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Preliminary observations on a hemolymph factor influencing the infectivity of *Schistosoma mansoni* miracidia*

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Summary

It has been demonstrated that the infectivity of *Schistosoma mansoni* miracidia for a highly susceptible strain of *Biomphalaria glabrata* was reduced markedly when the miracidia were pretreated with hemolymph from the snail *Helisoma caribaeum*. Although the *Helisoma* hemolymph factor(s) responsible for this phenomenon has not been identified with certainty, it appears to be a snail lectin associated with binding sites for n-acetyl-D-glucosamine on the surface of the miracidium. Hemolymph from uninfected and infected *B. glabrata* and from a resistant strain of *B. glabrata* did not reduce the infectivity of miracidia, nor did pretreatment with bovine plasma albumin. Likewise, we demonstrated that reduction of miracidial infectivity was not due to the presence of a miracidial immobilizing factor found in the hemolymph of many snail species. Differences in the infectivity between pretreated miracidia and controls were statistically significant ($P = 0.001$).

Key words: *Biomphalaria glabrata*; *Helisoma caribaeum*; miracidial infectivity; *Helisoma* hemolymph factor; snail lectins.

Introduction

It is generally recognized that the principal mean by which molluscs defend themselves against the insults imposed by parasites, microbial pathogens, and

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foreign bodies is through a series of cellular reactions (Michelson, 1975; Cheng, 1979). The role of humoral factors as a component of the overall defense mechanism is not clearly defined, although it is generally accepted that molluscs do not contain serum immunoglobulins, cannot produce antibodies to specific antigens, and show no evidence of immunologic memory (Bang, 1973; Cheng, 1979). However, in several species of molluscs, the hemolymph was found to contain substances which function as agglutinins, lysins, and opsonins (Gilbertson and Etges, 1967; Anderson and Good, 1976; Pemberton, 1974; Michelson and DuBois, 1977; Simina et al., 1979).

In a compatible snail host, the invading miracidia are recognized as "self" and elicit no cellular response unless dying or dead (Pan, 1965). However, in non-host species or in incompatible strains of a host species, the miracidia are quickly attacked by amoebocytes, surrounded by a fibrotic capsule, and soon destroyed (Newton, 1952; Sudds, 1960; Cheng and Garrabrant, 1977; Bayne et al., 1980). Lie et al. (1975) demonstrated that it was possible to alter the susceptibility in certain strains of *Biomphalaria glabrata* to infection with *Schistosoma mansoni* by pre-exposure of the snails to irradiated echinostome miracidia. Susceptible strains of *B. glabrata* may also be rendered resistant to infection with *S. mansoni* by exposing the snails, prior to miracidial challenge, to gamma-irradiation (Michelson and DuBois, 1981). The present report deals with the reduction in infectivity of *S. mansoni* miracidia for a highly susceptible strain of *B. glabrata* as a consequence of pretreatment of the miracidia with *Helisoma* hemolymph.

Materials and Methods

Miracidia used in this study were obtained from the livers of white mice infected, for 6–8 wk, with a Puerto Rican strain of *Schistosoma mansoni*. Snail hemolymph was collected, in accordance with techniques previously described (Michelson, 1966), from two strains of *Helisoma caribaeum* (257, CB) and from non-infected and infected *Biomphalaria glabrata*.

The experimental procedure for testing the treated miracidia was as follows: From a pool of miracidia, 5 were collected by means of a fine-tipped Pasteur pipet and deposited, in a small drop of filtered aquarium water, into a well of a plastic tissue culture plate (Linbro, Flow Lab., Inc.). To each well, 0.2 ml of *Helisoma* hemolymph was added and the miracidia were allowed to swim in the mixture for 10 min. At the end of this period, 0.8 ml of filtered aquarium water was added to each well. A 5–6 mm *B. glabrata* was then deposited into each well and allowed to remain in contact with the miracidial suspension for 3 h. The snails were transferred to 1 liter beakers, held for 14 days, and then crushed and examined for the presence of sporocysts. Each test was controlled by snails exposed to non-treated miracidia. For comparative purposes, miracidia were treated with hemolymph from normal and infected *B. glabrata* and with 0.1% bovine plasma albumin. Each test trial usually consisted of 10 test snails and 10 control snails. The strain of *B. glabrata*, PR-1, used in these experiments was highly susceptible to the strain of *S. mansoni* employed and was known to have a mean infection rate in excess of 85%.

The presence of miracidial immobilizing substances in the *Helisoma* hemolymph was tested in accordance with the techniques described by Michelson (1964). To ascertain if the *Helisoma* hemolymph factor (Hhf) was a lectin, we employed the mixed agglutination assay described by Yoshino et al. (1977). Miracidia were first immobilized by the addition of 2.5 volumes of 1.5% saline to the miracidial suspension, and then concentrated by centrifugation (150 g/5 min/–5°C). The concentrat-

ed miracidia were fixed in 1% glutaraldehyde in Chernin's Handling Solution (Chernin, 1963) for 12–18 h, at 5°C. The fixed miracidia were washed (5×) in phosphate buffered saline (pH 7.4) and stored at 5°C until required. To assay for lectin binding, fixed miracidia were placed in 2 volumes of *Helisoma* hemolymph for 1 h at 25°C, washed in phosphate buffered saline (5×), and then incubated (1 h at 37°C) in an equal volume of a 0.5% suspension of either human "A" or "O" erythrocytes. The miracidial-erythrocyte suspension was centrifuged (150 g/5 min) and the pellet resuspended in a small volume. A drop was removed to a glass slide and examined microscopically to determine if the erythrocytes agglutinated to the miracidia. Controls consisted of fixed miracidia which had not been pretreated with hemolymph, but were handled in a similar manner. In addition, the agglutinin titer of the hemolymph was determined with erythrocytes similar to those used in the assay and in accordance with the techniques described by Michelson and DuBois (1977).

Results

Our study demonstrated that the infectivity of *S. mansoni* miracidia, pre-treated with *Helisoma* hemolymph, was greatly reduced. The reduction in infectivity of pretreated miracidia was not due to failure of penetration, since active penetration was observed by microscopic examination. In a series of 8 experimental trials (Table 1), a total of 88 test snails survived and 48 (54.5%) were infected; whereas, 91 (94.8%) of 96 control snails were infected. When a t-test for paired data was applied to these trials, the differences between the two groups was found to be highly significant ($P = <0.001$). Trials which employed hemolymph from either normal or *S. mansoni* infected host snails, hemolymph from a strain of *B. glabrata* (S-3) resistant to *S. mansoni* infection, and trials in which 0.1% albumin was used instead of hemolymph, revealed no difference in the infectivity of miracidia so treated and those employed as controls (Table 2).

Miracidia held in *Helisoma* hemolymph for one hour and examined microscopically showed no evidence of shedding their ciliary plates. In addition, no evidence was found to suggest that the Hhf behaved as a miracidial immobilizing substance. In fact, it was observed that treated miracidia continued to

Table 1. Effect of pretreatment of *S. mansoni* miracidia with *Helisoma* hemolymph and their infectivity for *B. glabrata*

Trial	Controls No. and percent infected	Hemolymph treated No. and percent infected
1	10/10 (100%)	4/10 (40%)
2	8/10 (80%)	6/10 (60%)
3	17/18 (94.4%)	8/15 (53.3%)
4	19/20 (95%)	8/13 (61.5%)
5	8/9 (88.9%)	5/7 (71.4%)
6	9/9 (100%)	9/16 (56.3%)
7	10/10 (100%)	4/7 (57.1%)
8	10/10 (100%)	4/10 (40%)

t = 7.41 for 7 degrees of freedom

P = 0.001

Table 2. The infectivity of *S. mansoni* miracidia pretreated with selected snail hemolymphs and other proteins. Summary of experiments

Test group	Treated No. and percent infected	Controls No. and percent infected
Hemolymph, normal PR-1 <i>B. glabrata</i>	15/17 (88.2%)	18/19 (94.7%)
Hemolymph, infected PR-1 <i>B. glabrata</i>	4/5 (80%)	8/8 (100%)
Hemolymph, resistant S-3 <i>B. glabrata</i>	7/8 (87.5%)	10/10 (100%)
0.1% Bovine albumin	31/37 (83.8%)	21/23 (91.3%)

swim in an active manner and would penetrate the host snails. In 3 of 5 snails, in which treated miracidia were observed to have invaded the tissues, serial sections revealed that a fibrous reaction was initiated around the miracidia as early as 36 h post-infection.

Results of the mixed agglutination assay demonstrate that the agglutinin(s) previously detected in the hemolymph of *Helisoma* species (Michelson and DuBois, 1977) bound to receptor sites on the miracidia. When human "O" type erythrocytes were used in the assay, miracidia had 1+ to 3+ reactions. The *Helisoma* hemolymph used to pre-treat the miracidia gave 3+ to 4+ reactions at a titer of 1:256 against human "O" erythrocytes.

Discussion

Although there is no evidence that the host snails of schistosomes possess an immune system comparable to that found in higher animals, they do have the ability to discriminate between "self" and "non-self" (Tripp, 1961; Cheng and Galloway, 1970; Chorney and Cheng, 1980). The Hhf described in the present study appears to have the ability to significantly alter *S. mansoni* miracidia so that they appear to the compatible snail host as "non-self". The nature of the Hhf has not been defined at this time; however, evidence that the lectin(s) present in the hemolymph of *Helisoma* bind to reactive sites on the miracidia suggest that these substances may, in fact, be the Hhf. Yoshino et al. (1977) have demonstrated that *S. mansoni* miracidia bound *Dolichos* lectin and had receptor sites for n-acetyl-D-galactosamine. Earlier we reported (Michelson and DuBois, 1977) that the lectin(s) present in the hemolymph of *Helisoma* species exhibited a strong specificity for n-acetyl-D-galactosamine. Although only a weak agglutination response in treated miracidia was observed in the present study, it appeared comparable to that observed by Yoshino and his associates. The failure of miracidia treated with hemolymph from the S-3 refractory strain

of *B. glabrata* to become altered does not negate the premise that Hhf may be a lectin. It has been demonstrated (Michelson and DuBois, 1977; Abdul-Salam and Michelson, 1980) that the lectin(s) present in *B. glabrata* are quite different from those of *Helisoma*. *B. glabrata* lectin(s) show specificity for n-acetyl-D-glucosamine, glucose and a variety of other sugars, but not for n-acetyl-D-galactosamine. Likewise, they agglutinate human "A" and "B" cells, but not "O" and are found in the albumin gland and in eggs as well as in the hemolymph; whereas *Helisoma* lectin(s) are found only in the hemolymph and agglutinate all types of human erythrocytes. Moreover, susceptible and refractory strains of *B. glabrata* appear to have lectins which react in a similar manner with human erythrocytes.

How Hhf functions in reducing the infectivity of miracidia has not been determined. Evidence suggests that the Hhf is not a miracidial immobilizing substance. In some molluscan species it has been shown that lectins (i.e., agglutinins) may serve as opsonins and increase the phagocytic activity of the amoebocytes (Anderson and Good, 1976; Pauley et al., 1971). In *B. glabrata*, however, Abdul-Salam and Michelson (1980) were not able to demonstrate enhancement of phagocytosis in amoebocytes by the addition of homologous albumin gland-derived agglutinin. This does not rule out the possibility that heterologous *Helisoma* agglutinins may stimulate *B. glabrata* amoebocytes. It must also be remembered that other substances in the hemolymph, other than lectins, may serve as opsonins. In this regard, Sminia et al. (1979) have demonstrated a hemolymph factor in the snail *Lymnaea stagnalis* which enhances phagocytosis by amoebocytes, although most strains of *L. stagnalis* and other species of *Lymnaea* appear to lack agglutinins or show only slight reactivity with enzyme-modified erythrocytes (Lee-Potter, 1969; Pemberton, 1974). On the other hand, reaction to the pretreated miracidia may have nothing to do with opsonins, but merely reflect a non-specific response to a foreign protein with which the miracidia are coated. A definitive answer to the nature of the Hhf will require its isolation and purification.

Continued efforts to elucidate the factors which initiate defense responses in host snails and influence susceptibility will contribute to our understanding of the host-parasite relationship.

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