

Fish hypersensitivity

I. In vitro and oral challenge results in fish-allergic patients

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The purpose of this study was to determine whether patients allergic to one fish species can safely eat other fish species. Eleven atopic, food-allergic children and young adults with histories consistent with IgE-mediated fish hypersensitivity were skin prick tested to 10 fish species. Skin prick tests (SPTs) were positive to all 10 fish in eight of the 11 patients, and the remaining three patients had at least two positive fish SPTs. Positive oral challenges occurred to only one fish in seven of the patients, to two fish species in one patient, and to three fish species in two patients. One patient did not react to any of the fish tested. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot analyses were performed on raw and cooked protein extracts from nine of the 10 fish species used in SPTs. Several protein bands in the raw-fish extracts appeared to denature with cooking and form high molecular weight conglomerates. Immunoblot analyses with sera from documented fish-allergic patients demonstrated specific IgE binding to protein bands from fish to which patients were not clinically allergic, as determined by oral challenge. In ELISA-inhibition assays, the concentration of fish antigen required to achieve 50% inhibition was similar for fish to which the patients were clinically allergic as compared to fish to which they were clinically tolerant. SPT and in vitro evidence of IgE-specific cross-reactivity does not necessarily correlate with symptomatic fish allergy. In addition, these fish-hypersensitive patients were able to consume one or more other fish species without adverse allergic reactions. (J ALLERGY CLIN IMMUNOL 1992;89:730-7.)

Key words: Fish allergy, food hypersensitivity, double-blind placebo-controlled oral food challenges, immunoblot

Fish consumption is increasing in the United States. Fish are being fresh frozen and flown rapidly to cities far from the sea, allowing wide selections to appear nationwide on menus and in markets. Americans con-

Abbreviations used

SPT:	Skin prick test
DBPCFC:	Double-blind, placebo-controlled oral food challenge
SDS–PAGE:	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
PBS:	Phosphate-buffered saline
MW:	Molecular weight

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sumed 10.3 pounds of fish and other seafood per person per year in 1960, whereas the most recent statistics from 1990 indicate that this consumption has increased to 15.5 pounds of fish and other seafood per person (U.S. Department of Commerce, National Marine Fisheries Statistics. Personal communication.). Many physicians are recommending that patients at increased risk for ischemic heart disease increase their consumption of fish high in eicosapentaenoic acid be-

cause of its reported benefit in reducing coronary artery disease and because of a reduction in mortality among survivors of myocardial infarction who consume a moderate intake of fatty fish.^{1,2}

Unfortunately, fish is also a common cause of immediate food allergy in both the pediatric and adult populations. This problem has been more prevalent in Scandinavian countries in which large quantities of fish have been consumed for many generations. In Finland it was estimated that 3% of 3-year-old children were fish allergic,³ and fish antigens are so ubiquitous that they are found in house dust samples.⁴ Allergists and other practitioners generally recommend restricting a patient's diet of all edible fish, including both Osteichthyes (bony fish) and Chondrichthyes (sharks) when the patient has a history of an allergic reaction to any fish or has a positive RAST or SPT to a fish extract. However, a lack of consensus about this advice and recommendation has existed for at least 50 years.⁵⁻⁹

For a variety of reasons, many of our patients with previous histories of allergic reactions after ingesting fish were interested in eating fish again. In view of our earlier findings that multiple intrabotanical allergy among food families, including the legumes, is rare,¹⁰ a reappraisal of the traditional practice of total fish elimination diets appeared warranted. Furthermore, there is literature that suggests that total fish exclusion diets may be unnecessary. A recent retrospective study by de Martino et al.¹¹ found that some children with a history of codfish allergy had consumed other fish species without reaction. Another earlier retrospective study by Aas⁵ described 61 cod-allergic children, 34 of whom reported allergy to all fish, but 27 of whom reported that they had eaten one or more other fish species without difficulty.

MATERIAL AND METHODS

Challenge subjects

Eleven patients with histories of fish hypersensitivity were enrolled in the study. The patients ranged in age from 6 to 20 years (median, 11.2 years). All 11 patients were highly atopic; all patients came from atopic families, had asthma, allergic rhinitis, and atopic dermatitis and were previously documented by blinded challenge to be food allergic. SPTs with raw fish extracts of catfish, bass, perch, mackerel, tuna, salmon, trout, codfish, flounder, and sardine (1:20 wt/vol, Greer Laboratories, Lenoir, N.C.), histamine control (1 mg/ml), and saline control were applied to the forearms or backs of the patients by the prick method on the day of admission. The fish represented seven families and six orders of bony fish (Osteichthyes). The mean diameters of the wheal-and-flare lesions were recorded; a mean wheal at least 3 mm larger than wheal of saline control was considered positive. All tests were applied by means

of sterile bifurcated needles (Allergy Laboratories of Ohio, Columbus, Ohio), and measured by the same nurse-clinician.

Control subjects

SPTs with the 10 raw-fish extracts, histamine control, and saline control were applied to three groups of control subjects: 10 atopic food-allergic but nonfish-allergic patients, 10 atopic nonfood-allergic patients, and 10 nonatopic patients.

Procedures

Patients were admitted to The Johns Hopkins Pediatric Clinical Research Unit for double-blind, placebo-controlled oral food challenges and open oral challenges to four to six fish species. Because there are hundreds of commercially available fish species, species selected for oral challenge were primarily determined by patient and family preference and individualized local availability. Briefly, as previously described,¹² two double-blind challenges were performed each day containing 2 ounces (uncooked weight) of cooked, lyophilized fish or placebo substitute (blinded with Nutramigen; Bristol-Myers, Evansville, Ind., first five patients; blinded with lyophilized canned tuna, last six patients) in 100 ml of juice. Canned tuna was substituted for Nutramigen to create a better placebo after canned tuna was demonstrated in vitro to have decreased allergenicity. Randomization was performed by the research unit dietitian with a computerized program. The challenge liquid was administered during a 1-hour period in gradually increasing doses until symptoms developed or the entire amount was ingested. All negative-blinded challenges were confirmed by open challenge with 4 ounces of the cooked fish before discharge. After the second patient who entered into the study had a false-negative DBPCFC, the quantity of fish in the blinded challenge was increased to 3 ounces (uncooked weight) in 150 ml of juice. Symptoms were recorded on a standardized symptom-score sheet. All procedures were approved by The Johns Hopkins University School of Medicine Joint Council on Clinical Investigation (IRB).

Preparation of extracts

Ten grams of raw and cooked fish species (catfish, bass, perch, mackerel, tuna, salmon, trout, codfish, and flounder) were added separately to 40 ml of PBS in sterile centrifuge tubes with a mixing glass marble. The tubes were placed on a test tube rotator overnight in a cold room (4°C). The samples were centrifuged at 1250 g for 15 minutes and then clarified at 16,000 g for 15 minutes. The supernatants were removed and lyophilized. Protein determination of the nine raw and nine cooked lyophilized fish extracts were performed with the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.).

SDS-PAGE

Physicochemical properties of the various raw and cooked fish extracts were examined by SDS-PAGE, as previously described.¹³ Briefly 25 µg of each fish extract was

TABLE I. Fish SPT results in 11 patients

	No. patients with positive SPTs	Wheal of positive SPT (mm)	
		Median	Range
Catfish	9	16	4-22
Bass	9	12	3-23
Perch	9	12	9-26
Mackerel	8	10	6-26
Tuna	9	6	4-11
Salmon	9	10	4-19
Trout	9	13	5-18
Codfish	9	16	4-40
Flounder	9	11	4-17
Sardine	8	6	4-8

loaded into separate gel lanes and resolved in a discontinuous SDS-PAGE system.¹⁴ Proteins or protein subunits were electrophoretically transferred from the gels onto nitrocellulose paper at room temperature with 0.15 A in 50 mm/L of Tris-glycine buffer (pH 9.1) containing 20% methanol. The blots were either stained with a 0.1% amido black to define specific protein bands and to assure adequate transfer or blocked in PBS with 0.5% porcine gelatin and 0.05% Tween 20. As described previously,¹³ the blocked nitrocellulose blots were probed with a 1:25 dilution of sera from fish-allergic patients or control sera to determine antigen-specific IgE binding to the fish-protein bands. Bound human IgE was identified with absorbed, affinity-purified, biotinylated goat antihuman IgE (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.). The blots were then reacted with an enzyme horseradish peroxidase-avidin-D solution (Vector Laboratories, Inc., Burlingame, Calif.) and developed.

IgE ELISA-inhibition assay

Immunochemical analysis of the fish proteins were performed by an ELISA-inhibition assay. A cooked fish-protein extract was diluted in carbonate buffer (pH 9.6) to coat 500 ng of protein per well with a 96-well microtiter plate (Immunolon plates; Dynatech Laboratories, Inc., Chantilly, Va.), and the plates were incubated overnight at 4° C. The plates were washed with PBS-Tween and blocked for 1 hour at room temperature with 1% human serum albumin in PBS-Tween. The inhibition assay was performed by pipetting a fixed dilution (1:10 to 1:25) of fish-allergic patients' sera into several parallel series of Eppendorf (Netheler Hinz Gmb-H, Hamburg, Germany) tubes containing tenfold serial dilutions of a cooked fish extract from 500 µg/ml to 5 pg/ml. The antigen-antibody mixture was then incubated at 37° C for 2 hours. Cow's milk whey was used as the negative control protein antigen.

One hundred microliters of the antigen (fish extract)-antibody (sera) solution was added to microtiter wells previously coated with fish antigen, blocked, and incubated for 2 hours at 25° C. The plates were washed with PBS-Tween

and then incubated with a 1:1600 dilution of absorbed affinity-purified biotinylated goat antihuman IgE for 2 hours at 25° C. The washed plates were then reacted with horseradish peroxidase-avidin D enzyme solution for 30 minutes. After the wash step was repeated, the plate was developed and read on a kinetic microplate reader (Molecular Devices Corp., Mento Park, Calif.).

Statistical analysis

The sensitivity, specificity, and predictive accuracies were determined as previously described.⁹

RESULTS

SPTs to all 10 raw fish extracts (catfish, bass, perch, mackerel, tuna, salmon, trout, codfish, flounder, and sardine) were positive in eight of 11 patients. One patient had positive SPT to catfish, bass, and flounder; one patient had positive SPT to tuna, salmon, and codfish; and one patient had positive SPT to perch and trout. SPTs to the fish extracts were negative in the three control groups (Table I).

Oral challenge results

Each patient consumed four to six different fish species from at least four different fish families. A total of 41 DBPCFCs and nine primary open fish challenges were performed. All nine open challenges were negative. Eight of the 41 blinded challenges were positive and 33 were negative (Table II). These 33 negative-blinded challenges were followed by open challenges, seven (21%) of which were positive. The false-negative blinded challenges occurred in six of the 11 patients, involved different fish species (perch, salmon, codfish, flounder, and mahi mahi), and did not appear to be secondary to an insufficient quantity of fish antigen being consumed in the blinded challenge since symptoms often appeared after the first or

TABLE II. Results of the 50 oral fish challenges in 11 patients

Pts	Primary open challenge	No. of DBPCFCs	No. neg DBPCFCs	No. pos DBPCFCs	False-neg DBPCFCs	Total No. pos challenges
1	0	4	3	1	0	1/4
2	0	4	3	1	0	1/4
3	0	4	4	0	1	1/4
4	2 (neg)	3	2	1	0	1/5
5	2 (neg)	4	3	1	0	1/6
6	0	4	4	0	1	1/4
7	1 (neg)	4	4	0	1	1/5
8	1 (neg)	4	4	0	2	2/5
9	0	4	2	2	1	3/4
10	0	4	2	2	1	3/4
11	3 (neg)	2	2	0	0	0/5
Total	9	41	33/41 (80%)	8/41 (20%)	7/33 (21%)	15/50 (30%)

Pts, Patients; neg, negative; pos, positive.

TABLE III. Fish Challenge results in 11 patients by specie, symptom and corresponding skin test*

	1	2	3	4	5	6	7	8	9	10	11
Catfish											Neg
Black bass											Neg
Perch				OR/L				Neg	SK†		
Mackerel											
Tuna	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Salmon		OR/SK	OR/GI†		Neg		Neg	Neg			Neg
Trout	Neg			Neg	Neg	Neg	Neg		OR		
Codfish	OR/GI		Neg	Neg	Neg	Neg		OR†	OR		Neg
Flounder	Neg	Neg	Neg	Neg	OR/SK†	OR/SK†	OR/SK		R†	OR/SK	
Sardine		Neg			Neg						
Kipper								Neg			
Red snapper										OR	
Mahi mahi										OR†	

SK, Skin: urticaria, angioedema, pruritus, erythema; R, wheezing; GI, nausea, vomiting, diarrhea; L, laryngeal: tight and itchy throat;

OR, oral pruritus, lip angioedema, or hives.

*Boldfaced results correspond to a positive SPT.

†False-negative DBPCFC.

second bite in the open challenge. Overall, 15 of 50 oral challenges were positive. Positive oral challenges occurred to only one fish in seven of the patients, to two fish species in one patient, and to three fish species in two patients. One patient did not react to any of the fish tested. Overall, 26 negative oral fish challenges had corresponding positive SPTs (Table III).

Compared to the oral challenges, the overall sensitivity of the fish SPT was 100% and the specificity was 26%. (Only nine of the 35 negative challenges had a negative SPT.) The positive predictive accuracy was 37% (only 15 of the 41 positive SPTs resulted in

a positive oral challenge), and the negative predictive accuracy was 100%.

Gel results

The protein bands ranged in relative MW from >115 kd to <14 kd in each of the nine raw and nine cooked fish extracts (Fig. 1). With the exception of the raw and cooked tuna, all extracts had a prominent band migrating at approximately 13 kd that was believed to represent a protein fraction analogous to codfish Gad C1, known previously as allergen M. Seven of the nine fish species had a prominent band at ap-

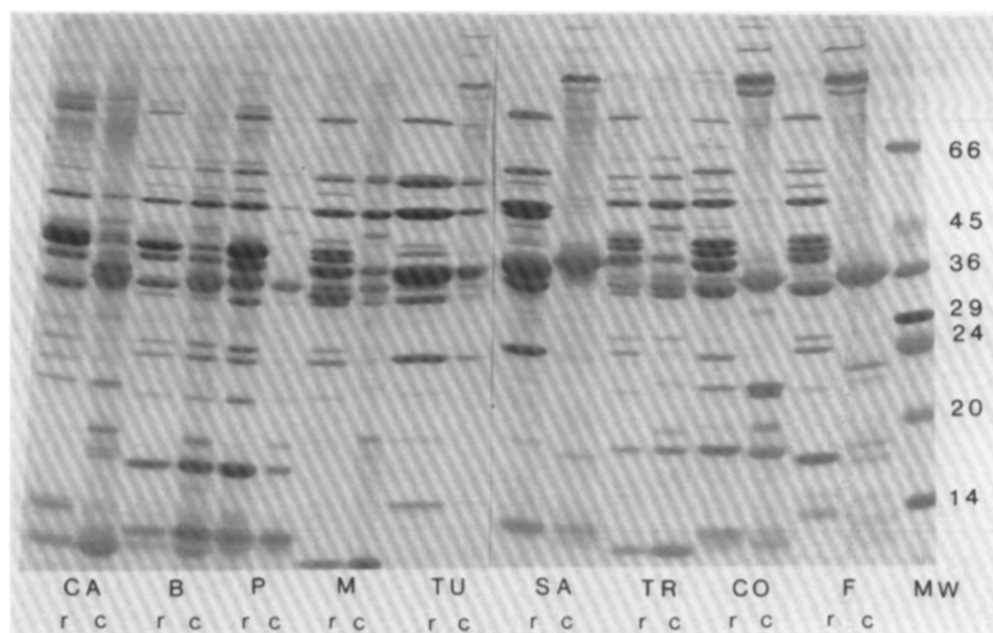


FIG. 1. Amido black-stained 15% SDS-PAGE of nine raw and nine cooked fish extracts; *r*, raw; *c*, cooked; *CA*, catfish; *B*, bass; *P*, perch; *M*, mackerel; *TU*, tuna; *SA*, salmon; *TR*, trout; *CO*, codfish; *F*, flounder; *MW*, molecular weight markers.

TABLE IV. The number of protein bands in each fish extract by SDS-PAGE

	Raw	Cooked
Catfish	29	24
Bass	29	24
Perch	28	15
Mackerel	26	21
Tuna	22	18
Salmon	29	21
Trout	25	20
Cod	25	23
Flounder	24	21

proximately 20 kd in the cooked, but not the raw, lanes (exceptions tuna and salmon). Four cooked fish extracts (tuna, salmon, codfish, and flounder) formed high (>115 kd) MW complexes that were not present in the raw fish lanes. The protein bands in the MW range of 40 kd to 90 kd appeared especially sensitive to heat denaturation, and many such bands present in the lanes containing raw fish were not found in the lanes containing cooked fish (Table IV).

Immunoblotting studies with the nine raw and nine cooked fish extracts were performed with sera from patients Nos. 1 through 6 (Table III). All the patients possessed IgE antibodies specific to various fish-protein fractions. The most prominent band that the IgE-

specific antibodies were directed against was the fraction that migrated at approximately 13 kd. This immunodominant fish protein appeared to be Gad C1 or its equivalent in other noncodfish species. Representative fish immunoblots from patients Nos. 3 and 5 who were allergic to salmon and flounder, respectively, are illustrated in Fig. 2. There is nonspecific binding of the goat antihuman IgE to two high MW bands and one band with a relative MW of 45,000 daltons in each fish extract. The nonspecific binding bands are indicated by *arrows*. There was no nonspecific binding to bands with relative MWs <45 kd. Sera from the five patients who had positive SPTs to all fish tested recognized the 13 kd protein in the fish extracts. Interestingly, sera of patient No. 4, who had positive skin tests only to perch and trout and was clinically allergic only to perch, did *not* have IgE binding to the 13 kd proteins. Sera from a nonatopic control patient and an atopic, nonfood-allergic patient with an IgE of 18,000 ng/ml had no detectable IgE antibodies to the fish-protein fractions.

ELISA-inhibition assays were performed with extracts from a fish that provoked an allergic reaction, a fish from the same order, and a fish from a different order to which the patient had previously demonstrated tolerance. The concentration of the different fish proteins required to achieve 50% inhibition were similar, and the assays had comparable inhibition curves. In addition, clinically nonallergenic fish extracts were

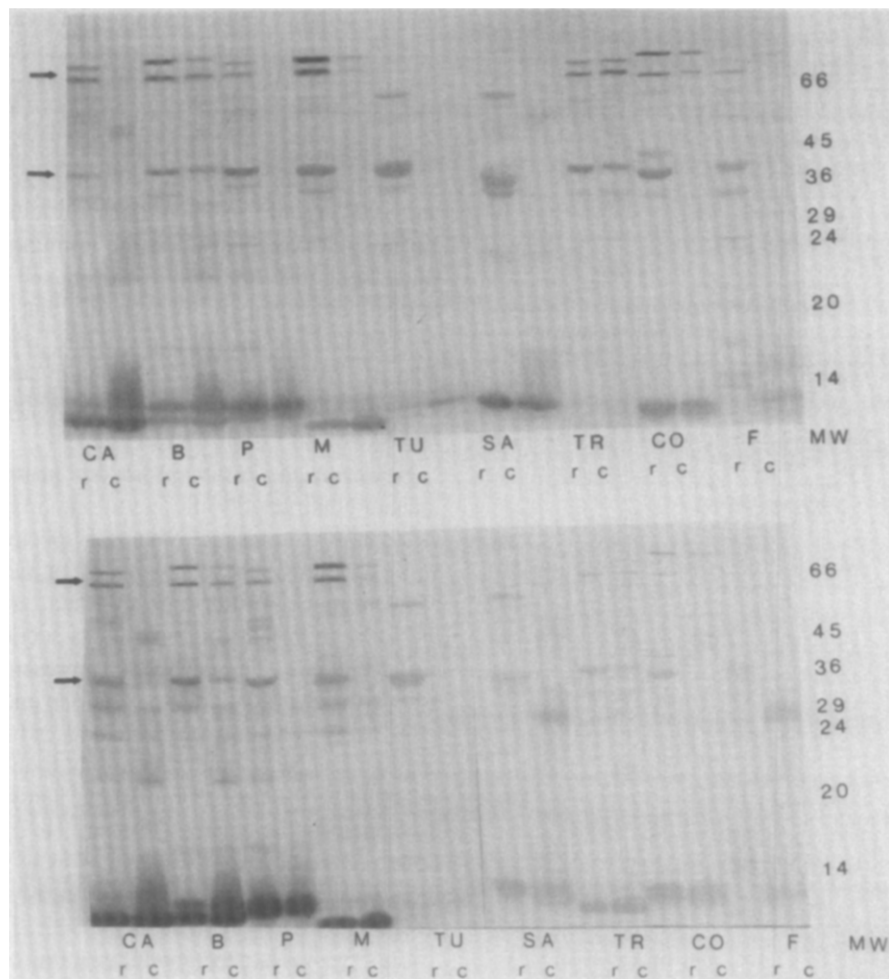


FIG. 2. Immunoblots of the nine raw and nine cooked fish extracts developed for IgE-specific binding. The **upper blot** is probed with sera from a salmon-allergic patient, and the **lower blot** with sera from a flounder-allergic patient.

able to achieve 100% inhibition in these assays. No inhibition was observed with an unrelated protein (whey). Representative IgE ELISA-inhibition curves from two salmon-allergic patients (Nos. 2 and 3) are illustrated in Fig. 3.

DISCUSSION

Fish consumption is growing in popularity in the United States (U.S. Department of Commerce, National Marine Fisheries Statistics. Personal communication.). However, fish is a frequent cause of adverse food reactions: toxic, infectious, and allergic. Scombrotoxic, or "histamine poisoning," from improperly handled fish (primarily tuna and mackerel) is frequently confused with IgE-mediated reactions. Physicians should be aware of the differences to avoid mislabeling a patient as fish allergic.^{15, 16} When proper diagnosis is assumed, the traditionally advocated pro-

cedure has been avoidance of all fish as soon as clinical hypersensitivity to any fish is suspected or proven.

There are no prospective studies in the literature on the species specificity of fish allergy, and there is a general lack of agreement in published articles on this issue. The earliest thorough discussion on the topic was by DeBessche⁶ in 1937, describing "fish asthma" in patients who wheezed after eating fish or breathing fish odors. DeBessche⁶ believed that most fish-allergic patients were allergic to all fish but that there were clearly patients who had species-specific fish allergy and other patients who reacted to fish odor but did not necessarily react to ingestion of the same fish. DeBessche⁶ also noted the common occurrence of clinically irrelevant positive fish skin tests. In 1940, Tuft et al.,⁷ using skin-testing data in fish-allergic patients, concluded that a common antigen existed among the various members of the true fish family and that this

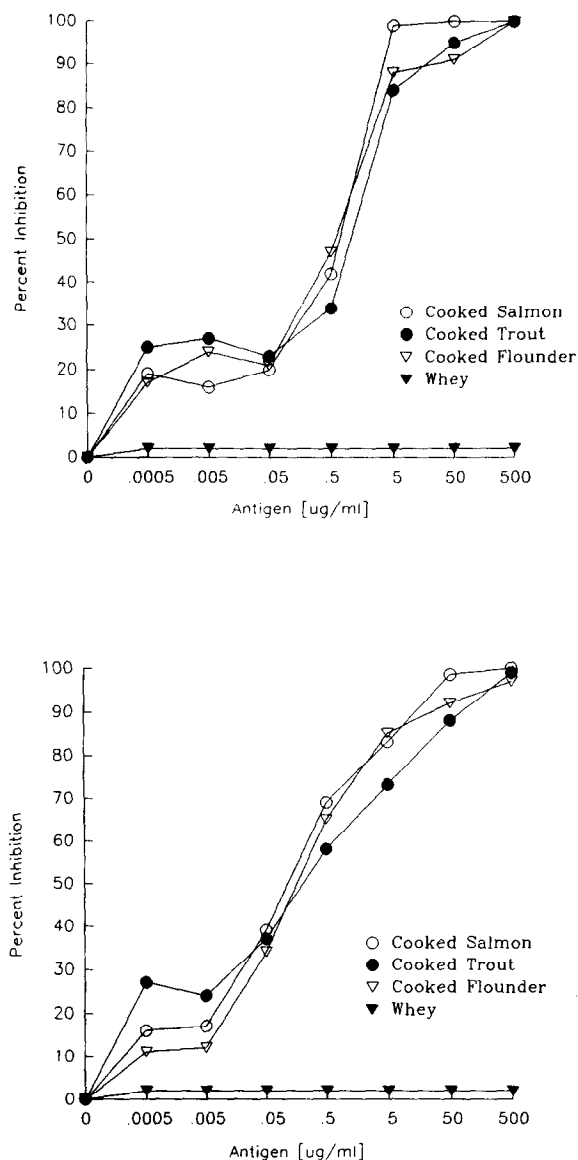


FIG. 3. IgE ELISA-inhibition assays of cooked salmon, trout, and flounder extracts and cow's milk whey performed separately with sera from two salmon-allergic patients.

common fish antigen explained the observation that fish-allergic patients are usually clinically sensitive to all fish.⁷ With a guinea pig sensitization/anaphylaxis model, Wenderoth and Bennecke⁸ attempted to demonstrate a common fish-family antigen to reconcile this debate, but its applicability to humans is not clear. During the last several decades Aas¹⁷ and Elsayed and Apold¹⁹ have done extensive work on codfish allergy.¹⁷⁻²⁰ These authors have elegantly isolated, defined, and characterized the major allergen in codfish, Gad C1, known previously as allergen M.¹⁷⁻²⁰ Gad C1 has been isolated in an allergenic form in breast milk

causing allergic sequela in the nursing infant, and cod-allergic patients react to <1 mg of the Gad C1 antigen concealed in 50 gm of meatballs.²⁰ The primary amino acid structure of Gad C1 was found to bind IgE, and short synthetic peptides of the Gad C1 also bind IgE. Gad C1 resists digestion, cooking, and proteolysis and is allergenic in its primary structure. For these various reasons, Gad C1 is the immunodominant antigen in codfish and may represent the common fish antigen described by Tuft et al.⁷ that was believed responsible for the clinical picture of multiple fish allergy. An analogous protein in carp has already been characterized and lends additional support to this theory.²¹

However, the results from our prospective study indicate that patients allergic to one or more fish species can often consume other fish species without adverse reactions. These patients often had a history of a severe reaction after ingesting fish, and we believe that they represent a typical cross section of fish-allergic patients. These patients represent a heterogeneous group with patient No. 4 at one end of the spectrum (only reacting to perch and only having two positive fish SPTs) and patient No. 9 at the other end (reacting to the ingestion of three fish species). Positive open challenge of patient No. 9 to trout may have been a respiratory or "fish asthma" reaction, since the fillet was brought to her room freshly cooked and steaming.

Concern has existed about histamine contamination of fish extracts causing false-positive results. Prick skin testing with these extracts in 30 control patients were all negative. Fish SPTs and oral challenge results are consistent with previous results on other food antigens.⁹ As with other foods, fish SPTs have excellent sensitivity and negative predictive accuracy, but poor specificity and positive predictive accuracy.

One area in which the oral fish challenges appear unique and do not correlate with our other extensive food-challenge data is the high (21%) rate of false-negative DBPCFCs. All other lyophilized food antigens used in DBPCFCs were associated with 1.8% (5/280) false-negative reactions.⁹ The false-negative fish DBPCFCs were an especially unexpected finding in view of the known characteristics of Gad C1, which is resistant to various processing treatments. To adjust for this problem, we have changed our fish-challenge protocol. Currently, we use cooked and pureed, *non*-lyophilized fish, blinded in a hamburger, with canned tuna in hamburger as the placebo control.

The patients had IgE-specific binding to protein bands of fish to which they were not clinically allergic. We have previously reported similar findings with legume immunoblot data.¹³ The failure of sera of patient

No. 4 to recognize the 13 kd protein is interesting in view of her selective positive SPT. Perhaps patients that are negative to Gad C1 or its analogous protein in other fish species are especially likely to have specie-specific fish allergy. The Gad C1 protein has been isolated, and this proposal could be tested.

IgE ELISA-inhibition assays failed to demonstrate a difference in the 50% inhibition concentration of fish species that provoked allergic reactions in these patients versus fish species that did not. In addition, there appeared to be no difference in the 50% inhibition potency between the index allergenic fish and close versus more distantly related fish. Interestingly, even the clinically nonallergenic fish were able to achieve 100% inhibition in these IgE-specific ELISA-inhibition assays.

In conclusion, clinical reactivity to fish is not diagnosed by IgE-specific antibodies against that fish species. It appears that, in some patients, fish allergy is species specific, and the history of a reaction to one fish species should not automatically result in a restriction from eating all fish. The patients in this study were all able to consume one or more other fish species without adverse reaction. We would recommend that fish-allergic patients interested in trying a fish species for the first time do so in a clinical setting appropriate for oral food challenges with resuscitation equipment and medication available.²² For the oral challenges, the clinician may want to select fish species to which the patient has a negative SPT, since all fish challenges with a corresponding negative SPT were negative.

Fish-allergic patients eating fish in restaurants must be aware of the possibility of cross-contamination with egg, milk, peanut, etc, during fish preparation. In addition, the Food and Drug Administration allows certain substitutions of fish species in fish-food products; therefore, fish-allergic patients may want to deal directly with a known fish market.

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