Supplementary material

Oral food challenges with shrimp

Shrimp was obtained from the sea by fisherman, who were instructed to not add any additives and to place the shrimp at -20oC immediately after catching. Shrimp was kept at -20oC, and at the time of use, shrimp was thawed, had their shell removed, and boiled in water for 10 minutes. For the double-blind-placebo-controlled shrimp challenge, shrimp was crushed using a food processor, and added to a mixture of hazelnut cream, oat flakes, milk-based ice cream with small bits of chocolate, powdered chocolate and vanilla extract, containing 15.4 % of fat. Prior to challenge, patients were asked about any reactions to each of the components of the mixture. The cumulative dose of 1g shrimp protein was the maximum we could use to allow shrimp taste to be disguised in the food matrix. Placebo challenges were carried out using the same mixture minus shrimp and similar timing schedule. For the open challenge, cooked shrimp prepared as described for the DBPCFC was lightly salted and seasoned with olive oil. Active and placebo challenges were performed one week apart.

IgE Immunoblots

Natural Lit v 1 and recombinant Per a 7 were prepared in our laboratory according to previously published methods (Santos et al). Protein concentration of shrimp extract, Lit v 1 and Per a 7 was measured by Bradford assay, using Bovine Serum Albumin (BSA) as standard. Fifty micrograms of protein/lane were applied to 10% Sodium Dodecyl Sulfate Polyacrylamide Electrophoresis (SDS-PAGE) gels. Electrophoresis was carried out under reducing conditions with beta-mercaptoethanol. Protein transferring to nitrocellulose membranes (Millipore, Bedford, MA) was confirmed by Ponceau S staining. Membranes were blocked with Phosphate Buffered Saline (PBS) + 0.1% Tween + 5% skim milk (blocking solution) for 1 hour at room temperature. After

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washing, sera were added at 1:10 dilution in blocking solution, and membranes incubated overnight at 4ºC under agitation. Bound specific IgE was detected by peroxidase-conjugated goat anti-human IgE antibody (Kirkegaard and Perry, Gaithersburg, MD) diluted 1:5,000 in blocking solution, using enhanced chemiluminescence solutions (GE Healthcare) and the ChemiDoc Imaging System equipped with the ImageLab software (Bio-Rad Laboratories, Hercules, CA).

Reference

Santos AB, Rocha GM, Oliver C, Ferriani VP, Lima RC, Palma MS, Sales VS, Aalberse RC, Chapman MD, Arruda LK. Cross-reactive IgE antibody responses to tropomyosins from Ascaris lumbricoides and cockroach. J Allergy Clin Immunol. 2008 Apr;121(4):1040-6

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