

Gelatin Prepared from Tuna Skin: A Risk Factor for Fish Allergy or Sensitization?

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Key Words

Food allergy · IgE · Tuna fish · Gelatin · Immunoblotting

Abstract

Background: Although fish gelatin may represent a useful alternative to bovine or porcine gelatins, the clearly recognized high prevalence of fish allergy could increase the risk of anaphylaxis to gelatin. The rationale for investigating tuna gelatin rather than gelatins from more allergenic fishes is the availability of an industrial gelatin under development. The infrequent occurrence of tuna allergy should influence the safety of a derived product. The present study investigated IgE antibodies to tuna-skin-derived gelatin in adults and children with documented fish allergy or sensitization. **Methods:** Serum samples were taken from 100 consecutive patients with fish allergy or sensitization and tested for IgE antibodies against hydrolyzed or nonhydrolyzed gelatin extracted from tuna skin as compared to extracts from tuna flesh, tuna skin as well as bovine or porcine gelatins. Patients with tuna allergies or sensitization were sensitive to the same tuna species (yellowfin) as that from which the gelatin was obtained. IgE antibodies to these various extracts were analyzed using SDS-PAGE and immunoblotting. **Results:** Only 3 of the 100 serum samples tested gave evidence of reactivity to gelatin extracted from tuna skin. Cross-reactivity between bovine/porcine and fish gelatins was not observed. **Conclusion:** The risk of ad-

verse reactions to tuna skin gelatin seems to be significantly lower than the risk of fish allergy. Fish gelatin may represent a valuable alternative to bovine or porcine gelatins.

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Introduction

Gelatin is widely used in many food and pharmaceutical preparations. Depending on the indication, it is used in the nonhydrolyzed gel-forming form or the hydrolyzed form. Beef and pork constitute the major sources of gelatin. Bovine gelatin can raise problems related to bovine spongiform encephalitis, while porcine gelatin raises religious problems. An alternative source of gelatin would be material prepared from fish.

Mammalian gelatins can cause severe anaphylactic reactions, as observed with gelatin used as a stabilizing agent in vaccines [1–3]. Bovine type I collagen is a major allergen in bovine gelatin allergy. Fish meat and skin also contain type I collagen. Recently, it was found that some fish-sensitive patients possessed IgE antibodies to fish type I collagen. IgE from pooled serum of these patients reacted with both the alpha-1 and alpha-2 chains of fish type I collagen in immunoblots [4]. As fish is a relatively frequent food allergen [5], the possibility of recognition of the gelatin prepared from tuna skin by the IgE of subjects sensitized to fish obviously represents an important issue

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to be studied. Tuna was selected because it is known to be less allergenic than other species and because tuna skin gelatin is a widely available industrial product.

Material and Methods

Patients

Serum samples from 100 consecutive patients with food allergy and presenting an allergy or simple laboratory signs of sensitization to fish without clinical reactivity, were used for this study. The distinction between allergy and sensitization was explained in previous studies [6, 7]. This series of subjects was part of a group of 1,000 consecutive patients, in whom a diagnosis of food allergy was made. These patients had been systematically tested for sensitivity to fish. The patients were distributed as follows: 43 children (9 girls and 34 boys) aged from 12 months to 15 years (mean age: 8 years) and 57 adults (27 women and 30 men) aged from 16 to 76 years (mean age: 35 years). Their symptoms consisted of urticaria and/or angioedema in 35 cases, atopic dermatitis in 39 cases, anaphylactic shock in 7 cases, gastrointestinal disorders in 10 cases and rhinitis and/or asthma in 9 cases. In 28 cases, the patients had a positive history of fish allergy, 5 cases experienced distaste for fish and 6 cases reported excessive consumption of fish.

Skin Tests

Skin prick tests were performed in the Allergy Unit, Department of Gastroenterology, Centre Hospitalier Lyon-Sud. The tests were performed on the flexor aspect of the forearm with a standardized prick needle (Stallerpoint, Stallergènes, Antony, France) using fish extracts (Stallergènes). Histamine dihydrochloride (10 mg/ml) was used as a positive control and the glycerol-containing diluent of the prick solution, as a negative control. The wheals were marked with a thin pen and then transferred onto transparent adhesive tape. Reactions were recorded after 15 min. A wheal with a diameter of at least 3 mm was considered positive.

Sera

Total IgE and anti-fish IgE assays were performed by the Pharmacia CAP SystemTM method (Pharmacia Diagnostics, Uppsala, Sweden). Anti-tuna IgE associated with anti-cod IgE was present in 50 patients. It was associated with anti-salmon IgE in 18 cases, anti-sardine or anti-trout IgE in 3 cases and anti-plaice, anti-herring or anti-mackerel IgE in 1 case. Anti-tuna IgE without anti-cod IgE was present in 11 patients, associated with anti-salmon IgE in 1 case. Anti-cod IgE without anti-tuna IgE was present in 33 patients, associated with anti-salmon IgE in 3 cases and anti-trout and anti-plaice IgE in 1 case. The mean anti-tuna IgE titer in the 61 patients sensitized to this fish was 3.34 kU/l (range: 0.36–57.7 kU/l). The mean anti-cod IgE titer in the 83 sensitized patients was 2.88 kU/l (range: 0.36–76.6 kU/l). Anti-salmon IgE alone was present in 3 patients with titers of 1.7, 1.05 and 2.29 kU/l; anti-sardine IgE alone was present in 1 patient (titer: 2.82 kU/l), anti-herring IgE alone was present in 1 patient (0.53 kU/l) and anti-plaice IgE alone was present in 1 patient (0.83 kU/l). Individual data are presented in table 1.

Sera were also obtained from 2 patients with IgE to bovine gelatin and no IgE to fish. These patients developed anaphylactic shock after infusion of modified fluid gelatin (Plasmion, Fresenius Kabi, France).

Preparation of Extracts of Tuna Flesh and Tuna Skin

The flesh and skin of tuna fish were prepared separately according to the technique described by Bugajska-Schretter et al. [8]. One gram of flesh or 1 g of skin from lacerated fresh Mediterranean tuna (yellowfin) was frozen in liquid nitrogen then dissolved in 1 ml of Laemmli buffer (Bio-Rad, Richmond, Calif., USA) containing 5% 2-mercaptoethanol (Sigma, St. Louis, Mo., USA), heated for 10 min at 100°C and centrifuged at 14,000 rpm for 2 min.

Preparation of Gelatins

Gel-forming gelatins from tuna (yellowfin), beef and pork and hydrolyzed gelatins from tuna (yellowfin) and pork were provided by Rousselot SAS. Gel-forming gelatins were prepared from tuna skins via the acid pathway according to the process described in European patent EP 1016 347 A1 and from the skins of cows or pigs according to the process described by Ward and Courts [9]. Non-gel-forming hydrolyzed gelatins were prepared in a similar way, but with an additional step of enzymatic hydrolysis by Novo neurase.

One hundred milligrams of each gelatin were dissolved in 1 ml of Laemmli buffer containing 5% 2-mercaptoethanol. After allowing the gelatins to swell, the solutions were heated for 10 min at 100°C and then centrifuged at 14,000 rpm for 2 min.

Protein Content of Extracts

Extracts of tuna flesh and tuna skin and gelatins were prepared in Laemmli buffer as described above, but without 2-mercaptoethanol to assay the proteins in solutions according to the method of Lowry (Bio-Rad DC Protein Assay, Bio-Rad). The concentrations of extracts prepared with 2-mercaptoethanol were adjusted to 1 g/l for tuna flesh and tuna skin and 3.5 g/l for gelatins, respectively. Aliquots were stored at –20°C.

Gel Electrophoresis

Proteins of the extracts were separated by SDS-PAGE on 12% polyacrylamide gel and 4% stacking gel. Ten microliters of extract or molecular weight marker (6.5–200 kD, SDS-PAGE standards or biotinylated SDS-PAGE standards in the case of transfer onto nitrocellulose membrane; Bio-Rad) was loaded. Migration was performed at 200 V for 50 min in Tris-SDS buffer, pH 8.3 (25 mM Tris, 192 mM glycine, 0.1% SDS) (Mini Protean II System, Bio-Rad). The molecular weights of the various proteins of the extracts were estimated after staining the gels for 1 h with Coomassie blue (Bio-Safe Coomassie Stain, Bio-Rad).

Immunoblotting

Proteins separated by SDS-PAGE were then transferred onto 0.2 µm nitrocellulose membranes (Bio-Rad) for 1 h at a constant voltage of 100 V. The transfer buffer used was Tris-glycine pH 8.3 buffer (25 mM Tris, 192 mM glycine) containing 20% methanol (Mini Transblot System, Bio-Rad). Membranes were then rapidly stained with Ponceau Red solution (Sigma) to visualize the protein profiles, and were cut into strips. Strips were then destained by washing in TTBS buffer (TBS buffer (20 mM Tris-HCl, 500 mM NaCl, pH 7.5) containing 0.1% Tween 20).

Unoccupied binding sites of the membranes were then blocked by casein for 1 h at 37°C in blocking buffer (TTBS buffer containing 5% nonfat milk powder) and blots were then incubated overnight at room temperature, on a rocking platform, with sera diluted in TTBS buffer with 1% nonfat milk powder (antibody buffer). The dilution depended on the antibody content of the serum. After incubation,

Table 1. Study population and specific fish IgE antibodies

Patient No.	Age	Sex	Symptoms	Total IgE kU/l	Specific IgE, kU/l								
					tuna	cod	salmon	sardine	trout	plaice	herring	mackerel	anchovy
1	26	F	urticaria	957	5.33	<0.35	ND	ND	ND	ND	ND	ND	ND
2	9	M	AD	1,707	11.90	4.91	7.60	ND	ND	ND	ND	ND	ND
3	1	M	AD	7	<0.35	1.14	ND	ND	ND	ND	ND	ND	ND
4	16	M	AO	590	3.80	0.64	ND	ND	ND	ND	ND	ND	ND
5	18	M	urticaria + AO	1,030	<0.35	0.36	ND	ND	ND	ND	ND	ND	ND
6	41	M	AD	17,046	0.90	1.80	ND	ND	ND	ND	ND	ND	ND
7	42	F	ana.shock	553	2.22	0.83	ND	ND	ND	ND	ND	ND	ND
8	47	F	urticaria	907	<0.35	<0.35	1.70	ND	ND	ND	ND	ND	ND
9	35	M	urticaria + AO	112	0.44	<0.35	<0.35	ND	ND	ND	ND	ND	ND
10	9	M	AD	1,600	<0.35	1.50	<0.35	ND	ND	ND	ND	ND	ND
11	76	F	GI disorders	7	0.73	<0.35	<0.35	<0.35	ND	ND	ND	ND	ND
12	34	M	ana.shock	295	<0.35	<0.35	ND	2.82	ND	ND	ND	ND	ND
13	56	M	urticaria	1,020	<0.35	1.30	<0.35	ND	ND	ND	ND	ND	ND
14	4	M	AD	2,800	<0.35	2.00	ND	ND	ND	ND	ND	ND	ND
15	20	F	urticaria + AO	566	<0.35	0.87	ND	ND	ND	ND	ND	ND	ND
16	1	M	AD	11,000	3.00	3.75	ND	ND	ND	ND	ND	ND	ND
17	11	M	rhinitis	90	<0.35	2.20	ND	ND	ND	ND	ND	ND	ND
18	25	M	AD	4,620	9.82	13.60	ND	ND	ND	ND	ND	ND	ND
19	31	F	urticaria + AO	3,436	<0.35	1.06	ND	ND	ND	ND	ND	ND	ND
20	23	M	urticaria	850	3.40	1.50	<0.35	ND	ND	ND	ND	ND	ND
21	44	F	rhinitis	2,150	<0.35	0.49	<0.35	ND	ND	ND	ND	ND	ND
22	4	M	GI disorders	143	2.40	3.03	1.28	1.41	ND	0.83	ND	ND	ND
23	11	M	asthma	970	<0.35	0.72	ND	ND	ND	ND	ND	ND	ND
24	20	M	GI disorders	900	0.55	1.30	<0.35	ND	ND	ND	ND	ND	ND
25	58	F	AD	6,200	1.90	2.10	0.87	ND	ND	ND	ND	ND	ND
26	8	M	urticaria + AO	348	2.10	2.10	ND	ND	ND	ND	ND	ND	ND
27	5	F	AO	865	<0.35	11.70	4.20	ND	ND	ND	ND	ND	ND
28	52	M	AD	326	0.97	1.51	0.47	<0.35	ND	ND	ND	ND	ND
29	13	M	urticaria + AO	133	1.53	2.07	4.20	ND	ND	ND	ND	ND	ND
30	45	F	urticaria	340	<0.35	1.10	ND	ND	ND	ND	ND	ND	ND
31	8	M	AD	42	0.50	0.91	0.53	ND	ND	ND	ND	ND	ND
32	14	M	ana.shock	367	5.00	4.50	3.30	ND	ND	ND	ND	ND	ND
33	7	M	urticaria + AO	389	0.55	0.78	ND	ND	ND	ND	ND	ND	ND
34	2	M	GI disorders	550	<0.35	0.50	ND	ND	ND	ND	ND	ND	ND
35	13	F	AD	1,020	5.40	17.50	13.50	ND	ND	ND	ND	ND	ND
36	30	F	AD	7,500	<0.35	0.59	ND	ND	ND	ND	ND	ND	ND
37	42	F	GI disorders	385	1.23	1.70	ND	ND	ND	ND	ND	ND	ND
38	17	F	GI disorders	117	1.32	1.07	ND	ND	ND	ND	ND	ND	ND
39	17	M	AD	899	<0.35	5.30	ND	ND	ND	ND	ND	ND	ND
40	20	F	AO	228	<0.35	1.40	<0.35	ND	ND	ND	ND	ND	ND
41	10	M	urticaria + AO	1,347	2.00	1.70	ND	ND	ND	ND	ND	ND	ND
42	24	F	AD	1,310	<0.35	0.57	ND	ND	ND	ND	ND	ND	ND
43	44	M	AD	758	1.51	3.88	ND	ND	ND	ND	ND	ND	ND
44	12	M	AD	1,070	<0.35	0.37	ND	ND	ND	ND	ND	ND	ND
45	20	M	AD	9,750	0.56	<0.35	<0.35	ND	ND	ND	ND	ND	ND
46	1	M	AD	4,966	1.16	3.13	ND	ND	ND	ND	ND	ND	ND
47	65	M	AD	>10,000	<0.35	4.32	4.39	ND	ND	ND	ND	ND	ND
48	5	F	AO	72	<0.35	0.99	ND	ND	ND	ND	ND	ND	ND
49	15	M	AO	556	1.95	1.42	0.37	ND	ND	ND	ND	ND	ND
50	23	F	urticaria + AO	2,292	19.90	2.37	0.90	4.02	0.79	ND	2.54	ND	ND
51	44	M	urticaria + AO	594	1.00	1.10	<0.35	ND	ND	ND	ND	ND	ND
52	31	F	GI disorders	25	0.82	<0.35	0.63	ND	ND	ND	ND	ND	ND
53	7	M	AO	944	1.30	<0.35	ND	ND	ND	ND	ND	ND	ND
54	14	M	AD	2,400	10.50	0.38	ND	ND	ND	ND	ND	ND	ND
55	16	M	AD	7,200	<0.35	0.41	ND	ND	ND	ND	ND	ND	ND
56	2	M	urticaria + AO	53	<0.35	<0.35	1.05	ND	ND	ND	ND	ND	<0.35
57	6	F	AD	2,173	<0.35	0.41	ND	ND	ND	ND	ND	ND	ND
58	35	M	asthma	272	0.75	1.36	ND	ND	ND	ND	ND	ND	ND
59	12	M	asthma	6,000	0.42	6.40	13.70	ND	ND	ND	ND	ND	ND
60	25	F	ana.shock	475	<0.35	<0.35	<0.35	ND	<0.35	ND	0.53	<0.35	ND
61	12	F	urticaria + AO	193	7.18	<0.35	ND	ND	ND	ND	ND	ND	ND
62	31	M	urticaria	537	0.94	<0.35	ND	ND	ND	ND	ND	ND	ND



Table 1 (continued)

Patient No.	Age	Sex	Symptoms	Total IgE kU/l	Specific IgE, kU/l								
					tuna	cod	salmon	sardine	trout	plaice	herring	mackerel	anchovy
63	6	F	AD	4,300	10.90	3.70	ND	ND	ND	ND	ND	ND	ND
64	52	F	ana.shock	14	6.67	9.29	ND	ND	ND	ND	ND	ND	ND
65	49	F	AD	660	4.40	0.38	<0.35	ND	ND	ND	ND	ND	ND
66	10	F	urticaria + AO	1,140	<0.35	6.90	2.10	<0.35	8.40	5.90	ND	ND	ND
67	37	M	urticaria	138	6.70	<0.35	ND	ND	ND	ND	ND	ND	ND
68	11	M	rhinitis	1,902	<0.35	<0.35	<0.35	ND	ND	0.83	ND	ND	ND
69	46	F	urticaria	74	<0.35	1.30	ND	ND	ND	ND	ND	ND	ND
70	6	M	urticaria + AO	149	<0.35	0.61	ND	ND	ND	ND	ND	ND	ND
71	15	M	asthma	17,900	1.18	2.20	ND	ND	ND	ND	ND	ND	ND
72	12	M	AD	22,100	<0.35	6.90	ND	ND	ND	ND	ND	ND	ND
73	15	F	AO	253	1.48	3.28	5.06	ND	ND	ND	ND	ND	ND
74	48	M	urticaria	12,000	<0.35	0.90	ND	ND	ND	ND	ND	ND	ND
75	19	F	GI disorders	325	57.70	76.60	59.00	ND	ND	ND	ND	ND	ND
76	1	M	AD	872	3.78	28.70	ND	ND	ND	ND	ND	ND	ND
77	56	F	GI disorders	25	0.89	<0.35	ND	ND	ND	ND	ND	ND	ND
78	53	F	AD	650	<0.35	0.47	ND	ND	ND	ND	ND	ND	ND
79	29	M	ana.shock	138	<0.35	3.70	<0.35	ND	ND	ND	ND	ND	ND
80	62	F	AD	5,020	2.39	2.50	ND	ND	ND	ND	ND	ND	ND
81	28	M	AO	825	0.67	<0.35	<0.35	ND	ND	ND	ND	ND	ND
82	31	M	AO	1,040	10.00	14.20	9.60	9.90	9.30	ND	ND	11.00	ND
83	25	M	AD	6,700	<0.35	0.93	ND	ND	ND	ND	ND	ND	ND
84	29	F	AO	6,600	0.36	5.90	<0.35	ND	ND	ND	ND	ND	ND
85	16	M	AO	1,650	0.99	0.69	0.95	ND	ND	ND	ND	ND	ND
86	35	M	ana.shock	320	0.92	0.55	1.30	ND	ND	ND	ND	ND	ND
87	4	M	AO	1,560	19.00	37.40	42.60	ND	52.80	ND	ND	ND	ND
88	12	M	AD	7,246	<0.35	<0.35	2.29	ND	ND	ND	ND	ND	ND
89	31	M	asthma	111	<0.35	2.47	1.04	ND	ND	ND	ND	ND	ND
90	39	F	AD	8,300	0.98	4.30	2.00	ND	ND	ND	ND	ND	ND
91	1	M	AD	1,685	3.88	7.65	ND	ND	ND	ND	ND	ND	ND
92	30	M	AD	3,780	1.20	0.89	ND	ND	ND	ND	ND	ND	ND
93	3	M	AD	8,100	0.91	1.82	ND	ND	ND	ND	ND	ND	ND
94	31	M	AD	17,500	1.31	1.90	ND	ND	ND	ND	ND	ND	ND
95	32	M	GI disorders	595	0.47	2.20	1.40	ND	ND	ND	ND	ND	ND
96	17	F	AD	780	0.48	6.60	10.50	ND	ND	ND	ND	ND	ND
97	3	M	AD	617	<0.35	1.60	ND	ND	ND	ND	ND	ND	ND
98	6	M	asthma	277	1.42	1.90	ND	ND	ND	ND	ND	ND	ND
99	3	M	AD	5,993	3.10	4.30	ND	ND	ND	ND	ND	ND	ND
100	12	F	AD	712	<0.35	0.61	ND	ND	ND	ND	ND	ND	ND

AD = Atopic dermatitis; AO = angioedema; GI disorders = gastrointestinal disorders; ana.shock = anaphylactic shock.

blots were washed 4 times for 10 min with TTBS buffer. Another blocking by casein was performed for 15 min at 37°C, and nitrocellulose strips were then incubated for 2 h at 37°C with biotinylated anti-IgE solution (goat biotinylated anti-human IgE, Vector, Burlingame, Calif., USA) in antibody buffer (1:400 vol/vol). After washing with TTBS buffer, the blots were incubated for 30 min at 37°C with peroxidase-labeled streptavidin solution (1:2,000 vol/vol) (CovalAb, Oullins, France) in antibody buffer.

The same procedure was performed using the two sera with IgE to bovine gelatin.

Visualization of Protein Allergens by Chemiluminescence

After washing in TTBS buffer then TBS, blots were immersed, for about 10 min, in a chemiluminescent solution (CovalAb). Mem-

branes were exposed on Kodak XAR 5 film (Kodak, Rochester, N.Y., USA). Films were then developed and fixed (Kodak GBX).

Diaminobenzidine Developing of Protein Allergens

After rinsing in TBS, blots were developed for several minutes in diaminobenzidine (DAB) (CovalAb). The reaction was stopped by washing in TBS. Antigen-IgE complexes were detected by the formation of brown precipitates. This method is less sensitive than the previous method, but allows good verification.

Inhibitions of Anti-Fish IgE

The specificity of revelation of allergens by chemoluminescence and DAB was confirmed by blotting with sera preadsorbed with tuna flesh, tuna skin, tuna gelatin, porcine or bovine gelatins.

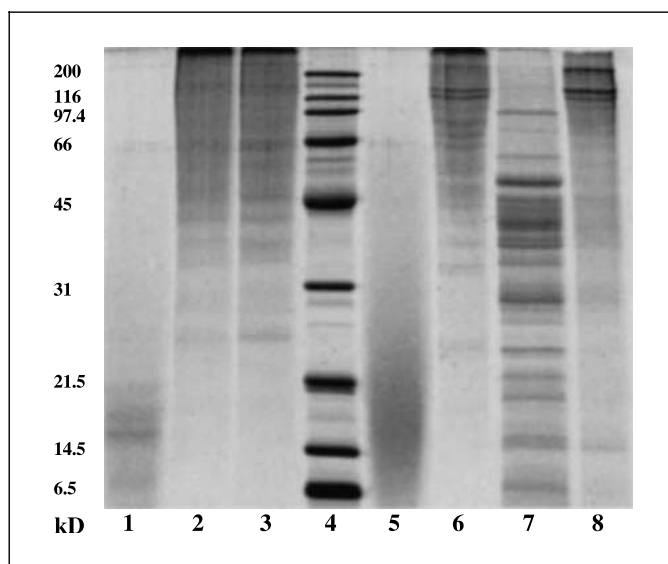


Fig. 1. SDS-PAGE protein profile, Coomassie blue staining. 1 = Hydrolyzed tuna skin gelatin; 2 = bovine gelatin; 3 = porcine gelatin; 4 = molecular-weight markers; 5 = hydrolyzed porcine gelatin; 6 = tuna skin gelatin; 7 = tuna flesh; 8 = tuna skin.

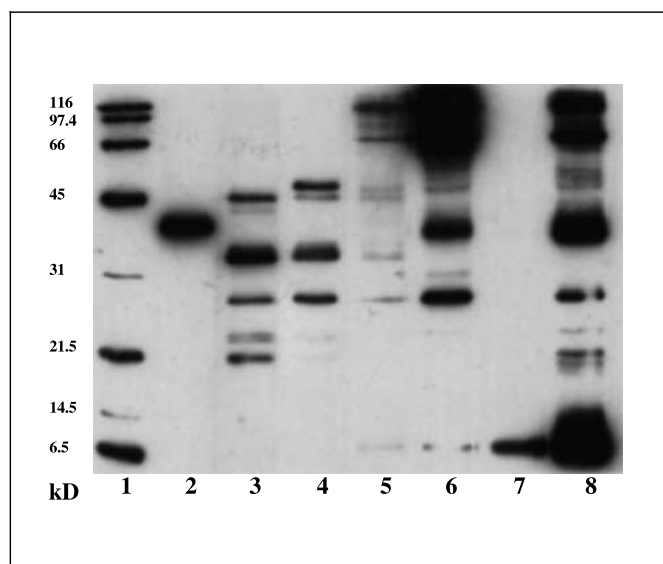


Fig. 2. Immunoblotting performed with tuna flesh. 1 = Molecular-weight markers; 2–8 = sera from patients sensitized and/or allergic to fish. The corresponding patient numbers in table 1 are No. 4, 96, 22, 26, 31, 32, 75.

Clinical Significance of the Presence of IgE Directed against Tuna Skin Gelatin

Subjects presenting IgE directed against tuna gelatin were subsequently submitted to the following investigations in order to determine the clinical significance of these laboratory signs of sensitization.

Skin prick tests were performed using glycerinated tuna skin gelatin extracts prepared by Stallergenes and appropriate positive (histamine) and negative (glycerol saline) controls. For solubility reasons, the concentration of the prick tests were 1:100 for nonhydrolyzed gelatins and 1:20 for hydrolyzed gelatins.

Single-blind food challenge was performed on an inpatient basis in the Allergy Unit, Department of Gastroenterology. The usual procedure was performed with 5 g of dry tuna gelatin masked in black-currant juice. As no threshold dose of oral gelatin was defined in the literature, a dose of 5 g was chosen because it corresponds to the intake of a child eating fruit gums. This dose is more than one thousand times greater than the threshold level for allergenic foods such as peanut flour, cow's milk, hen's egg or soy flour [10].

Results

Protein Bands Detected by SDS-PAGE

SDS-PAGE after Coomassie blue staining of the gels revealed proteins present in the extracts from tuna flesh, tuna skin and the various gelatins. Molecular weight markers were used to calculate the approximate molecular weights of these proteins (fig. 1).

Molecular weights for tuna flesh were about: >116, 116, 98.5, 77, 60, 53, 46, 43, 40, 38, 35, 30, 28, 25, 21, 19, 15 and 8.5 kD. The 8.5-kD band clearly corresponded to protein M (parvalbumin). For tuna skin, bands of molecular weights identical to those observed for tuna flesh were detected, except for the 60-, 35-, 30- and 21-kD bands. In addition, Coomassie blue staining also revealed the presence of several high-molecular-weight bands greater than 116 kD.

The molecular weights of gelatin proteins were more difficult to define.

Tuna gelatin showed molecular weight bands greater than 116 kD and other bands at 98, 88, 76, 66, 53, 48, 44, 42, 38, 34, 25 kD. Hydrolyzed tuna gelatin presented a continuum of molecular weights from 6.5 to about 31 kD.

Coomassie blue staining revealed molecular weights of about 197, 160, 130, 98, 85, 69, 53, 47, 43, 38, 27 and 19 kD in porcine gelatin, and bands of 160, 140, 112, 73, 69, 61, 43, 39 and 26 kD in bovine gelatin; a continuum from 6.5 to 31 kD was observed for hydrolyzed porcine gelatin as for tuna gelatin.

Protein Allergens Detected by Immunolabeling

Immunolabeling of the 100 sera showed the presence of allergenic bands for both tuna flesh and tuna skin, corresponding to the molecular weight bands >116, 116, 77,

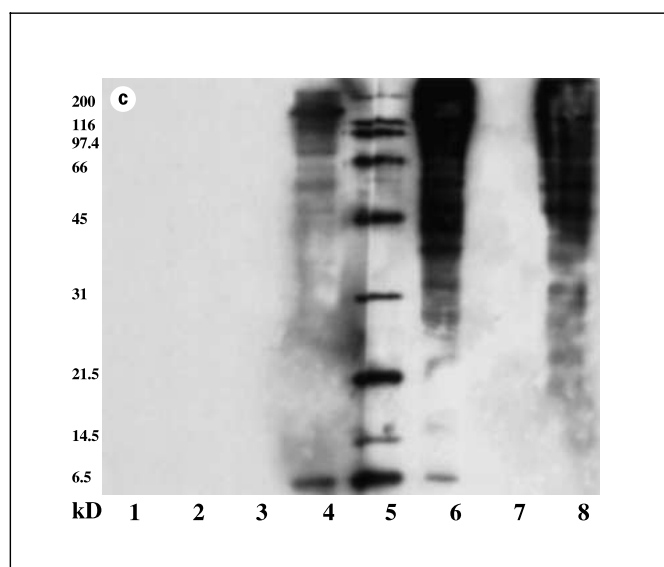
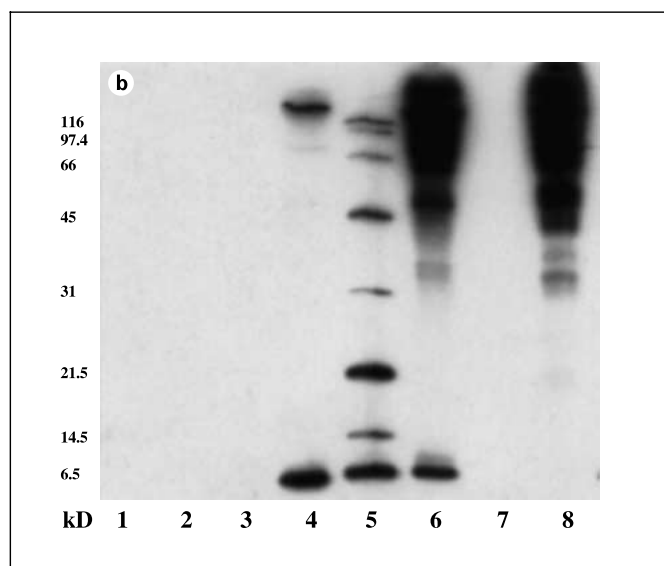
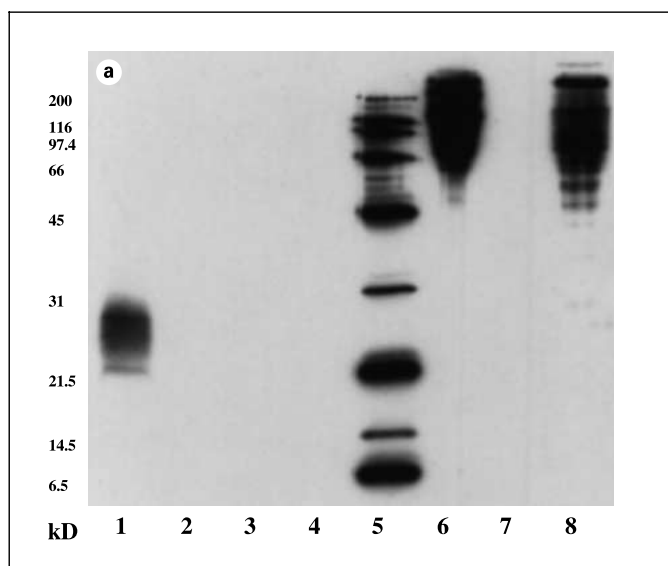


Fig. 3. SDS-PAGE performed with: 1 = Hydrolyzed tuna skin gelatin; 2 = bovine gelatin; 3 = porcine gelatin; 4 = tuna flesh; 5 = molecular-weight markers; 6 = tuna skin; 7 = hydrolyzed porcine gelatin; 8 = tuna skin gelatin. Immunoblotting of sera from patients No. 52 (a), 90 (b), and 99 (c).

53, 46, 38, 35, 28, 25, 21 and 8.5 kD (fig. 2). The most frequent and most intense bands were those with molecular weights of 46, 38, 35 and 8.5 kD. As this method is more sensitive than Coomassie blue staining of the gels, it more intensely revealed protein M and demonstrated allergens with molecular weights of 35 and 21 kD in tuna skin. Immunolabeling with sera preadsorbed with tuna flesh or tuna skin no longer revealed any band, confirming that these bands correspond to allergens.

None of the 100 sera tested revealed any allergenic activity in bovine or porcine gelatins or hydrolyzed porcine gelatin.

None of the sera with IgE to bovine gelatin revealed any allergenic activity in hydrolyzed gelatins and nonhydrolyzed gelatins extracted from tuna skin.

Three (sera corresponding to patients No. 52, 90, 99) of the 100 sera tested revealed an allergenic activity within the tuna gelatin continuum (fig. 3a–c). This immunobinding was totally inhibited by preincubation of the sera with tuna gelatin. It was not inhibited by preincubation of the sera with porcine and bovine gelatins (not shown). These inhibition tests are specific, as gelatin did not inhibit the visualization of the other bands which were inhibited by tuna flesh and tuna skin (fig. 4). In this inhibition experi-

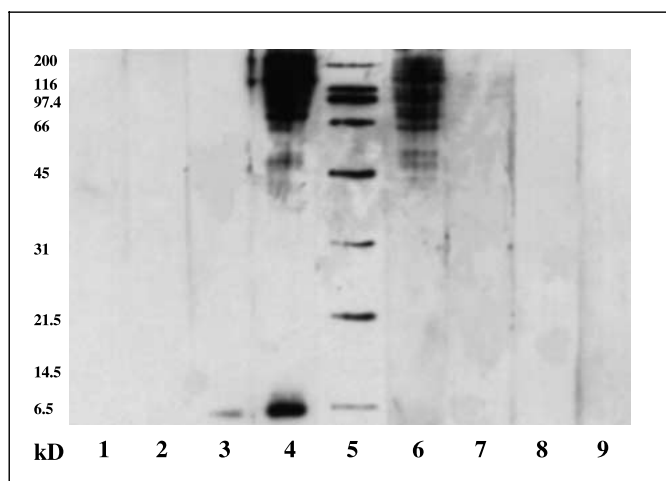


Fig. 4. SDS-PAGE performed with tuna skin (lanes 1–4), molecular-weight markers (lane 5) and tuna skin gelatin (lanes 6–9). Immunoblotting of serum from patient 90 (lanes 4 and 6), serum preincubated with tuna skin gelatin (lanes 3 and 7), serum preincubated with tuna skin (lanes 2 and 8), serum preincubated with tuna flesh (lanes 1 and 9).

ment there is strong IgE binding to allergens with high molecular weights present in extracts from tuna skin and gelatin and strong IgE binding to an allergen with low molecular weight present in tuna skin extract. Preincubation of one serum (patient No. 90) with tuna gelatin, tuna skin and tuna flesh totally inhibits IgE binding to these high-molecular-weight allergens in both extracts, but does not inhibit IgE binding to the low-molecular-weight allergen in tuna skin extract. This suggests that these high-molecular-weight allergens are collagen and the low-molecular-weight allergens are noncollagen fish allergens. One of these 3 sera revealed the presence of allergen within the hydrolyzed tuna gelatin continuum (fig. 3a).

There is no correlation between the level of IgE to tuna and the presence of IgE to fish gelatin. The levels of IgE to tuna in the serum from patients No. 52, 90 and 99 were only 0.82, 0.98 and 3.10 kU/l. Serum from patients with much higher levels of IgE to tuna, i.e. No. 2 (11.90 kU/l), No. 18 (59.82 kU/l), No. 50 (19.90 kU/l), No. 54 (10.50 kU/l), No. 63 (10.90 kU/l), No. 75 (57.70 kU/l), No. 82 (10.10 kU/l), No. 87 (19.00 kU/l) did not react with tuna skin gelatin.

Clinical Significance of the Presence of IgE Directed against Tuna Skin Gelatin

IgE directed against tuna skin gel-forming gelatin were detected in 3 subjects: patients No. 52, 90 and 99. IgE

directed against hydrolyzed gelatin were also present in only one of these patients (No. 52). This last patient, complaining of abdominal pain and angioedema, no longer ate fish. Subjects No. 90 and 99 suffered from atopic dermatitis and asthma and continued to eat fish with no clinical consequences.

Skin prick-tests for tuna skin gelatin were negative in these 3 subjects. A food challenge test with 5 g (dry weight) of tuna gelatin masked by blackcurrant juice was also negative. No food challenge was performed with tuna.

Discussion

Bovine gelatin can be responsible for various allergic reactions, such as those occurring after vaccinations [1–3] or after intravenous administration of liquid gelatin [11] or drugs, such as gelatin-stabilized erythropoietin [12]. It has also been established that consumption of bovine gelatin can be responsible for allergic reactions [13]. This first demonstration of bovine gelatin as a food allergen in adults [13] has subsequently been confirmed in children [14], in whom food allergy was observed in 5 out of 7 cases after parenteral vaccination and in 2 cases before vaccination, suggesting that sensitization to gelatin can occur via the oral and parenteral routes. Research for a nonmammalian source of gelatin therefore appears to be justified for safety reasons.

Tuna skin is a primary source of fish gelatin. The paper aimed at demonstrating the low level or absence of allergenicity of gelatin derived from tuna, a less allergenic fish species, as confirmed by the present observations. Out of 100 sera from fish sensitized or allergic patients, 61 had IgE specific for tuna, usually with a fairly low level.

The validity of the immunoblotting techniques used in this study was confirmed by testing these sera on samples of material composed of antigens derived from tuna flesh and tuna skin. Classical protein antigen bands were demonstrated. When the sera were used against tuna gel-forming gelatin, the presence of anti-gelatin IgE was detected on 3 occasions and the specificity of the reaction was demonstrated by appropriate inhibition experiments. Only one of these 3 sera revealed IgE specific for hydrolyzed tuna gelatin. None of the other 99 sera presented this reactivity.

Three subjects with IgE directed against tuna skin gelatin did not present any clinical consequences, as none of these 3 subjects presented cutaneous reactivity to tuna skin gelatin and the food challenge test performed with 5 g

of gelatin was also negative. Two subjects (No. 90 and 99) continued to eat fish with no clinical consequences and their negative reactivity was only to be expected as their anti-fish IgE levels were 4.30 PRU, i.e. very different from the levels of 20 PRU considered to be 95% predictive of clinical reactivity on a food challenge test. This value of 4.30 PRU is also lower than the 90% negative predictive value for clinical reactivity (5.0 PRU). The third subject (No. 52) presented an anti-fish IgE level of 0.82 PRU, i.e. lower than the 95% negative predictive value of 0.90 PRU [15]. Three of the 100 subjects studied also presented anti-fish IgE levels exceeding the 95% positive predictive value for clinical reactivity. Nine of the subjects presented anti-fish IgE levels exceeding the 90% positive predictive value (9.50 PRU) for clinical reactivity. None of these 12 subjects presented IgE directed against tuna skin gelatin.

When the material used was composed of porcine or bovine gel-forming gelatin, or hydrolyzed porcine gelatin, immunoblotting was constantly negative. The same finding was made when the material used was tuna fish gelatin and sera containing IgE to bovine gelatin. This confirms a

previous report [2] of a single case of a child reactive to fish gelatin with no cross-reactivity to bovine gelatin. The cross-antigenicity between mammalian gelatins, i.e. bovine, porcine, kangaroo, guinea pig, rat and mouse [2], does not extend to fish gelatins. This was confirmed by the present data and by 2 other cases from another study [4] which also demonstrated cross-reactivity among fish gelatins.

In conclusion, the use of gel-forming or non-gel-forming gelatin derived from tuna appears to present an extremely limited risk of anaphylactic reactions to fish, due to the manufacturing process which eliminates the allergens detected in fish from the gelatin. Tilapia skin is an alternative source of fish gelatin, but its allergenicity also needs to be investigated.

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