

Contact urticaria caused by heat-sensitive raw fish allergens

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Patients allergic to fish usually present with skin reactions after handling raw fish. Less frequently, these reactions are seen without symptoms after oral intake, often in chefs and food handlers. We have attempted to explain the skin selectivity of such reactions in a 36-year-old woman with contact urticaria after handling raw fish. We obtained aqueous extracts of raw and cooked fish (sole and hake) for in vivo (prick test) and in vitro (SDS-PAGE, IgE Immunoblot) tests. Prick-by-prick test, 20-min closed patch test, rub test with fresh and cooked fish (sole, hake and cod) and specific IgE (CAP-system) to sole, cod and hake were performed. The strength of positive reaction to raw fish was greater than to cooked fish on both prick and prick-by-prick testing. Rub tests showed positive responses only to raw fish. Specific IgEs to sole (45 KU/l), hake (66.9 KU/l) and cod (18.7 KU/l) were obtained. IgE immunoblot recognized 3 antigens of 25, 48, 56 kDa in raw sole and 1 of 42 kDa in raw hake extracts. No IgE binding was observed with the cooked extracts or control sera. Our findings strongly suggest a Type-I hypersensitivity to fish. Immunoblot analyses demonstrated a loss of specific IgE binding to cooked extracts. We have reported a case of contact urticaria caused by heat-sensitive raw-fish allergens in a patient who probably became sensitized via the cutaneous route.

Key words: contact urticaria; food allergy; allergenic fish proteins; heat-sensitive allergens.
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Fish represents a large part of many people's diet worldwide. Fish consumption has increased because of its reported cardiovascular benefits (1). Fish are a common cause of food allergy. Such reactions usually result from ingestion, but skin contact reactions have also been reported (2–7), as well as respiratory symptoms through airborne exposure during the cooking of fish (8) or environmental exposure (9). Fish may be an inhalant or skin contact allergen, mainly in patients previously sensitized via the gastrointestinal route. However, skin reactions with good tolerance to fish ingestion are often seen in food handlers (2, 3, 10). Most allergenic food proteins are heat and acid stable, resistant to proteolysis and to digestion, with IgE binding to their primary amino acid structures. Fish allergens have similar properties, and allergic reactions to heat-sensitive allergens are not well characterized.

Patient and Methods

A 36-year-old woman, with a history of atopic dermatitis, developed contact urticaria after handling raw fish. She had had pruriginous, papular, erythematous eruptions on the hands and forearms immediately after skin contact with raw fish, which resolved 20 min after ceasing contact with no late reactions. These symptoms were mainly induced by sole, hake and codfish. The handling of cooked fish induced no reaction. She had no symptoms after oral intake of fish, which did not even increase her atopic dermatitis lesions.

Preparation of fish extracts

Raw and cooked (at +100°C for 15 min) fish species (sole and hake) were extracted in phosphate buffered saline (PBS) by mixing 4 g in 40 ml of

PBS (pH 7.3) at laboratory temperature. After it had been stirred for 30 min, the solution was centrifuged at 3000 g for 10 min. The supernatant was centrifuged again and the solution then passed through a Millipore filter (0.22 μ) for sterilization (Millipore, Molsheim, France), with a final dilution of 1/10 w/v. The protein concentration of the final extracts, as determined by turbidimetric analysis (Boehringer, Germany), was 32 mg/dl, 66 mg/dl, 11 mg/dl and 24 mg/dl to the raw sole, cooked sole, raw hake and cooked hake, respectively.

Prick tests

Prick tests were performed with the allergy pricker (Dome-Hollister-Stier), as described elsewhere (11). Histamine phosphate (10 mg/ml) served as positive control and NaCl (0.9%) as negative control. The tests were read after 15 min. We considered a positive reaction to have a diameter at least 3 mm more than the negative control. Prick tests with a series of commercially available common fish (cod, hake, salmon, tuna, trout) (Bial-Artegui, Bilbao, Spain) and our own raw and cooked fish extracts were performed. Prick-by-prick tests (which consisted of pricking the food with the lancet immediately before pricking the skin of the patient) with raw and cooked cod, hake and sole were also performed.

Rub test

The skin of the flexor surface of the patient's forearms was rubbed with raw and cooked sole and hake for 20 s. Readings were made 20 min later and at 6 h and 24 h following immediate response.

Closed patch test

20-min closed patch tests with small pieces of raw and cooked sole and hake were applied to the patient's upper back. After removal of the materials, the tests were read at 15 min, 30 min, 1 h, 6 h and 24 h.

Specific IgE determinations

Total IgE and specific serum IgE antibodies against sole, hake and codfish were measured by CAP-FEIA for commercially available allergens, according to the manufacturer's instructions (Pharmacia Diagnostics AB, Uppsala, Sweden).

SDS-PAGE and immunoblotting

Gel electrophoresis of raw and cooked sole and hake extracts was carried out in 10% polyacryl-

amide gels in the presence of SDS (Bio-Rad, Hercules, CA, USA), according to Laemli (12), using a SE250 Mighty Small mini-vertical Unit (Hoefer Scientific Instruments, San Francisco, CA, USA). The samples were reduced with β -mercaptoethanol (Sigma, St Louis, MO, USA) before electrophoresis. Approximately 40 μ g of either boiled or non-boiled samples were applied to each lane. Rainbow molecular weight markers (Amersham, Buckinghamshire, UK) were used. After the separation, 1 set of proteins was stained with Coomassie Brilliant Blue (Sigma, St Louis, MO, USA) and 1 set was transferred by electroblotting into PVDF sheets (Immobilon, Amersham), following the method of Towbin et al. (13). Unreacted membrane sites were blocked for 1 h with TBS containing 3% bovine serum albumin and 0.1% Tween 20 (TBS-T-BSA). The membranes were then incubated for 1 h at room temperature with patient or control serum diluted 1/50. Extensive washes were performed in TBS 0.1% Tween. Afterwards, alkaline phosphatase-conjugated goat anti-human IgE (Tago, Burlingame, CA, USA), 1/200 in TBS-T-BSA, was incubated for 1 h at room temperature. Finally, the reaction was developed with nitro blue tetrazolium: 5-bromo-4chloro-3indolyl phosphate (NBT-BCIP) as substrate.

Results

Prick tests were positive to all commercially available fish extracts tested. Prick tests with our own extracts showed stronger reactions to the raw fish than to the cooked fish, and became negative with cooked sole extract. Prick-by-prick tests showed similar positive reactions to raw and cooked sole, hake and codfish. Rub tests confirmed our findings, showing immediate positive responses without late reactions to raw fish (sole and hake) and negative responses to the same cooked fish. 20-min closed patch tests were all negative. Total IgE was >2000 Ku/l. Specific IgEs to sole (45 KU/l), hake (66.9 KU/l) and cod (18.7 KU/l) were found.

Coomassie-Blue staining after SDS-PAGE separation of the extracts is showed in Fig. 1. Whereas similar patterns can be observed for sole and hake in the 2 situations tested, a striking difference in antigenic composition is induced by cooking for both species of fish. The raw extracts of both species contain proteins of MWs ranging between 100 and 26 kDa, whereas the 2 cooked extracts consist mainly of higher molecular weight proteins (more than 60 kDa), except for a notable 28 kDa band in cooked hake. IgE immunoblotting (Fig. 2) showed specific recognition of several bands by the patient's IgE. In raw sole, bands of 26, 32, 34, 40, 48 (the most intense), 56, 58 and 85 kDa contrasted

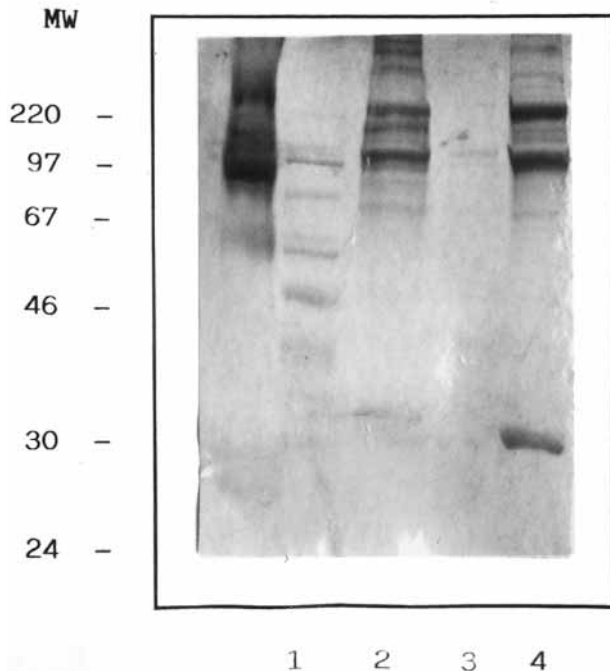


Fig. 1. Coomassie-Blue staining of a representative 10% SDS-PAGE gel after separation of the 4 fish extracts. Left lane: molecular weight standards (kDa). Lanes 1 and 2, raw and cooked sole respectively. Lanes 3 and 4, raw and cooked hake, respectively.

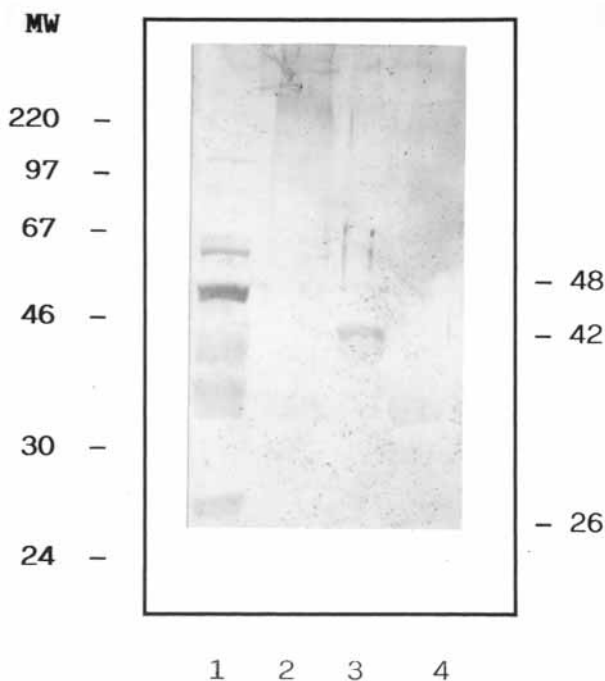


Fig. 2. IgE immunoblotting of the fish extracts after protein transfer to a PVDF membrane and incubation with the patient's serum and anti-IgE secondary antibody. Lanes 1 and 2, raw and cooked sole, respectively. Lanes 3 and 4, raw and cooked hake, respectively.

with the only intense band, of 42 kDa, in raw hake. A very slight reactivity with a band of 48 kDa can be also discerned, but faintly, in raw hake. In contrast, the cooked extracts showed a very weak reactivity of few bands which were also detected by the 2 control sera (not shown), indicating their non-specificity. There was no reactivity with secondary antibody alone (not shown).

Discussion

Fish constitute one of the main causes of allergic reactions to food, in adults as well as children (14, 15). Generally, the allergenic determinants of fish are heat-resistant, as reported in many studies (16–18). A clear example is that of cod major allergen, GadC1, a 12.3 kDa parvalbumin which is supposedly responsible for the cross-reactivity observed between different fish species (17, 18).

The vast majority of allergic reactions to fish occur after its ingestion, as with any other food allergen. However, fish allergens are ubiquitous and their presence has been demonstrated in house dust (19) as well as in open-air samples (20). Thus, fish may be either an aeroallergen or a contact allergen. In these 2 situations, the majority of patients have previously been sensitized via the gastrointestinal route, with a history of allergic reactions after fish intake. Many fish-allergic children show systemic symptoms after inhaling fish particles (21, 22) or after skin contact with fish allergens (23). These alternative routes might constitute a permanent and unknown source of exposure to fish allergens, and could explain the persistence of fish sensitization to an adult age in spite of strict avoidance of fish consumption.

Contact urticaria constitutes a common reaction in fish-allergic patients (6, 7, 23). However, there are cases of exclusive cutaneous involvement, often in food handlers and chefs (3, 10, 24). In these workers, fish represents one of the most important allergens and sensitization is effected through the skin by protein allergens. It appears that in contact urticaria to fish, the relevant antigens would be heat-labile, since the cooking of the fish results in decreased reactions in skin tests (25, 26).

We show a molecular basis for these observations, by *in vitro* assessment of the IgE reactivity to raw and cooked fish in our patient. In our case, the patient had cutaneous symptoms only in areas of contact with raw (uncooked) fish, with a good tolerance to its ingestion and with no respiratory symptoms due to inhalation during handling. The *in vivo* and *in vitro* tests that we have performed demonstrate a Type-I hypersensitivity as the underlying cause of contact urticaria from fish in our patient.

Besides, the near-loss of antigen recognition of the cooked extracts by our patient's serum IgE (as shown by immunoblotting) correlates well with the reduced allergenicity of these extracts in vivo (prick, prick-by-prick, rub tests). Cooking both fish before antigenic extraction induces a loss of medium-sized antigens (the ones that are allergenic) and the appearance of higher MW antigens which are not allergenic. Additionally, the allergenic epitopes are not determined by any conformation involving disulfide bonds, since β -mercaptoethanol did not alter IgE recognition. Thus, the epitopes are conformational but not dependent on SH₂ groups. The slight reactivity against a 48 kDa band in hake might explain some cross-reactivity with the equivalent band in the sole extract.

We show our patient recognizes heat-labile allergens. This is unusual, since most patients allergic to fish recognize heat and denaturation resistant allergens and have symptoms after oral ingestion (27, 28). It has been reported that antigens between 40 and 90 kDa are mainly heat-sensitive (28), in accordance with the antigens recognized by our patient's IgE. Our study suggests that the primary sensitization route might have been via the skin, since the allergenicity of the fish, for this particular patient, is greatly reduced after cooking and she does not consume raw uncooked fish.

In summary, we demonstrate that the reason for isolated contact urticaria without allergic symptoms after oral intake in this patient, is a Type-I hypersensitivity to heat-sensitive fish allergens.

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