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Do mice genetically selected for resistance to oral tolerance provide selective advantage for *Schistosoma mansoni* infection?

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Abstract

A highly evolved relationship exists between the parasitic flatworm *Schistosoma mansoni* and its vertebrate hosts that include the use of host immune signals by parasites. The *S. mansoni* infection was studied in two strains of mice genetically selected, over 18 generations of assortative mating, for extreme phenotypes of susceptibility (TS) and resistance (TR) to immunological tolerance. The objective was to observe whether the different host genetic backgrounds affected the outcome of experimental schistosomiasis. Fecal egg excretion, tissue egg count, worm recovery, and adult worm morphology and morphometry were monitored throughout the period of infection. TR mice presented total fecal egg excretion and thickness of tegument in adult male worms significantly higher than TS mice. Therefore, the comparative analysis of mice with extreme phenotypes of immunological response turns out to be useful in host–parasite relationship studies. Our results suggest that the TR mouse immunological profile provides a more favorable environment for the development of *S. mansoni*.

Index Descriptors and Abbreviations: *Biomphalaria glabrata*, Planorbidae; $CD4^+$ αβ, T-cells that carry the cluster of differentiation CD4; IL-7, Interleukin-7; *Schistosoma mansoni*, trematode; SCID mice, severe combined immunodeficiency; TNF-α, Tumor necrosis factor-α; TR strain, oral tolerance resistant mice; TS strain, oral tolerance susceptible mice

Keywords: *Schistosoma mansoni*; Morphometrical characteristics; Infection susceptibility; Oral tolerance; Host resistance; Mice; Experimental model

1. Introduction

Many helminths are capable of modifying their development in response to environmental and host factors. Responses of parasites at different stages of their life cycles could facilitate their survival under adverse conditions and increase the probability of transmission between hosts (Davies and McKerrow, 2003).

Variation of infection outcome is a hallmark of helminth infections (Maizels et al., 1993). Variability between individual hosts may contribute to the variation infection outcomes observed in an affected host population (Davies and McKerrow, 2003). Therefore, the infection outcome can alter according to the genetic characteristics of the host (Incani et al., 2001).

In murine models, *S. mansoni* is able to develop by using signals provided by the host's adaptive immune system (Davies and McKerrow, 2003; Hernandez et al., 2004).

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Growth of worms and fecundity are dependent upon modulatory signals supplied by $CD4^+$ αβ lymphocytes (Davies et al., 2001). Egg excretion is minimal in immuno-compromised mice and can be augmented by the transfer of sera and lymphocytes from infected animals (Doenhoff, 1997; Doenhoff et al., 1981), and T-cell deprivation causes a reduction in fecundity of *S. mansoni* (Harrison and Doenhoff, 1983). The parasite has adapted so successfully to its host that it uses host-derived immunoregulatory proteins as a signal for replication and transmission (Amiri et al., 1992). The influence of the immune factors on parasite development and egg transit through the wall of the intestine can also occur in humans. In a study with co-infected HIV patients, there was a correlation with the reduction in fecal egg output (Karanja et al., 1997).

Recently, we produced, by assortative mating, a new genetic model mouse using a two-way breeding selection for susceptibility (TS strain) or resistance (TR strain) to oral tolerance induction (da Silva et al., 1998). Oral tolerance, a major example of immunoregulation, is defined as a reduction in specific immune responsiveness after immunization with an antigen previously administered by the oral route. Oral administration of the antigen can lead to peripheral immunological suppression, inhibiting both humoral and cellular immune responses, and is a clinical strategy for allergic desensitization and treatment of inflammatory autoimmune diseases (Faria and Weiner, 1999). Active mechanisms of oral tolerance were shown to significantly reduce granuloma formation in mice infected with *S. mansoni* (Carvalho et al., 2002; Sadigursky et al., 1987).

To determine whether the different genetic backgrounds of TS and TR mice (now in the 18th generation of selective breeding) affect the outcome of experimental schistosomiasis, we monitored fecal and liver egg counts, infection rate, and the phenotypic features of *S. mansoni* adult worms analyzed under bright-field microscopy.

2. Materials and methods

2.1. Mice

Mice of both sexes from F18 generation of the TS and TR strains, obtained by two-way selection for susceptibility or resistance phenotypes to oral tolerance to egg-albumin were used. The original foundation population from which the TR and TS strains were derived consisted of an intercross of eight different inbred mouse strains (A/J, DBA/2J, P/J, SWR/J, SJL/J, CBA/J, BALB/cJ, and C57Bl/6J) (Fig. 1) (da Silva et al., 1998). The Commission for Care and Use of Laboratory Animals of the Rio de Janeiro State University, Brazil approved the protocols. The selective breeding for the strains susceptible and resistant to oral tolerance was carried out at the Cellular Biology and Genetics Department of the Rio de Janeiro State University.

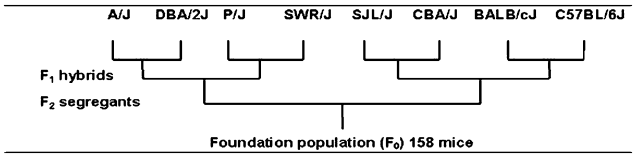

Fig. 1. Production of the foundation population from eight inbred mouse strains.

2.2. Experimental design

Six mice of each strain were individually infected with ~50 *S. mansoni* cercariae (BH strain, Belo Horizonte, Brazil) recovered by shedding from laboratory-reared *B. glabrata* (Paraense and Correa, 1989). This strain has been maintained at the Malacology Department (Oswaldo Cruz Institute, Rio de Janeiro, Brazil) by cycling in Swiss Webster mice and *B. glabrata* snails over 30 years. All the mice were 4–8 days old at the time of infection.

Stool samples were collected twice a week from each mouse from day 42 until 61 following cercariae exposure. The feces were processed and the number of eggs per gram (epg) was calculated by the Kato–Katz technique (Katz et al., 1972). Two slides per animal were counted. On day 63 post-infection, all mice were killed by cervical dislocation. Adult worms were recovered from the portal system and mesenteric veins, counted in a stereomicroscope and infection rate was assessed as a percentage of cercariae that had matured into adult worms (Freire et al., 2003).

The small intestine was removed from each mouse and a fragment (1 cm long) was cut from the central third and crushed between two glass slides for egg count under bright-field microscopy. Eggs were classified according to criteria described by Prata Prata (1957). The remaining intestine and part of the liver were dissolved in 4% KOH to determine the number of eggs, as previously described (Cheever, 1968).

2.3. Morphological study

The adult worms recovered were fixed in AFA (95% alcohol 70–3% formaldehyde and 2% acetic acid) stained with chloride carmine, cleared in methyl salicylate with Canada balsam (1:2), prepared as whole-mounts and examined microscopically to assess morphology (Neves et al., 1998). The images were taken by an analog camera (Sony, 640×480 pixels, RGB) using light microscopy (Olympus BX50). The images were then transferred to a computer-linked image analysis software (Image Pro Plus-version 3.0, Media Cybernetics, USA) for morphometric analysis.

Measurements were made of the area, diameter and perimeter of testicular lobes, uterine egg and spine,