

***Schistosoma mansoni*: Reinfections and Concomitant Immunity in Mice. Importance of Perfusion Time after Challenge Infection for Evaluation of Immunoprotection**

Paulo Marcos Z Coelho/⁺, Rômulo T Mello*, Teresinha EV Pollom

Departamento de Parasitologia, Instituto de Ciências Biológicas *Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Caixa Postal 486, 30161-970 Belo Horizonte, MG, Brasil

The concomitant immunity in the presence of repeated infections (with 15 cercariae) was studied in mice sacrificed on the 20th day after each infection. The comparison of the averages of immature worms, recovered from mice submitted to reinfection, with those of their respective controls (previously uninfected) showed a significantly lower worm recovery rate in the animals with previous infections (concomitant immunity). However, statistically significant differences could not be detected among the various groups of animals, when the mice that accumulated worms in this mature stage were perfused.

The theoretical projection based on the accumulation of young worms which developed to adult ones indicates a lower recovery rate of adult worms in the animals with concomitant immunity, but this projection was not corroborated by the experimental data. The visceral hemodynamic alterations that occurred in reinfections due to the pathogeny, favouring recirculation of the recent arriving worms to the portal system, could explain the lower recovery rate of immature worms, which could remain in other organs on the occasion of perfusion of the portal system.

These results suggest that special care should be taken when one wants to investigate concomitant immunity in mice based on the distinction of the immature worms from challenge infection and the mature ones from primary infection.

Key words: *Schistosoma mansoni* - concomitant immunity - perfusion time - immunoprotection

The concept of concomitant immunity in schistosomiasis *mansoni* was first described by Smithers and Terry (1967) establishing that the presence of adult worms induces a partial immunoprotective response against the juvenile forms of *Schistosoma mansoni*. The comprehensive review by Hogan and Wilkins (1993), dealing with this subject, shows an intense research activity on the same matter, the publication by Smithers and Terry (1967) being their starting point.

Assessment of concomitant immunity in experimental schistosomiasis *mansoni* is commonly achieved by means of a sole cercarial challenge in previously infected animals (single infection), worm recovery being performed on approximately day 20 post-challenge. At 20 days after reinfection, it is possible to distinguish the primary infection population, composed of fully developed worms, from the reinfection population, comprised of smaller and immature worms easily distinguishable from the older ones. The degree of protection

may be evaluated by comparison between the worm recovery rate of 20-day-old worms (challenge), in the animals with mature infection, and the worm recovery rate of immature worms, in the controls (uninfected animals). On the other hand, very few researchers who carried out work to investigate concomitant immunity in mice used repeated infections. It is important to point out that the present approach simulates what could occur with patients living in endemic areas, who might undergo several reinfections. On the other hand, hemodynamic alterations due to the disease could elicit recirculation of the immature worms. Thus, in the present study, a theoretical projection of the cumulative number of immature worms on mature ones was compared with the data experimentally obtained related to mature worms.

MATERIALS AND METHODS

Albino Swiss mice (outbred females) were infected with the LE strain (Belo Horizonte, MG, Brazil) of *S. mansoni* isolated from a patient and kept at the Schistosomiasis Research Unit - Prof. José Pellegrino Laboratory (Federal University of Minas Gerais) by passage in laboratory reared *Biomphalaria glabrata* and hamster, for more than 30 years. The animals were transcutaneously infected with ± 20 cercariae through the abdominal

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⁺Corresponding author

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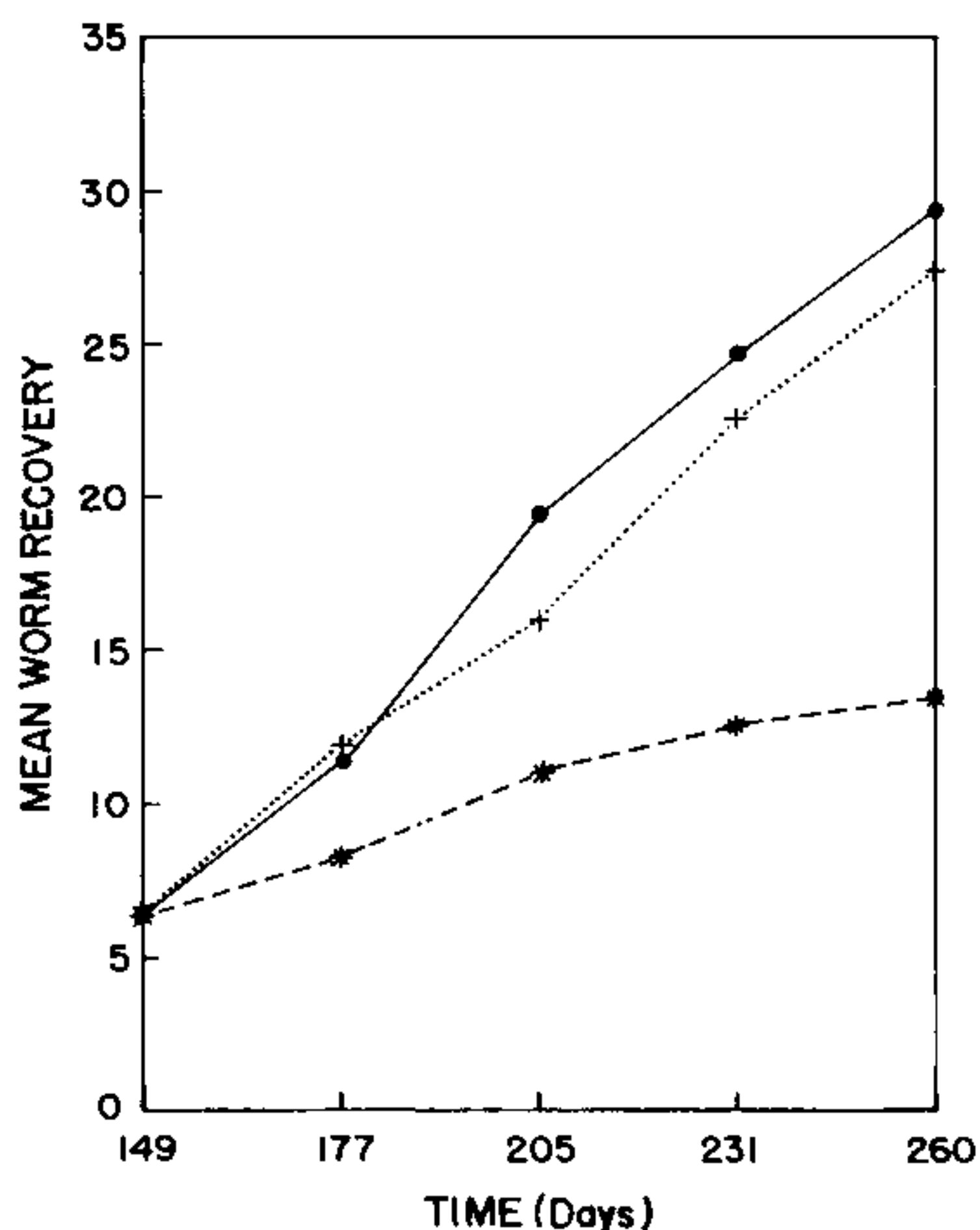
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skin, according to the technique described by Barbosa et al. (1978). Serial infections (with 15 cercariae) were carried out every 30 days, from day 120 after primary infection onwards. The following schedule was adopted: (a) group with the primary infection and 1 reinfection; (b) group with the primary infection and 2 reinfections; (c) group with the primary infection and 3 reinfections; (d) group with the primary infection and 4 reinfections; (e) group with the primary infection and 5 reinfections.

Dating from the first day of each reinfection, one control group (previously uninfected) was infected. Every 20 days after reinfections, the animals from the control and previously infected groups were sacrificed and perfused for worm recovery from the portal system, according in general terms with the technique described by Pellegrino and Siqueira (1956). Worm counts were done so as to separate the immature worms inherent to reinfection from the mature ones from prior infections.

RESULTS

As it can be seen in Table, a statistically significant lower worm recovery rate was detected in the animals with primary infection(s) in relation to the one observed in the controls (a),(c),(d) and (e), except group (b), ($p < 0.12$). Analysis was performed by means of the Student's *t* test. The theoretical projection connected with the cumulative



Theoretical and experimental graphic projections of worm recovery after reinfections. •Experimental data: adult worms recovered. +Theoretical projection: primary infection (adults) plus immature worms of reinfections in normal mice controls. *Theoretical projection: primary infection (adults) plus immature worms of reinfection in mice with concomitant immunity.

TABLE

Schistosoma mansoni: recovery of 20-day-old (immature) worms from the portal system of mice, after repeated reinfections with 15 cercariae, by transcutaneous route (and their respective controls)

	M±SD	No.	P<
Group (a) Primary infection plus 1 reinfection	1.3±1.3	12	0.001
Control g (a)	6.4±1.8	13	
Group (b) Primary infection plus 2 reinfections	2.8±1.5	12	0.12 (n/s)
Control g (b)	4.1±2.3	11	
Group (c) Primary infection plus 3 reinfections	1.5±1.2	12	0.001
Control g (c)	5.2±2.0	12	
Group (d) Primary infection plus 4 reinfections	1.0±1.2	12	0.02
Control g (d)	4.8±2.5	12	
Group (e) Primary infection plus 5 reinfections	2.3±1.9	12	0.001
Control g (e)	10.1±3.5	12	

effect of immature worms on the mature ones, in the previously infected animals, indicates that a marked lower adult worm burden should be detected (-*- Fig.), but this has not occurred as shown by the experimental data (-•-, Fig.). The theoretical projection dealing with the accumulation of immature worms on mature ones obtained from the uninfected control animals and recovered as adult worms (-+- Fig.), absolutely coincided with the experimental data recorded for the reinfected group (-•-, Fig.).

DISCUSSION

Concomitant immunity in experimental schistosomiasis is usually studied by means of an approach that utilizes perfusion for worm recovery carried out on approximately day 20 post-challenge, in order to ascertain protection. In fact, this methodology allows the distinction between 20-day-old worms (immature, small-sized) and those inherent to the initial population, which is composed of mature and larger size worms. In this approach, a control group of normal mice undergoes infection on the same date of challenge, perfusion being carried out on day 20 post-infection. The protection degree is evaluated by comparison of the averages of immature worms from the group bearing concomitant immunity and those of the control group.

In our experiment, using the above mentioned approach, statistical comparison of the averages of immature worm recovery between the groups with concomitant immunity and their respective controls always showed a decrease in those averages, that is, in the averages of recovery from immature worms coming from the challenge infection, in the animals previously showing mature worms (concomitant immunity) (Table). Nevertheless, as can be seen in Fig., there was accumulation of mature worms, in such a manner that everything occurred as if, in fact, concomitant immunity did not occur. Thus, the theoretical projection derived from the sum of the averages of adult worms (from the group with concomitant immunity) with the immature ones (from their respective controls) as a result shows a curve that practically superposes itself on the average of adult worms from the subsequent stage, which were experimentally obtained. On the other hand, still in connexion with that theoretical exercise of graphic projection, when the averages of adult worms from the group with concomitant immunity are added to the immature worms recovered from the same groups, it can be seen that the curve obtained shows a markedly lower worm recovery (Fig.).

The possible reasons that might explain this apparent paradox could base their fundamentals mainly on the research works by Harrison et al. (1982), Wilson et al. (1983), McHugh et al. (1987),

and Wilson (1990), who demonstrated that some hemodynamic alterations occur in the portal system in experimental schistosomiasis, thus allowing recirculation of the worms newly migrated from the lungs to the liver, due to shunts and anastomoses (mainly porto-cava). So, at the moment of perfusion (on day 20 post-infection) several immature worms would remain in other organs, recovery of these worms being not possible by means of portal system perfusion.

The results of the present study lead to the inference that, when studying concomitant immunity, the approach taken as a model by several investigators, i.e., perfusion of the challenged animals and their respective controls on dates that allow the separation of the former infection from the challenge one (based on the development of worms) should be analyzed with extreme caution, due to recirculation of immature worms in the animals with hemodynamic alterations caused by the pathogeny of schistosomiasis.

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REFERENCES

- Barbosa MA, Pellegrino J, Coelho PMZ, Sampaio IBM 1978. Quantitative aspects of the migration and evolutive asynchronism of *Schistosoma mansoni* in mice. *Rev Inst Med Trop S Paulo* 20: 121-132.
- Dean DA 1983. A review of *Schistosoma* and related genera: acquired resistance in mice. *Exp Parasitol* 55: 1-104.
- Hagan P, Wilkins HA 1993. Concomitant immunity in schistosomiasis. *Parasitol Today*, vol. 9, nº 1.
- Harrison RA, Bickle QD, Doenhoff MG 1982. Factors affecting the acquisition of resistance against *Schistosoma mansoni* in the mouse. IX - Evidence that the mechanism which mediate resistance during early patent infections may lack immunological specificity. *Parasitol* 84: 3-110.
- McHugh SM, Coulson PS, Wilson RA 1987. Pathologically induced alterations in the dimension of the hepatic portal vasculature of mice infected with *Schistosoma mansoni*. *Parasitol* 94: 69-80.
- Smithers SR, Terry RJ 1967. Host antigens in schistosomiasis. *Proc R Soc* 171: 483-494.
- Pellegrino J, Siqueira AF 1956. Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infectadas. *Rev Bras Malariol* 8: 589-597.
- Wilson RA 1990. Leaky livers, portal shunting and immunity to schistosomes. *Parasitol Today* 6: 354-358.
- Wilson RA, Coulson PS, McHugh SM 1983. A significant part of concomitant immunity of mice to *Schistosoma mansoni* is the consequence of a leaky hepatic portal system, not immune killing. *Parasite Immunol* 5: 595-601.