

## INTERACTION BETWEEN NEUTROPHILS AND *Schistosoma mansoni* LARVAE IN VIVO. A TRANSMISSION ELECTRON-MICROSCOPIC STUDY

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### SUMMARY

*Schistosoma mansoni* cercariae were inoculated into the peritoneal cavity of naive mice and recovered 30 minutes later. Ultrastructural studies showed that neutrophils adhere to the larval surface and participate in the removal of glycocalyx by phagocytosis. This finding suggests that the neutrophils can play a role on the cercaria-schistosomulum transformation process.

**KEY WORDS:** Neutrophils; *Schistosoma mansoni*; Cercaria transformation.

### INTRODUCTION

During the cercaria-schistosomulum transformation process, the larvae of *Schistosoma mansoni* undergo adaptation changes to survive in the vertebrate host<sup>23</sup>. The basic differences between cercaria and Schistosomulum were established by STIREWALT<sup>22</sup>, and subsequently by other investigators<sup>3,4,6,7,8,24,25</sup>. One critical step of the larval transformation is the loss of glycocalyx. This cercarial envelope activates the host complement system, by the alternate pathway<sup>12</sup>, representing an inconvenience when the larva enters the host.

*In vivo* studies of the cercarial transformation into the peritoneal cavity of naive mice, allowed the description of a rapid and transitory host cell adhesion to the larval surface<sup>15,16</sup>. This cell adhesion is parallel to the transformation process<sup>16,18</sup> and could be used to distinguish *in vivo*-obtained schistosomules from the *in vitro*-raised ones<sup>17</sup>. Histological and scanning electron-microscopic studies showed that the host cells able to adhere to the larval surface soon after inoculation are neutrophils. These cells spread on the cercarial surface, probably detaching when the transformation process is over<sup>18</sup>. Transmission electron microscopic studies could be worthy in elucidating the role played by the neutrophil on the cercarial surface. Therefore, the aim of the present report was to describe the ultrastructural aspects of the neutro-

phil adhesion to the *S. mansoni* larvae developing in the peritoneal cavity of naive mice.

### MATERIALS AND METHODS

Twenty outbred albino male mice, weighing about 20 g were inoculated with cercariae of the LE strain of *S. mansoni*, intraperitoneally (about 500 cercariae per animal). Thirty minutes after inoculation, the mice were killed by cervical dislocation, and the larvae were recovered from the peritoneal cavity with isotonic saline, according to PEREIRA et al.<sup>19</sup>. After being carefully selected under a stereomicroscope, the recovered larvae were concentrated by centrifugation at 1,000 RPM and fixed overnight in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3. After buffer washing and post-fixation in 1% buffered osmium tetroxide for 1 hour, they were dehydrated in graded ethanol and embedded in epon-araldite resin. At each step, centrifugation at 3,000 RPM, for 5 minutes, made it easy to handle the material. The ultrathin sections obtained with a diamond knife were routinely stained with uranyl acetate and lead citrate before examination in a Zeiss-EM-10.

### RESULTS AND DISCUSSION

The ultrastructural studies showed neutrophils tightly adhered to the larval tegument (Fig. 1). The

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apposed surface of most larva-adhered neutrophils presented several slender and long cytoplasmic processes in a labyrinth arrangement and the cells were highly vacuolar (Fig. 1 and 2). Material similar to cercarial glycoalyx was frequently seen inside the neutrophils vacuoles (Fig. 2 and 3), and engulfment of this material by neutrophil processes could also be observed (Fig. 3).

It is known that neutrophils represent the predominant cell type in the non-specific inflammatory response to primary infection<sup>26</sup>. Since these cells are very rare into the peritoneal cavity of naive mice, they are thought to be attracted by some chemotactic factors. The alternate pathway of complement activation could well provide such attractants<sup>20</sup>.

The present results suggest that the neutrophils can recognize molecules on the larval surface that

allow their adhesion and degranulation which causes the intense membrane folding and cytoplasmic vacuolation. In fact, neutrophils, under appropriate stimulation, undergo shape alterations involving degranulation that promotes best adhesion to the substrate or to themselves<sup>9</sup>. The neutrophil specific granules are specially important for this surface remodeling as they contribute with adhesion molecules to the cell surface<sup>21</sup>.

Neutrophils can also adhere to schistosomula of *S. mansoni in vitro* in the presence of specific antibody, complement, or both<sup>1,2,10,11,13</sup>, but killing was observed only under conditions of complement activation<sup>10</sup>. The phenomenon of neutrophil adhesion that we described occurs in absence of specific antibodies and so may involve different kinds of larval surface molecules. This adhesion may contribute to modify the larval surface by removing some molecules. In fact, the present results

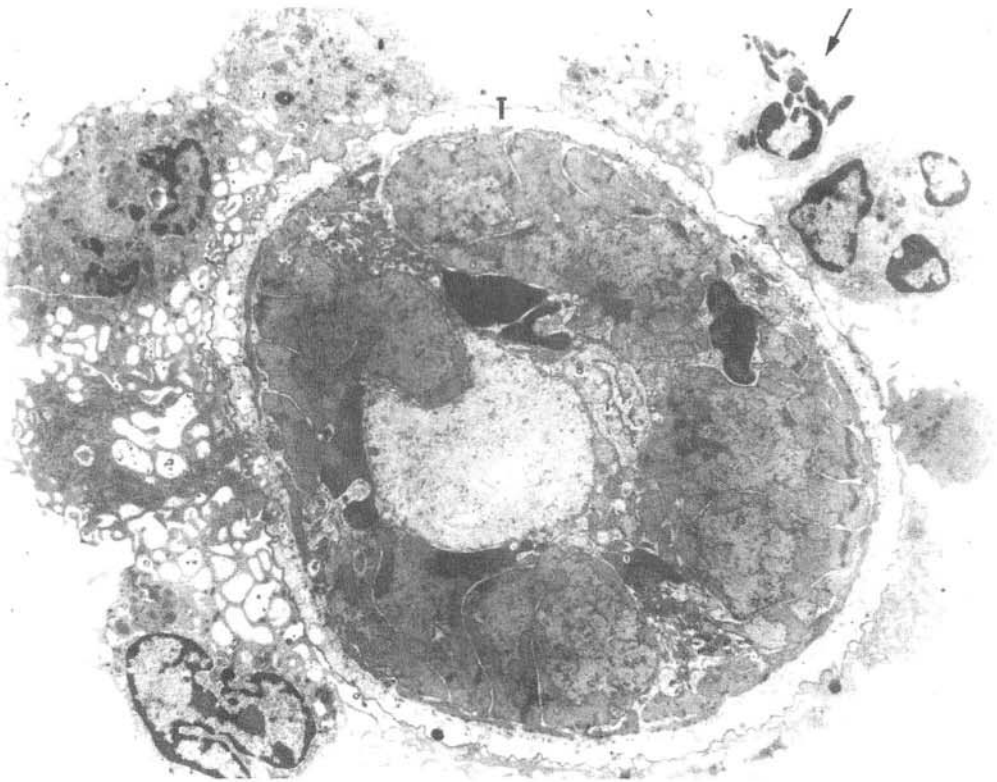


Fig. 1 - Electron micrograph showing a *Schistosoma mansoni* larva recovered from the peritoneal cavity of naive mice 30 minutes after cercarial inoculation. Neutrophils tightly adhered to the larval tegument (T) are highly vacuolar and exhibit slender cytoplasmic processes. An eosinophil is seen touching the neutrophil processes (arrow). X 5,700

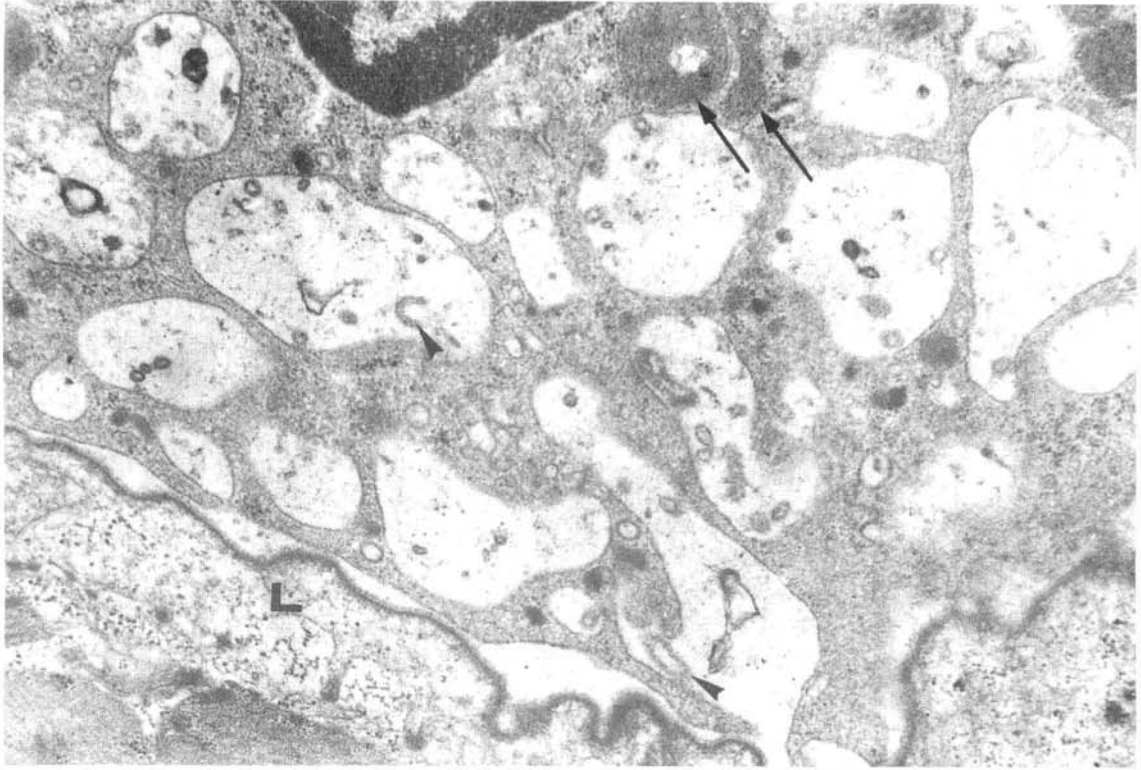
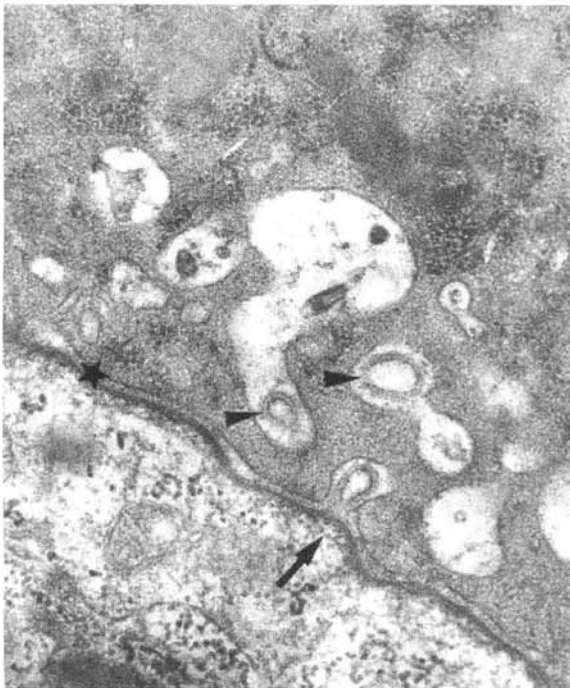


Fig. 2 - Detail of a highly vacuolar neutrophil adhered to the larva (L) surface exhibiting material similar to the glycocalyx (arrowheads) and debris in vacuoles and membrane folds. Note some neutrophil granules (arrows). X 27,000.



provide strong evidence for the participation of the neutrophils on the removal of the larval *glycocalyx*. However, it is not clear whether the neutrophils are responsible for the *glycocalyx* detachment or whether they withdraw fragments spontaneously shed by the parasite. This intense phagocytic activity contrasts with *in vitro* studies demonstrating that the rat neutrophils adherent to schistosomula exhibit phagocytic activity only when antigen-antibody complexes were present on the schistosomular surface<sup>11</sup>. The neutrophil phagocytic activity on the *S. mansoni* cercaria surface *in vivo* is a new finding that may be important as it indicates a role for neutrophils on the transformation process. Indeed, after losing the *glycocalyx*, the larvae now called schistosomula become resistant to cell mediated-killing<sup>5,14,20</sup>.

Fig. 3 - Detail of a larva-adhered neutrophil showing engulfment of cercarial glycocalyx (arrow) and the presence of glycocalyx fragments (arrowheads) inside the neutrophil vacuoles. Note the tight adhesion of the neutrophil to the larval glycocalyx (\*). X 40,000.

## RESUMO

### Interação entre neutrófilos e larvas de *Schistosoma mansoni* in vivo. Estudo ao microscópio eletrônico de transmissão.

Cercárias de *Schistosoma mansoni* foram inoculadas na cavidade peritoneal de camundongos normais e recuperadas 30 minutos depois. Estudos ultra-estruturais mostraram que neutrófilos aderem à superfície da larva, participando da remoção do glicocálice através de fagocitose. Este achado sugere que neutrófilos possam ter papel no processo de transformação de cercária em esquistossômulo.

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