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Immunologic evaluation of shrimp-allergic individuals

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Thirty-three individuals with a history of immediate hypersensitivity reactions after shrimp ingestion and 29 nonshrimp-sensitive control subjects were evaluated for evidence of crustacea-specific immunity by skin prick test titration end point, RAST, and ELISA, with extracts of shrimp, crab, crayfish, and lobster. Individuals were categorized as either atopic or nonatopic on the basis of history and skin test reactivity to common inhalant allergens. Most (28/33) shrimp-sensitive subjects had positive skin prick tests to shrimp extract, whereas skin tests were negative in 27/29 control subjects. Eighty-one percent of atopic and 41% of nonatopic shrimp-sensitive subjects had elevated shrimp-RAST ratios. The RAST ratios of atopic individuals were significantly higher than ratios of nonatopic individuals, and there was a significant correlation between shrimp-RAST ratios and historical clinical symptom scores. RAST determinations of all control subjects were negative. Shrimp-sensitive subjects also had significantly elevated serum levels of shrimp-specific IgG and IgA as compared to control individuals. Both IgG and IgA shrimp-specific reactivity demonstrated a significant positive correlation with shrimp-RAST ratios. These studies indicate that IgE-mediated, type I mechanisms, detected by positive shrimp skin tests and RASTs, appear to be operative in crustacea-sensitive individuals, particularly those with concurrent respiratory allergy. Although the role of shrimp-specific IgG and IgA antibodies in the immunopathogenesis of crustacea allergy remains unclear, such antibodies appear to represent increased immunologic recognition of shrimp allergens/antigens in shrimp-sensitive subjects. (J ALLERGY CLIN IMMUNOL 1987;80:716-22.)

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Abbreviations used
OD: Optical density
PBS: Phosphate-buffered saline
BSA: Bovine serum albumin

Allergic reaction after the ingestion of food is a commonly invoked clinical diagnosis. Since reactions to foods may result in a variety of symptoms, the

TABLE I. Symptoms associated with shrimp ingestion in shrimp-sensitive subjects

Symptom	Atopic n 16 (%)	Nonatopic n 17 (%)	Total n 33 (%)
Urticaria/angioedema	14 (88)	14 (83)	28 (85)
Gastrointestinal	7 (44)	6 (35)	13 (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)

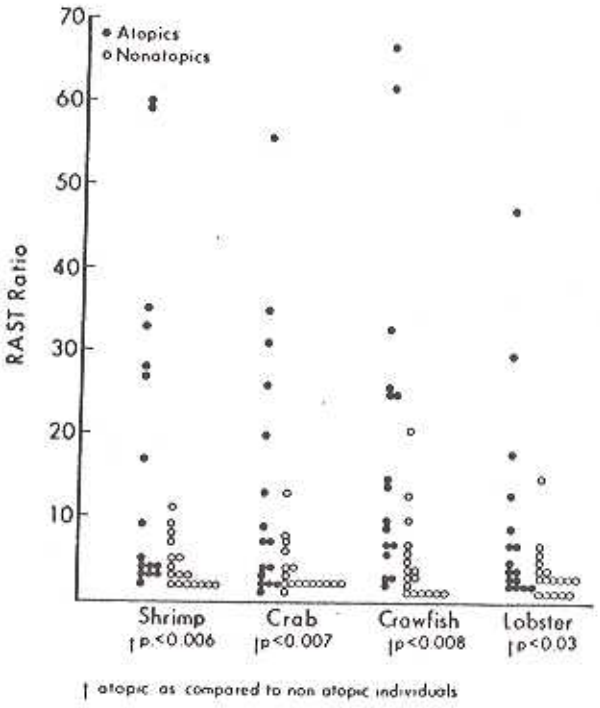

FIG. 1. RAST reactivity of shrimp-sensitive subjects to crustacea extracts. The p value indicates level of significance of the difference between atopic and nonatopic individuals for each extract.

actual mechanism is sometimes obscure. Although most confusion can be resolved by clearly delineating the characteristics of an allergic or hypersensitivity reaction,[1] there have been relatively few well-designed studies in this regard. Most substantiated food hypersensitivity reactions appear to be IgE-mediated.[2-5] Although immunologic mechanisms other than IgE have been suggested,[6-9] their role in food hypersensitivity reactions often remains unclear.

Studies of reactions to a particular food may provide a useful approach to the investigation of food allergy.[3] Although seafood is a commonly reported cause of food hypersensitivity,[10] it is rarely used as a model for elucidating immunopathogenic mechanisms of such reactions. Since crustacea are readily identified constituents of the diet, and in our geographic area a common dietary component, studies of reactions to crustacea may provide a unique opportunity to investigate immunologic correlates of clinical food reactivity. We have previously reported the use of crustacean extracts to identify crustacea-sensitive individuals.[11] We present in this article a more comprehensive investigation of immunologic reactivity to shrimp allergens (antigens) in a large group of subjects with a history of immediate sensitivity to shrimp.

MATERIAL AND METHODS

Study subjects

All individuals participating in this study were administered a questionnaire regarding personal and family allergic history, as well as reactions to crustacea. For the purposes of this study, subjects with two or more positive skin tests to a panel of 10 common inhalant allergens[11] and a personal or family (first degree relatives) history of allergy were designated as atopic.

Thirty-three adults with a clinical history suggestive of immediate hypersensitivity reactions after the ingestion of shrimp were enrolled. The median age of these shrimp-sensitive individuals was 35 years with a range of 22 to 58 years. Sixteen subjects (six male and 10 female) were atopic, and 17 subjects (four male and 13 female) were nonatopic by the criteria described above.

Twenty-nine healthy adult volunteers with no history of reactions to crustacea served as control subjects. These nonsensitive individuals to shrimp were either Medical Center personnel or volunteers referred to the Clinical Immunology Section for evaluation of rhinitis or asthma. The median age of this control group was 34 years with a range of 21 to 61 years. Thirteen subjects (seven male and six female) were atopic, and 16 subjects (six male and 10 female) were nonatopic.

In order to evaluate the intensity of allergic reactions to shrimp, specific symptoms reported to occur after its ingestion were given a weighted score (based on increasing clinical severity) as follows: urticaria, 1; angioedema, 2; gastrointestinal symptoms (nausea and vomiting to diarrhea), 3; pulmonary (shortness of breath or wheezing), 4; and generalized systemic anaphylaxis, 5; no score was given for symptoms other than symptoms listed. The cumulative total was designated as the clinical symptom score.

Crustacea allergens and skin testing

Water-soluble extracts of shrimp, crab, crayfish, and lobster were prepared as previously described[11]; concentrations of 1 μg/ml to 10 mg/ml in 50% glycerol were used to determine the prick skin test end point. The positive titration end point was the lowest allergen concentration

TABLE II. Incidence of positive skin test to crustacea extracts*

Extract	Crustacea-sensitive			Control subjects		
	Atopic n 16 (%)	Nonatopic n 17 (%)	Total (%)	Atopic n 13 (%)	Nonatopic n 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.

TABLE III. Shrimp skin test and RAST reactivity in all study subjects

	Shrimp-sensitive		Nonsensitive	
	Atopic n 16 (%)	Nonatopic n 17 (%)	Atopic n 13 (%)	Nonatopic n 16 (%)
ST+/RAST+	13 (81)	7 (41)	0	0
ST+/RAST−	2 (13)	6 (35)	2 (15)	0
ST−/RAST+	0	0	0	0
ST−/RAST−	1* (6)	4 (24)	11 (85)	16 (100)

ST+ = immediate wheal and flare at 10 mg/ml of shrimp extract; RAST+ = RAST ratio > 3.0 with shrimp extract.
*Delayed ST+ at 6 hours.

producing an immediate reaction with a mean wheal diameter of 3 mm greater than that of the control reaction (PBS in 50% glycerol).

Total IgE determinations (PRIST)

Total serum IgE levels were determined in duplicate with the commercially available PRIST kit (Pharmacia Diagnostics, Piscataway, N. J.). Briefly, 100 μl of serum was added to anti-IgE-coupled disks and incubated at room temperature. After washing, each disk was incubated overnight with 100 μl of ^{125}I-labeled anti-IgE at room temperature. The excess-labeled antibody was removed by washing, and the quantity of ^{125}I attached to each disk was counted. IgE serum concentrations were determined from the standard curve and expressed in kilo units per liter.

RAST

RAST ratios were determined on sera samples as previously reported.[11] Briefly, crustacea allergens or human serum albumin control was conjugated to CNBr-activated disks. All sera were tested in duplicate, and results were expressed as a ratio of mean counts per minute for crustacea disks divided by mean counts per minute for human serum albumin control disks. In this study a ratio of >3 was considered positive.

ELISA

Serum levels of shrimp-specific IgG, IgA, and IgM were quantified with a previously published ELISA method[12] modified to detect antishrimp reactivity. Briefly, wells of flexible microtiter plates were coated with shrimp extract (100 μg/0.2 ml) by overnight incubation at 4° C. Plates were washed five times with 0.15 mol/L of PBS, pH 7.4, containing 0.5% BSA (PBS-BSA). Sera were diluted 1:10 in PBS-BSA, and 100 μl was added to duplicate wells. The plates were incubated 1 hour at 37° C and again were washed five times. Column-purified peroxidase-labeled goat antiserum specific for human IgG, IgA, or IgM (Cappell Laboratories of Cooper Diagnostics, Inc., West Chester, Pa.) was added as developing antibody. After reaction with the peroxidase substrate (ABTS, Boehringer-Mannheim, Gambit, West Germany), the intensity of the reaction was determined by measuring the OD at 405 nm on an ELISA reader (BioTek Instruments, Inc., Burlington, Vt.). Results were recorded as Δ OD where Δ OD = OD experimental − OD diluent. Assays were standardized by including a serum with known reactivity on each plate; the Δ OD of this test serum varied <10% among assays.

Statistical analysis

Atopic and nonatopic subjects were compared with the Mann-Whitney U nonparametric test (for the immunoglobulin, RAST, skin test titration, and symptom score values) and Fisher's exact test (for prevalences of skin test sensitivity). Shrimp-specific IgG, IgA, and IgM values were compared with Student's t test. Spearman's correlation coefficient was used in testing for a nonparametric association between continuous variables (e.g., immunoglobulin determinations and RAST values).[13]

RESULTS

Clinical evaluation of study subjects

The prevalence of symptoms associated with shrimp ingestion are summarized in Table I. Sixteen (48%) of 33 crustacea-sensitive individuals and 13 (45%) of 29 control subjects were atopic. Both atopic and nonatopic, shrimp-sensitive individuals reported a spectrum of immediate (within 1 to 2 hours after ingestion) symptoms consistent with type I reactivity. The most common symptom (85%) was urticaria and/or angioedema. Gastrointestinal symptoms reported by 40% of the subjects included nausea, emesis, and diarrhea; pulmonary manifestations in 27% of subjects included chest tightness, shortness of breath, and frank asthma. Additionally, two nonatopic individuals reported generalized pruritus as their only symptom after shrimp ingestion. Only atopic subjects reported symptoms of systemic anaphylaxis that resulted in a trend toward a higher mean clinical symptom score in atopic subjects of 7.1 compared to 4.5 in the nonatopic individuals.

Skin test reactivity

Skin test reactivity to the battery of crustacea extracts are presented in Table II. Eighty-five percent of individuals reporting reactions to shrimp had positive skin tests to shrimp with titration end point concentrations ranging from 1 μg to 10 mg/ml. The one atopic individual with a negative immediate skin test to shrimp had a delayed positive reaction 6 hours later. Most individuals with a positive skin test to shrimp also had positive skin tests to other crustacea extracts. All atopic and most (82%) nonatopic sensitive subjects demonstrated a positive prick test to at least one of the crustacea extracts. Additionally, the incidence of positive skin tests to crayfish and lobster were significantly higher in atopic as compared to nonatopic subjects ($p < 0.01$).

No differences in skin test titration end points to each extract were detected between the atopic and nonatopic sensitive subjects. No correlation between the shrimp titration end point and clinical symptom score was noted. However, the titration end points to shrimp, crab, crayfish, and lobster correlated significantly ($p < 0.006$).

Two of the 29 control subjects had positive skin tests to shrimp at concentrations of 1.0 and 10 mg/ml, respectively. One of these individuals reacted to all crustacea allergens, and the other individual reacted to shrimp and crab. A third individual had a positive response to lobster. All positive skin tests to crustacea extracts occurred in atopic control individuals.

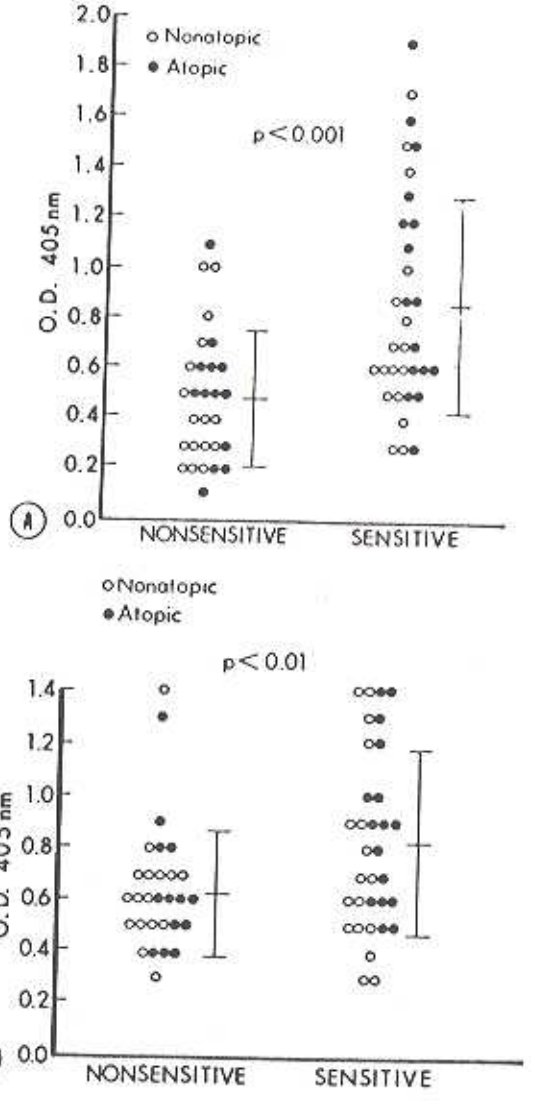

FIG. 2. A, IgG. B, IgA. Relative reactivity of serum shrimp-specific antibodies determined by ELISA. Sensitive subjects were subjects with a history of immediate adverse reaction to shrimp ingestion, whereas nonsensitive subjects had no reactions to shrimp or other crustacea. The p value indicates level of significance of the difference between sensitive and nonsensitive individuals.

Total and specific IgE determination

As expected, the serum IgE levels of atopic sensitive subjects (mean = 420 kU/L) were significantly higher ($p < 0.001$) than that of nonatopic sensitive individuals (mean = 147 kU/L). Although IgE level did not correlate with the clinical symptom score, a significant correlation was noted between serum IgE levels and RAST ratios for all crustacea extracts ($p < 0.001$ for each).

The RAST reactivity of shrimp-sensitive subjects

to crustacea extracts is illustrated in Fig. 1. Elevated shrimp RASTs (ratios ≥3) were detected in 60% of sensitive subjects but in none of the 29 control subjects. Significantly, more atopic subjects had elevated RAST ratios than nonatopic subjects ($p < 0.02$), and shrimp-RAST ratios of all shrimp-sensitive subjects correlated with clinical symptom score ($p < 0.012$). Furthermore, the RAST ratios of the atopic group were significantly higher than the ratios of the nonatopic group ($p < 0.03$). Although the shrimp RAST ratio did not significantly correlate with shrimp skin test titration end points, the RAST values to shrimp, crab, crayfish, and lobster correlated significantly with each other ($p < 0.001$), as previously noted with skin test titration end points.

Table III presents the incidence of positive shrimp skin test and RAST ratios in all study subjects. Eighty-one percent of atopic sensitive subjects had both positive skin tests and RAST, whereas only 41% of nonatopic sensitive subjects had similar reactivity. Conversely, 24% of nonatopic sensitive subjects were both skin test and RAST negative as compared to only 6% of the atopic group. Although there was no statistical relationship between atopic status and positive skin tests, there was clearly a significant relationship between history of shrimp hypersensitivity reaction and positive shrimp skin prick test.

Shrimp-specific IgG, IgA, and IgM

Serum levels of IgG and IgA measured by ELISA are illustrated in Fig. 2, *A* and *B*). The crustacea-sensitive individuals had significantly elevated shrimp-specific IgG ($p < 0.001$) and IgA ($p < 0.01$) as compared to control subjects. There were no differences in levels of shrimp IgG or IgA between the atopic and nonatopic crustacea-sensitive individuals. The shrimp-specific IgM in sensitive subjects was not different from control values (data not presented). Both shrimp-specific IgG and IgA reactivity correlated significantly with shrimp RAST ratios ($p < 0.01$ and 0.04, respectively), but not with clinical symptom scores or shrimp prick skin test titration end points.

DISCUSSION

This study extends our earlier observations describing immunologic reactivity of shrimp-sensitive individuals to crustacea. In this current study, as in the previous study, all our sensitive study subjects reported symptoms consistent with immediate, type I hypersensitivity reactions. When symptoms were analyzed with respect to atopy, generally, no significant differences were noted except that only atopic subjects reported anaphylaxis and that only nonatopic individuals reported generalized pruritus as their only shrimp-induced symptom. These results suggest our atopic subjects with shrimp-specific IgE-mediated disease are at greater risk for shrimp-induced systemic anaphylactic reactions similar to that reported for other allergic reactions.[14]

Prick skin test reactions to shrimp were positive in 85% of the population reporting shrimp sensitivity as compared to only 7.5% of the control population. The prevalence of skin test reactivity was greater in atopic sensitive individuals as compared to nonatopic sensitive subjects (94% versus 76%). These results indicate that the prick skin test correlates well with history of shrimp sensitivity and that a higher prevalence of positive reactions occurs in atopic subjects as compared to nonatopic subjects. Similar results were observed with the other crustacea extracts. Furthermore, no subjects had elevated RAST ratios when skin tests were negative to shrimp extract.

Clearly, the presence of shrimp-specific IgE would appear to be the pathologic mechanism in those subjects with positive prick skin tests and/or elevated RASTs to shrimp. Moreover, shrimp-specific serum IgE occurred primarily in atopic subjects, although it was also detected in the sera from 41% of nonatopic sensitive subjects. Previous studies of patients with positive food RASTs indicate that such patients, irrespective of food-induced symptoms that they report, tend to have higher serum IgE levels than patients with negative food RASTs[15] and are more likely to be atopic.[16] Similar results were observed in our study in that the serum IgE levels did correlate with RAST ratios, and atopic individuals did have significantly higher IgE levels than nonatopic individuals.

However, since a significant number of nonatopic subjects (24%) have neither skin test nor RAST reactivity to shrimp, what is the basis of their food sensitivity? In these RAST negative, skin test negative individuals, it is possible that there may be other immunologic mechanisms contributing to these reactions. The role of non-IgE antibodies, such as IgG or IgA, in food allergy is presently not well understood.[6, 17-20] It has been proposed that such antibodies in secretions may serve as a protective barrier by sequestering potential allergens, thus preventing the stimulation of IgE-specific responses by the process of immune exclusion at mucosal surfaces.[21] However, the presence of such shrimp-specific antibody in intestinal secretions has not yet been investigated.

Our studies demonstrate that shrimp-specific IgG and IgA are present in the sera of all subjects, but they are significantly higher in shrimp-sensitive individuals. The levels of these antibodies generally cor-

related with IgE antibody levels (RAST ratios), but not necessarily with clinical history or skin test titration end points. These findings are similar to studies of cow's milk allergy in children that demonstrated higher levels of food antibody in the sera of sensitive children[18, 19] and a parallel occurrence of IgE and IgG reactivity in most cases.[22] The presence of such elevated shrimp-specific IgG and IgA probably indicates a generally enhanced immunologic recognition of crustacea allergens (antigens) in persons with shrimp hypersensitivity. Whether this reflects a greater innate immunologic ability to produce increased quantities of such food-specific antibodies or indicates enhanced exposure to allergens, possibly either through permeability changes (local gut anaphylaxis) or intrinsic ability of low-molecular-weight allergens to penetrate gut mucosa in such individuals, has not been determined.

The exact role of such non-IgE antibody in the immunopathogenesis of food allergy is controversial. Antigen-specific serum IgG has been demonstrated to facilitate the uptake of antigen in the gut,[23, 24] and late reactions to milk have been associated with IgG reactivity (presumably IgG4 subclass).[9] However, high ratios of IgG/IgE has been associated with good tolerance.[22] Further evaluation of the exact IgG subclass responsible for the elevated shrimp reactivity noted in our shrimp-positive subjects may help clarify the proposed contradictory protective and pathogenetic roles for IgG4 subclass in food allergy.[25]

Based on this study, several conclusions may be drawn about shrimp hypersensitivity: (1) IgE-mediated, type I mechanisms are operative in most crustacea-sensitive individuals. (2) Although prick skin testing with shrimp extracts detects the presence of this hypersensitivity, shrimp RAST ratios appear to correlate better with the intensity of clinical symptoms after shrimp ingestion. (3) Atopic individuals demonstrated higher RAST ratios and a more severe spectrum of clinical symptoms, including anaphylaxis, than nonatopic shrimp-sensitive subjects. (4) Significant levels of shrimp-specific IgG and IgA reactivity are detected in shrimp-sensitive subjects.

Further studies with double-blind food challenges are currently in progress to delineate variables of prognostic significance as well as the role of non-IgE antibodies in shrimp-sensitive reactions. Of particular interest will be the role of non-IgE allergen-specific antibodies present in gastrointestinal secretions.

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