A Sea Urchin Roe Tropomyosin-Like Protein Is Recognized in Vitro by Shrimp-Allergic Individuals

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Shrimp is the most common cause of shellfish allergy. Several allergens have been described, the most allergenic of which is tropomyosin, which in addition is responsible for immunoglobulin (Ig) E cross-reactivity with crustaceans, other arthropods, and mollusks [1]. The safe ingestion of other marine invertebrates by crustacean-allergic patients has not been described, and the general recommendation is to avoid all shellfish, including sea urchins [2]. Sea urchins are marine invertebrates belonging to the phylum echinoderms. They are considered seafood, despite the fact that they are not related to fin fish, mollusks, or crustaceans. Their reproductive organs (roe) are used either raw or briefly cooked in Korean and Japanese cuisine (they are called *uni* in sushi) throughout the world. They are considered a delicacy and are becoming increasingly popular. Although delayed hypersensitivity skin reactions due to stings have been reported [3], immediate hypersensitivity is rare [4-6]. Recently the major allergen of sea urchin roe was identified as a major yolk protein (160 kDa) [7].

This study aimed to address whether shrimp-allergic patients recognize sea urchin allergens. Ten adult and pediatric shrimp-allergic patients with a positive double-blind, placebo-controlled food challenge with shrimp but no previous exposure to urchin roe were selected. IgE recognition of shrimp (*Litopenaeus vannamei*) and urchin roe extracts was evaluated as previously described [8]. Raw and boiled roe extracts were prepared from fresh green sea urchins obtained from a local store. Briefly, the roe was separated from the spiny shell and brown innards and ground. One portion was boiled in distilled water. Protein was extracted from manually homogenized raw and boiled roe by agitation in phosphate buffer saline–containing protease inhibitor cocktail without EDTA (Roche) and with 0.05% sodium azide overnight at 4ºC. The mixture was centrifuged at 3000 rpm for 10 minutes and at 12 600 rpm for 30 minutes at 4ºC. The pellets and supernatants were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Nupage 4-12% Zoom Gels; Invitrogen). The pellets showed the best protein discrimination and thus were used. Protein

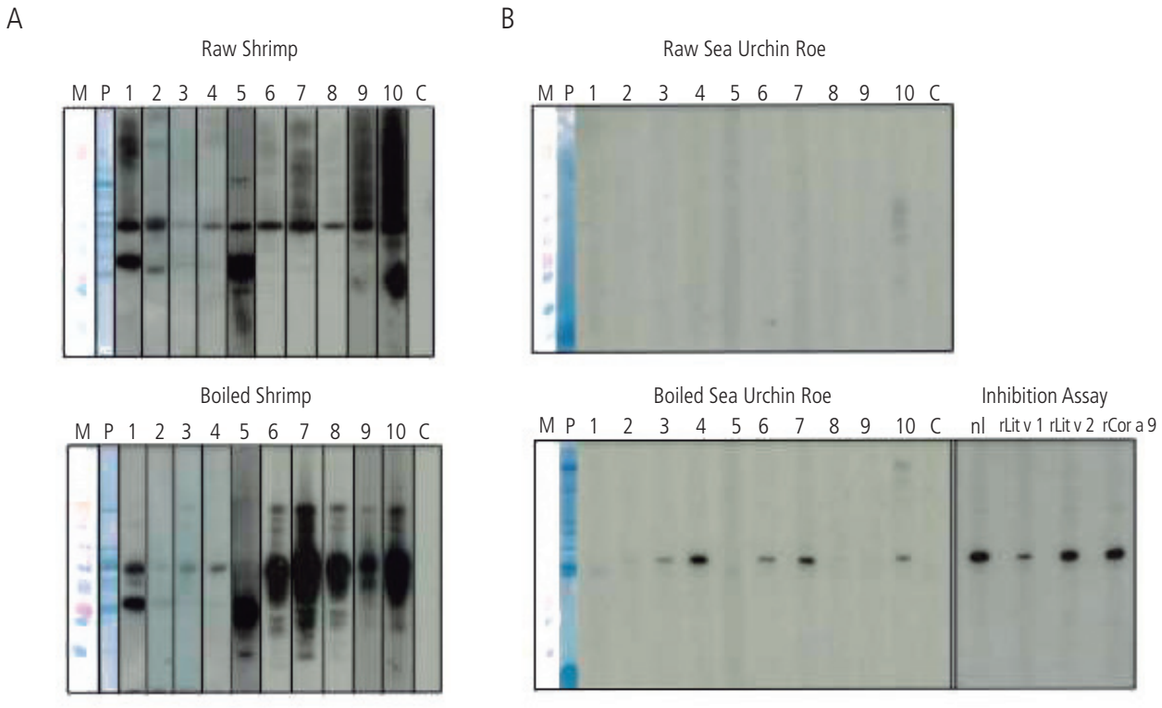

Figure. A, Immunoglobulin E immunoblotting with boiled and raw shrimp extracts. B, Immunoblotting with boiled and raw sea urchin roe extracts, and inhibition assay on boiled sea urchin roe extract. nl indicates noninhibited sera; M, molecular weight; P, protein staining.

concentration was determined with the Coomasie Plus Protein Assay (Pierce). The shrimp and roe proteins separated by SDS-PAGE were electrophoretically transferred onto Immobilon-P membranes (Millipore) [8]. After blocking, the membranes were incubated with sera from individual patients and a nonatopic control. Iodine 125–labeled goat anti-human IgE (DiaMed) was used as the secondary antibody. The membranes were exposed to Kodak Imaging Film (Carestream Health Inc) for 4 to 8 days.

All 10 individuals recognized multiple proteins in the shrimp extracts (Figure 1A), but only 6 showed IgE-binding to a 38-kDa protein in the boiled urchin roe extract. Just 1 individual showed faint recognition of a high molecular weight protein. Almost no protein was recognized in the raw urchin roe (Figure 1B). To identify the immunoreactive urchin protein, an inhibition assay was performed with pooled sera from 3 individuals who recognized the band. Tropomyosin and arginine-kinase, as shrimp allergens of a similar molecular weight that might be implicated in cross-reactivity, were used as inhibitors. The pool (1:20) was preincubated at room temperature for 2 hours with recombinant tropomyosin (Lit v 1), arginine-kinase (Lit v 2), and Cor a 9 as a control (100 ng/μL). Then IgE immunolabeling using a boiled sea urchin roe membrane was performed. Tropomyosin partially inhibited IgE binding (Figure 1B). Several isoforms of tropomyosin have been described in urchin eggs [9]. A tropomyosin-like protein of sea urchin (*Strongylocentrotus purpuratus*) (XP_001192266) showed only 22% sequence identity and 35% similarity with Lit v 1 (ACB38288.1). Such values are lower than those observed between mollusk and shrimp tropomyosins [1]. Since clinical cross-reactivity between shrimp and mollusks is estimated around 15%, for sea urchin it might be less.

Our study shows that some shrimp-allergic patients recognize 1 protein in boiled sea urchin roe and that this protein cross-reacts with shrimp tropomyosin. These patients, therefore, may be at risk of allergic reactions when consuming roe. Since most individuals did not recognize any proteins in raw roe, its ingestion may be safe. An oral challenge with boiled and raw forms of sea urchin roe would help to determine the clinical implications of these findings in shrimp-allergic subjects that wish to consume this food, especially in its boiled form.

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