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Short communication

Allergenic activity of *Molicola horridus* (Cestoda, Trypanorhyncha), a cosmopolitan fish parasite, in a mouse model

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Abstract

The cestode *Molicola horridus* is a muscle parasite of teleost fish. The ability of molecules present in this parasite to induce allergic response is not known yet. Since fish-borne parasitic allergens can induce allergic manifestations even when the parasitized fish is well cooked, the knowledge of potential allergens present in food is important in order to provide a save products for consumers. The aim of the study was to determine the allergenic potential of the components present in the crude larval extract (CLE) of *M. horridus*. Two mouse models were exposed to the CLE: adult BALB/c mice that were intraperitoneally (i.p.) immunized and newborn BALB/c mice that were orally exposed. Specific antibody levels in serum and faeces were measured by ELISA. The cellular immune response was determined by proliferation assay of splenocytes from sensitized mice. The protein profile of CLE was analysed by SDS-PAGE and western blot. In adult mice, specific IgG and IgA were detected in sera and faeces, whereas specific IgE were detected in sera only. In newborn mice, specific IgG were detected in sera and a low level of IgA was detected in faeces. SDS-PAGE revealed the CLE protein profile, with most of the proteins running from 15 to 50 kDa. Specific IgG recognized mainly the 26 and 75 kDa proteins and a molecular complex below 100 kDa by immunoblot. Specific IgE recognized the same 26 kDa protein as IgG did, and, with less intensity, another protein at 30 kDa. Splenocytes from CLE-immunized mice proliferated when stimulated with CLE in a dose-dependent manner. The crude larval extract from *M. horridus* has potential allergenic molecules which can represent a risk for fish consumers.

Keywords: Food allergy; Fish allergens; *Molicola horridus*; Swordfish; Mouse model; Balb/c mice

1. Introduction

The consumption of raw or undercooked parasitized sea-food can represent a serious public-health risk (Chai et al., 2005; Puente et al., 2008). Fish parasites can induce several pathologies, whose clinical manifestations can range from mild symptoms (e.g., diarrhoea) to severe symptoms (e.g., acute abdomen). In the already sensitized host, even the consumption of well-cooked parasitized fish can induce allergic manifestations, since some of the allergens involved in such reactions are thermo-stabile (Audicana and Kennedy, 2008; Puente et al., 2008).

To date, most attention has been focused on nematode worms of the genus *Anisakis* (Pellegrini et al., 2005), yet there are numerous sea-food-borne

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parasitic allergens (Martinez De Velasco and Cuellar, 2002; Chai et al., 2005). However, nothing is known about the allergenic capacity of another cosmopolitan Trypanorhyncha cestode, *Molicola horridus*, which parasitizes the liver and muscles of teleost fish. *M. horridus* has been frequently detected in swordfish (*Xiphias gladius*) and short moonfish (*Mola mola*) in the Mediterranean Sea and along the coasts of Australia, Canada, India, Japan, and New Zealand. This cestode has also been documented in the spiral valve of the elasmobranche shortfin mako (*Isurus oxyrinchus*), captured along the coasts of Japan and Southern California (Knoff et al., 2004).

Like other parasites infecting the somatic muscles of fish, the larval stage (known as ''plerocercoid'') of *M. horridus* develops into cysts that are easily visible to the naked eye, decreasing the edible portion and resulting in economic loss. The larvae, approximately 14 cm long, are localised in canalicols parallel to the backbone and spread in all muscles except the superficial lateral muscles. The scolex is a large structure with four bothridia and four contractile and retractable proboscids which are covered by numerous rows of hooks. *M. horridus* presents a sinusoidal chain of small hooks on the lateral surface of the proboscid. The distal part of the proboscid is completely covered by long hooks. Moreover, since there is no effective method for detecting the cysts without destroying the tissue, the parasitized fish can reach the consumer. This is particularly important in the industry of tinned and homogenized baby foods. In 2006, 105,805, 35,442 and 6518 tones of swordfish were fished worldwide, in the European Union, and in Italy, respectively (data from ISMEA, www.ismea.it, which are based on the FAO web site, www.globefish.org and on the IREPA web site, www.irepa.org).

The objective of the present study was to determine the allergenic capacity of the crude larval extract (CLE) from the plerocercoids of *M. horridus* and its ability to induce sensitization when administered orally. To this end, two mouse models were exposed to the CLE antigens: adult BALB/c mice, exposed intraperitoneally (i.p.), and newborn BALB/c mice, exposed per os.

2. Materials and methods

2.1. Parasites and soluble extracts

The plerocercoids of the parasite *M. horridus* were manually collected from the skeletal muscles of swordfish purchased at fish-markets in Italy, using forceps and scissors.

After the morphological identification, the plerocercoids were separated from fish tissues and ridden of their membranes by rinsing them several times in sterile 0.1 M phosphate buffered saline (PBS) pH 7.3 and then in PBS supplemented with 5% penicillin and 5% streptomycin, followed by three additional washes in sterile PBS. The plerocercoids were then homogenized on ice in a PTFE-on-glass tissue homogenizer for 1 min, with a 1-min pause, 20 times; they were then sonicated on ice for 30 s with 30 s pauses, six times. The suspension was centrifuged at 60,000 × *g* at +4 °C for 30 min. The supernatant was filtered using a 0.22 μm filter (MillexGV; Millipore, Molshe, France). The CLE protein content was estimated using Bradford's protein assay (Bradford, 1976). The same protocol was used to prepare a crude fish muscle extract (CME) which was used as control to treat per os the control mouse group and as antigen for control serological assays.

2.2. Animal model, parenteral immunization and oral administration

Ten-week-old (adult) and 1-week-old (newborn) BALB/c mice (purchased from Charles River Laboratories; Milan, Italy) were housed in the Animal Care Unit of the Istituto Superiore di Sanità and treated according to the European Directive 86/609 EEC.

Before each immunization, a suspension was prepared by mixing the CLE (50 μg/mouse) with the commercial alum gel suspension ($Al(OH)_3$, 13 mg/mL, 200 μL/mouse, Sigma–Aldrich, Saint Louis, MO, USA). In the first experiment, one group of adult BALB/c mice ($n = 7$) was inoculated i.p. with 50 μg of CLE in $Al(OH)_3$, on days 0 and 21 (Martínez de Velasco et al., 2003; Orlandi et al., 2004). Two control groups were included. The first control mouse group ($n = 7$) received i.p. $Al(OH)_3$ at the same times. The second control mouse group ($n = 3$) received i.p. 500 μg of peanut agglutinin (*Arachis hipogaea*, Sigma–Aldrich) on days 0, 7 and 21. Blood samples were taken by tail bleeding at 0, 7, 14, 21, 28 and 35 days after the first immunization. Individual sera were stored at −20 °C until analysis.

In the second experiment, one group of 1-week-old BALB/c mice ($n = 6$) received 4 weekly doses of 200 μg/50 μL of CLE by a bucco-gastric tube. The control group ($n = 3$) received the same dose of CME at the same times. Blood samples were taken by tail bleeding at 0, 7, 14, 21, and 25 days after the first treatment. Individual sera were stored at −20 °C until analysis.