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Differential expression and glycosylation of proteins in the rat ileal epithelium in response to *Echinostoma caproni* infection

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ABSTRACT

Echinostoma caproni (Trematoda: Echinostomatidae) is an intestinal trematode that has been used as experimental model to investigate the factors determining the expulsion of intestinal helminths. We analyze the changes in the protein expression and glycosylation induced by *E. caproni* in Wistar rat, a host of low compatibility in which the parasites are rapidly rejected. To determine the changes in protein expression, two-dimensional difference gel electrophoresis was employed using protein extracts from the intestine of naïve and infected rats. The patterns of glycosylation were analyzed by lectin blotting. Those spots showing differential expression or glycosylation were analyzed by mass spectrometry. A total of 33 protein spots differentially expressed were identified (26 were found to be over-expressed and 7 down-regulated). Moreover, *E. caproni* induced changes in the glycosylation status of 8 proteins that were successfully identified. Most of these proteins were related to the cytoskeleton and the maintenance of the functional integrity of the ileal epithelium. This suggests that the regeneration of the intestinal tissue is a major effector mechanism responsible for the early expulsion of this helminth. Furthermore, several proteins involved in the energy metabolism were also altered in the ileum of rats as a consequence of the *E. caproni* infection.

Biological significance
Our analysis provides essential new insights in the factors determining the natural expulsion of intestinal parasitic helminths from their hosts. The results obtained contribute to a better understanding of the effective mechanisms involved in the defense against the intestinal helminths. The identification of proteins in the intestine that become modified in their expression or glycosylation in hosts in which the parasite is rapidly rejected may serve for the development of tools for the control of these infections.

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1. Introduction

Echinostomes are cosmopolitan intestinal trematodes that infect a wide variety of warm-blooded host species, including humans [1]. They constitute an important group of food-borne trematodes with prevalences that reach up to 3% in several areas of Asia [2,3]. Apart from their interest as human pathogens, echinostomes are excellent models for the study of several aspects of the host-parasite interactions in the intestinal helminth infections [4]. Our group has used *Echinostoma caproni* as a model for the study of the factors determining the establishment of chronic infections or in contrast, the rejection of the intestinal worms. *E. caproni* is an intestinal trematode with no tissue phases in the definitive host [1]. After infection, the metacercariae excyst in the duodenum and the juvenile worms migrate to the ileum, where they attach to the mucosa [1]. *E. caproni* has a wide range of definitive hosts, although its compatibility differs considerably between rodent species on the basis of worm survival and development [4]. In hosts of high compatibility, such as mice, the infection becomes chronic, while in hosts of low compatibility, such as rats, the worms are expelled from 2 to 4 weeks post-infection (wpi) [5,6]. Because of these characteristics, the *E. caproni*-rodent systems are highly suitable for elucidating aspects of the host specific components that determine the course of infections with intestinal helminths [4].

Previous studies of our group have shown that the establishment of chronic infections is dependent upon the local production of IFN-γ [7]. The infection induces important inflammatory responses and a rapid and substantial injury in the ileal epithelium [6,8]. In contrast, the early rejection of *E. caproni* is associated with the development of a local Th2/Th17 local phenotype with elevated levels of IL-6, IL-13, IL-17 and IL-23 [7,9]. Furthermore, the inflammation and mucosal damage were significantly less than those observed in hosts of high compatibility [1,8]. The elevated level of IL-13 was regarded as the most relevant factor determining the worm expulsion, in relation to the alterations induced in the intestine by this cytokine [7]. However, the changes induced at the protein level in the intestine of rats by the infection are scarce. In this context, the analysis of the proteomic features of the infection combined with the known data on the immunological and pathological changes in the intestine of low-compatible hosts will be of great interest for the understanding of the factors determining the worm rejection.

Herein, we aimed to uncover the intestinal changes induced by *E. caproni* in rats at proteomic and glycoproteomic levels that lead to the worm expulsion. To this purpose, proteins that were differentially expressed as a consequence of the infection in the ileum of rats were identified using two-dimensional difference gel electrophoresis coupled to liquid chromatography and tandem mass spectrometry (LC–MS/MS). Furthermore, we also analyze the proteins that became glycosylated after *E. caproni* infection by lectin blotting after two dimensional separation of the samples. The results obtained combined with previous data on the responses to *E. caproni* infection may be of great help for the understating of the mechanism involved in the expulsion of the intestinal helminths.

2. Material and methods

2.1. Animals and infection procedures

Female Wistar rats weighing 80–100 g were used in the present study. The strain of *E. caproni* and the infection procedures has been described previously [7,10]. Briefly, encysted metacercariae of *E. caproni* were removed from kidneys and pericardial cavities of experimentally infected *Biomphalaria glabrata* snails and used for infections. A total of 12 rats were each infected by stomach tube with 100 metacercariae of *E. caproni*. Additionally, 12 rats were maintained uninfected and used as control animals. Positivity ante-mortem was determined coprologically. At 2 wpi (initiation of the worm expulsion phase) all the animals were sacrificed to obtain the tissue samples. The animals were maintained under standard conditions with food and water ad libitum. The study was approved by the institutional committee on animal care of the University of Valencia (Spain), in compliance with the European Agreement of Strasbourg.

2.2. Tissue collection

During necropsy of rats, sections of the ileum were removed. The epithelial cells from the intestines were obtained as described by Hansson and co-authors [11] with slight modifications. Briefly, the ileal sections were opened longitudinally and rinsed by gentle shaking in ice-cold Hank's balanced salt solution containing 2% heat-inactivated FCS (washing buffer). The supernatant was removed and fresh washing buffer was added. This was repeated (at least 4 times) until the supernatant remained clear. The tissue was cut into 1 cm lengths and gently shook at 37 °C for 20 min in HBSS containing 10% FCS, 1nM EDTA, 1 mM DTT, 100 units/mL penicillin and 100 μg/mL streptomycin (dissociation buffer). The supernatant was collected and placed on ice. The supernatant was filtered through a 100 mm strainer and cells were washed in HBSS by two centrifugations at 200 *g* for 10 min at 4 °C. The cells were resuspended in PBS.

The protein extraction was performed using the ProteoJET™ Mammalian Cell Lysis Reagent (Fermentas Life Sciences) following the manufacturer's instructions. Briefly, cell suspensions were centrifuged at 250 *g* for 5 min and the supernatant discarded. The cells were then rinsed with PBS and centrifuged to remove residual medium. The tissue powder was transferred to a microcentrifuge tube and the ProteoJET™ Mammalian Cell Lysis Reagent was added to the powder (20:1 V:V). The lysate was resuspended and, thereafter, clarified by centrifugation at 18,000 *g* for 15 min. The supernatants from each group of animals (uninfected and infected) were pooled and transferred to new tubes and stored at −70 °C until use.

2.3. Preparations for CyDye labeling for DIGE

CyDye DIGE Fluor minimal dyes (GE Healthcare) were reconstituted according to the manufacturer's instructions. 50 μg protein of each sample was labeled with either Cy3 or Cy5. The sample volumes were adjusted to 18 μL with labeling