

Allergy to different fish species in cod-allergic children: In vivo and in vitro studies

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The presence of a positive clinical history and skin test (ST) results for 17 fish species (anchovy, bass, carp, dogfish, eel, gilthead, mackerel, mullet, perch, red mullet, salmon, sardine, sole, tench, toothed gilthead, trout, and tuna) were investigated in 20 children with cod-positive clinical history, ST, and RAST, and in 40 children positive to one or more foods different from cod (cow's milk, chicken egg white, peanut, and tomato). In cod-positive children, positive clinical history (60%) and ST (85%) to fish species were more frequent than in cod-negative children (7.5% and 10% respectively). In cod-positive children, a high frequency of positive STs to eel (85%) and to bass, dentex, sole, and tuna (55%) was observed. Positivity to dogfish (10%) was the least frequent. RAST-inhibition experiments suggested the presence of cross-reacting antigen(s) in cod, bass, dentex, eel, sole, and tuna. Results of this study demonstrate that cod allergy might be, on the whole, a reliable index of fish allergy, but cod-positive children may perhaps tolerate some other species, which will have to be tested for possible inclusion in their diet. (J ALLERGY CLIN IMMUNOL 1990;86:909-14.)

Allergy to cod in children is not infrequent in fish-eating or fish-processing populations.¹⁻³ In our areas, we found IgE antibodies to cod in 18% of children with food allergy, two thirds of whom had clinical manifestations when cod was ingested or inhaled.^{4,5} The major cod allergen (allergen M) has been purified, and its role in determining allergic reactions in sensitive subjects has been clearly established.³

By contrast, little information is available on allergy to other fish species. Investigations performed some decades earlier led to conflicting results demonstrating a wide range of species specificity with varying patterns of cross-reactions.⁶⁻⁹ Most studies on fish allergy refer either exclusively to cod^{1,2} or generically to fish,^{10,11} which is surprising, since fish is by far the most heterogeneous group of recent vertebrates.¹² Testing for cod and advising positive patients to avoid

Abbreviation used

ST: Skin test

all fish is a common practical approach in many clinics. In the case of some botanical species, the practice of automatically placing patients allergic to one member of a food family on a diet avoiding all the members has already been refuted.¹³ The purpose of this study was to ascertain whether cod-allergic children have increased frequency of positive STs to other fish in the view of determining which species might be included in the diet of these children.

MATERIAL AND METHODS

Children

Sixty children were selected for this study. The patients had been referred for allergic disorders and had undergone routine STs for inhalant and food allergens. Foods routinely tested included cow's milk, chicken egg white, peanut, tomato, and cod (*Gadus morrhua*). The glycerin-preserved, food-allergen crude extracts, diluted 1:20 (wt/vol), were obtained from a commercial supplier (Hollister-Stier Laboratories, Spokane, Wash.).

Twenty children (17 male and three female children, aged 2 to 8 years; mean age, 5.1 years) had positive ST to cod, and this positivity closely correlated with clinical history, since one or more adverse reactions were clearly reported in all patients: urticaria-angioedema (8/20, 40%) worsening

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TABLE I. Children with positive STs to fish species

Fish species	Cod-positive children (n = 20) (%)	Cod-negative children (n = 40)
Eel	17 (85)	0
Bass	11 (55)	0
Dentex	11 (55)	1 (2.5%)
Sole	11 (55)	3 (7.5%)
Tuna	11 (55)	2 (5%)
Perch	8 (40)	0
Tench	8 (40)	0
Anchovy	7 (35)	0
Red mullet	7 (35)	0
Trout	7 (35)	0
Mullet	6 (30)	0
Carp	5 (25)	0
Gilthead	4 (20)	0
Mackerel	4 (20)	0
Salmon	4 (20)	0
Sardine	4 (20)	0
Dogfish	2 (10)	0

in atopic dermatitis (7/20, 35%), asthma (7/20, 35%), or vomiting after ingestion (5/20, 20%) when cod was ingested or inhaled.

Forty children (29 male and 11 female children, aged 2 to 8 years; mean age, 4.6 years) had positive ST and RAST to one or more foods (among that routinely tested) different from cod. They had been selected irrespective of referred adverse reactions after ingesting or inhaling fish. All patients had manifested adverse reactions after ingesting the food(s) to which they were ST and RAST positive.

In all patients, adverse reactions had occurred on at least two occasions, one occasion was within 4 months before the initiation of the study.

Parental consent was obtained for all children who entered the study, which was approved by a human safety committee.

Skin testing

STs were performed by an epicutaneous (prick) method on the palmar surface of the forearm, with 1 mm prick lancets (Hollister-Stier Laboratories). Both a positive (histamine dihydrochloride, 1 mg/ml) and a negative (allergen diluting fluid) control were included. Seventeen fish species—allergen crude extracts, obtained from a commercial supplier (Lofarma, Milan, Italy), were used: anchovy (*Engraulis encrasicolus*), bass (*Dicentrarchus labrax*), carp (*Cyprinus carpio*), dogfish (*Mustelus plebeius*), eel (*Anguilla anguilla*), gilthead (*Chrysophrys aurata*), mackerel (*Scomber scomber*), mullet (*Mugil cephalus*), perch (*Perca fluviatilis*), red mullet (*Mullus barbatus*), salmon (*Salmo solar*), sardine (*Sardina pilchardus*), tench (*Tinca tinca*), toothed gilthead (*Dentex dentex*), trout (*Salmo trutta*), tuna (*Thunnus thynnus*), and sole (*Solea vulgaris*).

TABLE II. RAST-inhibition experiments

Antigen coupled to RAST sphere	Inhibiting antigen (1 mg/ml)	% RAST inhibition (mean)
Bass	Cod	71.5
	Eel	70.7
	Dentex	53.1
	Sole	51.4
	Tuna	36.0
Cod	Eel	88.9
	Bass	74.7
	Dentex	63.4
	Sole	51.2
	Tuna	35.0
Dentex	Cod	86.1
	Eel	85.3
	Bass	85.3
	Sole	50.8
	Tuna	39.1
Eel	Cod	89.7
	Bass	86.4
	Dentex	81.7
	Sole	66.9
	Tuna	51.2
Sole	Eel	80.8
	Bass	79.8
	Cod	79.1
	Dentex	76.6
	Tuna	32.0
Tuna	Dentex	77.4
	Bass	64.9
	Sole	60.1
	Cod	55.9
	Eel	50.8

The fish-allergen extracts were diluted 1:20 (wt/vol) in glycerol solution. The most common fish in the normal diet in Italy are included among the species tested.¹⁴

Results were recorded after 10 minutes for positive and negative control solutions and after 15 minutes for allergen extracts. Two perpendicular diameters of the wheals were recorded, and their mean was calculated. The reactions of half or more of the size of the positive histamine reference reaction were considered positive.¹⁵ When (on one occasion) reaction occurred to the negative control, it was subtracted from the size of the fish-allergen reactions for subsequent analysis. Any medication was withdrawn at least 10 days before skin testing was performed.

A second ST was performed 1 year after the first evaluation in 18 cod-positive and in 33 cod-negative children.

Total and specific IgE levels

A radioimmunoassay technique was used according to the test procedure instructions to determine total (paper radioimmunosorbent test, Pharmacia Diagnostics AB, Upp-

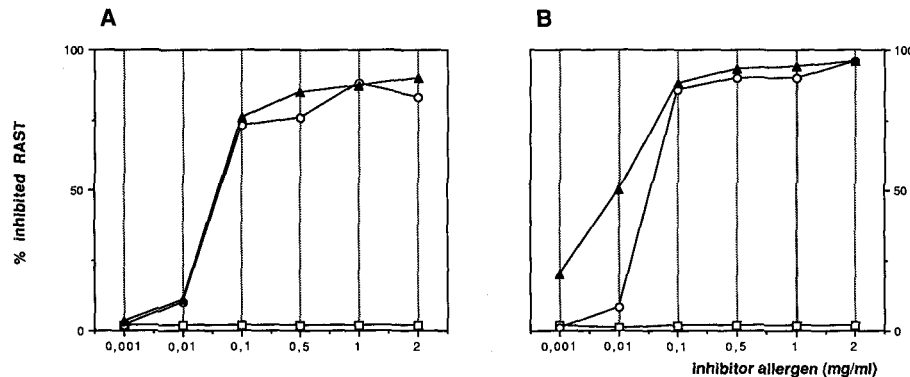


FIG. 1. RAST-inhibition experiments. **A**, RAST inhibition of RAST to cod by different dilutions of cod (▲), *D. pteronyssinus* (□), and eel (○) extracts. **B**, RAST inhibition of RAST to eel by different dilutions of eel (▲), *D. pteronyssinus* (□), and cod (○) extracts.

sala, Sweden) and specific IgE (RAST) levels to fish species (cod, bass, dentex, dogfish, eel, sole, and tuna) studied with commercially available kits (Sferi kit, Lofarma). Results were calculated as counts per minute and, according to the reference standards, positive sera bound $>2.7\%$ (i.e., >1315 cpm) added radioactivity (48,700 cpm). To rule out the possibility of a nonspecific IgE binding to fish allergens, RAST to fish species was also performed on five additional sera from children with high IgE levels (>1000 IU/L). These patients were ST negative to fish, but ST and RAST positive to inhalant allergens (*Dermatophagoides pteronyssinus* and grass pollen).

RAST-inhibition experiments

RAST-inhibition experiments were performed as were previously described.¹⁶ In brief, 20 μ l of serum and 30 μ l of saline (control test) or of the inhibiting allergen solution in saline (sample test) were incubated for 3 hours at room temperature. Thereafter, a RAST sphere was added, and the assay was continued with the standard RAST technique. Fish-allergen crude extracts (Lofarma) were used in six different dilutions (2, 1, 0.5, 0.1, 0.01, and 0.001 mg/ml). Reciprocal RAST-inhibition experiments were performed with bass, cod, dentex, dogfish, eel, sole, and tuna allergens. All sera RAST positive to both the inhibiting allergen and the allergen coupled to RAST sphere were studied. From seven to 17 sera (according to different combinations) were studied, with the exception of experiments with dogfish allergens, which were carried out on two sera.

In control experiments, sera of cod-allergic children were incubated with six different dilutions (2, 1, 0.5, 0.1, 0.01, and 0.001 mg/ml in saline) of *Dermatophagoides pteronyssinus* extracts (Pharmacia Diagnostics AB,) or of the same fish extract as extract coupled to the RAST sphere.

Results were calculated as counts per minute, and inhibition was expressed as $1 - \text{cpm sample test} / \text{cpm control} \times 100\%$.

Clinical history

Parents were questioned carefully whether their children (1) had eaten any of the fish studied and (2) had manifested

asthma, urticaria-angioedema, vomiting, or worsening of atopic dermatitis when they had eaten any of the fish studied.

Statistical methods

The chi-square test was used for differences between proportions. Differences in allergen-specific IgE levels were analyzed by Student's *t* test after $\log_{10}$ transformation of data. Correlations between variables were determined by calculation of linear regression (*r*).

RESULTS

Positive STs

The number of subjects with positive ST to one or more fish species was significantly ($p < 0.00001$) higher in children with allergy to cod (17/20, 85%) than in children negative to cod (4/40, 10%). In Table I, ST positivities to single fish species are reported. Positivity was recorded in 123 (36.1%) of the 340 STs performed on cod-positive children, but only in six (0.88%) of the 680 STs performed on cod-negative children ($p < 0.00001$). Cod-positive and cod-negative patients did not differ as to response to histamine positive control (mean $\pm$ SD of diameters, 7.6 ± 2.1 and 7.4 ± 1.9 mm, respectively).

Reproducibility of STs to fish

In 18 cod-positive and in 33 cod-negative children, ST to fish species were performed 1 year after the first evaluation. In no subject was modification in ST results observed.

Total and specific IgE levels

RAST to cod, bass, dentex, dogfish, eel, sole, and tuna confirmed results of ST in all children. Total IgE levels correlated to levels of specific IgE to cod ($r = 0.569$; $p < 0.01$) and eel ($r = 0.581$; $p < 0.01$). Levels of specific IgE to cod and eel correlated directly ($r = 0.577$; $p < 0.01$). No other correlation

TABLE III. Children with adverse reactions to fish species

Fish species	Sure history of			
	Having eaten that fish		Reactions to that fish	
	Cod-positive (n = 20) (%)	Cod-negative (n = 40) (%)	Cod-positive (n = 20) (%)	Cod-negative (n = 40)
Dogfish	20 (100)	40 (100)	0	0
Sole	20 (100)	40 (100)	11 (55%)*	2 (5%)*
Tuna	20 (100)	40 (100)	7 (35%)†	1 (2.5%)†
Dentex	4 (20)	9 (22.5)	1 (5%)	0
Mackerel	4 (20)	10 (25)	1 (5%)	0
Anchovy	3 (15)	8 (25)	1 (5%)	0
Sardine	2 (10)	7 (17.5)	1 (5%)	0
Red mullet	1 (5)	5 (12.5)	1 (5%)	0
Salmon	1 (5)	3 (7.5)	1 (5%)	0

* $p < 0.00001$.† $p < 0.001$.

in IgE levels to other fish species studied by RAST was observed.

RAST to fish species studied was constantly negative in control experiments performed on sera with high IgE levels from fish ST-negative children.

RAST-inhibition experiments

In control experiments, *D. pteronyssinus* extracts (at all dilutions) did not affect RAST to fish species studied (inhibition constantly <5%). The presence of cross-reacting antigen(s) among cod, bass, dentex, eel, sole, and tuna was suggested by results of RAST-inhibition experiments (Table II). In all cases, a statistically significant difference ($p < 0.05$) was observed between means $\pm$ SD of sample and control tests. A dose-dependent fashion of RAST-inhibition experiments was clearly present. In all different combinations of reciprocal RAST-inhibition experiments, an increasing inhibition from at least 73% up to 96% was observed when extract dilutions from 0.01 to 2 mg/ml were used. In Fig. 1, results of RAST-inhibition experiments between cod and eel extracts are illustrated. Results of positive (the same extract as that coupled to the RAST sphere) and negative (*D. pteronyssinus* extract) control experiments are also reported. In all combinations, a strictly comparable behavior was observed.

Experiments with dogfish extracts, performed on sera of two positive children, demonstrated inhibition (>85%) of dogfish RAST by cod, bass, dentex, eel, and sole allergens, whereas no effect was observed when tuna allergens were used. Dogfish allergens did not affect RAST to cod, bass, dentex, eel, sole, or tuna (inhibition, <4%).

Clinical history

All children (both cod positive and cod negative) were reported to have eaten fish on several occasions. All children had eaten cod, sole, tuna, and dogfish, and a portion of children were reported definitely to have eaten anchovy, dentex, mackerel, red mullet, sardine, and salmon (Table III). As regarding the remaining fish species, information was unreliable, but all parents consistently denied their children had ever eaten eel. The number of referred adverse reactions to fish is reported in Table III. In all cases, adverse reactions to a single fish species occurred in children with positive ST to that fish. Frequency of adverse reactions was significantly ($p < 0.00001$) higher in cod-positive (12/20, 60%) than in cod-negative children (3/40, 7.5%). Of the total of 29 adverse reactions that were reported in cod-positive children, urticaria-angioedema was experienced in 11 cases (37.9%), vomiting in seven cases (24.1%), asthma in six cases (20.6%), and worsening in atopic dermatitis in five cases (17.2%). No association was observed between the fish species ingested and single adverse reactions.

DISCUSSION

Results of the present study demonstrate that children with allergy to cod (i.e., patients with positive ST and clinical history to this fish) manifest a high frequency of positive ST to other fish species, most of which are common in dietary habits in Italy.¹⁴ Frequency was high, even when the frequency was compared with that observed in a highly selected population, such as children who were cod negative but affected by allergy to other food(s). Therefore, considering the close association of cod allergy and pos-

itive ST to different fish species, the low frequency of these positivities in children with allergy to other food(s) and the estimated incidence of food allergy in general allergy practice,¹³ it may be suggested that cod skin testing is a useful approach to fish allergy in a general allergist's clinical practice.

Besides the increased frequency of positive ST to fish species, a high frequency of adverse reactions to species other than cod was recorded in cod-positive children. Therefore, it is possible that the increased sensitivity to fish has clinical relevance in these patients, but caution must be used in interpreting data of this retrospective inquiry, since it is possible that parents of cod-allergic children remember reactions to fish more readily than parents of children who are not allergic to cod.

Cod-allergic children were not uniformly ST positive to all the fish species tested. A strong association was present between cod allergy and positive ST to bass, dentex, eel, sole, and tuna, but association with other species was lower, and only a minority of children were positive to dogfish. Thus, a corollary of our study is that, in cod-allergic children, to counsel a diet avoiding all fish may be incorrect without having tested other fish species that could perhaps be eaten safely. Aas⁹ observed that fish-allergic children are not uniformly sensitive to all species, and about half of these patients can tolerate one or more species. Similar conclusions have been reached in studies on legume allergy, which have demonstrated that hypersensitivity to one species does not automatically warrant dietary elimination of all legumes.¹³ These findings oppose the theory of food families that states that foods share antigenic similarities (and consequently, that crossed allergic reactions would occur) within taxonomically related botanical or animal species.^{17, 18} Exceptions in this rule may have relevance in managing diets, since the less foods that are eliminated, the more likely patients will be to comply with dietary restrictions and to maintain optimal nutrition.¹³ However, as to fish allergy, differences that have been reported⁹ in the allergenic activity of extracts from the same fish species produced in different laboratories must be considered. Consequently, caution must be used when ST results are being interpreted, the clinical relevance of which can be correctly assayed only by double-blind, placebo-controlled challenges.^{4, 5, 13}

Results of RAST-inhibition experiments suggest the possibility that some cross-reacting antigen(s) are present in cod, bass, dentex, eel, sole, and tuna. The presence of cross-reacting antigens is well-known in vegetables,^{16, 19} as well as in animals, such as mollusca, artropoda, birds, and mammals.²⁰⁻²³ In fish, varying patterns of cross-reactions have been re-

ported,⁶⁻⁹ but only immunoblotting methods¹⁹ can clarify the antigenic features of these common structures. There is a possibility that cod antigen M³ is at least one of the cross-reacting antigens, since a protein from carp very similar to antigen M has been already characterized.^{3, 24} The presence of cross-reacting antigens may have a role in sensitization, since in cod-allergic children, a high frequency of positive ST to eel was observed, even though parents resolutely denied their children had ever eaten this fish. In contrast, antigens of cod, bass, dentex, and sole were good inhibitors of IgE to dogfish, but not vice versa. According to other models,^{16, 19} it is possible that the common structure is a major allergen in dogfish, but a minor allergen in other fish. It is known that cod, bass, dentex, eel, and tuna belong to the *Actinopterygii* subclass, whereas these fish and dogfish zoologically belong to the remote *Osteichthyes* and *Chondrichthyes* classes.¹²

This study provides preliminary information on allergy to different fish species in children with allergy to cod. Skin testing for cod allergy is confirmed as a good approach to the diagnosis of allergy to fish, but it is suggested that cod-allergic children may not be sensitive to, and may tolerate, other fish species, particularly dogfish. Additional studies are required to define the patterns of common structures in edible fish.

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