Rev. Inst. Med. trop. São Paulo
36 (3): 193-198, maio-junho, 1994

EVALUATION OF THE RESISTANCE TO *SCHISTOSOMA MANSONI* INFECTION IN *NECTOMYS SQUAMIPES* (RODENTIA: CRICETIDAE), A NATURAL HOST OF INFECTION IN BRAZIL

Arnaldo MALDONADO Jr. (1), José Roberto MACHADO E SILVA (2), Rosângela RODRIGUES E SILVA (1), Henrique Leonel LENZI (3) & Luis REY (1)

SUMMARY

The development of resistance in three stages throughout an active infection (pre-ovular, acute and initial chronic stages) was studied, comparing the total number of adult worms recovered from the reinfected group and the control groups. It was shown that *Nectomys squamipes* was unable to develop resistance in the tested conditions and, on the other hand, reinfection in the pre-ovular period of the parasite led the rodent to present the phenomenonacilitation, with reduction of natural resistance and an increase in the parasite load. These results suggest the existence of other forms of immunity diverse from the concomitant immunity in the host-parasite relationship, according to the employed model.

KEYWORDS: *Nectomys squamipes*; Reinfection; Immunity; Wild rodent; Experimental schistosomiasis; *Schistosoma mansoni*.

INTRODUCTION

The semi-aquatic rodent *Nectomys squamipes* has been found infected by *Schistosoma mansoni* in several states of Brazil [2, 3, 13, 15, 16]. Its effective ability to keep the parasitic cycle in the absence of human infection has been studied [1, 5]. On laboratory-infected or non-infected animals captured in nature, it was shown that these rodents develop a percentually low parasite load (10%) when infected with 500 *S. mansoni* cercariae, whichever the parasite strain or the route of infection [11, 16]. This finding applies also to laboratory born animals [18]. Probably these rodents can tolerate schistosome infection and throughout the course of infection, may partially eliminate its parasite load [16, 17].

The concepts of immunity to *S. mansoni* have recently been discussed, being controversial in relation to the mouse model [12, 20] and the mechanisms involved. HAGAN & WILKINS [8] (1993) point out the great variation in host-parasite relationship among different experimental hosts and indicate the need for investigating other forms of immunity in schistosomiasis, since the available information does not allow analogies with the situation in man.

Based on the fact that rodents of this species participate in the natural cycle of *S. mansoni* and have semi-aquatic habits, they are daily exposed to the sources of

(1) Department of Medicine IOC/FIOCRUZ - Oswaldo Cruz Institute - Oswaldo Cruz Foundation. Av. Brasil 4365 Manguinhos. CEP 21045-900 Rio de Janeiro, RJ, Brasil.
(2) Department of Pathology IOC/FIOCRUZ Oswaldo Cruz Institute - Oswaldo Cruz Foundation.
(3) Department of Pathology and Services - States University of Rio de Janeiro - UERJ, Av. 28 de Setembro 87 fundos - Vila Isabel. CEP 20551-031 Rio de Janeiro, RJ, Brasil.
Address for correspondence: Dr Luis Rey, Departamento de Medicina Tropical - IOC/FIOCRUZ, Av. Brasil, 4365 - Manguinhos C.P. 926, 21045-900 Rio de Janeiro/RJ - Brasil

infection. The present paper intends to study the regulatory mechanisms of hiperinfection, according to the concept of concomitant immunity.

MATERIALS AND METHODS

Animals used: Sixty specimens of *Nectomys squamipes* (2n = 56 cromossomes), of both sexes, heterogenetic, aged two to three months and weighing 200 - 250 grams, obtained from the development of a colony originated from wild rodents captured in the District of Sumidouro, state of Rio de Janeiro.

Experimental Design: The animals were randomly divided in three groups of 18 animals, with three subgroups of 6 animals each. Groups I, II and III were reinfected in different periods from the first infection, respectively in the pre-ovular stage (third week), acute (seventh week) and initial chronic stage (fifteenth week), with the same number of cercariae used in the first infection. The animals were killed on the ninth week after reinfection, counting up to 12, 16 and 24 weeks from the first infection. Three subgroups acted as controls of the first infection and sacrificed within 12, 16 and 24 weeks of parasitism. The control animals of the reinfection were examined always nine weeks after infection. Other 6 animals (group IV) were also used as non-infected controls for comparison of the organs weight.

Experimental Infection: The animals were infected and/or reinfected with 500 cercariae, by the transcutaneal route, with a *Schistosoma mansoni* strain isolated from *Biomphalaria glabrata* naturally parasitized and collected in 1985 in the District of Ressaca (Belo Horizonte). The cycle is being maintained in the laboratory through passages in successive *B. grablata* and albino mice (*Mus musculus*). For that, the animals were anesthetized by intraperitoneal route with sodium pentobarbitate at the dose of 25 mg/kg weight and laid in dorsal decubit with the back paws immersed in a solution with 250 cercariae for each paw, for a thirty minutes period. To avoid differences that could be originated from different batches of cercariae, the infection were done according to the following schedule: The same cercariae were used for the primary infection of the respectively control and infected groups. Otherwise, the control animals of the reinfection and the challenged of each group received the same batch of cercariae according to the time of infection.

Analysis of Data: The number of adult worms recovered served as an evaluation criterion for measuring the degree of resistance developed upon reinfection and for this purpose the rodents were submitted, during autopsy, to portal-hepatic perfusion by the SMITHERS & TERRY [19] (1965) technique, followed by collection of the worms from mesenteric veins with a pointed tool. The weight of liver, spleen and intestine from all animals was compared to the average weight of the same organs from non-infected control animals.

In order to verify the degree of significance in the experiments, the Mann-Whitney test was employed to compare the number of worms and weight of the organs to the level of $p<0.05$ and the Chi-square test for counting the number of worms, considering $p<0.01$ as a significant difference.

TABLE 1

Recovery of adult *S. mansoni* worms in *N. squamipes* infected only once or reinfected in different periods.

Subgroups	Autopsiated after (weeks)	Number of cercariae used 1st (weeks)	3rd (weeks)	7th (weeks)	15th (weeks)	worms Total	recovery %
Infection controls	12	500	-	-	-	72	14.6
	16	500	-	-	-	54	10.6
	24	500	-	-	-	57	12.1
Reinfected	12	500	500	-	-	243	24.3
	16	500	-	500	-	94	9.4
	24	500	-	-	500	121	12.1
Reinfection controls	9	-	500	-	-	78	15.6
	9	-	-	500	-	21	4.2
	9	-	-	-	500	64	12.8

RESULTS

In all control groups of the first infection, there was a decrease in parasite load through time without significant differences in the counts (table 1). The average number of adult worms recovered from controls of the first infection was 58 worms, which means a recovery of 11.5% from the initial inoculum. On the other hand, the average recovery of adult worms in the reinfection controls was 54 worms, with a percentage of 10.8%, being similar to the rates obtained in first-infection controls. However, considering the average number of worms from all groups which received both infections, 152 worms were recovered, corresponding to 15.2% of infecting larvae.

Analysing data within each group, one can verify that when reinfections were carried out shortly after the first infection, i. e., on the third (group I) and seventh weeks (group II), 62% and 25% of the parasites were recovered, respectively, beyond the sum of the controls (Figure 1). However, when reinfection was performed on the fifteenth week (group III) the recovery of adult worms was proportional to the sum of recoveries obtained from the control groups. When the number of adult worms was compared within each group, it was possible to observe a statistically significant difference only on the group reinfected on the third week (group I), in relation to the controls ($p<0.05$). When the parasite load was estimated by Chi-square test using the sum of the means of each group, a significant difference was also seen only in the same group ($p<0.01$).

The relation between male and female worms in the groups which received only one infection (controls) presents an average of 1.0 : 1.0. As for the reinfected groups, the relation was 0.9 : 1.0; 0.9 : 1.0 and 1.0: 1.0 for groups I, II and III, respectively.

The increase on the liver weight, in all experimental groups, when compared with the negative control group (group IV), was not statistically significant. The weight of the intestine increased significantly on all studied groups ($p<0.05$), except on reinfected control group III. Since there has been an increase during the course of infection, the difference in intestinal weight between reinfected animals and those infected only once, was always small (table 2). All the infected animals presented an increase in spleen size, which was considerably higher after reinfection ($p<0.05$).

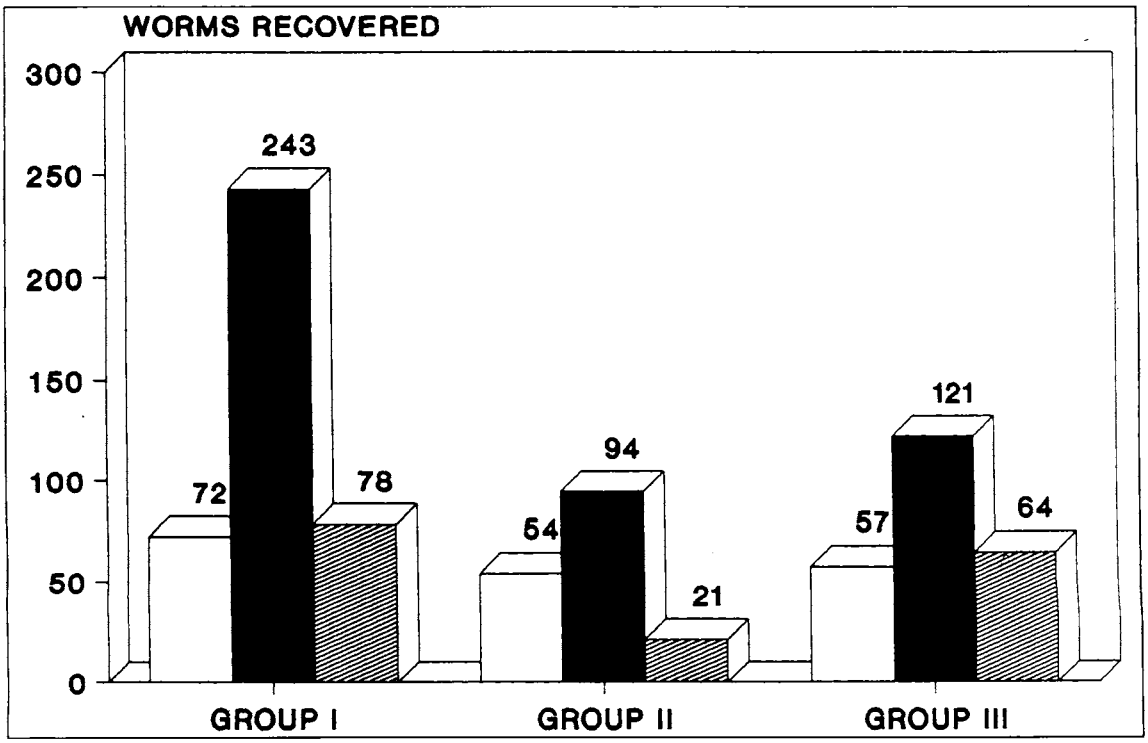

Figure 1 - Means of the worms recovered after portal and picking off from the mesenteric veins of the *N. squamipes* (□ infection controls, ■ reinfected and ▩ reinfection controls).

TABLE 2

Weight of spleen, liver and intestines of the *N. squamipes* at the end of experiments.

Subgroups	Spleen		Liver		Intestine		weeks after infection
	g	% (*)	g	%	g	%	
Infection controls	0.70	700	7.7	5	18.6	72	12
	0.98	980	7.7	5	17.0	57	16
	1.16	1160	9.8	34	22.8	111	24
Reinfection	3.70	3700	11.4	56	32.6	201	12
	1.87	1870	9.1	24	24.8	129	16
	2.47	2470	10.7	46	24.3	122	24
Reinfection control	0.81	810	8.3	13	18.1	67	9
	0.82	820	8.0	9	17.0	57	9
	0.51	510	9.3	27	19.0	75	9
Not infected	0.10	-	7.3	-	10.8	-	24

(*) Percentual increase related to the negative control

DISCUSSION

Our results showed that there was no reduction in the number of adult worms recovered after challenge inoculum in the three different times of infection tested. On the contrary, the rodents challenged on a pre-ovular period of infection presented a significant reduction in the resistance, increasing the number of adult worms recovered, 62% above the sum of the control groups.

Comparing the time of the secondary infections with the very well known life cycle of the *S. mansoni* in the mouse model, which is also probably reproduced in the *N. squamipes* model, it is possible to perceive different situation between the group I versus groups II and III. The skin schistosomula of the challenge infection in the group I were coincident with the beginning of the migration to portal veins by the young adult worms of the primary infection. Therefore, at the same time when schistosomula of the reinfection would be going through the lungs, worms from the first infection would be starting the migration to the mesenteric veins and production of eggs. Otherwise, the skin and lung schistosomules of the secondary infection in the groups II and III were concomitant with a very well defined acute and chronic pos-postural phases respectively.

LENZI [9] (1991) showed that the presence of antibodies against cercariae and eggs (soluble eggs antigen) in the mouse model appeared around 30 and 45 days of infection, respectively. Considering the emergence of antibodies in *N. squamipes* on the same period as for the mouse, it may be concluded that these antibodies, instead of provoking resistance, would be blocking defense mechanisms. Although, there are no sequential studies on the production of antibodies by *N. squamipes* during schistosomal infection. MALDONADO JR. & DE SIMONE (1991) have detected the presence of antibodies against adult worms on the 26th week of infection.

During the course of infection in *N. squamipes*, the phenomenon of facilitation was decreasing (reinfection on the seventh week presented only 25% facilitation rate) until its absence when reinfection was performed on the fifteenth week. It is possible that blocking antibodies might be present and may be replaced by other classes of antibodies during the infection, as described by BUTTERWORTH et al. [4] (1988a) for human infection.

The absence of the resistance phenomenon was also demonstrated by DETTMAN & OPTIZ [7] (1989) on the rodent *Mastomys coucha*. Although not discussed by the authors, it is possible to verify, according to the data shown, the occurence of the facilitation phenomenon when animals were challenged on the fourth week of infection, with an increase of 11% in relation to the sum of its controls. However, on the ninth week of the infection this phenomenon was not observed. The authors blame the functional inability of macrophage cells for this absence.

Resistance related to anatomic changes induced by egg-laying in the hepatic tissue, as described on the mouse [20], could not be seen on this rodent, probably due to the absence of portal hypertension development in *Nectomys* [16].

The differences on the percentage of the adult worms recovery was partially influenced by the distinct batches of cercariae. In the groups (I and II), when the facilitation phenomenon was detected, there was a more significant increase in the weight of the spleen and intestine when compared with the control groups.

Comparing our results with those obtained in other *S. mansoni* hosts where the same matter was studied, we conclude that they differ from those obtained in the mouse [6] and the hamster [14] but are similar to those occurring in *M. coucha* [7].

RESUMO

Avaliação da resistência na infecção por *Schistosoma mansoni* em *Nectomys squamipes* (Rodentia: Cricetidae) um hospedeiro natural da infecção no Brasil.

O desenvolvimento de resistência em três fases do transcurso de uma infecção ativa (fase pré-ovular, aguda e crônica inicial) foi estudada comparando-se o número total de vermes adultos recuperados no grupo reinfectado e o somatório dos grupos controles. Demonstrou-se que o *N. squamipes* não foi capaz de desenvolver resistência nas condições testadas e que ao contrário, a reinfecção no período pré-ovular do parasito levou o roedor a apresentar o fenômeno de facilitação, com redução da resistência natural e aumento da carga parasitária. Esses resultados sugerem a existência de outras formas de imunidade, distintas da imunidade concomitante, segundo o modelo empregado.

ACKNOWLEDGEMENTS

To Dra. Ligia Correa from the Department of Malacology, Oswaldo Cruz Institute/ FIOCRUZ who gave us the *S. mansoni* strain.

To Paulo Sérgio D'Andrea for keeping the *N. squamipes* colony in the laboratory.

To Mariza Soares from the Department of Biology/ FIOCRUZ and to Luiz Otávio of the FIOCRUZ Center for Health Information for guidance in statistical work.

REFERENCES

1. AMORIM, J. P. - Roedores selvagens como disseminadores de ovos de *Schistosoma mansoni*. **Rev. Inst. Med. trop. S. Paulo, 4**: 397-402, 1962.

2. AMORIM, J. P.; ROSA, D. & LUCENA, D. T. - Ratos silvestres reservatórios do *Schistosoma mansoni* no nordeste do Brasil. **Rev. bras. Malar., 6**: 13-33, 1954.

3. BORDA, C. E. - *Infecção natural e experimental de roedores por Schistosoma mansoni*. Belo Horizonte, 1972. (Dissertação de Mestrado da Universidade Federal de Minas Gerais).

4. BUTTERWORTH, A. E.; DUNNE, D. W.; FULFORD, A. et al. - Immunity in human schistosomiasis mansoni: crossreactive IgM and IgG2 anti-carbohydrate antibodies block the expression of immunity. **Biochimie, 70**: 1053-1063, 1988.

5. CARVALHO, D. M. - *Sobre a importância do Nectomys squamipes na epidemiologia da esquistossomose mansônica no Município de Sumidouro, RJ*. Rio de Janeiro, 1982. (Dissertação de mestrado - Escola Nacional de Saúde Pública).

6. DEAN, D. A.; BUKOWSKI, M. A. & CHEEVER, A. W. - Relationship between acquired resistance, portal hypertension and lung granulomas in the strains of mice infected with *Schistosoma mansoni*. **Amer. J. trop. Med. Hyg., 30**: 808-814, 1981.

7. DETTMAN, C. D. & HIGGINS-OPTIZ, S. B. - *Mastomys [Praomys] coucha* as a host for experimental schistosomiasis. 2. Failure to develop concomitant immunity against either homologous or heterologous challenge infection. **S. Afr. J. Sci., 85**: 732-736, 1989.

8. HAGAN, P. & WILKINS, H. A. - Concomitant immunity in schistosomiasis. **Parasit. today, 9**: 3-6, 1993.

9. LENZI, H. L. - *A dinâmica da resposta hematológica e celular na esquistossomose mansônica murina, com ênfase nas séries eosinofílica e mastocitária*. Belo Horizonte, 1991. (Tese de Doutorado - Universidade Federal de Minas Gerais).

10. MALDONADO Jr, A. & DE SIMONE, S. G. - Identification and characterization of sex-linked proteins of *Schistosoma mansoni*. **Mem. Inst. Oswaldo Cruz, 86**: 31-36, 1991.

11. MALDONADO Jr., A.; SILVA, J. R. M.; SILVA, R. R. & REY, L. - Infecção experimental prolongada de *Nectomys squamipes* por *Schistosoma mansoni*. In: CONGRESSO BRASILEIRO DE PARASITOLOGIA, 4, Rio de Janeiro, 1989. **Anais**. p. 84.

12. MEDEIROS, M. V. M. J. & ANDRADE, Z. A. - The importance of collateral portal veins for resistance to schistosomiasis in mice. **Braz. J. med. biol. Res., 19**: 85-88, 1986.

13. MELLO, M. D. - Parasitic diseases in Brazil and the role of wild mammals: an analysis based on leishmaniasis, Chagas'disease and schistosomiasis mansoni. **Ciênc. e Cult., 43**: 274-277, 1991.

14. MONGE, E. O.; COELHO, P. M. Z. & TAVARES, C. A. P. - Estudios inmunologicos en hamsters (*Cricetus auratus*) infectados con *Schistosoma mansoni*. **Rev. Inst. Med. trop. S. Paulo, 28**: 239-245, 1986.

15. PICOT, H. - *Holochilus brasiliensis* and *Nectomys squamipes* (Rodentia: Cricetidae) natural hosts of *Schistosoma mansoni*. **Mem. Inst. Oswaldo Cruz, 87** (suppl. 4): 255-260, 1992.

16. RODRIGUES E SILVA, R.; MACHADO-SILVA, J. R.; FAERSTEIN, N. F.; LENZI, H. L. & REY, L. - Natural infections of wild rodents by *Schistosoma mansoni*. Parasitological aspects. **Mem. Inst. Oswaldo Cruz, 87** (suppl. 1): 271-276, 1992.

17. SILVA, T. M. C. & ANDRADE, Z. A. - Infecção natural de roedores silvestres pelo *Schistosoma mansoni*. **Mem Inst. Oswaldo Cruz, 84**: 227-235, 1989.

18. SOUZA, V. A. M.; RODRIGUES E SILVA, R.; MALDONADO Jr., A.; MACHADO E SILVA, J. R. & REY, L. - *Nectomys squamipes* (Rodentia: Cricetidae) as an experimental model for schistosomiasis mansoni. **Mem. Inst. Oswaldo Cruz, 87** (suppl. 1): 277-280, 1992.

19. SMITHERS, S. R. & TERRY, R. J. - The infection of laboratory host with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. **Parasitology, 55**: 695-700, 1965.

20. WILSON, R. A. - Leaky liver portal shunting and immunity to schistosomes. **Parasit. today, 6**: 354-358, 1990.

Recebido para publicação em 25/10/1993.
Aceito para publicação em 08/02/1994.