

## Provocation-challenge studies in shrimp-sensitive individuals

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*Thirty individuals with history of immediate, objective, adverse reactions after shrimp ingestion underwent double-blind, placebo-controlled shrimp-food challenges. All individuals who did not exhibit a positive response (reproduction of objective symptoms) were administered an open challenge of 16 whole cooked shrimp. Positive challenge responses occurred in 9/30 subjects (30%); six of these subjects experienced a positive response during the double-blind phase. Of the 21 remaining subjects, 12 experienced generalized pruritus as their only symptom, whereas nine subjects had completely negative challenge responses. All placebo challenges were negative. Although a positive skin test was strongly associated with challenge symptoms ( $p < 0.001$ ), the shrimp prick skin test titration end points were not different among the challenge groups. The serum shrimp RAST percent was significantly higher in the positive challenge group ( $p < 0.02$ ). Mean levels of shrimp-specific serum IgG, IgA, and IgM levels were not different among the challenge groups. Although no single immunologic variable could be consistently used to identify subjects more likely to exhibit a positive challenge response, the composite of a positive shrimp prick skin test and elevated serum shrimp-specific IgE (RAST percent label bound  $>11\%$ ) demonstrated a correct predictive value of 87% in this group of shrimp-sensitive subjects. (J ALLERGY CLIN IMMUNOL 1988;81:1180-6.)*

It is well established that sensitized individuals who ingest crustacea can develop urticaria, angioedema, laryngospasm, asthma, and severe anaphylaxis.<sup>1,2</sup> Since most substantiated hypersensitivity reactions to foods appear to be IgE mediated,<sup>3,4</sup> positive immediate hypersensitivity skin prick tests are useful in predicting the potential for such allergic responses.<sup>1,3</sup> However, it is evident that these skin tests are not predictive of definite reactivity on direct challenge with the offending food.<sup>3,6</sup> At present, provocative challenge is considered the only method that can definitively identify the subject at greatest risk for food-induced hypersensitivity reactions.<sup>3,4</sup>

Our previous studies of shrimp-induced hypersensitivity reactions<sup>1,2</sup> have demonstrated a significant association between a history of immediate, type I

### Abbreviations used

- DBPCFC: Double-blind, placebo-controlled food challenge
- OFC: Open food challenge
- OD: Optical density
- GI: Gastrointestinal

anaphylactic symptoms and a positive shrimp prick skin test. Furthermore, several measures of shrimp-specific immunologic reactivity were more common in atopic shrimp-sensitive subjects. These atopic subjects had higher reported symptom scores, higher shrimp RAST values, and a greater incidence of both positive shrimp skin tests and RASTs than the non-atopic, shrimp-sensitive subjects. The RAST values correlated with the reported symptom scores and prick skin test titration end points. Additionally, shrimp-specific serum IgG and IgA reactivity was significantly higher in the shrimp-sensitive group, as compared to shrimp-tolerant control individuals, and correlated with the shrimp RAST reactivity.

To assess the relationship between such immunologic recognition of shrimp allergens/antigens and the propensity to respond to provocation challenge, 220 food challenges were performed on 30 subjects with

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a history of shrimp sensitivity. The results obtained support the usefulness of both skin test and RAST determinations in the evaluation of individuals with a history of immediate, adverse reactions to shrimp ingestion.

## MATERIAL AND METHODS

### Study subjects

One hundred eight individuals with suspected shrimp allergy completed a questionnaire eliciting information about past adverse reactions to crustacea, as well as personal and familial allergic histories. To evaluate the intensity of historical allergic reactions to shrimp, specific symptoms reported to occur after its ingestion were assigned a weighted score as follows: generalized pruritus, 1; urticaria, 2; angioedema, 3; GI symptoms (nausea, emesis, diarrhea), 4; pulmonary symptoms (shortness of breath or wheezing), 5; and generalized systemic anaphylaxis, 6. The cumulative total designated as the reported symptom score, was used to select 30 adult subjects with a strong history of objective adverse reactions within 1 hour after shrimp ingestion. Informed consent was obtained before all studies, and skin tests and venipuncture were performed before challenge. Subjects were prick skin tested with shrimp-allergen extracts prepared as described below, as well as commercial extracts of 20 common inhalant and 10 food allergens (Greer Laboratories, Lenoir, N.C., and Hollister-Stier, Spokane, Wash.). Subjects with history of milk sensitivity or positive skin prick test to milk were excluded from this study. As in previous studies,<sup>1,2</sup> subjects with two or more positive skin tests to common inhalant allergens and a personal or family history of respiratory allergy were designated atopic.

### Shrimp-allergen extracts

Water-soluble extracts of shrimp were prepared as previously described.<sup>1</sup> These extracts were used for skin testing, *in vitro* studies, and provocative challenges. In this study, all extracts were sterile filtered (0.45  $\mu$ m) before use and were negative for hepatitis B surface antigen.<sup>7</sup> For challenge studies, the amount of shrimp extract was expressed as shrimp equivalents where one shrimp equivalent (8 mg) equals the amount of extract obtained from a standard 4 gm medium-sized shrimp.

### Skin testing

Increasing tenfold concentrations of shrimp extract (1  $\mu$ g/ml to 10 mg/ml) in 50% glycerol were used to determine the prick skin test titration end point. Prick skin tests were performed with a straight taper point surgical needle (Richard-Allan Medical Industries, Richland, Mich.). The positive end point was the lowest allergen concentration producing a mean wheal diameter 3 mm greater than the control reaction (phosphate-buffered saline in 50% glycerol).

### Total and specific IgE determinations

Total serum IgE levels were determined in duplicate with a commercially available PRIST kit (Pharmacia Co., Pis-

TABLE I. Historical shrimp-induced symptoms in 30 challenged subjects

Symptom	n (%)
Pruritus	28 (90)
Urticaria	19 (61)
Angioedema	16 (52)
Pulmonary	13 (42)
GI	11 (35)
Shock	3 (10)

cataway, N.J.). Shrimp-specific IgE (RAST) was determined on duplicate serum samples by RAST with either shrimp allergens or human serum albumin conjugated to cyanogen bromide-activated disks, as reported previously.<sup>2</sup> Results, expressed as percent label bound, were calculated as mean counts per minute for shrimp disks divided by total counts per minute (<sup>125</sup>I-labeled anti-IgE) added to system times 100. In this study, >3% label bound was considered significant. This level of significance was determined by calculating the 99% confidence interval for the percent label bound obtained with a serum panel from skin test negative, shrimp-tolerant, atopic control subjects (N = 15). The mean percent label bound to human serum albumin conjugated disks was always <2%.

### ELISA

Serum levels of shrimp-specific IgG, IgA, and IgM were quantified in an ELISA as previously described.<sup>2</sup> Results were recorded as  $\Delta$  OD measured at 405 nm where  $\Delta$  OD = OD experimental serum - OD diluent. Assays were standardized by including a reference serum with known reactivity on each plate; the  $\Delta$  OD of this serum varied <10% among assays.

### Provocative challenge protocol

Challenges, performed in two phases, were completed within a single 8-hour testing period. The first phase was double-blind and placebo-controlled with either shrimp extract or placebo (phosphate-buffered saline) administered at hourly intervals. The shrimp extract or saline was masked in 200 ml of vanilla ice cream containing 40 ml Syralta (Emerson Laboratories, Texarkana, Texas) grape flavoring.

The dosages of shrimp administered in this challenge were 1, 4, and 16 shrimp equivalents, although up to 128 shrimp equivalents could be masked as described above. If these challenges were negative, the first challenge phase was followed by a second phase that was an OFC with 16 medium-sized boiled shrimp (approximately 64 gm of shrimp). A positive response was defined as reproduction of objective symptoms during the challenge procedure.

### Statistical analysis

Atopic and nonatopic subjects were compared with the Mann-Whitney U nonparametric test (for IgE, ELISA,

**TABLE II.** Results of oral shrimp challenge performed on 30 subjects with historical adverse reactions to shrimp

Group (n)	Shrimp challenge				Placebo challenges	Total challenges
	Positive	Negative	DBFC	OFC		
Positive (9)	11	21	28	4	25	58
Pruritus (12)	0	56	44	12	44	100
Negative (9)	0	36	27	9	27	63

DBFC = Double-blind food challenge.

RAST, skin test end points, and reported symptom score values) and Fisher's exact test (for prevalences of skin test sensitivity and RAST). The differences among the challenge response groups were analyzed in the same manner. Spearman's correlation coefficient was used in testing for a non-parametric association between continuous variables.<sup>8</sup>

## RESULTS

### Historical (questionnaire) shrimp-induced symptoms

The symptoms reported by our challenge group after shrimp ingestion, listed in Table I, were similar to those of other shrimp-sensitive subjects studied previously.<sup>1,2</sup> Pruritus, the least severe symptom, was also the most commonly reported (90%), although it always occurred in association with at least one of the listed objective symptoms. Urticaria was the next most common symptom; generalized anaphylaxis (shock) was reported by three subjects (10%). Sixty-one percent of subjects (19/30) had considered their shrimp-induced symptoms severe enough to warrant emergency room treatment.

### Shrimp-challenge responses

Results of the 220 food challenges performed in these 30 individuals are summarized in Table II. DBPCFCs were performed in 195 challenges; 99 of these challenges were with shrimp and 96 were with placebo. Whole shrimp OFC were performed on 25 occasions.

Eleven of the shrimp challenges were positive in nine subjects (30%), as determined by an objective change in their status during the challenge procedure. Of the 21 remaining challenge subjects, 12 experienced pruritus as their only symptom after shrimp challenge, whereas the remaining nine subjects had negative challenge responses, neither reporting nor manifesting signs or symptoms during the procedure. All 96 placebo challenges in these 30 individuals were negative.

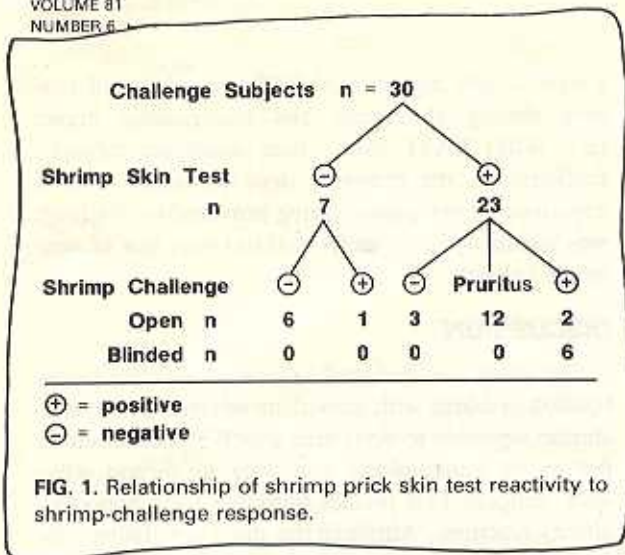
All 11 positive reactions occurred within 60 minutes of shrimp ingestion. Most symptoms resolved within

**TABLE III.** Comparison of historically reported symptoms with challenge-induced symptoms in positive challenge responses

Symptoms (n)	Produced during challenge	Reported history (n)	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

60 minutes, and by 2 hours, 91% had spontaneously cleared. Only two subjects required treatment, one for persistent vomiting and the other, for asthma. The dose of shrimp necessary to provoke an observable reaction varied among the nine individuals with positive reactions. Seven positive reactions (in a total of six subjects) occurred during the double-blind challenge, four reactions occurring at four shrimp equivalents, and three reactions, at 16 shrimp equivalents; no OFCs were performed in these six subjects. In three additional subjects, a total of four reactions occurred at the OFC with 16 whole shrimp (64 gm). The mean shrimp dose producing a positive reaction was 47 gm.

As summarized in Table III, the challenge-induced symptoms in the nine subjects experiencing a positive challenge response were similar in quality to historical studies. Generalized pruritus was experienced by all of these subjects in addition to an objective symptom(s) listed as part of their positive response. Although urticaria, angioedema, pulmonary, and GI symptoms were reported historically by these individuals with similar frequency, urticaria occurred less frequently than the other objective symptoms during provocative challenge. None of the positive challenge subjects had a history of generalized anaphylaxis, nor



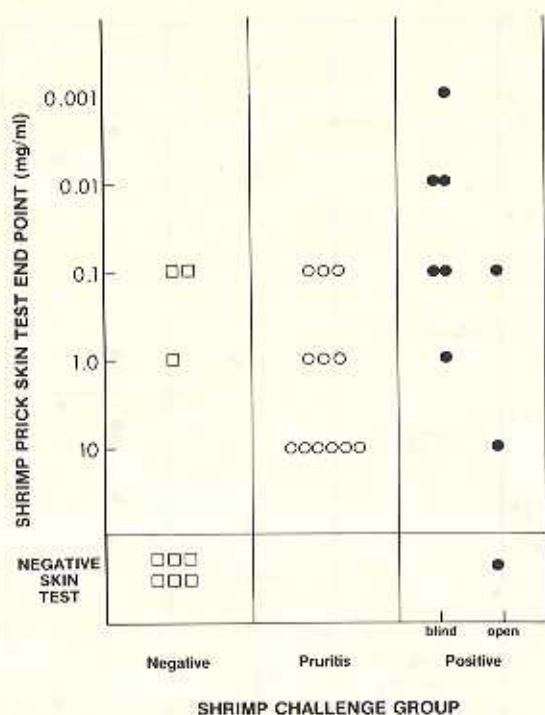
was this induced during the challenge procedure. Furthermore, 6/9 subjects (67%) with positive challenge responses reported the subjective symptom of pruritus at a lower shrimp dose than required to produce their objective symptoms.

In addition to the pruritus reported during the positive challenge responses, pruritus was reported as the only symptom in an additional 12 members of the challenge population; pruritus occurred only after shrimp ingestion and never after placebo ingestion. Pruritus was first experienced during the blind phase of the challenge in 58% of this subgroup; this symptom recurred with increasing shrimp-challenge doses. However, even on OFC, these 12 subjects only reported pruritus. Furthermore, the dose of shrimp necessary to induce pruritus (threshold dose) was significantly higher ( $p < 0.001$ ) for this group with pruritus than the dose of shrimp necessary to induce pruritus in the positive challenge group.

Additionally, the reported symptom score of the positive challenge group was significantly higher ( $p < 0.05$ ) than the other groups. This reported symptom score also correlated significantly with both the threshold dose ( $p < 0.025$ ) in both the group with pruritus and positive group, as well as with the objective symptom dose in the positive challenge group ( $p < 0.01$ ).

### The relationship of immunologic reactivity to challenge response

The relationship between the shrimp skin prick test reactivity and challenge response is summarized in Fig. 1. Of the seven individuals with negative skin prick tests to shrimp extract, all had negative challenges, except one who experienced a positive OFC resulting in hives. Of the 23 skin test positive subjects, three had negative challenges, 12 experienced pruritus only, and eight subjects had positive challenges. Six



**FIG. 2.** Relationship between shrimp skin prick test titration end point and shrimp-challenge response.

individuals had positive challenges during the double-blind phase and two, during the OFC.

The proportion of positive skin tests to shrimp extract was significantly higher ( $p < 0.001$ ) in both the positive group and group with pruritus, as compared to the negative challenge group. Shrimp prick skin test titration end points among the challenge groups (Fig. 2) demonstrated that most of the negative challenge group also had negative skin tests. Of those individuals with positive skin tests, there were no significant differences in the titration end points among the three challenge response groups. However, the skin sensitivity to shrimp allergens, as measured by the titration end point, correlated with the shrimp RAST percent bound ( $p < 0.001$ ).

The relationship of the shrimp RAST (expressed as percent label bound) to challenge response is illustrated in Fig. 3. The shrimp RAST reactivity was significantly higher ( $p < 0.02$ ) in the positive challenge group as compared to the other groups, although there was no significant difference between the group with pruritus and the negative challenge groups. RAST reactivity correlated with the reported symptom score ( $p < 0.05$ ), the shrimp titration end points ( $p < 0.001$ ), and both the threshold ( $p < 0.05$ ) and objective symptom shrimp dose ( $p < 0.001$ ). Although the magnitude of a given RAST alone was not

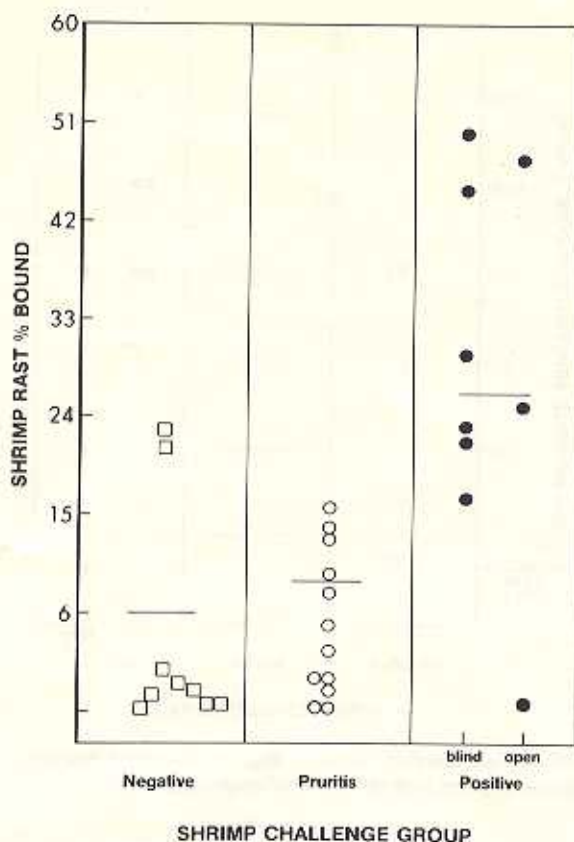


FIG. 3. Relationship between shrimp RAST percent label bound and shrimp-challenge response.

able to predict the occurrence of a positive challenge, the combination of a positive shrimp skin prick test and a shrimp RAST percent bound  $>11\%$  had a positive predictive value of 87%.

No differences in serum levels of shrimp-specific IgG, IgA, and IgM were noted that related to challenge response. Levels of shrimp-specific IgG, IgA, and IgM reactivity did not correlate with either the skin test titration end points, RAST values, threshold and objective symptom shrimp-challenge doses, or each other. There were no significant differences in the total IgE levels among the challenge groups.

#### The relationship of atopic diathesis to challenge response

Several differences were noted among the challenge groups when these were analyzed on the basis of atopic status of subjects within these groups. A greater proportion of atopic individuals were in both the positive challenge group and challenge group with pruritis; 8/9 and 10/12 subjects were atopic in these groups, respectively, whereas 4/9 in the negative challenge group were atopic. Atopic individuals had

a significantly higher ( $p < 0.002$ ) incidence of positive shrimp skin tests and significantly higher ( $p < 0.01$ ) RAST values than nonatopic subjects. Furthermore, the threshold dose of atopic subjects experiencing symptoms during provocative challenge was significantly lower ( $p < 0.01$ ) than that of nonatopic subjects.

#### DISCUSSION

Our study was designed to be a prospective investigation of adults with immediate adverse reactions to shrimp ingestion to determine which aspects of either history or immunologic reactivity to shrimp allergens/antigens best predict objective food hypersensitivity reactions. Although the study population consisted of subjects reporting adverse reactions to shrimp, only subjects with immediate objective signs or symptoms were accepted into the study to decrease the likelihood of false positive challenges. Therefore, our findings may not be valid for those with delayed onset of symptoms (beyond 60 minutes). Patients with previous systemic reactions (anaphylaxis) to shrimp were included in our study populations.

Thirty subjects participated in 220 oral food challenges (DBPCFC) and the outcome of these challenges were correlated with studies of serum shrimp-specific antibody reactivity and the results of standard skin prick testing to both inhalant and food allergens. Of the 124 shrimp challenges and 96 placebo challenges, 11 positive challenges occurred to shrimp ingestion in nine subjects, whereas all 96 placebo challenges were negative.

Several interesting features were noted. First, there was no correlation between either the time since first or last reaction to shrimp and the outcome of shrimp challenges. Furthermore, all positive challenge symptoms were similar to symptoms reported to occur in previous reactions, a finding previously observed in other food-challenge studies.<sup>5,9,12</sup> The intensity of provoked symptoms was only slightly less than previously reported reactions and resolved during a similar time frame. Most reactions began within minutes (15 minutes) of ingesting shrimp. In patients experiencing positive challenges, none of the patients developed delayed reactions or a recrudescence of symptoms after initial resolution. Of course, as mentioned above, the selection of this study population was biased against such events, as in similar food-allergy studies.

Oropharyngeal pruritis and occasional subjective throat-pharyngeal swelling was reported by most subjects with positive challenge at lower doses of shrimp than that provoking their positive objective response. Such subtle, unimpressive findings may be accentuated by studies such as ours that focus on reactions

to a particular food rather than food reactions in general, but similar subjective findings appear to be common to some food-challenge studies.<sup>10\*</sup> Furthermore, use of capsule rather than liquid vehicle for these oral challenges should significantly decrease allergen contact with the buccal mucosa and hence should influence the occurrence of these symptoms. We have considered the possibility that such pruritus may represent the precursor of a positive response for several additional reasons. The threshold shrimp dose in the group with pruritus was higher than that of the positive challenge group. This threshold dose also correlated significantly with the reported symptom score. Furthermore, the lack of objective symptoms in the group with pruritus may well be due to a final challenge dose below that necessary to induce such observable responses, since the maximum dose of shrimp in our present study was 64 gm of shrimp. Studies are currently under way to test this hypothesis.

Aside from the issue of pruritus, the patterns of organ involvement in shrimp challenge positive and negative groups were similar to those previously observed by Atkins et al.<sup>9, 10</sup> The subjects exhibiting positive challenge had significantly higher reported symptom scores and thus were more likely to have reactions that included GI, respiratory, and dermatologic complaints. However, the observed symptoms noted in positive challenge usually involved only one target organ, and this target organ was not uniform among positive reactors with similar historical symptoms. The preferential induction of GI and respiratory symptoms rather than urticaria during positive challenge is not dissimilar from other studies.<sup>9-13</sup> The reasons for this are not evident at present, but this finding appears to be valid for food-hypersensitivity reactions in general and may represent clinical correlates of immunopathogenic mechanisms. Organ-specific differences in the threshold concentration of food allergens provoking vascular permeability changes have been demonstrated,<sup>14</sup> which may involve local mast cell-mediator release.<sup>15</sup> Additionally, it has been suggested that organ-specific differences in allergen-induced histamine release or responsiveness to histamine may exist.<sup>13</sup>

High levels of serum shrimp-specific IgE were noted in our positive challenge group. High food RAST reactivity has been correlated with a history of anaphylaxis to a variety of foods<sup>16-19</sup>; in particular, level of allergen-specific IgE is a highly specific and sensitive index of hypersensitivity to cod fish.<sup>18, 20</sup> Although RAST does not appear to be equally reliable

for all foods,<sup>20-22</sup> this study indicates that shrimp RAST provides diagnostically useful information in shrimp-sensitive subjects.

In this study, shrimp prick skin test end point titration failed to identify those subjects with a positive challenge response. In contrast, a significant correlation between prick skin test end point titration and bronchial reactivity was demonstrated with aeroallergens.<sup>23</sup> Moreover, in that study, allergen-specific IgE levels provided a slightly but significantly better assessment of bronchial reactivity than did skin test titration.<sup>23</sup> In other aeroallergen studies, a strong correlation between RAST, intradermal end point titration, and positive provocation challenge has been demonstrated.<sup>24, 25</sup> Moreover, RAST reactivity appeared to be as sensitive a measure of clinical reactivity as carefully performed intradermal end point titration.<sup>24</sup> Thus, intradermal end point titration with shrimp extract may provide a more quantitative assessment of clinical shrimp sensitivity than prick titration but possibly not more than shrimp-specific IgE levels. However, intradermal testing is not recommended in food-allergic patients because of the perceived potential danger of systemic reaction in these subjects.<sup>26</sup>

Immediate reactions to foods reported by patients can be problematic for the clinician and are not uncommon in allergic subjects. Evaluation of such patients by double-blind food challenge is not practical in an office setting. However, studies such as this may provide some guidance in identifying patients at highest risk for subsequent reactions. In this evaluation of immediate adverse reactions to shrimp, subjects who developed objective symptoms on challenge, 30% of subjects challenged, almost always had positive prick skin tests to shrimp (8/9); furthermore, of subjects who developed pruritus, all had positive skin tests. Subjects with positive skin tests could be classified as having either high or low shrimp RAST reactivity; no subjects had negative skin test and positive RAST. Of particular interest is the observation that positive shrimp prick skin test and elevated RAST reactivity (<11% label bound) had an 87% correct predictive value of positive challenge in this study. Whether the subjects with positive skin tests and low RAST (essentially our group with pruritus) represent subjects at lower but definite risk of positive challenge remains to be established.

Clearly, most substantiated immediate objective reactions to shrimp are mediated through IgE mechanisms, and the patient at greatest risk can be identified by history, skin testing, RAST reactivity, and atopy. These findings are consistent with most studies of food-hypersensitivity reactions. Moreover, non-IgE

\*Bock A. Personal communications

(skin test and RAST negative) reactions are rare but can occur because 3% of our positive challenge subjects are in this category. Fortunately, such reactions appear to be mild (urticaria), but it is difficult to generalize from such a small percentage (representing one subject). However, the mechanisms and risk factors related to this less common form of shrimp sensitivity remain to be elucidated.

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