Identification of crab proteins that elicit IgE reactivity in snow crab–processing workers

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Background: The expanding snow crab–processing industry has resulted in increased numbers of workers at risk of occupational allergy.
Objective: Our study is to identify relevant allergenic proteins in cooked snow crab meat (CM) and crab water (CW) used for cooking for improved remediation, diagnosis, and treatment.
Methods: Extracts were prepared from CM extracts, CW extracts, and an air-filter collection near the crab cooker. Of the 207 workers, 24 with the highest IgE antibody reactivity to CM and CW extracts, as determined by using RASTs, were tested for reactivity to nitrocellulose membranes containing CM and CW proteins separated with SDS-PAGE. A 3-serum pool was similarly incubated against nitrocellulose-bound proteins from air samples collected near the crab cooker.
Results: Of the 207 sera tested, 27 and 39 sera exhibited positive IgE antibody reactivity (≥2%) to CM and CW extracts, respectively. Twenty-two of 24 sera with the highest RAST activity (≥3.5% binding) demonstrated IgE binding to multiple proteins (13.6-50 kd). A majority of the sera reacted to 4 proteins: 79% and 71% to a 34.0-kd protein, 79% and 42% to a 25-kd protein, 67% and 71% to an 18.5-kd protein, and 75% to a 14.4-kd protein in both CM and CW extracts, respectively. The pool of IgE-positive sera blotted against the air-filter extract reacted to 14.4-, 18.5-, 34.0-, 43.2-, and 50-kd proteins present in both crab extracts.
Conclusion: Four major IgE-reactive proteins were identified in CM extracts, CW extracts, and air-filter eluate. Analysis of any potential association of protein reactivity with disease suggested crab proteins at 34.0 and 14.4 kd might be more relevant.
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Key words: *Crab, occupational allergy, RAST, IgE reactivity, immunoblot*

The increased demand for healthy, protein-rich food in the modern diet has generated a substantial increase in the consumption of seafood. Americans alone consumed 1.612 trillion kilograms of crustaceans in 2005.[1] With this increased demand has come a dramatic growth in the harvesting and processing of seafood, including snow crab. A number of older, often poorly ventilated fish-processing plants in the Canadian provinces of Newfoundland and Labrador were converted to snow crab (*Chionoecetes opilio*)–processing plants to meet this demand during the past decade. By 2004, more than 400 shellfish-processing plants along the eastern Canadian coast used an estimated 22,000 laborers, making snow crab the second most commonly processed shellfish (approximately 100,000 metric tons in 2004) in Eastern Canada.[2]

The cleaning, steaming, washing, sawing, cracking, and crushing of snow crabs within these plants can routinely expose many workers to crab proteins in the form of dust, steam, vapor, and crab meat (CM).[3] These conditions place the plants' usually seasonally employed laborers at risk for occupational IgE-mediated allergic disease.[4-6] Respiratory illnesses in these workers negatively affect their quality of life and, for those forced to give up their jobs, imposes economic burdens on them and their families because of limited employment alternatives and infrequent access to health specialty services. Often rural and remote plants can result in affected workers remaining at their jobs, making it difficult for them to obtain a specific diagnosis and access to workers' compensation.[3]

Previous studies have shown the primary source of antigen exposure in seafood processing to be the inhalation of aerosolized proteins ranging in molecular weight from 10 to 70 kd.[7] In the crab industry these aerosols often contain primarily crab exoskeleton, muscle protein, gills, and internal organs with lesser amounts of background material, such as cellulose, synthetic fibers, and inorganic particles.[8] At least 30% of these particles have diameters of 5 μm or less and thus lie within the respirable range.[3] Collaboration with the snow crab–processing industry and regulatory agencies of Newfoundland and Labrador has helped to identify the sources and relative intensity of antigen exposure to modify

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Abbreviations used
CM: Crab meat
CW: Crab water
OA: Occupational asthma

plant design, processing procedures, and safety equipment, and practices to improve work safety are an important goal. This study is the first description assessment of the importance of snow CM and CW allergens in snow crab allergy and identification of aerosolized allergens from snow crab cooking water.

METHODS

Snow crab extracts

Cooked snow crab (908 g) purchased commercially (P. Janes & Sons, Hant's Harbour, Newfoundland, Canada) was blended with 500 mL of PBS (pH 7.2, 0.01 mol/L) in a commercial blender (3× for 1 minute each). The slurry was stirred for 1.5 hours at 4°C and then centrifuged at 10,000*g*. The supernatant was concentrated by means of ultrafiltration (YM1 filter; molecular weight >1000 d; Amicon Corp, Lexington, Mass) to a volume of 100 mL. Centrifugation of this concentrate at 80,000*g* and 4°C resulted in the recovery of 56 mL of supernatant (CM extract) at 33.6 mg/mL protein, as assayed by using the Bradford method.[9]

A 2-L water sample was taken from a boiler of a Newfoundland crab-processing facility after cooking 50,000 lbs of snow crab and freezing for shipment. The water was thawed and concentrated (YM1 filter), and the resultant concentrate was centrifuged (80,000*g*), all at 4°C. Sixty-five milliliters of supernatant-concentrated CW extract at 12.5 mg/mL total protein was recovered.[9]

Area air samples were collected with the Air Sentinel (Quan-Tec-Air, Inc, Rochester, Minn) at a flow rate of 3 L/s by using a polytetrafluoroethylene filter (0.3 μm at 99% efficiency) over the samples and placed near the cooker exhaust of a Newfoundland plant, allowing filter steam from the active cooker to be collected for 2 hours. The filter was removed and extracted overnight in 250 μL of 10% SDS solution (liquid filter extract).

Subject sera

In 2002-2003, a total of 215 workers (representing approximately 45% of the total workforce) from 4 snow crab–processing plants in Newfoundland and Labrador invited to participate in health assessments for occupational asthma (OA) and allergy consented to the use of their sera for linked research into OA and allergy to snow crab. The sera of 196 of these workers were analyzed in the present study. The comprehensive study performed in Newfoundland and Labrador was described elsewhere.[4] A diagnosis of highly probable OA was based on work-related respiratory symptoms, positive skin prick test results, or specific IgE measurements to crab allergens with or without positive peak expiratory flow monitoring; these criteria were met in 39 workers. The study protocols had been approved by the Ethics committee of Hôpital du Sacré-Coeur de Montréal and of Memorial University, St John's Newfoundland and Labrador.

In another survey done in Quebec (Iles de la Madeleine) in 1982 and 1984, we assessed 303 of 313 workers in 2 crab-processing plants. In 54 of these workers, the diagnosis of OA was confirmed by using specific inhalation challenges, a combination of positive peak flow monitoring and significant changes in bronchial responsiveness to histamine, or both.[5,6] The sera of 11 of these workers were used in the present study (206 from Newfoundland and 11 from Quebec).

Sera drawn for 207 of the snow crab processors included in those surveys were used.

RASTs

All 207 crab workers' sera were tested for IgE antibodies to CM and CW extracts by using a RAST, according to the method of Ceska and Lundkvist.[10] Crab proteins were coupled to cyanogen bromide–activated filter paper discs at 25 μg per disc, incubated with 100 μL of sera per disc, washed, and further incubated with iodine 125–labeled goat anti-human IgE (DiaMed, Windham, Me) at 15,000 counts per minute per disc. The assay was performed in duplicate with appropriate controls. The results are expressed as percentages of IgE bound to the individual discs.

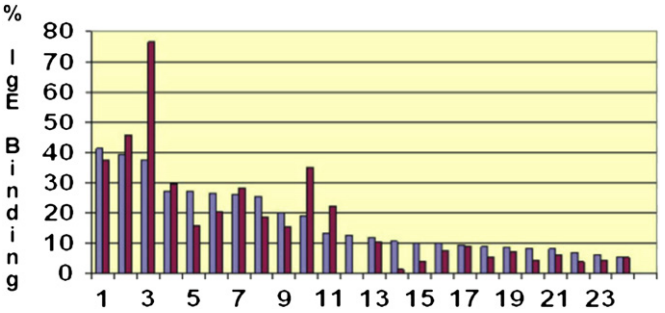

FIG 1. RAST values (percentage IgE binding) of snow crab workers to CM *(red)* and CW *(blue)* extracts.

Immunoblotting

The proteins of the CM, CW, and air-filter extracts were separated by using discontinuous SDS-PAGE and transferred to cyanogen bromide–activated nitrocellulose membrane (Scheicher & Schuell, Dassel, Germany). Of the 207 sera tested, the 24 sera with the most significant IgE-binding values were blotted against the meat and water membranes overnight. A pool of the 3 sera with highest IgE antibody reactivity to crab were blotted against the filter extract to accommodate the limited amount of extract available. Each membrane was cut into 5-mm-wide strips, washed, and incubated with 15,000 cpm per strip of iodine 125–labeled goat anti-human IgE for 24 hours. After washing and drying, the membrane strips were exposed to autoradiographic film (Amersham International, Little Chalfont, Bucks, United Kingdom) for 6, 10, and 18 days in the absence of light at −70°C to identify and grade all reactive bands. The varying periods of incubation were used so that individual bands to which reactivity occurred (and not obscured by more reactive bands with overexposure) could be detected, as well as to have a way of assessing the intensity of reactivity. Bands that were barely detectable or showed little reactivity even at the greatest exposure were given a grade of 1. Bands to which significant reactivity occurred but did not appear to be of the strongest intensity were given a grade of 2. Only those bands to which maximal reactivity occurred, generally in the earliest incubation period, were given a grade of 3. These blots were viewed and graded independently by 3 reviewers, and the grading is a composite of their review. Generally, the results of all the reviewers were in agreement; for the few bands in which this did not occur, the result with the greatest number of reviewers in agreement was chosen. Molecular weight markers and extracts were visualized separately by using Colloidal Gold Total Protein Stain (Bio-Rad Laboratories, Hercules, Calif).

Statistical analysis

Exploratory univariate linear regression analyses were performed to identify any potential associations between quantified subject protein reactivities and RAST scores using data from the 24 samples with significant IgE antibody reactivity to crab allergens on which immunoblotting was done. The proportions of individuals with high, medium, low, or no sera binding were computed for each protein studied. Statistical analyses were performed with SPSS 15.0 software (SPSS, Inc, Chicago, Ill). A P value of less than .05 was considered statistically significant.

RESULTS

Of the 207 sera tested, 27 and 39 exhibited positive IgE antibody reactivity (≥2% binding, as determined by means of RASTs) to the CM and CW extracts, respectively. Twenty-four sera with the