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SPECIFITY IN THE IMMUNE RESPONSE AGAINST SOMATIC ANTIGENS OF *Lagochilascaris minor* AND ITS RELATIONSHIPS WITH SIMILAR ANTIGENS OF *Ascaris lumbricoides* AND *Toxocara canis*

Clemencia E. Medrano*, Gilberto Payares** & Gregorio S. Volcán.

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RESUMEN

ESPECIFICIDAD DE LA RESPUESTA INMUNE FRENTE A LOS ANTIGENOS SOMATICOS DE *Lagochilascaris minor* Leiper, 1909, INTER-RELACIONES CON ANTIGENOS SIMILARES DE *Ascaris lumbricoides* Y *Toxocara canis*. *Lagochilascaris minor* es un nematodo parásito del hombre rural de la Región Neotropical, en el cual coloniza cavidades aéreas y tejidos de la cabeza y el cuello. Se ha estudiado en primer término, antígenos somáticos y de membranas en gusanos adultos, mediante el uso de detergentes de conocida eficacia. Se obtuvieron 3 extractos proteicos a 4°C y 50.000 g, y con igual técnica, extractos de adultos de *Ascaris lumbricoides* y *Toxocara canis,* nematodos ascaridios cosmopolitas que infectan también al hombre. Las concentraciones proteicas de los extractos fueron elevadas, así, para el N° 1 de *L. minor, A. lumbricoides* y *T. canis* alcanzaron 480, 1.920 y 1.305,g/ml, respectivamente, y para el N° 2 fue de 705, 1.980 y 1.785 g/ml, en el mismo orden. Anticuerpos homólogos para *L. minor* fueron obtenidos de una paciente con 12 años de enfermedad, y de roedores infectados experimentalmente, los cuales desarrollaron solamente estadios larvales, en sus tejidos. También se estudiaron los sueros de familiares y allegados a la paciente. Geles de Poliacrilamida en presencia de SDS (SDS-PAGE), fueron usados para el análisis electroforetico de los extractos, identificando los antígenos por la técnica de Immunoblotting. Los inmunocomplejos se identificaron por una prueba de proteína A-radioiodinada y auto-radiografía a -70°C. Los antígenos fueron enfrentados a los anticuerpos por la técnica de ouchterlony y solamente el suero de la paciente dio 3 bandas de precipitación, con los 3 extractos de *L. minor* y sólo 1 con los de *A. lumbricoides* y *T. canis*. Fue demostrado un antígeno dominante en *L. minor* de peso molecular (p. m.) 120.000, el cual fue reconocido por todos los sueros (con excepción de un suero humano control negativo), y otro de p. m. >200.000 el cual fue reconocido por el de la paciente, un tío, 2 hermanos y de un conejo. En la confrontación de los sueros frente antígenos de *A. lumbricoides*, hubo reacción con 3 de ellos, cuyos p.m. fueron >200.000, 120.000 y 65.000. Estos resultados demuestran la existencia de anticuerpos en la paciente, sus familiares y animales infectados experimentalmente, que no son detectados por ouchterlony, siendo probable que antígenos de p. m. >200.000 y 120.000, sean comunes a *L. minor* y *A. lumbricoides.*

*Departamento de Parasitología, Escuela de Medicina, Universidad de Oriente. Sección Parasitología, **Instituto de Zoología Tropical, Escuela de Ciencias, Universidad Central, Venezuela.

Brief communication

In the Neotropical region, the appearance of new cases of humans infected by the ascaridean *Lagochilascaris minor* has awakened increasing interest in this parasite (Fraiha Neto *et al.* 1989). Its behavior in invading the soft tissues of the neck and the cephalic cavities open to the air (mastoids, paranasal sinuses, and even the cranial cavity) along with its morphology and biological characteristics, differentiate this parasite from the cosmopolitan pathogenic ascarideans common to man.

It has been reported that nematodes colonizing the interior of the small intestine stimulate the response of IgA and the increase of basophils and mastocytes (Ogilvie *et al.* 1978), while those invading muscular tissue, such as *Trichinella spiralis* stimulate the increase of IgG, IgM, IgE, eosinophils and mastocytes (Kazura 1982), However, *L. minor* comes in contact with both epithelialized surfaces, and with muscle, fat, and connective tissue. Also, while carriers with long-standing infections liberate large numbers of fertils

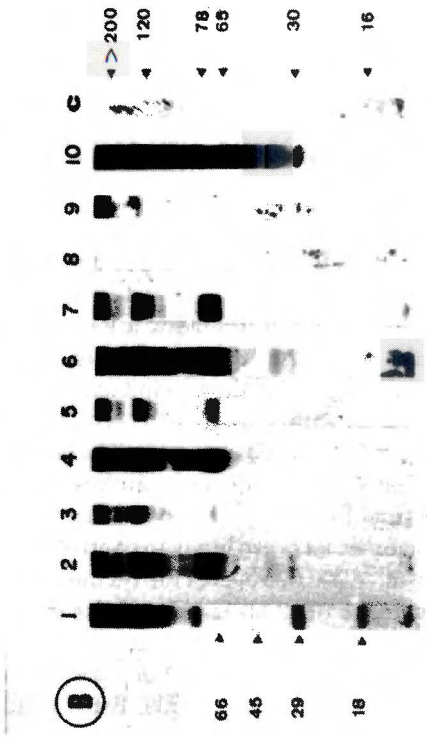

FIGURE 1-A. Identification and characteritation of membrane antigens from adult L. *minor*, with sera from the human patient (1), and family members: mother (2), uncle (3), sister (4), sister (5), sister (6), brother (7), cousin (8), and healthy control (c). Track 9 contained serum from a *Dasyprocta leporina* orally infected with eggs of *L. minor*, and track 10 and 11 contained sera from rabbits infected and sacrificed 60 days post-infection. Molecular weight markers were: myosin (200,000), phosphorylase B (92,500), bovine albumin (68,000), egg albumin (43,000), chymotrypsin (25,000), and ß-lactoglobulin (18,400). Except for track 2 (mother) and call the sera strongly recognized the polypeptide of molecular weight 120,000, which was strongly recognized by the serum of the patient (Track 1).

eggs in the feces and in nasal secretions, purulent or otherwise, they do not appear to infect their families or close companions.

While much is known about the composition and character of the antigens of *Ascaris lumbricoides* and *Toxocara canis,* nothing is known about the antigens of *L. minor*, due to the difficulty of obtaining parasitic material. However, the experimental infection of the sylvatic rodent *Dasyprocta leporina* (Volcán & Medrano 1990), and the development of a heteroxenous cycle in mice and domestic cats (Volcán 1990) has made possible the maintenance of a laboratory strain for study.

The first study of the antigenic composition of *L. minor* was made on somatic antigens that were proteins solubilized in an aqueous buffer by homogenizing and freeze-thawing adult parasites. Later, proteins bound to internal membranes and the cuticle were extracted with detergents of known effect (Helenius & Simons 1978); 3 protein extracts were obtained by centrifugation (50.000 g at 4°C). Protein concentrations obtained by detergent action were determined by the modified Lowry method (Dulley & Grieve 1975). The same procedure was used to obtain extracts from the related ascarids A. *lumbricoides* and *T. canis*. High concentrations were obtained: in the first procedure 480, 1920, and 1305 ug/ml were measured for *L. minor,* A. *lumbricoides* and T. *canis*, respectively, while the second procedure yielded 705, 1980, and 1785 ug/ml, in the same order.

Homologous antibody for *L. minor* was obtained from a human case of 16 years history. Sera were also taken from family members. The patient and family lived in a rural area with little sanitation; many of them had repeated infections of *A. lumbricoides* and were in

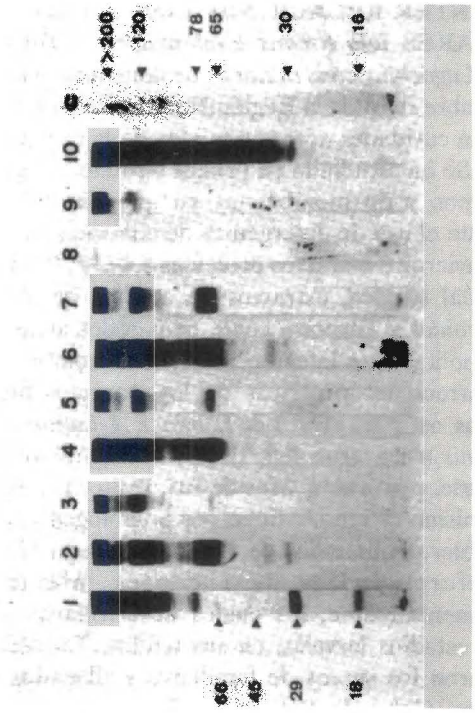

FIGURE 1-B. Identification and characterization of membrane antigens from adult *A. lumbricoides* with the serum from a patient with *lagochilascariasis* (1) and family members: mother (2), uncle (3), sister (5), sister (6), sister (4), brother (7), cousin (8), and a healthy control (c). Track 9 and 10 contain sera from infected rabbits, standard markers were: bovine albumin (66,000), egg albumin (45,000), carbonic anhydrase (29,000), ß-lactoglobulin (18,000). Tree major antigens were recognized by the majority of human and rabbit sera (>200,000, 120,000, 65,000). The *L. minor* infected patient recognized antigens of molecular weight >200,000, 78,000, 30,000, 16,000.

intimate contact with dogs having high infections of *T. canis*. In addition, sere were taken from rabbits, and from a D. leporina which had been infected with an oral dose of 1500 eggs of L. *minor*, leading to development of 3rd-stage larvae in muscle tissue. None of the animals had been exposed to the other ascarids. The polypeptids composition of the various extracts was determined by electrophoretic analysis in (12.5%) polyacrylamide gels in the presence of SDS (SDS-PAGE). Identification of antigens was carried out only on the membrane extracts (Nº 2) of *L. minor* and *A. lumbricoides,* by the technique of immunoblotting (Towbin *et al.* 1978). The immune complexes were identified by a Protein A-radioiodine probe, and by radioautography with an amplyfying screen at -70°C. Calculation of molecular weights was based on prestained proteins of BRL Laboratories and standards from Sigma Ltd.

The antigenic extracts were challenged with the antibody sera by immunodiffusion in agarose gel. The ouchterlony plates were charged with 8-14 g protein for L. *minor,* 22-44, g for A. *lumbricoides*, and 15-36 g for *T. canis* out of a total of 12 sera studied, including one from a human control uninfected with ascarids, and 3 experimentally infected animals, only the serum from the patient with *lagochilascariasis* gave 3 bands of precipitation with the 3 extracts of *L. minor* (not shown). Serum from the patient also reacted with the membrane antigen of A. *lumbricoides*, giving one band, and with the antigens 1 and 2 of *T. canis,* giving one band each. The membrane extracts of all 3 ascarids gave an immunologically identical band of precipitation against the patient serum. The other human and animal sera did not form bands of precipitation with any of the extracts (not shown).

Figure 1-A shows the antigens of *L. minor* recognized by the various sera tested. A major polypeptide of molecular weight 120,000 was recognized as the dominant antigen by all sera, except for that of the human control. This antigen reacted most intensely with serum from the human patient (track 1). Another antigen of high molecular weight (>200,000) was recognized by the serum of the patient and by the sera of an uncle and 2 sisters, and also by the serum of a rabbit (Fig. 1; track 3, 4, 6, 10, respectively). Still minor antigens, mol. wt. 55,000, 45,000, 21,000 and 16,000 were recognized by the sera of the patient and uncle.

Challenge of the sera from the patient, the family, and infected animals with protein extract from the membranes of A. lumbricoides showed reactions by the majority with 3 dominant antigens (mol. wts.) >200,000, 120,000, and 65,000); the last protein was not recognized by sera of the patient, of a cousin, or of the D. *leporina* (Figure 1-B).

Kennedy *et al.* (1989) have reported the antigenic homology between *A. lumbricoides* and *T. canis*, both in somatic and excretory/secretory antigenic proteins. The patient with L. *minor* has shown no infection with A. *lumbricoides* during 12 years observation (Volcan & Medrano, unpublished). However, the serum shows strong cross-reaction with the antigens from A. *lumbricoides* and *T. canis*. Results from the Immunoblotting test were obtained by analyzing electrophoretically the deoxycholate extract from the adults of *L. minor* and *A. lumbricoides*, given the recognized antigenicity of the membrane proteins of helminth parasites (Soulsby 1963). These results confirm the presence of antibodies in the sera of the patient's relatives and in the infected animals undetectable by the ouchterlony technique. Since sera from animals infected with *L. minor* recognize antigens of molecular weight >200,000 and 120,000 from both extracts of *L. minor* and *A. lumbricoides* it would appear that these 2 peptides are fundamentally responsible for the antigenic homology. It should be noted that while the antigen of 120,000 mol. wt. was recognized as dominant in the 2 parasites, the 65,000 mol. wt. hemiantigen (?) was identified only for *A. lumbricoides* by the sera of the family group. The allergenic polypeptide of 14,000 mol. wt., emphasized in importance by Kennedy & Qureshi (1986), was not detected.

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