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HOST CELL ADHESION TO Schistosoma mansoni LARVAE IN THE PERITONEAL CAVITY OF NAIVE MICE. HISTOLOGICAL AND SCANNING ELECTRON MICROSCOPIC STUDIES

Alan Lane de MELO (1), Conceição Ribeiro da Silva MACHADO (2) & Leógenes Horácio PEREIRA (1)

SUMMARY

Cercariae of **Schistosoma mansoni** inoculated into the peritoneal cavity of naive mice induced host cell adhesion to their surface, but after 90 minutes the number of adherent cells sharply decreased. The cell detachment is progressive and simultaneous to the cercaria-schistosomule transformation. The histological study showed mainly neutrophils in close contact with the larvae. Mononuclear cells and some eosinophils were occasionally seen surrounding the adherent neutrophils. The scanning electron microscopy showed cells displaying twisted microvilli and several microplicae contacting or spreading over the larval surface, and larvae completely surrounded by clusters of cells.

These results suggest that the neutrophils recognize molecules on the cercarial surface which induce their spreading.

KEY WORDS: Schistosoma mansoni; Cell adhesion; Neutrophils; Cercaria

INTRODUCTION

The cercariae of schistosomes during and just after their penetration into the skin of the vertebrate host undergo structural and physiological changes, originating schistosomules which mature to adult worms adapted to the blood stream. The difficulties in retrieving the schistosomules from the skin led to the development of several techniques for transforming cercariae into schistosomules *in vitro*[22].

The newly transformed schistosomules are susceptible to specific immune attack[3] and, therefore, have been widely used for studies on host-parasite interactions. However, schistosomules can be easily obtained *in vivo* by inoculating cercariae into the peritoneal cavity of mice[7,8,19,24]. We have recently focused on the use of the mouse peritoneal cavity for studying the cercaria-schistosomule transformation[12,14] as well as the effect of drug on such transformation[13,15,16]. During these studies, it was observed under stereomicroscope, that the living recovered larvae exhibited host cells adhered to their surfaces. In this paper, the kinetic of cell adhesion to *Schistosoma mansoni* larvae developing in the peritoneal cavity of naive mice was investigated, and the phenomenon was further studied by light and scanning electron microscopies.

MATERIAL AND METHODS

Albino mice (males, weighing 18-22 g) were inoculated intraperitoneally with **S. mansoni** cercariae (LE strain) shed by laboratory reared and infected *Biomphalaria glabrata* (Belo Horizonte strain). The organisms were concentrated by the method of PELLEGRINO & MACEDO[17], and 0.5 ml of well water containing about 500 cercariae was injected in each mouse with a Cornwall syringe, supplied with a 30 x 10 gauge needle.

The mice were killed by cervical dislocation 30, 60, 90, 120, 150, 180 minutes and 24 hours after cercarial inoculation. The larvae were collected by washing the peritoneal cavity with saline, and were concentrated by centrifugation[14]. The recovered larvae, resuspended in 1 ml of saline, were examined under a dissecting stereomicroscope and were counted as larvae (tailed or tailless), with or without adherent host cells. Isolated tails were also counted. For parasite counting, 120 naive mice inoculated with cercariae were used: four groups

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(1) Departamento de Parasitologia do Instituto de Ciências Biológicas da UFMG.
(2) Departamento de Morfologia do Instituto de Ciências Biológicas da UFMG.
Address for correspondence: Prof. Dr. Alan Lane de Melo - Departamento de Parasitologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Cx. P.2486, CEP 30161-970 Belo Horizonte - MG, Brasil.

with five mice per group at each time after inoculation, ranging from 30 to 180 minutes. Recovered organisms were tested for water intolerance by adding 5 ml of water to the Petri dishes containing the recovered larvae in 1 ml of saline.

The histological and scanning electron-microscopic studies were confined to the larvae recovered 30 minutes after cercarial inoculation.

For the histological studies the larvae were fixed in 10% formalin for 24 hours and processed for embedding in paraffin or glycol methacrylate. Five-µm-thick sections were stained with Wright's solution or by the Dominici's method.

For the scanning electron microscopy the recovered larvae were fixed in ice-cold 3% glutaraldehyde in 0.1M phosphate buffer, pH 7.3, for 60 minutes. After being washed in the buffer they were post-fixed in 1% osmium tetroxide in phosphate buffer for 90 minutes, dehydrated in graded ethanols and dried in a Balzers critical point drier, using liquid CO_2 as transitional fluid. After being mounted on specimen stubs, they were coated with gold by sputtering (Balzers sputter coater), and examined in a Zeiss Novascan 30.

RESULTS

STEREOMICROSCOPY

Our quantitative data on host cell adhesion to **S. mansoni** larvae into the peritoneal cavity of normal mice are summarized in Table 1. Thirty minutes after cercarial inoculation 65-75% of the recovered larvae exhibited cells attached to their surfaces. The number of larvae with adherent cells remained around 50% up to 90 minutes after inoculation and then dropped significantly to values around 5% by 120 minutes of infection. Cell adherence became more scarce at 180 minutes (0-2.4%), and was not observed 24 hours after inoculation. However, 91.5% and 100% of cast tails were covered by host cells 30 and 180 minutes after inoculation, respectively (not shown in the table).

Dead organisms (motionless) were not present till 90 min after inoculation and they were very rare afterwards.

The larvae free from adherent cells were tailed or tailless organisms, and several of them showed intolerance to hypotonic solution, as demonstrated elsewhere[14,15].

Table 1

Percentages of larvae showing cell adhesion after intraperitoneal inoculation of cercariae into naive mice. In parenthesis are the mean number of recovered larvae (with and without cell adhesion) plus standard deviation.

Time after inoculation	Percentages of larvae with cell adhesion			
	EXPERIMENTS			
	1	2	3	4
30min.	66.0 (229.4±16.3)	68.0 (210.4±3.7)	75.7 (209.6±7.1)	65.5 (194.4±18.5)
60min.	58.8 (111.8±7.7)	54.1 (252.4±10.5)	55.6 (162.4±7.0)	53.3 (137.8±15.2)
90min.	56.5 (166.6±8.3)	51.0 (154.4±7.3)	54.8 (130.2±8.0)	49.6 (173.2±13.7)
120min.	3.8 (181.0±4.6)	5.2 (102.4±8.6)	5.7 (175.2±8.2)	6.3 (167.8±9.8)
150min.	4.0 (206.0±18.8)	4.6 (190.8±12.8)	6.1 (198.6±16.5)	3.9 (174.8±5.1)
180min.	1.1 (190.8±15.3)	2.4 (205.6±8.0)	0.8 (221.2±12.9)	0.0 (126.4±33.1)

LIGHT MICROSCOPY

The material, recovered 30 minutes after the inoculation, was rich in peritoneal cavity cells, that is, mononuclear cells and mast cells. However, neutrophils were the most frequent cells in close contact with the *S. mansoni* larvae. Flattering of some of these cells was suggested by the close relationship of their nuclei with the larval surface. Sections of larval bodies and tails completely surrounded by one or several layers of round and flattened neutrophils were frequent (Fig. 1). In these clusters of cells some eosinophils were occasionally observed (not shown in the Fig. 1). Some small mononuclear cells were sometimes seen contacting the larval tegument.

SCANNING ELECTRON MICROSCOPY

Most tailed larvae, isolated bodies and tails recovered 30 minutes after inoculation were partial or totally covered by cells displaying twisted microvilli and several microplicae on their surface (Fig. 2 and 3). Most cells were round (Fig. 2a), but elongate ones (Fig. 3) and cells flattened against the larval surface (Fig. 2b) were seen. A few looked like moving cells because of a large tongue-like pseudopodium. However, several of them appeared as being in a process of spreading over the larval tegument (Fig. 2b and 3). At high magnification, the spread cells exhibited delicate pseudopods adjusted to the larval surface and showed membrane integrity at least on their convex non-adherent region. Frequently, spread cells or elongate ones connected each other by flattened

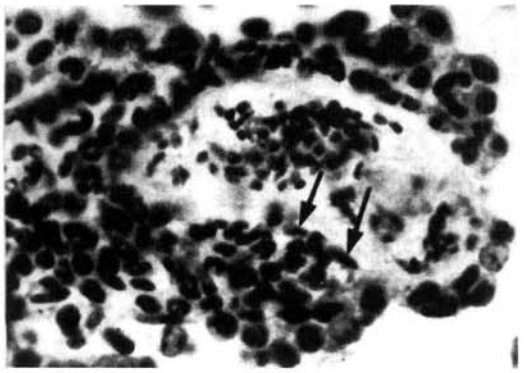

Fig.1 - Photomicrograph of *Schistosoma mansoni* larva recovered from the peritoneal cavity 30 minutes after cercarial inoculation. Neutrophils surround the parasite forming several layers. Most cells seem to be flattened against the larval tegument (arrows) or other neutrophils. Paraffin embedding, Dominici's method, X 480.

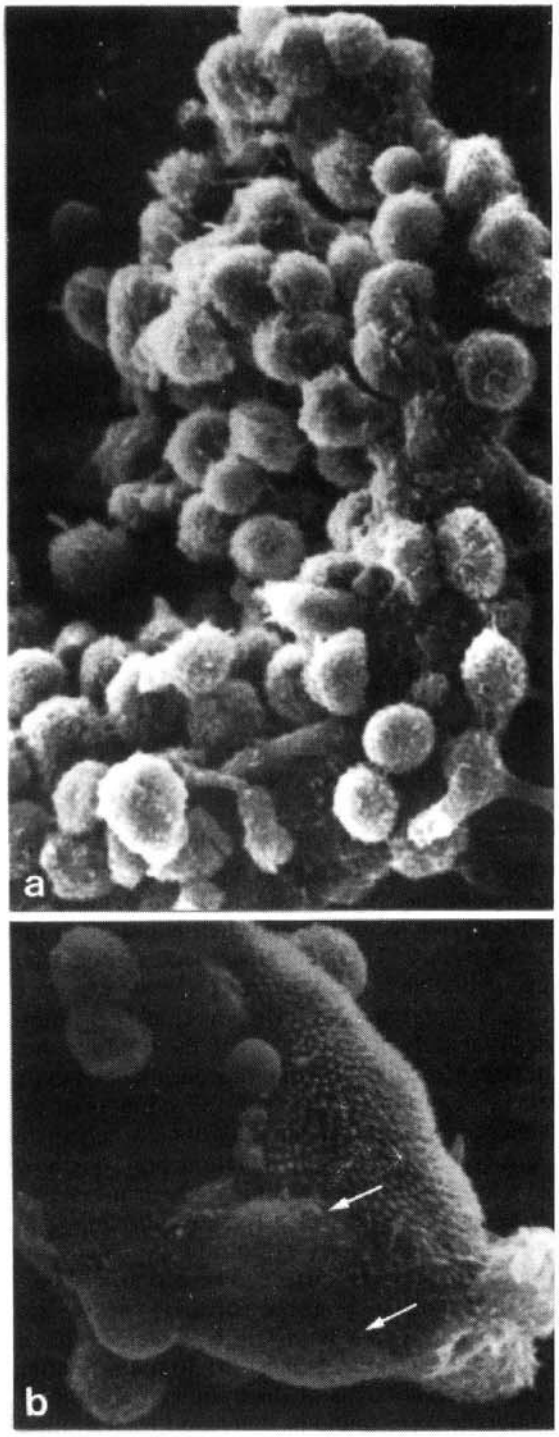

Fig.2 - Scanning electron micrographs *of Schistosoma mansoni* larvae recovered 30 minutes after the intraperitoneal inoculation. a) the parasite is hidden by many host cells (X 2000); b) the adherent cells are round or flattened (arrows) against the larval surface and all of them present the same surface aspect (X 2000).

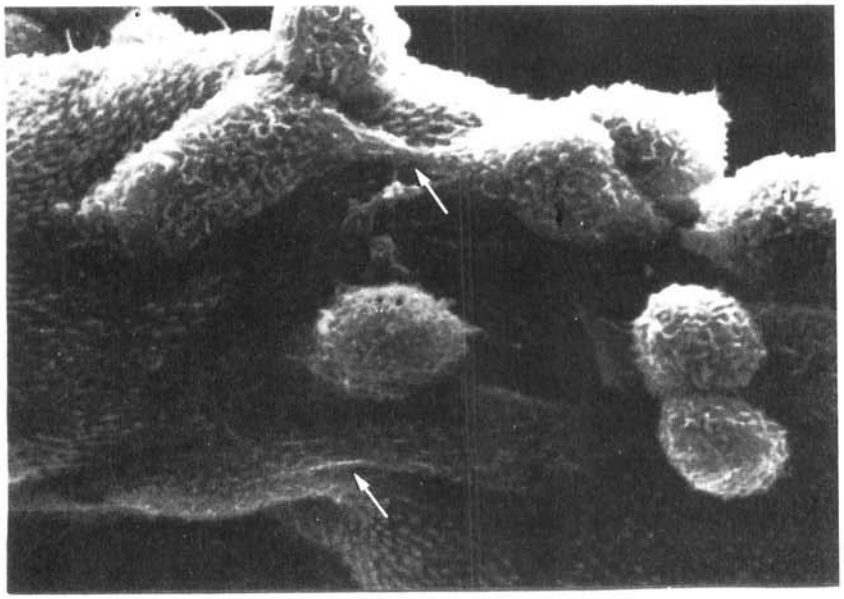

Fig.3 - Scanning electron micrograph *of Schistosoma mansoni* larvae showing details of the adherent cells. Note the integrity the larval and adherent cell surfaces. Elongate adherent cells are touching each other (arrows) and all adherent cells present microplicae and some microvilli on their surfaces. (X 4000)

processes (Fig. 3). Clusters of cells disclosing only a piece of larva were also seen.

DISCUSSION

The present investigation shows that the intraperitoneal inoculation of *S. mansoni* cercariae into naive mice triggers an afflux of neutrophils to the peritoneal cavity and their adhesion to the larval surface. This adherence occurs soon after inoculation and in absence of previous exposure to *S. mansoni* antigens. However, after 90 minutes it sharply decreases. Since the total number of larvae recovered from the peritoneal cavity remains constant at least till 180 minutes of infection, it seems that the cells which adhere to the larvae soon after inoculation are somehow detached later. The scanning electron microscopic-obtained data clearly show the adhesion and spreading of cells exhibiting the usual pattern of granulocyte surface. These findings suggest that molecules on the cercarial surface are recognized by the neutrophils which respond with shape changes. At least till 30 minutes after inoculation, no signs of disruption of neutrophil membrane were found as conveyed by transmission electron-microscopic studies of complement-mediated adhesion of neutrophils to schistosomules *in vitro*[10].

Studies on the kinetics of the cercaria-schistosomule transformation into the peritoneal cavity of mice[14,15] showed that there are only a few schistosomules 30 minutes after inoculation. Their number increases rapidly till 90 minutes, when living cercariae are rare. Thereafter, all recovered organisms are sensitive to water. Our data on water intolerance confirm these previous studies showing that the host cell adhesion to *S. mansoni* larvae are quite parallet to cercaria-schistosomule transformation. According to this assumption, schistosomules obtained *in vivo* fail to trigger cell adhesion[12], and isolated tails recovered 180 minutes after cercarial inoculation remain covered with cells.

Several studies on the cutaneous cellular response to schistosome invasion, have established that neutrophils comprised the major leukocytic component of the dermal infiltrate in naive animals[1,11,18,22,23]. The neutrophil number peak at 18 hours and these are the only cells in close proximity to the invading worms. This proximity is evident by 3 hours although cast cercarial tails are almost invariably surrounded by neutrophils at early times[18].

Host-cell adhesion to *S. mansoni* cercariae in the hypodermic tissue was also observed by using Algire chambers inserted in the dorsal skin of mice and cheek pouches of hamsters[2]. The adherent cells were identified as granulocytes. In these experiments, the host cellular response begins at the end of the first hour in both primary and challenge infection, peaking during the 3rd and 4th hours. The cel-

lular attachment to schistosomules, at the time the cercariae shed their tails, was found to be the same as prior to the shedding. Subsequently, it decreases to be null by the 2nd day. Thus, cell adhesion and cell detachment seem to occur in hypodermic tissue being, however, slower than in peritoneal cavity.

During cercaria-schistosomulum transformation, the cercarial glycocalyx is progressively lost and the trilaminar membrane of the invader changes to an heptalaminar membrane[22]. It seems very likely that the attachment and subsequent detachment of neutrophils to *S. mansoni* larvae *in vivo* are related to surface molecules that are lost during the process of transformation. In contrast to *in vivo*-obtained schistosomules, the 4-hour and 24-hour *in vitro*-obtained organisms were able to induce host cell adhesion when inoculated into the mice peritoneal cavity[12]. This finding reinforces the importance of the glycocalyx in the phenomenom of cell adhesion since comparative studies on the development of shistosomules produced by several techniques showed that the degradation of glycocalyx is made slow in artificially derived organisms, and may not be complete until 24 hours[6].

Since newly transformed schistosomules spontaneously lose surface antigens and C3 receptors during culture[18,20], neutrophils are not probably essential to the developmental changes which occur during cercaria-schistosomulum transformation. *In vivo*, neutrophils could accelerate the process, unless the peritoneal cavity environment by itself could speed the developmental changes. Our scanning electron microscopic studies showing that neutrophil-like cells can move on and spread over the larval surface without damaging the tegument endorse a role for the neutrophil in speeding the surface developmental changes. According to BLOCH[2], cell adherence to *S. mansoni* larvae is essentially a race between the rate at which cells make contact with the parasites and the rate of transformation of the parasite surface membrane. Our data reinforce this assumption, because some water intolerant organisms were found as early as 30 minutes after inoculation.

RESUMO

Adesão celular às larvas de *Schistosoma mansoni* na cavidade peritoneal de camundongos normais. Estudos histológicos e microscopia eletrônica de varredura.

A inoculação de cercárias de *Schistosoma mansoni* na cavidade peritoneal de camundongos normais induz uma aderência de células do hospedeiro a essas larvas. Essa adesão decresce rapidamente quando a larva infectante transforma-se em esquistossômulo. O destacamento das células é progressivo e simultâneo à transformação. Os métodos histológicos e a microscopia eletrônica de varredura mostraram que o neutrófilo é a célula predominante em estreito contacto com a larva. Células mononucleadas e eosinófilos foram observados rodeando o parasito, usualmente sem estar em contacto direto com a larva. Os resultados indicam que neutrófilos podem reconhecer, na superfície larvária, moléculas que induzem sua adesão e espalhamento.

REFERENCES

1. BENTLEY, A.G.; CARLISLE, A.S. & PHILLIPS, S.M. - Ultrastructural analysis of the cellular response to **Schistosoma mansoni**. II - Inflammatory responses in rodent skin. **Amer.J.trop.Med.Hyg.**, 30:815-824, 1981.

2. BLOCH, E.H. - *In vivo* microscopy of schistosomiasis. IV - Dynamics of the host parasite responses to *Schistosoma mansoni* in the hypodermic tissues as observed in transparent chambers in two susceptible hosts during primary and challenge infections. **Amer.J.trop.Med.Hyg.**, 33: 899-910, 1984.

3. BUTTERWORTH, A.E. - Cell mediated damage to helminths. **Advanc. Parasit.**, 23:143-235, 1984.

4. CAULFIELD, J.P.; KORMAN, G.; BUTTERWORTH, A.E.; KOOGAN, M. & DAVID, J.R. - The adherence of human neutrophils and eosinophils to schistosomula: evidence for membrane fusion between cells and parasites. **J.Cell Biol.**, 86: 46-63, 1980.

5. CAULFIELD, J.P.; KORMAN, G. & SAMUELSON, J.C. - Human neutrophils endocytose multivalent ligands from the surface of schistosomula *of Schistosoma mansoni* before membrane fusion. **J.Cell Biol.**, 94: 370-378, 1982.

6. COUSIN, C.C.; STIREWALT, M.A.; DORSEY, C.H. & WATSON, O.L.P.- *Schistosoma mansoni*: comparative development of schistosomules produced by artificial techniques. **J.Parasit.**, 72: 606-609, 1986.

7. CRAM, E.B. & BOZICEVICH, J. - Experimental *Schistosoma mansoni* infection by intraperitoneal injection. **Trop.Med.News**, 1: 16-17, 1944.

8. EVELAND, L.K. - *Schistosoma mansoni:* conversion of cercariae to schistosomula. **Exp. Parasit.**, 32: 261-264, 1972.

9. INCANI, R.N. & McLAREN, D.J. - Neutrophil-mediated cytotoxicity to schistosomula of *Schistosoma mansoni in vitro*: studies on the kinetics of complement and/or antibody-dependent adherence and killing. **Paras. Immunol.**, *3*: 107-126, 1981.

10. INCANI, R.N. & McLAREN, D.J. - Ultrastructural observations on the *in vitro* interactions of rat neutrophils with schistosomula of *Schistosoma mansoni* in the presence of antibody and/or complement. **Parasitology**, *86*: 345-357, 1983.

11. INCANI, R.N. & McLAREN, D.J. - Histopathological and ultrastructural studies of cutaneous reactions elicited in naive and chronically infected mice by invading schistosomules of *Schistosoma mansoni*. **Int. J. Parasit.**, 14: 259-276, 1984.

12. MELO, A.L.; MACHADO, C.R.S. & PEREIRA, L.H. - Differences between *in vitro* and *in vivo* obtained schistosomules. **Rev. Inst. Med. trop. S.Paulo**, 32: 84-85, 1990.

13. MELO, A.L. & PEREIRA, L.H. - Effect of oxamniquine on the detachment of the tail of *Schistosoma mansoni* cercaria. **J. Parasit.**, *66*: 1067-1068, 1980.

14. MELO, A.L. & PEREIRA, L.H. - Kinetics of the cercaria - schistosomulum transformation, *in vivo*. **Rev. Soc. bras. Med. trop.**, 18: 17-21, 1985.

15. MELO, A.L. & PEREIRA, L.H.- Kinetics of the cercaria-schistosomulum transformation, *in vivo*: 2- The effect of oxamniquine. **Rev. Soc. bras. Med. trop.**, *18*: 251-255, 1985.

16. MELO, A.L.; PEREIRA, L.H. & CORREA, M.C.R. - *In vivo* cercaria-schistosomulum transformation: inhibitory effect of oxamniquine. **Trans. roy. Soc. trop. Med. Hyg.**, 72: 158-159, 1978.

17. PELLEGRINO, J. & MACEDO, D.G. - A simplified method for the concentration of cercariae. **J.Parasit.**, *41*: 329-330, 1955.

18. PEARCE, E.J. & McLAREN, D.J. - Schistosoma mansoni: the cutaneous response to cercarial challenge in naive guinea pigs and guinea pigs vaccinated with highly irradiated cercariae. **Int. J. Parasit.**, *16:* 491-510, 1986.

19. PEREIRA, L.H.; PELLEGRINO, J.: VALADARES, T.E.; MELLO, R.T. & COELHO, P.M.Z. - A new approach for screening prophylactic agents in schistosomiasis. **Rev. Inst. Med. trop. S. Paulo**, *16*: 123-126, 1974.

20. SAMUELSON, J.C.; SHER, A. & CAULFIELD, J.P. - Newly transformed schistosomula spontaneously lose surface antigens and C3 acceptor sites during culture. **J. Immunol.**, *124*: 2055-2057, 1980.

21. SAVAGE, A.M. & COLLEY, D.G. - The eosinophil in the inflammatory response to cercarial challenge of sensitized and chronically infected CBA/J mice. **Amer. J. trop. Med. Hyg.**, *29*: 1268-1278, 1980.

22. STIREWALT, M.A. - *Schistosoma mansoni*; cercaria to schistosomule. **Advanc. Parasit.**, *12*: 115-182, 1974.

23. VON LICHTENBERG, F.; SHER, A.; GIBBONS, N. & DOUGHTY, B.L. - Eosinophil enriched inflammatory response to *Schistosoma* in the inflammatory response to schistosomula in the skin of mice immune to *Schistosoma mansoni*. **Amer. J. Path.**, *84*: 479-500, 1976.

24. YOLLES, T.K.; MOORE, D.V. & MELENEY, H.E. - Post cercarial development of *Schistosoma mansoni* in the rabbit and hamster after intraperitoneal and percutaneous infection. **J. Parasit.**, *35*: 276-294, 1949.

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