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Crawfish and Lobster Allergens: Identification and Structural Similarities with Other Crustacea[1]

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Abstract. Antigenic and allergenic components in crawfish and lobster extracts were studied using crossed immunoelectrophoretic techniques. Crossed immunoelectrophoresis with rabbit antisera revealed 23 antigens in crawfish and 17 antigens in lobster extracts. Both extracts exhibited structural similarities in antigens mutually and with other crustacea in crossed-line immunoelectrophoresis. Crossed radioimmunoelectrophoresis (CRIE) demonstrated 6 crawfish and 4 lobster allergens when individual or pooled sera from radioallergosorbent test (RAST)-positive crustacea-sensitive subjects were used. Since radiostaining was also observed with sera from RAST-negative nonsensitive subjects, specificity of IgE binding was tested using CRIE-inhibition. Preincubation of RAST-positive sera with crawfish or lobster extract decreased radiostaining in CRIE, while no changes occurred when using control sera. These results confirmed the presence of IgE-mediated mechanisms in seafood allergy and demonstrated a number of shared antigenic determinants among crustacea allergens.

Introduction

Seafood, including crustacea, are among the most frequently mentioned foods which are claimed to cause adverse reactions in food-sensitive individuals [1–5]. Adverse reactions to foods may be mediated by nonimmunologic mechanisms, such as natural toxins, contaminants and preservatives present in the food, as well as by immunologic mechanisms [3]. Most adverse reactions to shrimp have been shown to be due to type-I immediate hypersensitivity as determined by skin test and radioallergosorbent test (RAST) [1, 4, 5].

Members of the order Decadoda, including shrimp, crab, lobster and crawfish (crayfish), are closely related to each other. Consequently, similar antigenic structures are expected, and human IgE antibodies against antigens in one crustacean species may cross-react with those from other species. For example, individuals with claimed shrimp hypersensitivity often complain of adverse reactions following ingestion of other shellfish [4] and they have been shown to exhibit positive skin and RAST reactions when tested with crab, crawfish and lobster extract [4, 5].

Previously, we have studied the antigenic and allergenic composition of boiled shrimp extract and have shown common structures in shrimp and other crustacea antigens/allergens by RAST inhibition and crossed-line immunoelectrophoresis and crossed radioimmunoelectrophoresis [6–8]. The purpose of this study was to identify the antigenic and allergenic components in two other crustacea extracts: crawfish and lobster, and to study further the structural similarities in crustacea antigens/allergens.

[1] This study was presented in part at the 12th International Congress of Allergology and Clinical Immunology, Washington, 1985.

Materials and Methods

Extracts

The crustacea studied, crawfish *(Procambarus clarkii)*, spiny lobster *(Panulirus argus)*, *white shrimp (Penaeus setifecus)* and blue crab *(Callinectes sapidus)*, were extracted as follows: boiled (15 min), peeled crustacea were homogenized in 0.01 mol/l sodium phosphate-buffered saline (PBS), 0.015 mol/l, pH 7.2 (in an approximate crustacea: buffer ratio of 1:2 for 3 min using Waring blender homogenizer), mixed overnight at +4°C, and centrifuged (16,000 *g*, +4°C, 30 min). Supernatants were concentrated by ultrafiltration (Amicon YM2, molecular weight exclusion, 2,000 daltons) to approximately one tenth of their original volume and dialysed against PBS (Spectrapor 3, molecular weight exclusion 3,500 daltons) for 3 days at +4°C, changing the buffer twice daily. The dialysates were centrifuged (66,000 *g*, +4°C, 30 min), dry weights were estimated, and the extracts were aliquoted and stored at –20°C until used. Additionally, crawfish boiling water was also concentrated, dialysed, and stored as described for crustacea extracts.

Timothy pollen extract (Allergopharma, FRG) and coffee bean extracts, prepared as described previously [9], were used as controls.

Antibodies

Antibodies against crawfish, lobster, shrimp or crab antigens were raised in New Zealand white rabbits. Three rabbits/extract were immunized by 5 biweekly subcutaneous injections of 10 mg extract emulsified in 50% complete Freund's adjuvant. After the last injection, rabbits were bled weekly for 2 months and sacrificed by cardiac puncture under anesthesia. All sera from each of 3 rabbits were combined and concentrated by precipitating the serum immunoglobulins with $(NH_4)_2SO_4$ at 40% saturation. After centrifugation, the pellet was suspended in PBS, dialysed against PBS for 10 days, changing the buffer twice daily, centrifuged, and the volume was adjusted to one tenth of the original volume. Antisera were stored at –20°C until used.

Patient Sera

Sera from 23 individuals with a clear cut history of adverse reactions (i.e., anaphylactic, gastrointestinal or respiratory) to one or more crustacea, by questionnaire, and a positive skin prick test to crawfish and lobster, were screened using RAST. A mean wheal of 2 mm equal to or greater than the negative control, elicited by the crustacea extract (maximum concentration of 10 mg/ml), was considered a positive skin prick reaction. Fifteen of the 24 individuals were atopic, exhibiting a personal and/or family history of allergy by questionnaire, and 2 or more positive skin prick reactions to common inhalant allergens. A crustacea-positive serum pool was prepared from equal amounts of 10 crawfish and lobster RAST-positive sera. Each serum sample had a RAST result (expressed as a percent of total radioactivity added) greater than 18%. A crustacea-negative sera pool from 10 crustacea skin test-negative, nonsensitive atopic individuals was used as a negative control. Additionally, sera from nonsensitive, nonatopic subjects as well as a high total IgE serum (2,200 kU/l) from a crustacea nonsensitive atopic individual were also used as controls.

Radioallergosorbent Test (RAST)

Antigens from crawfish and lobster extracts were coupled to CNBr-activated filter paper discs (Whatman 50) using 50 μg (dry weight) extract per disc [10]. Crawfish- and lobster-specific IgE was measured using the double overnight RAST procedure, employing 100 μl of patient serum and 100 μl ^{125}I-labelled (25,000 cpm) anti-IgE (Pharmacia Diagnostics, N.J.). All RASTs were performed in duplicate and the results expressed as mean values. For this study, the percentage of total radioactivity added binding to the discs greater than 5% was considered positive.

Total IgE

Serum total IgE concentrations were determined using the Phadebas PRIST kit (Pharmacia Diagnostics, N.J.).

Immunoelectrophoretic Methods

Crossed immunoelectrophoresis (CIE) [11], crossed-line immunoelectrophoresis (CLIE) and CIE with intermediate gel (CIE-WIG) [12], and crossed radio immunoelectrophoresis (CRIE) [13–15] were performed. The experiments were performed on 5 × 7 cm glass plates or on 4.9 × 6.3 cm hydrophilic plastic plates (Gel Bond, Pharmacia Diagnostics, N.J.). Specific information for each run is contained in the legends of the figures.

CRIE results were semiquantitated using the CRIE reference system [16], modified for use with intensifying screens and exposure at –70°C. CRIE classes form A to G were scored according to the appearance of radiostaining on an X-ray film, caused by specific IgE-binding on the native CIE plate, compared to the appearance of the radiostaining from a series of reference discs after different times of exposure at 70°C. CRIE class A represents the highest radiostaining (visible radiostaining from the sample plate prior to that from the highest reference disc), and CRIE-class G the weakest degree of radiostaining. In autoradiography, AGFA X-ray film and DuPont intensifying screens were used. The reference discs were obtained from Pharmacia. ^{125}I-labeled anti-IgE was from Pharmacia (from rabbit) with the exception of some experiments where ^{125}I-labeled anti-IgE from Kallestad (from horse) was used. Inhibition of CRIE was performed by preincubation overnight at +4°C with the interacting antigen extract, centrifuged and a normal CRIE was performed.

Results

Antigenic composition of the crawfish and lobster extracts were analyzed using CIE. Twenty-three separate antigens in crawfish (fig. 1) and 17 antigens in lobster (fig. 2) extracts were distinguished when CIEs of the two extracts against the respective rabbit antisera were performed. Under the experimental conditions used all antigens in both extracts migrated towards the anode. The reference patterns, obtained through several experiments varying the antigen/antibody concentrations, were numbered arbitrarily according to the mobility of antigens in the first dimension electrophoresis from anode to cathode.

Comparison of antigens in crawfish and lobster extracts, mutually and with shrimp and crab antigens, was performed directly using CLIE and indirectly by

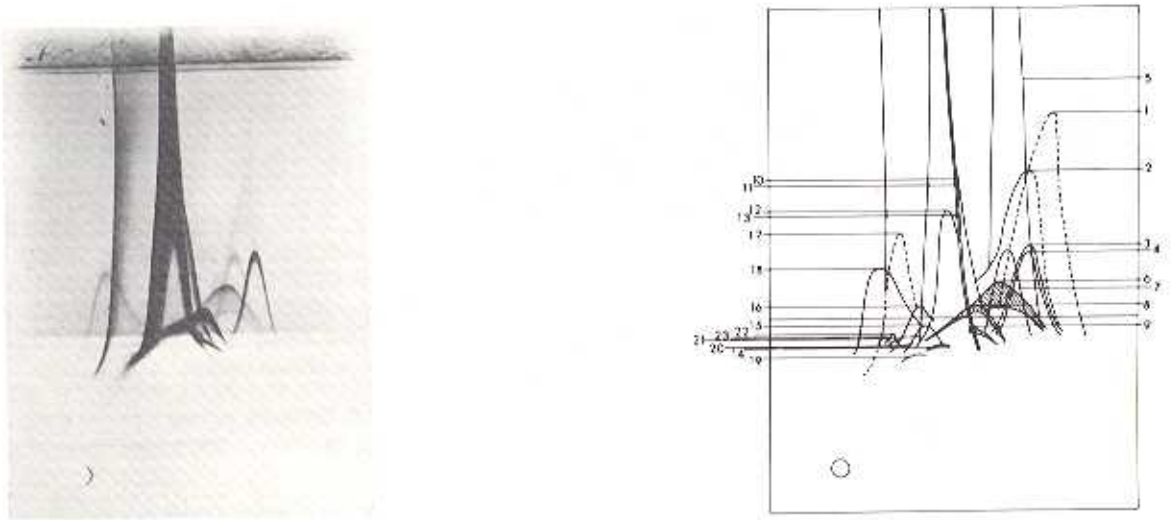

Fig. 1. CIE precipitation pattern of crawfish antigens. First dimension electrophoresis was performed with 75 μg crawfish extract at 10 V/cm, 5 °C, for 35 min and second dimension electrophoresis was performed with gel containing 75 μl rabbit antisera to crawfish extract at 2 V/cm/5 °C, for 18 h.

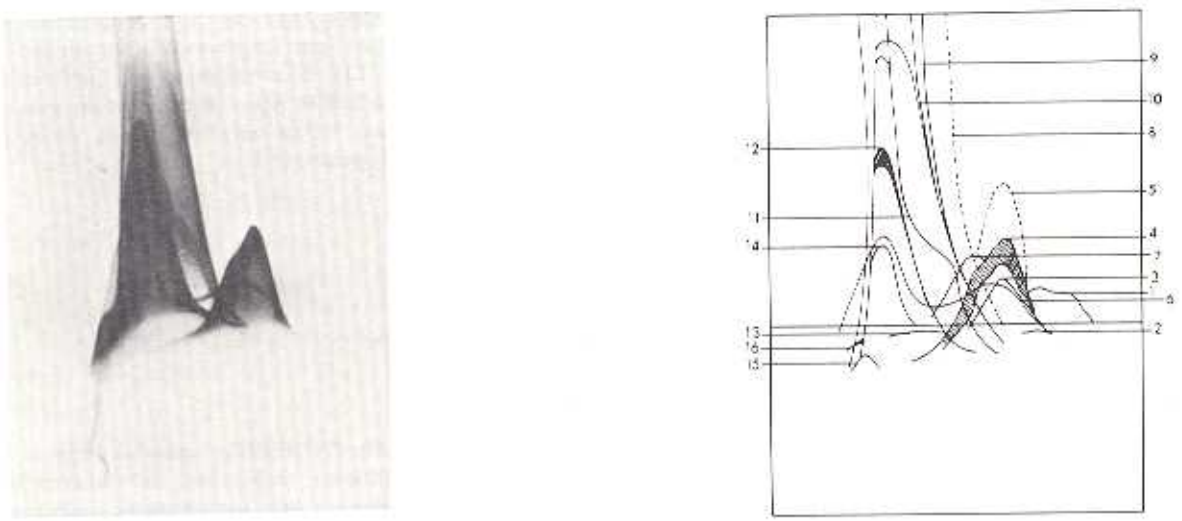

Fig. 2. CIE precipitation pattern of lobster antigens. First dimension electrophoresis was performed with 15 μg of lobster extract at 10 V/cm, 5 °C, for 35 min, and second dimension electrophoresis was performed with an agarose gel containing 100 μl of concentrated rabbit antisera to lobster extract at 2 V/cm, 5 °C, for 18 h.

CIEWIG. Some examples of the CLIE and CIEWIG experiments for crawfish are demonstrated in figures 3 and 5 and for lobster in figures 4 and 6. Frequent shared determinants in antigens present in all four crustacea extracts could be demonstrated in crawfish (table I) and lobster (table II). Crawfish or lobster antigens, which are immunologically identical or partially identical to antigens from the other crustaceans tested, exceed in number those unique to crawfish or lobster and include all the predominant antigens. In CLIE experiments, when concentrated boiling water was introduced into the intermediate gel in both crustacea/anti-crustacea systems studied, common antigenic epitopes were observed (fig. 7).

Allergenicity of distinct crawfish and lobster antigens (identified by CIE) was demonstrated by CRIE analyses using 14 crawfish and lobster RAST-positive sera. Examples of crawfish and lobster CRIEs are shown in figure 8. The results are summarized in table III. Eleven and 13 crawfish and lobster RAST-posi-

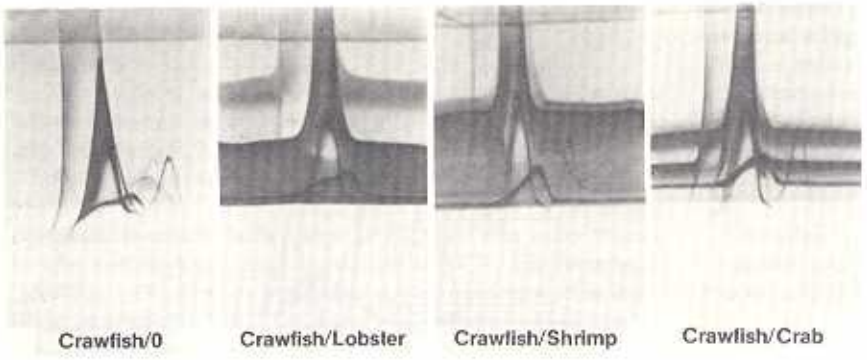

Fig. 3. The comparison of crawfish antigens with antigen from lobster, shrimp and crab by CLIE. Electrophoresis was performed as described in figure 1. Intermediate gels contained 250 μg of lobster, 250 μg of shrimp, or 250 μg of crab extract.

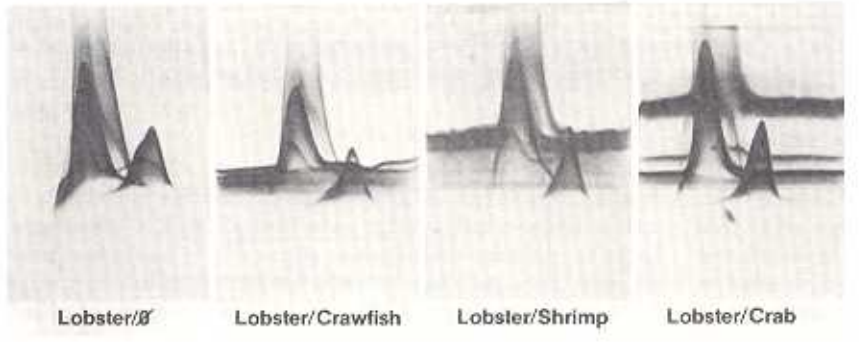

Fig. 4. The comparison of lobster antigens with antigens from crawfish, shrimp and crab by CLIE. Electrophoresis was performed as described in figure 2. Intermediate gels contained 100 μg crawfish, 100 μg shrimp, or 250 μg crab extract.

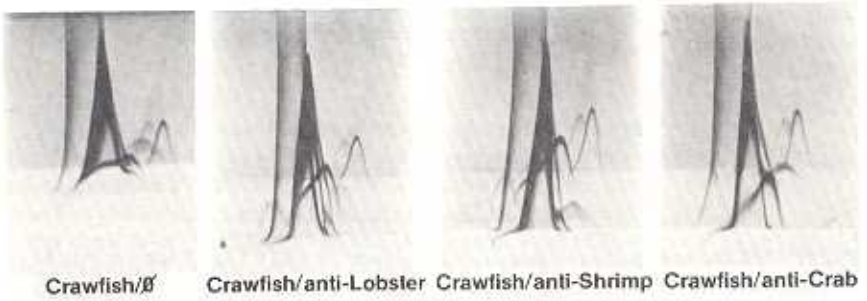

Fig. 5. CIEWIG of crawfish antigens with anti-lobster, anti-shrimp, and anti-crab. Electrophoresis was performed as described in figure 1. The intermediate gel contained 50 μl rabbit antisera to lobster, 50 ml rabbit anti-shrimp, or 50 μl rabbit anti-crab.

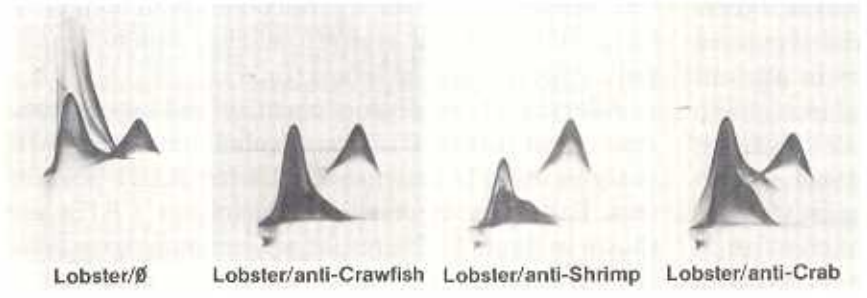

Fig. 6. CIEWIG of lobster antigen with anti-crawfish, anti-shrimp, and anti-crab. Electrophoresis was performed as described in figure 2. Intermediate gels contained 50 μl rabbit antisera to crawfish, 50 μl rabbit anti-shrimp, or 50 μl rabbit anti-crab.

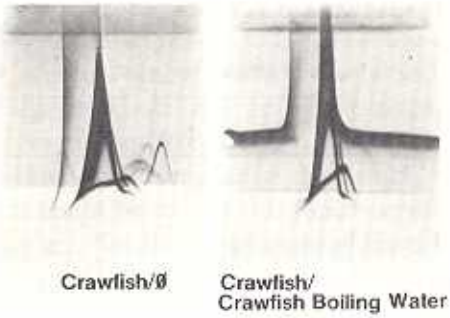

Fig. 7. Crawfish CLIE with crawfish boiling water. Electrophoresis was performed as described in figure 1. The intermediate gel contained 250 μg of extract from the crawfish boil water.

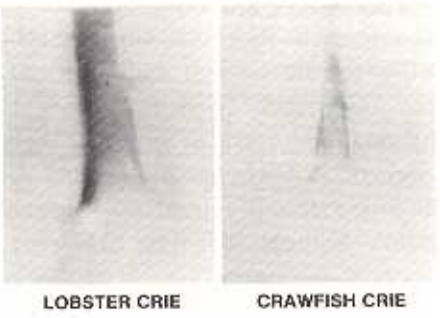

Fig. 8. Crawfish and lobster CRIE. Electrophoresis was performed as described in figure 2. Plates were then washed, incubated with the subject's serum, and CRIE performed as described in Materials and Methods.

Table I. Immunological identity/partial identity of crawfish antigens with antigen from lobster, shrimp and crab

Crawfish antigen number	Lobster	Shrimp	Crab
1	×	×	×
2			
3			
4			
5	×	×	×
6	×	×	×
7			×
8	×	×	×
9	×	×	×
10	×	×	×
11	×	×	×
12	×	×	×
13			×
14	×	×	×
15			
16	×	×	×
17	×	×	×
18	×	×	×
19	×	×	×
20	×	×	×
21	×	×	×
22	×	×	×
23	×	×	×

Table II. Immunological identity/partial identity of lobster antigens with antigens from crawfish, shrimp and crab

Lobster antigen number	Crawfish	Shrimp	Crab
1	×	×	×
2	×	×	×
3			
4			
5	×	×	×
6	×	×	×
7		×	
8	×	×	×
9	×	×	×
10	×	×	×
11	×	×	×
12	×	×	×
13	×	×	×
14	×	×	×
15	×	×	×
16	×	×	×

tive sera, respectively, reacted in CRIE. Crawfish antigen (ag 11) appears to be the main allergenic component in crawfish, as it binds IgE from 9 of the 11 CRIE-positive sera, corresponding to CRIE classes A–E.

In CRIE class F, weak radiostaining from ag 11 was observed with all sera used, including controls, and therefore class F/ag 11 was excluded from the data. Antigen 12 also gave frequent radiostaining but this can be a result of co-precipitation, since ag 12 is located completely under the precipitation arc of ag 11. Additionally, 4 different antigens showed radiostaining, to various degrees, with the sera used, so that a total of 6 allergens were identified in crawfish.

In the lobster extract, 4 antigens were shown to have IgE-binding capacity with 13 of the 14 sera

Table III. Crawfish and lobster CRIE results

Patient	Crawfish antigen no.						Lobster antigen no.			
	6	8	10	11	12	13	3	6	8	13
S.A.			E	A	B		F		A	B
M.A.T.		F		D	D	F		D	E	
H.I.		F	F	C	E		F		B	E
Mc.M.				C	D	F	F		D	D
S.H.				C	D	D			D	
S.T.		F		D		E	F		D	D
H.A.		F		D	E	F	F		F	
A.I.				E	F	D	F			D
A.M.					D	D			D	
M.A.		F						E		
J.E.									F	
B.E.	D	E		D	F					
M.A.							E		E	
B.A.							F			

tested. Antigens 8 and 13 gave both the strongest and the most frequent radiostaining; the other 2 antigens, ag 3 and 6, showed weak radiostaining with only 8 and 2 sera, respectively. Antigen 13 gave weak radiostaining with all sera, including the controls, in class F and the class was again excluded.

Controls gave rise to weak, but distinct radiostaining in CRIE class G, crawfish ag 11 and lobster ag 13 already in class F. The high total IgE serum control reacted similarly to other negative control sera in crawfish and lobster CRIE.

In the CRIE inhibition experiment, a reduction in radiostaining was achieved in lobster/antilobster system by preincubation of a patient sera (RS) with lobster or crawfish extract. Timothy pollen extract and coffee bean extract induced no changes in radiostaining. Radiostaining from control plates (nonsensitive, nonatopic individual serum) was not affected by preincubation with any of the mentioned interacting antigens.

To determine whether the observed fairly early radiostaining was due to a nonspecific reaction between the crustacea antigens and some antibodies in rabbit serum, some CRIE experiments using ^{125}I-labelled anti-IgE raised in horse were performed. No changes were observed in radiostaining from a sample plate or from the control plate.

Discussion

The recognition of crustacea as a group causing adverse reactions in sensitive individuals has a basis in the close phylogenic relationship of shrimp, crab, crawfish, and lobster. Patients often complain of adverse reactions to all above-mentioned crustaceans and have positive skin test reactions not only to shrimp, but also to the other three crustacea, even in absence of a previous contact [4, 5]. RAST inhibition and crossed-line immunoelectrophoretic studies [6–8] indicate common allergic epitopes in shrimp and other crustacea.

In the present study, evaluation of crawfish and lobster antigens and allergens, as well as a comparison of distinct antigens and allergens with those from shrimp and crab were performed by crossed immunoelectrophoretic techniques. As expected, a close relationship between the identified crawfish and lobster antigens and other crustacea was found. In fact, most of the antigen present in crawfish or lobster were fully or partially identical within the crustaceans studied, as determined by CIE and CIEWIG. Furthermore, the predominant allergens, by CRIE, were among the common antigens.

The demonstration of similar structural entities in crawfish and lobster extracts and in concentrated

crawfish boiling water, including two crawfish and one lobster allergen, indicates that the crustacea antigens are heat-stable, water-soluble and can enter the atmosphere in steam aerosols from the cooking process. Some sensitive individuals complain of adverse reactions following only the 'smell' of crustacea, which could be due to the vapor containing allergen in the air. Similar observations have been reported with hypersensitivity to bony fish [17] and crabs [18].

CIE of crawfish and lobster antigens revealed several asymmetric precipitates, suggesting pH heterogeneity in the antigens. Also molecular heterogeneity in many antigens in both extracts was observed, as immunologically identical or partially identical precipitates situated beneath one another were frequently found. The presence of multiprecipitates could be due to artificial proteolytic degradation in the antibody containing gel during the second dimension electrophoresis or during the course of the extraction process. However, in some CLIE experiments, shared antigenic epitopes with only one precipitate of the multiprecipitates were demonstrated, suggesting the true presence of heterogeneous protein units.

An explanation of the observed heterogeneity in antigens could be the aggregation of antigens. Consistent with the results by Hoffman et al. [19] for fractionation of shrimp extract, in our attempts to separate crawfish antigens by gel filtration (Biorad A-0.5, operation range 5,000–500,000 daltons), the main allergenic activity was found in void volume (data not shown), suggesting the presence of aggregated proteins. The dissociation of these aggregates could give rise to the observed multiprecipitates. Heterogeneity in crawfish and lobster antigens could also be due to variable degrees of antigen denaturation. Since extracts were prepared form boiled crustacea, antigenic determinants may have been partly denatured when boiled, and partially identical antigens could occur. The partial heat denaturation of allergens could explain why some individuals, who claim to be crustacea-sensitive, occasionally tolerate these foods [4].

By CRIE, the IgE-binding capacity of 6 crawfish and 4 lobster antigens was demonstrated, suggesting that crawfish extract ag 11 and 12 and lobster ag 8 and 13 are the main allergenic components and that these are shared by other crustacea as revealed by CRIE-CLIE. The correlation between CRIE and RAST is significant and the sensitivity of these two methods is considered to be of the same order [18]. In this study, however, the correlation was less than expected, since one third of the RAST-positive sera failed to react in CRIE. Although the cut-off for a positive test may have been too low, additional explanations are needed, since the magnitude of the RAST values and CRIE classes did not always correlate. A lack of specific rabbit antibodies against some allergens present in crustacea extracts studied could explain this discrepancy.

Additionally, possible interference of IgG antibodies in CRIE should be considered. IgG antibodies against foods are frequently found, even in normal population [20] and elevated levels of crustacea-specific IgG and IgA have been reported in crustacea-sensitive individuals [5]. The effect of IgG antibodies on CRIE is not clear.

In conclusion, our studies confirm the presence of IgG-mediated mechanisms in seafood allergy and demonstrate a number of shared antigenic determinants among crustacea allergens. Crustacea allergens appear to be heat-stable, water-soluble molecules, and in the case of crawfish a number are present in the boil fluid as well. Further studies are directed at isolation and characterization of major allergens that are present in all crustacea.

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