

Species-specific shrimp allergens: RAST and RAST-inhibition studies

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Two edible shrimp species are widely available in Louisiana, Penaeus setiferus (white shrimp) and Penaeus aztecus (brown shrimp). Some sensitive individuals report only occasional allergic symptoms after shrimp ingestion, suggesting that there may be species-specific allergens. To investigate this possibility, we evaluated shrimp species-specific reactivity in 31 individuals with a history of immediate hypersensitivity reactions after shrimp ingestion with skin prick tests and RASTs with white and brown shrimp extracts. On selected individuals, RAST-inhibition studies were performed with white shrimp and/or brown shrimp-coupled disks, with white and/or brown shrimp extracts as inhibiting allergen. Positive skin tests to both types of extract were observed in 77% (23/30) of the subjects; one individual reacted to brown shrimp extract only. Elevated RASTs to both extracts were observed in 16/31 study participants; one subject reacted only to white shrimp extracts and two subjects to brown shrimp extract alone. Sera from two individuals tested by RAST inhibition recognized qualitatively different allergens in brown and white shrimp extracts, supporting the hypothesis that there are species-specific shrimp allergens. Species specificity is important because it may explain the intermittent symptoms of some study subjects. The percentage of shrimp-sensitive subjects testing positive by skin test and RAST can be increased by use of extracts from more than one species of shrimp. (J ALLERGY CLIN IMMUNOL 1989;83:1112-7.)

Crustacea (shrimp, crab, lobster, and crawfish [crayfish]) are a commonly reported cause of food hypersensitivity.¹ Therefore, the characterization of crustacean allergens, in particular, allergens in shrimp, has been the subject of a number of studies.²⁻⁶ In these studies, shared antigenic/allergenic determinants have been demonstrated among various members of the class crustacea. However, there are also allergens unique to separate genera, for example, shrimp-specific allergens.² In crustacea-sensitive subjects, IgE to either unique or shared allergens may explain an individual's clinical sensitivity to one or more members of this taxonomic class. Certain of the shrimp-sensitive subjects that we have studied have

Abbreviations used

PBS: Phosphate-buffered saline
HSA: Human serum albumin
IC₅₀: Concentration of extract yielding 50% inhibition of RAST

histories of intermittent reactivity,⁷ suggesting that there may be allergenic variability among the shrimp being ingested. To test this hypothesis, we investigated the possibility that there are species-specific allergens in the two major shrimp species, white (*Penaeus setiferus*) and brown (*Penaeus aztecus*), which are harvested in Louisiana.

MATERIAL AND METHODS

Study subjects

Thirty-one volunteers with a history of immediate, adverse reactions after ingestion of shrimp were enrolled in this study. Eleven of these subjects had reported occasions when they had eaten shrimp with no adverse effects. The median age was 36 years with a range of 20 to 49 years. There were 12 male subjects and 19 female subjects. Seventy-one percent (22/31) (10 male and 12 female subjects) were atopic, defined by two or more positive skin tests to inhaled allergens and a personal history of allergy.

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The control group consisted of 13 atopic, crustacea-tolerant individuals, median age 32 years, with a range of 23 to 61 years. There were seven male and six female subjects. Informed consent was obtained before entry into the protocol, at which time study subjects donated a sample of blood, completed a questionnaire, and were skin tested.

Shrimp-allergen extract preparation

Water-soluble shrimp extracts were prepared as described previously.² Briefly, locally purchased shrimp were boiled in deionized water for 15 minutes without spices. The meat was removed from the shell and ground with a Waring blender (Waring Products Div., New Hartford, Conn.) in PBS, pH 7.2, 0.1 mol/L. The slurry was stirred overnight at 4° C and centrifuged at 27,500 g. The supernatant was recovered, concentrated with an Amicon YM5 (Amicon Corp., Danvers, Mass.) (molecular weight cut off 5000), centrifuged at 105,000 g to remove remaining insoluble material, and dialyzed against PBS.

Brown and white shrimp were extracted separately. Multiple extractions of each species of shrimp were performed. Each extract was numbered sequentially with Roman numerals, aliquoted, and stored at -20° C.

Skin testing

Skin prick tests were performed on the forearm of each subject with a 1:20 wt/vol dilution of 10 common airborne allergens: house dust, the pollens of live oak, pecan, Bermuda and Johnson grasses, ragweed, English plantain, *Aspergillus* sp, *Alternaria* sp, and *Fusarium* sp, and a saline-glycerol control (Hollister-Stier Laboratories, Spokane, Wash.). An immediate wheal with a mean diameter 2 mm greater than control wheal occurring 15 minutes after testing was scored as a positive reaction. The shrimp-sensitive study subjects were also skin prick tested with one white and one brown shrimp extract at a maximum concentration of 10 mg/ml. In this case, an immediate wheal with a mean diameter 3 mm greater than control wheal occurring 15 minutes after testing was scored as a positive reaction.⁸

RAST

Shrimp-specific IgE levels were determined on duplicate serum samples with RASTs. Filter paper disks were activated after the method of Ceska and Lundkvist⁹ with cyanogen bromide (Sigma Chemical Co., St. Louis, Mo). Activated disks were coupled with 10 mg/ml of shrimp extract or, as a control, human serum albumin (Miles Laboratories, Elkhart, Ind.) in borate buffer (pH 8.0) overnight on a rotator at room temperature, washed three times in ethanolamine (1 mol/L, pH 8.0), three times with assay buffer (500 ml 0.2 mol/L of Tris buffer, pH 7.5; 500 ml of 1.8% NaCl [wt/vol], 10 ml of 5% NaN₃, 5 ml of Tween 20, 2 gm of bovine serum albumin), and stored in assay buffer at 4° C until use.

For the test, 100 µl of serum were added to tubes containing either antigen or human serum albumin-coupled disk, incubated overnight at room temperature on a rotator,

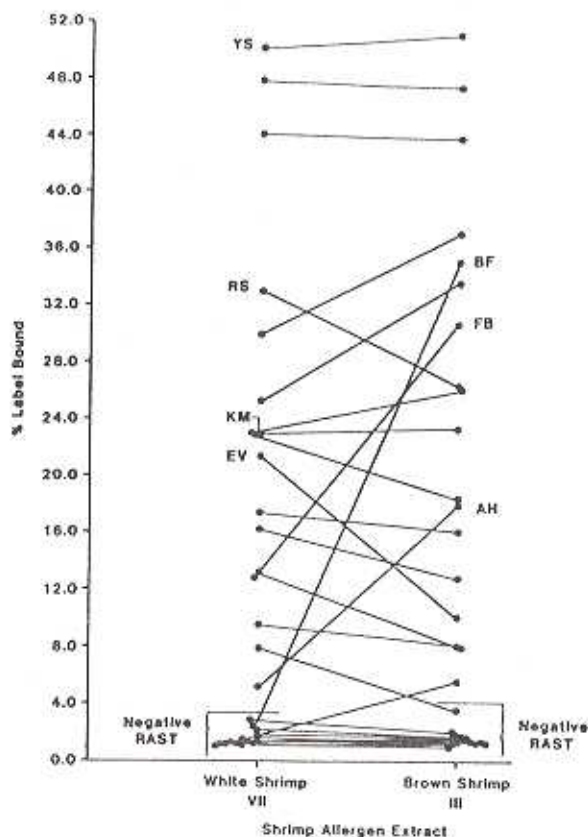


FIG. 1. Shrimp-specific IgE to white and brown shrimp extracts demonstrated by direct RAST. Initials identify those sera used in RAST-inhibition studies. See text for definition of negative RAST.

and washed three times with 2.5 ml of physiologic saline to remove unreacted serum. One hundred microliters of ¹²⁵I-labeled anti-IgE (Pharmacia Fine Chemicals, Piscataway, N.J.) containing approximately 25,000 cpm were added, the incubation and washing steps were repeated, and tubes were counted on a gamma counter. Results are expressed as mean percent of label bound.¹⁰ RASTs were negative when they were within the 99% confidence interval calculated on the percent label bound of the 13 atopic, noncrustacea-sensitive individuals.

RAST inhibition

Seven serum samples were evaluated further with RAST inhibition. Those serum samples with a higher percent label bound to brown shrimp extract were tested with brown shrimp-coupled disks; conversely, serum samples with higher reactivity to white shrimp were assayed with white shrimp extract-coupled disks. If serum reactivity was similar to both brown and white shrimp extract, then RAST inhibition was performed with both disks. Inhibiting antigens were brown shrimp extract, white shrimp extract, and peanut extract (unrelated allergen control). Fifty microliters

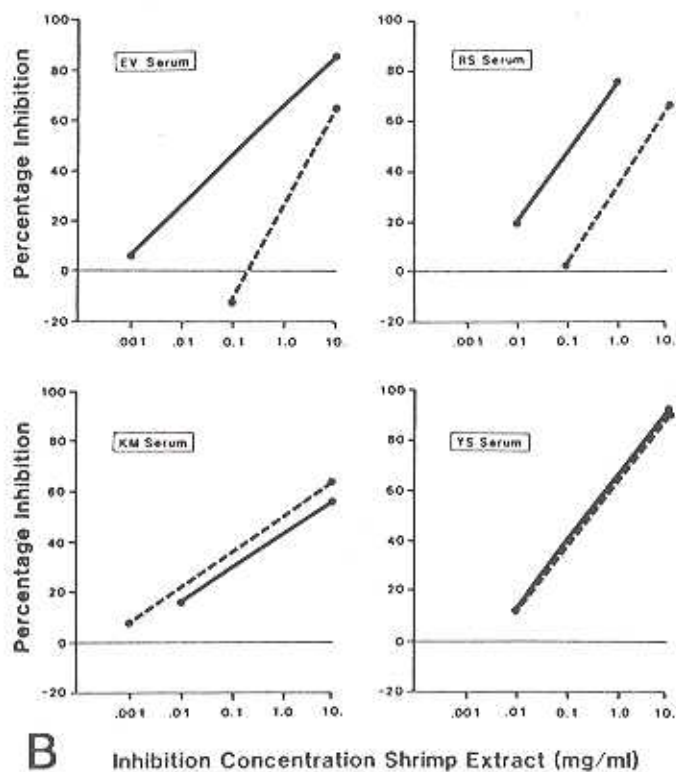
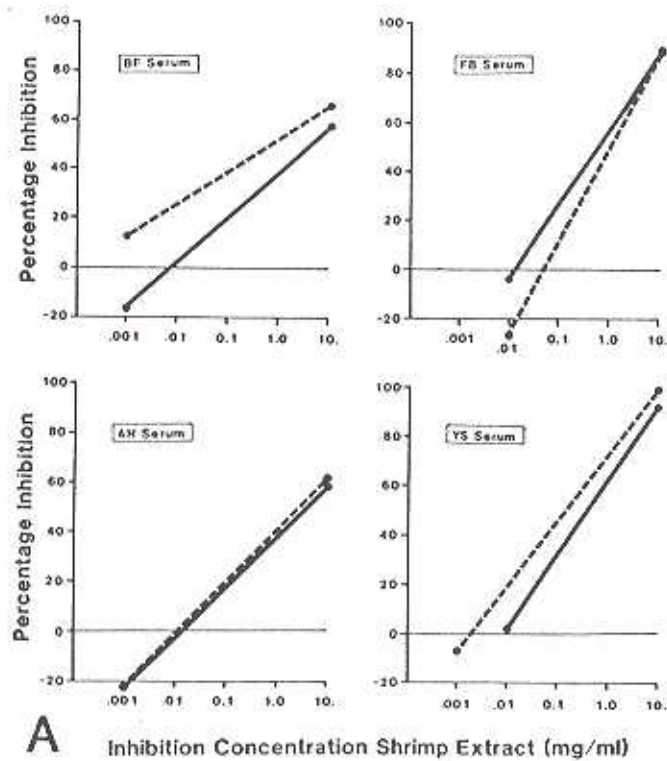


FIG. 2. A, Linear regression analyses of RAST-inhibition studies with brown shrimp-coupled disks and brown (---) and white (—) shrimp extracts as inhibiting allergens. **B**, Linear regression analyses of RAST-inhibition studies with white shrimp-coupled disks and white (—) and brown (---) shrimp extracts as inhibiting allergens.

TABLE I. Analysis of RAST-inhibition studies demonstrating the IC_{50} s and the results of the comparisons of the slopes and the intercepts of the dose-response curves

Serum	Extract coupled to disk	IC_{50} shrimp extract (mg/ml)		Significance of statistical analysis	
		Brown	White	Slopes	Intercepts
B. F.	Brown IV	0.55	3.47	SIG	SIG
F. B.	Brown IV	0.98	0.52	NS	NS
A. H.	Brown IV	2.49	3.65	NS	NS
Y. S.	Brown IV	0.14	0.36	NS	NS
E. V.	White III	4.18	0.16	SIG	SIG
R. S.	White III	3.20	0.12	NS	SIG
K. M.	White III	1.03	3.58	NS	SIG
Y. S.	White III	0.30	0.25	NS	NS

SIG, significant; NS, not significant.

of serum and 50 μ l of increasing concentrations of the inhibiting antigen (0.01, 0.1, 1.0, and 10 mg/ml of PBS), added concomitantly, were incubated with allergen-coupled disks, as for the RAST.

To evaluate RAST-inhibition dose-response curves, the percent inhibition was fitted to the log concentrations (milligrams per milliliter) of inhibiting antigen with least-squares regression lines. Slopes and intercepts of dose-response curves were compared with multiple regression methods.¹¹ An alpha level of $p < 0.05$ was considered significant. Concentrations of extracts yielding IC_{50} were determined from the regression lines.

RESULTS

Skin tests

Thirty of the shrimp-sensitive individuals were skin prick tested with brown shrimp III and white shrimp VII extracts. Of these subjects, six (20%) subjects were negative to both extracts, 23 (77%) subjects were positive to both extracts, whereas one individual (M. S.) was positive to brown shrimp extract but not white shrimp extract.

RAST

Of the 31 individuals who reported adverse reactions after shrimp ingestion, 16 (51.6%) had positive RAST reactivity to both brown and white shrimp extracts. No RAST-positive individual was skin test negative. One individual reacted to white shrimp extract only, whereas two individuals reacted to brown shrimp extract alone (Fig. 1).

A value $>4.13\%$ label bound was positive for brown shrimp-coupled disks, whereas $>2.51\%$ label bound was positive for white shrimp-coupled disks. All 13 of the atopic, shrimp-tolerant individuals were RAST negative. The mean percent label bound to HSA-coupled disks was always $<2\%$.

RAST inhibition

For two (B. F. and A. H.) of the three serum samples with the higher RAST to brown shrimp extract, the IC_{50} s for brown shrimp extract were lower than IC_{50} s for white shrimp extract when extract was tested against brown shrimp-coupled disks (Fig. 2, A, and Table I). In one individual (B. F.) the slopes of the regression lines were significantly different, suggesting that there was a qualitative difference in the allergens recognized.

Serum samples with higher RASTs to white shrimp extract than to brown shrimp extract were evaluated with white shrimp-coupled disks. For two of these sera (E. V. and R. S.), the IC_{50} s were lower for white than for brown shrimp extract. However, for the third serum sample (K. M.) the IC_{50} was lower for brown shrimp extract than for white shrimp extract (Fig. 2, B and Table I). Analysis of the regression lines demonstrated that serum from E. V. recognized qualitatively different allergens, whereas serum from R. S. and K. M. reacted with quantitatively different allergens.

When the serum sample (Y. S.) with similar brown and white shrimp extract RASTs (50.1% and 51.0% label bound, respectively) was used, there was no significant difference in the ability of the brown or white shrimp extract to inhibit IgE reactivity to either disk.

Results presented were from a series of experiments with one brown shrimp and one white shrimp extract. Other experiments, with additional brown and white shrimp-extract preparations, confirmed these results (data not presented). Experiments with B. F. serum demonstrated that all brown shrimp extracts evaluated were more inhibitory than white shrimp extracts. Furthermore, RAST inhibition with serum from Y. S.

with all combinations of two white and two brown shrimp extracts (coupled to disks or as inhibitory antigens) elicited IC_{50} s that were not different among any of the brown or white shrimp extracts.

DISCUSSION

Circulating IgE antibodies to shrimp (either white or brown) can be detected in the serum of most (19/31, 61.2%) study participants with a history of shrimp sensitivity. Most of these subjects (16/19, 84.2%) reacted to both white and brown shrimp extracts. However, since one person had IgE only to white shrimp extract, whereas two reacted to brown shrimp extract alone, we hypothesized that there were species-specific shrimp allergens. This was further supported by a number of shrimp-sensitive subjects who had quantitatively different RAST values to brown and white shrimp extracts. RAST-inhibition studies with selected serum samples suggest that there may be qualitative and quantitative differences in allergenic determinants of brown and white shrimp extracts. Specifically, two of the serum samples (B. F. and E. V.) tested with RAST inhibition reacted to qualitatively different allergens in brown and white shrimp extracts.¹²

One notable finding was that one subject (B. F.) had a negative RAST to white shrimp-coupled disks, but white shrimp extract was capable of inhibiting the subject's brown shrimp RAST. A possible, although it is unlikely, explanation is that this inhibition is nonspecific. More probably, white shrimp extract has a constituent that is capable of eliciting an IgE response but which does not couple to a CNBr-activated disk. Thus, specific IgE to this white shrimp component cannot be detected by RAST.

Two serum samples (R. S. and K. M.) tested by RAST inhibition demonstrated quantitative differences in the brown and white shrimp extracts. One of these samples (K. M.) was tested because the original RAST percent label bound was higher to white extract than to brown. However, the RAST-inhibition studies demonstrated that the reactivity of this serum sample to white shrimp extract was better inhibited by brown rather than white shrimp extract. Two other serum samples (A. H. and B. F.) with higher specific IgE levels to brown shrimp were inhibited equally by both brown and white shrimp extract. These results imply that there are quantitative differences in the allergen content of different shrimp extracts.

There were no positive RASTs to shrimp extracts in skin test-negative subjects. Of the two subjects (B. F. and M. S.) with positive brown and negative white shrimp RASTs, only one (M. S.) had similarly discordant skin test results. This subject was not cho-

sen for RAST-inhibition studies because of a relatively low RAST value to brown shrimp extract. The reason for B. F.'s concordant skin test results but discordant RASTs is obscure. We have hypothesized that B. F. responds to a constituent of white shrimp that does not couple to a RAST disk. The presence of such a constituent would also explain her skin test reactivity in the absence of RAST reactivity. There were six other study participants who had positive skin tests and negative RAST results. This finding has been reported previously^{7, 10, 13} and may be due to the fact that the two assays (skin tests and RASTs) detect different pools of IgE or, again, that not all allergens are coupled to RAST disks.

What is the use of these observations? Use of both white and brown shrimp skin tests and RASTs detect more positive individuals than by either extract alone. Our present study confirms and extends earlier studies^{7, 10, 13} that reported that not all individuals with a history of shrimp sensitivity have positive skin tests and RASTs. The reasons for this lack of immunologic reactivity have been discussed previously^{7, 13} and include the possibilities that some food-hypersensitivity reactions may be mediated by non-IgE mechanisms,⁸ that individuals may be reacting to other allergens, such as spices and/or food additives that are used routinely to season seafood,^{14, 15} and that certain shellfish contain toxins that may elicit adverse reactions by nonimmunologic mechanisms.¹⁶ To this list we can add species variability of shrimp allergens.

We have reported that to be predictive of a positive response to shrimp food challenge, positive skin tests need to be associated with elevated (>11% bound) RASTs.¹⁰ Thus, as expected, one study subject (B. F.) with a positive skin test and negative RAST to white shrimp extract (2.30% label bound) did not respond to challenge with white shrimp extract.¹⁰ Although she has not been formally challenged with brown shrimp extract, her immunologic reactivity, positive skin test, and an elevated RAST (35.1% label bound) is predictive of a positive challenge response. Based on these findings, the species specificity of her serum IgE response could be responsible for her history of intermittent shrimp sensitivity.

It is necessary to place our current observations in the context of previous studies of the antigenic/allergenic similarity among the class crustacea.^{2, 4, 7, 13} With serum samples from eight shrimp-sensitive individuals, seven shrimp allergens were identified by crossed radioimmuno-electrophoresis.² Three of the allergens were designated as major allergens.² All the major shrimp allergens were present in at least two of the three other crustacea tested, crawfish [crayfish], crab, or lobster.² Four allergens, designated minor, appeared

to be shrimp specific. One serum failed to react to any of the major allergens but reacted to three minor allergens. Thus, although a crustacea-sensitive population may exhibit remarkable cross-reactivity among various members of this taxonomic class, there is considerable variability in allergen recognition by individual serum samples.

In conclusion, there are qualitative differences in the allergenic determinants of extracts from white and brown shrimp. Thus, species-specific sensitivity may explain the intermittent nature of the clinical histories in certain patients. Therefore, the use of extracts from more than one shrimp species may increase the correlation between history and immunologic reactivity determined by skin test or RAST.

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