
Specific IgE determination to Ani s 1, a major allergen from *Anisakis simplex*, is a useful tool for diagnosis

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Background: The most serious limitation of the serodiagnosis of parasitoses is the occurrence of cross-reactions.

Objective: The possible use of *Anisakis simplex* major allergen Ani s 1 for diagnosis.

Patients and Methods: Forty-nine non-fish-allergic patients with *Anisakis simplex* hypersensitivity, 21 patients without allergic episodes suffering intestinal anisakiasis with obstruction of the intestinal lumen, and 10 unrelated sera as a control were included in this study to determine specific immunoglobulin (Ig)E and IgG to *Anisakis simplex* major allergen Ani s 1 by immunoblotting.

Results: Eighty-six percent of patients with *Anisakis simplex* hypersensitivity showed specific IgE directed to Ani s 1. Identical result was obtained for IgG detection in this group. Among patients with intestinal anisakiasis, 86% showed specific IgE, but only 29% had specific IgG ($P < 0.001$, two-tailed Fisher exact test). One of the 10 control subjects was positive both for IgE and IgG ($P < 0.001$).

Conclusions: Determination of specific IgE directed to *Anisakis simplex* major allergen Ani s 1 is a useful tool for the diagnosis of hypersensitivity and intestinal anisakiasis. Further, measurement of specific IgG directed to *Anisakis simplex* major allergen Ani s 1 is only valid for *Anisakis simplex* allergy.

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INTRODUCTION

Anisakis simplex is a nematode parasite with a wide geographic distribution and frequently found in a great diversity of fish and cephalopod species commonly consumed by man. It causes a gastrointestinal disease and /or acute allergy symptoms such as urticaria, angioedema, and anaphylactic reactions.¹ Determination of specific antibodies, especially specific immunoglobulin (Ig)E, has been used for diagnosis,^{2,3} but all commercial diagnostic assays are performed with crude parasite extracts. The occurrence of cross-reactions is a limitation of the serodiagnosis of parasitoses. For this reason, tests using recombinant antigens are being developed for diagnosis of several parasitoses,^{4–7} but, at the moment, no recombinant antigen for diagnosis of *Anisakis simplex* sensitization is available.

In a previous study,⁸ we found that allergen Ani s 1, an excretory gland protein with a molecular weight close to 25 kD isolated and purified from the fish parasite *Anisakis simplex*, had a high clinical relevance. Of 20 symptomatic subjects, 85% had detectable levels of specific IgE directed against this protein. Further, approximately 5% of these individuals detected only this protein in the crude parasite

extract. The aim of this paper is to evaluate the use of Ani s 1 for diagnosis of the *Anisakis simplex* infection in the clinical practice.

MATERIAL AND METHODS

Sera

Forty-nine non-fish-allergic patients with symptoms of urticaria/angioedema after fish ingestion, 21 patients without allergic episodes suffering intestinal anisakiasis with obstruction of the intestinal lumen, and 10 unrelated sera as a control were included in this study.

Antigens

Anisakis simplex extract was prepared with larvae obtained from muscle tissue of *Micromesistius poutassou*. Approximately 300 larvae were ground in a mortar with 10 mL of phosphate-buffered saline. The mixture was centrifuged for 5 minutes at 1,000 *g*, and the pellet was discarded. The protein content of the supernatant was 2 mg/mL, as measured by the method of Bradford⁹ with bovine serum albumin as a standard.

Anisakis simplex major allergen Ani s 1 was isolated from larvae and purified from the crude parasite extract by ethanol precipitation and reversed-phase high-power liquid chromatography according to the method described in a previous work.⁸

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Immunoblotting

Ani s 1 (0.1 mL of a 100 μ g/mL dilution) or crude *Anisakis simplex* extract (0.1 mL of a 1 mg/mL dilution) were mixed with 0.1 mL of sample buffer and subjected to electrophoresis in a 16% acrylamide minigel (150 V) under standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis conditions and transferred by diffusion to two nitrocellulose sheets for 18 hours. The next day, after blocking in 3% Nonidet P-40 for 30 minutes, the membranes were placed on a Mini-Protean II Multiscreen (Bio-Rad Lab, Hercules, CA). The different sera were diluted in incubation buffer and 0.6 mL of the mixture was incubated overnight in each independent chamber. For specific IgE determination, the different sera were diluted 1:6 in incubation buffer. After incubation and washing, the membranes were incubated for 3 hours with 10 mL of a 1:1,000 dilution of a monoclonal anti-IgE antiserum (Ingenasa, Madrid, Spain). After new washes, the membranes were incubated with 10 mL of a 1:4,000 dilution of an alkaline phosphatase-labeled goat anti-mouse antiserum (Biosource, Camarillo, CA). Finally, the membranes were washed, and the signal was visualized by alkaline phosphatase-5-bromo-4-chloro-3-indolyl phosphate/4-nitroblue tetrazolium system (Amresco, Solon, OH) for 30 minutes.⁸

The presence of specific IgG in the sera was tested using the same method with some modifications. For specific IgG determination the different sera were diluted 1:100 in incubation buffer. After incubation for 1 hour and washing, the membranes were incubated for 1 hour with 10 mL of a 1:4,000 dilution of a goat anti-human IgG (alkaline phosphatase-labeled; Tago, Burlingame, CA) and the substrate was added as described above.

RESULTS

The analysis of specific IgE and IgG to Ani s 1 by immunoblotting showed the distribution presented in Figure 1. Eighty-six percent of patients (42 of 49) with *Anisakis simplex* hypersensitivity showed detectable levels of specific IgE directed to Ani s 1. Identical result was obtained for patients

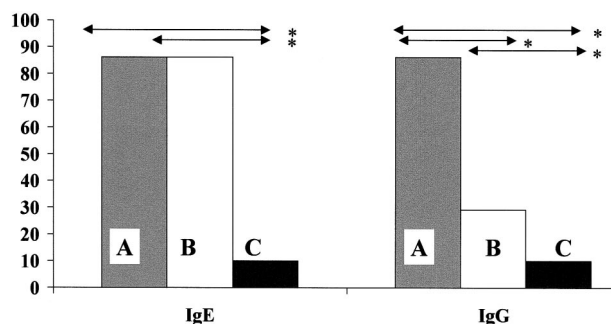


Figure 1. Distribution of specific IgE and IgG to Ani s 1. (A) Patients with *Anisakis simplex* hypersensitivity; (B) patients with intestinal anisakiasis; and (C) unrelated sera as a control. *, $P < 0.001$, two-tailed Fisher exact test.

with intestinal anisakiasis, although these patients did not remember any allergic episode. Only 1 of the 10 control subjects had specific IgE to Ani s 1 ($P < 0.001$, two-tailed Fisher exact test).

The absence of allergic symptoms in the patients with intestinal anisakiasis could perhaps be explained by the presence of a high amount of specific antibodies belonging to the IgG isotype. However, when specific IgG to Ani s 1 was measured in these patients, only 29% (6 of 21) had specific IgG antibodies in contrast with 86% (42 of 49) found for patients with *Anisakis simplex* hypersensitivity ($P < 0.001$). The control subject with positive specific IgE to Ani s 1 also had specific IgG antibodies.

Although patients with allergic symptoms and intestinal anisakiasis patients analyzed in our study had similar specific IgE recognition patterns with a parasite crude extract (Fig 2), we observed that patients with *Anisakis simplex* hypersensitivity had both IgE and IgG specific antibodies directed to Ani s 1, whereas patients with intestinal anisakiasis had IgE more frequently directed to Ani s 1 than IgG.

DISCUSSION

At present, specific IgE detection by immunoblotting is a relevant approach to diagnose *Anisakis simplex* allergy.² This method offers the advantage of giving information about the presence of specific IgE directed to the different parasite antigens.

The existence of immunologic cross-reactivity among nematodes and cross-reactivity between proteins from *Anisakis simplex* and arthropods has been described.¹⁰⁻¹³ Epidemiologic studies performed using quantification of specific IgE antibodies against *Anisakis simplex* crude extracts have shown an alarming prevalence of sensitization.¹⁴⁻¹⁶ Further, positive skin test and CAP values (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden) have been found in many asymptomatic subjects, normal population, and patients suffering urticaria and angioedema without a clear relationship to fish consumption.^{2,3,17} All these data suggest that crude *Anisakis simplex* extracts should not be used for prevalence studies because of their low specificity.

In our previous work,⁸ *Anisakis simplex* major allergen Ani s 1 showed a very high clinical relevance and the following features that could increase the specificity of the diagnosis assays. 1) Its N-terminal amino acid sequence showed no homology to previously described proteins. 2) This protein was detected by immunohistochemistry only in the parasite excretory gland, making it therefore an excretory/secretory antigen.

For these reasons, we tried to determine the value of Ani s 1 for diagnosis of *Anisakis simplex* infection. According to the results obtained in the present study, patients with *Anisakis simplex* hypersensitivity and patients with intestinal anisakiasis had similar specific IgE recognition patterns with a parasite crude extract. Both groups had IgE and IgG specific antibodies directed to Ani s 1, but patients

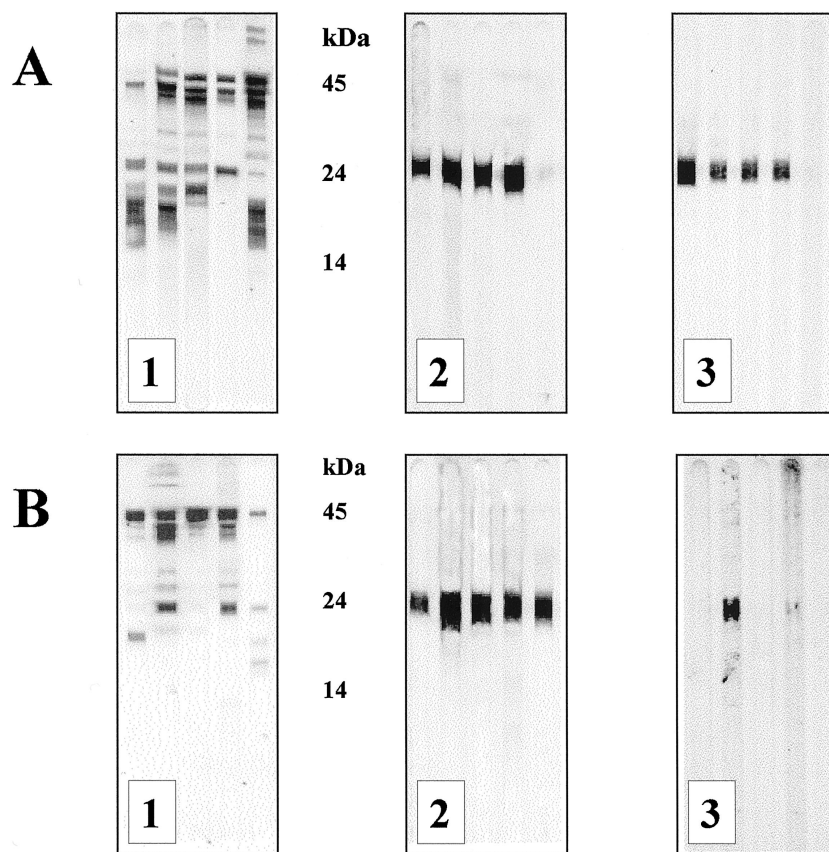


Figure 2. Immunoblotting of some representative sera from patients with *Anisakis simplex* hypersensitivity (A), and sera from patients with intestinal anisakiasis (B). Specific IgE determination using *Anisakis simplex* crude extract (1); specific IgE determination using Ani s 1 (2); and specific IgG determination using Ani s 1 (3).

with intestinal anisakiasis had IgE more frequently directed to Ani s 1 than IgG. Del Pozo et al¹⁸ showed that specific IgG measurement using crude parasite extracts was not sufficiently reliable because of the high cross-reactivity observed and, as a result, this determination has not been considered.

One of the 10 control subjects was positive for both IgE and IgG. This fact is in agreement with the frequency of sensitization found among the control group in our study performed with sera from different geographic regions.¹⁹

Because both groups of patients had IgE antibodies to Ani s 1, we suggest that specific IgE determination to Ani s 1 could be a useful tool for the diagnosis of *Anisakis simplex* infection. Further, serologic assays based on specific IgG detection to Ani s 1 could be developed for *Anisakis simplex* allergy. For technical reasons, these assays could require a shorter incubation time than these based on specific IgE determination.

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