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Echinostoma caproni (Trematoda): Differential in vivo cytokine responses in high and low compatible hosts

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ABSTRACT

In order to investigate the factors determining the expulsion of intestinal trematodes, we have analyzed the in vivo cytokine responses at several levels and the local responses against *Echinostoma caproni* (Trematoda) in two host species displaying different compatibility with the parasite. The response of the high compatible host (mice) is characterized by a mixed Th1/Th2 phenotype in the spleen, Peyer's patches and mesenteric lymph nodes. At the intestine, a marked Th1 response with a marked increase of IFN-γ together with elevated number of mucosal neutrophils and expression of induced nitric oxide synthase were observed. The responses in the host of low compatibility (rats) with the parasite at the spleen, Peyer's patches and mesenteric lymph node did not show clear differences with regard to the mice. However, the response in the intestine was markedly different. In rats, a Th2 response with increase in the levels of IL-5, IL-6 and IL-13 expression was detected. According to these results, the local production of IFN-γ and the local inflammatory responses with neutrophilic infiltration are associated with the development of chronic infections, whereas the worm expulsion is related with the development of Th2 responses and appears to be based on effects on non-bone narrow-derived cells.

1. Introduction

Echinostoma caproni (Trematoda: Echinostomatidae) is an intestinal trematode with no tissue phase in the definitive host (Fried and Huffman, 1996). Although *E. caproni* is able to parasitize a wide range of laboratory rodent hosts, its compatibility differs considerably between rodent species. In highly compatible hosts, such as mice and hamsters, the infection becomes chronic, while in hosts of low compatibility, such as rats and jirds, the worms are expelled a few weeks after infection (wpi) (Odaibo et al., 1988, 1989; Christensen et al., 1990; Mahler et al., 1995; Toledo et al., 2004a, 2009; Toledo and Fried, 2005). Because of this characteristic, our group has used the *E. caproni*/rodent model to study several aspects of the host/parasite interactions, with emphasis in the host-specific components that determine early rejection of the worms or the establishment of chronic infections. Several studies have shown that the characteristics of the immune responses against *E. caproni* largely depend on the compatibility of the host species with the parasite (Toledo et al., 2006a, 2009). The establishment of chronic infection has been related with the development of rapid and strong antibody responses both at local and systemic levels together with increases in local populations of mucosal neutrophils and mononuclear inflammatory cells in the mesentery. Surprisingly, the early rejection of the worms has been associated with weak antibody responses and low levels of local neutrophils and inflammatory cells (Toledo et al., 2004b, 2005, 2006b; Muñoz-Antoli et al., 2007; Sotillo et al., 2007). These results make difficult the ascertaining of the effector mechanism involved in worm rejection. In this context, the analysis of cytokine responses may be essential for understanding the mechanisms implicated in worm rejection. However, the existing literature provides little information on the cytokine profiles in echinostome infections and most of the studies are based on in vitro observations on cultures of spleen cells or cells from mesenteric lymph nodes (MLN) and further studies on this topic are required for the understanding of the factors determining the course of the infection.

In the present study, we compared the in vivo cytokine profile in several immune active tissues against *E. caproni* in two hosts (mice and rats) in which the parasite survival markedly differs.

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Furthermore, we also analyze the pathological changes induced by the parasite in each host species. The results obtained may be of interest to gain further insight into the host–parasite relationships in intestinal trematode infections and into the factors and effector mechanisms involved in worm rejection.

2. Materials and methods

2.1. Parasites and experimental infections

The strain of *E. caproni* has been described previously (Fujino and Fried, 1993). Encysted metacercariae of *E. caproni* were removed from kidneys and pericardial cavities of experimentally infected *Biomphalaria glabrata* snails and used to infect ICR male mice and albino Wistar rats (Wistar). 36 ICR mice, weighing 30–35 g, and the same number of rats, weighing 80–100 g were each infected by stomach tube with 75 and 100 metacercariae of *E. caproni.* Both, mice and rats were randomly allocated to group A (18 mice and 18 rats), B (6 mice and 6 rats), and C (12 mice and 12 rats). Group A was used to study the worm recovery and its variations over the course of the infection in both definitive hosts. Group B was used to analyze the pathology induced by *E. caproni* in each host species. Finally, group C was used to investigate the cytokine profile induced by the parasite in both host species. Moreover, a total of 4 mice and 4 rats were left uninfected and used as controls in the studies on pathology and cytokines. All the animals were maintained under conventional conditions with food and water *ad libitum.*

2.2. Worm survival

This experiment was designed to compare the kinetics of worm survival of *E. caproni* adults in these two different hosts: mice and rats. At each 2 wpi, 3 mice and 3 rats of group A were necropsied and the number of worms recovered per host was recorded.

2.3. Histology

Histopathological responses to *E. caproni* infections in mice and rats were evaluated at 2 and 4 wpi. At each time post-infection, 3 mice and 3 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, PAS, Alcian Blue, and Toluidine Blue. All the cell counts were expressed as the number of cells per villus-crypt unit (VCU). Results are expressed as the mean number of cells per VCU ± standard deviation.

2.4. Total RNA extraction

At each 2 wpi, 3 rats and 3 mice belonging to the group C were necropsied. Total RNA was extracted from excised spleen, Peyer's patches (PP) and MLN and from full-thickness sections of ileum at the sites where the worms were located. Total RNA from each tissue obtained from rats and mice was isolated using Rapid Total RNA Purification Kit (Marligen Biosciences Inc.) according to the manufacturer instructions. The cDNA was synthesised using SuperScript III First-Strand Synthesis System (Invitrogen).

2.5. Real-time PCR and relative quantification analysis

For quantitative PCR, 40 ng total RNA reverse transcribed to cDNA was added to 10 μL of TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2×), 1 μL of the specified TaqMan® Gene Expression Assay, and water to final reaction volume of 20 μL. Reactions were performed on the Abi Prism 7000 (Applied Biosystems), with the following thermal cycler conditions: initial setup of 2 min UNG activation at 50 °C, followed by 10 min at 95 °C, and 40 cycles of 15 s denaturation at 95 °C and 1 min of anneal/extend at 60 °C each. Samples were amplified in a 96-well plate. On each plate endogenous control, samples and negative control were analyzed in triplicate. All TaqMan® Gene Expression primers and probes for iNOS and cytokines, except those for IL-6 in rats, were designed by Applied Biosystems and offered as Inventoried Assays. Table 1 shows each assay ID details, the housekeeping β-actin, iNOS and cytokine mRNAs. Each assay contains two unlabeled primers and one 6-FAM™ dye-labeled, TaqMan® MGB probe. Primers and fluorescent FAM-labeled probes of rat IL-6 were designed in-house from published sequences using the PrimerExpress software from Applied Biosystems. The sequences for primers and the fluorescent FAM-labeled probe were:

5′-ACCCACAACAGACCAGTATATACCA-3′ (sense)
5′-CATTTCCAAGATCTCCCTGAGAACA-3′ (antisense)
5′-CACAAGTCGGAGGCTTA-3′ (probe)

Cycle threshold (Ct) value was calculated for each sample, housekeeping and uninfected control. To normalize for differences in efficiency of sample extraction or complementary DNA synthesis we used β-actin as housekeeping gene. To estimate influence of infection in the expression levels we use a comparative quantification method ($2^{-\Delta\Delta C_T}$ – method) (Livak and Schmittgen, 2001). The method is based on the fact that the difference in threshold cycles (ΔCt) between the gene of interest and the housekeeping gene is proportional to the relative expression level of the gene of interest. The fold change in the target gene was normalized to β-actin and relative the expression at time 0 (uninfected animals) to generate relative quantification of expression levels (Klein, 2002).

2.6. Statistical analysis

To analyze the effect of worm age and the host species on the survival, the kinetics of cell populations in the intestine of mice and rats and the kinetics of each cytokine in each tissue, a two-factor ANOVA with interaction was used with the worm age and host species as independent variables. Moreover, when a significant worm age-host species interaction was detected, the Bonferroni *t*-test of the difference between means was performed as a post hoc analysis to determine if there are significant differences due to the host species within the same time post-infection. $P < 0.05$

Table 1
Applied Biosystems Inventoried assays used for β-actin, cytokines and induced nitric oxide synthase (iNOS).

	Assay ID details	
	Rats	Mice
β-actin	Rn00667869_m1	Mm01205647_g1
IL-4	Rn99999010_m1	Mm00445259_m1
IL-5	Rn01459975_m1	Mm99999063_m1
IL-6	Custom Assay*	Mm00446190_m1
IL-10	Rn00563409_m1	Mm00439614_m1
IL-13	Rn00587615_m1	Mm99999190_m1
IFN-γ	Rn01420317_m1	Mm99999071_m1
TNF-α	Rn99999017_m1	Mm99999068_m1
Eotaxin/CCL11**	Rn00569995_m1	Mm00441238_m1
iNOS	Rn00561646_m1	Mm01309897_m1

* Non-inventoried assay available.
** Chemokine (C–C motif) ligand 11.