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Oestradiol and progesterone differentially alter cytoskeletal protein expression and flame cell morphology in *Taenia crassiceps*

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ABSTRACT

We examined the effects of oestradiol (E_2) and progesterone (P_4) on cytoskeletal protein expression in the helminth *Taenia crassiceps* — specifically actin, tubulin and myosin. These proteins assemble into flame cells, which constitute the parasite excretory system. Total protein extracts were obtained from E_2- and P_4-treated *T. crassiceps* cysticerci and untreated controls, and analysed by one- and two-dimensional protein electrophoresis, flow cytometry, immunofluorescence and videomicroscopy. Exposure of *T. crassiceps* cysticerci to E_2 and P_4 induced differential protein expression patterns compared with untreated controls. Changes in actin, tubulin and myosin expression were confirmed by flow cytometry of parasite cells and immunofluorescence. In addition, parasite morphology was altered in response to E_2 and P_4 versus controls. Flame cells were primarily affected at the level of the ciliary tuft, in association with the changes in actin, tubulin and myosin. We conclude that oestradiol and progesterone act directly on *T. crassiceps* cysticerci, altering actin, tubulin and myosin expression and thus affecting the assembly and function of flame cells. Our results increase our understanding of several aspects of the molecular crosstalk between host and parasite, which might be useful in designing anthelmintic drugs that exclusively impair parasitic proteins which mediate cell signaling and pathogenic reproduction and establishment.

1. Introduction

Due to the difficulties of working with the natural hosts (pigs and humans) of *Taenia solium*, we used an experimental cysticercosis model to examine the complex host–parasite relationship in cysticercosis. Murine intraperitoneal cysticercosis is caused by the taeniid cestode *Taenia crassiceps* and has been used to study physiological host factors that are associated with porcine cysticercosis and, to some degree, with human neurocysticercosis (Larralde et al., 1990).

Intraperitoneal *T. crassiceps* cysticercosis in mice (Larralde et al., 1990; Sciutto et al., 1990, 2011) is amenable to controlled and reproducible experimentation, generating numerical data on parasite loads in individual mice weeks after infection. The similarities with other forms of cysticercosis have been strengthened by similar recent results in other mouse models and parasite strains (Dorais and Esch, 1969; Hildreth and Granholm, 2003), demonstrating the extensive sharing of antigens and high DNA homology with other taeniid cestodes such as *Taenia saginata and T. solium* (Morales-Montor et al., 2004b).

These characteristics render murine cysticercosis a convenient instrument to test vaccine candidates (Sciutto et al., 2011), new drugs and other therapeutic approaches against cysticercosis. Also, several features of natural cysticercotic disease have been identified by extrapolation from studies in experimental murine cysticercosis (Morales-Montor et al., 2008).

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In *T. crassiceps* cysticercosis, the females of all mouse strains develop more extensive infections than males, but during chronic infection (more than 4 weeks p.i.), this difference wanes and BALB/c mouse males undergo feminization, characterised by high serum oestradiol levels and a decrease in serum testosterone of 90% (Morales et al., 1996). Because gonadectomy alters this pattern of susceptibility and equalises infection intensities between genders (Morales-Montor et al., 2002), sex steroid hormones have been implicated in the establishment of *T. crassiceps* infection.

Moreover, sex steroids have direct effects on parasite reproduction. Both oestradiol and progesterone are key immunoendocrine factors that promote parasite growth by interfering with thymus-dependent cellular immune mechanisms that restrict parasite growth or by directly increasing cysticerci reproduction in vitro (Bojalil et al., 1993; Terrazas et al., 1994; Escobedo et al., 2010). Thus, the effects of the host's endocrine system on *T. crassiceps* must be determined to increase our understanding of the coevolution of the host–parasite relationship.

Microtubules are cylindrical structures in eukaryotic cells that mediate many processes including mitosis, ciliary and flagellar motility, and intracellular transport of vesicles and organelles. In addition, they are the most common component of the cytoskeleton, governing cell morphology (Valiron et al., 2001). In addition to β-tubulin, α-tubulin is a major building block of microtubules. These two components are similar in mass and form a heterodimer (Wade, 2007). In most eukaryotes, α- and β-tubulin undergo various post-translational modifications; therefore, they exist as families of related isoforms (Janke and Bulinski, 2011).

In cestodes, actin, tubulin and myosin form a complex that supports the morphology of flame cells (FCs). FCs are ciliated cells in the basal matrix of neodermal tissue in Cestoda. They are considered terminal cells in flames and bulbs, and the basic units of the protonephridial system (PS) in invertebrates (Rohde et al., 1992; Valverde-Islas et al., 2011). In cestodes, as in all parasitic platyhelminths, the PS is an excretory system that allows parasites to conserve water and eliminate salts.

To survive in the intestine or body cavities of a host, the parasite's PS acts as an osmoconformer (Rohde et al., 1992; Valverde-Islas et al., 2011). Parasites must also maintain osmotic pressure in their tissues within physiological limits against that of the host environment (Smyth and McManus, 1989).

FCs have a typical morphology: by light microscopy, they appear comet-like, with an anterior end that corresponds to the cell body and a distal end that contains the tuft of cilia, also known as the flames. The FCs of several helminths show similar morphologies by transmission electron microscopy (TEM), comprising a single nucleus and classical cilia with the arrangement of 9+2 axonemes. A plasma membrane surrounds the cell body that extends through the tip of the tuft of cilia and is apparently in close interdigitation with the membrane of adjoining cells (Valverde-Islas et al., 2011). Whereas the bodies of FCs are embedded in the parenchymal tissue, the cilia tufts extend to inside the PS tubules (Valverde-Islas et al., 2011).

In tapeworms, FCs appear to beat continuously and their cilia give the impression of flickering flames inside the cell (Valverde-Islas et al., 2011). Cytoskeletal proteins such as polymerized actin were recently described to form FCs in the cestode *Diphyllobothrium dendriticum* (Wahlberg, 1998) and the monogenean *Gyrodactylus rysavyi* (Arafa et al., 2007) by fluorescent phalloidin staining. In other parasites such as the cestode *Gymnorinchus gigas* (Moreno et al., 2001) and the trematode *Schistosoma mansoni* (Bahia et al., 2006), tubulin localises to the FC cilia.

FCs have been extensively studied, mainly as key regulators of parasite metabolism, drug excretion and interaction with the host (Kusel et al., 2009), however, to date there are no published data regarding the effects of host factors such as sex steroids on FC morphology or their function in parasite physiology. In tapeworm infections, as in those that are caused by the metazoan *T. crassiceps*, the parasite's FCs mediate survival in the host, because they perform specific functions such as detoxification and transformation of substances that are potentially harmful to the parasite (Smyth and McManus, 1989; Kusel, 2009).

Thus, the aim of this study was to examine the in vitro effect of oestradiol and progesterone on cytoskeletal protein expression and FC morphology at the metacestode stage in *T. crassiceps*. By two-dimensional protein analysis, ultrastructural morphology, immunofluorescence, flow cytometry and videomicroscopy, we found that oestradiol and progesterone differentially modified the distribution and expression of actin, myosin and tubulin in the tegumental tissue in *T. crassiceps*, also affecting FC morphology. These findings increase our understanding of host-parasite molecular crosstalk and should guide the design of drugs that specifically arrest the activity of important parasite molecules such as proteins that mediate the establishment, growth and reproduction of parasites in an immunocompetent host.

2. Materials and methods

2.1. Ethics statement

The Animal Care and Use Committee at the Instituto de Investigaciones Biomédicas evaluated animal care and experimentation practices according to the official Mexican regulations (NOM-062-ZOO-1999). These regulations are in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH and The Weatherall Report) of the USA, to ensure compliance with established international regulations and guidelines. The Ethics Committee of the Instituto de Investigaciones Biomédicas approved this protocol (Permission Number: 2009-16).

2.2. Parasites

Cysticerci were recovered from the peritoneal cavity of BALB/c mice 3 months p.i. and placed in tubes containing sterile PBS supplemented with 100 U/ml of antibiotics – fungizone (Gibco, Grand Island, USA). The tubes were centrifuged for 10 min at 800*g* and 4 °C, and the supernatant was discarded. Packed cysticerci were incubated in serum-free DMEM (Gibco 12491), and washed three times with centrifuging for 10 min at 800*g*. After the final wash, the number of viable cysticerci (complete, translucent and motile cystic structures) was counted under a binocular microscope.

Ten viable non-budding cysticerci, approximately 2 mm in diameter, were selected, plated in a 24-well culture plate (Falcon, Becton Dickinson Labware, New Jersey, USA) that contained 1 ml of DMEM, and incubated at 37 °C with 5% CO_2. Cultures were checked daily and the medium was replaced every 24 h or when it turned yellow.

2.3. Taenia crassiceps cysticerci culture and hormone treatment

Culture-grade 17-β oestradiol (E_2) and progesterone (P_4) were obtained from Sigma, (USA). For the in vitro tests, water-soluble E_2 and P_4 were dissolved in serum-free DMEM. Each hormone was prepared as a 10 mg/ml stock and sterilised by passage through a 0.2 μm filter (Millipore Co., México).

To generate concentration–response curves, the parasites were treated with: (i) DMEM (control group), (ii) 5, 10, 20 or 40 μg/ml of E_2, or (iii) 1, 10, 15, 40 or 80 μg/ml of P_4. Optimal concentrations of E_2 and P_4 were determined from these curves (data not shown) and used in the time-response curves. The final concentrations in the time-response curves were: 40 μg/ml of E_2 and 40 μg/ml of P_4 for 5 days.

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