

Reactivity of IgE antibodies with crustacea and oyster allergens: Evidence for common antigenic structures

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IgE-antibody reactivity to oysters and crustacea of sera from six oyster-sensitive, seven oyster- and crustacea-sensitive, and 12 crustacea-sensitive subjects was investigated. All six subjects with a history of only oyster sensitivity had minimal RAST reactivity (ratios 2 to 5) to extracts of raw or boiled oysters. Three of the seven oyster- and crustacea-sensitive subjects and six of the 12 crustacea-sensitive, oyster-tolerant or unexposed subjects had elevated RAST ratios to oyster (14 to 41). Generally, elevated oyster RAST correlated with skin prick test reactivity to oyster but not with total serum IgE levels. The oyster RAST values of the 19 crustacea-sensitive subjects (with or without oyster sensitivity) correlated with crustacea RAST reactivity (crab RAST, most significant; shrimp RAST, least significant). Rabbit antisera to crustacea extracts detected precipitating antigens present in extracts of raw or boiled oysters. Significant inhibition of the oyster RAST was obtained with oyster or crustacea extracts. These studies suggest that in the diagnosis of oyster sensitivity the RAST may not be useful and that oyster and crustacea contain common antigenic structures. (J ALLERGY CLIN IMMUNOL 1987;80:133-9.)

Shellfish are very popular cuisine in New Orleans and the rest of the country and yet can elicit profound allergic reactions in sensitized individuals.¹⁻³ We have been investigating allergic reactions to crustacea, primarily to shrimp. Most of our patients present with symptoms of immediate hypersensitivity, and many have elevated skin and RAST tests to crustacea, specifically to shrimp, crab, lobster, and crayfish.⁴⁻⁶ Further studies indicated that a number of crustacea allergens share common antigenic structures with one another.^{4, 5, 7-9}

Abbreviations used

PBS: Phosphate-buffered saline
HSA: Human serum albumin

Allergic reactions to oysters are of interest because of a number of our crustacea-sensitive patients report hypersensitive reactions on their ingestion. It is well acknowledged that oysters can cause reactions in sensitized subjects (although it is not documented in the literature) and cause occupational reactions in sensitized workers,^{10, 11} and yet, there has been no thorough study of IgE-antibody reactivity to oyster allergens in sensitized individuals. Therefore, the following study investigated IgE-antibody reactivity to oyster extracts in oyster-tolerant and oyster-sensitive subjects and the relationship of this reactivity to crustacea-specific responses.

MATERIAL AND METHODS

Study subjects

Three groups of study subjects were chosen on the basis of sensitivity to oysters or crustacea. The first group was comprised of six adults (29 to 40 years of age, 5/6 female

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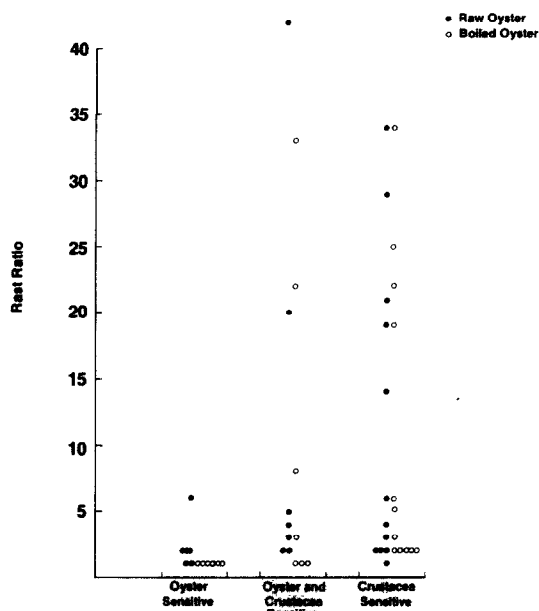


FIG. 1. RAST reactivity of oyster- and crustacean-sensitive individuals to oyster extracts. IgE antibody reactivity (expressed as RAST ratio) to extracts of raw and boiled oysters are presented in relationship to histories of only oyster sensitivity, oyster and crustacea sensitivity, or only crustacea sensitivity.

subjects) who reported sensitivity only to oysters. All symptoms reported were only gastrointestinal in nature, such as nausea, vomiting, diarrhea, and abdominal cramps. The second group was comprised of seven adults (21 to 49 years of age, 5/7 female subjects) who reported sensitivity to both oysters and crustacea. Five of the seven subjects complained of gastrointestinal symptoms (the only reported symptom in 4/5) similar to symptoms described in the first group, and 2/4 reported pulmonary symptoms such as wheezing and shortness of breath, of which one subject also had urticaria and angioedema. The third group of 12 study subjects (17 to 57 years of age, 8/12 female subjects) reported either a lack of prior exposure or hypersensitivity to oysters. However, on ingestion of crustacea, they reported pulmonary symptoms, gastrointestinal symptoms, urticaria, angioedema, and/or laryngospasm.

Informed consent was obtained from all subjects, and a history was taken of general allergy reactions. Allergic reactions to oysters and/or crustacea were noted. Subjects (required to omit any antihistamine drugs for at least 24 hours before testing) were skin prick tested with 10 common inhalant allergens (Hollister-Stier, Seattle, Wash., or Greer Laboratories, Lenoir, N. C.), and extracts of crustacea or oysters were prepared at Tulane Medical Center. Subjects were skin prick tested, and any resulting wheal and flares were measured 15 minutes later. A positive control consisted of a solution of histamine diphosphate (1 mg/ml), and the negative control was 0.1 mol/L of PBS, pH 7.2, in 50% glycerol. All test subjects exhibited a positive reaction to histamine and a negative reaction to the PBS control. A serum sample was obtained from all participants in the study.

Oyster and crustacea extracts

Crustacea were purchased locally. White shrimp (*Penaeus setiferus*), blue crab (*Callinectes sapidus*), spiny lobster (*Panulirus argus*), crayfish (*Procambarus clarkii*), and oyster (*Crassostrea virginica*) were boiled for 15 minutes in ion-depleted water. The meat of raw and boiled crustacea was removed and extracted in PBS for 3 minutes in a Waring (Waring Products Division, New Hartford, Conn.) blender. After mixing overnight at 4° C on a shaker, extracts were centrifuged (27,000 × g to 44,000 × g), and supernatants were concentrated on an Amicon YM2 filter (Amicon, Danvers, Mass.) (molecular weight exclusion >2000 daltons). Concentrates were dialyzed (Spectrapor 3, Spectrum Medical Industries, Inc., Los Angeles, Calif., molecular weight exclusion 3500 daltons) against PBS and recentrifuged (78,000 × g). Dry weights were determined, and aliquots of the supernatants were stored at -20° C until further use.⁴ Extracts of raw and boiled oysters, as well as the cooking water obtained from the boiled oyster and the shell fluid from the raw oyster, were dialyzed (Spectrapor 3) against deionized water and were centrifuged 80,000 × g, and supernatants were lyophilized. The extracts were stored under desiccation at -20° C until further use.

Rabbit antisera

Antisera to each crustacea extract were raised in three New Zealand female white rabbits (2.5 kg). Rabbits were bled (preimmune sera) and then subcutaneously injected biweekly with 10 mg/ml of crustacea extract emulsified in an equal volume of complete Freund's adjuvant for a total of five injections. One week after the last antigen injection, immune sera were obtained from the marginal ear vein at weekly intervals. Antisera per crustacea were pooled and concentrated by $(\text{NH}_4)_2\text{SO}_4$ precipitation (dialyzed against PBS) and Amicon ultrafiltration (XM 50 filter; molecular exclusion >50,000 daltons) to 1/10 original volume.

Specific total IgE determinations

Filter paper discs were activated for RAST according to the method of Ceska and Lundkvist¹² and coupled with crustacea extracts, oyster extracts, or HSA control (10 mg/ml) as reported previously.⁴ One hundred microliters of test serum was added to discs coupled with crustacea extract or HSA and incubated overnight at room temperature on a clinical rotator. After washing three times with 0.9% saline, 100 μ l of ¹²⁵I-labeled rabbit antihuman IgE (Pharmacia, Uppsala, Sweden), containing approximately 25,000 cpm, was added to each tube, incubated, and washed as above. Discs were counted for 5 minutes on a gamma counter. Tests were performed in duplicate, and results were expressed as a ratio of the mean average counts per minute of the crustacea or oyster-coated discs divided by the HSA control.

IgE PRIST was performed according to the Pharmacia procedure.

RAST inhibition

A pool of sera from four oyster RAST positive individuals was used in the RAST inhibition. Oyster, crustacea, or

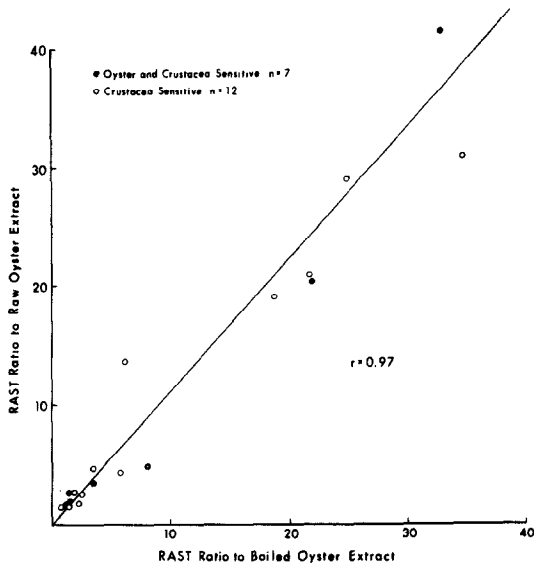


FIG. 2. Raw oyster RAST compared to boiled oyster RAST. RAST ratios to extracts of raw and boiled oysters were correlated for individual sera; r value = 0.97 indicates a significant correlation (p value < 0.001).

peanut extracts were tested only at 10,000 $\mu\text{g/ml}$ or at increasing (tenfold) concentrations (0.1 to 10,000 $\mu\text{g/ml}$) to determine the 50% inhibitory dose. Fifty microliters of the serum pool and PBS, oyster, crustacea, or peanut extracts were added to a raw or boiled oyster disc coupled at 10,000 $\mu\text{g/ml}$. The procedure then followed was the same as for the RAST. The percent RAST inhibition was expressed as:

$$1 - \frac{\text{cpm test sample}}{\text{cpm PBS control}} \times 100\%$$

Immunodiffusion

Immunodiffusion was performed in 0.4% agarose in deionized water containing 0.1% sodium azide. Oyster extracts (raw, boiled, shell fluid, and boiling water) were tested for reactivity to crustacea-specific rabbit antisera (boiled shrimp, crab, lobster, and crayfish). The center well contained 150 μl of rabbit antisera to shrimp or lobster or 50 μl of rabbit antisera to crab or crayfish. The peripheral wells contained 20 μl of either the homologous antigen or oyster extracts at a concentration of 10 mg/ml. Preimmune sera were tested as above. After 24-hour incubation at room temperature, plates were washed with 0.17 mol/L of citrate buffer, and results were recorded.

RESULTS

RAST reactivity to oyster extracts is summarized in Fig. 1. The results demonstrate that sera from oyster-sensitive subjects had little or no RAST reactivity. One of the six oyster-sensitive subjects had an elevated RAST ratio of 6 to raw oyster, whereas all others had essentially no reactivity (ratio < 2). Three

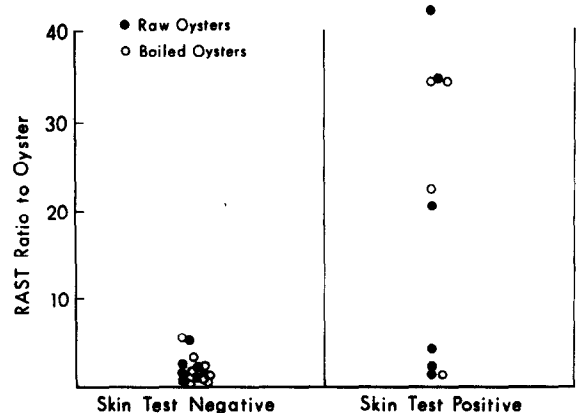


FIG. 3. Skin and RAST reactivity of oyster- and/or crustacea-sensitive subjects. RAST ratios to extracts of raw and boiled oysters are expressed according to skin test reactivity to boiled oyster.

of the seven oyster- and crustacea-sensitive subjects had elevated IgE antibodies (RAST ratios 7 to 41) to extracts of both raw and boiled oysters. Significant RAST reactivity to oyster extracts was also detected in sera from crustacea-sensitive subjects. Six of the 12 individuals had elevated IgE antibodies to extracts of both raw and boiled oysters with RAST ratios ranging from 6 to 34. In general, individual sera appeared to react similarly to extracts of both raw and boiled oysters, suggesting a similar allergenic content. When RAST reactivity to extracts of both raw and boiled oyster for individual sera was analyzed by linear regression (Fig. 2), the correlation coefficient ($r = 0.97$) was highly significant at a p value of < 0.001. For this reason, most subsequent experiments used only extracts of boiled oyster. In general, atopic subjects (two or more positive skin tests to common inhalants and a personal and/or family allergy history) had a greater incidence and magnitude of RAST reactivity than nonatopic subjects. All RASTs for 20 skin test negative control subjects were negative; RAST ratio was < 2.

In order to assess the specificity of IgE antibody reactivity to oyster extracts, the relationship of oyster RAST ratios to total serum IgE levels and to skin test reactivity for individuals was analyzed by linear regression. The results (not presented) demonstrated that there was essentially no correlation of RAST reactivity with total serum IgE levels. The correlation coefficient ($r = 0.037$) was not significant at p value of < 0.05. Comparison of skin and RAST reactivity, however, did suggest specificity, as presented in Fig. 3. These results indicated that skin test negative individuals, in general, had little or no RAST reactivity to oyster RAST discs, whereas sera from a number of skin test positive individuals had elevated IgE

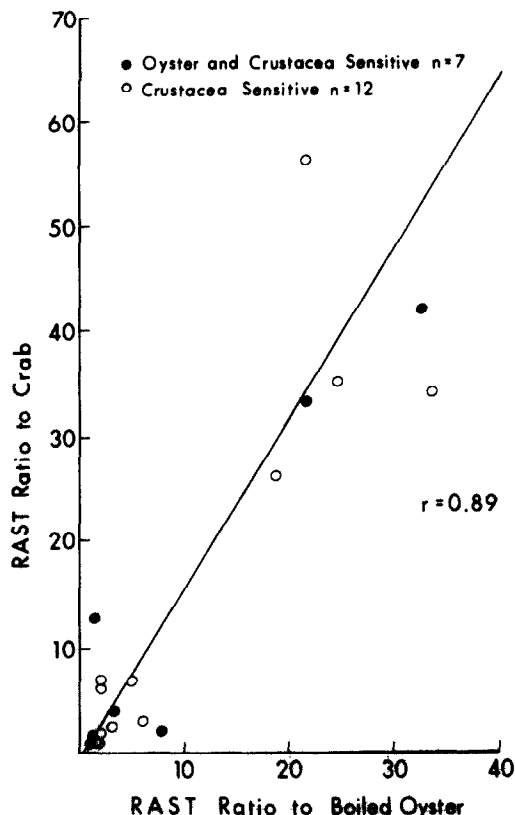


FIG. 4. Correlation of RASTs to crab and oyster. Crab and oyster RAST ratios were compared for individual sera from oyster- and crustacea-sensitive ($n = 7$) or only crustacea-sensitive ($n = 12$) subjects.

antibodies to oyster extracts. These results suggest that positive oyster RASTs are specific immunologic reactions rather than nonspecific binding of IgE to oyster discs.

Since most of the sera used in the study react to crustacea allergens, the relationship of RAST to crustacea and oysters of individual sera was of interest. Therefore, further studies compared reactivity of IgE antibodies in individual sera to oyster and crustacea extracts, and results were analyzed by linear regression. The comparison of crab and oyster RASTs is presented in Fig. 4. The correlation coefficient ($r = 0.89$) was highly significant at a p value of <0.001 . These results indicate a significant correlation of crab and oyster RASTs.

The results of linear regression analysis comparing all crustacea to oyster RASTs are summarized in Table I. These results demonstrate that significant correlation coefficients were obtained on comparison of boiled oyster RAST with raw oyster RAST or crab RAST (as presented in Figs. 2 and 4). A significant correlation was also demonstrated (Table I) between oyster RASTs and RASTs to crayfish, lobster, and

TABLE I. Relationship of IgE antibody reactivity to oyster and crustacea extracts

Test extract RAST	Correlation coefficient to cooked oyster RAST	Level of significance (p value)
Raw oyster	0.97	<0.001
Crab	0.89	<0.001
Crayfish	0.84	<0.001
Lobster	0.82	<0.001
Shrimp	0.65	<0.01

shrimp (correlation coefficients of 0.84, 0.82, and 0.65, respectively). Although shrimp and oyster RASTs had the lowest correlation coefficient ($r = 0.65$), it was still significant at a p value of <0.01 . These results indicate that IgE antibodies in individual sera appear to react similarly to oyster and crustacea allergens and support the hypothesis of common antigenic epitopes in oyster and crustacea antigens.

Antigenic relationships of oyster and crustacea were further studied by RAST inhibition, and results are summarized in Table II. Initial tests analyzed the ability of different oyster fractions obtained from raw oysters, boiled oysters, boiling water, or shell fluid to inhibit the oyster RAST. The results demonstrate that extracts of both raw and boiled oysters significantly inhibit the raw and boiled oyster RASTs. Furthermore, the shell fluid from raw shucked oysters also demonstrated significant inhibition, indicating the presence of soluble oyster allergens. In addition to testing various oyster extracts, different crustacea preparations were tested and demonstrated to significantly inhibit the oyster RAST. Inhibition of RASTs to either raw or boiled oyster was equally as effective with crustacea extracts. The least effective inhibitor was uncooked shrimp, and the most effective was the crab extract. The negative control, peanut extract, demonstrated essentially no inhibition of RASTs to either raw or boiled oysters.

In order to analyze further the inhibition of oyster RAST by oyster and crustacea, increasing concentrations of extracts of raw or boiled oysters, shrimp, crab, lobster, or crayfish were tested for their ability to inhibit the oyster RAST reactivity of a pool of RAST positive sera. Peanut extract was again used as the negative control. The results, summarized in Fig. 5, indicate that significant inhibition of the oyster RAST was obtained with oyster extracts. Of interest was the fact that the crustacea extracts were more potent inhibitors of the oyster RASTs than the oyster extracts. The control peanut extract that contains potent peanut allergens demonstrated minimal inhibition. These re-

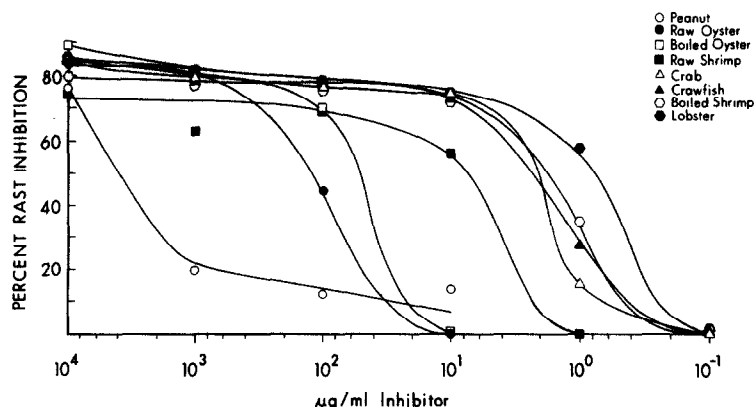


FIG. 5. Inhibition of RAST to boiled oyster with oyster or crustacea extracts. Increasing concentrations (0.1 to 10,000 $\mu\text{g/ml}$) of oyster or crustacea extracts were tested for the ability to inhibit the oyster RAST.

TABLE II. Inhibition of oyster RAST with oyster and crustacea extracts

Inhibiting antigen	% Inhibition	
	Raw oyster	Cooked oyster
Raw oyster	71	78
Cooked oyster	57	83
Cooking water	64	92
Shell liquid	36	45
Uncooked shrimp	56	52
Cooked shrimp	85	88
Lobster	85	89
Crab	88	90
Crayfish	85	90
Peanut	0	3

TABLE III. Dose of oyster or crustacea extracts inhibiting 50% of the oyster RAST

Inhibitors	Dose yielding 50% RAST inhibition boiled oyster (μg)
Boiled oyster	50.0
Raw oyster	120.0
Raw shrimp	7.8
Shrimp	1.6
Crab	2.2
Lobster	0.7
Crayfish	2.3
Peanut	3700.0

sults suggest that oyster and crustacea allergens share common antigenic epitopes.

Another method of expressing RAST inhibition is to calculate graphically that dose of test material that would inhibit 50% of the oyster RAST, and these results are summarized in Table III. The less material necessary to inhibit 50% of the RAST, the more potent the inhibitory activity of the extract. The results indicate that the extract of boiled oysters was three times more effective in inhibition of the boiled oyster RASTs than raw oyster extracts. Crustacea extracts demonstrated significant inhibition of the boiled oyster RASTs. Lobster extract, the most potent inhibitor of all materials tested, was approximately seventyfold more effective than boiled oyster extract in inhibition of the oyster RAST. These results, taken together, suggest that oyster and crustacea allergens share common antigenic epitopes.

Crustacea and oyster extracts were further assessed

for common antigenic structures by testing for reactivity of oyster extracts with rabbit antisera to crustacea. Results (Fig. 6) demonstrate that, although major precipitating antigens were detected only in homologous systems (i.e., shrimp versus antishrimp), shrimp-, crab-, lobster-, and crayfish-specific antisera all react with oyster antigens. The most significant reactions were obtained with antisera to shrimp, and the least was obtained with antisera to crab.

DISCUSSION

Although oysters have been frequently referred to as inducing adverse reactions on ingestion by sensitive subjects, there has been little investigation of the reactivity of IgE antibodies with oyster allergens. The following study was undertaken in order to assess the immunologic reactivity of oyster-sensitive patients to oyster allergens and the relationship of this reactivity to reactivity to crustacea allergens. Our results indicate that skin and RAST reactivity to oysters does not correlate with oyster sensitivity. Based on these re-

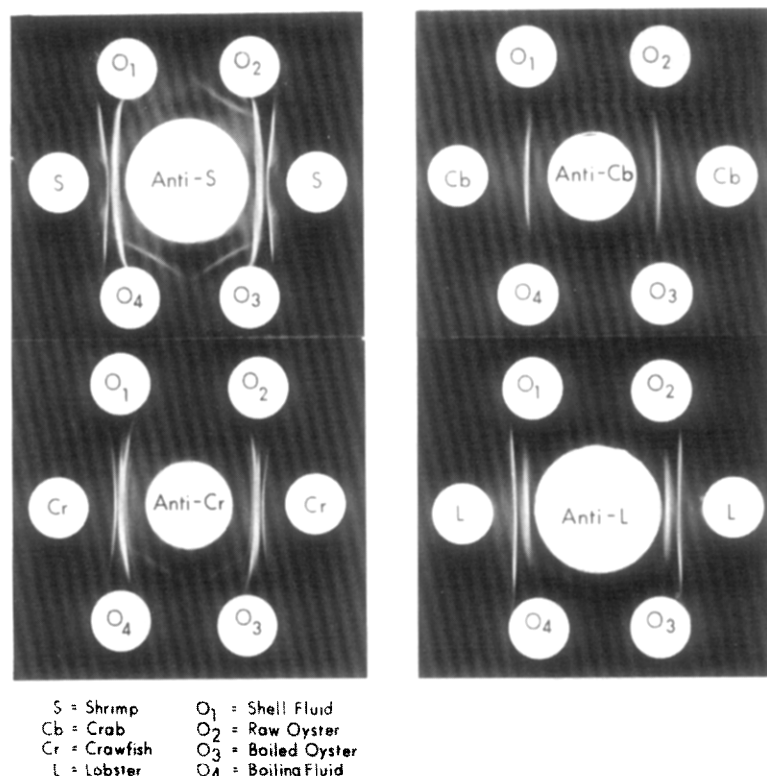


FIG. 6. Reactivity of crustacea-specific rabbit antisera with crustacea and oyster extracts. Rabbit antisera to shrimp (Anti-S), to crab (Anti-Cb), to crayfish (Anti-Cr), and to lobster (Anti-L) were tested for the ability to react with homologous crustacea or oyster extracts.

sults, caution should be used in assessing oyster sensitivity by skin and RASTs alone. Our interpretations should be tempered by the fact that they may be biased by the small number of subjects tested, and more extensive studies are needed. Obviously, a greater number of individuals must be tested in order to reach a definitive conclusion.

An interesting finding in our studies is the fact that skin and RAST reactivity to oysters can occur in crustacea-sensitive subjects. This reactivity does not appear to correlate with sensitivity to oysters but does correlate significantly with IgE antibody reactivity to crustacea extracts. This reactivity is not due to non-specific binding of IgE antibodies in that it does not correlate with total IgE levels but does correlate with oyster skin test reactivity. Thus, these IgE antibody responses appear to react specifically with allergens present in oyster extracts.

Do crustacea-specific IgE antibodies react to oyster allergens? Our studies indicate that there is a significant correlation of IgE antibody reactivity to oyster with that to crustacea allergens. Based on the fact that crustacea inhibit oyster RASTs and that rabbit antisera to crustacea also react with oysters, we conclude that

oyster and crustacea allergens share common antigenic epitopes. Although common antigenic epitopes have been reported for other foods, in particular our studies of crustacea,^{4,5} they were unexpected in this study because oyster and crustacea are not closely related in that they belong to different phylum (oysters, Mollusca; crustacea, Arthropoda). How can common antigenic structures exist in such organisms that lack a close phylogenetic relationship? At this time this observation is unexplained. It is possible that these common antigenic structures are related to primitive molecular entities that have been conserved in both phyla. Another explanation is based on the fact that oysters are filter feeders, and it is possible that crustacea larva may be part of their diet. Thus, the ingestion of small crustacea by oysters might lead to the incorporation of their allergens.

The most important question raised by our studies concerns the immunopathogenesis of allergic reactions to oysters and the role of IgE antibodies in sensitive patients. Our initial results suggest that IgE antibody reactivity is not significantly related to all cases of oyster sensitivity. This is based on the fact that a number of individuals who claim intolerance to crus-

tacea and yet report no intolerance to oysters, have elevated levels of oyster-specific IgE antibodies in their serum. These results suggest that IgE antibodies to oysters are not involved in immunopathogenesis of all cases of oyster allergy. However, further analysis and testing of additional oyster-sensitive subjects is necessary in order to substantiate this finding.

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