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Clinical manifestations of allergy to *Anisakis simplex*

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Introduction

The sea-fish parasite *Anisakis simplex* (*As*) is the causative agent of anisakiasis, or anisakidosis, and induces IgE production. In 1985, Desowitz et al. described a method to detect specific IgE against *As* (1). In 1990, two clinical reports confirmed the allergenic potential of *As* (2, 3). In 1994, we observed that *As* was able to induce type I hypersensitivity as well. After studying a case of anaphylaxis caused by *As* (4), we reported 28 patients with immediate hypersensitivity developed after ingestion of parasitized fish (5), and we now have more than 60 allergic cases. Each case was diagnosed by suggestive anamnesis, positive skin prick tests with three *As* extracts (frozen, heated, and boiled), specific IgE detection in serum (CAP System), and exclusion of fish allergy.

The 10 first cases: IgE against *Ascaris lumbricoides* and *Toxocara canis*

After the first case in our department, several cases of *As* allergy were diagnosed. Because *Ascaris lumbricoides* (*Al*) sensitization suggested *As* responsibility, we started to study sensitization to other Ascaridoidae order parasites including *Al* and *Toxocara canis* (*Tc*).

We have studied our first 10 patients showing immediate hypersensitivity to *As* after ingesting fish infected with this parasite (6). Clinical manifestations were as follows: urticaria/angioedema in all 10 patients, digestive symptoms in three, respiratory symptoms in three, and anaphylactic shock in one. Paradoxically, the patients reported tolerance to the ingestion of the same kind of fish between and after the allergic episodes.

Type I hypersensitivity reaction to *As* was demonstrated by means of the positivity of the following tests:

1) skin prick tests (SPT) to three self-prepared extracts from *As* larvae: stored at −40°C, and heated for 10 min at 40°C and for 20 min at 100°C.
2) histamine release with the same extract in the first patient
3) detection of specific IgE (mean 57 kU/l) (CAP System).

A. simplex extract and parasite identification

Larvae collected from muscle tissue of hake, obtained locally, were kindly identified by Dr David Gibson, of the Natural History Museum of London, as those of *As* (Rudolphi, 1809) third-stage larvae. Figs. 1 and 2 show a free larva and a section of parasitized hake muscle tissue. A self-prepared extract of a sample of these was made (8 mg/ml protein, Lowry's method), according to Desowitz et al. (1), and stored at −40°C until required. A 1:100 dilution in saline was used for a SPT on the patient and controls. In order to prove that it did not induce irritant (false positive) reactions, it was also applied on 150 persons in the control group. The same extract heated for 10 min at 40°C and for 20 min at

100°C was also tested, but only on patients in the study group.

Positive SPTs with the three *As* extracts were observed in all patients, but no reactions occurred in more than 100 controls. The results of the SPT with the heated extracts revealed no differences. Specific IgE against *Al* and *Tc* was demonstrated in 10/10 (mean 4.45 kU/l) and 2/10 (mean 1.38) cases, respectively, by commercial CAP.

The possibility of sensitization to the fish's own proteins was ruled out by the above-mentioned tests, which were negative in all patients.

It was proved that the allergen/s concerned may be resistant to cooking and deep-freezing. Hence, anaphylactic reactions could result either from infection by or from mere exposure to the allergen.

Sixty-seven case reports

We have now recorded 67 patients with immediate hypersensitivity to *As*, developed after ingestion of parasitized fish. Each case was diagnosed by four criteria:

1) suggestive anamnesis of urticaria/angioedema or anaphylaxis after fish ingestion
2) SPT with a self-prepared *As* extract
3) specific IgE detection in serum (CAP System)
4) exclusion of fish allergy and other sensitizations.

Forty-two (67%) of these 67 cases concerned urticaria/angioedema. There were 18 (27%) cases of anaphylaxis (with more than one organ involved), and drug allergy was suspected in seven cases. There were 38 female and 28 male patients. Their ages ranged from 12 to 71 years, with an average of 48. The mean number of episodes per patient was 2.5. Paradoxically, most patients reported tolerance to ingestion of the same kind of fish between and after the allergic episodes. Sensitization to the proteins of the fish themselves was ruled out by negative reactions of the above-mentioned tests in all cases.

Figure 1. Free larva of *A. simplex*.

The clinical manifestations can range from acute urticaria/angioedema to anaphylactic shock. Urticaria and/or facial angioedema were registered in all 67 patients. The second system involved was the gastrointestinal (vomiting, diarrhoea, and abdominal pain as symptoms) in 40% (27/67) of patients. Respiratory symptoms were the third kind, affecting 18% of patients (12/67). In eight patients, anaphylactic shock was diagnosed in the emergency room. More than 50% of patients required treatment by emergency services. Five had to be hospitalized; two because of severe angioedema, one for persistent shock, and one, our most severely affected patient, for respiratory arrest.

The SPT were positive in all patients with our somatic extract of *As*. Total IgE was more than 100 kU/l in 88% of patients with a high dispersion of values (median: 315, range: 76–17.047, SD 230). Values higher than 0.7 kU/l of specific IgE were considered positive. Most values were between classes 3 and 5. The median was 32.5 (range 1.4–100, SD 34.3).

We describe now several representative patients: a hypothetic typical patient, our most severely affected patient, two who presented arthralgia, and, for comparison and contrast, a parasitized patient without allergic symptoms.

Typical patient

The "typical" patient is middle-aged (average 48 years) without atopic antecedents, and reports more than one episode of urticaria/angioedema and/or anaphylaxis, from a few minutes to 6 h after eating fish or cephalopods. Sometimes the patient associates the episode with drugs, and often pruritus awakens the patient at night, because in Spain it is very common to have fish for the evening meal.

Most severely affected patient

A 53-year-old woman who ate at the evening meal raw anchovies marinated in vinegar and fried hake awoke 3 h later with palmar itching, dizziness, and dyspnea. At presentation in the emergency department of our hospital, she was unconscious with undetectable blood pressure, extremely dyspneic, and cyanotic. Epinephrine was injected subcutaneously, leading to dramatic improvement. Urticaria was then observed on the trunk and limbs. Cutaneous and serologic tests were positive against *As*.

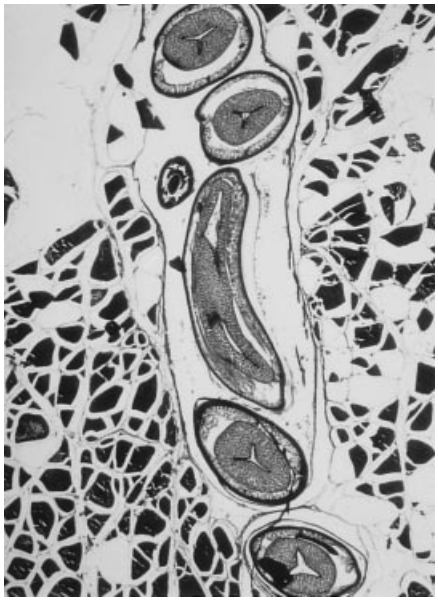

Figure 2. Section of parasitized hake muscle tissue (Masson's trichrome stain).

Arthralgia/arthritis

Patient 1

In 1994, a 33-year-old man developed acute arthritis of the elbow and knee 8 days after a process characterized by nausea, vomiting, and epigastric pain related to eating hake. Analytic findings included leukocytosis with eosinophilia (26%; 3484/mm^3) and elevated acute reactants (protein C reactive and sedimentation rate). Biochemistry, proteinogram, and immunoglobulins were normal except for total IgE, which was 835 IU/ml. Coprocultive, urocultive, and faeces analyses for parasites were negative. Antinuclear antibodies, rheumatoid factor, and serologic tests for *Yersinia* and *Salmonella* and HLA-B27 were negative. Three months later, values returned to normal, and the patient remained asymptomatic.

In 1996, the patient developed acute urticaria/angioedema and epigastric pain, without articular symptoms, 60 min after ingestion of hake. Positive SPT with *As* extracts, and IgE (>100 kU/l) confirmed *As* allergy. Anisakidae larvae were isolated from a fresh piece of the hake implicated in the second episode of the patient.

Patient 2

A 60-year-old woman was admitted to our hospital with urticaria and arthralgia 60 min after eating hake. Cutaneous tests with *As* were positive. Total IgE was 88 and specific IgE 6.62 kU/l. These observations are consistent with previous reports associating myalgia/arthralgia and anisakidosis (7, 8), but acute reactive arthralgia or arthritis and IgE response to *As* were not described in those reports.

Nonallergic parasitized patient

For contrast, we describe the clinical and serologic features of a patient infected by *As* who produced IgE antibodies without allergic symptoms. A 74-year-old man complained of severe epigastric pain and nausea about 8 h after eating hake undercooked in a microwave oven (30 s). He was admitted to our hospital, and gastroendoscopy revealed acute gastritis. Two worms were removed from the stomach wall with a biopsy clipper.

SPT were negative with our *As* extracts.

In this case, monoclonal antibodies were studied by Ubeira and colleagues, confirming a serologic pattern similar to those of confirmed anisakidosis from Japanese sera.

Fish species implicated

The fish species most commonly implicated were hake (*Merluccius merluccius* L) (23), anchovy (*Engraulis encrasicholus* L) (16), and cod (*Gadus morhua* L) (7).

Thirty-six patients developed symptoms after ingestion of raw fish, five with tinned fish and the remainder with cooked seafood.

In this particular "food allergy", it is worth emphasizing the advanced age of the patients and the prevalence in the nonatopic population. In view of the severity and the apparently high frequency of the reactions, we consider it imperative that the findings be brought to the attention not only of allergists but also of general practitioners, intensive-care practitioners, those working in emergency rooms, and fish inspectors who are veterinary professionals (9).

It is becoming increasingly likely that the impact of disease caused by *As* in man has been underestimated in its allergic aspects. We suggest that *As* allergy be considered in the differential diagnosis of patients presenting with a range of allergic disorders from acute, sporadic urticaria to anaphylactic shock, especially if these patients had recently eaten seafood.

Prevalence rates of allergy and sensitization in patients with acute episodes of urticaria/angioedema/anaphylaxis compared with controls

Currently, we are focusing in our clinical work on *As* allergy. Surprisingly, *As* has proved to be the source of one of the most important food allergens in Spain (10). Japanese authors have described a positive rate of AlaSTAT (excretory/secretory antigens from *As* larvae) of 87.5% in gastric anisakiasis patients who were endoscopically diagnosed, and 75% in mackerel-

induced urticaria patients, while in patients with urticaria of unknown origin and normal controls, the rates were 8.3% and 10%, respectively (11).

In view of these data, we designed a study to determine the percentage of subjects sensitized to *As* among healthy donors and patients with acute urticaria, and the percentage in whom this sensitization is relevant. This study was sponsored by the Spanish Foundation of Allergy and Clinical Immunology in 1995.

We studied 150 patients presenting acute urticaria/angioedema outbreak/s and 150 healthy subjects from the blood bank by the following methods:

1) questionnaire
2) SPT to *As* somatic extract and fish
3) determinations of total IgE and specific IgE to *As* in sera.

Among 150 cases of acute urticaria/angioedema and/or anaphylaxis, positive IgE values were shown by 26% (39/150), but only 31% (12/39) fulfilled the clinical criteria of true allergy. Surprisingly, in 150 controls (healthy blood donors without allergic or urticarial antecedents) matched by age and sex, 12.6% (19/150) were positive for specific IgE to the parasite.

According to these data, the percentage of subjects sensitized to *As* among the patients was 26%, a figure similar to that detected by Japanese authors among atopic dermatitis patients. The IgE detection was relevant in only 31% of sensitized patients. Detection of specific IgE is useful to detect sensitization to *As*, but not to determine true allergy.

In our study, the prevalence of *As* allergy (12%) was similar to that found to Rosaceae species plus nuts and shellfish. This illustrates the relevance of *As* to the pathogenesis of acute urticaria/anaphylaxis. Furthermore, we may note that these patients would have previously been labelled idiopathic because of the lack of recognition of the inciting agent if *As* had not been investigated.

Fish is clearly one of the foods most commonly implicated in sensitization in populations in which fish is a staple food (12–14). In our study, allergy to *As* was even more frequent than fish allergy (12% *vs* 1.3%), and sensitization to *As* (CAP $>$ 0.7 kU/l) was much higher than to fish (26% *vs* 3%). Spain is one of the countries with the highest fish consumption (89 g per person per day) (15). The mean rate of fish consumption among our patients was 2.54 times a week, and all of them ate fish at least once a week. The prevalence of fish allergy was lower than expected (12, 13) but was similar to that observed by Joral et al. also in northern Spain (14).

IgE immunoblotting in diagnosis of *A. simplex* allergy

After study of our first case of anaphylaxis caused by *As* (4) and a series of 67 patients, IgE antibodies (CAP) were found in a higher number of subjects, even without congruent symptoms, suggesting that the sensitization is more widespread. A positive SPT and the presence of very high levels of specific IgE to *As*, when accompanied by an unequivocal anamnesis, are valuable tools for the diagnosis of *As* allergy. Negative results of these tests are also important for the exclusion of such a diagnosis. In our experience, the diagnosis of *As* allergy should not be based on the mere presence of positive SPT and/or CAP value because of the high frequency of false positives found in normal populations: 26% in this study and 10% in a Japanese report (11).

At present, the best method of confirming the diagnosis of food allergy is the double-blind, placebo-controlled food ingestion challenge (16). This test has been evaluated in adult and paediatric populations; it correlated well with the results of SPT and measurement of specific IgE, as well as with basophil histamine-release assays (12–14, 17). As ethical reasons preclude the challenge test with a parasite, we lack a reference standard for the diagnosis of *As* allergy.

After previous IgE immunoblotting confirmed specific bands in an *As* extract with sera from allergic patients (18), a study by IgE immunoblotting was performed in order to define the differences in the pattern of IgE recognition between allergic and nonallergic patients. We have studied 61 patients with acute symptoms of urticaria/angioedema or anaphylaxis and positive specific IgE to *As*. According to the anamnesis, the time interval between the ingestion of the fish and the clinical onset, and the exclusion of other causes of allergy, three different groups of patients were established: group A (allergic), group NA (nonallergic), and group D (doubtful). Fifty-one healthy donors were included as controls (group C). IgE immunoblotting with a self-prepared *As* somatic extract was performed in all patients and controls.

Four types of immunoblotting pattern were observed:

1) with a group of several bands of medium molecular mass and others of low mass
2) two or more bands of medium mass
3) only one band of medium mass
4) without any band (19) (Fig. 3).

There was a significant difference in the predominance of type 1 immunoblotting in allergic patients (group A) from the other groups. The frequency of type 4 immunoblotting had a significant predominance (78%) in controls (group C).

Eight out of 20 patients (40%) of the doubtful group had type 1 immunoblotting, but they were not classified as allergic because they did not remember eating fish within the 4 h before the clinical onset. Although we could not perform a challenge test to determine whether these patients were allergic, we believe that they should be considered as such. Therefore, these patients should

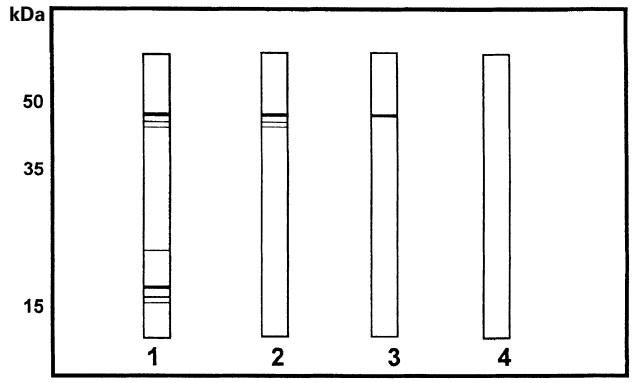

Figure 3. Specific IgE immunoblotting patterns.

be advised to avoid fish because they could be in progression toward allergy.

In group NA, only two (12.5%) patients showed type 1 immunoblotting. They did not present any clinical manifestation although they continued to eat fish, but this fact was irrelevant, since there was no evidence that the fish eaten was parasitized.

The proteins detected by subjects showing types 2 and 3 immunoblotting might have been responsible for the positive results in the SPTs and the high IgE values. We cannot rule out the possibility that these types of immunoblotting were the mere expression of an initial sensitization to *As* or of cross-reactivity with other parasites and/or other antigens. At present, the clinical relevance of these blots is not clear, and we think such patients should be monitored in order to detect possible evolution to true allergy.

SPT and IgE to *As* should be considered insufficient to diagnose *As* allergy because of the high frequency of false positives. On the other hand, negative results of SPTs and IgE detection would have a high predictive value in excluding this sensitization.

Conclusions

1) The presence of IgE antibodies against *As* in patients suffering from acute urticaria is similar to that found by Japanese authors in atopic dermatitis (20).
2) The finding of sensitization in healthy controls (25%) is higher than expected when compared to known data in Japan (10%) or in Sweden, where it is absent.
3) Sensitization to *As* in the absence of any relevant symptoms was frequently found. Specific IgE against *As* not only is unsuitable as a diagnostic tool to confirm allergy, but also appears to be misleading, since it was detected in 25% of the healthy controls. A possible explanation for the finding of specific IgE against *As* without symptoms could be cross-reactivity with other nematodes (21–23) or with the so-called panallergens, such as tropomyosin, which is found not only in *As* but also in crustaceans, insects, and mites (24, 25). Another possibility is cross-reactivity of IgE with *O*-linked or N-linked glycans, which are frequently found in the glycoproteins of other nematodes (26). Finally, this phenomenon may be explained by the conservation of molecules such as biotinyl-enzymes (26), which can stimulate the production of IgE in some patients.
4) Although IgE immunoblotting appears to be a useful diagnostic tool, and other authors have obtained good results with the histamine-release test in diagnosis (27), none of these tests can be used routinely for a definitive diagnosis.
5) The exposure test to *As* was not performed for ethical reasons. However, our preliminary results for allergy to *As* in acute urticaria/angioedema and/or anaphylaxis are similar to those for other well-known causes of food allergy (such as fruits and nuts).
6) Accurate diagnosis of *Anisakis* infection may be difficult, and we assume that this disorder is often undiagnosed. It is perhaps time to consider more sweeping measures than the ones currently in force to protect public health.

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