

Hypersensitivity reactions to ingested crustacea: Clinical evaluation and diagnostic studies in shrimp-sensitive individuals

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Adverse reactions to ingested crustacea are common and may be life-threatening. We studied 14 individuals with histories of such reactions to shrimp by immediate skin tests and RAST with extracts of shrimp, crab, crayfish, and lobster. Nine of these subjects (8/8 atopics and 1/6 nonatopics) had positive immediate skin tests (wheal ≥ 2 mm) and RAST (ratios >3.0) to shrimp. Their skin tests and RAST ratios to the other crustacea were also frequently positive even, in several cases, in the absence of prior exposure. In contrast, only 1/10 volunteers with no history of intolerance to crustacea had a weak positive skin test to raw shrimp. These studies suggest that both skin tests and RAST are useful in the confirmation of hypersensitivity to shrimp in atopic individuals and that cross-reactivity among crustacea may exist. (J ALLERGY CLIN IMMUNOL 76:440-5, 1985.)

In our region of the country (Louisiana), adverse reactions to ingested crustacea are common and not infrequently serious. We have been impressed with the variety of observed and reported symptoms that occur in sensitive individuals after ingestion of crustacea.¹⁻³ Most reactions have been ascribed to IgE antibodies, but some symptoms reported suggest other mechanisms.^{3, 4} Although crustacea have been reported to be one of the more common causes of allergic reactions to foods,¹⁻³ no study of seafood-induced hypersensitivity reactions has been performed specifically to investigate this problem. There is certainly need for such a study not only to clarify the immunopathogenesis of these allergic reactions but also to provide reliable characterized antigens that can be used in the diagnosis and study of crustacea-sensitive patients. The only allergens thus far identified in crustacea have been heat-stable and heat-labile allergens present in shrimp studied by Hoffman et al.⁵

As an initial step in the investigation of reactions to crustacea, we prepared extracts of shrimp, crab, crayfish, and lobster, useful for both skin and RAST

studies.⁶ With these extracts we evaluated 14 individuals with convincing histories of reactions to shrimp and 10 volunteers who ate shrimp frequently without difficulty. Our studies suggest that allergic reactions to crustacea are due to both IgE and non-IgE-mediated mechanisms. Furthermore, since some patients with classical immediate hypersensitivity to shrimp have IgE antibodies to crustacea not previously eaten, it appears that shared allergens exist among the crustacea tested.

MATERIAL AND METHODS

Study populations

Fourteen persons with convincing histories of untoward reactions to shrimp responded to an advertisement for study subjects allergic to shrimp. Ten nonsensitive laboratory personnel who frequently ate shrimp also volunteered to participate in this study. Each participant was requested to stop taking his/her antihistamines at least 24 hours before testing. Each person signed appropriate consent forms for skin testing and venipuncture and responded to a questionnaire administered by an investigator. The questionnaire included information on personal and family allergic status and contact with crustacea.

Crustacea extract preparations

Crustacea purchased locally, white shrimp (*Penaeus setiferus*), boiled and uncooked; blue crab (*Callinectes sapidus*), boiled; spiny lobster (*Panulirus argus*), parboiled and frozen; and crayfish tails (*Procambarus clarkii*), parboiled and frozen, were extracted in 0.01 mol/L phosphate-buffered saline (pH 7.2) (0.15 mol/L NaCl) by homogenizing the edible portion for 3 minutes in a Waring blender (Waring

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TABLE I. Characteristics of study population

Sensitivity to shrimp by history	Mean age	M	F	Atopic	Nonatopic
Sensitive (n = 14)	36.9	4 (28.6%)	10 (71.4%)	8 (57.1%)	6 (42.9%)
Nonsensitive (n = 10)	35.4	4 (40.0%)	6 (60.0%)	6 (60.0%)	4 (40.0%)

TABLE II. Symptoms after shrimp ingestion in sensitive subjects

Symptoms	Atopic (n = 8) (%)	Nonatopic (n = 6) (%)	Total (n = 14) (%)
Urticaria	7 (88)	5 (83)	12 (86)
Angioedema	4 (50)	4 (67)	8 (57)
Chest tightness or shortness of breath	2 (25)	2 (33)	4 (29)
Nausea, vomiting, or diarrhea	3 (38)	3 (50)	6 (43)
Fainting with documented hypotension	2 (38)	0 (0)	2 (14)
Other (chills, fever, abdominal discomfort, pain, finger stiffness)	3 (38)	1 (17)	4 (29)

Products Div., New Hartford, Conn.). After an overnight mixing on a shaker at 4° C, extracts were centrifuged (27,000 × g), and supernatants were concentrated on an Amicon YM5 filter (Amicon Corp., Danvers, Mass., exclusion >5000 daltons). Extracts were dialyzed (Union Carbide Corp., San Diego, Calif., exclusion 8000 to 12,000 daltons) and recentrifuged (78,000 × g). Dry weights were determined, and aliquots of the supernatants were stored at -20° C until further use.

Skin tests

Skin prick tests were performed on the forearm of each subject with 10 common airborne allergens: house dust mite, the pollens of live oak and pecan trees, Bermuda and Johnson grasses, ragweed, English plantain, and mold allergens *Aspergillus*, *Alternaria*, and *Fusarium* (all obtained from Hollister-Stier, Spokane, Wash., and used at 1:20 w/v). An immediate wheal and flare with the mean wheal diameter ≥2 mm occurring 15 minutes after testing was scored as a positive test. For purposes of this study, subjects with two or more positive skin tests to inhalant allergens and a personal and/or family history of allergy were called atopic.

Skin prick tests were then performed with extracts of uncooked shrimp, cooked shrimp, crab, crayfish, and lobster. The extracts were applied to the subject's back after it was cleansed with alcohol and marked in a grid with a felt tip pen. The crustacea extracts were applied to the upper part of the back at a beginning concentration of 1 ng/ml. At 15-minute intervals a log incremental concentration of each extract was applied until either a wheal of 2 mm or more in mean diameter was produced or a concentration of

10 mg/ml was tested. Control prick tests of histamine (1.0 mg/ml) and saline were also applied. All subjects had wheal-and-flare reactions to histamine but not to saline.

RAST

Filter paper discs were activated with cyanogen bromide according to the method of Ceska and Lundkvist.⁷ Cyanogen bromide-activated discs were coupled with the crustacea extracts or human serum albumin, and serum samples were tested in duplicate as previously reported.⁸ Results are expressed as a ratio obtained by dividing the mean counts per minute bound to the crustacea discs by the mean counts per minute bound to the human serum albumin control.

RESULTS

Clinical characteristics of study subjects

The characteristics of the 24 study subjects (14 with a history of shrimp sensitivity and 10 with no history of sensitivity) in our study are summarized in Table I. The group with a history of shrimp sensitivity (14/24) had a mean age of 36.9 years and were predominantly female and atopic. The nonsensitive group (10/24) had a mean age of 35.4 years and were also predominantly female and atopic.

The symptoms associated with shrimp ingestion as reported by the shrimp-sensitive group are summarized in Table II. Since the possibility of different mechanisms among atopics and nonatopics was considered, we subdivided subjects (with a history of shrimp sensitivity) for the purpose of analysis. Both

TABLE III. Time interval between crustacean ingestion and symptoms

Atopic status	Time to reaction	Offending crustacea*			
		Shrimp (%)	Lobster (%)	Crab (%)	Crayfish (%)
Atopic	<60 min	6/8 (75)	2/4 (50)	3/4 (75)	3/3 (100)
	>60 min†	2/8 (25)	2/4 (50)	1/4 (25)	0/3 (0)
Nonatopic	<60 min	5/6 (83.3)	1/2 (50)	3/4 (75)	4/5 (80)
	>60 min†	1/6 (16.7)	1/2 (50)	1/4 (50)	1/5 (20)

*Cooked.

†Less than 120 minutes.

TABLE IV. Time interval since last ingestion of offending crustacea

Last exposure	Atopic status	Boiled shrimp (%)	Boiled lobster (%)	Boiled crab (%)	Boiled crayfish (%)
<2 mo	Atopic	2/8 (25)	0/4 (0)	0/4 (0)	0/3 (0)
	Nonatopic	3/6 (50)	1/2 (50)	1/4 (25)	1/5 (20)
2 mo to <6 mo	Atopic	1/8 (12.5)	0/4 (0)	0/4 (0)	0/3 (0)
	Nonatopic	2/6 (33.3)	0/2 (0)	2/4 (50)	0/5 (0)
6 mo to <12 mo	Atopic	0/8 (0)	0/4 (0)	0/4 (0)	0/3 (0)
	Nonatopic	0/6 (0)	0/2 (0)	0/4 (0)	0/5 (0)
1 yr to <5 yr	Atopic	4/8 (50)	2/4 (50)	4/4 (100)	3/3 (100)
	Nonatopic	1/6 (16.7)	1/2 (50)	1/4 (25)	3/5 (60)
>5 yr	Atopic	1/8 (12.5)	2/4 (50)	0/4 (0)	0/3 (0)
	Nonatopic	0/6 (0)	0/2 (0)	0/4 (0)	1/5 (20)

atopic and nonatopic individuals reported experiencing similar symptoms. Most individuals reported urticaria or angioedema. Twenty-five percent to 33% of each group reported respiratory symptoms, i.e., wheezing or shortness of breath; whereas 38% of the atopic and 50% of the nonatopic subjects reported gastrointestinal symptoms. Table III summarizes the time interval between shrimp ingestion and the onset of symptoms as reported by study participants. Most subjects experienced symptoms within 1 hour of ingestion. Some study subjects had histories of reactions to other crustacea in addition to shrimp. The period of time from ingestion of these other crustacea to reaction is generally similar to that for shrimp.

The time interval since the last ingestion of crustacea for each sensitive subject is listed in Table IV. For the purpose of this study, those individuals who had never eaten a particular crustacea were included in the "more than 5 years since exposure" group. Most subjects who reported shrimp sensitivity (57%) had eaten shrimp in the year before evaluation.

Skin testing and RAST determinations

Twelve (86%) of the 14 individuals with histories of shrimp sensitivity had positive skin prick tests to one or more of the crustacea extracts (Table V). Concentrations eliciting positive skin tests range from 0.1 µg/ml to 10,000 µg/ml. Positive reactions at lower concentrations of crustacea extract were presumed to indicate greater skin sensitivity in these individuals. Seven of eight (88%) sensitive atopic subjects had positive skin tests to lower concentrations of extract (0.1 to 100 µg/ml), whereas only 33% of nonatopic sensitive individuals had positive skin tests at these concentrations. Of the 10 individuals studied with no previous history of reactions to shrimp or other crustacea, only one had a positive skin test to any of the extracts tested and that was to raw shrimp at 10,000 µg/ml (highest concentration). This individual was an atopic woman with respiratory allergy.

RAST ratios obtained by use of crustacea extracts are illustrated on Table VI. Nine of the 14 (64%) subjects with history of shrimp sensitivity had RAST

TABLE V. Skin reactivity to crustacean extracts of subjects with histories of shrimp sensitivity

Study subject	Atopy	Minimal crustacean extract ($\mu\text{g/ml}$) inducing wheal ≥ 2 mm				
		Raw shrimp	Boiled shrimp	Boiled lobster	Boiled crab	Boiled crayfish
S. A.	+	1	10	10	10	100*
F. B.	+	$>10,000^\dagger$	10,000	10,000	1,000	10,000
J. C.	+	1	100	100	0.1	10
F. M.	+	10	10	10*	10	10*
A. M.	+	1	1	1*	1	1*
H. M.	+	10	10	10	10	10
R. S.	+	10	10	10	10	10
W. S.	+	10	10	1	1	10
G. C.	—	$>10,000$	$>10,000$	$>10,000$	$>10,000$	$>10,000$
A. C.	—	10	10	10,000*	10	10
R. D.	—	$>10,000$	$>10,000$	$>10,000$	$>10,000$	$>10,000$
D. F.	—	$>10,000$	10	10,000	1	10,000
S. L.	—	$>10,000$	10,000	$>10,000$	$>10,000$	$>10,000$
J. W.	—	10,000	10,000	10,000	10,000	10,000

*Positive tests in individuals who claimed never to have eaten crustaceans.

 † Negative skin test reactions at 10,000 $\mu\text{g/ml}$.**TABLE VI.** RAST reactivity to crustacea of 14 individuals with histories of shrimp sensitivity

Study subject	Atopy	RAST ratio				
		Uncooked shrimp	Boiled shrimp	Boiled lobster	Boiled crabs	Boiled crayfish
S. A.	+	22.3	33.0	6.6	7.1	24.6*
F. B.	+	5.6	17.4	3.4	12.5	14.5
J. C.	+	3.1	4.4	1.5	4.4	6.7
F. M.	+	2.0	4.2	1.6	6.9	7.2*
A. M.	+	3.4	8.8	1.6	8.9	10.0*
H. M.	+	2.6	3.8	1.6	4.4	3.8
R. S.	+	27.2	35.3	13.1	26.4	32.8
W. S.	+	22.3	27.7	9.0	31.4	24.6
G. C.	—	2.0	1.6	1.2	1.5	1.0
A. C.	—	2.0	1.7	0.9	1.5	3.9*
R. D.	—	2.6	2.1	2.5	1.8	1.2
D. F.	—	2.4	4.6	1.1	1.8	1.9
S. L.	—	1.9	1.6	1.1	1.5	1.1
J. W.	—	1.8	1.5	1.0	1.3	0.9

*Positive reactions in individuals with no known prior exposure.

ratios of ≥ 3 to at least one of the crustacea tested. Of these 14 subjects, all shrimp-sensitive atopic individuals demonstrated positive RAST ratios to boiled shrimp, whereas only one (17%) of the nonatopic sensitive subjects had ratios ≥ 3 . The RAST ratios in the nonatopic group were generally much lower than those of the atopic group. Of the 12 shrimp-sensitive individuals with positive skin tests to shrimp,

most (nine) had positive RAST ratios to shrimp. All RAST ratios of sera from nonsensitive subjects were <3 .

DISCUSSION

Individuals with histories of shrimp sensitivity were selected and tested for skin and RAST reactivity to shrimp, crab, lobster, and crayfish. Results of our

investigation suggest a dichotomy among such patients. Atopic study subjects with a history of shrimp sensitivity generally had positive skin tests (with lower concentrations of crustacea extract) and positive RASTs to shrimp and clinical symptoms common to systemic IgE-mediated allergic reactions. For the most part these symptoms were reported to occur shortly after ingestion of shrimp, although this was not inevitably the case. Nonatopic individuals with histories of shrimp sensitivity usually had negative skin tests to shrimp (or positive reactions only at the highest test concentrations) and negative or minimal RAST reactivity.

The lack of skin test and RAST reactivity in nonatopic patients with histories of shrimp sensitivity could not be explained by the fact that a longer time had elapsed since they had last eaten shrimp. In fact, most of these patients had reported eating and reacting to shrimp in the 2- to 6-month period before investigation. By contrast, several atopic patients avoided contact with any crustacea for more than a 2-year period and yet still demonstrated significant skin and RAST reactivity to many of the crustacea extracts tested. Furthermore, negative skin and RAST tests in nonatopic sensitive subjects can not be due to inadequate crustacea antigens because atopic patients consistently reacted to these extracts. Non-IgE-dependent mechanisms may be responsible for the reactions in nonatopic subjects. Because the possibility of nonatopic patients being negative on challenge has not been eliminated, future studies with challenges and assessment of other immune mechanisms should help resolve this issue.

An important concern of this study was testing techniques for diagnosis of shrimp and other crustacea allergies by use of *in vitro* assays. Do skin tests and RASTs properly identify such sensitive individuals? In assessing our results it was important to define that group of sensitive individuals that we were detecting. Our studies suggest that skin tests and RASTs can identify atopic patients sensitive (by history) to shrimp but are less useful in identifying sensitivity in nonatopic patients. Another important consideration was the dose of extract necessary to elicit a skin response. For example: 2/6 nonatopic subjects not only had positive skin reactions only to the highest concentrations of extract but also had negative RASTs. In fact, even one of our RAST-negative control individuals (an atopic woman with asthma and hayfever who ate shellfish) demonstrated a positive skin test to raw shrimp extract only at the highest concentration tested. This observation has been reported by others⁴ and empha-

sizes the need for quantitative as well as qualitative analysis of RAST and skin test results when crustacea sensitivity is assessed.

Another observation relevant to shrimp-allergy diagnosis is that two of the shrimp-sensitive (by history) subjects with positive skin and RAST reactions have since knowingly eaten shrimp, without our permission, and without incident. The variability of clinical symptoms of some food allergies (assessed by challenge and skin reactivity) has been established in children and may be reflected in these two adults.^{9, 10} Their food sensitivity may depend not only on the presence of specific IgE antibodies but also on other factors such as changes in intestinal permeability. Obviously, further assessment of their current immunologic reactivity to shrimp as well as double-blind challenge of these individuals is needed.

Interestingly, four individuals demonstrated significant skin test and RAST reactivity to some crustacea extracts without prior history of exposure to these crustacea. For example, although there is no lobster native to Louisiana, three shrimp-sensitive volunteers, who had never eaten lobster, had positive skin tests and RASTs with lobster extract. Conversely, some subjects who had come to New Orleans from other parts of the country where crayfish is considered inedible and had not eaten crayfish in New Orleans, had positive tests with crayfish extract. It is possible that these subjects had been inadvertently exposed to crayfish and/or lobster, as crustacea are mixed in preparations of a variety of dishes, particularly in the city of New Orleans; however, a more plausible explanation is that there are cross-reacting allergens present in crustacea. Indeed, clinically, there has been a notion for some time that this might be the case, since some individuals report sensitivity to all crustacea rather than to just a few of them. We are now studying the possibility of cross-reacting crustacea allergens and have some evidence by immunoprecipitation and RAST inhibition to support this.¹¹ The documentation of shared allergens among various crustacea could have obvious clinical importance, and this warrants continued evaluation.

In conclusion, there appear to be two types of shrimp-sensitive individuals, those who are atopic and have positive skin and RASTs to crustacea and those who are nonatopic, most of whom have negative or minimal skin test reactivity and RASTs to crustacea. The possibility that these findings may reflect different immunologic or even nonimmunologic mechanisms resulting in adverse reactions to crustacea allergy is currently being investigated.

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Cholinergic nasal hyperreactivity in atopic subjects

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Increased nasal secretions are a fundamental component of allergic rhinitis. In order to analyze various parameters of nasal secretions, a relatively nontraumatic method for collecting nasal secretions was required. A small, flexible rubber catheter connected to a vacuum and inserted 4 cm into the nose proved to be an efficient method for recovering secretions produced from a series of nasal washes. An average of 67% of the washings were recovered and analyzed for protein content. Topical methacholine (5 to 100 mg) stimulated a dose-related increase in the amount of protein secreted with atopic patients demonstrating significantly more responsiveness than nonatopic patients (29.9 times the prechallenge production of protein for atopic patients and 4.8 times for nonatopic patients). Pretreatment with atropine (10 µg) reduced the effects of methacholine in atopic subjects, indicating that the secretory activity was in response to muscarinic receptor stimulation. Therefore, in addition to the array of autonomic abnormalities already recognized in atopic patients, these subjects are also hyperresponsive to nasal cholinergic stimulation. (J ALLERGY CLIN IMMUNOL 76:445-52, 1985.)

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Abbreviation used

r_s: Spearman's rank coefficient

Atopic subjects express an array of abnormal autonomic responses.¹ Hyperreactivity to cholinergic stimuli is one such abnormality observed in subjects with either allergic rhinitis or allergic asthma. The extent of these reactions has been determined with