Allergy to fish parvalbumins: Studies on the cross-reactivity of allergens from 9 commonly consumed fish

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Background: Fish-hypersensitive patients can probably tolerate some fish species while being allergic to others.
Objective: To determine the allergenic cross-reactivity between 9 commonly edible fish: cod, salmon, pollack, mackerel, tuna, herring, wolffish, halibut, and flounder.
Methods: Sera from 10 patients allergic to fish and rabbit antisera against 3 parvalbumins (Gad c 1, Sal s 1, and The c 1) were used. Cross-reactivity was investigated by SDS/PAGE and IgE immunoblotting, IgG ELISA, IgE ELISA inhibition, and skin prick test (SPT).
Results: Cod (Gad c 1), salmon (Sal s 1), pollack (The c 1), herring, and wolffish share antigenic and allergenic determinants as shown by immunoblots and IgE ELISA, whereas halibut, flounder, tuna, and mackerel displayed lowest cross-reactivities. The highest mean IgE ELISA inhibition percent of 10 sera was obtained by Gad c 1, followed by The c 1, herring, Sal s 1, wolffish, halibut, flounder, tuna, and mackerel with the least inhibition. Nine of the 10 patients showed positive SPT to cod, salmon, and pollack; 8 patients reacted to recombinant (r) Sal s 1. Positive SPTs to rGad c 1 and rThe c 1 were demonstrated in 1 patient.
Conclusion: Gad c 1, Sal s 1, The c 1, herring, and wolffish contained the most potent cross-reacting allergens, whereas halibut, flounder, tuna, and mackerel were the least allergenic in the current study. The latter could probably be tolerated by some of the tested patients. (J Allergy Clin Immunol 2005;116:1314-20.)

Key words: *Fish allergy, cross-reactivity, parvalbumin, recombinant allergen, cod, pollack, salmon*

Abbreviations used
DBPCFC: Double-blind, placebo-controlled food challenge
r: Recombinant
SPT: Skin prick test

Fish plays an important role in the human food, providing a valuable source of highly assimilated proteins, but it is also among the most common causes of food allergy.[1-3] Atopic allergy to fish is particularly common in children and young adults. The clinical symptoms related to fish allergy might be manifested in a variety of symptoms (eg, urticaria, allergic contact dermatitis, rhinoconjunctivitis, asthma, oral allergy syndrome, diarrhea, or anaphylaxis). Fish hypersensitivity is frequently encountered in coastal countries like Norway, where considerable numbers of the population work in the fish industry, and fish is constantly consumed. Exposure to fish allergens can be through inhalation of airborne allergens during outdoor drying, skin contact while filleting and cooking fish, or ingestion of fish meals. Fish allergy has been reported to occur in about 0.1% of the Norwegian population.[1] Most of the patients allergic to fish do not tolerate cod; therefore, this is usually used as reference to which other fish allergens are related. Codfish hypersensitivity has been extensively studied, and the major allergen Gad c 1 (allergen M) has been found to be a parvalbumin.[4-8] Fish muscle parvalbumin is a stable acidic Ca^{2+} binding protein (12 kd), resistant to heat, chemical denaturation, and proteolytic enzymes.[9-11] Parvalbumins are present in high amounts in white muscles of lower vertebrates[12] and in lower amounts in fast twitch muscles of higher vertebrates.[13] It has been demonstrated that parvalbumin is present in white muscle of many fish species; thus, cross-reactivity among different fish species might exist.[1,3,11,14-17] However, patients allergic to codfish can ingest some other species without risk of allergic symptoms, as shown by some previously reported studies.[15,16,18-20] In the current study, the cross-reactivity between Gad c 1 parvalbumin and 8 of the most commonly edible fish species in Norway was examined by several *in vitro* assays and skin prick test (SPT).

METHODS

Patients with fish allergy and controls

Twelve patients were recruited from the ambulant patients routinely examined at the Laboratory of Clinical Biochemistry and

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TABLE I. Summary of clinical histories and laboratory data*

Patient no.	Age	Sex	Symptoms	Total serum IgE	Specific IgE CAP FEIA, kU/L				
					Gad c 1	Sal s 1	Tuna	Herring	Mackerel
1	43	F	Anaphylaxis	56	10.30	12.90	4.42	20.40	2.88
2	33	F	Anaphylaxis	121	2.52	1.33	0.38	9.86	<0.35
3	42	F	Anaphylaxis	159	0.52	0.50	<0.35	0.95	<0.35
4	37	F	Anaphylaxis	67	1.39	<0.35	<0.35	<0.35	<0.35
5	27	M	Oral allergy syndrome, contact urticaria	79	13.80	22.20	7.45	19.00	4.29
7	25	M	Anaphylaxis	134	0.95	0.79	0.45	1.55	<0.35
8	21	F	Oral allergy syndrome	487	13.20	8.75	4.97	14.10	3.56
9	55	F	Anaphylaxis, oral allergy syndrome, contact urticaria	1712	>100	78.20	25.20	>100	17.10
11	53	F	Anaphylaxis, contact urticaria	86	4.46	10.90	0.95	7.89	1.43
12	30	F	Abdominal pain, flushing, dyspnea	308	5.18	3.04	1.08	6.50	0.85

*Specific IgE CAP-FEIA expressed in kU/L. All of the patients have IgE-mediated allergy.

the Centre for Occupational and Environmental Allergy, Haukeland University Hospital, Bergen, Norway. Two of them were excluded from this report because they did not show IgE-mediated allergy to fish. Ten patients, 8 women and 2 men, age 21 to 55 years, had histories of generalized or anaphylactic reactions after intake of cod on at least 2 occasions. The clinical data supporting allergy and serum total and specific IgE values as well as the CAP-FEIA classes are given in Table I. Seven have histories of generalized anaphylaxis after ingestion or direct contact with fish fillet and cooking fish. Five patients have variable gastrointestinal tract manifestations after fish meals. Ten patients have relatively high values of serum total (mean 312 kU/L) and specific IgE for cod and salmon. One patient (#9) strongly reacted to tuna and mackerel; 3 (#1, 5, and 9) strongly reacted to herring. Patient #9 has very high total and specific IgE against 5 fish species and intensive IgE-mediated anaphylactic response to most of the fish sorts tested. No double-blind, placebo-controlled food challenge (DBPCFC) could possibly be performed on the tested population because of an inherent risk of anaphylaxis. Ten control subjects (tolerating fish) were included. Informed consent was obtained from each volunteer, and the study was approved by the Regional Committee for Medical Research Ethics in Western Norway (REK Vest).

Rabbit IgG

Rabbit polyclonal antibodies against Gad c 1, Sal s 1, and The c 1 parvalbumins were usually raised in rabbits at the University of Bergen, Animal House (Vivarium), by the methods described previously.[7,21,22]

Preparation of fish extracts and fish parvalbumins

Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), Atlantic mackerel (*Scomber scombrus*), tuna (*Thunus albacares*), herring (*Clupea harengus*), wolffish (*Anarhichas sp*), halibut (*Hippoglossus hippoglossus*), and flounder (*Platichthys flesus*) were purchased from the fish market in Bergen. Alaska pollack (*Theragra chalcogramma*) was purchased as frozen fillet produced by FRoSTA AG, Bremenhaven, Germany. Fish extracts and parvalbumins were obtained by using update laboratory instrumentation and methodology by the methods classically described elsewhere.[4,23]

Recombinant fish parvalbumins

The production of the recombinant (r) parvalbumins rGad c 1, rSal s 1, and rThe c 1 has been described previously.[21,24,25]

IgG ELISA

ELISA was performed by using highly purified polyclonal rabbit IgG.[22] Briefly, 96-well, flexible round bottom microtiter plates (Dynatech Laboratories Inc, Chantilly, Va) were coated with 0.5 μg Gad c 1 in 100 μL buffer, pH 9.5. Coating was performed overnight at 4°C. This was followed by washing (Tris-Tween buffer, pH 7.4), and purified polyclonal IgG against Gad c 1, Sal s 1, and The c 1 (diluted 1.10^{-4}) was added and incubated for 2 hours at room temperature. After another washing, antirabbit IgG alkaline phosphatase conjugate (Sigma Chemical Co, St Louis, Mo) was used for incubation for 2 hours. Finally, after another wash, the color was developed by incubation with 100 μL/well Tris buffer pH 9.5, containing 1 mg/mL *p*-nitrophenylphosphate (Sigma). Absorbance was read at $\lambda = 405$ nm after 10 minutes.

IgE ELISA inhibition

IgE ELISA inhibition was performed as described previously.[21,24,25] Briefly, plates were coated with 1 μg Gad c 1 (100 μL/100 mmol/L sodium bicarbonate buffer, pH 9.6). Patients' sera (50 μL) were inhibited by incubation with 100 μg/100 μL Gad c 1, Sal s 1, and The c 1 parvalbumins, or purified allergen of halibut, mackerel, herring, wolffish, flounder, tuna, and rGad c 1, rSal s 1, and rThe c 1.

SDS-PAGE and specific IgG/IgE–immunoblotting

Fish extracts were separated by SDS-PAGE. The samples along with molecular weight standards were resolved in a 15% separating gel at 200 V. Proteins were visualized by Coomassie brilliant blue R-250 staining (Sigma). For immunoblot analyses, proteins were transferred onto nitrocellulose membranes (0.45 μm) using a minitrans-blot cell (BIO-RAD Laboratories, Richmond, Calif) for 1 hour at 100 V. Immunodetection of cross-reactivities between allergens was performed with the serum pool of patients allergic to fish or polyclonal rabbit IgG. After antibody binding, the color reaction was developed with SIGMA FAST BCIP/NBT tablets (Sigma).[21,25]

SPT

SPTs were performed in duplicate according to the guidelines of the European Academy of Allergology and Clinical Immunology Subcommittee on skin tests[26] with native and recombinant Gad c 1, Sal s 1, and The c 1 (1 mg/mL), dissolved in sterile physiological saline solution. Reactions were recorded after 15 minutes by