

IgE sensitization in snow crab-processing workers

André Cartier, M.D.,* Jean-Luc Malo, M.D.,* Heberto Ghezzi, Ph.D.,*
Marjorie McCants, B.A.,** and Samuel B. Lehrer, Ph.D.** Montreal, Canada

Occupational asthma is a highly prevalent disease among snow crab-processing workers, but its immunologic mechanism has not been identified. Prick skin tests with snow crab-meat extract, commercial extracts from other crab genera, and snow crab cooking water collected in 1984 were performed on 119 workers. Crab-specific IgE was assessed by RAST in sera from 115 workers with meat and water extracts. Both skin and RAST tests were performed in 58 individuals. Diagnosis of occupational asthma had previously been confirmed in 54 individuals. A highly significant relationship was demonstrated between the presence of immediate skin reactivity or increased serum levels of specific IgE to crab extracts and the occurrence of occupational asthma. There was good agreement between the results of skin and RAST tests with extracts of either meat or snow crab cooking water. Cooking water and snow crab-meat extracts were more sensitive than commercial preparations. Water extract was more potent and more sensitive than meat extract. We conclude that there is evidence that occupational asthma in snow crab-processing workers is mediated through an IgE mechanism. (J ALLERGY CLIN IMMUNOL 78:344-8, 1986.)

In a previous study,¹ a high prevalence (15%) of occupational asthma among snow crab-processing workers in two different plants of Îles de la Madeleine, Quebec, was documented. By the end of 1982, the diagnosis of occupational asthma had been confirmed in 46 of 303 workers who were investigated, this latter figure corresponding to 97% of all the exposed individuals. Occupational asthma was diagnosed by specific inhalation challenges and/or a combination of monitoring of peak expiratory flow rates and significant changes in bronchial responsiveness to histamine as well as in spirometry after reappearance of symptoms on return to work. The presence of immediate skin reactivity to a snow crab cooking-water extract and to three commercial-crab extracts prepared from other genera of crabs was highly associated with the occurrence of occupational asthma. This finding suggested an IgE sensitization to crab in these workers.

A group of workers was restudied in 1984 with

Abbreviations used

COM: Commercial extracts

HSA: Human serum albumin

different snow crab extracts for skin testing and specific IgE measurements. The aim of the present study was to determine the prevalence of IgE sensitization to snow crab extracts in these snow crab-processing workers with and without occupational asthma.

MATERIAL AND METHODS

Subjects

In 1982, 303 workers were prick skin tested with extracts of snow crab cooking water and commercial crabs and bled, and sera were stored at -20°C . In 1984, 119 of the 303 workers had prick skin tests with snow crab-meat extract and a new extract of snow crab cooking water (WATER-84). RAST studies with the latter extracts were done with sera from 115/303 workers with the 1982 serum; 58 workers had both tests done.

In 1982, the diagnosis of occupational asthma was confirmed in 46 workers.¹ After 1982, eight other workers developed the condition as confirmed by similar means previously described.¹ Eighteen subjects who had a history suggestive of occupational asthma (diagnosis not confirmed because of insufficient data) and four subjects who had dermatographism were excluded in the present article. All 54 individuals with occupational asthma were skin tested, and 48/54 had RAST studies.

From the *Department of Chest Medicine, Hôpital du Sacré-Coeur, Montreal, Canada, and the **Department of Medicine, Tulane University School of Medicine, New Orleans, La.

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Reprint requests: André Cartier, M.D., Department of Chest Medicine, Hôpital du Sacré-Coeur, 5400 W. Gouin, Montreal, Canada H4J1C5.

TABLE I. Occupational asthma and skin test results in 1982 and 1984

Skin tests										
1982						1984				
WATER-82		COM		Total	WATER-84		MEAT		Total	
+	-	+	-		+	-	+	-		
Asthma										
+	19	35	30	24	54	37	11	37	11	48
-	15	214	23	206	229	17	35	19	33	52
Total	34	249	53	230	283	54	46	56	44	110
	$\chi^2 = 27.0$		$\chi^2 = 49.4$			$\chi^2 = 20.6$		$\chi^2 = 17.2$		
Sensitivity:	35%		56%			Odds for the presence of occupational asthma in a subject with positive skin test:				
						69%		66%		
Specificity:	93%		90%			Odds for the absence of occupational asthma in a subject with negative skin test:				
						76%		75%		

Skin tests

These tests were done by the prick method.² The size of the wheal reaction was assessed 10 minutes after the introduction of the antigen by measuring the average value of two perpendicular diameters. A positive reaction was set at a diameter ≥ 2 mm above the reaction obtained with a control solution of 50% glycerin in normal saline (Bencard Allergy Service, Weston, Ontario). In addition, all subjects studied in 1984 had a control phosphate-buffered saline (50%)-glycerol (50%) skin test because the snow crab extracts prepared in 1984 were dissolved in this solution. Finally, all latter subjects had a positive reaction to histamine phosphate (1 mg/ml).

Allergen extracts

COM for prick tests were obtained from Hollister-Stier (Mississauga, Ontario), Bencard, and Omega (Montreal, Quebec). According to the manufacturers, these extracts were prepared from different genera of crabs (Alaskan king crab, Dungeness crab, and stone crab). In 1981, cooking water was collected from the cooking pots in one of the plants and frozen at -20°C . In 1982, this water (WATER-82) was thawed, filtered through a 22 μm Millipore membrane (Millipore Corp., Bedford, Mass.), and then dialyzed for 24 hours. The dialyzed material was then heated at 56°C for 1 hour, HSA was added (concentration of 0.03%), and it was then used for the skin tests.

In 1984, two additional extracts were prepared. One extract (MEAT) was obtained from snow crab meat removed from boiled claws and body, homogenized in phosphate-buffered saline, and centrifuged, and the supernatant fluid was concentrated by ultrafiltration on an Amicon YM 2 filter (molecular weight exclusion 1000) (Amicon Corp., Lexington, Mass.). Another extract (WATER-84) was prepared

from snow crab cooking water collected in the cooking pots in one plant and centrifuged, and the supernatant fluid was concentrated (Amicon YM 2). Extracts used for skin tests were sterile filtered, dry weight was determined, and concentrations were adjusted to 10 mg/ml concentration. These extracts were stabilized with 50% sterile glycerol.

Specific IgE assessment

RAST tests were used to quantitate circulating IgE in the test sera. Serum samples were coded, and the workers responsible for the tests (M. M. and S. L.) were not aware of the clinical findings at the time of the analysis. Ten mg/ml of the antigens (preparation described above) was coupled to CNBr-activated filter paper discs prepared by the method of Ceska and Lundkvist.³ Ethanolamine was used to block the unreacted sites. For the RAST, 100 μl of test serum was added to the tubes containing an antigen-coupled disc or a controlled disc to which HSA had been coupled. Tubes were incubated overnight at room temperature on a rotator and then washed three times in 2.5 ml of physiologic saline to remove unreacted serum. One hundred microliters of ^{125}I -labeled anti-IgE containing approximately 20,000 cpm (Pharmacia, Piscataway, N. J.) was added to the tubes. They were again incubated overnight at room temperature on a rotator, washed three times in 2.5 ml of saline, and counted for 5 minutes on a Beckman (Beckman Instruments, Palo Alto, Calif.) bio-gamma counter. Counts were adjusted to counts per minute, and the counts per minute obtained with the tests discs were divided by the counts per minute obtained with HSA-coupled discs.

Statistical analysis

Because the presence or absence of occupational asthma had been set before undergoing skin and RAST tests, the

TABLE II. Occupational asthma and RAST results

	WATER-84 RAST					MEAT-RAST				
	Ratio > 2		Ratio > 4.5		Total	Ratio > 2		Ratio > 4.5		Total
	+	-	+	-		+	-	+	-	
Asthma										
+	31	6	25	12	37	22	15	11	26	37
-	26	47	11	62	73	17	57	3	71	74
Total	57	53	36	74	110	39	72	14	97	111
	$\chi^2 = 24.5$		$\chi^2 = 30.7$			$\chi^2 = 14.2$		$\chi^2 = 14.0$		
	Odds for the presence of occupational asthma in a subject with positive RAST:					Odds for the presence of occupational asthma in a subject with positive RAST:				
	54%		69%			56%		79%		
	Odds for the absence of occupational asthma in a subject with negative RAST:					Odds for the absence of occupational asthma in a subject with negative RAST:				
	89%		84%			79%		73%		

TABLE III. RAST results and skin tests

	RAST results								
	WATER-84					MEAT			
	Ratio > 2		Ratio > 4.5		Total	Ratio > 2		Ratio > 4.5	
	+	-	+	-		+	-	+	-
Skin tests									
+	40	4	29	15	44	24	20	12	32
-	3	11	0	14	14	2	12	0	14
Total	43	15	29	29	58	26	32	12	46
	$\chi^2 = 23.9$		$\chi^2 = 25.1$			$\chi^2 = 7.7$		$\chi^2 = 7.6$	

Results of the usual and not of the maximum χ^2 are presented.

epidemiologic design of the study was of the case-control type.⁴ Therefore, odds ratios, instead of sensitivity and specificity used in cohort studies, were assessed for the relationship between skin tests, RAST results, and occupational asthma. Chi-square and maximum chi-square analysis⁵ were used to assess the relationship between skin tests, RAST results, and occupational asthma. The kappa statistics were used to assess the agreement and biases between the skin wheal diameter as well as the RAST ratios obtained with the different extracts.⁶

RESULTS

Table I illustrates that there was a strong association between the results of skin tests with WATER-82, COM, WATER-84, and MEAT extracts and the presence of occupational asthma. The range of wheal diameter with one or the other of the extracts varied from 2 to 18 mm. Table II presents information about

the relationship between RAST results and occupational asthma. For the sake of analysis, positive RAST ratios were defined as >2 or >4.5 . When individual RAST ratios were compared to the presence of occupational asthma, the maximum chi-square was obtained when the cutoff point for the ratio was set at 4.5. Again, there was a strong association between a positive RAST and the presence of occupational asthma. RAST ratios ranged from >2 to 41.

The relationship of RAST to skin test sensitivity of subjects to WATER-84 and MEAT extracts was analyzed, and results are summarized in Table III. There was better agreement between RAST and skin tests obtained with WATER-84 than with RAST and skin tests obtained with MEAT extracts. Further analysis compared each extract in terms of the skin wheal diameter (Table IV). There was good agreement between skin reactivity obtained with the WATER-84 as

TABLE IV. Correlation between skin test results with WATER-84 and MEAT extracts

	WATER-84 skin weal diameter					
	<2 mm	2 mm	3 to 4 mm	5 to 6 mm	7 to 8 mm	≥9 mm
MEAT skin weal diameter						
<2 mm	48	4	0	0	0	0
2 mm	2	1	2	2	1	0
3 to 4 mm	3	0	11	7	3	0
5 to 6 mm	1	0	4	8	4	4
7 to 8 mm	0	0	0	4	1	4
≥9 mm	0	0	0	1	2	2
Agreement = 60%; $p < 0.001$						
Positive bias, $p = 0.03$						

Numbers in bold characters represent perfect agreement.

TABLE V. Correlation between RAST results with WATER-84 and MEAT extracts

	WATER-84 RAST ratio				
	>2	2 to 2.4	2.5 to 4.4	4.5 to 9.5	>9.5
MEAT RAST ratio					
<2	52	5	13	4	1
2 to 2.4	1	2	3	3	0
2.5 to 4.4	0	1	1	8	6
4.5 to 9.5	0	0	0	1	1
<9.5	0	0	0	1	12
Agreement = 59%; $p < 0.001$					
Positive bias, $p < 0.001$					

Numbers in bold characters represent perfect agreement.

compared to that obtained with MEAT extract. Perfect agreement (equal diameter) occurred in 60% of cases ($p < 0.001$), the direction of bias being significant ($p < 0.05$) toward the WATER-84 extract. Perfect agreement of skin test reactivity in the comparisons between WATER-84 or MEAT extract on one hand and WATER-82 and COM extracts on the other hand varied from 48% to 55% (p values varying from <0.05 to <0.001), a significant ($p < 0.001$) bias being detected in favor of WATER-84 and MEAT extract.

The relationship between RAST results with MEAT and WATER-84 is presented in Table V. Perfect agreement was observed in 59% of observations ($p < 0.001$) with a positive bias in favor of the WATER-84 extract ($p < 0.001$). It is to be noticed that in this specific case the statistical significance of the results might have been overestimated because most subjects were either in the <2 or >9.5 RAST ratio scores.

From a questionnaire administered at the time of

the 1984 survey, it was observed that 12 subjects, all with occupational asthma, also had a history of food-allergy manifestations such as urticaria, angioedema, or gastrointestinal symptoms when they ate crab.

DISCUSSION

Some types of occupational asthma have been associated with a high prevalence of IgE sensitization: platinum salts,⁷ laboratory animals,⁸ trimellitic anhydride,⁹ and *Bacillus subtilis*.¹⁰ Evidence of IgE sensitization has also been documented with other agents, although it was to a lesser degree: red cedar¹¹ and isocyanates.¹² IgE sensitization has also been documented for occupational asthma in the fishery industry: oyster¹³ and prawn.¹⁴ Orford and Wilson¹⁵ have demonstrated positive skin tests and precipitins in some workers exposed to Alaskan king crab. However, in the latter work, no RAST studies were done.

Our study confirms previous finding on the association between occupational asthma and the presence of skin reactivity to crab in snow crab-processing

workers.¹ In the latter study we performed skin tests with commercial crab preparations that did not contain snow crab extract and a crude snow crab cooking-water extract. In the present article, we used a more specific (i.e., snow crab) and a more active water extract. The satisfactory association between the results obtained with COM containing different genera of crab but not snow crab and our snow crab extract suggests that one or more common constituents are shared by different genera of crab. Apart from the presence of immediate skin reactivity to crab extracts, IgE sensitization was also confirmed in the present article by RAST studies that correlated to the presence of occupational asthma on one hand and the skin test results on the other hand.

There was a satisfactory association between skin and RAST results with WATER-84 and MEAT extract. This suggests that one or several constituents common to both extracts cause the reaction. However, water was more sensitive and potent than the MEAT extract. One possible explanation for this could be that the constituents, probably heat stable or denatured proteins, responsible for the reaction are water soluble and are present in higher concentration once they are solubilized in water. The association between RAST and skin test results to crab extracts that we found could have been even better if the skin tests had been carried out at the time the sera were obtained. There was an interval of 2 years between these maneuvers, and some individuals might have acquired immunologic sensitization and developed skin reactivity to crab in the meantime.

The findings presented here are not only important to studies of occupational allergy but also for investigation of food allergy for several reasons. First, they clearly demonstrate that sensitization to a food allergen can occur through inhalation. Not only is this important for the work place, but it also lends credence to reports by some food-sensitive subjects of adverse reactions on exposure to food by merely smelling it. Conceivably, these individuals are being exposed to aerosolized droplets containing food allergens. Second, since 12 sensitive workers, all with occupational asthma, reported subsequent food allergy after oc-

cupational sensitization, this must result from an immune response stimulated to inhaled allergens. Thus, it is possible that food-allergic reactions can result not only from local IgE production in the gut but also systemic IgE production. It is possible that other allergic reactions to foods may also result from stimulation to inhaled or ingested allergens as well.

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