Biomphalaria tenagophila/Schistosoma mansoni Interaction: Premises for a New Approach to Biological Control of Schistosomiasis

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Biomphalaria tenagophila *is very important for schistosomiasis transmission in Brazil. However its mechanisms of interaction with* Schistosoma mansoni *are still scantly studied. Since this snail displays strains highly susceptible or completely resistant to the parasite infection, the knowledge of that would be a useful tool to understand the mechanism of snail resistance. Particularly, the Taim strain consistently shows absolute resistance against the trematode, and this resistance is a dominant character.*

A multidisciplinary research group was created aiming at studying B. tenagophila/S. mansoni *interaction. The possibility for applying the knowledge acquired to obtain a biological model for the control of* S. mansoni *transmission in endemic areas is discussed.*

Key words: *Biomphalaria tenagophila/Schistosoma mansoni* interaction - innate defense - schistosomiasis mansoni - biological control - mechanism - dominant resistance heritage

The interaction of *Schistosoma mansoni* and the intermediate host has been the object of several studies, most of them using the *S. mansoni/Biomphalaria glabrata* model. These studies show that the innate defense system is basically composed of phagocitary cells named hemocytes and their soluble products. Parasite recognition and hemocyte activation are mainly mediated by lectins (Bayne 1983, 1990, Bayne et al. 1984, Zelck & Becker 1990, Richards & Renwrantz 1991, Johnston & Yoshino 1996). Besides lectins, the hemocytes in *B. glabrata* also produce some proteins, which are similar to mammal cytokines, such as TNF-α (Boyer 1994), that is depleted in *S. mansoni* infections, and IL-1 "like", that was also found in *B. glabrata* and is associated to the activation and cellular proliferation (Raftos et al. 1992), as well as to the increase of phagocitary activity of hemocytes (Burk & Watkins 1991, Beck et al. 1993) and to the production of super-oxydes (Granath et al. 1994). *B. glabrata* snails pertaining to the most resistant strains to *S. mansoni* present, before and after exposition to *S. mansoni*, higher levels of IL-1"like" in hemolymph in relation to other strains, which are susceptible to the parasite. These results show that the intern defense system in *Biomphalaria* is more complex than it was supposed. It is also noted that *B. glabrata* species was the target of observations in these studies. However, there are no *B. glabrata* strain completely resistant to *S. mansoni* infection, after laboratory testing. The snail strain isolated from Dique do Tororó, Bahia, which was reported as resistant to *S. mansoni*, posteriorly proved to be susceptible to this parasite, when juvenile specimens were used (Richard 1977).

B. tenagophila is the second more important species related to the transmission of schistosomiasis in Brazil, and on the contrary of *B. glabrata*, there are strains that are highly susceptible to *S. mansoni* infection or, conversely, very resistant to the parasite (Paraense & Corrêa 1978). *B. tenagophila* Taim (coming from the Ecological Station at Taim, state of Rio Grande do Sul, Brazil), studied by our research group, proved to be sistematically resistant to *S. mansoni*. This strain has been challenged with different strains of *S. mansoni*, and with variable burdens of miracidia, showing always absolute resistance to infection. Several studies in which the Taim strain was challenged to infection were published or submitted for publication by the authors, as well as unpublished data of laboratory challenges with miracidia (more than 100 attempts to achieve infection, throughout more than two decades) have showed, sistematically, the resistance of this strain to *S. mansoni* (Santos et al. 1979, Bezerra et al. 1999, 2003, Martins-Souza et al. 2003, Rosa et al. 2004b). These studies have been conducted mainly in the laboratory of the Schistosomiasis Research Group, Institute of Biological Sciences, Federal University of Minas Gerais and in the laboratories of the Research Center René Rachou-Fiocruz, Brazil. Histological examinations of this strain carried out by Dr Zilton A Andrade show that para-

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site death occurs rapidly (within a 24-hour-period). Besides, the results obtained by other researchers (Barbosa 2001) demonstrated that transplantation of the hematopoietic organ from Taim strain to another susceptible snail strain (Cabo Frio) showed as a result an absolute resistance to the parasite in the receptors, that presented hemocytes with the typical molecular marker of the donor, i.e., of *B. tenagophila* Taim. Moreover, the transference of the hemolymph soluble fraction (without hemocytes) from Taim lineage to other susceptible strains also resulted in significant protection against *S. mansoni* infection (Pereira et al., manusc. in preparation). These results indicate that the resistance to *S. mansoni* observed in the Taim lineage is not merely the result of physiological incompatibility between the parasite and its host, but is due to components of the innate defense system of the snail. Studies aiming at breaking the resistance of this lineage to the parasite show that suppression of this resistance is very difficult to obtain. In this way, the use of silica inoculation in *B. tenagophila* reduced the number of phagocitary hemocytes and increased Cabo Frio susceptibility to *S. mansoni* infection, but it was not sufficient to promote infection in Taim lineage (Martins-Souza et al. 2003). In the same manner, even very high doses of gamma radiation (10 Krad) were not able to promote neither *S. mansoni* infection nor inhibition of the phagocytic capacity of the hemocytes from *B. tenagophila* Taim (Bezerra et al. 2003).

These findings show that the association of *B. tenagophila* and *S. mansoni* is very interesting and peculiar, different from *B. glabrata/S. mansoni* interaction, which does not show absolute resistance to *S. mansoni*, even in artificially selected lineages in laboratory. This model, besides offering better conditions for the study of the parasite/host interactions, as far as susceptibility and resistance are concerned, also allows the inference of a possible applicability of these findings in a model of biological control. Thus, the mass introduction of the Taim lineage in areas where the transmission of schistosomiasis is only due to *B. tenagophila* could be successful in the transmission control of this endemia. Recent results obtained by our research group corroborate this possibility. The cross-breeding between *B. tenagophila* of Taim lineage (resistant to *S. mansoni* infection) and the albino strain isolated from Joinville, state of Santa Catarina, Brazil (highly susceptible) resulted in an F1 generation resistant to the parasite. Out of 220 F1 snails, that were challenged in two separated experiments, only one presented low cercarial production showing self-cure posteriorly. The cross-breeding F1 x F1 resulted in an F2 generation with infection rates, in general terms (considering mortality), according to the classic Mendelian genetics. On the other hand, F2 individuals, that were infected, produced a cercarial burden 1/5 lower, when compared with their parental susceptible Joinville lineage (Rosa 2002, Rosa et al. 2004a). These findings allow some speculation about the possible existence of a more important dominant gene and secondary one(s) related to *S. mansoni* resistance.

We were able to identify a molecular marker of the Taim lineage, represented by a 350 base pair band of the ribosomal RNA internal transcribed spacer region (ITS), which is peculiar to the Taim lineage, since it does not appear in other different geographic strain of this species, that were studied in our laboratory. This band also shows a dominance character, according to Mendelian segregation. This marker, which does not present any relationship with the resistance aspect of the parasite, will be an excellent tool to observe the insertion of the genetic heritage from Taim lineage into the local population after intervention(s) dealing with introduction of the resistant strain in endemic regions (Rosa et al. 2004b).

Recently, a multidisciplinary research group constituted of experienced professionals, working with different disciplines, such as Parasitology, Epidemiology, Immunology, Pathology, Histology, Genetics, Systematics, and Molecular Biology, was created aiming at studying more thoroughly the mechanisms connected with *S. mansoni/B. tenagophila* interactions resulting from cross-breeding with susceptible strains. The biological control method, using introduction of the Taim lineage in areas where *B. tenagophila* is responsible for the transmission of the disease, will be carrtied out. An experimental field test using the Taim strain will be conducted in areas where the transmission is due to *B. tenagophila*, such as Bananal and Caraguatatuba (state of São Paulo). An accord between Sucen (Superintendência de Controle de Endemias do Estado de São Paulo) and CPqRR/Fiocruz (Centro de Pesquisas René Rachou/Fiocruz) has been prepared and the working up of this project is at the final stage. On the other hand, the authors of the present study reckon upon the financial support of a Pronex project (Fapemig/CNPq) already approved, dealing with basic studies on *S. mansoni/B. tenagophila* interaction, which will provide the necessary support for field studies.

The biological control model proposed is based on the premise that the mass introduction of Taim lineage, after applying molluscicide, would obligate the remaining local population to cross-breed with the introduced Taim lineage. Paraense (1955) demonstrated, in a classical paper, that snails of *Biomphalaria* genus, which are hermaphrodite, only perform self-fecundation when they are isolated. In this way, the snails always prefer cross-breeding to self-fecundation. Thus, the F1 population would have the desired dominant character of resistance to the parasite, inherited from Taim snails, and the adaptability to the local ecosystem inherited from snails of the area under intervention. As far as the environmental impact of the introduction is concerned, it must be emphasized that the same species of that one already existing in the area would be introduced. The aquatic environment, in endemic areas, has been rather modified by human activities, such as pollution due to domestic drain, changes in the aquatic course and vegetation, etc. Basically, our proposal in dealing with the mentioned model would be to accomplish the transference of the dominant gene(s) related to resistance from the Taim lineage to the snails of the endemic area. The presence of *S. mansoni* infected snails causes a devastating effect, producing high rates of mortality and resulting in the interruption of egg-laying, so the potentially susceptible snail could suffer a negative selection by parasitism pressure. The future event would be the presence of resistant descendants to

the parasite dominating the ecosystem.

The biological control model differs completely from the other ones, which have been used in schistosomiasis mansoni, since they were based in the introduction of other species, which would act as competitor or predator species.

As a secondary alternative, and certainly more ambitious but feasible, considering the human resources potentiality of the research group, would be the identification and cloning of the gene(s) linked to resistance to the trematode. After that, our goal would be the generation of transgenic *B. glabrata* endowed with the resistance gene to *S. mansoni*. Studies aiming at the transgenic technique for *B. glabrata* have already been carried out with classic insertions, e.g., luciferase, in order to make this technique available for the moment of the possible isolation and cloning of the gene(s). Finally, considering that the transgenic *B. glabrata* descendants would keep the genetic dominant character of resistance in future generations appearing in the habitat, and also considering all the reasoning elaborated for the *B. tenagophila* Taim model, we could obtain a valuable tool for an efficient biological control related to the most important transmitter of schistosomiasis mansoni in South America and in Caribbean states.

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