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Morphological tegument alterations of adult *Schistosoma mansoni*, harbored in non anti-helminthic treated, high-immune-tolerogenic and low-inflammatory mice

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ABSTRACT

The present study exhibits original results of *S. mansoni* tegumental alterations due to contact with the immune system of non anti-helminthic treated mice. We compared, by SEM, the tegument of adult worms recovered from strains of mice genetically selected to extreme phenotypes of resistance (TR strain) and susceptibility (TS strain) to egg-albumin oral tolerance (OT). The parasites recovered from TR mice displayed no morphologic alteration, while specimens collected from TS mice presented tubercle swelling with blunted and shortened spines in lower density, increased sensory organelle numbers, fusion and tegumental ridge peeling. These tegument alterations were similar to those described for Artemether or Praziquantel treatment, supporting observations that the host immune system influences the development and function of the tegument of worms harbored in both anti-helminthic treated and non-treated mice. Our results are indicative that the development and function of the worm tegument depend on the immune regulatory capacity of each individual host.

1. Introduction

The *Schistosoma mansoni* tegument is the major interface between the parasite and host. It comprises a double membrane structure involved in the absorption of nutrients, with secretory functions crucial for modulation of the host immune response for parasite survival, thus a prime target for vaccine studies (Lima et al., 1994). As such, many structural and molecular characteristics of the tegument, in conjunction with the host environment, are necessary for parasite development and survival. The physiological and reproductive status of the worm is strongly influenced by the host (Cioli et al., 1977; Senft et al., 1978), and as the parasite responds differently in the various life cycle stages, possibly facilitating survival under adverse conditions (Davies and McKerrow, 2003), gene regulation affords parasite adaptation to distinct environments associated to morphological and biochemical transitions among these different stages (Jolly et al., 2007).

The genetic variability among individual hosts may contribute to the outcome of infection (Davies and McKerrow, 2003), and the characteristics of the parasite can change according to the genetic profile of the host (Incani et al., 2001). The parasite takes advantage of the signals provided by the host immune system (Davies and McKerrow, 2003; Hernandez et al., 2004) for replication and transmission (Amiri et al., 1992).

Worm tegument is often approached as a drug target in schistosomiasis (Shaohong et al., 2006). However, many studies report that the tegument of parasites from resistant infections is less susceptible to praziquantel (PZQ) treatment, suggesting worm resistance to the drug (William et al., 2001). Chemotherapy proved to modulate cytokine responses, leading to protection in schistosomal infection (Martins-Leite et al., 2008). Thus, the efficacy of praziquantel would be dependent on host immune response (Farah et al., 2000; Ribeiro et al., 2004). Specific antibodies against antigens expressed on *S. mansoni* tegument were described to cause damage in adult worms

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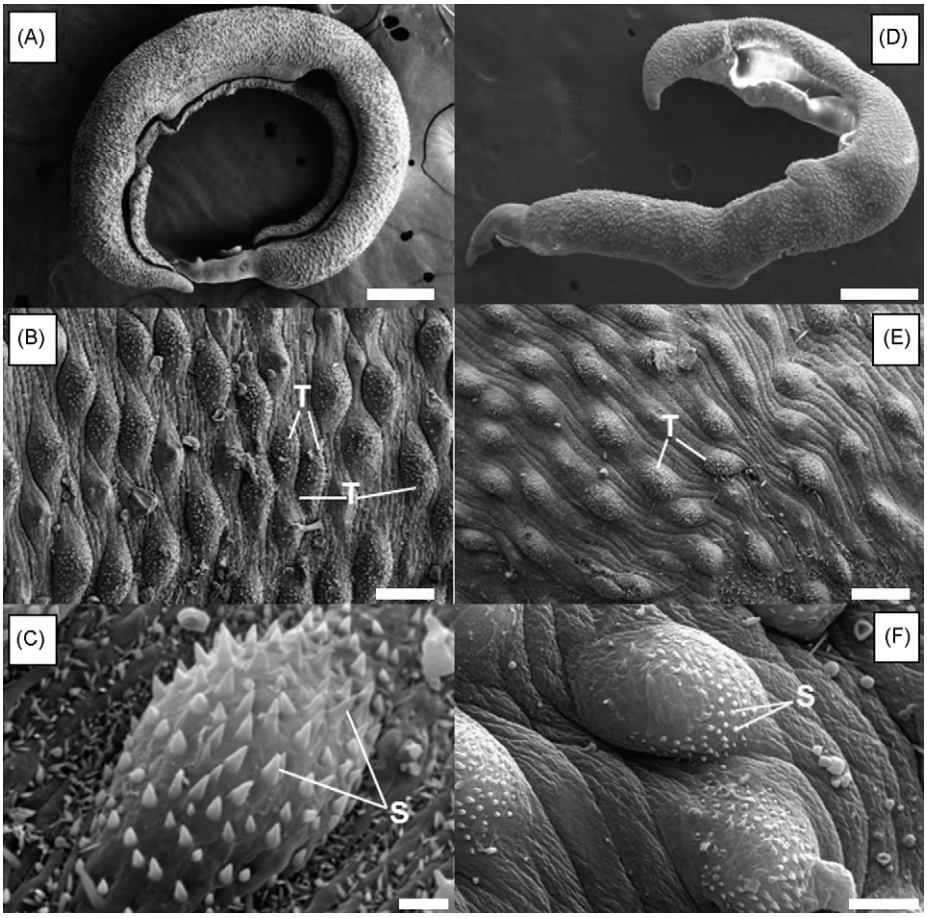

Fig. 1. Micrographs of adult male *S. mansoni* tegument recovered from genetically selected mice. A–C: Representative figures of male worms recovered from TR mice, showing tubercles (T) and spines (S). D–F: Representative figures of male worms recovered from TS mice, showing tegumental ridge swelling and fusion, lower concentration and severe swelling of the tubercles (T), and spines (S) flattened and in smaller number. Five TR or TS mice of each sex were used. Bars: A and D: 250 μm; B: 25 μm; C: 2.5 μm; E: 25 μm; F: 5 μm.

recovered from mice, and the combined exposure of praziquantel and antibodies induces greater morphological damage with enhanced severity than antiserum or the drug alone (Modha et al., 1990).

Previously, we produced by bidirectional genetic selection two strains of mice with extreme phenotypes of susceptibility (TS strain) and resistance (TR strain) to egg-albumin (OVA) oral tolerance (Silva et al., 1998). TS is a low-inflammatory strain producing high levels of cytokines such as IL-10 and IL-4, opposed to TR, a high-inflammatory strain, void of inhibitory responses (Kamphorst et al., 2004; Silva et al., 2004). The percentage of $CD4^+CD25^+$ and $CD4^+Foxp3^+$ spleen cells and IL-10 expression by $CD4^+$ cells is significantly higher in TS mice (Silva et al., 2010), the naive TS mice possessing a 20-fold lower serum IgE level and two- to threefold diminished mast cell numbers in stomach, intestine, lung, peritoneal cavity and mesentery tissues when compared to TR mice, which are highly susceptible to allergic inflammation and anaphylactic shock (Silva et al., 2006). As the TR and TS phenotypes were selected based on modification of the immune function, these animals demonstrate altered responses to infectious agents (Silva et al., 2001; Tavares et al., 2006). One advantage of adopting these strains of mice is the possibility of reconstituting F2 populations with a normal phenotype distribution in a genetically heterogeneous background resembling a natural population.

Consequently, the diverse immunoregulatory profile of these mice could present different conditions for adaptation and survival of the parasite. Previous studies have indicated increased thickness of *S. mansoni* tegument recovered from TR mice as well as greater fecal egg excretion (Machado-Silva et al., 2005). The present study aims to determine by SEM whether or not the *S. mansoni* tegument structure may be modified in the environments of these mice presenting different immune-regulatory capacities.

2. Materials and methods

2.1. Animals

TR and TS strains of mice from the F_{25} generation, obtained by two-way genetic selection according to susceptibility (TS) or resistance (TR) to OVA oral tolerance, were adopted. The original foundation population, from which the TR and TS strains were derived, was achieved by the balanced intercrossing of eight inbred mouse strains (Jackson Laboratory, Bar Harbor, ME) of distant origins (A/J, DBA/2J, P/J, SWR/J, SJL/J, CBA/J, BALB/cJ, and C57BL/6J) (Silva et al., 1998). The selective breeding that gave rise to these strains was carried out in the Genetics Department of the Rio de Janeiro State University where the mice were lodged together in our animal facilities. The Committee for the Care and Use of Laboratory Animals of the Rio de Janeiro State University, Brazil, approved the protocols of the experiments described in this paper.

2.2. Parasites and infection

Eight week old mice of TR and TS strains were exposed to 50 transcutaneous cercariae obtained from *Biomphalaria glabrata*, infected with *S. mansoni* (BH strain, Brazil) maintained in the Refer-