

Allergenic cross-reactivity between third stage larvae of *Hysterothylacium aduncum* and *Anisakis simplex*

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Anisakis simplex third stage larvae (L3) are a source of hidden allergens in fish. Hypersensitivity to the allergens of this nematode is common in patients who have urticaria/angioedema, anaphylaxis, or both after the ingestion of fish and who have negative skin test responses to fish proteins.^{1,2} The diagnosis of hypersensitivity to *A. simplex* is hampered by the current lack of provocation studies and the limitations in establishing a direct cause and effect.

Hysterothylacium aduncum is another anisakid nematode that commonly parasitizes fish. A study in Spain investigated the presence of larval anisakids in the fish market in Granada and found that 39.4% of the fish examined (*Trachurus trachurus* [horse mackerel]) contained larval anisakids; 26.1% were infected with L3 of *A. simplex* and 31.1% with L3 of *H. aduncum*.³

The aims of this study were to establish the prevalence of specific IgE to *H. aduncum* in 19 patients evaluated for a suspected *A. simplex* sensitivity and to assess the cross-reactivity between extracts of L3 of these fish nematodes. All the patients had positive skin test responses to *A. simplex* and experienced an allergic reaction (urticaria, angioedema, asthma, and/or anaphylaxis) after eating fish.

METHODS

Larvae of *H. aduncum* and *A. simplex* were collected from *Micromesistius poutassou* (Bacaladilla, Couch's Whiting) with forceps and carefully identified and separated by expert parasitologists from the Faculty of Pharmacy at the University of La Laguna (Tenerife, Canary Islands, Spain). A list of fish in which both parasites have been described is included in Table I. Five hundred milligrams of L3 from *A. simplex* and *H. aduncum* were lyophilized and extracted (1:20 wt/vol) in ammonium bicarbonate (0.125 mol/L, pH 8.2). After 16 hours of extraction at 4° C, the suspension was centrifuged, the pellet was discarded, and the remaining solution was dialyzed in 3.5 kd membranes and lyophilized. The lyophilized material was dissolved in distilled water to a final concentration of 10 mg/ml. Protein concentra-

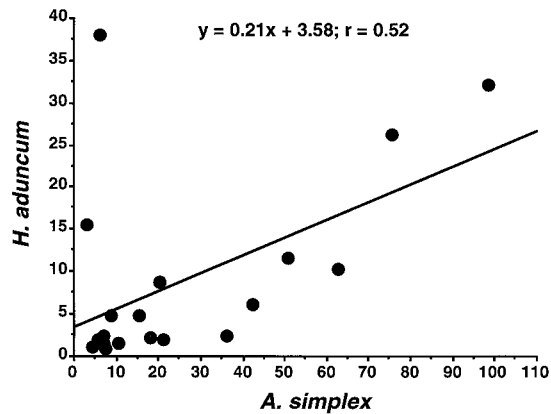


FIG. 1. Regression analysis of specific IgE (ELISA) determinations. $p = 0.02$.

TABLE I. Scientific, Spanish, and English names of fish species in which both *A. simplex* and *H. aduncum* have been identified

Latin	Spanish	English
<i>Sardina pilchardus</i>	Sardina	Pilchard, sardine
<i>Conger conger</i>	Congrio	Conger eel
<i>Lophius piscatorius</i>	Rape	Angler fish, fishing frog
<i>Micromesistius poutassou</i>	Bacaladilla	Couch's whiting
<i>Trisopterus luscus</i>	Faneca	Bib
<i>Pollachius pollachius</i>	Abadejo	Pollack
<i>Gaidropsarus mediterraneus</i>	Barbada	Shore rockling
<i>Trachurus trachurus</i>	Chicharro	Scad, horse mackerel
<i>Scomber scombrus</i>	Caballa	Common mackerel
<i>Boops boops</i>	Boga	Bogue
<i>Hyperoplus lanceolatus</i>	Piñón	Greater sand eel
<i>Lepidorhombus wiffiagonis</i>	Gallo	Meagrim
<i>Lepidorhombus boscii</i>	Gallo	Four-spot meagrim
<i>Microchirus variegatus</i>	Golleta	Thickback sole

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tions in both extracts were approximately 50% of the total dry weight.

Specific IgE was determined by direct ELISA and allergenic cross-reactivity by ELISA inhibitions. Plastic microtiter plates (Immulon 4, Chantilly) were coated with 1 µg of protein per well in carbonate/bicarbonate buffer (pH 9.2). A result was considered positive when a serum bound four

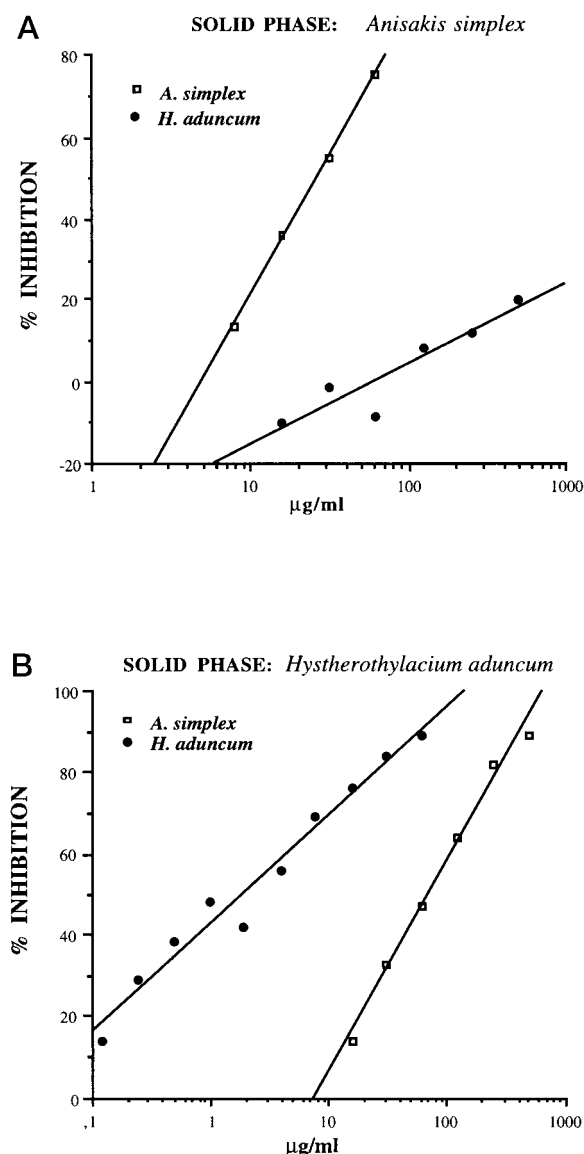


FIG. 2. ELISA inhibition assays with *A. simplex* (A) and *H. aduncum* (B) as solid phases. X-axis shows amount of inhibitor used expressed in log scale. Y-axis represents percent inhibition achieved with extracts of both parasites as inhibitors.

times more specific IgE than the mean titer of five atopic negative controls.

RESULTS

Fig. 1 shows a regression analysis of the results ($r = 0.52$, $p = 0.02$). Eighteen individuals (94.7%) had a positive ELISA to *A. simplex*, and 10 (52.6%) had a positive ELISA to *H. aduncum*.

A serum pool containing high titers of specific IgE to *A. simplex* and *H. aduncum* was used in the inhibition experiments. Minimal-to-moderate cross-reactivity was observed between *A. simplex* and *H. aduncum* (Fig. 2, A and B). When *A. simplex* was used in the solid phase, *H. aduncum* was able to inhibit 20.2% of the specific IgE

binding to *A. simplex*. When *H. aduncum* was used in the solid phase, *A. simplex* inhibited 89% of the specific IgE binding to *H. aduncum* but required an allergen concentration 37.7 times greater than that required by *H. aduncum* to achieve 50% inhibition.

DISCUSSION

The results suggest that both Anisakid larvae are the source of common and species-specific antigens. This fact is demonstrated by the differences in the degrees of inhibition achieved and the different slopes of the inhibition lines. It seems that in the event that these species share allergenic epitopes, these are present in larger quantities in *A. simplex* than in *H. aduncum*. This could also be due to a greater avidity of IgE antibodies for the *Anisakis* antigens. The specific IgE determination results may reflect cross-reactivity between *A. simplex* and *H. aduncum* or cosensitization to both nematodes. Sensitization to species-specific antigens is also possible. Two patients in this series had strongly positive responses to *H. aduncum* and negative or slightly positive responses to *A. simplex*.

Antigenic cross-reactivity has been described between *A. simplex* and *Ascaris lumbricoides*, *Ascaris suum*, *Toxocara canis*, *Blattella germanica*, and *Chironomus* spp.^{4,5} Our results extend these cross-reactivity findings to *H. aduncum*.

Sensitivity to fish parasites might be more common than previously suspected. In northern Spain, published rates of sensitivity to *A. simplex* have been as high as 52% in patients allergic to food.²

The identification of the parasites responsible for clinical symptoms is necessary for an accurate diagnosis of this kind of food-induced allergic disease. Although *A. simplex* is thought to be the main fish parasite causing allergic symptoms, other fish parasites should be considered in the evaluation of allergic reactions occurring after fish consumption.

REFERENCES

- Kasuya S, Hamano H, Izumi S. Mackerel-induced urticaria and *Anisakis* [letter]. *Lancet* 1990;335:665.
- Audicana M, Fernández de Corres L, Muñoz D, Fernández E, Navarro JA, Del Pozo MD. Recurrent anaphylaxis caused by *Anisakis simplex* parasitizing sea fish. *J Allergy Clin Immunol* 1995;96:558-60.
- Adroher FJ, Valero A, Ruiz-Valero J, Iglesias L. Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada, Spain. *Parasitol Res* 1996;82:319-22.
- Kennedy MW, Tierney J, Puseng Ye, McMonagle F, McIntosh A, McLaughlin D, et al. The secreted and somatic antigens of the third stage larvae of *Anisakis simplex* and antigenic relationship with *Ascaris suum*, *Ascaris lumbricoides*, and *Toxocara canis*. *Mol Biochem Parasitol* 1988;31:35-46.
- Pascual CY, Crespo JF, San Martín S, Ornia N, Ortega N, Caballero T, et al. Cross-reactivity between IgE-binding proteins from *Anisakis*, German cockroach, and chironomids. *Allergy* 1997;52:514-20.