

Laboratory determinations in *Anisakis simplex* allergy

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Background: Anaphylactic reactions caused by the fish nematode, *Anisakis simplex*, after ingestion of parasitized fish, have been described.

Objective: This study was undertaken to confirm, by histamine release tests, that *A. simplex* is able to trigger IgE-mediated reactions and to describe the serologic profiles in this sensitization.

Methods: Twelve patients who had anaphylactic symptoms after ingestion of cooked fish and positive prick test results and determinations of IgE to *A. simplex* were studied by indirect IgG ELISA and IgG and IgE immunoblotting. Sera from subjects parasitized with other nematodes, patients with fish allergy, and healthy donors were included as controls. A histamine release test was performed in a representative case.

Results: IgE immunoblotting was a specific test to detect *A. simplex* allergy. IgE-reacting bands were found in serum samples from 11 of our patients. Specific IgG antibodies were found by ELISA and immunoblotting, but this response was less specific. Histamine release was positive with *A. simplex* extract and negative with fish.

Conclusion: A specific and intense immune response to an *A. simplex* extract was found in our patients. *A. simplex* is able to elicit anaphylactic reactions, and *A. simplex* allergy should be suspected in patients with allergic symptoms after ingestion of fish. A positive prick test response to *A. simplex* and a negative response to fish is a good indication for a diagnosis of *A. simplex* allergy. (*J ALLERGY CLIN IMMUNOL* 1996;97:977-84.)

Key words: Parasite, *Anisakis simplex*, anaphylaxis, fish, allergy, IgE, IgG, immunoblotting

Human anisakiasis is a parasitic infection of the gastrointestinal tract caused by the third-stage larva of *Anisakis simplex*. It is acquired by eating raw (sushi or sashimi) or undercooked seafood or cephalopods. Although most of the cases have been described in Japan,¹⁻⁴ in the last few years they have increased in other countries, such as France and the United States.^{5,6} Clinical manifes-

Abbreviations used

AU:	Arbitrary units
BSA:	Bovine serum albumin
PBS:	Phosphate-buffered saline

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tations may mimic other gastrointestinal disorders such as gastric ulcers, tumors, acute appendicitis, or rectal carcinoma. Fiberoptic gastroscopy or exploratory laparotomy is required for diagnosis when anisakiasis is suspected. In view of these problems, a number of techniques for immunodiagnosis have been investigated,⁷⁻¹¹ and although they have provided better serologic diagnoses, difficulties with cross-reactivities with other nematodes, such as *Ascaris lumbricoides* or *Toxocara canis*,¹² remain.

However, allergic and anaphylactoid reactions caused by *A. simplex* have been described.^{13,14} In

TABLE I. Clinical findings and skin test and specific IgE and IgG results

Patient No.	Age (yr)	Symptoms	Skin prick test (mm)		Specific IgE (kU/L)			Specific IgG (AU/ml)
			<i>A. simplex</i>	Fish	<i>A. simplex</i>	Fish	<i>Ascaris</i>	<i>A. simplex</i>
1	52	U/AE/GI	7 × 8	Neg	90.8	Neg	4.01	50
2	49	U/AE/GI	4 × 5	Neg	97.3	Neg	12.4	45
3	58	U/GI	5 × 5	Neg	24.4	Neg	1.89	>100
4	53	U/AE	3 × 4	Neg	>100	Neg	2.25	35
5*	18	U	5 × 5	8 × 8	2.34	3.8	7.51	16
6	12	U	6 × 4	Neg	4.53	Neg	6.29	16
7	61	U/AE	6 × 5	Neg	55.1	Neg	5.71	>100
8	48	U/AE	5 × 5	Neg	>100	Neg	1.86	>100
9	42	U/AE	4 × 4	Neg	33.4	Neg	1.82	45
10*	46	AE	3 × 4	Neg	65.4	Neg	0.794	67
11	31	U/AE/GI	3 × 4	Neg	49.1	Neg	Neg	>100
12	58	AE/D	7 × 6	Neg	30.6	Neg	0.39	>100

Patients 10, 11, and 12 were originally from the prick test survey.

U, Urticaria; AE, angioedema; GI, gastrointestinal manifestations; D, dyspnea.

*Atopic antecedents.

Japanese studies, specific IgE has been detected in 75% of patients with mackerel-induced urticaria¹⁵ and in 33% of patients with atopic dermatitis.¹⁶ In a prior article, we reported a case of anaphylaxis caused by *A. simplex*,¹⁷ and afterward, we studied several new cases. Like Kasuya et al.,^{13,14} we suspect that hypersensitivity to *A. simplex* is relevant among patients allergic to fish.

We report 12 patients, who had allergic symptoms after ingestion of cooked fish, who were diagnosed as having *A. simplex* hypersensitivity by prick test and specific IgE determination (CAP system; Pharmacia, Uppsala, Sweden). These patients were studied by means of indirect IgG ELISA and IgG and IgE immunoblotting in order to establish the serologic profiles that this sensitization produces. A histamine release test was performed in one case to confirm that *A. simplex* is able to elicit IgE-mediated anaphylactic reactions.

METHODS

Subjects and serum samples

The study was performed in 12 patients. The inclusion criteria were: (1) one outbreak of urticaria or angioedema after fish ingestion, (2) positive prick test response to *A. simplex* extract, (3) positive specific IgE antibodies to *A. simplex* in sera. In most of the patients, the ingestion of the same fish did not always produce this reaction. Nine of these patients were specifically referred to us because of allergic symptoms after fish ingestion. The other three (patients 10, 11, and 12) were selected, because of positive prick test results, from among 150 subjects referred to our allergy department

who were prick tested with *A. simplex* extract to prove that it did not induce irritant (false-positive) reactions. Afterward, the other two inclusion criteria were confirmed in these three patients. No history of surgical interventions or gastrointestinal disorders, suggesting undiagnosed anisakiasis, was given by the patients. All of our patients had high levels of total serum IgE, from 263 to 1058 kU/L, with a mean value of 667 kU/L. Clinical manifestations are shown in Table I.

Serum samples from 40 supposedly healthy donors and from 30 subjects with enzymeallergosorbent test responses to *Ascaris* equal to or greater than class 2 were used as two additional control populations for indirect IgG ELISA. Sera from 10 African subjects with filariasis and four subjects allergic to cod were included in immunoblottings in order to prove the specificity of the assay. Total IgE from patients with filariasis was 825 to 7500 kU/L, with a mean value of 2470 kU/L.

Antigen

A. simplex extract was prepared with the procedure used by Desowitz et al.⁹ Two hundred milligrams of live larvae, manually extracted from the flesh of hake (*Merluccius merluccius* L), in 2 ml of phosphate-buffered saline (PBS) were centrifuged for 15 minutes at 1500 g. Two milliliters of saline solution was added, and the solution was centrifuged again for 30 minutes at 1500 g. The supernatant was finally filtered through a 0.45 µm filter (Flow Pore D 26; Flow Laboratories GmbH, Meckenheim, Germany). Protein concentration, measured by the Lowry method,¹⁸ was 8 mg/ml, with bovine serum albumin (BSA) as a standard. This extract was stored at -40° C until needed.

A sample of larvae preserved in formaldehyde was sent to Dr. David I. Gibson of the Natural History

Museum of London, who identified it as *A. simplex* (Rudolphi, 1809) third-stage larvae.

Skin tests

Skin prick tests were performed on the volar aspect of each patient's forearm by using Allergy pricker lancets (DHS, Madrid, Spain) with 1:100 dilution in saline solution of *A. simplex* extract; the same extract heated to 40° C during 10 minutes and 100° C during 20 minutes; commercial extracts of cod, fish mix, and shellfish mix (Bencard, Bayer, Madrid, Spain); and finally implicated fresh fish (hake, tuna, and salmon). Histamine, 10 mg/ml, and saline solution were used as positive and negative controls, respectively. Skin tests were read after 15 minutes. Test sites with a wheal diameter of 3 mm larger than that of the negative control were regarded as positive.

Specific IgE determination

Specific IgE determination was performed by radioimmunoassay (CAP system). For this purpose, commercially available immunoCAPs (cod, hake, *A. simplex*, and *Ascaris*; Pharmacia) were used. According to the manufacturer's instructions, results greater than 0.35 kU/L were considered positive.

IgG indirect ELISA

Flat-bottomed microtiter plates (Costar Corp., Cambridge, Mass.) were coated, overnight at room temperature, with 0.1 ml of a 1:1000 dilution in PBS of *A. simplex* extract. The plates were blocked with 1% BSA in PBS for 1 hour. One hundred microliters of 1:500 dilution in 1% BSA of sera from patients, *Ascaris*-positive subjects, and control subjects was added to the wells. A standard curve was introduced in each plate with several twofold dilutions of a pool of the IgE-positive sera. Incubation lasted 1 hour at room temperature. Afterward, the plates were incubated for 1 hour with a 1:2000 dilution in 1% BSA of a peroxidase-labeled goat anti-human IgG antiserum (Tago, Burlingame, Calif.). Finally, o-phenylenediamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.) was added as substrate. After every incubation period, four washes with saline solution were performed.

To estimate the specific IgG levels of the different sera, a standard curve was used. The first point (1:500 dilution of this pool) was considered to have 100 arbitrary units (AU)/ml. The cutoff point was arbitrarily set at 35 AU/ml. Student's *t* test was used to compare the mean absorbance of the different groups.

Specific IgE detection by immunoblotting

Sodium dodecylsulfate polyacrylamide gel electrophoresis was performed according to the discontinuous system of Laemmli¹⁹ with 12% acrylamide resolving and 4% stacking gels. Two hundred microliters of 1:2 dilution of *A. simplex* extract in nonreducing sample buffer, without heating, was added. The electrophoresis was

performed at 150 V in a Mighty Small System (Hoefer Scientific, San Francisco, Calif.). After sodium dodecylsulfate polyacrylamide gel electrophoresis was performed, the transfer procedure of Petit et al.²⁰ with some modifications²¹ was used. After blocking in 10 mmol/L Tris, 0.15 mol/L NaCl, and 3% BSA (pH 7.4), nitrocellulose sheets were placed in a blotter device (mini Protean II; Bio-Rad, Richmond, Calif.) and incubated overnight with 500 µl of 1:5 dilutions in incubation buffer of sera from the 12 patients, two *Ascaris*-positive subjects, two control subjects with high IgG levels as determined by ELISA, 10 control subjects with negative IgG as determined by ELISA, four patients allergic to fish, and 10 African subjects. After three washes, the membranes were taken out of the device and incubated for 3 hours with a 1:100,000 dilution of a biotin-labeled monoclonal anti-IgE (Ingenasa, Madrid, Spain). After washing, the membranes were incubated for 1 hour with a 1:2000 dilution of streptavidin-alkaline phosphatase (Pierce Chemical Co., Rockford, Ill.). Finally, after new washes, Nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate (Bio-Rad) was added as substrate, according to the manufacturer's instructions.

Specific IgG detection by immunoblotting

The transfer procedure was the same as described above. The same serum samples in a 1:200 dilution in incubation buffer were incubated for 1 hour. After washes, the membranes were incubated for 1 hour with a 1:2000 dilution of a goat anti-human IgG labeled with alkaline phosphatase (Tago). Then, the membranes were washed, and the substrate (Nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate) was added.

Histamine release test

A histamine release test was performed with a fluorometric method and washed whole blood, as described by Moneo et al.,²² with six 10-fold dilutions of the *A. simplex* extract and four 10-fold dilutions of a commercial fish skin prick test extract. Releasability of human basophils was measured with a monoclonal anti-IgE.

RESULTS

Skin tests

Results of skin prick tests with *A. simplex* extract were positive in all patients (Table I). Results were the same with the heated extract (data not shown). One hundred and fifty skin prick tests were performed in control subjects, and four more positive test results were obtained. Three patients were included as cases (patients 10, 11, and 12) because of clinical symptoms compatible with *A. simplex* allergy and positive specific IgE to *A. simplex* as determined by CAP. The fourth was a patient allergic to fish who was excluded because of in vitro negative specific IgE to *A. simplex*. Results of

TABLE II. Means of IgG values in the different groups

	Mean	SD	Range	Percent >35 AU/ml
Patients	85.33	58.69	16 to >100	83.3
Control subjects	23.74	24.23	9 to >100	12.5
<i>Ascaris</i> -positive subjects	16.17	26.11	2.5 to >100	6.6

TABLE III. Comparison of IgG mean values (Student's *t* test)

Group	Difference	SE	<i>t</i>	<i>p</i> Value
Patient/Control	61.58	17.37	3.54	0.0040
Patient/ <i>Ascaris</i> -positive	69.15	17.60	3.92	0.0020
<i>Ascaris</i> -positive/Control	-7.56	6.05	-1.25	0.21

SE, Standard error.

prick tests with fish extracts were positive only in patient 5.

Specific IgE determinations

Positive results for *A. simplex* (>0.35 kU/L) were obtained in all 12 patients. IgE levels found in patients 5 and 6 (2.34 and 4.53 kU/L, respectively) were lower than those in the rest of the patients (range, 24.4 to >100 kU/L). Specific IgE to *Ascaris* was positive in all patients except patient 11. It is interesting that all patients had levels of specific IgE to *A. simplex* higher than those of specific IgE to *Ascaris*, except for patients 5 and 6, in whom the ratio was inverted. Specific IgE to the different fish was negative in all of the patients, once again with the exception of patient 5.

Indirect IgG ELISA

The results of indirect IgE ELISA, expressed in arbitrary units per milliliter, in the 12 patients are shown in Table I. All of our patients had high titers of IgG (≥ 35 AU/ml) to *A. simplex*, except patients 5 and 6, with 16 AU/ml.

Among the *Ascaris*-positive serum samples, two had high IgG levels (>100 AU/ml). Each *Ascaris*-positive serum sample was studied by determination of specific IgE to *A. simplex* (CAP system). The two serum samples with high titers of IgG were positive (3.68 and 0.87 kU/L), and we obtained 10 additional positive results (specific IgE ranging from 0.36 to 16 kU/L). In the control group levels of IgG higher than 35 AU/ml were obtained in five serum samples. Two of these samples (having the highest values) were studied by determination of specific IgE to *A. simplex* (CAP system), and results were negative.

IgG mean values in the different groups and

their comparison are shown in Tables II and III, respectively. There were statistically significant differences between the group of our patients and the *Ascaris*-positive and control groups ($p = 0.002$ and 0.004 , respectively). However, no difference was observed between the *Ascaris*-positive and control groups ($p = 0.21$).

Specific IgE detection by immunoblotting

Fig. 1 shows the different profiles obtained. Several bands were identified by antibodies present in the sera of 11 of our patients. Most of them showed a repetitive pattern. A group of four bands of medium molecular weight (from 30 to 45 kd) could be seen in each serum sample. Several bands of lower (14 to 30 kd) and higher molecular weights (>45 kd) were recognized by IgE in sera from most of the patients. No band was noticed in serum from patient 5 (Fig. 1, *A*, lane *E*) and (Fig. 1, *A*, lane *F*) only one weak band was recognized in serum from patient 6 in the group of medium molecular weight.

Two weak bands were seen in serum from a patient allergic to fish and one in another (Fig. 1, *B*, lanes *Q* and *S*). No positive bands were seen in the rest of the control sera, even in sera from African subjects and the two *Ascaris*-sensitive subjects (with positive IgE values to *A. simplex* by as determined by the CAP system).

Specific IgG detection by immunoblotting

Fig. 2 shows the position of IgG-reacting bands. Four components of medium molecular weight were seen in sera from 10 of our patients. They were coincident with bands detected by IgE immunoblotting. Several bands of higher molecular weight, one of them expressed more strongly, were

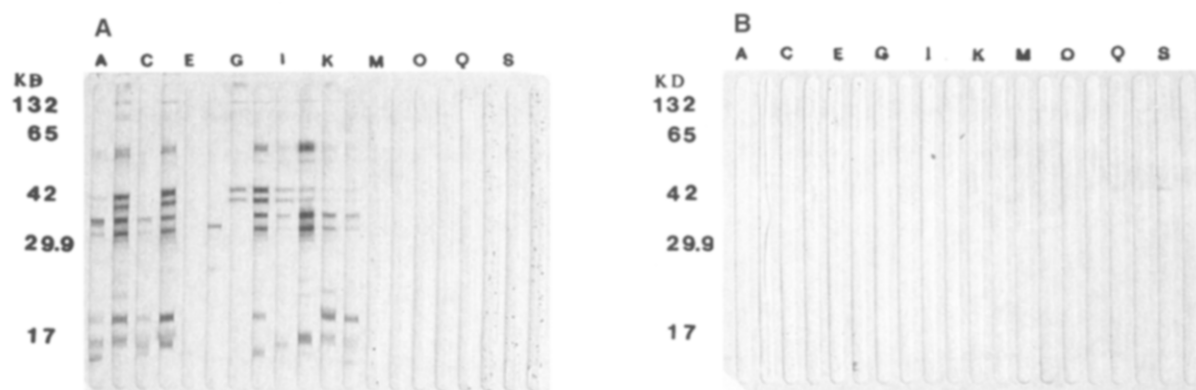


FIG. 1. IgE immunoblotting. **A**, Lanes A to L, Sera from patients; lanes M and N, sera from control subjects with high levels of IgG to *A. simplex* as determined by ELISA; lanes O and P, sera from *Ascaris*-positive subjects with high levels of IgG to *A. simplex* (ELISA); lanes Q to T, negative controls to *A. simplex* in IgG ELISA. **B**, Lanes A to F, Negative controls to *A. simplex* in IgG ELISA; lanes G to P, sera from African subjects; lanes Q to T, sera from patients allergic to fish.

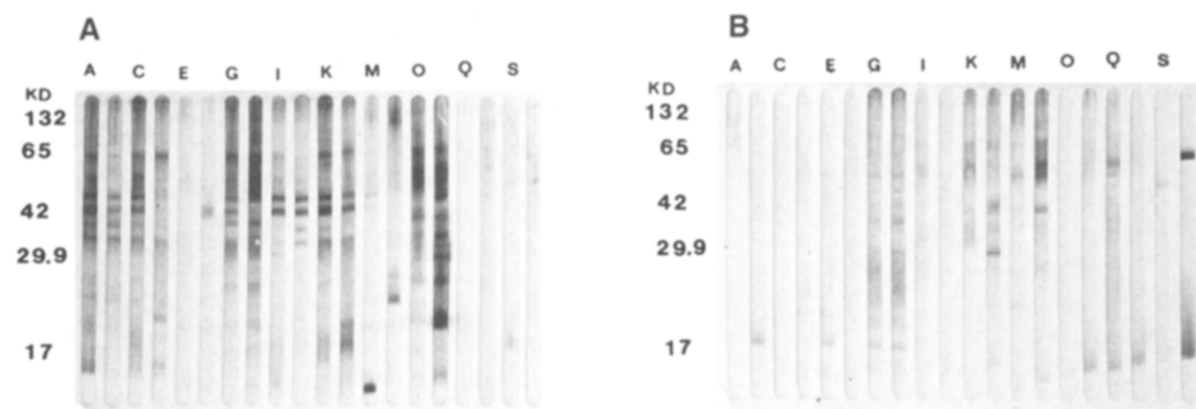


FIG. 2. IgG immunoblotting. **A**, Lanes A to L, Sera from patients; lanes M and N, sera from control subjects with high levels of IgG to *A. simplex* as determined by ELISA; lanes O to P, Sera from *Ascaris*-positive subjects with high levels of IgG to *A. simplex* (ELISA); lanes Q to T, negative controls to *A. simplex* in IgG ELISA. **B**, Lanes A to F, Negative controls to *A. simplex* in IgG ELISA; lanes G to P, sera from African subjects; lanes Q to T, sera from patients allergic to fish.

also detected in sera from all these subjects. However, only in some of the sera were weaker low molecular weight bands also observed. In patient 6's serum a single band of medium molecular weight was detected, and no bands were observed in serum from patient 5.

Multiple bands were seen in the rest of the sera, especially in that from the African group (Fig. 2, B, lanes G to P) and in that from the two *Ascaris*-positive subjects (Fig. 2, A, lanes o and p). Serum samples from two control subjects with high levels of IgG as determined by ELISA recognized two to three bands (Fig. 2, A, lanes M and N). Single bands were detected by some negative controls to *A. simplex* in IgG ELISA (Fig. 2, A, lanes Q to T,

and B, lanes A to F) and fish-allergic sera (Fig. 2, B, lanes Q to T).

Histamine release test

A representative case is shown in Fig. 3. Histamine release was positive, as much as 70%, with the 1:10,000 dilution of *A. simplex* extract. No release was obtained with the fish extract.

DISCUSSION

A. simplex larvae not only cause anisakiasis but allergic reactions as well. Mackerel-induced urticaria has been described by Kasuya et al.,¹³ and one case of anaphylaxis has been recently demonstrated by us.¹⁷ In our patients urticaria or angio-

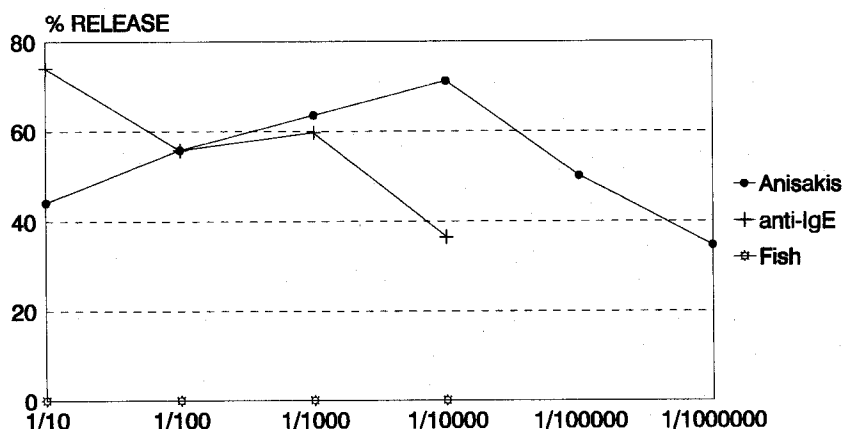


FIG. 3. Results of histamine release test performed with *A. simplex* and fish extracts in a representative case.

edema is the most frequent manifestation, but gastrointestinal symptoms are often reported also. In view of these data, we think that *A. simplex* is a potent antigen able to produce severe anaphylactic reactions. However, in some patients who were first seen with intense abdominal pain, a direct harmful action of the parasite against the gastrointestinal tract could also exist. Because gastroscopy was not performed and fish could have been undercooked, this possibility cannot be discarded.

Positive prick test results were obtained in all of the patients. Among the 150 subjects referred to our allergy department who were prick tested to control the extract, we obtained four positive test results. Three of them were included as cases (patients 10, 11, and 12) because of compatible clinical symptoms and positive specific IgE levels to *A. simplex* as measured by the CAP system. The other one was excluded from the study because of negative specific IgE to *A. simplex* as determined by the CAP system. This subject was a patient allergic to fish, and so this false-positive prick test result could be explained by contamination of the extract with fish proteins. These results demonstrated the high specificity of this simple test. The presence of fish antigens in our extract, however, could produce false-positive skin test results in patients allergic to fish.

No differences were observed in results of prick tests with the heated extract. This confirms the thermostability of the allergens and explains the clinical manifestations after ingestion of cooked fish. Stability to canning, however, has not been studied.

Presence of specific IgE antibodies to *A. simplex* was confirmed in our patients by several techniques (prick test, CAP system, and immunoblot-

ting), and a good concordance between these methods was obtained. IgE immunoblotting, as performed by us, showed high specificity and sensitivity. A similar profile of bands was found in most serum samples. It is remarkable that sera from patients 2, 4, and 8 had an almost identical pattern, with more bands than the others, and that this was coincident with high IgE levels as determined by the CAP system. Furthermore, they had clinical manifestations related to fish ingestion frequently for a long time. Two of them had received a diagnosis of idiopathic urticaria. The serum of patient 6 detected a single band. Only in patient 5, in whom specific IgE antibodies were detected by prick test and the CAP system, was blotting unable to detect IgE-reacting bands.

Some single bands were found in sera from patients allergic to fish. This could be due to fish proteins present in our extract. No bands were observed with the other control sera, including an African group with filariasis and the two *Ascaris*-positive sera with high titers of IgG and positive titers of IgE to *A. simplex* (CAP system), even though cross-reactions could be expected. Thus IgE immunoblotting was highly species-specific. Only concomitant fish allergy may interfere with the results.

The profiles of bands found in sera of our patients were different from those obtained by Akao et al.¹⁰ when studying patients with anisakiasis. The different blotting conditions (we used Nonidet P40 [Sigma Chemical Co., St. Louis, Mo.] as a renaturing agent and transfer by diffusion) could be the main reason, but it is possible that patients allergic to *A. simplex* and patients with anisakiasis show different IgE immune responses.

IgG antibodies were also detected by ELISA in sera from 10 of our patients. Positive results were also obtained in two *Ascaris*-positive sera and five control sera. Some of these sera were studied by means of the CAP system with discordant results, suggesting that specific IgG measurements do not have a predictive value for detecting patients allergic to *A. simplex*. However, when mean values of IgG levels in the three different groups were studied by Student's *t* test, statistically significant differences were observed between the group of our patients and the groups of *Ascaris*-positive and control subjects, respectively, but not between the *Ascaris* group and control groups. This fact suggested that patients with *A. simplex* hypersensitivity, as a group, had higher amounts of antigen-specific IgG antibodies than the other two groups of patients.

Results of IgG immunoblotting were concordant with results of indirect IgG ELISA but showed higher sensitivity, recognizing one band in serum from patient 6. No bands were noticed in serum from patient 5. Multiple bands were also observed in some control sera and in most of the sera from African subjects. These data demonstrated again that specific IgG measurement is not a diagnostic method for *A. simplex* allergy in the same way that it has not been useful for diagnosing the infection either.^{9, 10}

Finally, a histamine release test was performed in a representative case. Results were positive with *A. simplex* extract and negative with fish extract, confirming that *A. simplex* is able to elicit an IgE-mediated anaphylactic reaction.

Patient 5 had the only case of fish allergy, whereas patient 6 had an incipient case because he had only one outbreak of urticaria, 3 weeks before the study. It is interesting to point out some differences between these two patients and the others. Both were the youngest in the series (average 3.2-fold younger) and had the lowest levels of specific IgE (average 14.6-fold lower). No bands were detected by IgE and IgG immunoblotting in serum from patient 5 and only a single band was detected in serum from patient 6. Fewer exposures to *A. simplex* because of their age could explain these findings. In patient 5, the possible contamination of the *A. simplex* extract with fish proteins could explain the positive results of the skin prick test and specific IgE determination, or this patient could represent a mixed case of fish and parasite allergy. False-positive skin prick test results and determinations of IgE to *A. simplex*, caused by contamination of the extract with fish proteins could not be discarded.

A. simplex should be considered as a new agent in food allergy and should be suspected in those patients with anaphylactic symptoms after fish ingestion, more so if the same fish has been tolerated on other occasions. In such cases a positive prick test response to *A. simplex* and a negative response to fish is a good indication for a diagnosis of *A. simplex* allergy.

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REFERENCES

1. Kagei N, Isogaki H. A case of abdominal syndrome caused by the presence of a large number of *Anisakis* larvae. *Int J Parasitol* 1992;22:251-3.
2. Matsumoto T, Iida M, Kimura Y, Tanaka K, Kitada T, Fujishima M. Anisakiasis of the colon: radiologic and endoscopic features in six patients. *Radiology* 1992;183:97-9.
3. Ikeda K, Kumashiro R, Kifune T. Nine cases of acute gastric anisakiasis. *Gastrointest Endosc* 1989;35:304-8.
4. Kim LS, Lee YH, Kim S, Park HR, Cho SY. A case of anisakiasis causing intestinal obstruction. *Kisaengchunghak Chapchi* 1991;29:93-6.
5. Petithory JC, Marty B. L'anisakiase en France. *La Lettre de l'Infectiologue*. 1988;2:96-9.
6. Deardorff TL, Kayes SG, Fukumura T. Human anisakiasis transmitted by marine food products. *Hawaii Med J* 1991;50:9-16.
7. Petithory JC, Rousseau M, Siodlak F. Données séroépidémiologiques sur l'anisakiase: conséquences prophylactiques pour les produits de la pêche. *Bull Acad Natl Med* 1991;175:273-9.
8. Petithory JC, Lapierre J, Rousseau M, Clique MT. Diagnostic sérologique de l'anisakiase (granulome eosinophile digestif) par précipitation en milieu gélifié (ouchterlony, électrosynérèse, immunoélectrophorèse). *Med Mal Infect* 1986;3:157-62.
9. Desowitz RS, Raybourne RB, Ishikura H, Kliks MM. The radioallergosorbent test (RAST) for the serologic diagnosis of human anisakiasis. *Trans R Soc Trop Med Hyg* 1985;79:256-9.
10. Akao N, Ohyama T, Kondo K. Immunoblot analysis of serum IgG, IgA and IgE responses against larval excretory-secretory antigens of *Anisakis simplex* in patients with gastric anisakiasis. *J Helminthol* 1990;64:310-8.
11. Iglesias R, Leiro J, Ubeira FM, Santamarina MT, Sanmartín ML. *Anisakis simplex*: antigen recognition and antibody production in experimentally infected mice. *Parasite Immunol* 1993;15:243-50.
12. Kennedy MW, Tierney J, Ye P, et al. The secreted and somatic antigens of the third stage larva of *Anisakis simplex*, and antigenic relationship with *Ascaris suum*, *Ascaris lumbricoides*, and *Toxocara canis*. *Mol Biochem Parasitol* 1988;31:35-46.
13. Kasuya S, Hamano H, Izumi S. Mackerel-induced urticaria and *Anisakis*. *Lancet* 1990;335:665.
14. Kasuya S, Hamano H, Izurru S. Gastric anisakiasis with anaphylactoid reactions. *ACI News* 1989;1:13-4.
15. Kasuya S, Koga K. Significance of detection of specific IgE in *Anisakis*-related diseases [Abstract]. *Alerugi* 1992;41:106-10.
16. Lindqvist A, Iezawa Z, Tanaka A, Yman L. Seafood specific IgE in atopic dermatitis [Abstract]. Presented at

- American College of Allergy and Immunology; November 14-18, 1992; Chicago, Ill. Abstract 57.
17. Audicana M, Fernández de Corres L, Muñoz D, Fernández E, Navarro JA, Del Pozo MD. Recurrent anaphylaxis caused by *Anisakis simplex* parasitizing fish. J ALLERGY CLIN IMMUNOL 1995;96:558-60.
 18. Lowry O, Rosenbough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
 19. Laemmli UK. Cleavage of structural proteins during the assembly of the bacteriophage T4. Nature 1970;227:680-5.
 20. Petit C, Sauron ME, Gilbert M, Theze J. Use of immunoblotting to detect idiotypic determinants on monoclonal antibodies. In: Langone JJ, ed. Antibodies, antigens and molecular mimicry. San Diego: Academic Press 1989:144-51. Methods in Enzymology vol. 178.
 21. Moneo I, Alday E, Sanchez-Agudo L, Curiel G, Lucena R, Calatrava JM. Skin prick tests for hypersensitivity to α -amylase preparations. Occup Med 1995;45:151-5.
 22. Moneo I, Puente S, Sanchez-Agudo L, et al. Estudio de la liberación de histamina en sujetos normales, atópicos y parasitados. Inmunología 1993;12:54-8

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