Experimental Parasitology 172 (2017) 23–29

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Protein profiles of *Taenia solium* cysts obtained from skeletal muscles and the central nervous system of pigs: Search for tissue-specific proteins

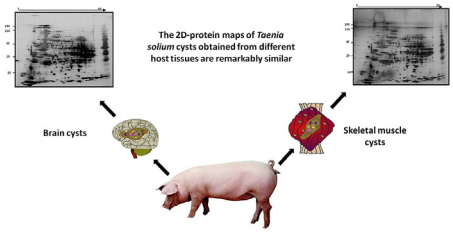
José Navarrete-Perea [a, 1, 2], Bárbara Moguel [a, 1], Raúl José Bobes [a], Nelly Villalobos [b], Julio César Carrero [a], Edda Sciutto [a], Xavier Soberón [c, d], Juan Pedro Laclette [a, *]

[a] Dept. of Immunology, Institute for Biomedical Research, Universidad Nacional Autónoma de México, México D.F, 04510, Mexico
[b] Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México D.F, 04510, Mexico
[c] Instituto Nacional de Medicina Genómica, Periférico Sur No. 4809, Col. Arenal Tepepan, Delegación Tlalpan, México, D.F, C.P. 14610, Mexico
[d] Instituto de Biotecnología, Universidad Nacional Autónoma de México, Avenida Universidad 2001, Cuernavaca, Morelos, C.P. 62210, Mexico

HIGHLIGHTS

- Protein maps from *T. solium* cysts from different tissues and different pigs are remarkably similar.
- Few proteins, among them paramyosin, were specifically associated with the skeletal muscle localization of the cysts.
- No protein was found significantly associated to the central nervous system localization of the cysts.
- However, the use of an insoluble protein fraction of cysts allowed preliminary identification of tissue-specific antigenic bands.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 October 2016
Accepted 29 November 2016
Available online 29 November 2016

Keywords:
2D-PAGE
Canonical discriminant analysis
Cysticercosis
Principal component analysis
Tissue-specific antigens

ABSTRACT

Taeniasis/cysticercosis caused by the tapeworm *Taenia solium* is a parasite disease transmitted among humans and pigs, the main intermediate host. The larvae/cysts can lodge in several tissues of the pig, i.e. skeletal muscles and different locations of the central nervous system. The molecular mechanisms associated to tissue preferences of the cysts remain poorly understood. The major public health concern about this zoonosis is due to the human infections by the larval form in the central nervous system, causing a highly pleomorphic and debilitating disease known as neurocysticercosis. This study was aimed to explore the 2DE protein maps of *T. solium* cysts obtained from skeletal muscles and central nervous system of naturally infected pigs. The gel images were analyzed through a combination of PDQuest™ and multivariate analysis. Results showed that differences in the protein patterns of cysts obtained from both tissues were remarkably discrete. Only 7 protein spots were found specifically associated to the skeletal muscle localization of the cysts; none was found significantly associated to the central nervous system. The use of distinct protein fractions of cysts allowed preliminary identification of several tissue-specific antigenic bands. The implications of these findings are discussed, as well as several

* Corresponding author. Dept. of Immunology, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México D.F, 04510, Mexico.
E-mail address: laclette@biomedicas.unam.mx (J.P. Laclette).
[1] Both authors made equal contributions to this work.
[2] Present address: Instituto Nacional de Medicina Genómica, Periférico Sur No. 4809, Col. Arenal Tepepan, Delegación Tlalpan, México, D.F. C.P. 14610, Mexico.

http://dx.doi.org/10.1016/j.exppara.2016.11.006

strategies directed to achieve the complete characterization of this parasite's proteome, in order to extend our understanding of the molecular mechanisms underlying tissue localization of the cysts and to open avenues for the development of immunological tissue-specific diagnosis of the disease.

1. Introduction

Human and porcine cysticercosis is caused by the larval stage of *Taenia solium*. Cysts (larval stage) can establish in different tissues of the intermediate host (usually pig), including the skeletal muscles (SM) and different locations of the central nervous system (CNS). In humans, the CNS localization of the cysts cause a serious and pleomorphic disease called neurocysticercosis (NC) (Sciutto et al., 2000). Variations in the clinical presentation of NC have been associated to the number of cysts, localization in different tissues and compartments of the CNS, as well as to parasite's heterogeneity (Fleury et al., 2010). In particular, genetic differences in the mitochondrial cytochrome B gene of *T. solium* cysts recovered from the CNS of humans, have been proposed to play a role in NC heterogeneity (Palafox-Fonseca et al., 2013). Other studies have been performed to investigate antigenic variations between *T. solium* cysts; early observations reported that only 30–50% of the antigenic content is shared between extracts of cyst samples from several endemic areas (Yakoleff-Greenhouse et al., 1982); more recently, a considerable variability in the antigen recognition by pig's sera from several endemic areas were also documented using vesicular fluid (VF) samples as the source of parasite material (Esquivel-Velázquez et al., 2011; Ostoa-Saloma et al., 2013). In contrast, CNS cysts obtained from humans and pigs have been found very similar at genetic level (Hinojosa-Juarez et al., 2011). All these studies have disregarded the molecular diversity among cysts collected from different tissues in the same host (for example CNS or SM).

Current proteomic tools allow the identification of a large number of proteins in a single assay, permitting a more extensive analysis of the protein expression in cysts under different conditions. Detailed studies of the parasite's proteome, including cysts obtained from different tissues could contribute to the identification of the parasite's proteins specifically associated with a tissue preference of the cysts. This is particularly interesting because if an antigenic signature could be identified for cysts obtained from each tissue, a tissue-sensitive immunodiagnostic test could be developed. It is also conceivable the identification of drugs targeted to specific parasite's proteins that are required for the establishment or permanence of cysts in a given tissue.

This study was designed to explore the protein differences between cysticerci obtained from SM and CNS of naturally infected pigs, using detailed 2D-PAGE protein maps for the VF and for whole cysts tissue extracts. Our results indicated that protein changes occurring among cysts collected from SM or CNS in the same pig, as well as among cysts from different pigs of the same endemic area were remarkably discrete.

2. Materials and Methods

2.1. Protein extracts

T. solium cysticerci were obtained from the skeletal muscle (SM) and the brain parenchyma (CNS) of four naturally infected pigs bred in rural areas from Cuentepec, state of Morelos, Mexico. Each protein extract was done using 5 cysticerci. The cysts were washed 3 times in sterile ice-cold PBS pH 7.3. In a Petri dish, the bladder wall was sectioned using a scalpel, the released VF was collected and diluted 1:2 in a solubilization buffer (7 M urea, 2 M thiourea, 4% CHAPS, 10 mM Tris pH 7.3, complemented with protease inhibitors 12.5 mM EDTA, 1 μM pepstatin, 1 mM PMSF and 0.1 mM leupeptin). The cysts tissues were transferred into a microcentrifuge tube and washed several times with PBS added with a mixture of protease inhibitors. Then, the cyst were transferred into a new tube and homogenized in a solubilization buffer (1:2 w/v) using a Teflon pestle; then vortexed and freeze-thawed 3 times. The extracts were centrifuged for 15 min at 14,000 g and the supernatants were collected.

The protein extracts were cleaned up using the ReadyPrep Clean-up kit (BioRad) according to manufacturer's instructions and the protein concentration in all extracts was determined using the NI Protein Assay (GBiosciences, USA).

2.2. Two dimensional SDS polyacrylamide gel electrophoresis (2D-PAGE)

Samples of 150 μg of each protein extract were diluted to a final volume of 120 μL in rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS) in 50 mM DTT, bromophenol blue and 0.2% IPG buffer for a 3–10 pH gradient. Non-linear, 7 cm IPG strips, pH 3–10 (BioRad, CA-USA) were rehydrated overnight at 20 °C with one of these protein mixtures. All samples were focused in a Protean IEF Cell (Bio-Rad CA-USA); the voltage profile was: 1. fast increase from 0 to 250 V for 30 min, 2. 250 V for 50 min, 3. fast increase from 500 to 1000 V for 30 min, 4. 4000 V for 120 min and 5. 4000 V for 10,000 Vh at a maximal current of 50 μA/strip. After focusing, the strips were reduced in equilibration buffer (6 M urea, 0.05 M Tris pH 8.8, 2% SDS and 30% glycerol) containing 2% DTT during 15 min and then alkylated using the same equilibration buffer, added with 2.5% iodoacetamide for 15 min. The samples were electrophoresed (in triplicates) in 12% Precast gels (Bio-Rad CA-USA) using a Mini Protean cell (Bio-Rad CA-USA) at 200 V. After electrophoresis, the 2-D gels were fixed (50% methanol, 10% acetic acid in ultrapure water) overnight for silver staining.

2.3. Silver staining

The fixed 2-DE gels were washed using 100 mL of an aqueous solution of 5% methanol and 1% acetic acid (v/v) for 15 min and then in 100 mL of 0.02% sodium thiosulfate during 90 s, before soaking three times with 100 mL of MilliQ deionized water. After, incubation of the gels during 40 min in 0.2% w/v silver nitrate in water, were washed three times as above and then transferred to 100 mL of a developing solution containing 6% (w/v) sodium carbonate, 50 μL formaldehyde (37%) and 25 μM sodium thiosulfate. Development was stopped with 7% v/v acetic acid and gentle shaking for 5 min. Finally, gels were washed three times in water.

2.4. Western blotting of antigenic fractions of T. solium cysts

The insoluble and soluble fractions of CNS and SM cysts were obtained as previously described (Navarrete-Perea et al., 2015); ten