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Role of porcine serum haptoglobin in the host-parasite relationship of *Taenia solium* cysticercosis

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ABSTRACT

Human and porcine cysticercosis is a parasitic disease caused by the larval stage (cysts) of the tapeworm *Taenia solium*. Cysts may live in several host tissues such as skeletal muscle or brain. We have previously described the presence of host haptoglobin (Hp) and hemoglobin (Hb) in different protein extracts of the *T. solium* cysts. Here, we report the binding of host Hp and Hb to a number of cyst proteins, evaluated through measuring electrophoretic and light absorbance changes.

In the sera obtained from 18 cysticercotic pigs, Hp-Hb complexes were abundant, whereas free Hp was undetectable. In contrast, in the sera from non 18 cysticercotic pigs, Hp-Hb and free Hp were found. In the soluble protein fraction of cysts tissue, free Hp was detected showing a considerable Hb-binding ability, whereas in the vesicular fluid, Hp is mainly bound to Hb. Interestingly, assays carried out with the insoluble fraction of *T. solium* cysts tissue, showed binding of Hp and Hp-Hb in a saturable way, suggesting the existence of specific interactions. Our results suggested that the parasite can take advantage of the uptaken host Hp and Hb, either free or in complexes, as a source of iron or as a way to modulate the inflammatory response surrounding the *T. solium* cysts.

1. Introduction

The host-parasite relationship in *Taenia solium* cysticercosis is multifactorial and involves a number of complex aspects. An example is the acquisition of all nutrients from host tissues by this cestode parasite; among others, *T. solium* cysts are able to uptake host fatty acids through a couple of specialized proteins: the fatty acid binding proteins [1,2]. It can also uptake glucose using at least two glucose transporters [3]. Moreover, cysts are able to uptake intact and functional host proteins including IgG [4], albumin [5], and other serum proteins like haptoglobin (Hp) and hemoglobin (Hb) [6].

The presence of host proteins has been reported in different protein extracts of *T. solium* cysts: total extracts [7], vesicular fluid (VF) [6], excretion/secretion products [8], as well as in the soluble and insoluble extracts of cysts tissue [9]. The role of these proteins in the physiology of the cysts remains unclear, although in the case of albumin it has been proposed that this host protein aids the maintenance of the parasite's osmotic pressure [5] and in the case of IgG, as a source of amino acids for protein biosynthesis [10]. If *T. solium* cysts are able to use these host proteins remains as a question that needs to be investigated.

A crucial element in the context of a number of infectious diseases (to both, pathogen and host) is iron. In vertebrate hosts, pathogens can obtain iron from different host sources including erythrocytes, serum Hb, Hp-Hb complexes, hemopexin, transferrin, lactoferrin, among others [11]. Iron acquisition is well understood in some bacterial infections; however, each pathogen develops specific ways to uptake iron. For instance, it has been reported the presence of Hp, Hb, heme groups, transferrin and lactoferrin receptors in different types of bacteria (reviewed in [12]).

The iron metabolism in the host-parasite relationship in human and porcine cysticercosis, to our knowledge, has never been explored, in spite of the presence of different proteins associated with iron metabolism/transport in the protein extracts of *T. solium*

Abbreviations: Hp, haptoglobin; Hb, hemoglobin; Hp-Hb, haptoglobin-hemoglobin complexes; VF, vesicular fluid.

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cysts. In the case of E/S products of *T. solium* cysts, the presence of Hp, Hb, hemopexin and transferrin have been reported [8] and in the case of the VF of *T. solium* cysts, the presence of Hp and Hb have also been documented [6]. The presence of Hp was reported in the soluble and insoluble fractions of *T. solium* cysts tissue; free Hp was detected in the soluble fraction and the Hp-Hb complex were detected in the insoluble fraction [9]. In addition, serum Hp has been proposed as a serum biomarker for cysticercotic pigs [13], although its role in the host-parasite relationship is unknown.

Serum Hp is a glycoprotein mainly produced by the liver that is considered as an acute phase reactant mainly induced by IL-6. Hp is involved in the binding and removal of free Hb from circulation, after formation of the Hp-Hb complex, it can bind the scavenger receptor CD163 on the surface of macrophages [14]. Some pathogens have developed the ability to uptake Hp-Hb and use it as a source of iron [12].

The aim of this study was to investigate the changes of serum Hp and Hb in cysticercotic pigs, to explore the role of these host proteins in parasite's physiology, with special emphasis on iron acquisition.

2. Materials and methods

2.1. Protein extracts

Soluble and insoluble fractions of *T. solium* cysts were obtained as described previously [9]. Briefly, *T. solium* cysts were obtained from the skeletal muscle of naturally infected pigs bred in remote rural areas of Mexico, sacrificed following the humanitarian procedures approved by the Biomedical Research Institute and the School of Veterinary Medicine (UNAM). Cysticerci were washed five times with sterile ice cold phosphate buffered saline pH 7.3 (PBS), and the bladder wall was sectioned in a Petri dish in order to collect the VF. The obtained VF was diluted 1:2 in 50 mM Tris pH 7.3 complemented with protease inhibitors (12.5 mM EDTA, 1 μM pepstatin, 1 mM PMSF and 0.1 mM leupeptin) and store at −70 °C until use. The cyst's tissue was transferred into a microcentrifuge tube and washed several times in PBS added with the same protease inhibitors, then the tissue and homogenized in a new microcentrifuge tube using a Teflon pestle in (1:2 w/v) 50 mM Tris pH 7.3, with the same protease inhibitors; vortexed and freeze-thawed 3 times. The extracts were centrifuged for 1 min at 14,000*g* and the supernatants collected and centrifuged again for 60 min. After centrifugation, the soluble fraction was precipitated and solubilized in 7 M urea, 2 M thiourea and 4% CHAPS. The insoluble fraction was washed several times with 50 mM Tris and solubilized in 7 M urea, 2 M thiourea, 2% CHAPS, 1% Triton X-100, 1% ASB-14 and 5 mM TBP in 10 mM Tris, pH 7.3. The protein concentration in all fractions was determined using the NI Protein Assay (GBiosciences, USA).

2.2. Electrophoretic pattern of serum Hp and Hb from pigs

Sera samples were obtained from 18 cysticercotic and 18 non-cysticercotic pigs and diluted 1:80 in 50 mM Tris pH 7.3; 5 μL sera samples were then resolved by SDS-PAGE (12%) or native-PAGE (8%). Electrophoresis was performed at 120 V at room temperature (SDS-PAGE) or 90 V at 4 °C (native-PAGE). Afterwards, the gels were transferred onto a nitrocellulose membrane at 120 V during 70 min. The membranes were blocked overnight using 10% skim milk in PBS. The primary antibody was incubated 2 h at RT in 10% skim milk in PBS. Polyclonal anti-human Hp and anti-albumin (Abcam) sera were used in 1:5000 dilutions; in the case of polyclonal anti-Hb epsilon chain serum (Abcam), dilution was 1:3000. After incubation with the primary antibody, the membranes were washed 3 times with 0.1% Tween 20 in PBS (PBS-T) and secondary antibodies (anti-sheep IgG was used 1:50,000 (Abcam) and anti-mouse IgG was used 1:30,000 (Sigma)) were incubated during 2 h a RT. The membranes were washed and the antibody-antigen interaction was visualized using the West-femto Quimioluminiscence kit (Thermo).

2.3. Haptoglobin-hemoglobin binding studies through UV-spectra analysis

The Hp-Hb binding was performed as previously reported [15]; in our case the Hp sources were a pool of sera from 5 cysticercotic pigs, a pool of sera from 5 non cysticercotic pigs, the soluble extract of cysts tissue and the VF. The presence of Hp in protein extracts of *T. solium* cysts has been recently documented [6,9]. A met-hemoglobin solution (obtained from an erythrocyte lysate treated with sodium cyanide), was incubated with one of the different Hp sources and the change in the UV-spectra (350–500 nm) was analyzed in a nanodrop spectrophotometer (Thermo).

2.4. Hemoglobin-binding capacity of T. solium cysts protein extracts through western blot

The soluble fraction obtained from cysts tissue, VF, as well as a commercial human haptoglobin (Abcam, pool of the three Hp forms), were incubated with hemoglobin during 30 min at RT; then the appropriate volume of non-reducing loading buffer 5× was added and the mixture was loaded into a native-PAGE. Western blot was performed as described above.

2.5. Affinity chromatography

The insoluble fraction of the cyst's proteins and the anti-human Hp antibody (Dako) were separately coupled to Sepharose 4B after activation with BrCn following the manufacturer's instructions (Sigma). The two columns were washed using 20 vols of PBS, then, the sera samples from cysticercotic pigs were diluted to a final volume of 10 mL in PBS. After applying the sera samples, both columns were washed with 10 vols of PBS and the bound fractions were eluted using 0.1 M glycine pH 2.3 until the O.D. at 280 nm was near to cero, the columns were washed with 10 vols of PBS and stored in 1 M NaCl in 20% ethanol in water until used.

2.6. Binding of serum Hp by the proteins of the insoluble fraction

In order to explore the binding of the serum Hp by cysts proteins, a previously reported method was slightly modified [16]. Briefly, 100 μL of a solution of insoluble proteins of *T. solium* cysts (15 μg/mL) in carbonate buffer pH 9.6 was used to cover each well of a microtiter plate and incubated overnight at 4 °C with mild agitation. Then, the plate was washed three times during 5 min, using 0.05% of Tween 20 in PBS and blocked with 1% albumin in 0.05% of Tween 20 in PBS. Several dilutions of the purified Hp (see above) were prepared and incubated overnight 4 °C with mild agitation. Afterwards, the plate was washed, and incubated with an anti-Human Hp hyperimmune serum (Abcam) during 60 min at RT. As secondary antibody, an anti-sheep IgG (Abcam) was used diluted 1:1000. The reaction was developed using OPD and stopped with 3 N HCl.

2.7. Sugar oxidation by sodium metaperiodate treatment

In order to explore the role of glycoprotein carbohydrates on the binding of Hp/Hp-Hb by cysts proteins, a sodium metaperiodate oxidation was performed [17] with slight modifications. Each well was coated with 100 μL of insoluble proteins of *T. solium* cysts (15 μg/mL), then the plate was incubated overnight at 4 °C with