radiolabeled anti-IgE, autoradiographs revealed that there were at least eight allergen bands corresponding to proteins between 20 and 72 kDa. The 36-kDa protein band was recognized by 12/13 (92%) sera in one group and by 18/25 (72%) in the other; in the latter group, 8/18 (44%) reacted exclusively with this protein. Although the 36-kDa band was clearly the most important allergen, other bands also bound IgE. The 20-kDa band bound IgE antibody from 10/13 (77%) and 6/25 (24%) samples; the 42/45 kDa band reacted with 9/25 (36%); and the 66/72 kDa band with 7/25 (28%).

Since the 36-kDa protein was such an important allergen, it was isolated for further study using preparative SDS-PAGE. This protein constitutes 20% of the soluble protein in crude cooked shrimp extract and can inhibit the crude shrimp extract RAST by 75%. The isoelectric point of this allergen is 5.2; and carbohydrates constitute

2.9% of its composition. It is rich in acidic amino acids; and the amino acid composition is similar to Antigen II (52) and Sa-II (53) (Table 4). The 36-kDa protein has been designated *Pen a* I or *Pen s* I, depending on the species of shrimp from which it is isolated. These data, taken together, suggest that *Pen a* I, Antigen-II, and Sa-II are similar if not identical allergens.

Pen a I was studied further following digestion with endopeptidase LYS-C. The amino acid sequence of a 21 residue peptide was compared with 2.5 million proteins in the Swiss protein database. The highest degree of homology (87%) was observed with the fruit fly muscle protein tropomyosin (57). A lesser degree of homology (60–62%) was found with tropomyosin from human, chicken, mouse, and rat. Further, analysis of rabbit tropomyosin on SDS-PAGE yielded a 36-kDa polypeptide chain (58). Thus, *Pen a* I appears to be the shrimp muscle protein homologous to tropomyosin.

Snow Crab Allergens

Many of the same techniques used to isolate and characterize allergens in shrimp extracts have also been used to evaluate snow crab (*Chionoecetes opilis*) extracts (Bush, Cartier, and Lehrer, unpublished results). Evaluation of snow crab extracts clearly demonstrated the presence of heat-labile and heat-stable allergens in shellfish. At least 36 and 34 separate protein bands were observed following SDS-PAGE separation of raw and boiled snow crab extract, respectively. The most prominent protein bands following Coomassie blue staining of the gel were of approx 36 and 45 kDa. There were 23 and 18 protein bands in raw and cooked extracts, respectively, that bound IgE antibodies. It is noteworthy that of 18 allergens present in boiled crab extract, the major allergen was not the 36-kDa protein, but rather a 14,750 Da protein. Fourteen of 18 (77%) serum samples obtained from RAST positive individuals reacted with that protein band, whereas only 4/18 (22%) of sera tested reacted with bands between 35 and 40 kDa.

Crossreactivity Among Shellfish

Crossreactivity Among Crustacea

Clinical histories of immediate hypersensitivity to multiple types of shellfish are commonly observed in our shrimp-sensitive patients (5). Further, individuals with a history of shrimp sensitivity demon-

Table 4
Amino Acid Composition of the Major Shrimp Allergen
as Isolated by Different Investigators

	<i>Pen a I</i>	Number of residues	
		Antigen II (52)	Sa II (53)
Mol wt (kDa)	36	38	34
Aspartic acid	40	58	39
Threonine	12	12	9
Serine	14	15	12
Glutamic acid	80	61	75
Proline	2	6	3
Glycine	10	20	6
Alanine	33	31	21
Cysteine	ND	2	3
Valine	13	19	15
Methionine	8	9	6
Isoleucine	6	12	6
Leucine	35	30	30
Tyrosine	4	7	6
Phenylalanine	4	9	6
Histidine	1	4	3
Lysine	26	27	27
Arginine	26	19	30
Tryptophan	ND	ND	4
Total	312	341	301

strated reactivity to other species of crustacea, even with no known prior exposure to a particular species (5). Prick skin test titration end points and RASTs to shrimp, crab, crawfish, and lobster extracts were highly correlated, with alpha values of <0.006 and <0.001 , respectively (9). Although these data are highly suggestive of cross-reactivity among various species of shellfish, neither skin testing nor RAST can eliminate the possibility of concomitant sensitivities.

RAST inhibition using pools of sera from shellfish hypersensitive subjects (33) and crossed-immunoelectrophoretic techniques (33,51,59) were used initially to determine whether crustacea have shared antigenic and allergenic determinants. The presence of shared allergenic determinants among white shrimp (*P. setiferus*), blue crab (*Callinectes sapidus*), crawfish (*Procambarus clarkii*), and lobster (*Panulirus sapidus*) was clearly demonstrated using RAST inhibi-

tion (Fig. 3). However, the ability to inhibit specific RASTs by 50% varied among the extracts, suggesting differences in potency. For example, shrimp was the most potent inhibitor of the shrimp RAST; it was followed in order of potency by crawfish, lobster, and crab (33).

Crossed immunoelectrophoretic techniques also demonstrated the presence of shared antigenic determinants among shrimp, crab, crawfish, and lobster (51), as well as the presence of genus-specific antigens (33). Of the 18 precipitins present in shrimp extract (59), 6–7 antigens were shared with crawfish and lobster, whereas crab had only 2 shared antigenic determinants with shrimp (33). Seven of the shrimp precipitins were allergens, as determined using crossed radioimmunoelectrophoresis; of these, 2 appeared to be common to shrimp, crab, crawfish, and lobster; 1 was also present in crawfish and lobster; 1 other was present only in crawfish and shrimp; and 4 were shrimp-specific (33). Unfortunately, the dependence of these techniques on rabbit hyperimmune sera limits their utility in studies of human allergic disease.

More recent studies from our laboratories have used *Pen a I* in RAST inhibition and blotting experiments to assess further crossreactivity among species of crustacea. Shrimp, blue crab, and crawfish extracts inhibit *Pen a I* RAST to a similar extent. Further, results from SDS-PAGE and immunoblot studies demonstrate that sera that identify *Pen a I* can also detect a 36-kDa protein present in crab, crawfish, and lobster extracts. The frequency with which sera react with this protein in the different species is identical, suggesting significant crossreactivity. It is probable that human IgE, although polyclonal, recognizes common epitopes present on the 36-kDa protein of each crustacea species tested.

To study further the epitope structure of *Pen a I* in relationship to allergens from other species of crustaceans, murine monoclonal antibodies (mAbs) were raised against *Pen a I*. Five of 6 mAbs had significant levels of reactivity with the 36-kDa shrimp allergen; several of these mAbs also recognized shrimp proteins at 29, 30, 45, and 66 kDa. Epitopes from *Pen a I* were identified in extracts of crawfish, crab, and lobster; mAbs also reacted with several other bands from extracts of these species (Fig. 4). Together, results obtained from blotting with human sera and mouse mAbs strongly suggest the presence of a common 36-kDa allergen, as well as other shared allergens, among species of edible crustaceans.

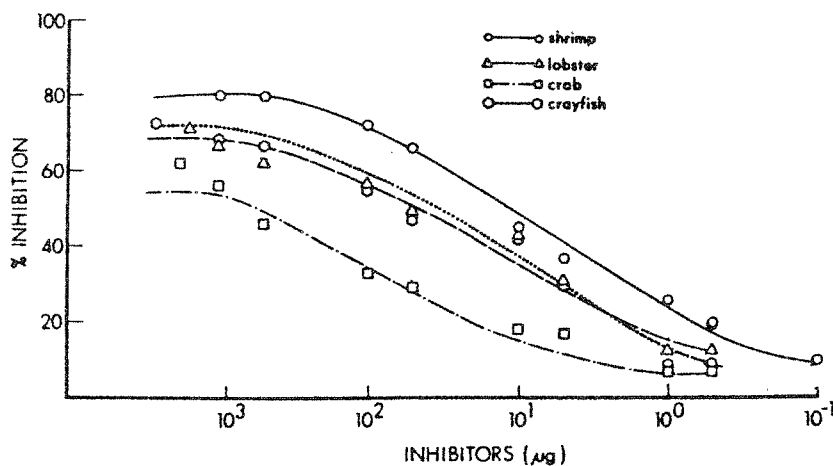


Fig. 3. Inhibition of shrimp RAST by shrimp, crab, crawfish, and lobster. From Lehrer, S. B.. The complex nature of food antigens: Studies of crossreacting crustacea allergens. *Ann Allergy* 57, 267–272, 1986. Reprinted with permission.

Crossreactivity of Crustacea with Other Arthropods

Crossreactivity of crustaceans with other groups of arthropods, particularly midges (60) and cockroaches (61), has also been reported. Recently, it has been demonstrated that *Pen a I* has allergenic epitopes shared with fruit fly (*Drosophila melanogaster*) tropomyosin (62). This shared allergenicity of tropomyosin between members of Arthropoda may explain those reports.

Homologous and Heterologous Crossreactivity Among Mollusks

Allergic reactions to mollusks, and consequently mollusk allergens, have not been as well studied as those of crustaceans. However, it has recently become apparent that mollusks are significant food allergens in exposed populations (6–8,32). Reports by some of our shrimp hypersensitive patients that they were also oyster intolerant prompted us (32) to assess potential crossreactivity between these two groups of shellfish. Shrimp, crab, lobster, and crawfish were highly crossreactive with oyster. Other investigators have confirmed this crossphyletic relationship. Using sera from limpet sensitive patients, a limpet RAST was significantly inhibited by round clam,

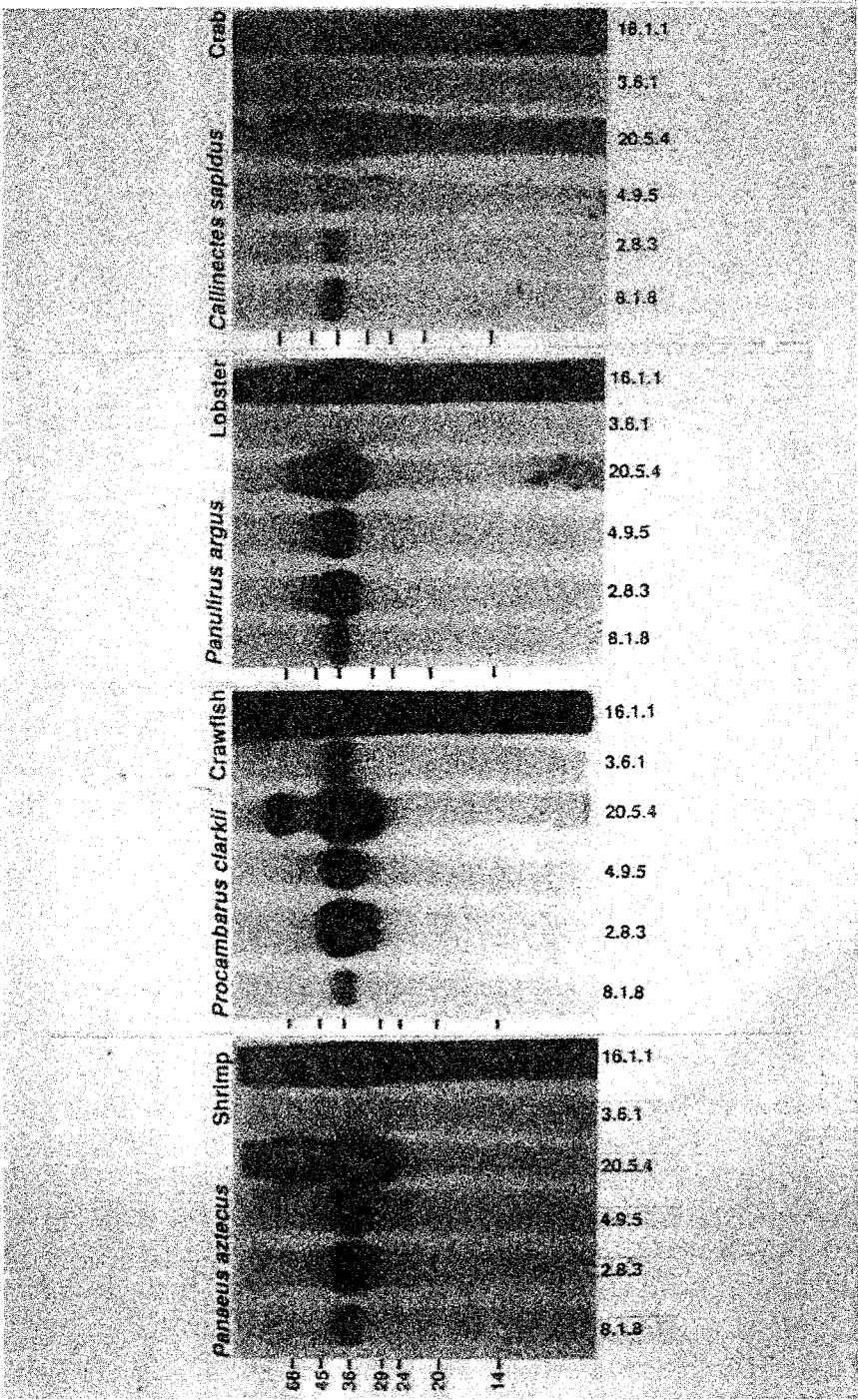


Fig. 4. Monoclonal antibody reactivity with shrimp, crawfish, lobster, and crab. *Int. Arch. of Allergy and Appl.* (in press)
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snail, oyster, shrimp, lobster, crawfish, and *D. pteronyssinus*. The RAST was not inhibited by mussel or squid extract. The apparent crossreactivity between mite and limpet was unexpected; and the authors hypothesized that limpets, which are filter feeders, may have ingested mite-like animals, which in turn were extracted with the limpets. In another study, sera from squid-allergic patients also demonstrated crossreactivity with other species of mollusks and arthropods (8). Overall, these data suggest that different species of mollusks share antigenic determinants with other species of mollusks, but also with several species of arthropods (7). However, since the source of these crossreacting antigens is not clear, particularly for filter feeding mollusks, clearly, these findings need to be confirmed and their clinical relevance established.

Summary and Future Directions

Crustacea and mollusks contain important allergens that can stimulate hypersensitivity responses in some individuals. Although the exact prevalence of reactivity to shellfish is not known, up to 250,000 individuals in North America alone may be at risk of developing disease. Anaphylactic reactions may follow ingestion of shellfish or inhalation of cooking vapors by sensitive individuals. The majority of adverse reactions appear to be IgE-mediated. Double-blind placebo-controlled food challenge should be used to confirm the diagnosis of shellfish hypersensitivity. Evidence from our studies of shrimp hypersensitive subjects suggests that, in some atopic individuals, a combination of a positive prick skin test and a RAST value >11% label bound may substitute for challenge.

Several investigators have isolated heat-stable shrimp allergens. Of these, *Pen a* I is the most important and has been the most thoroughly studied. This allergen is a 36-kDa glycoprotein exhibiting significant amino-acid sequence homology with the muscle protein tropomyosin. Evaluation of allergens in other species of crustacea, for example snow crab, has demonstrated the presence of other major shellfish allergens. Species of crustacea are highly crossreactive; and a 36-kDa protein is common to shrimp, crab, crawfish, and lobster. Crustacean allergens also share common allergens with mollusks; however, the exact nature of this phenomenon and its clinical implications have not been established.

Since shrimp hypersensitive subjects are usually easy to identify and a major shrimp allergen has been isolated and partially characterized, we believe that shrimp can be used as a model to study more fully food hypersensitivity reactions in general. Future directions in our laboratory will include further characterization of the epitopes of *Pen a* I, and other important shrimp allergens, and immunotherapy of shrimp hypersensitive individuals with these purified allergens. Additional studies will include further characterization of hypersensitive subjects who report only pruritus, the natural history of adverse reactions to shrimp, and uptake of allergens by the gut mucosa.

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