Hemocyte production in Biomphalaria glabrata snails after exposure to different Schistosoma mansoni infection protocols

Produção de hemócitos de caramujos da espécie Biomphalaria glabrata após a exposição a diferentes protocolos de infecção por Schistosoma mansoni

Producción de hemocitos de caracoles de la especie Biomphalaria glabrata luego de la exposición a diferentes protocolos de infección por Schistosoma mansoni

ABSTRACT

The objective of this work was to determine the profile of the cellular defense system during mansonic infection. Specifically, this study assessed the number of hemocytes that were produced and released into the hemolymph in response to the parasitic infection. The quantification of the Biomphalaria glabrata hemocytes was performed on groups of snails at 1, 5, 10, 15, 20 and 30 days post-infection that had been individually infected with 5, 10, 15 or 30 Schistosoma mansoni miracidia. The results revealed that B. glabrata possesses a cellular defense mechanism that is characterized by the release of hemocytes into the hemolymph. The maximum peak of cellular production occurred 24 hours after infection, and there was a significant reduction in the hemocyte concentration over the following 10 days. However, at 15 days post-infection, there was a second increase in the cellular hemocyte production, although this was not as strong as the primary peak. At 30 days post-infection, there was another moderate rise in the cellular hemocyte production. Based on this cellular response profile, the defense system of the snail appears to be effective immediately following infection, but the response does not ensure the destruction of all parasites during the course of the infection.

Keywords: Biomphalaria; Schistosoma mansoni; hemocytes.

INTRODUCTION

The resistance of Biomphalaria snails to parasitic infection with Schistosoma mansoni is directly related to the hemocyte capacity of the vector to phagocytose and destroy newly penetrated parasites. The hemocytes are the mollusk’s main line of defense against parasites and bacteria, and these cells are believed to originate from the amoebocyte-producing organ, although some authors have suggested that these specialized cells may have a multicentric origin.

Miracidium encapsulation occurs immediately after parasite penetration and determines the life or death of the parasite in its host. Basch demonstrated that this process can be altered by numerous factors, such as the vector's nutritional state, the virulence of the parasitic strain, the hemocyte quantity in the hemolymph, certain direct or indirect environmental conditions and the number of miracidia that manage to penetrate the host.

The objective of the present study was to evaluate the hemocyte production profile of captive Biomphalaria glabrata snails after experimental infection with different numbers of S. mansoni miracidia. The characterization of the variability in hemocyte production is fundamental for understanding the resistance mechanisms of different Biomphalaria species for infection with S. mansoni.
MATERIAL AND METHODS

Brazilian albino (non-pigmented) and pigmented (wild type) strains of B. glabrata differ in their susceptibility to S. mansoni infection. The B. glabrata specimens used in this study descended from snails captured in the Bragantine region in Pará State, Brazil. This snail colony was kept in captivity and was bred in specialized tanks at the Laboratory of Intestinal Parasitosis and Malacology at the Instituto Evandro Chagas, (Pará State, Brazil). The S. mansoni strain used was extracted from infected mice at this laboratory, but it was initially isolated from infected snails captured at freshwater reservoirs in the City of Belém, Pará State, Brazil.

The livers of the infected mice were macerated, filtrated through gauze and subjected to spontaneous sedimentation. The sediment was exposed to light for 45 min, and the miracidia were collected with micropipettes using a stereomicroscope (Zeiss, Stemi SV 11).

A total of 752 snails, which were approximately 8 months of age and had a shell diameter of 1.5 cm, were individually infected in glass vials filled with 2 mL of declorinated freshwater that contained 5, 10, 15 or 30 miracidia. The infection was considered successful if no miracidia were visualized in the recipient after 90 min of light exposure. Following this procedure, the mollusks were transferred to special aquariums that were labeled with their infection protocol. The control group consisted of 52 uninfected snails from the same initial colony.

Hemolymph samples (20μL) were collected by direct puncture from each snail at 1, 5, 10, 15, 20 and 30 days post-infection to evaluate the quantity of the circulating hemocytes. The samples were mixed with Turk’s solution (0.1% crystal violet in 1% glacial acetic acid) at a 1:1 ratio, and 5μL of the resulting solution were placed in each side of a Neubauer chamber. As a result, each hemolymph sample was analyzed in duplicate using an optical microscope (Leitz, Dialux 20 EB) at 100x and 400x magnification.

The results are presented as the mean values and the standard deviations. The differences between the values from each group were evaluated using an analysis of variance (ANOVA) and Student’s t test (α = 0.05). All statistical analyses were performed using the BioEstat 5.0 software (IDSM/MCT/CNPq).

RESULTS

Of the 752 infected snails, 8% died during the course of the infection, and there was no correlation found between the mortality rate and the number of miracidia used for the infection. As a result of this snail death, 710 snails were used for the quantification of circulating hemocytes. These snails were divided into 24 groups of approximately 30 individuals, and the control group consisted of 52 specimens. A summary of these results is presented in table 1.

There was a discrete increase (9.7%) in the circulating hemocyte count in the group that was infected with five miracidia 24 hours post-infection. At 5 days post-infection, there was a significant increase (41.6%) in the hemocyte count in the group infected with 10 miracidia compared to the control group. The hemocyte count continued to increase at 10 days post-infection, with a significant increase (58.4%) in the group infected with 15 miracidia. At 15 days post-infection, the hemocyte count continued to increase, with a significant increase (63.9%) in the group infected with 30 miracidia. The hemocyte count continued to increase at 20 days post-infection, with a significant increase (76.8%) in the group infected with 30 miracidia. At 30 days post-infection, the hemocyte count continued to increase, with a significant increase (82.6%) in the group infected with 30 miracidia.

Table 1– Hemocyte counts per group

<table>
<thead>
<tr>
<th>Time of infection</th>
<th>5 miracidia</th>
<th>10 miracidia</th>
<th>15 miracidia</th>
<th>30 miracidia</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>222.81 ± 99.8 (24)</td>
<td>442.33 ± 332.06 (29)</td>
<td>426.39 ± 218.59 (27)</td>
<td>393.57 ± 70.59 (14)</td>
</tr>
<tr>
<td>5 days</td>
<td>175.43 ± 89.91 (29)</td>
<td>248.25 ± 153.47 (20)</td>
<td>275.63 ± 138.89 (24)</td>
<td>250.26 ± 95.08 (29)</td>
</tr>
<tr>
<td>10 days</td>
<td>146.18 ± 58.44 (34)</td>
<td>172.18 ± 93.86 (39)</td>
<td>163.97 ± 84.12 (34)</td>
<td>208.36 ± 99.66 (32)</td>
</tr>
<tr>
<td>15 days</td>
<td>234.88 ± 178.18 (20)</td>
<td>343.79 ± 235.07 (29)</td>
<td>270.3 ± 140.89 (25)</td>
<td>276.72 ± 135.58 (29)</td>
</tr>
<tr>
<td>20 days</td>
<td>176.41 ± 79.84 (32)</td>
<td>170.17 ± 68.56 (29)</td>
<td>258.67 ± 107.45 (30)</td>
<td>369.38 ± 281.94 (24)</td>
</tr>
<tr>
<td>30 days</td>
<td>312.6 ± 181.04 (25)</td>
<td>267.59 ± 166.2 (28)</td>
<td>239.79 ± 137.73 (24)</td>
<td>236.96 ± 138.43 (28)</td>
</tr>
<tr>
<td>Mean</td>
<td>211 ± 59.49</td>
<td>274.05 ± 104.94</td>
<td>272.46 ± 85.72</td>
<td>289.21 ± 75.18</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (n) ; Control group = 192.26 ± 153.71 (52).
miracidia, but these values were not significant when they were compared with the control group. There were, however, very sharp increases in hemocyte production at 24 h post-infection in the groups that were infected with greater numbers of miracidia. The hemocyte production was increased by 130.07%, 121.78% and 104.71% in the groups receiving 10, 15 and 30 miracidia, respectively. After five days of infection, there was a significant reduction in the number of hemocytes in each of the snail groups, although the group that had been infected with five miracidia had a smaller production rate than the other experimental groups (p < 0.05).

At 10 days post-infection, there were the fewest numbers of circulating hemocytes in all groups, and the group that had been infected with 30 miracidia experienced the smallest reduction.

At 15 days post-infection, the circulating hemocyte count increased proportionally to the initial parasitic load, and this trend continued through day 20 post-infection. At day 30, the cellular production stabilized, and there were no significant differences between the experimental groups. These results are shown in figure 1.

\[ r = 0.99; p < 0.01 \]
\[ r = 0.91; p < 0.05 \]
\[ r = 0.97; p < 0.05 \]

Figure 1– The hemocyte quantity following different protocols of infection

After 24 h of infection, there was a strong inverse correlation between the circulating hemocyte count and the number of miracidia used for the infection \( (r = 0.99; p < 0.01) \). This relationship was inverted at day 10 post-infection \( (r = 0.91; p < 0.05) \) and was maintained until the last phase of the infection \( (r = 0.97; p < 0.05) \) (Figure 2).

DISCUSSION

Hemocytes, which are mobile amoeboid cells, are critical constituents of the Biomphalaria snail's defense against infection with S. mansoni miracidia\(^3\). These cells form the primary barrier against invading parasites and bacteria\(^1\); their cytoskeletal mobility and the adaptations of their plasma membranes encapsulate foreign organisms\(^1\), and these cells also cooperate with several humoral defense factors. There have been many studies that have attempted to elucidate the complex parasite/host interaction in this model\(^6,17,18,19\).
In this paper, we have evaluated variations in the circulating hemocyte counts of individual *B. glabrata* snails following exposure to different numbers of *S. mansoni* miracidia. The main finding of this study was that there was a significant increase in hemocyte production only when more than five miracidia penetrated the snail. This increase was followed by a dramatic decrease in the hemocyte count at 24 h, which persisted during the first 10 days of infection. At 15 days post-infection, a recovery in the hemocyte cell production was initiated, and the hemocyte level became stable at 30 days post-infection. Moreover, this was independent of the initial parasitic load. There was a strong negative correlation between the hemocyte count and the number of miracidia used for the infection at 24 h post-infection, but this relationship was inverted at 10 and 20 days post-infection.

After 24 h of infection, the mean increase in the number of circulating hemocytes was 93.11%. Stumpf and Gilbertson\(^{19}\) reported a twofold increase in the *B. glabrata* hemocyte count at 2 h after exposure to the miracidia. Similar results were obtained by Joky et al.\(^{20}\) and Jeong et al.\(^{\text{a}}\); there was an increase in the *B. glabrata* hemocyte count immediately following exposure to a smaller number of miracidia, which was followed by the peak of the cellular production on the third and fourth days and a marked decrease in the hemocyte counts on the sixth and the seventh days. Martins-Souza et al.\(^{21}\) characterized the live hemocytes present after schistosomal infection using a cytomeric analysis and identified the peak hemocyte production to be 24 h post-infection.

These data support our results in regard to the rise in the hemocyte count during the first hours after miracidia penetration, as well as to the subsequent decline in the circulating hemocyte numbers.\(^{22-24}\)

This study has also demonstrated that following exposure to *S. mansoni*, the hemocyte level in the hemolymph was maintained to a greater extent than in the control group and for a longer period of time than that reported by previous papers.\(^{25}\) This finding supports the potential existence of a long-term effect on the hemocytes, which most likely consists of humoral factors, after the snail has reacted to the invading organisms.

Our study has also demonstrated that the number of infectious miracidia is a deterministic factor for the magnitude of the cellular response to schistosomal infection. For example, the number of hemocytes produced in the first 24 h after infection was much greater in mollusks infected with 10, 15 or 30 miracidia than in those infected with five of these larvae.

In regard to the dynamics of the snail’s hemocytic cellular response to the invading miracidia, previous papers\(^{9,10}\) have reported that the hemocytes interact with or adhere to the parasite’s surface 3 h after the penetration of the larvae. Seven and a half hours after this contact, the hemocytes phagocytize the microvilli on the parasite surface. Twenty-four hours later, there is great hemocyte activity, and encapsulation processes and the formation of large phagosomes have been observed. Forty-eight hours later, capsules are found along with large numbers of hemocytes that contain numerous phagosomes. On the fourth day, however, these capsules are much scarcer.

The results obtained by Cheng and Garrabrant\(^{12}\) on two different strains of *B. glabrata* (PR-albino and 10-R2) infected by *S. mansoni* demonstrated that the hemolymph acid phosphatase levels increased eightfold after 24 h of infection with the 10-R2 strain when compared to the non-infected control group, but that there was only a discrete increase in these levels at the same time period for the group infected with the PR-albino strain. In regard to the hemocyte numbers, the results were completely reversed. The PR-albino strain induced a peak of hemocyte production during the first 12 h of the infection, which was immediately followed by a marked reduction in the circulating hemocyte numbers, while the 10-R2 strain did not significantly alter the hemocyte count during the infectious process, which indicated that acid phosphatases may have an important role in the regulation of hemocyte production following parasite exposure.

**CONCLUSION**

The dynamics of the phagocytosis process, which were observed through the fourth day post-infection, suggest that the mobilization of these defensive cells is only effective during the first phase of the infectious process after a large number of parasites have penetrated the snail. Moreover, this mobilization does not guarantee the elimination of the larvae during the course of the infection. The mollusk’s defense response against the parasitic larvae could be more effective if it were not only regulated by a cellular process, but also by biochemical mechanisms that would directly stimulate the hematopoietic organs to produce more hemocytes.

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RESUMO
O objetivo deste artigo foi determinar o perfil do sistema de defesa celular durante a infecção mansônica. Especificamente, este estudo avaliou o número de hemócitos produzidos e liberados na hemolinfa em resposta à infecção pelo parasita. A quantificação dos hemócitos de Biomphalaria glabrata foi realizada em grupos de caramujos previamente infectados com 5, 10, 15 ou 30 miracidíos de Schistosoma mansoni nos dias 1, 5, 10, 15, 20 e 30 pós-infecção. Os resultados revelaram que B. glabrata possui um mecanismo de defesa celular caracterizado pela liberação de hemócitos na hemolinfa. O maior registro de produção celular ocorreu 24 h após a infecção e houve uma redução significante na concentração de hemócitos durante os 10 dias seguintes. No entanto, no dia 15 pós-infecção, houve um segundo aumento na produção de hemócitos, porém não tão acentuado como o primeiro pico. No dia 30 pós-infecção, foi observado outro aumento moderado da produção de hemócitos nas células. Com base neste perfil de resposta celular, o sistema de defesa do caramujo apresenta ser eficiente nos momentos imediatamente posteriores à infecção, mas essa resposta não assegura a destruição de todos os parasitas no curso da infecção.

Palavras-chave: Biomphalaria; Schistosoma mansoni; hemócitos.

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RESUMEN
El objetivo de este artículo fue el de determinar el perfil del sistema de defensa celular durante la infección mansónica. Especificamente, este estudio evaluó el número de hemocitos producidos y liberados en la hemolinfa como respuesta a la infección por el parásito. La cuantificación de los hemocitos de Biomphalaria glabrata se realizó en grupos de caracoles previamente infectados con 5, 10, 15 o 30 miracidios de Schistosoma mansoni en los días 1, 5, 10, 15, 20 y 30 pos infección. Los resultados revelaron que B. glabrata posee un mecanismo de defensa celular caracterizado por la liberación de hemocitos en la hemolinfa. El mayor registro de producción celular ocurrió 24 h luego de la infección y hubo una reducción significante en la concentración de hemocitos durante los 10 días siguientes. Sin embargo, al 15º día pos infección, hubo un segundo aumento en la producción de hemocitos, aunque no tan acentuado como el primer pico. Al 30º día pos infección, se verificó otro aumento moderado de la producción de hemocitos. Con base en este perfil de respuesta celular, el sistema de defensa del caracol aparente ser eficiente en los momentos inmediatamente posteriores a la infección, pero esa respuesta no asegura la destrucción de todos los parasitos en el curso de la infección.

Palabras clave: Biomphalaria; Schistosoma mansoni; hemocitos.

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