Compatibility of *Biomphalaria tenagophila* with *Schistosoma mansoni*: a study of homologous plasma transference

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This study aims to investigate the importance of the serum factors present in the plasma of resistant Biomphalaria tenagophila *snails, when transferred to susceptible conspecific. Susceptible* B. tenagophila *(CF) received plasma from resistant* B. tenagophila *(Taim), and both were later infected with* Schistosoma mansoni. *We noticed that the plasma transfer showed an increase on the resistance of susceptible snails of about 86% when compared to the non-immunized group ($p < 0.001$).*

Key words: *Biomphalaria* - *Schistosoma mansoni* - plasma

Among the endogenous factors involved in snail-trematode interaction, the most important is in relation to the gastropods defense system. This system is different from the vertebrate immune system, as the lymphocytes, immunoglobulins specific antigen are lacking. Nevertheless, the differentiation between the "self" and the "not self" is kept (Bayne 1983). The defense system is made by cellular and humoral elements. The cellular defense system is operated by hemocytes (moving cells), which have phagocytic capacity. The humoral immunity is measured by lectins and opsonins, which are proteins synthesized by hemocytes with specific affinities with carbohydrates (Zelck et al. 1995). Bayne et al. (1980) showed the serum transfer effect of resistant *Biomphalaria glabrata* on the increase of phagocytic capacity of hemocytes from susceptible snails, demonstrative of interaction between plasmatic factors and the hemocytes during the encapsulation and phagocytic process, that occurred few hours after the miracidium penetration. Although the model *B. glabrata*/*S. mansoni* has been the object of several studies, the association *B. tenagophila*/*S. mansoni* is also a very interesting model because it shows absolute resistance of the snail to the parasite (Coelho et al. 2004). The aim of this study was to evaluate the occurred changes after plasma transference from resistant to susceptible *B. tenagophila* snails following infection with *Schistosoma mansoni*.

All snails used came from Brazil. Susceptible *B. tenagophila* from Cabo Frio - state of Rio de Janeiro and resistant *B. tenagophila* from Taim - state of Rio Grande do Sul. The *S. mansoni* LE strain was obtained after passage in hamsters and *B. glabrata*, at the Federal University of Minas Gerais. Parasites and hosts were kept in the Snail Biology and Parasite Research Laboratory at the Federal University of Ceará, Brazil. The protocol was basically the same used by Granath and Yoshino (1984), with minor changes, briefly: all *B. tenagophila* Taim and Cabo Frio strains were anesthetized with sodic pentobarbitol (Nembutal®), 100 mg/2ml) (Martins-Sousa et al. 2001), for 6 h, to relax all the musculature. The plasma for the transference was collected from a hemolymph pool of five *B. tenagophila* - Taim snails. After centrifugation of 80 g for 10 min to remove all the hemocytes, it was diluted in sterile PBS in 1:2 proportion. The *B. tenagophila* - Cabo Frio were inoculated with 5 µl of the diluted serum in the cephalopodal region using a 25 µl Hamilton Microliter® syringe. The control groups *B. tenagophila* (Taim and Cabo Frio) received the same volume of sterile PBS. Twenty-four hours later, the three groups were individually infected with ten *S. mansoni* miracidia. Six hours later, the groups were replaced in separate aquariums with aeration by pumping and fed with lettuce ad libitum. All the groups were once a week analyzed concerning the cercarial release up to 60 days after infection. The protocol was executed in duplicate. All data were analyzed using chi-square test (Zar 1996).

In this study, we were successful in passively transferring resistance to *S. mansoni* from the resistant *B. tenagophila* (Taim) to the susceptible *B. tenagophila* (CF) through the plasma (cell-free hemolymph) injection. After 60 days post-infection 86.2% of the susceptible strains, which received serum from the resistance, were completely protected of a primary infection with the parasite. By the other hand, only 36% from the non-immunized susceptible snails were not infected, and the control (*B. tenagophila*-Taim) showed no infection index (Table).

Loker and Bayne (1982) obtained in vitro 57% of protection when they joined hemocytes of susceptible snails and the serum from the resistant ones. Granath and Yoshino (1984) working with susceptible M-line albino *B. glabrata* that received serum from resistant 10-R2, obtained more than 60% of protection against *S. mansoni*. Rosa et al. (2005) showed that F1 generation of *B. tenagophila* generated by crossbreeding from *B. tenagophila* Taim and *B. tenagophila* Joinville (albino) were almost 100% resistance to the LE strain of *S. mansoni*.

The plasma of *B. glabrata* and other planorbid pulmonates contains factors which agglutinate mammalian

Financial support: Funcap
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Received 8 December 2005
Accepted 31 January 2006

TABLE

Percentage of infection in susceptible *Biomphalaria tenagophila* Cabo Frio strain after plasma transference from resistance *B. tenagophila* Taim strain. Snails were individually infected with 10 miracidia of *Schistosoma mansoni* and exposed 30 and 60 days post- infection to cercarial release

			B. tenagophila		
Number of snails	Days post-infection	Treatment schedule	% infected snails	% not infected snails	Significance χ^2 (p)
29	30	Imunized	6.9	93.1	43.1 (< 0.001)
		Control	20	80	18 (< 0.001)
25	60	Imunized	13.8	86.2	30.41(< 0.001)
		Control	64	36	3.92 (> 0.05)

erythrocytes. These invertebrate hemagglutinins are, in general, multivalent lectins agglutinating red blood cells by linking carbohydrate moieties on neighboring cells, and are likely to function as recognition factors (Loker et al. 1984). Two kinds of recognition receptors have been shown to be present on surfaces of invertebrate hemocytes: receptors which bind directly to foreign cells without mediation of serum factors, and receptors which bind only to particles coated with opsonizing molecules (Renwrantz 1990). Acting in vitro in hemocytes from susceptible snails plasmatic factors will favour the parasite destruction by citotoxicity (Bayne et al. 1980). Fryer and Bayne (1989) detected the agglutinin presence only in the resistant snail's plasma. Zelck and Becker (1992) observed that the pre-incubation of target cells in homologous plasma of resistant snails results in an increase of phagocytic activity hemocytes, even in the plasma absence during the standard analysis. Based on these results we believe that existence of a transferable factor in the serum of *B. glabrata* resistant snails, which specifically activates the hemocytes occurs in *B. tenagophila* as well. It makes them able to encapsulate and to destroy the parasite.

ACKNOWLEDGEMENTS

To Dr J Russell Stothard and Dr Fiona Allan from the Natural History Museum, London for their improvement of the English version of the manuscript, and also to Dr Rene D Martins for the statistical analysis support.

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