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Schistosoma mansoni: HOST CELL ADHESION TO THE DIFFERENT STAGES OF THE PARASITE, IN VIVO[1]

Alan L. MELO (2), Leógenes H. PEREIRA (2) & Conceição R. S. MACHADO (3)

SUMMARY

The peritoneal cavity of laboratory mice was used to study the phenomenon of host cell adhesion to different evolutive stages of the **Schistosoma mansoni** (cercaria, adult worm, developing and mature eggs, miracidium, young and mature daughter sporocysts). Material recovered from the peritoneal cavity 30 and 180 min after the inoculation of each evolutive form was examined with the help of a stereomicroscope. The free swimming larvae (cercaria and miracidium), and the evolutive forms producing such larvae (mature egg and mature daughter sporocyst) elicited the host cell adhesion phenomenon. In all forms but cercariae the adherent cells remained as so till 180 minutes after inoculation.

KEY WORDS: Schistosoma mansoni; Cell adhesion; Peritoneal cavity.

INTRODUCTION

The peritoneal cavity of mice has been proved to provide a useful, rapid, simple and reproducible system for **in vivo** studies of phenomena affecting the infective **Schistosoma mansoni** larvae. These studies include the transformation of cercaria into schistosomule[12, 20, 22, 24] and a rapid screening of chemoprophylactic compounds[27]. This procedure was also used to investigate the effect of an antischistosomal compound on the process of cercaria-schistosomulum transformation[19, 21, 23].

When cercariae are inoculated into the peritoneal cavity of naive mice they induce host cell adhesion to their surface. Host cell adhesion is strongly directed against the cercarial, decreasing as the transformation process progresses, and being practically absent against well characterized schistosomules[19, 24].

The purpose of this paper is to verify whether other **S. mansoni** evolutive stages elicit the phenomenon of cell adhesion, when inoculated into the mouse peritoneal cavity.

MATERIALS AND METHODS

Outbred albino mice were used for intraperitoneal inoculations with the evolutive forms of **Schistosoma mansoni** (LE strain).

Adult worms were collected after saline perfusion of the portal system of infected mice[26] and concentrated to about 200 worms per ml. **S. man-**

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(2) Grupo Interdepartamental de estudos Sobre Esquistossomose/Departamento de Parasitologia and 3 — Departamento de Morfologia — Instituto de Ciências Biológicas da UFMG — Caixa Postal 2486 CEP 30161 Belo Horizonte, Brazil.

soni eggs obtained from the livers of infected hamsters[30], with suppression of detergent (to prevent the foam), miracidia (taken from **S. mansoni** eggs and concentrated following CHAIA's procedure[5]) and cercariae, were concentrated to about 1,000 organisms per ml[25]. Mature daughter sporocysts were obtained after dissection of infected **Biomphalaria glabrata** and the young daughter sporocysts were recovered as described elsewhere[28] and concentrated to about 200 organisms per ml. Carefull washings of all evolutive forms were performed to avoid host contaminants and debris as much as possible.

Different groups of mice received intraperitoneally, by a Cornwall syringe, supplied with a 30 x 12 gauge needle, 0.5 ml of each of the evolutive stage suspension, and sacrificed 30 and 180 min later, by cervical dislocation. Organisms were recovered by gentle washing of the peritoneal cavity with isotonic saline, and then concentrated by centrifugation at 1,000 rpm during 2 minutes[20].

Fresh resuspended material was examined under the stereomicroscope for counting the organisms with and without adhered cells and for establishing cell adhesion indices. According to the number of host cells attached to the parasite stages, the following indices were used: 1 (no host cell), 2 (1 to 10 cells), 3 (11 to 50 cells), 4 (over 50 cells, but with visualization of the organism), 5 (clusters of host cells surrounding the parasite in so large numbers the organism was eclipsed).

RESULTS

The data on host cell adhesion to different stages of the parasite is summarized in Table 1. Cell adhesion was rarely observed when immature eggs, young daughter sporocysts, and adult worms were used in the experiment. In contrast, high percentages of the recovered mature eggs, miracidia, mature daughter sporocysts and cercariae showed host cell adhesion.

As can be seen in Table 2, all evolutive stages but cercariae, are able to elicit cell adhesion and presented large number of adherent cells (indices 4 and 5) till 180 minutes after inoculation. Regarding the cercariae, the tails remained surrounded by host cells till 180 minutes but the larval bodies were practically free of host cells by this time.

Contrasting with the cercarial inocula, sporocysts and miracidia were usually dead when recovered 180 minutes after the inoculation.

TABLE 1
Host cell adhesion to different evolutive forms of **Schistosoma mansoni**, recovered from the peritoneal cavity of naive mice 30 and 180 minutes after the inoculation.

Evolutive forms	% recovered with cell adherence*	
	30 min.	180 min.
Immature eggs	0.2 ± 0.44	0
Mature eggs	99.6 ± 0.56	98.4 ± 2.07
Miracidia	99.0 ± 1.76	100
Young sporocysts	0	0
Mature sporocysts	98.8 ± 1.30	99.2 ± 0.41
Cercariae	75.7 ± 13.60	3.6 ± 2.92**
Adult worms	0	0

* mean and SD from 2 experiments with 5 mice/each evolutive form.
** for cercarial bodies, since host cells remain attached to the tails.

TABLE 2
Percentages of the evolutive forms of **Schistosoma mansoni**, recovered from the peritoneal cavity of naive mice 30 and 180 minutes after the inoculation, exhibiting the different indices of host cell adhesion.

Time and evolutive forms	Cell adhesion indices				
	1	2	3	4	5
30 min					
Mature eggs	0.8	5.3	17.2	37.9	38.8
Miracidia	0.4	8.9	21.7	42.3	26.7
Mature sporocysts	3.0	7.5	15.7	34.2	39.6
Cercariae	32.4	6.2	11.6	35.8	15.6
180 min					
Mature eggs	1.4	5.6	15.1	44.1	33.8
Miracidia	0.0	4.1	19.4	59.9	16.6
Mature sporocysts	0.0	8.7	18.3	26.1	46.9
Cercariae*	98.2	0	1.8	0	0

* for cercarial bodies, since host cells remain attached to the tails.

DISCUSSION

Our results demonstrate that some larval stages of **S. mansoni**, when inoculated into the peritoneal cavity of naive mice, elicit a host response resulting in cell adhesion to parasite (cercaria, mature egg, miracidium, mature daughter sporocyst). In sharp contrast, no remarkable cell adhesion was observed to occur with adult worm, immature egg and young daughter sporocyst. Then, differences in surface components could be suspected to occur between these two stage groups.

Using different techniques, several workers showed that some **S. mansoni** antigens were shared among the evolutive stages, particularly between cercariae and adult worms[2, 3, 16, 32, 33, 36] or between larva and egg[10]. On the other hand, stage specific antigens are also found in cercariae, adult worms, and eggs[11, 17, 38, 39].

Recently, HARN and co-workers[13] showed that anti-egg monoclonal antibody E1, can recognize a membrane epitope of cercariae, newly in vitro-transformed schistosomula and ciliary plates of miracidia. However, this antibody fail to bind to the lung recovered schistosomulum or adult worm. It has been also demonstrated that miracidium possess a glycocalyx similar to that of the cercaria, both in its structure and antigenicity[7]. The data presented here are in close agreement with these recent findings. In fact, cercariae, miracidia, mature sporocysts (containing cercariae) and mature eggs (containing miracidia), are all organisms in which the glycocalyx is found.

Cercaria and miracidium are both free-living and water-adapted larvae, that must cash out their envelopes (glycocalyx and the ciliated integument respectively), was well as the content of their penetration glands, in order to adapt themselves to their hosts. It is well known the cercarial glycocalyx is highly antigenic[14, 15, 37], and presents immunogenic glycoproteins[8]. Besides, the cercarial glycocalyx is able to activate complement by the alternative pathway[4, 6, 18, 34, 37]. Although this activation is reported to be provoked by the **in vitro** schistosomulum[9], it does not occur with **in vivo**-obtained schistosomulum. Interestingly, differences between **in vivo** and **in vitro**-produced schistosomules have been also reported regarding the induction of cell adhesion[24]. Only **in vitro**-obtained schistosomules are able to induce host cell adhesion probably because they still present some of the cercarial surface molecules.

The host cell adhesion during the crucial cercaria-schistosomulum transformation into the peritoneal cavity, may be critical for the parasite survival, since this cell adhesion do not kill the larvae. Ultrastructural studies (MELO et al, submitted) showed that the adherent cells are mainly neutrophils that may contribute for the rapid degradation of cercarial surface components as they presented signs of marked phagocytosis and intracellular digestion of the cercarial glycocalyx.

The exact physiological mechanism of the host cell adhesion to the larval stages of the parasite remain to be fully elucidated. Considering the neutrophils can express C3 receptors on their surface as well as Fc receptors[31], the binding of these cells to the parasite could be achieved by complement in the presence or absence of antibodies[1, 29, 35]. Since we used naive mice, antibodies against the parasite can be practically excluded. Besides we have reasons to disregard the participation of CR3 and CR4. These receptors by requiring the divalent cations calcium and magnesium can be blocked by EDTA[31, 40], which had no effect in the cell adhesion we are now describing (A. L. MELO, unpublished data).

RESUMO

Schistosoma mansoni: **adesão de células do hospedeiro aos diferentes estádios do parasito,** in vivo.

A cavidade peritoneal de camundongos foi utilizada para estudos de adesão celular a diferentes estádios evolutivos do **Schistosoma mansoni** (cercária, verme adulto, ovos imaturos e maduros, miracídio, esporocisto jovem e esporocisto maduro). O material recuperado da cavidade peritoneal 30 e 180 min após o inóculo, foi examinado com auxílio de estereomicroscópio. As formas livres (cercária e miracídio) e as formas evolutivas que produzem tais larvas (ovo maduro e esporocisto maduro) apresentam células do hos-

pedeiro aderidas à superfície. Em todas as formas, exceto cercária, as células permanecem aderidas pelo menos até 180 min após o inóculo.

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