

IgE antibody to fish gelatin (type I collagen) in patients with fish allergy

Masahiro Sakaguchi, PhD,^a Masako Toda, PhD,^a Testuya Ebihara, MS,^b Shinkichi Irie, PhD,^b Hisae Hori, PhD,^c Atsushi Imai, DVM,^d Makoto Yanagida, PhD,^d Hiroshi Miyazawa, PhD,^e Hiroyuki Ohsuna, MD,^f Zenro Ikezawa, MD,^f and Sakae Inouye, MD^g Tokyo, Gunma, and Yokohama, Japan

Background: Most children with anaphylaxis to measles, mumps, and rubella vaccines had shown sensitivity to bovine gelatin that was included in the vaccines. Recently, it was found that bovine type I collagen, which is the main content in the gelatin, is a major allergen in bovine gelatin allergy. Fish meat and skin also contain type I collagen.

Objective: The present study was designed to investigate IgE antibody to fish gelatin in children with fish allergy.

Methods: Serum samples were taken from patients in 3 groups: (1) 10 patients with fish allergy and specific IgE to fish meat; (2) two patients with allergies to both fish meat and bovine gelatin and specific IgE to fish meat and bovine gelatin; and (3) 15 patients with atopic dermatitis and specific IgE to fish meat. Various fish gelatins (type I collagen) were prepared from fish skin. IgE antibody to fish gelatin was analyzed by using ELISA and immunoblotting.

Results: Of 10 patients with fish allergy, 3 had specific IgE to fish gelatin. Of two patients with fish allergy and bovine gelatin allergy, all had specific IgE to fish gelatin. Of 15 patients with atopic dermatitis and specific IgE to fish meat, 5 had specific IgE to fish gelatin. Furthermore, IgE from pooled serum of the patients reacted with both the $\alpha 1$ and $\alpha 2$ chains of fish type I collagen in immunoblots. There is cross-reactivity among gelatins from various fishes, but there is little cross-reactivity between fish and bovine gelatins.

Conclusion: Some fish-sensitive patients possessed IgE antibody to fish gelatin. Fish gelatin (type I collagen) might be an allergen in subjects with fish allergy. (*J Allergy Clin Immunol* 2000;106:579-84.)

Key words: Allergen, fish allergy, gelatin, IgE

Fish is one of the most commonly allergenic foods.¹ It has been reported that many fish cause food allergy.² The major codfish allergen, Gad c 1, has been purified and characterized.³ Other allergens have been shown in the fish extracts by immunoblotting with the sera of patients who have fish allergy.⁴⁻⁶ None of these allergens had been purified.

Kelso et al⁷ reported that a child with anaphylaxis to combined measles, mumps, and rubella vaccine had IgE antibody to gelatin. In Japan, most of the live vaccines had been shown to contain bovine gelatin.⁸ Vaccines available in Japan have not contained fish gelatin. Also, in previous studies⁹⁻¹¹ we found that most of the children who had the systemic immediate-type reactions, including anaphylaxis, to measles, mumps, and rubella vaccines had anti-gelatin IgE. Thus bovine gelatin included in the live vaccines is considered as the allergen.¹²⁻¹⁴ Anaphylactic reactions to recombinant human erythropoietin were reported.¹⁵ Recently, we found that bovine gelatin in erythropoietin products caused anaphylaxis.¹⁶ Furthermore, it has been reported that the gelatin causes food allergy.¹⁰

In our previous study we reported that IgE in bovine gelatin-sensitive children reacted with gelatin derived from various animals (eg, rat, mouse, chicken, codfish, salmon, and shark).¹⁷ We found that one of 10 children who had anti-bovine gelatin IgE also showed a strong IgE reactivity to fish gelatin and that there is no cross-reaction between fish and bovine gelatin.¹⁷ It is possible that fish gelatin might be one of the allergens in fish allergy.

The present study was designed to investigate the presence of specific IgE to fish gelatin in children with fish allergy.

METHODS

Patients

In this study patients were classified into 3 different groups: (1) 10 patients with fish allergy and specific IgE to fish meat (Table I); (2) two patients with allergies to both fish meat and bovine gelatin and specific IgE to fish meat and bovine gelatin (Table II); and (3) 15 patients with atopic dermatitis and specific IgE to fish meat (Table III). We measured specific IgE to cod and tuna meat in 65 patients with atopic dermatitis and selected 15 patients who had specific IgE to cod or tuna.

From ^athe Departments of Immunology and ^gInfectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo; ^bNippi Research Institute of Biomatrix, Tokyo; ^cthe Division of Adult Diseases, Medical Research Institute, Tokyo Medical and Dental University, Tokyo; ^dPharmaceutical Development Laboratory, Kirin Brewery Co Ltd, Gunma; ^ethe Department of Medical Technology, Kyorin University School of Health Science, Tokyo; and ^fthe Department of Dermatology, Yokohama City University School of Medicine, Yokohama.

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Reprint request: Masahiro Sakaguchi, PhD, Department of Immunology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan.

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TABLE I. Reactivity to fish meat and gelatin in patients with fish allergy

Patient No.	Age	Sex	Type of fish	Fish meat IgE*	Fish gelatin IgE†	Fish allergy symptom
1	8 y	M	Tuna	37.5	4.05	Urticaria, cough
			Saurel	127	4.82	
2	8 y	M	Tuna	3.60	6.30	Wheezing
3	26 y	M	Cod	0.50	0.45	Itching in mouth
			Saurel	0.45	0.58	
			Salmon	0.66	0.50	
4	28 y	F	Cod	18.9	<0.35	Urticaria, cough
			Salmon	21.0	<0.35	Stomach ache
5	40 y	M	Tuna	74.6	<0.35	Urticaria, hypotension, airway obstruction, diarrhea
6	1 y 4 m	M	Saurel	97.1	<0.35	Urticaria, angioedema
7	4 y	F	Salmon	2.17	<0.35	Urticaria
8	2 y 6 m	M	Salmon	1.27	<0.35	Urticaria
9	5 y	F	Cod	13.5	<0.35	Urticaria
10	4 y	F	Mackerel	1.94	<0.35	Urticaria

*CAP (Ua/mL).

†ELISA (U/mL).

TABLE II. Reactivity to fish meat and gelatin in patients with fish and bovine gelatin allergy

Patient No.	Age	Sex	Type of fish	Fish meat IgE*	Fish gelatin IgE†	Fish allergy symptoms	Bovine gelatin IgE*	Gelatin allergy symptoms
11	2 y 10 m	F	Cod	11.4	7.02	Urticaria	5.10	Urticaria to measles vaccine
			Salmon	4.50	6.23			
12	7 y	M	Cod	8.89	51.5	Urticaria, cough, wheezing, vomiting	3.14	Asthma to gelatin-containing food

*CAP (Ua/mL).

†ELISA (U/mL).

Gelatin

Gelatin was used after denaturation of type I collagen at 100°C for 10 minutes. Type I collagens from fish species (eg, tuna, salmon, saurel, and mackerel) and bovine gelatin were prepared from skin dermis by 0.5 mol/L acetic acid extraction and purified by differential salt precipitation.¹⁸ Identification and assessment of purity were routinely achieved by using SDS-PAGE (Fig 1).¹⁹

Bovine gelatin (Wako Pure Chemical Industries, Osaka, Japan) and cod gelatin (Sigma Chemicals, St Louis, Mo) were commercially purchased.

SDS-PAGE and immunoblot

SDS-PAGE was performed according to the procedure of Laemmli.²⁰ The sample was applied to SDS-PAGE (5% gel) under reducing conditions. The gel then was stained with Coomassie blue, or the proteins in the gel were transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, Calif). The membrane was rinsed in PBS and blocked with 1% BSA in PBS. After another rinse with PBS containing 0.02% Tween-20, it was cut into strips that were incubated overnight with the pooled serum of patients diluted 1:2. After washing, the strip was incubated with anti-human IgE antibody-conjugated alkaline phosphatase (Quidel, San Diego, Calif) overnight and stained with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium.

Measurement of specific IgE antibody to gelatin

The Pharmacia CAP system (Pharmacia, Uppsala, Sweden) was

used to determine the concentration (in units per milliliter) of IgE antibody to bovine gelatin and fish meat.⁹

Specific IgE to fish gelatin was determined by using a fluorometric ELISA, as described previously.^{10,21} Microplates were coated with each bovine or fish gelatin (10 µg/mL). After incubation overnight at 4°C, the serum sample was added to the wells. After incubation for 3 hours at room temperature, anti-human IgE antibody conjugated with β-D-galactosidase (Pharmacia) was added. The enzyme reaction substrate was 0.1 mmol/L 4-methylumbelliferyl-β-D-galactoside (Sigma Chemicals). Fluorescence units (FU) were measured on a fluorometric microplate reader (Fluoroskan; Flow Laboratory, McLean, Va). The levels of the specific IgE antibody were expressed as arbitrary units per milliliter. The units of each serum in the ELISA were calculated from a standard titration curve of the anti-bovine gelatin reference serum with known CAP units corresponding to the CAP values.²¹

Inhibition of gelatin-specific IgE

The antigenic cross-reactivity among fish and bovine gelatins was analyzed by using fluorometric ELISA inhibition, as previously described.^{16,21} Briefly, each kind of gelatin (5 µg/mL) was adsorbed to microplate wells. The serum was mixed with gelatin (100 µg/mL) as an inhibitor, and then the mixture was added to the wells. After 3 hours of incubation at room temperature, anti-human IgE antibody conjugated with β-D-galactosidase was added. The following procedures were the same as those in the abovementioned fluorometric ELISA. The percentage of inhibition was calculated as follows:

$$\left(1 - \frac{\text{FU in presence of gelatin}}{\text{FU in absence of gelatin}}\right) \times 100 = \% \text{ inhibition.}$$

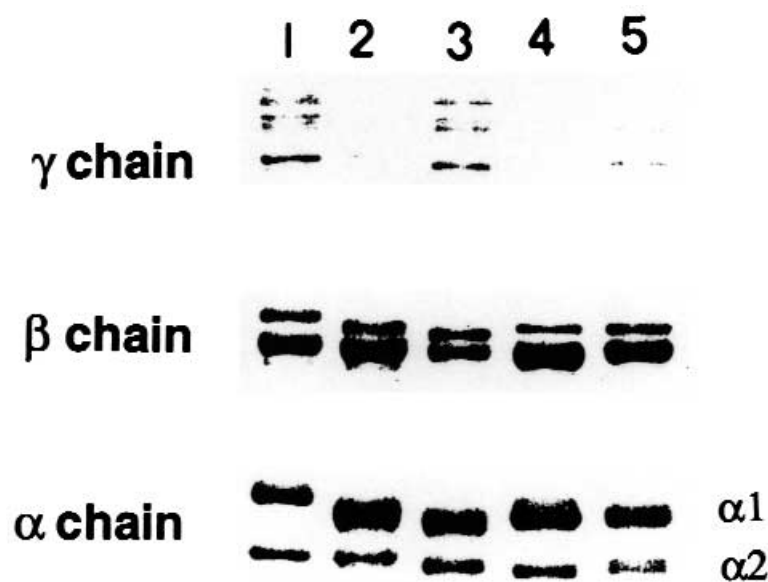


FIG 1. SDS-PAGE of denatured (100°C for 10 minutes) bovine and fish type I collagen. *Lane 1*, Bovine; *lane 2*, tuna; *lane 3*, salmon; *lane 4*, saurel; *lane 5*, mackerel. Type I collagens of salmon and mackerel are unique in having $\alpha 3$ as the third subunit and exist as a heterotrimer composed of $\alpha 1$, $\alpha 2$, and $\alpha 3$ chains.²⁹ Under electrophoretic conditions, in this study $\alpha 1$ and $\alpha 3$ chains show the same molecular weight.

RESULTS

Purification of type I collagen from bovine and fish skin

Each type I collagen was heat denatured at 100°C for 10 minutes and electrophoresed in 5% gel SDS-PAGE (Fig 1). Bovine type I collagen is a heterotrimer composed of two $\alpha 1$ chains and one $\alpha 2$ chain. The α chains from fish type I collagen showed two bands; the higher band was the $\alpha 1$ chain, and the lower band was the $\alpha 2$ chain. The β chains showed two bands; the higher band consisted of two $\alpha 1$ chains, and the lower band consisted of $\alpha 1$ and $\alpha 2$ chains. In spite of heat treatment and reduction, a small amount of γ chain in some fish was detected. Commercial bovine and cod gelatins showed smear bands (data not shown).

Specific IgE to fish meat and gelatin in patients with fish allergy

We measured specific IgE to fish meat and gelatin in 10 children with fish allergy. All the children had specific IgE to fish meat (Table I). Of 10 children, 3 had specific IgE to fish gelatin (Table I). None of the children had anti-bovine gelatin IgE (data not shown). IgE from pooled serum of two patients (patients 1 and 2) reacted with the $\alpha 1$ and $\alpha 2$ chains of type I collagen as tuna gelatin on immunoblotting (Fig 2). Furthermore, the IgE reacted with two β chains. As a negative control, IgE from pooled normal serum reacted with no tuna type I collagen chains (data not shown).

TABLE III. Reactivity to fish meat and gelatin in patients with atopic dermatitis

Patient No.	Age	Sex	Type of fish	Fish meat IgE*	Fish gelatin IgE†
13	20	M	Cod	13.3	49.9
			Tuna	20.2	35.6
14	14	F	Cod	0.50	2.41
			Tuna	0.46	3.88
15	45	M	Cod	13.2	1.29
			Tuna	14.9	2.53
16	10	M	Cod	4.41	1.54
			Tuna	2.58	1.30
17	19	F	Cod	1.54	0.50
			Tuna	1.50	0.95
18	25	F	Cod	4.96	<0.35
			Tuna	<0.35	<0.35
19	8	F	Cod	6.94	<0.35
			Tuna	0.43	<0.35
20	23	M	Cod	5.66	<0.35
			Tuna	6.34	<0.35
21	28	M	Cod	0.79	<0.35
			Tuna	<0.35	<0.35
22	23	M	Cod	<0.35	<0.35
			Tuna	0.48	<0.35
23	30	M	Cod	0.56	<0.35
			Tuna	<0.35	<0.35
24	25	M	Cod	0.48	<0.35
			Tuna	1.45	<0.35
25	45	F	Cod	0.59	<0.35
			Tuna	1.45	<0.35
26	21	M	Cod	2.08	<0.35
			Tuna	1.35	<0.35
27	20	M	Cod	3.18	<0.35
			Tuna	1.08	<0.35

*CAP (Ua/mL).

†ELISA (U/mL).

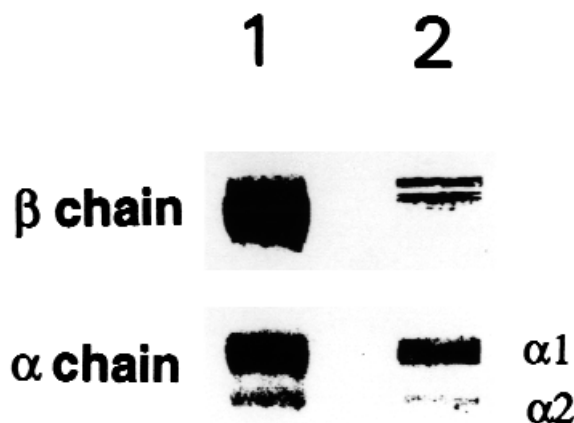


FIG 2. SDS-PAGE of denatured type I collagen from tuna. Coomassie blue staining (lane 1) and immunoblot analysis (lane 2).

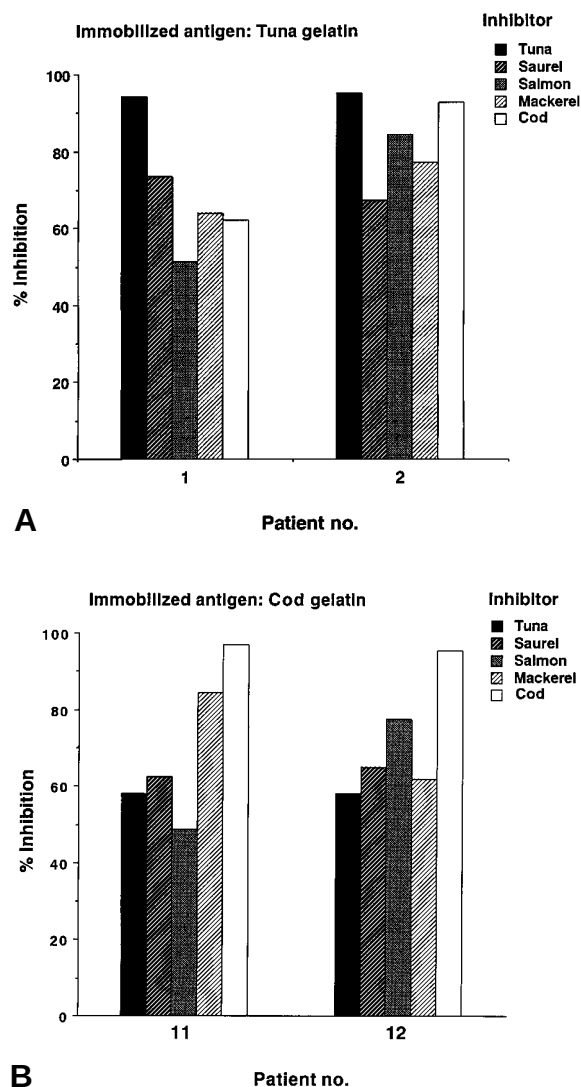


FIG 3. Antigenic cross-reactivity among fish gelatins as determined by using ELISA inhibition. **A**, Immobilized antigen: tuna gelatin; **B**, immobilized antigen: codfish gelatin.

Specific IgE to fish meat and gelatin in patients with both fish and bovine gelatin allergy

We measured specific IgE to fish meat and gelatin in two patients who had fish and bovine gelatin allergy. Both patients had specific IgE to fish meat and gelatin (Table II). They showed bovine gelatin allergy as follows: patient 11 had urticaria to measles vaccine containing gelatin (1 mg/dose), and patient 12 often experienced asthma after consuming gelatin-containing food. Patient 12 underwent no controlled food challenge or bronchial challenge tests. They had specific IgE to bovine gelatin (Table II).

IgE antibody to fish meat and gelatin in patients with atopic dermatitis

We measured specific IgE to cod and tuna meat in 65 patients with atopic dermatitis. Of the 65 patients, 15 had specific IgE to cod or tuna meat (Table III). Of these 15 patients, 5 had specific IgE to cod or tuna gelatin. The 5 patients had no specific IgE to bovine gelatin (data not shown).

IgE antibody to other fish gelatins in patients with fish allergy

We measured specific IgE to 5 kinds of fish gelatins in 5 patients who showed IgE reactivity to fish gelatin in Tables I and II. These patients had specific IgE to other gelatins (Table IV).

Antigenic cross-reactivity among fish gelatins as determined by ELISA inhibition

Cross-reactivity among fish gelatins was studied by using ELISA inhibition. In two patients (patients 1 and 2) with tuna allergy, IgE binding to tuna gelatin was inhibited by saurel, salmon, mackerel, and codfish gelatins (Fig 3, A). Similarly, in two patients (patients 11 and 12) with codfish allergy, IgE binding to cod gelatin was inhibited by tuna, saurel, salmon, and mackerel gelatins (Fig 3, B). Therefore a strong cross-reactivity was observed among fish gelatins.

Antigenic cross-reactivity among fish and bovine gelatins as determined by ELISA inhibition

Cross-reactivity between bovine and fish gelatins was studied by using ELISA inhibition. In patients (patients 11 and 12) with both anti-bovine and anti-fish gelatin IgE, IgE binding to bovine gelatin was not inhibited by codfish gelatin (Fig 4, A), and no reciprocal inhibition was observed (Fig 4, B). These results indicate that there is little cross-reactivity between bovine and fish gelatins.

DISCUSSION

Kelso et al⁷ and Wahl and Kleinhans²³ reported that gelatin causes food allergy. Also, we reported that 26 children had anaphylaxis to vaccine and anti-bovine

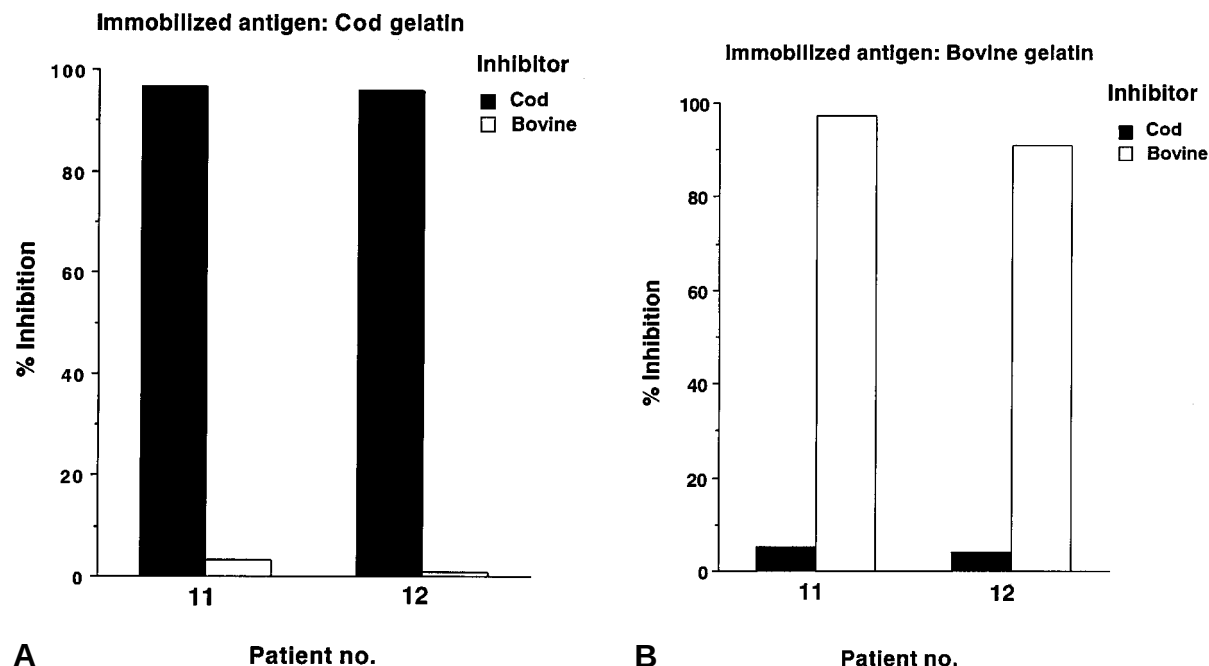


FIG 4. Antigenic cross-reactivity among fish and bovine gelatins as determined by using ELISA inhibition. **A**, Immobilized antigen: cod gelatin; **B**, immobilized antigen: bovine gelatin.

gelatin IgE, and 7 had food allergy to bovine gelatin.¹⁰ In our previous study we investigated IgE reactivity to gelatins from various animals in the bovine gelatin-sensitive children.¹⁷ We found that one child showed IgE reactivity to fish gelatin. It is possible that fish gelatin might be one of the allergens in fish allergy.

In this study we found that some patients with fish allergy had specific IgE to both fish meat and fish gelatin. Furthermore, IgE from pooled serum of patients reacted with tuna collagen on immunoblotting. Two patients with allergies both to fish meat and bovine gelatin had specific IgE to fish gelatin. From these results, we found that fish gelatin is an allergen in patients with fish allergy. In this study we found no patient with fish allergy who demonstrated IgE to fish gelatin but not to fish meat because meat of various species of fishes contains the collagen in the ranges from 0.34% to 2.19% of wet tissue and from 1.6% to 12.4% of the total protein.²⁴ The vaccines available in Japan have not contained fish gelatin. It is likely that the patients may become sensitized by fish gelatin, which is derived from fish meat and skin.

In a previous study children sensitized with bovine gelatin strongly reacted with type I collagen, which is the main content of gelatin.²² It is found that bovine type I collagen is a major allergen in bovine gelatin allergy. Type I collagen is composed of two α 1 and one α 2 chains.²⁵ However, commercial bovine and cod gelatins showed smear bands, and few α 1 and α 2 chains of collagen are detected in SDS-PAGE (data not shown) because commercial gelatin has been isolated from bone or skin under severe conditions. Furthermore, we found that the children with anaphylaxis to vaccines who had specific IgE to gelatin also demonstrated specific IgE to the α 2

TABLE IV. IgE reactivity to fish gelatin in patients with fish allergy

Fish gelatin	Patient No.				
	1	2	3	11	12
Tuna	4.05*	6.30	0.50	10.0	24.1
Cod	3.07	6.65	0.45	7.02	51.5
Salmon	3.20	4.83	0.50	6.23	34.5
Saurel	4.82	6.04	0.58	8.08	25.9
Mackerel	5.91	6.13	<0.35	9.58	35.2

*ELISA (U/mL).

chain (molecular weight, ~100 kd) of bovine type I collagen.²² We found that homology (98%) between α 1 chains of human and bovine type I collagen is higher than that (93%) between α 2 chains of human and bovine type I collagen.²² These homologic data might suggest that the α 2 chain has higher allergenicity than the α 1 chain. In this study IgE reacted with both α 1 and α 2 chains of tuna fish collagen type I. At present, the amino acid sequence of fish type I collagen (α 1 and α 2 chains) has not yet been analyzed. It is suggested that homology between human and fish type I collagen is lower than that between human and bovine type I collagen.

Cross-reactivity among fish species has been reported.²⁶⁻²⁸ Gad c 1 is a cross-reactive fish allergen found in several different species of fish.²⁸ In this study we found cross-reactivity among fish gelatins by using ELISA inhibition. In contrast, there is no cross-reactivity between bovine and fish gelatins. Similarly, in our previous study we found that there is no cross-reactivity between bovine and fish gelatins.²²

In conclusion, we found that some fish-sensitive patients showed IgE reactivity to fish gelatin. Fish gelatin (type I collagen) might be an allergen in fish allergy. The analysis of the purified allergen from fish is expected to facilitate further studies on the immunologic responses to fish allergens in patients with fish allergy.

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