

Codfish allergy in adults: IgE cross-reactivity among fish species

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Background: Fish is reported to be one of the most common causes of food allergic reactions. Species specificity and patterns of cross-reactivity are still to be defined.

Objective: To demonstrate the immunologic reactivity of clinically codfish-allergic adults to four species of fish: cod, mackerel, herring, and plaice.

Methods: IgE reactivity was measured in eight clinically codfish-allergic adult patients, confirmed by double-blind, placebo-controlled challenges with fresh raw codfish, and in 30 codfish-tolerant control subjects, by means of skin prick test, histamine release test, specific IgE tests, SDS-PAGE, and immunoblotting.

Results: All eight patients had positive skin prick tests to plaice and herring, seven of eight to mackerel, whereas fish-induced histamine release from basophil leukocytes was positive in five, four, and six of six patients, respectively. Elevated specific IgE to the fish species was found in all eight, and reactions among the control subjects using an in-house method, the Maxisorp RAST, with freshly prepared fish extracts, were fewer ($n = 8$) than found with Phadebas RAST ($n = 12$) and Pharmacia CAP System ($n = 11$). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting revealed individual and common antigenic proteins in freshly prepared extracts. Sera from all eight patients recognized a protein in the area of 11 to 14 kD in all fish species. This is believed to be a protein fraction analogous to Gad cI. Inhibition of the codfish Maxisorp RAST was obtained with mackerel, herring, and plaice. No cross-reactivity to shrimp or milk was shown.

Conclusion: This study suggests that serologic cross-reactivity to different fish species in clinically codfish-allergic adults exists, and that cod, mackerel, herring, and plaice share a common antigenic structure.

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INTRODUCTION

IgE-mediated reactions to fish are among the most commonly encountered food allergies in adults. The sensitivity often appears at an early age, and in most patients, persists lifelong. It is well documented that sensitized individuals who ingest fish can develop immediate allergic reactions such as urticaria, angioedema, asthma,

rhinitis, vomiting, diarrhea, and anaphylaxis.¹⁻⁷ Previously, a significant relation between a history suggestive of fish allergy and elevated specific IgE or skin reactions has been described, and based on these findings, patients rarely have been challenged but treated with elimination diets.

Until recently, only few studies have been performed to explore the degree of species specificity and pattern of cross-reactions. Generally, most studies of fish allergy and cross-reactivity among fish species have been carried out in children.^{1,3,5,8-12} The advice usually given to patients who have experienced allergic reactions to one kind of fish has been to avoid all kinds of fish, although some studies have shown that patients who are clinically

sensitized to one species of fish can tolerate other species.^{5,9,10,13} RAST-inhibition or SDS-PAGE/immunoblotting studies have suggested that the current recommendation, ie, that fish-sensitive individuals should avoid all species of fish, should be re-evaluated due to clinically irrelevant but serologically evident allergenic cross-reactivity.⁵

Some of the most commonly consumed fish species in Scandinavia have been chosen for this study: cod (Gadiformes), mackerel (Perciformes), herring (Clupeiformes), and plaice (Pleuronectiformes). Based on our previous studies of fish allergy,^{6,14} we considered specific IgE as a prerequisite for a reaction to a certain fish. We therefore investigated IgE reactivity to mackerel, herring, and plaice extracts in clinically codfish-allergic patients and codfish-tolerant subjects in order to describe the degree of common reactivity and whether a common structure in the fish species might account for the binding. Furthermore, we investigated by inhibition techniques whether such common reactivity could be due to cross-reactivity. Finally, we determined whether the subjects' basophils reacted with histamine release to the different species of fish.

MATERIAL AND METHODS

Patients and Control Subjects

A total of 38 adults were investigated. Eight clinically codfish-allergic adults (ranging in age from 21 to 31 years) participated in this study. They had all been challenged previously with codfish using titrated, oral double-blind, placebo-controlled food challenge (DBPCFC), as described in detail elsewhere.^{6,14}

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Control subjects were 30 adults of whom three presented convincing medical histories indicating type I codfish allergy upon ingestion but were found to be clinically tolerant to codfish by DBPCFC.⁶ The remaining 27 adults, who reported a frequent intake of fish with no symptoms, included three subgroups: eight nonatopic subjects; seven pollen-allergic subjects (proven by case histories of seasonal rhinoconjunctivitis and positive skin prick tests or specific IgE tests); and 12 with atopic dermatitis,¹⁵ six having serum-IgE concentrations > 2000 kIU/L and six < 2000 kIU/L. Since these 27 control subjects ingested codfish regularly without any adverse reactions they did not undergo DBPCFC but were classified as challenge-negative/fish-tolerant subjects.

All patients and control subjects had undergone detailed questioning, skin prick test, histamine release test, and specific IgE tests with respect to codfish as described earlier.^{6,14} The demographic characteristics of the whole study group are listed in Table 1. Blood was drawn from all 38 subjects at their initial visit: the heparinized whole blood was used within 24 hours for histamine release test, and the sera were stored at -20 °C for fish-specific IgE analyses, immunoblotting, and RAST-inhibition.

Total IgE

Total serum-IgE was measured in all patients and control subjects by the PRIST kit (Pharmacia, Uppsala, Swe-

den) and concentrations were expressed as kIU/L.

Allergen Extracts

Commercial, glycerinated extracts of codfish (726), mackerel (723), herring (725), and plaice (724) (Soluprick, ALK Hørsholm, Denmark) 1:20 wt/vol were used for histamine release test and skin prick test.

Fresh, raw white meat of codfish, mackerel, herring, and plaice was stored at -20 °C for up to seven days before use. The fish meat was homogenized in PBS (10 mM, pH 7.4) or distilled water (50 mg wet weight/mL) for 2 × 3 minutes using a thermostatically controlled Waring blender (Waring Products Division, New Hartford, CT). For coating in Maxisorp RAST the extracts were centrifuged (1800 g, 15 minutes), while suspensions were used for histamine release test, SDS-PAGE, immunoblotting, and RAST inhibition. Dilutions were performed in PBS with azide (MAXI, RAST inhibition, SDS-PAGE, immunoblotting) and PIPES buffer (histamine release test).

Skin Prick Test

Skin prick tests were performed in quadruplicate on the forearms of all 38 subjects according to the European Academy of Allergology and Clinical Immunology Guidelines.¹⁶ Fish extracts used throughout the study were from the same batch. A positive control consisted of histamine dihydrochloride, 10 mg/mL, and a diluent of

glycerol/sodiumchloride was used as the negative control. Skin prick test was carried out according to diagnostic principles outlined previously.⁶ The reaction was considered positive when the wheal induced by the fish extracts had a net diameter 3 mm larger than that of the negative control in all four punctures.

Histamine Release Test

Histamine release from basophil leukocytes was performed as previously described.^{17,18} Heparinized blood was drawn from each patient and control subject, stored at room temperature, and used within 24 hours. Commercial codfish, mackerel, herring, and plaice extracts were tested at six 3.5-fold dilutions, starting with 1:10 of the stock solution. Measurements with fresh codfish, mackerel, herring, and plaice (50 mg wet weight/mL) were performed in 12 dilutions with a dilution factor of 3.5.

A histamine release of 20 ng/mL whole blood or more was considered positive. Subjects with non-responding basophils, ie, a histamine release of less than 20 ng/mL following anti-IgE challenge, were classified as inconclusive.

Commercially Available Tests for Specific IgE

Specific IgE to codfish (f3), mackerel (Rf206), herring (Rf205), and plaice (Rf254) was measured by two commercially available kits: Phadebas RAST (PHA) (measuring range 0.35 to

Table 1. Characteristics of Study Group. Number of Positive Test Results (codfish)

	Age, yr	Sex	Total IgE, kIU/L		SPT	HR-C	HR-F	PHA	CAP	MAXI
			Mean	Range						
8 DBPCFC+ (codfish)	21-31	6F/2M	2315	29-14600	8	5*	6*	8	8	8
3 DBPCFC- (codfish)	23-28	1F/2M	5362	83-15900	1	0	0	1	2	0
8 nonatopic subjects	26-43	7F/1M	20	3-72	0	0	0	0	0	0
7 pollen-allergic subjects	25-48	2F/5M	106	12-401	0	0	0	0	0	0
12 atopic dermatitis										
6 < 2000 kIU/L	17-32	5F/1M	808	5-1883	0	0	0	0	0	0
6 > 2000 kIU/L	20-38	3F/3M	8208	2153-15834	0	0	0	2	2	1

* Two inconclusive. Abbreviations: DBPCFC, double-blind, placebo-controlled food challenge; SPT, skin prick test; HR-C, histamine release test using commercial extract; HR-F, histamine release test using fresh extract; PHA, Phadebas RAST; CAP, Pharmacia CAP System; MAXI, Maxisorp RAST; F, female; and M, male.

17.5 PRU/mL) and the Pharmacia CAP System (CAP) (measuring range 0.35 to 100 kU/L) both from Pharmacia Diagnostics, Uppsala, Sweden. Both tests were performed according to the instructions of the manufacturers. All samples were tested in duplicate.

Maxisorp RAST

The Maxisorp RAST (MAXI) was performed according to the method previously described.^{14,19} In brief, test tubes (Maxisorp polystyrene test tubes, Nunc, Roskilde, Denmark) were coated and incubated with diluted fresh allergen. The tubes were washed in PBS including Tween. Serum samples were diluted 1:10 (in PBS 1/15 mol/L, pH 7.0, containing azide 0.01% and Tween 20 0.1%) and added to the allergen-coated tubes. Finally ¹²⁵I anti-IgE (Pharmacia, Uppsala, Sweden), diluted 1:12 (approximately 10000 cpm), was added. A pool of RAST-negative sera from blood donors served as a negative reference serum (sera were negative to pools of pollen, fungi, and animal dandruffs).

Results were obtained from duplicate analyses and means were expressed as % binding = sample/total cpm.

Dose-response experiments showed the optimal allergen concentration for coating the surface of the test tubes at: 100 µg/mL fresh codfish and herring and 167 µg/mL of fresh mackerel and plaice.

SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as previously described^{14,20} with 16% separation gels and 5% stacking gels in a vertical electrophoresis chamber. Samples were diluted in a sample buffer containing 5% mercaptoethanol and were boiled for five minutes. An amount of 0.9 mg (wet weight) of codfish, mackerel, herring, and plaice extracts in 30 µL were loaded onto 0.5 cm of gel. Molecular weights were determined using prestained molecular weight markers (Amersham, 14.3 to

200 kD). Electrophoresis was performed at 13 mA overnight.

The gels were either stained by Coomassie for protein determinations or electroblotted for subsequent determination of IgE-binding.

For the immunoblotting experiments, a radioactive molecular weight marker (CFA. 756, Amersham) was used for estimation of the molecular weights.

Electroblotting and Detection of Specific IgE

Immunoblotting was performed as previously described.¹⁴ Briefly, fish proteins separated by SDS-PAGE were transferred onto nitrocellulose paper (pore size 0.2 µm, Schleicher & Schuell, Germany) by a semidry electroblotter (JKA-Biotech, Denmark) at 0.8 mA/cm² for one and one-half hours in a discontinuous buffer system at room temperature. Nitrocellulose was cut into strips of 0.5 cm (0.4 in mackerel, herring, plaice) and blocked in Tris-buffered saline containing 3% wt/vol powdered milk and 1.5% Tween 20 (blocking solution). For IgE detection, strips were incubated with 1 mL of serum diluted 1:10 (one patient reacted very strongly, and had to be diluted 1:200) in blocking solution. Bound antigen-specific IgE was detected by ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia, approximately 200,000 cpm) diluted 1:12 in blocking solution. Hyperfilm (Amersham, MP, RPN. 6) was exposed to the air-dried nitrocellulose strips for 3, 7, and 14 days at -80 °C.

Control strips were tested with a pool of serum from normal patients and a buffer control, respectively. These controls were invariably negative.

The IgE-stained protein bands were visually scaled to two intensity classes representing strong and weak IgE-binding (after seven days of exposure).

RAST Inhibition

RAST inhibition was performed in all subjects with elevated specific IgE antibodies to codfish (in MAXI) according to the MAXI procedure.¹⁹ Maxi-

sorp test tubes were coated overnight at 37 °C with 300 µL of fresh codfish extract, 1:500. For inhibition, freshly prepared allergen extracts of codfish, mackerel, herring, plaice, shrimp, and milk were used (undiluted fish/shrimp = 50 mg/mL, then diluted 1:5 and 10-fold diluted to 1 ng/mL). Four hundred microliters of diluted (in PBS + Tween) patient's serum was preincubated with 400 µL of these extracts overnight at 4 °C. The MAXI-RAST procedure was then carried out as described above. The uninhibited binding for each serum was determined by adding saline instead of allergen extract. A pool of RAST-negative sera from blood donors served as negative reference serum. All inhibitions were performed in duplicate and the mean cpm was used for further calculations. The percentage inhibition in the binding of IgE antibodies was calculated as follows: % inhibition = 100 - 100 × (cpm sample - cpm normal serum)/(cpm uninhibited - cpm normal serum). For each inhibiting extract, the 50% inhibitory dose was calculated (graphically) and expressed relatively to the codfish inhibition. Inhibitor doses ≥ 1 mg/mL were excluded from calculations.

RESULTS

Case History

All DBPCFC-positives (eight patients) and the three challenge-negatives to codfish had a positive medical history to codfish, while the remaining control subjects (n = 27) ingested fish without any adverse reactions. Table 2 shows the results of the subjects' case histories regarding adverse reactions after ingestion of mackerel, herring, and plaice. Most of the clinically codfish-allergic patients reported type I adverse reactions (such as oropharyngeal itching and swelling, vomiting, diarrhea, urticaria, rhinoconjunctivitis, and asthma) after ingestion of these fish species, while few did not know, due to avoidance (some had reacted to contact or to the smell of the fish).

Table 2. Case History

Case History	Codfish			Mackerel			Herring			Plaice		
	+	–	ne*	+	–	ne	+	–	ne	+	–	ne
8 DBPCFC + (codfish)	8	0	0	6	0	2	5	0	3	6	0	2
3 DBPCFC– (codfish)	3	0	0	0	0	3	0	0	3	1	1	1
27 other control subjects	0	27	0	0	27	0	0	27	0	0	27	0

* +, positive reaction; –, tolerates; and ne, never eaten.
DBPCFC, double-blind, placebo-controlled food challenge.

Skin Prick Test

Of the eight clinically codfish-allergic patients, all of whom were skin prick-test positive to commercial codfish extract, seven had positive skin prick tests to mackerel, and all eight to herring and plaice (Table 3). One challenge-negative patient (to codfish), having a positive skin prick test to codfish, also had positive skin prick tests to herring and plaice, while no reaction was seen at all among the other control subjects tested with mackerel, herring, and plaice.

Histamine Release Test

Table 3 shows the outcome of the histamine release test using commercially and freshly prepared extracts. Using commercial extracts, six of six clinically codfish-allergic patients were positive to mackerel, four of six to herring, and five of six to plaice, while fresh extracts in histamine release test showed positive reactions in four of six to mackerel, and six of six to herring and plaice. Two codfish-allergic patients had non-responding basophils (ie, release from basophils < 20 ng histamine/mL following anti-IgE challenge). Two positive reactions were

seen in histamine release tests among the codfish-tolerant subjects: one patient with a positive case history to codfish (the one with positive skin prick test to herring and plaice) reacted to fresh mackerel extract, and a control subject with atopic dermatitis (total IgE = 5210 kIU/L) reacted to commercial mackerel.

Tests for Specific IgE

Specific IgE reactivity to the fish extracts is summarized in Table 3. The results demonstrate that sera from codfish-sensitive subjects have specific IgE to the other species tested. By Phadebas RAST (PHA), all eight clinically codfish-allergic patients were positive to the 3 fish species. In CAP, eight of eight were positive to mackerel and herring, while seven of eight had positive reactions to plaice. Reactions were seen among the codfish-tolerant subjects who had positive case histories (PHA 1-2/3, CAP 1/3) and among those who had atopic dermatitis (and high total IgE) (PHA 2-3/12, CAP 1-2/12). In MAXI with freshly prepared mackerel, herring, and plaice, all eight DBPCFC-positives to codfish reacted positively, and one to three of 30

codfish-tolerants had a positive response (Table 3).

SDS-PAGE

Figure 1 shows the Coomassie-stained protein bands ranging in molecular weight from <14.3 to 200 kD. Protein profiles and intensities of the fish species appeared almost identical within the larger proteins (>25 kD), while differences were seen in the lower molecular weight proteins. The fish extracts comprised approximately 25 to 30 bands, of which about 11 were of identical molecular weight and were the most prominent: 16 to 18, 21, 27, 31, 37–39, 43, 52, 90, 150, ~200. Furthermore, the prominent band migrating at approximately 11 to 14 kD was found in extracts of codfish, mackerel, and herring (although very weak in mackerel) whereas for plaice two bands were seen in that region.

Immunoblotting

The allergenic profiles of the four fish were visualized by immunoblotting in order to evaluate the possible common reactivity among the fish extracts (Figure 2 A–D, not all control subjects shown). The immunoblottings of these

Table 3. Number of Positive Test Results Using Mackerel, Herring, and Plaice in Skin Prick Test, Histamine Release Test, and Tests for Specific IgE

	SPT			HR-C			HR-F			PHA			CAP			MAXI		
	M	H	P	M	H	P	M	H	P	M	H	P	M	H	P	M	H	P
8 DBPCFC + (codfish)	7	8	8	6*	4*	5*	4*	6*	6*	8	8	8	8	8	7	8	8	8
3 DBPCFC – (codfish)	0	1	1	0	0	0	1	0	0	1	1	2	1	1	1	1	0	2
8 nonatopic subjects	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7 pollen-allergic subjects	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 atopic dermatitis																		
6 < 2000 kIU/L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 > 2000 kIU/L	0	0	0	1	0	0	0	0	0	0	2	3	2	1	1	2	1	1

* Two inconclusive. C, codfish; M, mackerel; H, herring; P, and plaice. For other abbreviations, see legends to Tables 1 and 2.

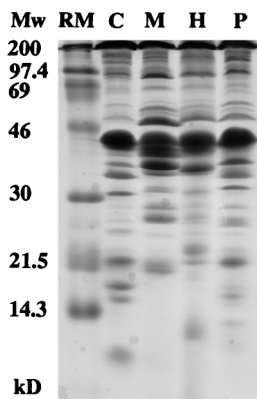


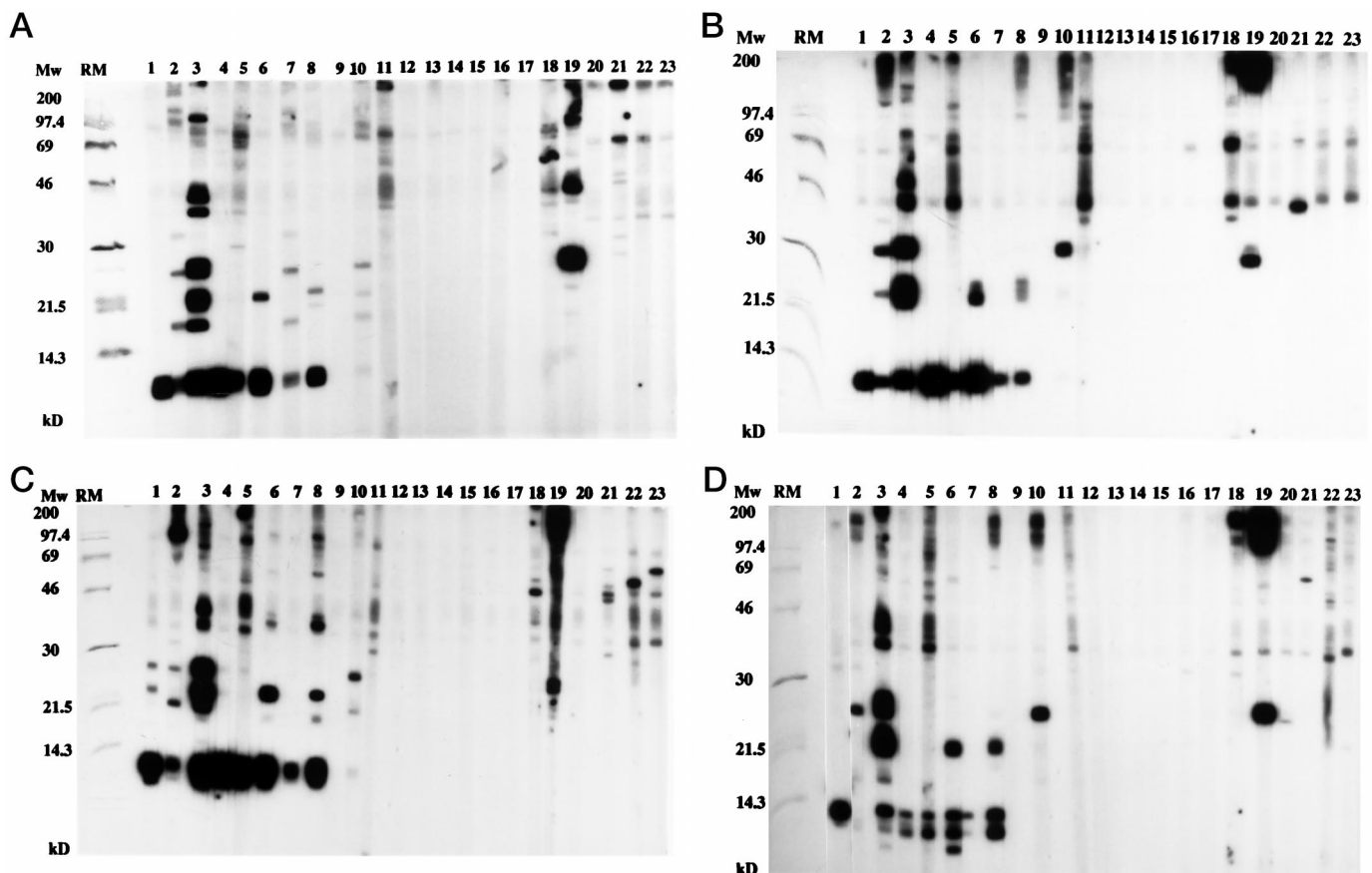
Figure 1. Coomassie stained 16% separation SDS-polyacrylamide gel showing the protein pattern of freshly prepared extracts of codfish (C), mackerel (M), herring (H), and plaice (P). RM, molecular weight (Mw) marker in kiloDaltons (kD). Loading dose: 0.9 mg/30 μ L. Sample buffer containing mercaptoethanol.

fish showed more or less homology and a total of approximately 17 various IgE-stained bands were seen. Immunoblotting demonstrated that the 11 to 14-kD band bound IgE-antibodies most efficiently and all eight DB-PCFC-positives to codfish reacted to a band in that area for both mackerel, herring, and plaice. In plaice five of eight reacted to two bands, two of eight to three bands, and one of eight showed a weak binding of one band (Fig 2 D). Two of the eight clinically codfish-allergic patients only showed binding in that area of all fish extracts, whereas the remaining also bound proteins at other molecular weights. One DBPCFC-negative to codfish showed a very weak reaction to mackerel and herring in the area < 14.3 kD. Three to

seven of the remaining 27 codfish-tolerant control subjects (especially those with atopic dermatitis) reacted to other bands mainly of higher molecular weights.

RAST Inhibition

Concentrations of fish extracts required to achieve 50% inhibition of the codfish RAST were calculated (relative to codfish); the extent of inhibition is shown in Table 4. A small amount of the inhibitor needed to inhibit the RAST by 50% indicates a high inhibitory value of the extract, calculated in relation to codfish. Herring, plaice, and mackerel were capable of inhibiting reactivity to codfish in the clinically codfish-allergic patients, except in two: codfish RAST was not inhibited by herring in one, and by plaice in the



Figures 2 A-D. Immunoblotting showing IgE binding to freshly prepared extracts of codfish (A), mackerel (B), herring (C), and plaice (D). Sera were diluted 1:10, patient number 3, 1:200. Mw, radioactive molecular weight marker (RM) in kiloDaltons (kD). 1-8: DBPCFC-positive patients (codfish). 9-23: control subjects (9-11: DBPCFC-negative patients (codfish), 12-14: nonatopic subjects, 15-17: pollen-allergic subjects, 18-23: atopic dermatitis, 5 of these with total IgE > 2000 kIU/L).

Table 4. Cross-Reactivity with Codfish. The Ability of Fresh Codfish, Mackerel, Herring, Plaice, Shrimp, and Milk to Inhibit Binding of Specific IgE to Codfish

	Codfish	Mackerel	Herring	Plaice	Shrimp	Milk
Patient 1	*****	****	*****	*****	0	0
Patient 2	*****	****	*****	*****	0	0
Patient 3	*****	****	0	*****	0	0
Patient 4	*****	***	***	*	0	0
Patient 5	*****	**	*****	*****	0	0
Patient 6	*****	*****	*****	0	0	0
Patient 7	*****	*****	*****	*****	0	0
Patient 8	*****	***	***	*****	0	0
Control subj.†	*****	0	***	*****	0	0

† Control subject with atopic dermatitis. The extent of inhibition calculated in comparison with codfish: ***** $\geq 1/2$, **** $\geq 1/10$, *** $\geq 1/100$, ** $\geq 1/1000$, * $\geq 1/10000$, and $\geq 1/100000$.

other. No cross-reactivity to shrimp or milk was seen. Figure 3 shows an example of RAST-inhibition results in a clinically codfish-allergic patient (number 8). Median extent of inhibition was calculated and ranked: herring > plaice > mackerel. Serum from one control subject with elevated specific IgE to codfish (having atopic dermatitis and total IgE of 15834 kIU/L) was inhibited by plaice and herring.

The dose of each fish extract yielding 50% inhibition of raw codfish in the eight DBPCFC-positives to codfish varied from 0.01 to 93 $\mu\text{g/mL}$ of codfish, 0.015 to 800 $\mu\text{g/mL}$ of plaice,

0.005 to 255 $\mu\text{g/mL}$ of herring, 0.7 to 686 $\mu\text{g/mL}$ of mackerel, while shrimp and milk never achieved 50% inhibition in 1 mg/mL.

DISCUSSION

IgE-mediated reactions to fish are among the most commonly encountered food allergies in adults. Our study was undertaken in order to assess the immunologic reactivity of clinically codfish-sensitive adult patients to various fish species, and especially the possible cross-reactivity between codfish, mackerel, herring, and plaice extracts.

The existence of common reactivity to various fish species in eight clinically codfish-allergic adults was suggested by means of case histories, skin prick test, histamine release test, specific IgE tests, and by applying fresh fish extracts in histamine release test, specific IgE test, and immunoblotting.

All eight patients had positive skin prick tests to herring and plaice, while positivity to mackerel was found in seven of the eight. Furthermore, all eight had strongly elevated specific IgE to all fish species tested, and four to six of six reacted in histamine release test depending on the fish species. All patients were positive in at least one of the various tests. No patient had a negative test result in more than one test with the same fish extract. Measuring IgE-reactivity in control subjects tolerant to fish showed that positive reactions occurred among subjects with atopic dermatitis and those who had a positive case history, but who were DBPCFC-negative, whereas all tests were negative in pollen-allergic and nonatopic volunteers. These results show a common reactivity in clinically codfish-allergic patients which suggests the existence of cross-reactivity.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting were also used to evaluate common proteins and allergens. All species showed protein bands ranging in molecular weight from <14.3 to 200 kD. Several of the bands were able to bind specific IgE from the sera of codfish-allergic patients. Approximately 17 components, ranging in molecular weight from <14.3 to 200 kD, showed IgE-binding activity. The component with a molecular weight of approximately 11 to 14 kD showed the highest frequency of IgE binding and was considered to represent a protein fraction analogous to codfish Gad cI, the major allergen in codfish. Immunoblotting is acceptable for screening for codfish allergy when using DBPCFC as the "true diagnosis." Specific IgE-binding within the 11 to 14-kD area in immunoblotting may be a good indicator of clinical fish allergy. In

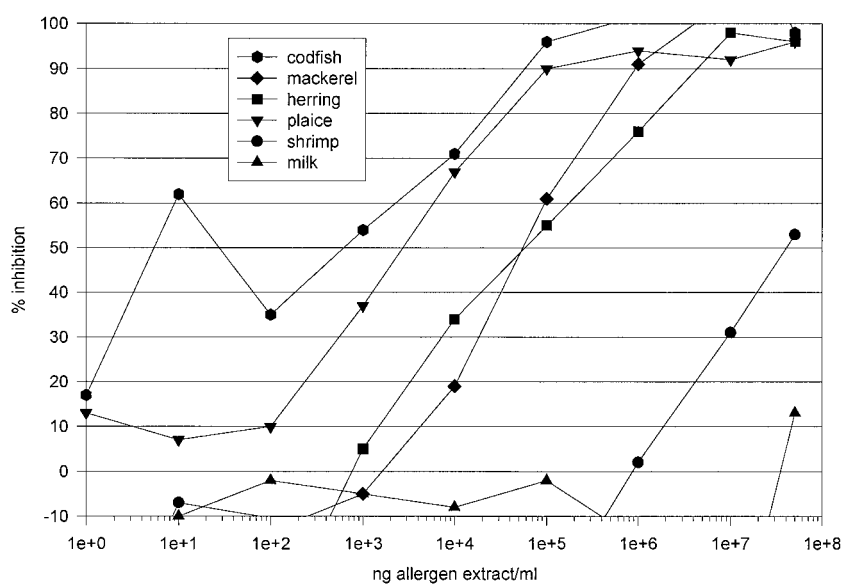


Figure 3. RAST-inhibition assay of freshly prepared extracts of codfish, mackerel, herring, plaice, and shrimp, together with cow's milk carried out with sera from patient number 8, one of the clinically codfish-allergic patients (DBPCFC-positive).

conclusion, the immunoblotting experiments indicated the existence of both general fish allergens and species-specific allergens.

In order to confirm whether mackerel, herring, and plaice shared allergenic determinants with codfish (ie, whether the common reactivity is due to cross-reactivity), inhibition studies were performed with sera from all subjects with specific IgE to codfish using MAXI. These RAST-inhibition experiments demonstrated that the three other fish species were capable of inhibiting reactivity to codfish. Cross-reactivity in a varying degree with these extracts was seen in all eight adults except with herring in one patient and plaice in another. Those with a lower degree of cross-reactivity had a positive outcome in all the other tests performed. The rank of extracts able to inhibit codfish RAST varied slightly from patient to patient. Generally, herring and plaice were slightly more potent (ie, stronger cross-reacting) than mackerel. Cross-reactivity of the clinically codfish-allergic patients who denied or were not sure of having ever eaten mackerel, herring or plaice, was also observed. The RAST inhibition experiments thus confirmed the hypothesis of cross-reacting antigens between codfish and mackerel, herring, and plaice.

By means of skin prick test^{7,10,11} and RAST^{10,11} it has been demonstrated that common fish allergens may be present in varying amounts in different species (for example between codfish and flounder, salmon, mackerel; codfish and eel, bass, sole; codfish and hake, whiff), contributing to differences in allergenic activity. To confirm serologic and skin prick test results of cross-reactivity in non-characterized patients, clinical investigations should be performed.²¹⁻²³

Earlier, cross-allergenicity among various species of fish and the clinical significance has been studied. Some studies have described the existence of serologic cross-reactivity that did not necessarily correlate with clinical allergy to individual fish species, whereas other studies have shown the

presence of both serologic and clinical cross-reactivity between fish species.

As early as in 1937,¹³ de Besche reported that patients hypersensitive to a single species of fish (codfish) could tolerate other species of fish (salmon and trout). Aas described cross-reactivity among fish species in children who were clinically sensitized to codfish, but could tolerate other species than codfish (salmon).⁹ His study indicated that some fish-allergic children react to species-specific antigens in fish while others react to common or closely related allergens in different species.

Recently, Bernhisel-Broadbent⁵ showed that clinically fish-allergic patients had positive skin prick test and that their sera contained specific IgE antibodies to fish species that were clinically tolerated. Furthermore, the dose of each fish extract required to achieve 50% inhibition in inhibition assays was equal for fish eliciting a clinical allergy as compared with fish that were tolerated. To evaluate cross-reactivity among the fish extracts, SDS-PAGE and immunoblotting were used. All fish extracts (except tuna) in the study had a prominent band at approximately 13 kD and immunoblotting demonstrated that this band bound IgE-antibodies most efficiently. Not all DBPCFC-positive individuals in their study had IgE binding to this band. Compared with our study, the fish investigated (catfish, bass, perch, mackerel, salmon, trout, and flounder) had more or less identical pattern in SDS-PAGE to codfish and they all contained an IgE-binding band which might be analogous to Gad cI. Our inhibition studies showed that several common antigens among the fish species were present but mackerel had fewer allergens in common than in the other fish, which might explain the differences in allergy.

Our studies indicate that there are both general fish allergens and species-specific allergens, and that the common fish reactivity found can be related to cross-reactivity among fish species. Whether this cross-reactivity correlates with clinical hypersensitivity is unknown and will remain so until DBPCFC with mackerel, herring, and plaice have been carried out. Neverthe-

less, a major part of the clinically codfish-allergic patients reported adverse reactions to the other species of fish (Table 2).

Our interpretations should be tempered by the fact that only a small number of subjects have been tested and more extensive studies are needed. Obviously, clinical reactivity to mackerel, herring, and plaice (established by means of DBPCFC) in a greater number of individuals must be available in order to reach a definitive conclusion.

In conclusion, this study demonstrated that all clinically codfish-sensitive adults reacted to other fish species by several of the following tests: skin prick test, specific IgE tests, histamine release test, and immunoblotting. RAST-inhibition documented that cross-reactivity between codfish and other fish species (herring, plaice, and mackerel) is present. From a practical/clinical point of view, general caution is advisable for codfish-allergic adults ingesting other species of fish or fish-based products. Confirmation of immunologic reactivity has to be established by controlled challenges.

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