

SENSIBILITY OF DIFFERENT LARVAL STAGES OF SCHISTOSOMA MANSONI TO THE LARVAE DISAPPEARING REACTION (LDR) IN MURINE SCHISTOSOMIASIS (1)

Alan Lane de MELO (2), Leógenes Horácio PEREIRA (2) & Munir CHAMONE (3)

S U M M A R Y

The delay produced by drug, in the process of cercaria-schistosomulum transformation, was used to verify the sensibility of different larval stages to the host cell immune responses, *in vivo*. The peritoneal cavity of mice, a model used for *in vivo* observations, was choiced for the experiments. As well characterized schistosomules, cercariae and larvae in the process of transformation were coated and arrested by host cells, and could not be recovered by simple saline washings. After 10^{-2} M EDTA saline washings, they were released alive, with good vitality and movements. Thus, different kind of larvae in the process of adaptation of the cercaria to the host are strongly coated by immune cells, but these fail to kill the invading organisms, at least during a few hours after invasion.

KEY WORDS: Murine schistosomiasis — *S. mansoni*; Larval stages
— Larvae disappearing reaction.

I N T R O D U C T I O N

In nature, animals chronically infected with any of a diverse group of pathogens can develop resistance to reinfection. The schistosome, because of its sophisticated defense against the host immune system, has proven to be a worthy adversary to immunologists. Since significant advances have been made towards understanding and enhancing acquired resistance, several mechanisms that participate in immunity to reinfection have been suggested (SMITHERS & TERRY¹³).

The cercariae of schistosomes during and just after their penetration into the skin of the vertebrate undergo structural and physiological changes to be adapted from water free-living larvae to the tissues and later to the blood system of the host (STIREWALT¹⁵). This period is critical not only regarding to the changes

themselves as well as facing the host defenses. The skin, as a natural barrier against the parasite, was stressed by GORDON & GRIFFITHS³ and CLEGG & SMITHERS¹. This period is even more critical when cercariae penetrate hosts with a previous *S. mansoni* infection (SMITHERS & TERRY¹²).

When different *in vitro* systems are used, several immune mechanisms have been demonstrated to act against the newly transformed schistosomulum, but the *in vivo* studies of immune mechanisms and correlation of these experiments with the protection, largely remain to be shown. Observations of the dynamics of the process were carried out mostly *in vitro* owing to difficulties for direct observations *in vivo*. Fast recovery of schistosomula from the peritoneal cavity was reported by EVE-

(1) This study was supported by CNPq-Brazil, and by the World Health Organization — Switzerland

(2) Grupo Interdepartamental de Estudos sobre Esquistossomose e Departamento de Parasitologia, ICB/UFMG

(3) Departamento de Bioquímica-Imunologia, ICB/UFMG

Address for reprints: Prof. Alan Lane de Melo — GIDE — ICB/UFMG — Caixa Postal 2486, 30000 Belo Horizonte, MG, Brasil

LAND² and PEREIRA et al.¹⁰. This procedure was also used by MELO et al.^{5,7}; MELO & PEREIRA⁶ for *in vivo* quantification of several phenomena in the cercaria-schistosomulum transformation, including some answers of the host immune system against the early developing forms of the parasite. In fact, in mice with previous infection, larvae were arrested by immune cells and were not recovered by washings of the peritoneal cavity with saline, the finding being reported as a larvae disappearing reaction (LDR) by MELO et al.⁷ This LDR was not related to the possible death of the organisms in the observation period, since the use of a chelator (EDTA) released the living larvae. These could not be recovered following washings of the peritoneal cavity with saline without EDTA because, due to the immune response to a previous infection, they were retained or adhered to the visceral peritoneum. Since the removal of the larvae from mice was carried out 3 hours after inoculation (MELO et al.^{5,7}), and by that time all organisms were recovered as newly transformed schistosomula, it was not possible to say LDR involved, besides schistosomula, other larval stages found in the process of cercaria-schistosomulum transformation.

In the present report, the delay of the steps involved in the process of transformation, following the use of high doses of oxamniquine, was used to clarify this point.

MATERIALS AND METHODS

Infection of animals

Albino mice (males, weighing about 20 g) were inoculated subcutaneously or percutaneously with 30 to 40 *Schistosoma mansoni* cercariae (LE strain) shed by laboratory-reared and infected *Biomphalaria glabrata* (Belo Horizonte strain). The infection was carried out 88 to 92 days before intraperitoneal inoculation with a suspension of about 500 cercariae in 0.5 ml of spring water.

Drug administration

Appropriate doses of oxamniquine were mixed with polyethyleneglycol in a mortar and injected (0.1 ml) intramuscularly in the hind leg. The drug was administered one hour be-

fore intraperitoneal inoculation of cercariae. Doses of 500, 1,000, 2,000 and 4,000 mg/kg were given to four groups of five animals each. One untreated group served as control.

The Larvae Disappearing Reaction

Three hours after inoculation mice were killed by cervical fracture and the larvae, recovered from the peritoneal cavity with saline, were concentrated by centrifugation and counted under a dissecting microscope. A second washing of the peritoneal cavity of mice with 10⁻²M EDTA in isotonic saline solution was performed according to MELO et al.⁷. LDR controls were carried out with animals without a first infection.

Worm collections

Recovery of adult schistosomes from the first infection was performed by perfusing the portal system of mice (PELLEGRINO & SIQUEIRA⁸).

The experiments were performed four times.

RESULTS

All mice receiving a first infection showed male and female worms, with full oviposition.

LDR data are summarized in Fig. 1. As can be seen, LDR was found positive in all experiments with mice harbouring a mature schistosomal infection.

LDR controls (mice without a previous *S. mansoni* infection):

LDR controls carried out in mice without a previous *S. mansoni* infection, showed a good recovery of larvae (about 40%) from intraperitoneally injected cercariae in the same way.

The delay produced by oxamniquine in the transformation of cercariae into schistosomules was according to previous report by MELO et al.⁵. 500 mg/kg body weight of oxamniquine did not change the pattern of larvae recovery. Higher doses (1,000, 2,000 and 4,000 mg/kg) of the same compound produced a characteristic delay in the process, and living cercariae, dead

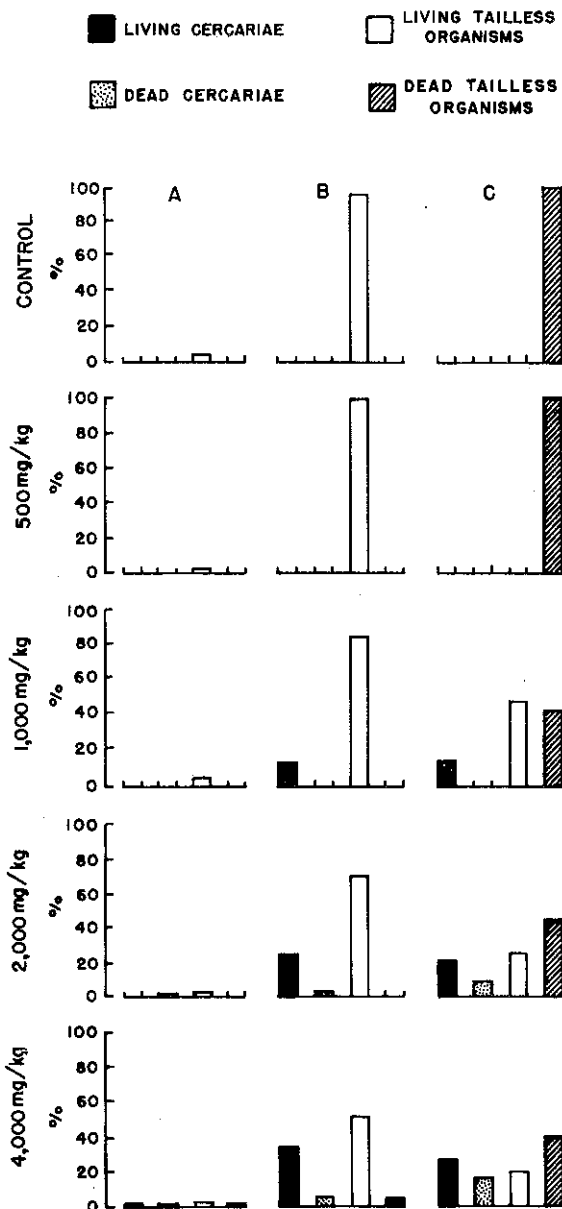


Fig. 1 — Recovery of *Schistosoma mansoni* larvae from the peritoneal cavity of mice, following intrabdominal injection of cercariae into animals harbouring a previous schistosomiasis infection. The animals were treated intramuscularly with different doses of oxamniquine to produce a delay in the cercaria-schistosomulum transformation. A second washing using saline-EDTA was used to recover the retained larvae (B). Recovered larvae were submitted to hipotonic solutions to separate tailless cercariae from true schistosomules (C).

Larvae found in positive LDR (mice with mature *S. mansoni* infections):

In the absence of the drug, after a first saline washing, very few larvae were recovered from the peritoneal cavity. The number of larvae recovered increased after EDTA administration. Tailless and worm-like moving organisms died, showing intolerance to hipotonicity after water addition to the Petri dishes. These larvae were regarded as true schistosomules. The same picture was found using the compound dosed at 500 mg/kg. From 1,000 to 4,000 mg the compound, as expected (MELO et al.⁵), produced a delay in the process of transformation, and besides schistosomules, living cercariae and cercarial bodies (without tail) were recovered. The higher doses (2,000 and 4,000 mg/kg) produced some dead cercariae. Some tailless larvae did not show the typical movements of schistosomules and were not killed by hipotonicity. They were regarded as cercarial bodies (larvae in the process of transformation).

DISCUSSION

In vivo studies of immune response have not yet clearly demonstrated effector mechanisms of protection in intact hosts. The development of techniques enabling the recovery of schistosomes from different sites of the host, at various times after infection, should help to resolve *Schistosoma mansoni* infections in laboratory hosts (SMITHERS & GAMMAGE¹⁴). Although the peritoneal cavity of mice is not a natural site of infection it has been used for studies of evolution of *S. mansoni* larvae in normal mice (PEREIRA et al.²; EVELAND²), effects of drugs (PEREIRA et al.^{10,11}; MELO et al.⁵; MELO & PEREIRA⁶) and immunological phenomena (KASSIS et al.⁴; MELO et al.⁷).

In a previous work we verified a marked reduction or absence of recovery of *S. mansoni* larvae injected into the peritoneal cavity of mice, harbouring a first mature infection with the same parasite. That was described as a Larvae Disappearing Reaction (LDR) and reported by MELO et al.⁷. The reaction itself involved:

cercariae, cercarial bodies and true schistosomules were found after saline washings.

a) injecting cercariae of *S. mansoni* (about 500 larvae) into the peritoneal cavity of

mice harbouring a previous *S. mansoni* infection;

- b) washing the peritoneal cavity with 0.85% saline with 10^{-2} M EDTA three hours later;
- c) centrifugation and counting of larvae under microscope.

The retaining of alive larvae as reported was suggested by the authors as an useful model to study immune response to early invading forms of *S. mansoni* in the vertebrate host, *in vivo*. When LDR occurs, larvae are presumably arrested by immune cells and then the captive larvae seem to be attached to the peritoneum. Although suspected, the attachment of larvae to the peritoneum (and not their death or migration through adjacent tissues) was only clear when EDTA saline was used for a second washing of the peritoneal cavity (MELO et al. 7). Since all the recovered larvae (3 hours after infection) have been recovered as schistosomules, no information of participation of other kind of larvae was given. Since the transformation process is fast (after 1 hour), an artificial delay by drug administration was used in this paper.

The main conclusion is other larvae from cercariae-schistosomules transformation are also arrested by the immune defenses (the cercaria itself and the tailless cercarial body). These larvae were recovered in very low numbers after saline washings (A), but released by the use of EDTA (B), many still presenting tolerance to hypotonicity (C). It is worth to mention, as well as the true schistosomules, these intermediate larvae were released from cell attachment by EDTA, without any cell coating them, and showing in general a good vitality and characteristic movements.

In mice harbouring a previous infection, immune responses affect several kind of larvae as showed in this paper, in the peritoneal cavity. Such responses are large enough to arrest living larvae and presumably act as a "glue" between them and the visceral peritoneum, but they fail to kill the larvae, at least during the periods observed.

RESUMO

Sensibilidade de diferentes estádios larvares de *Schistosoma mansoni* à Reação de Desapareci-

mento de Larvas (LDR) na esquistossomose murina.

O retardo, induzido pela oxamniquine, no processo de transformação da cercária-esquistossômulo, foi utilizado para verificar a sensibilidade de diferentes estádios larvares à resposta celular do hospedeiro, *in vivo*. A cavidade peritoneal do camundongo, um modelo utilizado para observações *in vivo*, foi escolhida para estes experimentos. Esquistossômulos, cercárias e larvas no processo de transformação foram sequestrados pelas células do hospedeiro, não sendo recuperados através de perfusão da cavidade peritoneal com salina. As larvas somente foram liberadas vivas e com movimentos após o uso de 10^{-2} M de EDTA em salina. Os diferentes estádios larvares, no processo de adaptação ao hospedeiro, são intensamente recobertos por células que, entretanto, não conseguem matar os organismos invasores, pelo menos nas primeiras horas após a invasão.

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Recebido para publicação em 2/12/1985.