

Original article

Cross-reactivity to eel, eelpout and ocean pout in codfish-allergic patients

Fish allergy is one of the most common food allergies in both children and adults and patients with allergic reactions to one fish species have in many cases been given the advice to avoid all fish, without further evaluation. The possible common reactivity between different fish species is not well studied. Because of this and a possible exploitation of fish species hitherto not much used in the Scandinavian diet ocean pout, eelpout and eel were evaluated. We examined the serological and biological cross-reactivity of these species in double-blind challenged-confirmed codfish-allergic patients using CAP, Maxisorp-radio allergosorbent test (RAST) inhibition, western blot, skin prick test (SPT) and histamine release (HR). All 18 codfish allergic patients had specific IgE to ocean pout, eelpout and eel determined by Maxisorp-RAST. All four fish species could induce basophil HR using blood from 16 of 18 patients and all patients tested reacted in SPT. This study demonstrates that patients with a verified clinical allergy to codfish in a high frequency express biological cross-reactivity to other fish species. By RAST inhibition this common reactivity was shown to be a true cross-reactivity.

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Fish allergy is one of the most common food allergies especially among adults and can be significant in populations where fish is a major component in the food. The clinical manifestations are ranging from local oral allergy syndrome over urticaria to anaphylaxis (1–3). Patients found allergic to one fish species have in many cases been advised to avoid all kinds of fish without further evaluation. In the literature reports of extensive clinical cross-reactivity between fish species are common (4). For instance has serological cross-reactivity between codfish and various other fish species been suggested by means of skin prick test (SPT), histamine release (HR), radio allergosorbent test (RAST) and immunoblotting by Hansen et al. (2). There have, however, lately been several reports of patients reacting to only one or a few species (5–8).

Because of a possible introduction of fish species hitherto not much seen on the Scandinavian market and the inconsistency in the literature regarding the

cross-reactivity between different kinds of fish we have evaluated the IgE-mediated cross-reactivity of ocean pout, eelpout and eel.

Methods

Patients

Eighteen patients (aged 16–44 years, median 31) diagnosed with codfish allergy, according to the guidelines published by The European Academy of Allergy and Clinical Immunology (9, 10), were recruited. All had a positive SPT to codfish and were positive in double-blind placebo-controlled food challenge (DBPCFC), except where their case history indicated that they had suffered anaphylaxis or a very severe reaction upon ingestion.

This study was approved by the local ethical committee (Den videnskabetiske komité for Vejle og Fyns amter) and informed consent was obtained from the patients.

Fishes and extracts

Ocean Pout was purchased from Aqua Bounty Canada Inc. (St John's NF, Canada) while codfish, eel and eelpout were bought locally.

Abbreviations: CPM, counts per minute; Ig, immunoglobulin; FCS, fetal calf serum; HR, histamine release; RAST, radio allergosorbent test; SPT, skin prick test.

The extracts were produced by homogenizing 1 g fish/ml H₂O. They were then centrifuged at 1850 × *g* for 10 min to remove insoluble debris and stored at -20°C until use.

Protein content of extract

The protein content of the extracts was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin (BSA) as standard. Ten microlitre of standard or sample was placed in microtitre plate-wells and 200 µl of Bio-Rad dye were added to each well. The plate was incubated at room temperature for 5–15 min and analysed in a spectrophotometer Tim-200 (Teknunc, Roskilde, Denmark) at 600 nm. Standard curves were plotted as absorbance vs BSA concentration and the protein concentration of the extracts were calculated in µg/ml.

CAP

The CAP analyses were performed in duplicates in accordance with the manufacturers (Pharmacia, Uppsala, Sweden) recommendations.

Maxisorp-RAST

The direct Maxisorp-RAST was performed as previously described (11). Briefly, Maxisorp tubes (Nunc A/S, Roskilde, Denmark) were coated with 250 µl of allergen extract (codfish, eel, eelpout or ocean pout) diluted to 2 µg protein/ml in phosphate-buffered saline (PBS), at 37°C overnight. The tubes were washed and incubated for 2 h in 37°C with 250 µl of RPMI 1640 including 5% fetal calf serum (FCS). After being washed the tubes containing 250 µl sample (serum) diluted 10 times in washing buffer (10 mM PBS, 0.1% Tween) were incubated at room temperature on a vibrator overnight. The next day 250 µl of anti-IgE-I¹²⁵ (Pharmacia) diluted 12 times in washing buffer was added to the washed tubes, which were incubated overnight at room temperature on a vibrator. After washing the γ -radiation was measured on a 1470 Wizard counter (Wallac, Allerd, Denmark) for 2 min. All measurements were performed in duplicate.

RAST inhibition

The RAST inhibitions were performed in the same way as the Maxisorp-RAST described above. Extract of codfish or ocean pout was used for coating. The serum diluted 10 times was incubated overnight at 4°C together with allergen extracts (codfish, eel, eelpout or ocean pout 20 ng/ml to 200 µg/ml) and centrifuged at 3000 *g* for 15 min, before use. Inhibition curves were plotted as percentage inhibition against the concentration of the inhibiting extract. The inhibition for each concentration was calculated by dividing the counts per minute (CPM) value of the inhibited sample with the CPM value of the uninhibited sample. For all values the background, determined by a pool of serum from nonallergic persons, was subtracted. The amounts of extract needed for 50% inhibition (IC₅₀) were calculated.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis, protein transfer and immunoblotting

The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blots were performed in accordance with the protocols described by Laemmli (12) and Hansen (2). Briefly, the

extracts were electrophoresed in a 1.0 mm 16% gel with a 4% stacking gel. The samples were diluted in a reducing buffer and boiled before use. Ten microlitre of sample or Rainbow marker (Amersham Pharmacia Biotech, Uppsala, Sweden) was added to each well. The proteins were blotted on to a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA) for 1.5 h (*I* = 1.0 mA/cm²). The membranes were blocked with tris buffered saline (TBS) with 0.5% Tween (TBST) and 5% BSA for 2 h at room temperature before incubating overnight at 4°C with the sera. For inhibition studies the sera were preincubated with extract (2 mg/ml) overnight. After wash, the membranes were incubated with anti-IgE Horse-radish peroxidase (HRP) (Dako A/S, Glostrup, Denmark) for 2 h and washed again. ECL Western blotting detection reagent (Amersham Pharmacia Biotech) was used as substrate. Some gels were stained with GelCode® Blue stain reagent (Pierce, Rockford, IL).

Histamine release

The HR was performed by the glass fibre technique according to the manufacturer (RefLab, Copenhagen, Denmark) (13, 14). Each extract was tested in six different 3.5-fold dilutions, C1–C6 (stock solution 10% w/v). Histamine release results were expressed in Class C1–C6 corresponding to the lowest dilution inducing a release at > 15 ng histamine/ml blood.

Skin prick test

The SPT was performed in duplicate according to EAACI (15) with freshly thawed ocean pout, eelpout and eel, and commercial, glycerinated allergen of codfish, 1 : 20 (w/v; Soluprick, ALK-Abello) (10, 16, 17). The SPT with a weal net diameter 3 mm larger than that of the negative control (a diluent of glycerol/NaCl) was considered positive. The area of the weals were determined either using a scanner and a personal computer calculation program (patients 1–5) (18) or by measuring the mean, D_{mean} , of the longest diameter and the mid-point orthogonal diameter and use the formula $\text{Area} = \pi \times (D_{\text{mean}}/2)^2$ (patients 6–18).

Results

The optimal concentration of the coating extract used for Maxisorp-RAST was determined to be 2 µg/ml by the performance of a dose-response curve (data not shown). Sera were defined positive if the response was three times above the background. The background was determined as the corresponding value for a pool of sera from nonallergic donors employing identical conditions. All 18 sera had specific IgE to all four fish species. Figure 1 shows the titration curves for two representative sera to the four extracts. In Table 1 the mean cpm values with SD for all 18 sera diluted 10 times are shown. The amount of specific IgE were in all cases higher for codfish compared with the other fish species. The two main patterns for the IgE-binding to ocean pout, eelpout and eel was either similar amount of binding to all three or a lower binding to eel compared with ocean pout and eelpout. All 18 sera were positive in CAP (Pharmacia) to codfish (Table 2). The cpm values for Maxisorp-RAST with codfish correlates with the CAP-values.

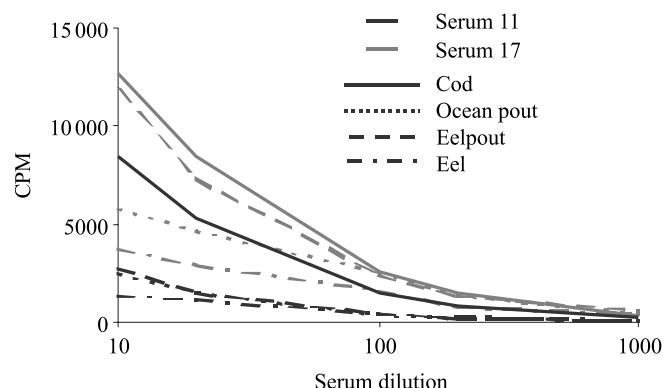


Figure 1. Titration curves of fish-specific IgE for serum nos 11 and 17 analysed by Maxisorp-radio allergosorbent test (RAST).

As shown in Table 3 all tested (17 of 18) patients reacted in SPT to all fish species with generally large weals. Patient no. 4 were not tested because of his/her high amount of specific IgE and the large reactions seen with the other patients, e.g. severe swelling of SPT area on the arm, itching in hands and feet, indisposition and asthma. Average weal area was $163 \pm 110 \text{ mm}^2$, $191 \pm 104 \text{ mm}^2$, $228 \pm 157 \text{ mm}^2$ and $255 \pm 337 \text{ mm}^2$ respectively; codfish, ocean pout, eelpout, eel. In nine of 18 cases the weals for ocean pout, eelpout, eel were larger than the weal for codfish. The results of the SPT were confirmed by measuring the amount of histamine released from patients' basophiles sensitized with fish extract (Table 4). The extract concentration needed for a release of $>15 \text{ ng histamine/ml}$ was in 14 cases lower for all three species ocean pout, eelpout, eel compared with codfish. Two patients (ns 7 and 17) were negative in HR to ocean pout and eelpout and reacted only with high amounts of eel protein (C1).

The cross-reactivity was studied by preincubating the sera with extract from codfish, ocean pout, eelpout or eel and determine the remaining binding to codfish or ocean pout by Maxisorp-RAST. Figure 2 shows the IgE-specific binding, to codfish (A) and ocean pout (B), of two representative sera preincubated with the four different fish extracts. All extracts were able, with dose-dependency, to inhibit the binding to codfish and ocean pout for all sera at least to some extent. Using extract dilution from 20 ng/ml to $200 \text{ } \mu\text{g/ml}$ an increasing inhibition of the binding to ocean pout up to at least 92% was observed using ocean pout (positive control) or eelpout as inhibitors. Using eel the inhibition was at least 82% except for three sera (32, 54 and 70%) and with codfish as inhibitor above 83%. When inhibiting the binding to codfish, preincubation with the same codfish extract gave at least 96% inhibition except with one sera which had a maximum at 78%. For the other three extracts the inhibition of the codfish-specific binding was at least 77% with the exception of; for ocean pout five sera (42, 58, 62, 68, and 71%); eelpout three sera (49, 73, and 75%) and

Table 1. CPM values for the IgE-specific binding to the fish extracts determined by Maxisorp-radio allergosorbent test (RAST) and the IC_{50} values for the inhibition of cod and ocean pout-specific binding for all 18 patients

Patient	Direct Maxisorp-RAST*					RAST inhibition of the IgE-binding to ocean pout					RAST inhibition of the IgE-binding to Cod				
	Cod	Ocean pout	Eelpout	Eel		IC_{50} (ng/ml)	Ocean pout	IC_{50} (ng/ml)	Eelpout	IC_{50} (ng/ml)	Cod	IC_{50} (ng/ml)	Ocean pout	IC_{50} (ng/ml)	Eel
1	1208 $\pm$ 72	340 $\pm$ 21	437 $\pm$ 27	505 $\pm$ 16		<20	+	<20	+	425	+	<20	+	<20	+
2	492 $\pm$ 3	373 $\pm$ 34	403 $\pm$ 3	338 $\pm$ 7		<20	+	<20	+	<20	+	<20	+	<20	+
3	885 $\pm$ 40	321 $\pm$ 6	678 $\pm$ 5	618 $\pm$ 3		<20	+	<20	+	<20	+	<20	+	<20	+
4	20 946 $\pm$ 67	11 935 $\pm$ 472	13 216 $\pm$ 175	8996 $\pm$ 89		277	+	68	+	91	+	296	+	562	+
5	686 $\pm$ 20	266 $\pm$ 17	370 $\pm$ 29	211 $\pm$ 20		<20	+	<20	+	<20	+	7.4 $\mu\text{g/ml}$	+	47 $\mu\text{g/ml}$	+
6	6903 $\pm$ 107	4551 $\pm$ 1112	1937 $\pm$ 37	3777 $\pm$ 92		28	+	<20	+	<20	+	3.1 $\mu\text{g/ml}$	+	100	+
7	2958 $\pm$ 123	2200 $\pm$ 161	2488 $\pm$ 18	2052 $\pm$ 71		<20	+	<20	+	316	+	13 $\mu\text{g/ml}$	+	63 $\mu\text{g/ml}$	+
8	7241 $\pm$ 612	5814 $\pm$ 325	5300 $\pm$ 165	3087 $\pm$ 150		<20	+	<20	+	<20	+	<20	+	<20	+
9	646 $\pm$ 12	336 $\pm$ 42	362 $\pm$ 30	417 $\pm$ 10		<20	+	<20	+	<20	+	<20	+	<20	+
10	14 160 $\pm$ 5	12 104 $\pm$ 683	13 603 $\pm$ 471	3006 $\pm$ 337		220	+	74	+	20	+	34	+	73	+
11	8476 $\pm$ 325	2705 $\pm$ 18	2456 $\pm$ 155	1359 $\pm$ 20		<20	+	<20	+	200	+	>200 $\mu\text{g/ml}$	+	>200 $\mu\text{g/ml}$	+
12	1178 $\pm$ 3	657 $\pm$ 18	535 $\pm$ 25	346 $\pm$ 36		<20	+	<20	+	349	+	688	+	>200 $\mu\text{g/ml}$	+
13	3434 $\pm$ 147	2167 $\pm$ 122	2345 $\pm$ 98	1089 $\pm$ 51		<20	+	<20	+	181	+	25 $\mu\text{g/ml}$	+	20	+
14	5310 $\pm$ 432	2858 $\pm$ 243	748 $\pm$ 143	695 $\pm$ 60		<20	+	<20	+	121	+	<20	+	94	+
15	9443 $\pm$ 315	7539 $\pm$ 570	2134 $\pm$ 300	885 $\pm$ 22		52	+	396	+	52	+	33	+	20	+
16	4191 $\pm$ 246	1369 $\pm$ 28	348 $\pm$ 20	308 $\pm$ 6		44	+	331	+	44	+	100	+	158	+
17	12 712 $\pm$ 593	11 785 $\pm$ 368	5728 $\pm$ 333	3701 $\pm$ 46		36	+	193	+	55	+	1 $\mu\text{g/ml}$	+	422	+
18	5419 $\pm$ 101	1486 $\pm$ 32	1499 $\pm$ 27	2729 $\pm$ 2		<20	+	<20	+	<20	+	11 $\mu\text{g/ml}$	+	25	+
						91	+	62	+	<20	+	3.4 $\mu\text{g/ml}$	+	88	+

* Counts per minute (CPM) values for sera diluted 10 times. IC_{50} concentration used for 50% inhibition.

Table 2. Detection of specific IgE to codfish using CAP

Patient	CAP (kUA/l)
1	2.0
2	1.3
3	1.4
4	>100
5	1.8
6	34.8
7	7.2
8	24.3
9	1.5
10	71.5
11	24.6
12	3.4
13	8.8
14	17.9
15	75.0
16	11.1
17	66.8
18	8.7

Table 3. The mean weal areas of two pricks with fresh eel, eelpout, ocean pout and commercial codfish-extract

Patient	Skin prick test (SPT, mm ²)			
	Codfish	Eel	Eelpout	Ocean pout
1	180 ± 15	292 ± 97	323 ± 21	285 ± 115
2	62 ± 4	61 ± 17	81 ± 41	87 ± 3
3	93 ± 42	118 ± 36	167 ± 124	80 ± 17
4	100 ± 1	ND	ND	ND
5	135 ± 23	105 ± 18	79 ± 32	82 ± 70
6	60 ± 5	287 ± 84	189 ± 34	262 ± 30
7	221 ± 28	111 ± 46	161 ± 40	195 ± 26
8	214 ± 0	173 ± 57	235 ± 48	159 ± 78
9	91 ± 18	57 ± 9	247 ± 10	64 ± 0
10	138 ± 72	322 ± 238	270 ± 180	355 ± 35
11	543 ± 231	1565 ± 733	787 ± 576	352 ± 196
12	211 ± 124	140 ± 52	139 ± 37	114 ± 27
13	276 ± 10	162 ± 55	263 ± 51	214 ± 37
14	113 ± 13	133 ± 14	295 ± 96	314 ± 0
15	134 ± 78	291 ± 32	203 ± 114	287 ± 156
16	106 ± 38	202 ± 36	149 ± 23	214 ± 0
17	170 ± 81	130 ± 46	201 ± 96	100 ± 19
18	79 ± 22	179 ± 106	76 ± 27	75 ± 5

eel five sera (32, 42, 56, 57, and 59%). The IC₅₀ values for all sera and extracts are shown in Table 1. For patient no. 7, who was negative in HR with ocean pout and eelpout, the cross-reactivity between codfish and the other three species and between ocean pout and eel seems low in regard to the high IC₅₀ values. Patient nos 10 and 15 who had lower amounts of specific IgE to eel compared with the other fishes also show a low cross-reactivity between eel and both codfish and ocean pout. For patient no. 18 the opposite is true, meaning that the RAST value for eel is higher compared with the other species and the cross-reactivity to codfish is higher. To get a clearer

Table 4. Histamine release results (class C1–C6) for all 18 patients to the different fish species

Patient	HR			
	Codfish	Eel	Eelpout	Ocean pout
1	C3*	C8	C9	C9
2	C3	C6	C5	C5
3	C2	C4	C5	C5
4	C3	C7	C8	C9
5	C3	C3	C4	C5
6	C3	C6	C7	C8
7	C3	–	–	C1
8	C3	C6	C6	C8
9	C3	C4	C4	C5
10	C3	C9	C9	C9
11	C3	C7	C6	C8
12	C2	C3	C4	C4
13	C3	C3	C3	C4
14	C3	C5	C5	C5
15	C3	C7	C8	C9
16	C3	C6	C6	C8
17	C1	–	–	C1
18	C2	C5	C5	C7

* C1–C6, class corresponding to the lowest dilution inducing release at >15 ng histamine/ml blood. 3.5-fold dilutions of a 10% (w/v) stock solution.

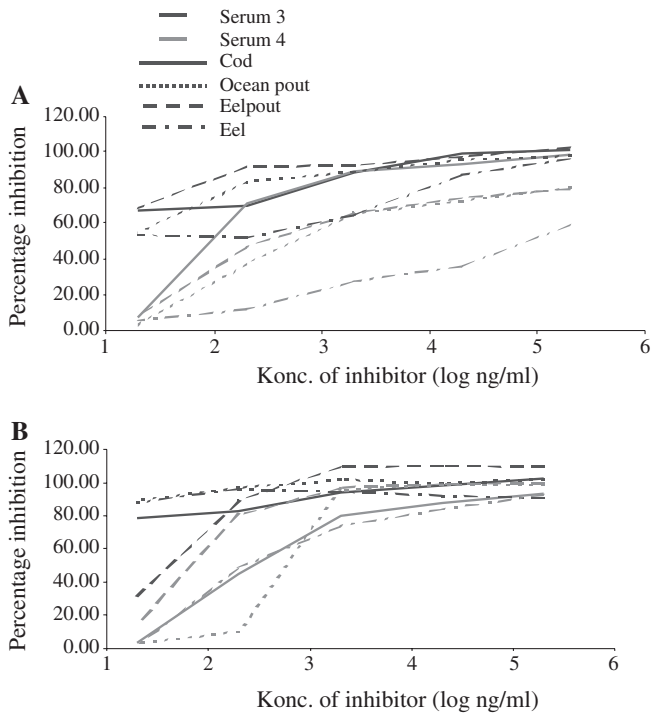


Figure 2. Inhibition of the (A) codfish-specific and (B) ocean pout-specific IgE-binding of serum nos 3 and 4 using Maxisorp-radio allergosorbent test (RAST).

picture of the cross-reactivity, devoid of the differences in cpm values between sera, the IC₅₀ values were plotted in six figures (Fig. 3A–F). The sera were divided into classes

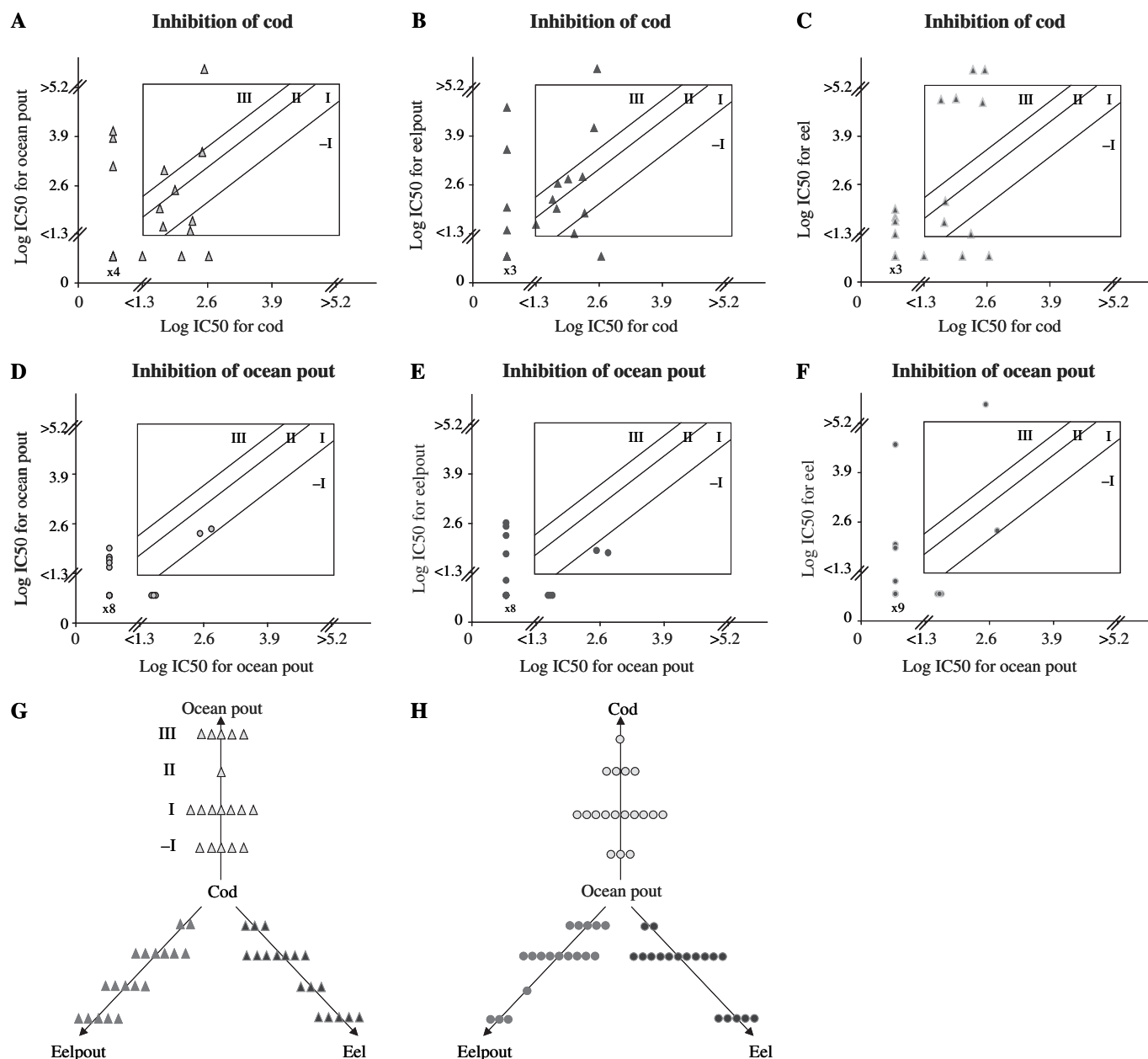


Figure 3. Plots of the IC₅₀ values for the inhibitions (A–F). The sera are divided into classes (–I–III), depending on the cross-reactivity between the two extracts, indicated in the graphs as lines. The serum classification are exhibited in G (inhibition to codfish) and H (inhibition to ocean pout) where each dot represents one sera.

dependent on the IC₅₀ values for each fish divided by the IC₅₀ for the extract used for coating (codfish A–C or ocean pout D–F; class –I < 1/3; I = 1/3–3; II = 3–10; III > 10). Sera with lower class shows higher cross-reactivity. In the figures, values in the box fall within the detection range set by the concentrations of the inhibitor. The number of sera in class –I–III are plotted in Fig. 3G and H, where the extract in the middle is the one to which the IgE-specific binding were inhibited. In general, the cross-reactivity between ocean pout and the rest seems larger compared with the cross-reactivity of codfish.

To compare the four extracts the proteins were separated on a 16% SDS-PAGE gel which were stained with GelCode® Blue (Fig. 4A). No major differences were seen except for a protein at 17 kDa which was missing in the eel extract. In Western blot, detecting IgE binding to the extracts, four bands appeared at approximately 12, 17, 40 and 55 kDa (Fig. 4B). One sera only bound to the protein at 40 kDa (patient no. 5) and five sera only to the protein at 12 kDa (Gad c1) (see patient no. 11 Fig. 3B) while the rest bound to two or three proteins in different combinations. Two sera did not bind to Gad c1 (patient

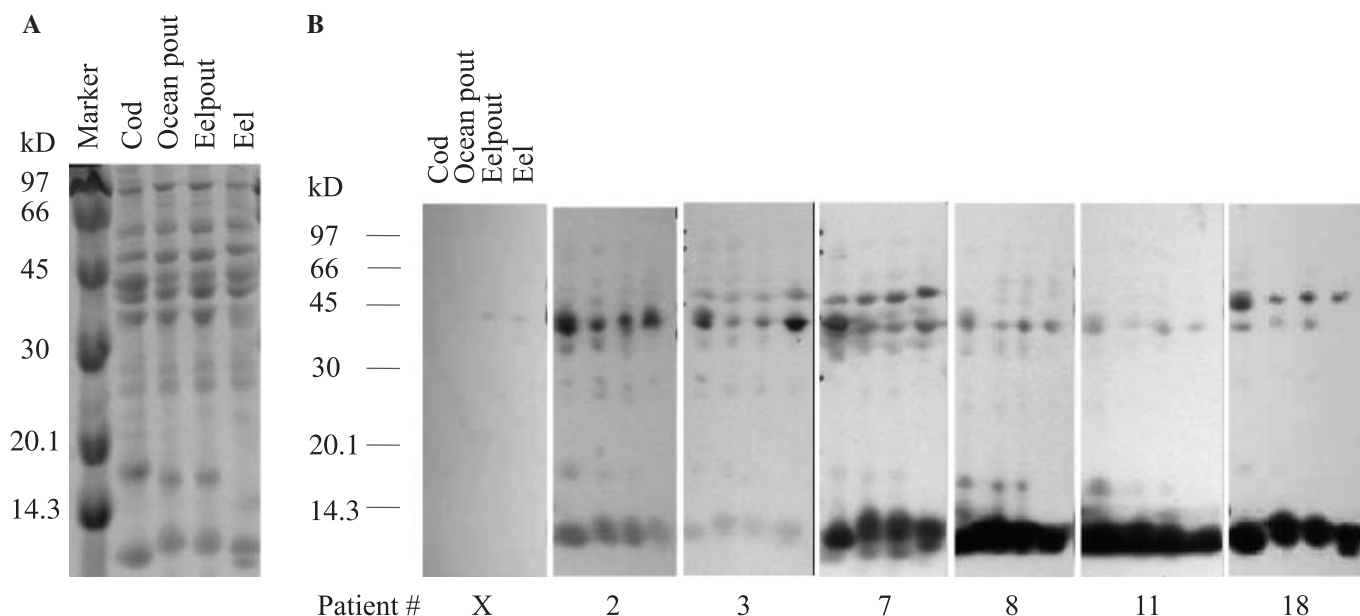


Figure 4. (A) Protein staining of codfish, ocean pout, eelpout and eel extracts run on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and (B) immunoblots detecting specific IgE in six representative patients serum to the same extracts. X is a pool of sera from nonallergic patients.

nos 3 and 5). No differences in RAST inhibition pattern could be detected for these two. The only difference seen comparing the binding to the four extracts on behalf of each sera were the missing band at 17 kDa in eel. Five of the sera were preincubated, with the four extracts respectively, before immunoblotting. Using 2 mg extract/ml most of the IgE-binding to all extracts was inhibited apart from some binding to the 40 kDa protein. Three other sera were preincubated only with cod with the same result.

Discussion

We have evaluated the biological reactivity to ocean pout, eelpout and eel in codfish allergic patients and investigated the serological cross-reactivity using RAST inhibition and Western blot analysis. The results of the RAST, SPT and HR show that there is a high serological and biological sensitivity to other fish species in these patients. It is, however, not advisable to exclude all fish species from the diet based on a single positive test result to codfish as SPT, and *in vitro* evidence of IgE-specific cross-reactivity not necessarily correlate with symptomatic fish allergy. Despite a 90–97% specificity, a positive HR, SPT and/or RAST result is only suggestive of the presence of clinical reactions (10, 19, 20). The predictive accuracy of SPT and RAST compared with DBPCFC has, in fish allergic adults, been shown to be 84% and 78% respectively (7). A negative SPT is, however, considered to be an excellent means of ruling IgE-mediated food allergies

out. Sampson et al. (20) have shown that it is possible to identify a subset of patients with fish-specific IgE Ab levels >95% positive predictive value who are extremely likely to experience a clinical reaction if they ingest fish. Despite this, it is in most cases necessary to perform a DBPCFC, not only to be sure of a clinical reaction but also to determine the threshold dose and the severity of symptoms as these do not correlate with the level of specific IgE (7, 16, 19–21).

It has been reported that differences in cross-reactivity patterns, such as those seen in our study, might be of importance when it comes to clinical symptoms. Gad c1, a 12 kDa protein, is the major codfish allergen and it has been suggested that patients with specific IgE to Gad c1 are more likely to react upon ingestion of other fish species which contain Gad c1 homologues (2, 5, 6, 16, 22, 23). In a study by Bugajska-Schretter et al. (24) where the cross-reactivity between codfish and five other fish species was studied, Gad c1 was found to be the major cross-reactive allergen. In eel, Gad c1 was the only IgE-binding protein in three of four patients, shown by Western blot. For these three, of which two had clinical symptoms, preadsorption with codfish protein gave at least 80% inhibition while it in the fourth case was 50%. This patient also had specific IgE to a protein at 66 kDa which was not inhibited. In our study no correlation between the band pattern and the IC_{50} values could be seen. Neither was any major differences seen in the inhibition pattern when the sera were preincubated with the different extracts before immunoblotting. Most of the IgE-binding was inhibited except for some of the binding to the 40 kDa protein. Reports of monosensitivity to one

fish species indicate that these patients do not have IgE-specific to Gad c1. Monosensitivity to swordfish has been shown to be due to a species-specific allergen at 25 kDa (8) and monospecific allergy to tropical sole was associated with IgE-binding to proteins at 6–7 and 40 kDa (6). The allergen found at 40 kDa in this study as well as in our might be identical to the 41 kDa protein, homologous to an aldehyde phosphate dehydrogenase, reported by Das Dores et al. (25) to be an allergen to which four of 13 codfish-allergic patients had specific IgE. It seems that monosensitized patients are less likely also to react in skin tests to other species (5, 6, 8). Our patients were all highly reactive in the skin to all species which indicates that they are not monosensitized. In addition, most of them had reactivity to Gad c1. This is, however, not a particularly suitable evidence in the light of the fact that patients, despite clinical symptoms to one or more fish species, have been able to consume other fish species without adverse reactions and that the reactions at least in part were related to Gad c1. It should be noted that the two patients without any Gad c1 specific IgE had skin reactions to codfish in the same range as to the other three species.

The higher cpm values for codfish, seen in this study, compared with the rest of the species could be a result of differences in the extracts although the protein content were determined and adjusted and no major differences were seen when the extracts were run on SDS-PAGE. In the more biological assays, e.g. SPT and HR, the reactions to codfish were not larger but on the contrary in many cases smaller. The results of the SPT should, however, be seen in the light of the circumstance that the tests with codfish were performed with extract while the

others were performed with fresh fish. The cross-reactivity to codfish seems, in reference to the RAST inhibition results, to be higher compared with the cross-reactivity to ocean pout. This might although reflect the fact that these patients were selected for being allergic to codfish as well as the unlikelihood that they have ever eaten ocean pout. Bernhisel-Broadbent (5) noted that the IC₅₀ values for fish to which the patients had clinical symptoms were similar compared with fish to which they were clinically tolerant.

Despite that no large differences regarding the IgE-binding could be detected in Western blot the inhibition with eel are in some cases lower compared with the other fish species. In a study with codfish allergic children that had never eaten eel, the inhibition to eel was found to be as high as 89%. The concentration of the inhibitor was, however, higher (up to 2 mg/ml) in this study compared with the present study (up to 0.2 mg/ml) (26). In the study by Bugajska-Schretter (24) referred to above the inhibition results were more similar to ours. Ocean pout and eelpout are more closely related compared with eel which might influence the protein content of the different fish species.

We demonstrate that patients with a confirmed allergy to one fish species in a high frequency show biological cross-reactivity to other fish species according to RAST, SPT, HR and Western blot results.

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