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Echinostoma caproni: Intestinal pathology in the golden hamster, a highly compatible host, and the Wistar rat, a less compatible host

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Abstract

The histopathological changes induced by *Echinostoma caproni* (Trematoda: Echinostomatidae) in a high (golden hamster) and a low compatible host (rat) were compared at 15 and 30 days post-infection. Infection of rats was characterized by a progressive increase in erosion of villi and elevated numbers of goblet cells, which could be related to the early expulsion of the parasite in a host of low compatibility. In contrast to rats, the number of goblet cell in *E. caproni*-infected hamsters was low, but increased numbers of neutrophils and mesenteric inflammatory cells were observed. This indicated that local inflammatory responses in hamsters were greater than in rats. An immunohistochemical study using polyclonal IgG anti-*E. caproni* excretory–secretory antigens demonstrated a greater level of passage of *E. caproni* antigens through the intestinal mucosa in hamsters than in rats, probably in relation to the greater inflammatory response. Our results indicate the fact that early inflammatory responses could be important for the establishment of *E. caproni* chronic infections in highly compatible hosts.

Index descriptors and Abbreviations: Echinostoma; *Echinostoma caproni*; Hamster; Rat; Intestinal pathology; Inflammation; IFN-γ, interferon-γ; H–E, hematoxylin–eosin; VCU, villus-crypt unit; HPF, high power field; ES, excretory/secretory; IL-13, interleukin-13

1. Introduction

Echinostoma caproni (Trematoda: Echinostomatidae) is an intestinal trematode with no tissue phases in the definitive host (Fried and Huffman, 1996). After infection, metacercariae excyst in the duodenum of the definitive host, and the juvenile worms migrate to the posterior third of the small intestine where they attach to the mucosa by the ventral sucker (Fried and Graczyk, 2004; Fried and Huffman, 1996). *E. caproni* has a wide range of definitive hosts, although its compatibility differs considerably between different rodent species on the basis of worm survival and development in each host species. In highly compatible hosts, such as hamsters and mice, *E. caproni* induces chronic infections and the parasite can survive for more than 25 weeks, while in hosts with low compatibility, such as rats, the worms are rapidly expelled a few weeks after infection (Christensen et al., 1990; Hansen et al., 1991; Odaibo et al., 1988, 1989; Toledo et al., 2004a). Moreover, it has been shown that the dynamics of growth and fecundity of *E. caproni* adult worms depend greatly on the host species (Toledo et al., 2004a). Because of such characteristics, the *E. caproni*/rodent systems are highly suitable models for elucidating aspects of the host specific components that determine the course of infections with intestinal helminths (Toledo and Fried, 2005).

Significant differences in relation to several immunological parameters during *E. caproni* infections have been also detected between low and high compatible host species.

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Hamsters and mice develop strong systemic antibody responses to *E. caproni* infections (Agger et al., 1993; Graczyk and Fried, 1994; Toledo and Fried, 2005; Toledo et al., 2004b, 2005). Furthermore, these hosts showed high levels of *E. caproni* antigens in the serum during the infection (Toledo et al., 2004b, 2005). In contrast, rats only develop weak serum antibody responses to *E. caproni* infections and the levels of seroantigens were low (Toledo et al., 2003a, 2004b). These discrepancies were attributed to differences in the local inflammatory responses to *E. caproni* infection in each host species. This may be of importance in determining the different course of the infection in each host. In intestinal nematodes, local inflammation, mediated by interferon-γ (IFN-γ) and other pro-inflammatory cytokines, inhibits protective immunity resulting in chronic infections (Finkelman et al., 1997; Hayes et al., 2004; Maizels and Holland, 1998; Maizels and Yazdanbakhsh, 2003). It has been reported that the establishment of *E. caproni* chronic infections in mice is biased toward a Th1 phenotype and related to elevated levels of IFN-γ during the first weeks of the infection (Brunet et al., 2000). It is therefore likely that inflammatory responses at the intestinal level during the first weeks post-infection are involved in the establishment of chronic infections in highly compatible hosts and the earlier worm rejection in less compatible hosts. However, the intestinal pathology elicited by *E. caproni* infections has only been studied in highly compatible hosts, i.e., mainly in mice (Fujino and Fried, 1993; Fujino et al., 1993, 1996).

In this paper, the intestinal pathology of *E. caproni* in two hosts (golden hamster and rat) with different compatibilities to the worm has been studied for the first 4 weeks of the infection to gain further insight in host–parasite relationships in intestinal trematode infections. An emphasis is placed on implications of the pathological effects on the different course of the *E. caproni* infection in each host species.

2. Material and methods

2.1. Parasite and experimental infections

The strain of *E. caproni* has been previously described (Hosier and Fried, 1991). Encysted metacercariae of *E. caproni* were removed from the kidneys and pericardial cavities of experimentally infected *Biomphalaria glabrata* snails and used to infect golden hamsters (*Mesocricetus auratus*) and albino Wistar rats (*Rattus norvegicus*). Each of six outbred male golden hamsters, weighing 45–60 g, and six rats, weighting 110–150 g, was infected by stomach tube with 100 metacercariae of *E. caproni*. Moreover, three hamsters and three rats were left uninfected and used as controls. The animals were maintained under conventional conditions with food and water ad libitum. The worm egg release was investigated daily in each infected animal as described previously (Toledo et al., 2003b).

2.2. Histology

Histopathological responses to *E. caproni* infections in rats and hamsters were evaluated at 15 and 30 days post-infection. At each time post-infection, three hamsters and three rats were necropsied and intestinal sections of 0.7–1 cm in length from the sites where the worms were located were obtained from each animal and fixed in 4% buffered formalin. After embedding in paraffin wax, serial 4 μm sections were cut from each tissue block. Intestinal sections were stained with Hematoxylin–eosin (H–E), Giemsa, P.A.S., Alcian blue, and Toluidine blue.

Sloughing of the villi tips was considered as an histological criterion of *E. caproni* induced mucosal damage as in previous studies (Bindseil and Christensen, 1984). Ten randomly selected low power (100×) fields of each section were examined and the numbers of destroyed or eroded villi, and the total number of villi were recorded. Three sections of each rat and hamster were examined and the results expressed as the percentage of destroyed or eroded villi.

All the cell counts were expressed as the number of cells per villus-crypt unit (VCU), except those of the mesenteric cells which are expressed in number of cells per high power field (HPF) (400×), studied over 10 selected HPFs. Results are expressed as the mean number of cells per VCU or HPF ± standard deviation.

2.3. Polyclonal IgG anti-E. caproni excretory–secretory antigens

To obtain excretory/secretory (ES) antigens of *E. caproni*, we followed the methodology described in a previous study (Toledo et al., 2003a). Adult worms were collected from the small intestines of rats 4 weeks after experimental infection with 100 metacercariae of *E. caproni*. After repeated washings with phosphate-buffered saline (PBS, pH 7.4), the adult worms were maintained in PBS at concentrations of 10 worms/ml for 12 h at 37 °C in PBS containing 0.8 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, Missouri), 100 U penicillin (Sigma), and 100 μg/ml streptomycin (Sigma). The medium was collected and centrifuged at 15,000*g* for 30 min at 4 °C and the supernatant was collected. The protein content was measured by the Bio-Rad protein assay and adjusted to 1 mg/ml using an ultrafiltration membrane. The antigens were stored at −20 °C until use.

Polyclonal IgG anti-*E. caproni* ES antigens were obtained by inoculating two 3 kg New Zealand white rabbits with ES trematode products obtained from rats. Three hundred micrograms of ES antigen were emulsified in 0.3 ml of Freund's complete adjuvant and were used to hyperimmunize each rabbit by giving several inoculations. Rabbits were given similar inoculations on four occasions, with 2 weeks intervals, using Freund's incomplete adjuvant. Blood was drawn 21 days after the final inoculation. Serum was pooled and the anti-*E. caproni* ES antigens globulins containing IgG were obtained by precipitation with 50%