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An Update on Hemocytes in *Biomphalaria* Snails

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Abstract :

The hemocyte is a major immunological cell of molluscs. Much of the immunological phenomena associated with molluscan immunology can be attributed to cellular immunity associated with these cells suspended in the hemolymph. These cells are often referred to as amoebocytes or hemocytes. Such cells are of great importance to immune mechanisms associated withBiomphalaria snails. The Biomphalaria snail is the main vector of the important trematode parasite Schistosoma mansoni. This is a waterborne parasite that affects about 200 million people globally and puts countless other millions at risk of infection. Larval stages of the parasite are released from the snail in tainted waters and the larval cercarial stage actively penetrates the skin of humans and other vertebrates. Larvae migrate via the venous system to vital organs associated with the heaptic portal and mesenteric blood vessels. Larvae develop into sexually mature male and female adult worms that live in major venous blood vessels. The worms mate and produce eggs that lodge in major organs such as the spleen, liver, and intestines. Eggs produce extensive granulomas that cause cirrhosis and other pathological conditions in the affected organs.

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Introduction:

The hemocyte is a major immunological cell of molluscs. Much of the immunological phenomena associated with molluscan immunology can be attributed to cellular immunity associated with these cells suspended in the hemolymph. These cells are often referred to as amoebocytes or hemocytes. Such cells are of great importance to immune mechanisms associated with *Biomphalaria* snails. The *Biomphalaria* snail is the main vector of the important trematode parasite *Schistosoma mansoni*. This is a waterborne parasite that affects about 200 million people globally and puts countless other millions at risk of infection (Lewis and Tucker, 1). Larval stages of the parasite are released from the snail in tainted waters and the larval cercarial stage actively penetrates the skin of humans and other vertebrates. Larvae migrate via the venous system to vital organs associated with the heaptic portal and mesenteric blood vessels. Larvae develop into sexually mature male and female adult worms that live in major venous blood vessels. The worms mate and produce eggs that lodge in major organs such as the spleen, liver, and intestines. Eggs produce extensive granulomas that cause cirrhosis and other pathological conditions in the affected organs. A recent book on *"Biompalaria Snails and Larval Trematodes"* (Fried and Toledo, 2) is concerned with all aspects of the biology of these snails. The chapter in that book of great interest to this review is the one by Yoshino and Coustau (3) on the *"Immunobiology of Biomphalaria-trematode Interactions."* That chapter provides excellent coverage on hemocytes up to about the year 2010. The purpose of this review is to update the rapidly growing literature in the field, particularly on hemocytes and related phenomena, from about 2010 to the present. My coverage is to 11 papers in this time frame and is given in reverse chronological order (from the latest to the earliest salient references). Because all of these papers refer to hemocytes either directly or tangentially, I have found no need to arrange this short review according to topics.

Biomphalaria glabrata and *B. straminea* serve as intermediate hosts for *Schistosoma mansoni*. Several studies reported two cell types in the hemolymph of *B. glabrata* (hyalinocytes and granulocytes). There are no studies describing the hemocytes of *B. straminea*. With the aim of describing the hemocytes of *B. glabrata* and *B. straminea* (Cavalcanti *et al.*, 4), we conducted a detailed study using optical microscopy and transmission electron microscopy. Based on the morphological characteristics of the cells, they identified the same types of hemocytes in these two species of molluscs, i.e., blast-like cells, granulocytes, type I hyalinocytes, type II hyalinocytes and type III hyalinocytes. Blast-like cells had a spherical profile with a central nucleus filling most of the cell. Granulocytes contained a variable numbers of granules. Type I hyalinocytes were the most abundant cell type and had various cytoplasmic projections. Type II and type III hyalinocytes were not reported previously; they were few in number and contained eccentric nucleus. The authors concluded that there are five types of cells in the hemolymph of *B. glabrata* and *B. straminea* and suggested that further studies are needed to identify the role of hemocytes in the immune response of biomphalarid snails.

Bakry *et al.* (5) did this study to evaluate the immunological and physiological responses of *Biomphalaria alexandrina* snails to the effect of methanol extracts of *Azadirachta indica* plants. In this study, haemolymph samples were collected from snails treated with LC25 from methanol extracts for 1 month and untreated snails. The collected hemolymph samples from treated and untreated snails with tested plant were used for flow cytometric analysis of the cell cycle. The results indicated that hemolymph samples from *B. alexandrina* snails contained two morphologically distinct types of hemocytes, designated as hyalinocyte and granulocyte cells. The number of hyalinocytes and

granulocytes in both snails and the mortality rates were significantly increased following treatment with *A. indica* extract. Phagocytosis in the group treated with the tested plant was significantly increased than the control one indicating a high increased response of the snails against the treatment. The lipid peroxide and glucose levels in the hemolymph of treated snails were elevated while the protein and glycogen contents showed a decrease in soft tissues when compared with the control group. Additionally, the activity level of some enzymes representing glycolytic enzymes such as hexokinase (HK), pyruvate kinase (PK), phosphofructokinase (PFK), lactate dehydrogenase (LDH),and glucose phosphate isomerase (GPI); glycogenolytic enzymes as glycogen phosphorylase, glucose-6-phosphatase (G-6-Pase); gluconeogenic enzymes as fructose-1-6 diphosphatase (F-D-P ase), phosphoenolpyruvate carboxykinase (PEPCK) were also significantly reduced in response to treatment. In conclusion, the authors noted that the application of methanol extracts of *A. indica* plants may be helpful in snail control since it interferes with the immunology and physiology of the snails.

In Egypt, *Biomphalaria alexandrina* is the intermediate host of *Schistosoma mansoni*. The outcome of infection by *Schistosoma miracidia* in the snails varies between different species of *Biomphalaria*. The internal defense system is one factor that influences the susceptibility pattern of the snails. The interaction between *Biomphalaria* snails and *S. mansoni* needs to be identified for each species, and even between the members of the same species with different degrees of susceptibility. In this study, Abou-El Naga (6) studied the first generation of susceptible and resistant parents of *B. alexandrina* by histological means at 30 days post exposure. Their study included a characterization of the immune response, as expressed by tissue reactions, of susceptible and resistant *B. alexandrina* snails against *S. mansoni*. Their study was also designed to determine the impact of the resistance increase in parent snails, on the mechanisms of interaction of their offspring against infection. Their results showed that the infection rate of the offspring from the susceptible parents was 92%. No susceptible offspring was produced from the resistant parents. When the parents were of equal number of susceptible and resistant snails, they gave an offspring with an infection rate of 20%. Susceptible snails that had susceptible parents showed a higher degree of susceptibility than those that had both susceptible and resistant parents. A common feature of the resistant snails was the absence of any live trematodes. The tissue reactions of the resistant snails having only resistant parents occurred at the site of miracidial penetration. In resistant snails for which susceptible ones were included in their parents, the reactions occurred in the deep tissues. The authors' results characterized the immune response of *B. alexandrina* snails against *Schistosoma* infection which occurred by two different mechanisms. One type of defense occurred in highly resistant snails, and used direct miracidial destruction soon after larval penetration. The other type occurred in less resistant snails where a delayed resistance development occurred after the spread of sporocysts in the snail tissues. The *B. alexandrina* snails responded similarly to *B. glabrata*. The results of the study also showed that the immune response of the internal defense system increased with an increased number of the inherited resistant genes.

Schistosomiasis is a parasitic disease that is highly prevalent, especially in developing countries. Nacif-Pimenta *et al*. (7) noted the importance of *Biomphalaria tenagophila* is a major vector of *Schistosoma mansoni* in Brazil. They also noted that some strains (e.g. Cabo Frio) are highly susceptible to the parasite, whereas others (e.g. Taim) are resistant to infection. *B. tenagophila* is an important research model for studying immune defense mechanisms against *S. mansoni*. The internal defense system (IDS) of this snail consists of hemocytes and hemolymph factors acting

together to recognize self from non-self molecular patterns to eliminate the infection. The authors did experiments to understand the cellular defenses related to resistance or susceptibility of *B. tenagophila* to *S. mansoni*. During the early stages of infection, fibrous host cells of both snail strains were arranged as a thin layer surrounding the sporocysts. At later stages of infection, the cellular reactions in resistant snails were considerably more intense, with thicker layers surrounding the larval trematodes, in contrast to the susceptible strains. All trematodes were damaged or destroyed inside the resistant snails after 10 hrs of infection. In contrast, larval trematodes inside susceptible snails were morphologically healthy. The authors did experiments on isolated hemocytes from the two strains that interacted with sporocysts. Hemocyte attachment started as early as 1 hr after initial infection in both strains, but the killing of sporocysts was exclusive to hemocytes from the resistant strain and was time course dependent. The resistant snail strain was able to kill all sporocysts. The authors concluded that their study showed important aspects of the initial process of infection related to immune defense responses of strains of *B. tenagophila* that were resistant to *S. mansoni* compared to strains that were susceptible. They noted that information is relevant to the survival or death of the trematodes and therefore is important in the development of control measures against *S. mansoni*.

Previous studies have reported that the trematode *Plagiorchis elegans* decreased the fecundity and survivorship of the incompatible snail host *Biomphalaria glabrata*. A prior infection with *P. elegans* made the snails resistant to the compatible trematode *Schistosoma mansoni*. In this study, Daoust *et al*. (8) tested the hypothesis that infection with *P. elegans* stimulates the immune system of *B. glabrata*. The authors' indicated that infection with *P. elegans* significantly increased the number of free hemocytes in the hemolymph of *B. glabrata* by an average of about 60%. This immuno-stimulated state lasted from the first day post-infection (PI) to between 7 and 21 days PI. This is one of a few studies that indicated that a trematode could stimulate the immune response of an incompatible host.

Mohamed AH (9) did this study to elucidate the cellular mechanisms of *Biomphalaria alexandrina* snail hemocytes against sublethal concentration (10 mg/L) of the herbicide Roundup (48% Glyphosate) and/or *Schistosoma mansoni* infection during 7 days of exposure. The results of the study indicated that herbicide treatment and/or infection led to a significant increase ($P<0.05$) in the total hemocyte counts during the exposure period. Examination of hemocyte monolayers resulted in the observation of 3 morphologically different cell types, i.e., round small, hyalinocytes and spreading hemocytes. The spreading hemocytes was the dominant, more responsive, and highly phagocytic cell type in all the experimental groups. Additionally, the exposure to herbicide, infection, or both together, led to a significant increase ($P<0.05$) of in vitro phagocytic activity against yeast cells during 7 days of exposure. Moreover, flow cytometric analysis of cell cycle and comet assay, resulted in DNA damage to the *B. alexandrina* hemocytes exposed to herbicide and/or *S. mansoni* infection when compared to the control group. The immunological responses as well as the molecular aspects in *B. alexandrina* snails have been suggested to be biomarkers of exposure to environmental pollutants.

Mohamed *et al.* (10) studied snail susceptibility to infection with *Schistosoma mansoni* by observing infection rates, total cercarial production and tissue responses of the first generation (F1) of *Biomphalaria alexandrina* snails, originally collected from different Egyptian governorates (Giza, Fayoum, Kafr El-Sheikh, Ismailia and Damietta). Responses were compared between groups. The emergence of cercariae for a 3-

month period and the calculation of survival and infection rates, in control (Schistosome Biological Supply Center; SBSC) and infected snails were evaluated. SBSC and Giza snails showed greater susceptibilities to infection and lower mortality rates. Additionally, at 6 and 72 hr post- miracidial exposure all the snail groups showed no differences in the anatomical locations of sporocysts. These larvae were found in the head-foot, the mantle collar and the snail tentacles. Sporocysts showed normal development with low tissue reactions in SBSC and Giza snail groups infected with *S. mansoni* miracidia. In Fayoum, Kafr El-Sheikh, Ismailia and Damietta snail groups, variable tissue responses were observed in which numerous hemocytes made direct contact with *S. mansoni* larvae and formed capsules. The findings of this study suggested that different responses of *B. alexandrina* snail hemocytes towards *S. mansoni* are related to the degree of susceptibility of these snails. This information is important in planning the strategy of schistosomiasis control.

Ottaviani *et al*. (11) noted that the circulating phagocytic immune cell is considered to be the main effector of the invertebrate defense system, including in molluscs, is involved in both immune and neuroendocrine responses. These cells show functional characteristics of vertebrate macrophage. Various names have been used for these cells in different taxa i.e., hemocyte, celomocyte, amebocyte, and plasmatocytes. Regardless of the terminology, these cells perform the same immune function, and possess very similar morphology. For these reasons, it is suggested that the general term immunocyte be used to describe these cells in invertebrates.

de Mattos, Alves Ana Carolina (12) noted that the outcome of the interaction between *Biomphalaria* and *Schistosoma mansoni* depends on the response of the host internal defence system (IDS) and the escape mechanisms of the parasite. The aim of their study was to evaluate the responsiveness of the IDS (hemocyte and soluble hemolymph factors) of resistant and susceptible *Biomphalaria tenagophila* and *Biomphalaria glabrata* lineages in the presence of in vitro-transformed primary sporocysts and secondary sporocysts obtained from infected *B. glabrata*. To do this, they assayed the cellular adhesion index (CAI), analysed viability/ mortality, used fluorescent markers to evaluate the tegumental damage and transplanted secondary sporocysts. *B. tenagophila*, Taim strain was more effective against primary and secondary sporocysts than the susceptible lineage and *B. glabrata*. Compared with secondary sporocysts exposed to *B. tenagophila*, primary sporocysts showed a higher CAI, a greater percentage of dead sporocysts and were labelled by lectin from Glycine max and Alexa-Fluor 488 fluorescent probes at a higher rate than the secondary sporocysts. However, the two *B. tenagophila* lineages showed no cercarial shedding after inoculation with secondary sporocysts. The authors' hypothesis that secondary sporocysts can escape the *B. tenagophila* IDS cannot be confirmed by the transplantation experiments. Their data suggested that additional mechanisms are involved in the lower susceptibility of *B. tenagophila* to *S. mansoni* infection.

Garcia et al. (13) identified and characterized a Macrophage Migration Inhibitory Factor (MIF) in *Biomphalaria glabrata*. In mammals, MIF is a widely expressed pleiotropic cytokine with potent pro-inflammatory properties that controls cell functions such as gene expression, proliferation or apoptosis. The authors showed that the MIF protein from *B. glabrata* (BgMIF) is expressed in circulating immune defense cells (hemocytes) of the snail as well as in the *B. glabrata* embryonic (Bge) cell line that has hemocyte-like features. Recombinant BgMIF (rBgMIF) induced cell proliferation and inhibited NO-dependent p53-mediated apoptosis in Bge cells. Moreover, knock-down of BgMIF expression in Bge cells interfered with the *in vitro* encapsulation of *S. mansoni* sporocysts. The *in vivo* knock-down of BgMIF also prevented the changes in

circulating hemocyte populations that occur in response to an infection by *S. mansoni* miracidia and led to a significant increase in the parasite burden of the snails. The results of the Garcia *et al*. (13) study provide the first functional evidence that a MIF ortholog is involved in an invertebrate immune response towards a trematode infection and highlight the importance of cytokines in snail-parasite interactions.

Oliveira *et al*. (14) studied the variation in the number of hemocytes for 24 hr after exposing larval trematodes to various strains of *Biomphalaria tenagophila*, susceptible or not to infection with *Schistosoma mansoni*. The differences were analyzed in regard to the variations in the number of hemocytes in molluscs susceptible or not to infection by *S. mansoni*. The hemolymph of the selected and non-selected snails was collected, and hemocytes were counted using a Neubauer chamber at six designated periods: 0 hr (control, non-exposed individuals), 2 hr, 6 hr, 12 hr, 18 hr and, 24 hr after larval trematode exposure. Samples of hemolymph of five selected snails and five non-selected snails were separately used at each counting time. There was a significant variation in the number of hemocytes between the strains, which indicated that defense cells have different behaviors in resistant and susceptible snails.

This review has covered the literature until 2012. Additional studies on this topic have appeared from 2013 to 2015. Rather than name the new studies I am referring the reader to a very significant review by Coustau et al (15). This review provides good coverage of the papers that were published after my review was submitted.

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