CASE REPORT

Multiple Acute Parasitization by *Anisakis simplex*

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Abstract

Hypersensitivity to *Anisakis* is an increasingly prominent medical problem throughout the world, due to a better understanding of diseases induced by parasites and to modern culinary habits of eating raw or undercooked fish.
We describe the case of a patient who presented epigastric pain, wheals, erythema, and pruritus 3 hours after the ingestion of fish. More than 200 larvae were obtained by endoscopy. However, the patient only developed an immune response with specific immunoglobulin E and eosinophilia peaking at day 18 and decreasing during the 17-month follow-up. Only eosinophilia reached normal limits.

Key words: Parasitization. *Anisakis simplex*. Ani s 1. Ani s 4. Recombinant allergen.

Resumen

La hipersensibilidad a *Anisakis simplex* es un problema médico de distribución mundial cada vez con mayor relevancia debido tanto al mejor conocimiento de las enfermedades ocasionadas por parásitos como a los modernos hábitos culinarios de ingesta de pescado crudo o poco cocinado.
Describimos el caso de una paciente que presentó epigastralgia, eritema, pápulas y prurito tres horas después de la ingesta de pescado. Se extrajeron más de doscientas larvas mediante endoscopia oral. A pesar de ello, sólo desarrolló una respuesta inmune con IgE específica frente a Anisakis simplex y eosinofilia, alcanzándose la máxima respuesta a los 18 días tras la ingesta y descendiendo durante un seguimiento de 17 meses, aunque las cifras de eosinófilos retornaron al rango de normalidad.

Palabras clave: Parasitación. *Anisakis simplex*. Ani s 1. Ani s 4. Alérgeno recombinante.

Introduction

Anisakis simplex is a nematode that can infect humans who eat raw or undercooked fish containing live L3 larvae. Larvae invading the gastrointestinal mucosa secrete proteins implicated in the pathogenesis of anisakiasis, which can induce immunoglobulin (Ig) E–mediated symptoms, with clinical manifestations ranging from urticaria or angioedema to anaphylaxis [1]. On infection by the parasite, humans develop specific IgE to parasite allergens. Further contact induces basophil and mast cell degranulation, thus leading to symptoms.

When an *Anisakis* infection is suspected, correct diagnosis is based on a clinical history that is suggestive of fish ingestion, with oral panendoscopy as the test confirming diagnosis and, as in our case, enabling a therapeutic intervention to be made. Skin prick tests and determination of specific IgE to *Anisakis* are also routinely performed [2].

We report the case of a patient infected by a large number of *A simplex* larvae. Despite the quantity of allergen, she developed only minimal allergic symptoms and a weak immune response.

Case Description

A 37-year-old woman with no personal history of interest, presented epigastric pain, pruritus, diffuse erythema, and wheals (face, pressure points, and flexures) 3 hours after ingestion of fried hake and fish ova. She did not experience nausea or vomiting. Two hours later, she was admitted to

the emergency room (5 hours after ingestion) where she was treated with intravenous dexchlorpheniramine (5 mg), methylprednisolone (40 mg), and pantoprazole (40 mg).

Fourteen hours after ingestion, due to the persistence of epigastric pain (no cutaneous symptoms), fiberoptic endoscopy revealed an accumulation of parasites accompanied by erosion of the gastric mucosa. Many parasites were removed during this 75-minute procedure. A second endoscopy was performed under sedation to extract the remaining parasites. More than 200 parasites were obtained from the cardia and fundus (Figure 1) in both endoscopies. Analysis of the biopsy specimen revealed chronic superficial antral gastritis, the presence of *Helicobacter pylori*, and anisakiasis. The microbiology department reported that all of the parasites were *A simplex*. An allergologic study with skin prick tests designed for urticaria-angioedema (including food and fish extracts, aeroallergens, *Anisakis*, latex, and *Saccharomyces cerevisiae*) was negative. The results of complementary tests (including hepatitis serology and stool parasite testing) were all normal. Chest X-ray and abdominal ultrasound results were unremarkable.

Total serum IgE was measured using enzyme-linked immunosorbent assay (ELISA) (Immulite, DPC 1000; DPC; Los Angeles, California, USA) following the manufacturer's instructions. Specific IgE to parasites was measured using CAP FEIA (ImmunoCAP 250, Phadia, Uppsala, Sweden) following the manufacturer's instructions. An ImmunoCAP value equal to or greater than 0.35 kU_A/L was considered positive. Specific IgG and IgA to parasites was also measured using CAP FEIA following the manufacturer's instructions. Tryptase and eosinophilic cationic protein were measured using ELISA (UniCAP 100, Phadia, Uppsala, Sweden).

Specific IgE to crude *A simplex* extract, enriched fractions, and recombinant parasite protein measurements was determined by immunoblotting using previously described methods [3-8].

Total serum IgE was in the normal range, specific IgE to *A simplex* was 2.06 kU_A/L, and specific IgE for *Toxocara canis* was 9.75 kU_A/L. The results for the remaining parasites studied (*Ascaris lumbricoides* and *Echinococcus granulosus*) were negative. Sera were negative for hepatitis A virus, hepatitis B virus, hepatitis C virus, human immunodeficiency virus, *Treponema pallidum*, *E granulosus*, and *T canis*. The patient had no contact with animals and did not present symptoms compatible with toxocariasis, since serum IgG for *T canis* was negative.

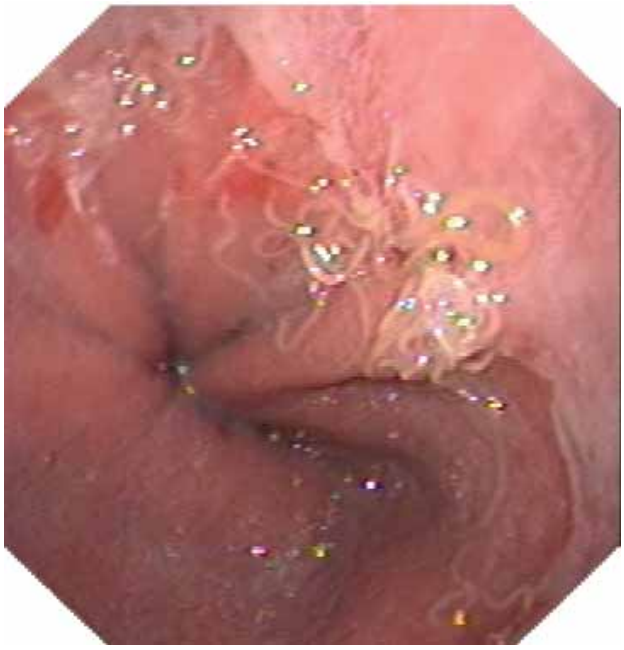

Figure 1. Multiple parasites forming a nest in the gastric mucosa.

One-dimensional electrophoresis (sodium dodecyl–

Table. Main Immunological Findings

	Baseline	4 Days	18 Days	46 Days	7 Months	17 Months
Total IgE[a], kU/L	48	37	344	140	83	48
IgE *Toxocara canis*[b]	9.75			5.92	3.83	5.86
IgE *Ascaris*[b]	<0.35			<0.35		<0.35
IgE *Echinococcus*[b]	<0.35			<0.35		<0.35
IgE *Anisakis*[b]	2.06	1.41	10.3	5.27	7.42	1.23
IgG *Anisakis*[c], mgA/L	4.77	5.99	55.3	44.7	13.10	6.62
IgA *Anisakis*[c], mgA/L	2.76	2.45	8.55	5.55	3.48	2.50
Tryptase[d], µg/L	2.75	2.54	2.86	2.14	1.44	1.28
ECP[e], µg/L	2.84	8.24	28.1	6.71	5.68	5.38

Abbreviations: ECP, eosinophil cationic protein; Ig, immunoglobulin.
[a]Normal value, <200.00 kU/L.
[b]Normal value, <0.35 kU_A/L.
[c]Normal value, <20.00 mg_A/L.
[d]Normal value, <11.00 µg/L.
[e]Normal value, <12.00 µg/L.

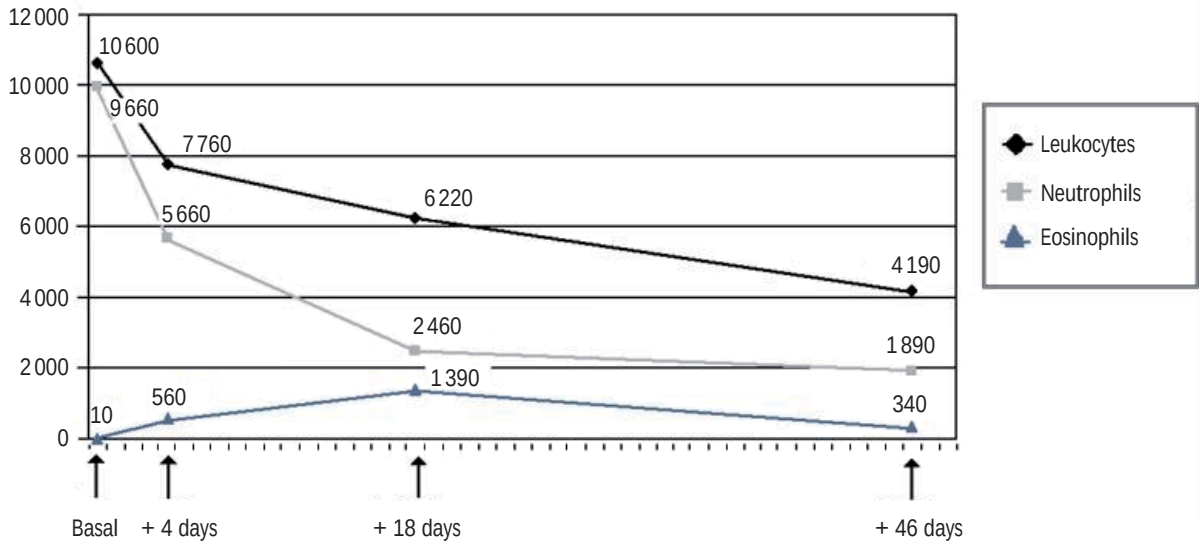

Figure 2. Total leukocytes, neutrophils and eosinophils after the acute episode, per mm^3.

polyacrylamide gel electrophoresis) and IgE-immunoblotting were used for better characterization of the specific immune response. The results were negative for the crude extract and some allergen-enriched fractions and for Ani s 1 and Ani s 4 (both clinically relevant secretory allergens from *A simplex*).

After this acute episode, the patient was followed up for 17 months. During this period, total and specific IgE values reached their maximum at day 18 and gradually decreased (Table), although they never reached normal limits. The measurement of specific IgG and IgA to *Anisakis* showed the same pattern. No elevation of tryptase or eosinophil cationic protein levels was observed.

Specific IgE measurement by immunoblotting was positive only in the sample obtained on day 18, revealing several *Anisakis* allergens with moderate intensity. No clinically relevant allergens, such as Ani s 1 or Ani s 4, were detected (data not shown).

After the acute episode, the white cell count was 10 600/µL, and this gradually decreased to 4190/µL at the last determination. Neutrophils decreased from 9660/µL on admission to 2460/µL on day 18. More remarkably, slight eosinophilia was detected on day 4, peaked at day 18 (1390/µL), and returned to normal levels on day 46 (Figure 2).

Discussion

Infection by *Anisakis* L3 larvae induces increases in total and specific IgE values, and these determinations seem to be useful for the diagnosis of acute episodes during follow-up. Daschner et al [9] found that 85.36% of patients had an increase in their initial total IgE value and 90.24% had higher specific IgE values 1 month after the acute episode. Endoscopy performed during the acute episode usually reveals the presence of a single larva.

Massive infection by *A simplex* is seldom reported. Kargei et al [10] described one case of a 58-year-old woman with gastric anisakiasis who presented epigastric pain and nausea after eating sashimi. Fifty-six larval nematodes were removed directly from the greater curvature of the stomach with a gastroendoscopic biopsy clipper and were identified as larvae of *A simplex*. Daschner et al [11] described a 66-year-old man with gastroallergic anisakiasis who presented intense epigastric pain and generalized urticaria. Twenty larvae of *A simplex* were extracted using endoscopy. López-Serrano et al [12] described a 63-year-old woman with urticaria, angioedema, and diarrhea after ingestion of fresh fish. More than 10 parasites were removed by oral endoscopy. Noh et al [13] reported a 68-year-old man with epigastric pain and vomiting 1 hour after eating anchovies. Four nematode larvae were found anchored in the gastric mucosa, and these were subsequently identified as L3 larvae of *A simplex*. We found no further articles on multiple parasitization by *A simplex*. Unfortunately no follow-up data were available from these cases.

We expected the patient to develop a strong IgE response to *Anisakis* allergens. Patients experiencing systemic reactions after eating infected fish usually have specific IgE values that exceed the upper limit of detection (100 kU_A/L), total IgE values higher than 1000 kU/L, and a strong presence of multiple allergens, especially Ani s 1 and Ani s 4, in the IgE-immunoblotting assay. None of these values were observed in our case. The poor immune response of our patient to this allergenic stimulus could be explained by some kind of immune deficiency. The patient did not report a history of infections, allergic diseases, or any other abnormalities.

A second hypothesis is that the patient experienced a modulated immune response induced by the parasite during

the acute infection. This could lead to an ineffective immune response by the host, thus allowing the parasite to evade the consequences of a vigorous defensive attack. If IgE responses can be considered protective, at least in some parasitoses [14-16], then any mechanism that could block IgE production would be an advantage for the parasite. In this regard, it is important to remember that no IgE response to 2 relevant secreted allergens (Ani s 1 and Ani s 4) was demonstrated using recombinant allergens.

This poor IgE response has been fully demonstrated previously in experimental infection models. In fact, several years ago Amano et al [17] reported that rats infected with low numbers of *Anisakis* L3 larvae developed strong IgE responses, but rats infected with a high number of larvae did not show this specific IgE response. The present case could be considered to represent the human counterpart of this experimental approach.

Toxocariasis is more common in children under 12 years old, and having a young puppy at home is a risk factor. Growing up in a low-income neighborhood has been associated with a higher rate of seropositivity for toxocariasis than being raised in a middle-income area. Adult patients institutionalized for mental retardation have also been reported to be at high risk [18]. Seroprevalence to *Toxocara* in children was reported to be over 1% in children at University Hospital La Paz, Madrid, Spain [19]. Larva migrans affects humans alone and is caused by the larvae of *T canis*. The condition most commonly occurs in children who have had close contact with household pets or who have frequented areas such as public parks where the ground is contaminated by dog feces. Covert toxocariasis has been described as the most common presentation [20], although in this case report, IgG *Toxocara* serology was negative. Various studies have reported cross-reactivity between *Anisakis* and *Toxocara* [21], which may explain our positive IgE results; however, it was not possible to complete the study in vitro due to the lack of additional sera.

We present the case of a patient with minimal and evanescent symptoms of allergy, despite intense parasitization by *A simplex* larvae. The specific humoral response to *Anisakis* was weak, and this observation seems consistent with the results of previous experimental animal models, in which high parasite loads led to poor IgE responses. We were unable to find similar cases of massive infection.

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